Prenatal Stress and Vagal Tone in Infancy

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Abstract

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Background. The fetal origins hypothesis poses that adverse intrauterine conditions predispose to cardiovascular and metabolic diseases in adulthood. Evidence is accumulating that similar mechanisms to those identified for physical disorders may also apply to psychiatric disorders. Focusing on the activity of neurophysiological systems thought to regulate emotions from very early in life may be key to understanding how maternal stress in pregnancy impacts on the developing baby with possible long-lasting consequences for behaviour and psychopathology. Respiratory Sinus Arrhythmia (RSA), "vagal tone" is thought to reflect autonomic regulatory capabilities that may underpin emotion regulation. However, little is known about possible fetal origins of vagal tone. Animal studies increasingly point to sex differences in the effects of prenatal stress, and this is supported by human studies of the prenatal origins of cardiovascular functioning and psychopathology. The current investigation examines whether prenatal depression and anxiety predict vagal tone in infancy, and whether the associations are modified by infant sex.

Method. Two hundred mothers and infants from a high-risk consecutive community sample were examined prospectively from the first trimester of pregnancy until 29 weeks postnatal. Maternal self-reports of stress (EPDS and STAI) were collected in pregnancy (20 and 32 weeks) and postnatally (5 weeks and 29 weeks). Vagal tone was ascertained across five procedures, the "Helper-Hinderer" social evaluation task, toy exploration and the "Still Face" paradigm (2 minutes of social engagement, followed by 2 minutes of maternal unresponsiveness and concluded by 2 minutes of social reunion).

Results. Principal Component Analysis of the RSA scores yielded a one-factor solution explaining over 70% of the variance, and so mean of RSA scores was used as the index of overall vagal tone, and the difference between overall and RSA during the Still Face as the estimate of vagal withdrawal. There were no main effects of prenatal maternal depression or anxiety on vagal tone or vagal withdrawal. However, there were significant prenatal stress by sex of infant interactions. Follow-up analyses revealed that increasing maternal depression and anxiety at 20 weeks gestation were associated with decreasing vagal tone in males and increasing vagal tone in females. Vagal withdrawal in response to the still face showed similar patterns i.e. decreased in males and increased in girls with elevated maternal anxiety at 32 weeks gestation. These associations were not explained by possible confounding variables assessed in pregnancy, nor by postnatal maternal depression and anxiety.

Conclusions. The findings support the fetal origins hypothesis for vagal tone and vagal withdrawal, but only in interaction with sex of the infant. Longitudinal study is required to determine conditions under which increasing vagal tone and withdrawal in girls associated with prenatal depression and anxiety, and decreasing vagal tone and withdrawal in boys, are associated with later resilience or vulnerability to psychopathology.

Declaration

No portion of the work of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Copyright Statement

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Dedication

I would like to dedicate this PhD thesis to my dear wife Carmen, who has been my source of strength throughout this three-and-a-half year journey in spite of the tough times she has been through. Without her incredible and unconditional love, care and support, this dream would have never come true or even started.

I also dedicate this work to my parents, and dear Olga.

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Chapter 1

Background

1.1 Outline of the Structure of the Thesis

The main focus of this thesis is the study of the association between maternal stress during pregnancy and vagal tone functioning measured in the infant. Chapter 1 presents the theoretical background for the major topics of study together with a rationale for the examination of the proposed associations. First, the topic of prenatal stress is introduced and the newly emerged theory of fetal origins hypothesis (Barker, 1995) is presented as a possible model of development of psychopathology. This sets the scene for emphasising the importance of conducting such investigations like the current one. Then, some methodological considerations in human studies of prenatal stress are presented, followed by showing the existing human and animal evidence for the proposed link. Second, the construct of vagal tone is presented, embedded in its original theoretical model developed by Porges (1995) and known as the polyvagal theory. Evidence is then presented on links between vagal tone and behaviours and later psychopathology in human studies with infants and children. This is followed by a brief section on the rationale for including the vagal tone measure in a study which aims to link prenatal stress to behavioural and emotional problems in children. Third, the topic of sex differences in behaviours and psychopathology is introduced. The term *sex* is preferred to that of *gender* as it is considered to reflect more accurately the biological nature of the concept, rather than its psychological connotations, which is closer to the topic of the current investigation. Besides, most of the studies reviewed in this thesis seem to prefer using the term "sex differences". Because the issue of sex differences in studies of human psychopathology appears to be less established, corroborative evidence is presented from animal studies, as well as from human investigations in the connected field of cardiovascular correlates of acute stress in prenatally stressed individuals. Finally, at the end of the first chapter, the aim and hypotheses for the current study are listed.

Chapter 2 is the method section in which detailed information is presented about the design of the study, approach to statistical analyses, sample and the measures that were used. The design explains how the current study is part of a larger longitudinal investigation on the earliest origins of conduct problems in childhood. The statistical analyses section details the step-by-step approaches employed in the examination of longitudinal maternal stress data and the cross-sectional infant outcome variables and how predictions to outcomes are assessed. Then, the sampling procedure is described together with the inclusion criteria of the participants, followed by descriptions of the follow-up assessments and sample characteristics at each phase of the study. At the end of the section descriptions of all measures used in the study are provided.

The results of the current investigation are presented in chapters 3 and 4. Chapter 3 shows preliminary analyses of the variables of interest and basic associations between them and confounders. Also, a novel approach to analysing the vagal tone variables is presented. Chapter 4 focuses on testing the hypotheses of the study by examining predictions from indices of prenatal stress to the outcome variables, together with the role of sex of infant in the proposed link.

Finally, chapter 5 contains the interpretations of the findings in the context of the relevant human and animal literature, with a special focus on possible connections with later psychopathology. Also, the vagal tone findings are discussed in relation to previous infancy studies of vagal tone. Methodological strengths and limitations are presented too. At the end, directions for future research are discussed and conclusions of the study are drawn.

The bibliography is listed at the end of the thesis, together with appendices containing histograms with distributions of relevant data, copies of the ethical approval forms, consent forms, parent information sheets and the questionnaire measures that were used in the study.

1.2 Prenatal Stress and Links to Outcomes in the Offspring

1.2.1 Introduction to the Topic

There are writings dating back from ancient times which intuitively envisaged a connection between the mother's state during pregnancy and the physical and psychological health of the developing baby (Ferreira, 1965). Recent advances in the medical and psychological sciences have contributed to a revitalised interest in the topic of effects of prenatal stress and the past two decades have become relatively abundant in such studies with both animal and human subjects.

It is thought that a range of risks in pregnancy may have consequences for the fetus and the future baby and that some of these consequences can be long-lasting and spanning as far as into late adulthood. Both influences and effects can be physical and psychological and it is believed that variations even within normal range can have effects. There is evidence suggesting that more disruptive stressors may be associated with more severe outcomes. For example, life-threatening traumas such as famine and war have been linked with schizophrenia (Hoek et al., 1996) and congenital problems of the central nervous system (Stein, Susser, Saenger, & Moraolla, 1975). On the contrary, other studies (e.g. DiPietro, Novak, Costigan, Atella, & Reusing, 2006) have found that mild to moderate stress in pregnancy may associate with enhanced motor and cognitive performance. Although the existent evidence generally seems to support the idea that elevated risk is linked with less optimal outcomes at all ages, little is known about how specific risk factors and forms of stress may be associated with specific outcomes in the offspring. Also, manifestations of stress differ in animals and humans and it may be difficult to assess how useful the animal models are for the study of effects in humans.

Specifically, in animals, stress has typically been induced in the form of crowding, electric shocks, bursts of unexpected noise or flashes of lights, saline injections or restraint, or combinations of these stressors, while in humans some of the most studied symptoms have been feelings of depression and anxiety and life events.

Another important aspect of stress is its timing in pregnancy. Because different phases of fetal maturation take place at different stages in pregnancy it is reasonable and potentially beneficial to assess how disruptions at certain points in time may be linked with certain types of problems and whether certain timings may be associated with higher risk or impairment in the offspring. For example, there is some evidence (Khashan et al., 2008) suggesting that risk for schizophrenia may be higher if severe life events took place in the first semester of pregnancy. However, it may be difficult to know whether effects associated with milder forms of stress acting at the same timing would be similar or not. Isolating the effects of different stresses of different intensities and at different stages in pregnancy may be difficult, particularly in studies with humans.

There is now clear evidence that sub-optimal physical development of the fetus evidenced in the form of low birth weight is associated with adversity during pregnancy (e.g. Rahman, Bunn, Lovel, & Creed, 2007). This well-documented finding raises the possibility that such physical indices are markers of poor intrauterine conditions for growth of the fetus and may have an adverse influence on development in the postnatal life. This idea is at the core of an influential theory developed in the early 90's called the *fetal origins* hypothesis, which postulates that certain forms of adult disease may have their roots in the adverse prenatal environment.

1.2.2 The Fetal Origins Hypothesis

Much of the work on possible effects of prenatal stress on the fetus has been informed by the fetal origins hypothesis (Barker, 1995). Barker's theory poses that there are physical diseases with an onset in adulthood that appear in connection to developmental changes in the body occurring during fetal life. He presents human epidemiological evidence which links low birth weight (an indicator of non-optimal environment while in the womb) with later cardiovascular disease, non-insulin dependent diabetes and the insulin resistance syndrome. Although much of the mechanisms that contribute to these associations remain unknown, it is thought that under adverse intra-uterine conditions (e.g. under-nutrition) the fetal morphology and physiology goes through a number of adaptive modifications that prepare the organism for future life based on predictions of harsh outside conditions (Bateson et al., 2004). This is made possible due to plasticity in the future organs and brain which allows for these changes to take place, particularly during windows of intense developmental change called *sensitive* periods.

Although adaptive in the uterus, these morphological and functional alterations (which are sometimes referred to as *programming*) can become non-adaptive after birth if the "weather forecast" (Bateson et al., 2004) does not prove accurate. For example, a fetus that is provided with limited amounts of nutrients is likely to develop into a small and low birth baby with slow metabolism in "anticipation" of the lack of resources after birth. However, if the postnatal environment is not under-resourced, normal dietary intake by the underweight infant can result at some point in life in her or him becoming overweight, which is a condition potentially associated with cardiovascular problems and non-insulin dependent diabetes.

Animal studies with several species generally support the fetal origins hypothesis. For example, Gardner et al. (2005) found impaired glucose tolerance (a syndrome characterised by disturbance in glucose-insulin homeostasis) in adult sheep of low birth weight. Also, numerous studies in rats conducted by the Langley-Evans group (for a review, see Langley-Evans, 2001) show consistent evidence of a link between maternal under-nutrition and elevated blood pressure in offspring in adulthood.

Similarly, in humans, evidence exists that in both males and females risk for cardiovascular disease later in life is inversely associated with weight at birth and that risk is particularly high in the low birth weight individuals who become heavier as adults (Eriksson, Forsen, Tuomilheto, Osmond, & Barker, 2001). These effects cannot be explained by other confounding variables such as socio-economic status (see, e.g. Leon et al., 1998). Although it is plausible to think that there may be genetic influences in predisposition to adult disease, some evidence from twin studies (e.g. Gluckman & Hanson, 2004) suggests that it is unlikely that the effects are attributable to shared genes.

Evidence is accumulating that similar mechanisms to those identified for physical disorders may also apply to psychiatric disorders. For example, Costello, Worthman, Erkanli, and Angold (2007) found that low birth weight girls were at a much higher risk for developing depression in preadolescence and that the risk associated with childhood adversities was greater in the low birth weight that the normal birth weight group. This suggests that low birth weight was not just one in a series of risks that contributed to the illness, but rather a marker that predisposed to depression in the presence of later adversity. Interestingly, in the absence of any psychological risks, none of the low birth weight girls were diagnosed with depression, but with just one adversity present, risk for depression increased sharply. It may be that, in the case of psychiatric disorders, adverse

intra-uterine conditions acting *together* with postnatal adversities associate with problems. Although this is in line with the fetal origins hypothesis, it also raises the possibility that, at least in depression, there are other factors that need to be considered in conjunction with the prenatal environment.

The fetal origins hypothesis may therefore become part of an explanatory model for the development and, possibly, intergenerational transmission of mental illness. Low birth weight is thought to be a reliable index of elevated maternal stress in pregnancy and has been identified as a predictor for later externalising and internalising problems (e.g. Kelly et al., 2001; Liu, Sun, Neiderhiser, Uchiyama, & Okawa, 2001). An examination of the evidence on effects of prenatal stress in animal and human models is provided below with the aim of assessing the robustness of the proposed link to mental health processes.

1.2.3 Human Studies of Effects of Prenatal Stress

1.2.3.1 Methodological Considerations

The study of consequences of prenatal stress in humans poses some methodological challenges, which is largely due to the intricate nature of the processes involved and the obvious ethical constraints (Huizink, Mulder, & Buitelaar, 2004).

First, unlike animal research, experimental manipulation of stress is not possible in humans thus studies have to focus on naturally occurring stressors in the women's lives, such as relationship difficulties, daily hassles, life events, depressive mood or pregnancy-related anxiety. This often leads to variations in type, intensity and frequency of stressors, and very little is known on how these variations may result in differences in the measured outcomes, or how stressors with specific characteristics may be associated with specific types of maladaptive functioning.

Second, stresses in humans usually occur together with other difficulties, for example the co-occurrence of depression and partner violence (Campbell, 2002), and therefore it is hard to disentangle specific actions. In fact, it is possible that certain effects that are attributed to one factor are sometimes linked with the presence of other factors acting concomitantly with the other. Smoking in pregnancy represents an excellent example of presenting one risk as causal, whereas it can be an innocent bystander.

Third, it is not possible to prescribe timing of stressors in pregnancy. The fact that stresses can occur at random can impede thorough investigation of timing effects, as may

do the accumulation of stresses in pregnancy (e.g. a late pregnancy effect may in reality reflect cumulative risk from the whole pregnancy).

Fourth, prenatal stresses are often correlated with postnatal stresses and this overlapping makes it difficult to discern between prenatal and postnatal influences on the outcome (Robinson et al., 2008). For example, mothers who are depressed in pregnancy may continue feeling down after the baby's birth, which results in a continuum of low mood but with different mechanisms of impacting on the baby. This symptomatological flow can pose considerable challenge in isolating pre- and postnatal effects.

Fifth, a lot of stresses may occur together with genetic risk for certain conditions which can be transmitted from parent to child. Controlling for genetic factors may require complex designs and sufficient power to be able to detect interaction effects.

Summing up, the investigation of prenatal stress effects in humans can be a complex venture with likely methodological obstacles resulting from the multitude of co-occurring processes and the ethical constraints that do not permit manipulation of conditions.

1.2.3.2 Evidence on Links Between Prenatal Stress and Behaviour and Psychopathology in Humans

A small number of studies have examined prospective investigations of stress in pregnancy and behaviour in humans. Part of the reason for this fact lays in the complexity of the topic and the methodological challenges that such studies may pose (see Section 1.2.3.1 above). Moreover, few of these studies have progressed beyond early childhood. Most of the investigations that focused on early development found that

stress (mostly self-reported maternal anxiety and depression) was linked with less optimal behavioural scores in the fetus and the newborn, as well as poor cognitive outcomes and more neurodevelopmental problems in infants and children (for a review, see e.g. Van den Bergh et al., 2005; Talge et al., 2007). However, for the purposes of this thesis, a selection of relevant studies has been made to comprise investigations which focused on emotionality in infancy and behavioural and emotional problems in children. Information on sample size and characteristics, method for sample analysis and relevant statistical indices are also provided in order to be able to assess the strength of the design and the generalizability of results. Importantly, all the reviewed studies controlled for postnatal effects of maternal mood (depression, anxiety or both), while some have also controlled for other possible confounders (e.g. smoking, socio-economic deprivation).

In one of the earliest investigations of effects of prenatal stress on infant temperament, Van den Bergh (1990) measured state and trait anxiety using the State-Trait Anxiety Scale (Spielberger, C., Gorsuch, R., & Lushene, R., 1970) in 70 primiparous mothers and then assessed their infants using a temperament questionnaire at 10 weeks and 7 months postnatal. High anxiety scores in the antenatal period were positively correlated with perception of difficult temperament at both postnatal points in time.

More recently, Huizink, de Medina, Mulder, Visser, and Buitelaar (2002) examined 170 pregnant women of a total of 640 who were eligible. Mothers were administered questionnaires for pregnancy-specific anxiety and perceived stress in early pregnancy. Ratings of difficultness of their infants' temperament were provided at 3 and 8 months. Also, observations of infants' regulation of attention were conducted at the same ages. Results showed that pregnancy anxiety was linked with regulation of attention at both ages, and perceived stress was related with the same infant measure at 8 months only.

Moreover, perceived stress predicted temperamental difficulty at both 3 and 8 months and explained 8% and 2%, respectively, of the variance. In spite of the relatively large sample, generalizability of results may be limited because of the high participation refusal rate and the less established stress measures that were used.

Similarly, Davis et al. (2004) tested for the effect of maternal anxiety and depression during the third trimester of pregnancy on negative emotionality at 4 months in a small sample of 22 low risk dyads. Prenatal anxiety and depression separately predicted the infant outcome (i.e. negative behavioural reactivity in response to novel stimuli) in the expected direction and no effects were found postnatally (unique variance explained was 3% and 2%, respectively).

In a study with a large sample of 247 mothers and their full-term infants, Davis et al. (2007) tested for the effects of maternal depression, anxiety and cortisol levels at three points during pregnancy on infant temperament report (i.e. the fear subscale of the Infant Behaviour Questionnaire) at 8 weeks. The results showed that mean prenatal depression score (ΔR^2=.02, $p<.05$) across pregnancy and level of maternal cortisol (ΔR^2=.05, $p<.01$) in the third trimester of pregnancy independently predicted the infant outcome. This is one of the very few human studies suggesting a link between maternal depressed mood and the activity of the hypothalamic-pituitary-adrenal (HPA) axis in pregnancy and negative emotionality in the infant, and its results are likely to be generalizable due to its large community-based consecutive sample.

On a slightly different line, Werner et al. (2007) examined 50 dyads and used diagnostic interviews to identify mothers with depression and anxiety disorder in the second trimester of pregnancy and measured change in fetal heart rate from a resting state to

during a stroop task. Also, a temperament questionnaire was completed by mothers and observations of motor and behavioural reactivity at 4 months were coded. The results showed that change in heart rate during the stroop task (thought to reflect maternal cognitive effort and possibly stress) was associated with an 11-fold increase in the chance to exhibit high motor reactivity as an infant. Also, infants whose mothers were diagnosed with depression and/or anxiety in pregnancy were 4 times more likely to match a profile of high cry reactivity than the infants whose mothers were well. The results in this study show consistency with previous investigations in which maternal mental health problems are associated with negative emotionality in infancy. Moreover, heart rate changes in the fetus associated with maternal mental effort or stress may be an intrauterine marker for high motor activity in infancy.

In an investigation testing for the role of attachment in the link between prenatal stress and observed negative emotionality in infancy in a sample of 123 mothers and infants recruited consecutively, Bergman, Sarkar, Glover, and O'Connor (2008) found a main effect in the expected direction of prenatal stress (measured here as frequency of prenatal stressful life events), as well as an interaction effect of prenatal stress and attachment classification. Specifically, the infants classed in the Strange Situation procedure (a widely-used standard observational measure of attachment in the child yielding a secure type and 3 sub-types of insecure attachment) as insecure-ambivalent (a sub-type characterised by overt distress in the child following separation from the caregiver followed by clinging, resistance to soothing but also hostility when the interaction resumes) had increased fearfulness in response to being presented with an unpredictable toy. This suggests that certain postnatal interaction processes or factors may still shape the effects of prenatal stress on the infant and, as the authors speculate, it may be that the "programming window" spreads in the postnatal life.

Several investigations have found links between maternal depression and anxiety in pregnancy and emotional and behavioural problems at later ages (e.g. childhood). These child data have been collected mainly through maternal reports. For example, in the Avon Longitudinal Study of Parents and Children study (ALSPAC; O'Connor, Heron, Golding, Beveridge, & Glover, 2002; O'Connor, Heron, Golding, & Glover, 2003), which started with a large cohort of over 14,000 pregnant women, maternal anxiety at 32 weeks gestation predicted behavioural and emotional problems at ages 47 and 81 months, after controlling for a number of confounders as well as anxiety and depression at several points in the postnatal period. Moreover, the reported odd ratio statistics from 47 to 81 months in the ALSPAC were at comparable levels for most of the outcomes. These findings coming from the study of an impressively large sample provide strong evidence of long-lasting effects of stress in pregnancy on later mental health problems, and there is no indication that the strength of effects diminishes in time.

In a similar prospective investigation with a sample of 72 mothers and their children, Van den Bergh and Marcoen (2004) found links between maternal state anxiety at 12-22 weeks gestation and ADHD, externalising and anxiety problems at age 8-9 years.

Focusing on biological measures (i.e. diurnal salivary cortisol) of stress and outcomes, Gutteling, de Weerth, and Buitelaar (2005) found that higher levels of maternal cortisol at 16 weeks gestation predicted higher cortisol levels on school days in the 5-year-old offspring. Besides, prenatal psychosocial stress, measured through fear of bearing a handicapped child, also predicted higher cortisol in the child. These findings suggest that the activity of biological mechanisms of stress regulation in the offspring may be linked with prenatal maternal biological and psychosocial functioning and lend support for the study of possible underlying structures of emotion and behaviour.

In an attempt to disentangle environmental and genetic influences in the associations between late pregnancy psychosocial stress and childhood psychopathology, Rice et al. (2010) examined over 700 pregnant women who used in vitro fertilization. Of these women, 200 were not related genetically with their babies, while the rest of 500 were. Findings were that prenatal stress predicted hyperactivity only in the related group, implying the presence of a genetic link, while for antisocial behaviour associations with prenatal stress were found for both the related and the unrelated children, thus suggesting an environmental effect. Child anxiety was also linked with prenatal stress in both groups, but the effect was rather attributable to postnatal influences, as when controlling for postnatal mood the prenatal effect dropped to non-significant level. This study emphasises the need to consider prenatal, postnatal and genetic influences and raises the possibility that different outcomes can arise from each of these influences.

Some inconsistencies exist with regard to effects of prenatal stress in humans. Generally, there are three kinds of studies that contribute to these inconsistencies in findings. First, there are studies that found no associations. For example, in the study of a community-based sample of over 1000 from South Africa, Sabet et al. (2009) found no links between low birth weight, a commonly accepted index of prenatal stress, and total, externalising or internalising problems at age 2 years. Second, there are investigations that have found links at one age, but not another, as in that conducted by Ramchandani, Richter, Norris, and Stein (2010), in which predictions were found from prenatal stress to risk for behaviour problems at age 4, but not at age 2. Finally, there are even studies that have reported associations in the opposite direction to what was expected. For example, DiPietro et al. (2006) found that maternal stress in the second and third trimesters of pregnancy was associated with higher motor and mental development indices and better regulation of behaviour in preschoolers. There may be methodological differences (like

sample characteristics, or type of outcome that was studied) between studies which may explain these inconsistencies. However, the findings from DiPietro et al. are striking and one of the possible explanations given by the authors is that mild or moderate levels of stress (which was rather characteristic for their low-risk sample) may be associated with good developmental performance. This raises the possibility that an optimal level of stress in pregnancy, in contrast to high stress, may in fact prove beneficial for the baby.

1.2.3.3 Conclusions of Human Studies

There is evidence of links between prenatal maternal stress and child outcomes at various ages, with some studies suggesting a positive association between the levels of stress and levels of behavioural and emotional problems. However, there are also inconsistencies in findings. Also, with regard to the effect of timing of stress in pregnancy, the results are mixed, with links being reported at various stages in pregnancy. Clearly, more research is needed in order to understand, for example, how specific forms of stress may or may not have different effects in the offspring, whether specific links between timing of stressor and different outcomes may be established, whether there may be optimal levels of stress, or whether there are infant or environmental factors that may moderate or mediate the relationship.

1.2.4 Animal Studies of Effects of Prenatal Stress in the Offspring

Increasing evidence suggests that animal behaviours and some of their structural and functional biological underpinnings can be affected by stress induced in the pregnant mother (Weinstock, 2001). Most studies have experimented on rodents, although non-human primate studies have also found differences between the offspring of stressed and non-stressed mothers in behaviours such as neuromotor performance and attention (e.g. Schneider, Coe, & Luback, 1992; Schneider, Roughton, Koehler, & Lubach, 1999).

Behavioural modifications in rodents following prenatal stress include locomotor impairment, increased anxiety and depressive-like behaviours, altered learning behaviour, reduced exploration inside mazes and changes in sexual behaviour (for a review, see e.g. Kofman, 2002). Although there is variation in findings which may be attributable to factors such as strain of rodent, type, timing and severity of stressor, or age and sex of offspring, the overall conclusion is that prenatal stress is associated with sub-optimal development and that altered behaviour is likely to be paralleled by modifications in brain structures and stress response systems.

Of interest for the current study is the review of findings on emotionality. A number of studies have found elevated levels of anxiety and an inhibition of play or exploratory behaviour (thought to be expressions of depressive-like behaviours) following exposure to prenatal stress, particularly under stressful situations. For example, Pfister and Muir (1992) and Fride, Dan, Feldon, Halevy, and Weinstock (1986) found lower motor behaviour and increased defecation (an indicator of increased anxiety) in novel situations. Also, avoidance in entering the open arms of a maze was found in the investigation conducted by Vallee et al. (1997). Similarly, a reduction in play behaviour and social

interactions (e.g. Takahashi et al., 1992) and decreased movement in an activity wheel (Lambert et al., 1995) were found. In short, these rodent studies indicate reliable modifications in motor behaviours and emotionality in offspring of mothers who were stressed in pregnancy, although extrapolation of findings from rodent studies to humans requires careful consideration as human processes are likely to be different or of more complex nature.

One of the advantages of conducting studies of effects of prenatal stress in animals is that there is potential for a more direct exploration of possible mechanisms. Biological mechanisms for a direct effect have been proposed in both humans (for a review, see e.g. Van den Bergh et al., 2005) and animals (for a review, see e.g. Weinstock, 2001), with the most studied one being the HPA axis. It is plausible to look at the HPA axis as a mediational candidate as it is considered to be the main neuroendocrine stress response system in the body (Gunnar & Quevedo, 2007). Besides, disturbances in the activity of the HPA axis occur in depression and anxiety in the adult (Pariante & Lightman, 2008; Gunnar & Quevedo, 2007) while in the child altered HPA responses has been associated with conditions such as anxiety disorder and post-traumatic stress disorder (Pfeffer, Altemus, Heo, & Jiang, 2007). A description of the HPA axis together with evidence from animal and human studies for its involvement in the link between prenatal stress and offspring adaptation are presented in Section 1.2.5 below.

1.2.5 The HPA Axis as Possible Mediator in the Link Between Prenatal Stress and Offspring Development

The HPA axis is the main stress reactivity and regulation system in mammals and is under cortical and sub-cortical control. Perception of stress triggers secretion of corticotrophin releasing hormone (CRH) from the hypothalamus, then CRH stimulates secretion of adreno-corticothopin hormone (ACTH) from the pituitary gland, which in turn stimulates the release of glucocorticoid hormones (corticosterone in rodents and cortisol in primates) from the adrenal cortex. Glucocorticoids play important roles in the body, including maturation of most parts of the central nervous system and modulation of reactions to stress. They then act at the glucocorticoid and mineralocorticod receptors from different parts of the brain providing negative feedback to the hypothalamus and the pituitary gland thus inhibiting secretion of CRH and ACTH and shutting off the stress response.

An adaptive reaction to stress includes a swift and potent stimulation of production of glucocorticoids followed by a relatively rapid decrease in secretion once the stressor has ceased its action. Abnormal patterns of regulation of the HPA axis, for example, a chronically high and flattened profile, or a less powerful response seem to have an impact on the normal functions of several of the brain structures, including those involved in regulating emotions (e.g. hypothalamus, hippocampus), and may underpin emotional dysregulation and other affective problems. Therefore, both under- and over-regulation of the HPA axis may not be beneficial for the body and have been associated with anxiety and depressive-like behaviours in animals, and depression, post-traumatic stress disorder, anxiety disorder and chronic fatigue syndrome in humans.

Several animal and human studies report findings suggesting the implication of the HPA axis in the link between prenatal stress and later adaptation. For example, in both animal and human models, administration of synthetic glucocorticoids to pregnant mothers has been associated with behaviours and changes in fetal brain which are similar to those occurred after prenatal exposure to stress (e.g. Oliveira et al., 2006; Huang et al., 1999; Trautman, Meyer-Bahlburg, Postelnek, & New, 1995). Also, in animals, studies conducted by Barbazanges, Piazza, Le Moal, and Maccari (1996) and Maccari et al. (2003) found increased levels of glucocorticoids and a decrease in glucocorticoid receptors (linked with prolonged secretion of corticosterone) following prenatal stress. Moreover, maternal adrenalectomy and substitution with maintenance levels of corticosterone were found to prevent effects of prenatal stress that are usually seen in the offspring (Zagron & Weinstock, 2006). Consistent with the results from the animal work, in humans, Van den Bergh, Van Calster, Smits, Van Huffel, and Lagae (2008) found that the HPA axis mediated the link between antenatal anxiety and depressive symptomatology in adolescent females, while Gutteling et al. (2005) reported higher levels of cortisol in the children of mothers with over-reactive HPA axis and pregnancy-related anxiety in the prenatal period.

Overall, these animal and human studies suggest that there may be biological mechanisms involved in which these changes account for the long-term effects. The HPA axis is of interest as it is implicated in the expression of over 1000 genes (O'Donnell, O'Connor, & Glover, 2009) and provides an example on how maternal stress can influence the offspring behaviour. Nevertheless, it should be borne in mind that the studies providing with most of the evidence on possible mechanisms were conducted with animals. Processes in humans are likely to be more complex or at least different since there may be an interplay between pre- and postnatal and genetic influences. In support for the

complexity of processes that need to be taken into account when discussing about mechanisms in humans, Hay, Pawlby, Waters, Perra, and Sharp (2010) propose five possible pathways (direct and indirect and involving biological effects, previous environmental risk, exposure to long-term maternal mental illness and genetic components) linking prenatal maternal depression to antisocial and violent behaviour in adolescence, and provide evidence for each of them from previous investigations or their own research.

1.2.6 Conclusion to the Chapter

There is some evidence on effects of prenatal stress on behaviour in both humans and animals, although there are inconsistencies also. These effects in humans are not well understood. The HPA axis has been studied extensively, particularly in animals, as a possible mechanism in the proposed link and provides an important background to the current study. However, another plausible biological mechanism is explored in the current thesis. Vagal tone has been linked with emotional regulation and mental health problems, but little is known about possible links with prenatal stress. The next chapter presents the topic of vagal tone and explains how a focus on it may be relevant for the understanding of effects of stress in pregnancy.

1.3 Vagal Tone and the Polyvagal Theory

1.3.1 Introduction

Vagal tone is thought to reflect a portion of the parasympathetic influence on the heart which is exerted through motor fibres of the cardiac branch of the tenth cranial nerve originating in the nucleus ambiguus. However, a good understanding of the concept requires some basic conceptualization of the structures and functioning of the autonomic nervous system (ANS).

The ANS is the part the nervous system which regulates the cardiovascular system, and controls the activity of certain groups of muscles, and of the visceral organs. It comprises two subsystems (i.e. the parasympathetic and the sympathetic nervous systems) that have complementary (and typically antagonistic) actions on the organs they innervate. For example, parasympathetic motor fibers have inhibitory action on the heart and constrict the bronchiolar diameter, while the sympathetic fibers speed up the cardiac rate and dilate bronchioles when the need for oxygen increases. Thus, the two systems permanently modulate the activity of the vital organs, usually in opposition one to the other, in order to maintain homeostasis of the body.

The heart, like all internal organs, is connected to the ANS through efferent and afferent sympathetic and parasympathetic fibers. The sympathetic nervous centres are situated at the level of the spinal cord and have a mobilising activity on the heart. This results in increased blood outflow to the vital organs and the large muscles, thus preparing the individual for fight/flight behaviours when faced with threatening situations. On the

other hand, the effect of the parasympathetic system is to slow heart rate via activity of the vagus nerve, and hence reduce blood flow to the periphery.

1.3.2 The Polyvagal Theory

The polyvagal theory (Porges, 1995; 2001; 2007) has been influential in bringing the study of the ANS to one of the leading positions in the field of neuropsychophysiology and making vagal tone one of the important topics in emotion regulation over the past couple of decades. Central to the theory is the proposal that the vagus nerve has evolved, structurally and functionally, to provide neural regulation of the heart in the context of increasingly complex emotions, decisions and communication in mammals. The vagus is the tenth of twelve cranial nerves and the main parasympathetic structure that innervates facial and head structures and some of the viscera. According to the theory, in phylogeny, two distinct neuroanatomical structures of the vagus with different roles have emerged that are involved in the expression of different psychological processes. The term *polyvagal* refers to the two branches of the vagus and is used to emphasise the differences between their structures, roles and origins in the brainstem.

Porges states that there are three structures of the ANS (two of parasympathetic origin and the sympathetic nervous system) with each of them employing different behavioural strategies of coping when the individual is in front of a danger or life threatening situation. Table 1.1 presents these structures together with their neuroanatomical origins and the behaviours that they are thought to promote when activated.

Table 1.1 Neural Control of the Heart in Phylogeny as Proposed in the Polyvagal Theory (Adapted from Porges, 2003)

Evolutionary stage	ANS structure	Behaviour	Lower motor neurons
III	Myelinated vagus	Social communication, self-soothing and calming	Nucleus ambiguus
II	Sympathetic-adrenal	Mobilization (fight/flight)	Spinal cord
I	Unmyelinated vagus	Immobilization (death feigning, freezing)	Dorsal motor nucleus

In Porges's view there is an older (or, "vegetative") part of the vagus compounded of unmyelinated fibers that originates in the dorsal part of the vagal complex (i.e. the dorsal motor nucleus) and whose activation during life-threatening situations leads to immobilising behaviours (i.e. feigning death, freezing behaviours). By contrast, a more recently-evolved component is the myelinated "smart" vagus originating in the ventral part of the vagal complex (i.e. the nucleus ambiguus). The functional role of the smart vagus during stress-free periods is to promote growth and metabolic recovery in the body (Porges, 2007). This is made possible through an increased action of the myelinated motor fibers on the sino-atrial and atrial-ventricular nodes, which results in bradicardia, reduced sympathetic flight/fight behaviours, attenuation of the stress-response system of the HPA-axis (e.g., lower secretion of cortisol), and an overall calming self-soothing state in the individual (Porges, 2007).

The evolution and differentiation of these distinct autonomic systems allow the individual to assess whether the surroundings are safe or threatening (in Porges, 2004, this process of evaluation is referred to as *neuroception*) and consequently employ the most adaptive response (social engagement, fight/flight, freezing). If the environment is perceived as threat-free the nucleus ambiguus becomes the primary system to regulate the cardiac vagal input and, through parallel activation of other cranial nerves, promotes calm states in the individual which facilitate social communication. However, in the case of detection of danger in the environment the action of the smart vagus is removed and the sympathetic system is recruited whose actions, although requiring high metabolic resources, may prove adaptive by adopting the fight or flight stance. And, if level of threat in the environment is perceived as potentially lethal, the third and most primitive autonomic system of coping, which originates in the dorsal motor nucleus, is engaged and whose action promotes immobilization strategies to ensure survival.

Porges (2001) theorises that the brainstem nuclei that regulate the activity of the myelinated vagus have become connected phylogenetically with the nearby nuclei that control the activity of the muscles of the head and face. He argues that during these states of growth and restoration of bodily resources, the individual becomes more available to interact socially, because of the coupled activation of the social engagement system (Porges, 2001). This system comprises the nervous circuitry of several cranial nerves (i.e. V, VII, IX, X and XI) that regulate the activity of the muscles of the face and of the larynx which are involved in expressing emotions and in emitting vocalizations, the neck muscles for head turning to social stimuli, the muscles involved in opening the eyelids, the muscles of the middle ear employed to heighten auditory attention and to extract human voices from the environment, and the visceral organs (e.g. heart, bronchi) involved in mobilising of metabolic resources.

Thus high vagal tone is generally seen as adaptive in humans for purposes of optimising social interactions and the capacity of orienting in the environment. However, reduction of vagal tone is required when the individual faces novel or challenging situations, and resources have to be mobilised without necessarily recruiting the costly fight or flight response. Porges (2007) argues that the smart vagus through its myelinated fibers has evolved into a remarkably fast and efficient system in order to respond to environmental demands in the social world. Under normal safe environmental conditions the smart vagus exerts a tonic influence on the heart which maintains its beat rate at a level below that provided through the sino-atrial node. By contrast, when facing moderate challenges (e.g. when a child meets a new person, or when the individual is trying to solve a problem) the cardiac action of the vagus can be withdrawn quickly, which increases heart rate to its intrinsic level without the intervention of the sympathetic system but still releasing the required supplemental resources. The re-establishing of the previous levels of safety or familiarity with the environment, or the solving of the problem, is usually followed by the return of the tonic vagal influence on the heart. Porges, Doussard-Roosevelt, Portales, and Greenspan (1996) use the metaphor *vagal brake* to describe this functional role of releasing and re-applying of the action of the smart vagus to constantly adjust to the environment and allocate appropriate levels of resources. Homeostatic and environmentally-challenging needs can compete with each other, since vagal tone is involved in both processes. Studies involving preterm babies (e.g., Lester, Zachariah-Boukydis, & LaGasse, 1996) suggest that engaging with external stimuli can be associated with some degree of homeostatic instability.

1.3.3 How Do We Measure Vagal Tone?

The measurement of vagal tone requires special consideration due to the intricate nature of the neurophysiological processes embedded in this concept. Literally, as stated in the polyvagal theory (Porges, 2007), cardiac vagal tone is conceptualised as the parasympathetic influence exerted on the heart through the branch of the vagus nerve originating in the nucleus ambiguus. However, an invasive method for measuring this strictly-delineated portion of vagal efference is not possible to use for obvious reasons. Therefore, one has to rely on indirect measures of heart activity, such as heart rate variability, that is, differences in beat-to-beat (or heart period) intervals. But, heart rate variability data alone cannot provide with an accurate image of the parasympathetic activity because of other factors affecting variability, such as sympathetic motor fibers or the baro-receptor reflex (Beauchaine, 2001). Currently, the most widely accepted method for measuring cardiac vagal tone involves quantification of the amplitude of the respiratory sinus arrhythmia (or, RSA), which is the variability in beat-to-beat intervals that occur at respiratory pace. Typically, heart rate speeds up during inhalation and slows down during exhalation and vagal tone is involved in regulation of both heart rate and respiration. Although there may be several potential confounders (e.g., respiratory pace, or posture) that can affect RSA, it is considered in the literature as a good, though not exact, measure of cardiac vagal tone (Beauchaine, 2001). Studies that used pharmacological blockade (i.e. on beta-adrenergic receptors to measure sympathetic influences, and on muscarinic receptors for isolating parasympathetic efference) found moderate to strong correlations between RSA and cardiac vagal tone, with Pearson's *r* ranging from .5 to .9 (see Grossman & Taylor, 2007) after controlling for respiratory parameters. Throughout the thesis I will use the terms vagal tone and RSA interchangeably depending on context (e.g. vagal tone will be used predominantly for

theoretical considerations, while RSA will be primarily referred to in relation to measurement) and I will assume that RSA it is a moderately good index of vagal tone.

1.3.4 Two Dimensions of Vagal Tone

As described in Section 1.3.2, Porges (2007) proposes that the smart vagus has evolved to meet the emotional and social demands in the mammalian world. However, vagal tone is thought to be involved not only in providing the individual with the appropriate allocation of resources to engage with and function in the environment. Porges et al. (1996) propose that the ANS plays a major role in maintaining homeostasis in the organism and that in the absence of environmental demands vagal tone functions at a relatively constant level to promote bodily growth and restoration. Porges refers to this characteristic level of vagal efferent output on the heart in conditions of low threat and demand as *baseline* vagal tone. By contrast, the decrease in cardiac vagal input, thought to be adaptive in the presence of a moderate threat or challenge because it triggers rapid release of metabolic resources, is described as vagal *withdrawal*. The next two sub-sections focus in depth on each of these two constructs.

1.3.5 Baseline Vagal Tone – What Does It Reflect? Links to Adaptation

The construct of baseline vagal tone is thought to reflect the tonic vagal influence from nucleus ambiguus to the heart occurring in the absence of psychological challenge (Porges, 1995). There is extensive evidence linking baseline vagal tone to a diverse range of psychological processes or constructs, such as attention abilities or temperament, but before presenting this body of research I would consider useful making a few remarks on the conditions of its measurement.

1.3.5.1 Issues in Measurement – Can There Be a Baseline or "Resting" Measure?

Despite the fact that the vagal tone construct has a theory behind it which has been on the arena for several decades and has been studied extensively in laboratories throughout the world, there has not yet been formulated a consensual view on the state in which the individual should be during the measurement of vagal tone (further details on the actual procedure for collecting the RSA data are provided in Section 2.6.1 in the Method chapter).As a result to this, baseline RSA data has been collected in several types of quiet states ranging from deep sleep (Feldman & Eidelman, 2007) to active sleep (Porges, Doussard-Roosevelt, Stifter, McClenny, & Riniolo, 1999) to quiet sleep and calm alert state without stimulation (Arditi, Feldman, & Eidelman, 2006) and to calm alert state while watching a video of neutral content (Calkins, 1997).

Porges (2007) argues that the baseline measure of vagal tone is reflected during quiet alert state and in the absence of intense stimulation and concentration. However, it is unlikely that this state can always be maintained for a long time. This is because during alert quite state there still are variations regarding the individual's level of alertness and stimulation, engaging with the environment and emotional arousal, all of which have been found to be associated with variation in RSA levels. Moreover, young children are likely to change states fairly soon if required to sit still and prevented from interactions. Sleeping may be one of the conditions apparently showing little change, but there is evidence suggesting that cardiac vagal input is lower during sleep than when awake and RSA may become markedly changed during rapid eye movement phases (Valladares, Eljammal, Motivala, Ehlers, & Irwin, 2008). As a result to this, very little research has conducted measurements of baseline vagal tone in sleeping states. At young ages, baseline RSA has typically been recorded while maintaining the children's attention engaged with stimuli of

apparently neutral emotional content (e.g. while watching cartoon movies, as in Calkins, Graziano, & Keane, 2007), but there are also studies that do not describe what the child was doing during baseline vagal tone, possibly assuming that the apparent absence of a challenge is the baseline.

Evidence suggests that there are RSA differences between conditions used as baseline. For example, Porges et al. (1999) found differences in RSA between quiet sleep and active sleep, with newborns in the former situation having greater RSA than during the latter. Similarly, Arditi et al. (2006) found differences in newborns' RSA in quiet sleep versus calm alertness in a study which examined visual attention, although no differences were found between the same conditions in a study which focused on pain reactivity and which involved a sample with very similar characteristics to the one used in their visual attention study. Calkins (1997) notes that, although watching a movie during quiet awake state may not represent a genuine baseline condition, keeping 2-year-olds calm and still for several minutes without exposing them to any form of stimulation can be challenging for researchers. Moreover, several studies (e.g. Smith, Dmochowski, Muir, & Kisilevsky, 2007) have conducted measurements of baseline RSA in the fetus, although it may be difficult to understand perception of threat in the fetus. Therefore, agreement does not exist with regard to the individual's state under which baseline RSA should be measured and differences were found across conditions used as baseline.

1.3.5.2 Processes Associated With Vagal Tone

Beauchaine (2001) reviewed the infant literature linking psychological processes to baseline vagal tone and concluded that high baseline vagal tone is indicative of a high "capacity for active engagement of infants with the environment" (page 194). This

concept of capacity includes attentional capacity, ability to engage socially and appropriate expression of affect, either positive or negative, depending on the nature of the context. Porges (2007) proposes that vagal tone facilitates the individual's interaction with the environment and supports metabolic demands for reacting to stimuli.

1.3.5.3 Vagal Tone, Adaptive Reactivity and Behaviour Regulation

High baseline RSA has been associated with more crying in response to pain caused by circumcision (Porter, Porges, & Marshal, 1988) and after a pacifier withdrawal procedure (Stifter, Fox, & Porges, 1986), and also with higher behavioural distress during gavage feeding, a procedure which involves insertion of liquid food in the stomach through a tube (DiPietro & Porges, 1991), and with elevated levels of cortisol following a heel-stick procedure for drawing blood, suggesting integrated autonomic reactions to the painful stimulus (Gunnar, Porter, Wolf, Rigatuso, & Larson, 1995). By contrast, Huffman et al. (1998) found that 3-month infants with high baseline vagal tone were less behaviourally negative and required less calming intervention for finishing laboratory procedures. Huffman et al. interpret these findings to mean that high baseline RSA is related to high behavioural and emotional reactivity, whether negative or positive, whichever is most appropriate in response to the environment. For an infant who is subjected to painful stimulation or whose movements are restricted, the most adaptive reaction seems to be through expressing negative emotions and behaviours (e.g. crying, anger), while an unintrusive and pleasant social interaction normally elicits more positive or regulated responses.

The interpretation of the findings from early infancy is not straightforward and may seem somewhat contradictory as long as high RSA seems to be associated with adaptive

behaviours of either positive or negative emotionality depending on the context. This may be further complicated by findings suggesting that high RSA may be associated with negative emotionality in infancy but with positive outcomes reflecting high regulation in childhood. Evidence supporting the idea of a possible developmental shift (Beauchaine 2001) in regulating behaviours from infancy to childhood suggesting that highly reactive infants can achieve better regulation by attracting more support from the caregiver in modulation of state is shown in a longitudinal investigation conducted by Porges, Doussard-Roosevelt, Portales, and Suess (1994). Specifically, high RSA was associated with difficult temperament at 9 months, but also with a less difficult temperament and fewer behaviour problems at age 3. It should be pointed out that this study used a small sample and there do not appear to have been any attempts to replicate these findings. The authors speculate that high negative reactivity in infancy can be more adaptive because it supports the development of social skills through more frequent and more demanding interactions with the parents, who have become more actively and earlier involved in addressing their infant's assertive responses.

Investigations at later ages and longitudinal studies from infancy to early childhood may be relevant for illustrating links between vagal tone and adaptation and understanding reactivity processes. Calkins (1997) conducted a cross-sectional study with preschoolers aged 2 to 3 using observational measures of positive (e.g. latency to smile, duration of smiling) and negative (e.g. latency to frown, duration of frowning) emotions in a standardised measure involving social interaction with an examiner. RSA scores were positively correlated with positive emotionality (r=.45, p<.01), and negatively correlated with negative emotionality (r=-.34, p<.05), but not to behavioural regulation scores (for which statistical data are not provided). The negative association between vagal tone and negative reactivity reported in this study (i.e. children with high baseline vagal tone were

exhibiting less negative reactivity to a frustration task) is contrary to the findings from an earlier study conducted with 5-month-olds by Stifter and Fox (1990) in which high vagal tone correlated positively with negative behavioural reactivity measured in an arm-restraint procedure, r=.28, $p<.05$. There may be several explanations for these contradictory findings. The age of the participants, as well as the nature of the tasks used for eliciting negative reactivity might have contributed to the differences. It may be that infants during the first year of life are more prone to exhibiting negative reactions (Fox & Davidson, 1984), or maybe restraining arms at 5 months is a more frustrating experience than not being able to reach a toy at 2 or 3 years. Also, there may be situations in which emotional responding is adaptive, as in Huffman et al. (1998). And, another possible interpretation relates to the idea of the developmental shift proposed by Beauchaine (2001) and presented above in this section in which highly reactive infants acquire better regulation skills in childhood and learn how to modulate their responses.

1.3.5.4 Vagal Tone and Attentional Processes

High baseline RSA has also been linked with high attentional capacities in infants. In a study conducted by Linnemeyer and Porges (1986), 6-month-old infants with high vagal tone who had to achieve a 5-second period of looking to a stimulus succeeded doing that more rapidly than their low vagal tone counterparts. They also performed more efficiently during the familiarization phase of the experiment, and had a higher preference for novel stimuli. Richards (1985; 1987) examined 14-, 20-, and 26-week-old infants cross-sectionally and found that high baseline vagal tone was related to less distraction during a presentation of visual stimuli at 26 weeks, while at earlier ages it differentiated amongst infants in terms of efficiency in processing visual information. Despite the evidence from the infant studies mentioned above, Arditi et al. (2006) found no relations

between baseline vagal tone and performance during a visual attention task in healthy newborns. The authors conclude that this may be explained by the immaturity of the visual attention system at birth; therefore more research is needed into the developmental processes linking vagal tone and the emergence of early visual attention capacities.

1.3.5.5 Vagal Tone and Social Engagement

Some interesting results on baseline RSA and the capacity to interact socially in relation to later adaptation have come from studies involving preterm newborns and infants. Doussard-Roosevelt, Porges, Scanlon, Alemi, and Scanlon (1997) found that very low and extremely low birth weight infants who showed higher RSA between 33 to 35 weeks were rated as having fewer behaviour problems at age 3. More evidence supporting the idea that high RSA may represent a protective factor in preterm babies for more optimal developmental outcomes has been provided more recently by Feldman and Eidelman (2007) in a study which examined maternal behaviour and synchrony of infant-parent relationship in early infancy. They found that premature babies (with gestational ages between 29 and 33 weeks) with high RSA assessed at 37 weeks postconceptional age attracted more maternal behaviour (e.g. positive affect, gaze toward infant's face, and talking to the baby), and had more synchronous interactions with mothers and fathers at 3 months than the low RSA babies. The authors argue that this may be due to the fact that infants with lower autonomic maturity (e.g. lower baseline RSA) are less capable of eliciting maternal attention, and subsequently receive less maternal contact because of their limited abilities to display social cues and interact socially. Also, differences in RSA accounted for the amount of affectionate touch received at 3 months corrected age, in that the high RSA premature infants received a greater amount of touch than the low RSA infants. This study supports Porges's hypothesis that elevated vagal tone is

associated with a greater capacity for social engagement. This investigation and a number of related studies seem to suggest that higher vagal tone may be protective in preterm infants.

1.3.5.6 Summary of Findings Related to Processes Linked With Vagal Tone

Overall, findings from the studies that examined baseline vagal tone and links with adaptation suggest associations with temperamental reactivity (both positive, and negative) and, generally, with the ability of the individual to engage with and attend appropriately to the environment (Calkins, 1997). In these studies, temperament has been assessed based on maternal reports and laboratory procedures. Another consistent finding is that in infancy, high vagal tone seems to be adaptive and predicts good developmental outcomes at later ages. However, there are also some contradictory findings between studies that were carried out at different ages, with high vagal tone infants predominantly exhibiting higher negative reactivity, while their 2- and 3-year-old counterparts were less likely to show negative reactions to frustration tasks. A possible explanation for this difference is likely to be the lack of agreement on what baseline vagal tone is and how to measure it. Another interpretation might include the possibility of a developmental shift in emotion reactivity referred to in Section 1.3.5.3, and that of developmental change in vagal tone, which is discussed later on in Section 1.3.9. Conducting longitudinal studies from infancy to preschool age on larger samples and with measures that are appropriate for the developmental stage of the participants, and which are likely to elicit responses of comparable quality and intensity may prove useful in addressing these inconsistencies.

1.3.6 Vagal Withdrawal – What Does It Reflect? Links to Adaptation

As outlined earlier in Section 1.3.4, the process of withdrawal of vagal tone, also referred to as withdrawal of the vagal brake, is thought to quickly facilitate mobilization of resources through increased arterial blood flow and raised alertness in response to environmental threat or challenge (Porges 2007). Therefore a lower level of RSA relative to an earlier measurement under less challenging conditions seems adaptive.

1.3.6.1 Issues in Measurement of Vagal Withdrawal

As with the issue of measuring baseline vagal tone referred to in Section 1.3.5.1, it would be important first to try to establish under which conditions or states it would be expected to encounter vagal withdrawal. Clarification concerning this issue is bound up with the issue of what is baseline discussed earlier because vagal withdrawal is calculated by subtracting RSA in response to a challenge from a previous condition. Several investigators (e.g. Moore et al., 2009) have proposed that the initial RSA from which subtraction of current RSA is believed as more appropriate would be the baseline RSA, irrespective of when in the experimental sequence was it measured. By contrast, Moore (2010) argues that the most accurate measure of vagal withdrawal in response to a stressor is the difference between RSA during the presentation of the stressor and the episode immediately preceding it. Therefore there is not a unanimous view on how RSA withdrawal should be computed. The lack of established methods for measuring baseline and withdrawal of RSA may therefore lead to inconsistencies in RSA withdrawal findings.

A second potential source of error in measuring vagal withdrawal may be the limited understanding on how different types of challenges are associated with different rates of

withdrawal or lack of withdrawal. Only a few studies have investigated this problem. For example, Calkins et al. (2007) measured baseline RSA in preschoolers while they were watching a cartoon with neutral content and then RSA withdrawal during a series of five episodes of executive functioning, attention persistence, positive surprise and frustration tasks and found lower RSA scores compared to baseline (indicating vagal withdrawal) in four of them . Other studies that focused on emotional challenges (see the Still Face studies summarised in Table 3.17 in Section 3.4.2 in the Results I chapter) have found reliable RSA responses in samples of diverse characteristics. Generally, vagal withdrawal has been noted as a reliable process across various tasks and challenges. Therefore, agreement regarding under which conditions to expect vagal withdrawal seems to exist, in spite of the lack of consistency in conditions used for measuring baseline vagal tone.

1.3.6.2 Vagal Withdrawal – Individual Differences and Links With Behaviour Regulation

Although vagal withdrawal seems to be a reliable response in whole sample analyses under certain conditions, there is also evidence suggesting individual differences. Research across all age ranges (i.e. from birth to adulthood) has revealed that some individuals fail to show vagal suppression in stressful situations or show very low withdrawal compared to others (see, for example, Arditi et al., 2006; Calkins, 1997; Graziano, Keane, & Calkins, 2007). Other studies have shown that some individuals exhibited large withdrawal (e.g. Calkins et al., 2007), which in some cases may indicate sympathetically-mediated fight/flight behaviours (Beauchaine, 2001), while others did show a pattern of change, but in an unexpected direction – they exhibited vagal *augmentation*, that is, increased vagal tone in response to an obvious challenge (e.g. Moore & Calkins, 2004). These individuals who did not show vagal withdrawal also had poor

self-regulating skills and were behaviourally dysregulated, as identified through parental reports or laboratory procedures.

Evidence on the fact that vagal withdrawal is likely to reflect emotional responsiveness and also self-regulatory and social skills comes from Calkins and colleagues in studies involving infants and preschoolers. Some of these infant studies used the Still Face procedure (Tronick, Als, Adamson, Wise, & Brazelton, 1978), which is also the focus of the study reported in this thesis. The Still Face consists of a sequence of three episodes of social engagement and total unresponsiveness initiated by the adult in interaction with the infant (i.e. engagement with infant – still face behaviour – re-engagement with infant) and is used for measuring a variety of outcomes ranging from social engagement skills to emotional and physiological regulation to behavioural synchrony in the dyad (for a full description of the procedure, see Section 2.5.2.4 in the Method chapter). For instance, Moore and Calkins (2004) found in a study involving 73 3-month-old infants of postnatally depressed and not depressed middle- to upper-class mothers that mean RSA during still face was significantly lower than in the baseline condition (measured with the infant sat in a high chair without toys and not interacting with the mother or the experimenters). During the social reunion episode, which succeeded after the still face, the mean RSA returned to a level which was comparable with the mean baseline RSA. In a contrast of "suppressors" (infants with RSA withdrawal scores above the median) and "non-suppressors" (those with RSA withdrawal below median), the suppressors had increased levels of positive engagement with their mothers during normal play and at reunion, while the non-suppressors continued to show a high level of negative affect during the reunion. Results point toward a carry-over behavioural effect in the non-suppressor group, showing that these children experienced a harder time recovering following the distress (i.e. the still-face episode).

Similarly, Bazhenova, Plonskaia, and Porges (2001) examined the behavioural and physiological responses of 41 5-month-old infants of low risk mothers while engaged in a sequence of object-mediated and person-mediated tasks including the still face. Behaviours were quantified in terms of positive engagement (quietly attending with objects or persons), negative signalling (getting upset, fussy or disorganised), and motor activity (slight, moderate or considerable body movements). Results showed that mean RSA followed a similar pattern of change as to that reported in Moore and Calkins (2004), decreasing during the still-face from the previous episode, and then returning to the baseline level during social interaction. Baseline RSA was recorded in the same way as described in Moore and Calkins, with the infant sat in a high chair and the parent and experimenters out of the child's view. Further analyses revealed that the infants who did not show a pattern of RSA withdrawal during still-face followed by RSA increase in the social reunion received higher scores on negative signalling during the interaction which followed the distressing episode, which is consistent to what Moore and Calkins found at 3 months of age, namely that poor autonomic regulation can parallel behavioural dysregulation. More evidence in support of the account that the ability to suppress vagal tone during challenges is related to "good" developmental outcomes comes from Porges et al. (1996) in a longitudinal study in which children who did not suppress vagal tone from baseline condition (with the infant calm and sat on the mother's lap) to during a cognitive ability exam at age 9 months had higher problems scores on several scales of the Child Behaviour Checklist (CBCL) at age 3 years.

In another study involving 99 2-year-olds, Calkins and Dedmon (2000) examined physiological, emotional, and behavioural regulation in relation to risk for early conduct problems. Children were screened for externalising problems, and were divided in two groups (high versus low risk) based on their scores on the parent reported CBCL's

externalising scale. Physiological measures included baseline RSA (with the infants watching a cartoon with neutral content similar to that used in the study by Calkins et al. 2007 presented in Section 1.3.5.1) and change RSA, as assessed during a sequence of 2-minute-long episodes designed to elicit emotional and behavioural responses (i.e. joy, fear, problem-solving strategies, empathy and frustration). Analyses were conducted to address the issue of RSA suppression across tasks in the whole sample. Results showed that children from both low and high risk groups consistently suppressed RSA during the fear, problem-solving, and frustration episodes, but not when positive emotions like joy and empathy were elicited, which is consistent with Porges's polyvagal theory in that one typically exhibits vagal withdrawal in challenging situations. Further results revealed that high-risk children showed lower RSA suppression across tasks than their low-risk counterparts. However, correlations computed across the five episodes between RSA suppression and behavioural and emotional regulation showed a relatively strong relation only during the problem-solving task (r=-.28, p<.01), with higher RSA suppression being associated with lower behavioural and emotional dysregulation. This suggests that the processes linking physiological support to regulatory behaviours at preschool age might be more complicated. The authors concluded that a longitudinal observation might provide with a clearer picture on how these systems work together at this early age.

In another recent study involving a large-scale sample (N=341) of 5 ½ -year-olds, Graziano et al. (2007) examined vagal tone regulation in relation to how children were perceived by their peers in terms of preference and sociability. Two questions were addressed: first, whether vagal regulation was related to the sociometric ranking (i.e. how highly was a child liked or disliked by his/her kindergarten group peers), and second, could mediators of this link, such as social skills, be identified. Vagal tone was assessed during a baseline condition (while watching the same neutral content cartoon) and during

a sequence of tasks that required executive control and problem-solving skills. Behaviour problems and social skills were assessed through teacher questionnaires and peer reports. In multiple linear regression vagal withdrawal (i.e. suppression from baseline to cognitive tasks) predicted social preference. The children who were rated by their peers as more sociable and were more preferred than others had higher vagal tone suppression during the laboratory tasks. This replicates the findings from a study conducted by Stifter and Corey (2001) in which 12-month-old infants who displayed higher vagal suppression during a cognitive test were rated as more sociable by the experimenters who came into contact with the children.

1.3.7 Is There an Optimal Level of Vagal Tone and Vagal Withdrawal?

If vagal tone is implicated in adaptive responses to the environment the question arises as to whether there may be optimal levels of vagal tone and withdrawal, and hence risks for psychopathology arising from "too high" as well as "too low" levels. This is consistent with a literature (e.g. Beauchaine, 2001) suggesting that children with anxiety problems may show excessive vagal regulation, often resulting in large withdrawal of the vagal brake and possible activation of fight/flight behaviours. The study conducted by Calkins at al. (2007) using a 3-group design is relevant to this issue. They compared children with both externalising and internalising problems, children with externalising problems only, and children without externalising or internalising problems. The design of the study was that children were selected and included in a particular group based on their CBCL scores with the threshold set at 60, the score corresponding to the lower limit of the borderline clinical band. The children who scored under 60 on both the externalising and internalising scales were placed in the low-risk problem group (N=240), those who scored at least 60 on the externalising scale and under 60 on the internalising scale were

assigned to the externalising group (*N*=47), while the children who scored at least 60 on both externalising and internalising scales formed the group at risk for mixed problems (*N*=28). The laboratory assessment consisted of a series of short episodes designed to elicit executive control, attention, positive response, and frustration. Baseline and change RSA data were collected. The results indicated a main effect of problem group on RSA withdrawal, with the children at risk for mixed problems displaying the highest RSA withdrawal. Children at risk for externalising problems showed the smallest decreases in RSA, while the low-problem children had their RSA withdrawal scores in between those in the symptomatic groups. The findings of this study are novel compared to all the other studies reviewed up to here because they: 1) showed that high vagal withdrawal is not necessarily associated with good outcomes, and; 2) launched the possibility that a moderate, rather than high level of vagal withdrawal being associated with low risk for mental health problems in childhood.

1.3.8 Evidence on the Association Between Baseline Vagal Tone and Vagal Withdrawal

More recently, studies have started to examine both baseline vagal tone and vagal withdrawal, but the association between the two processes has still received little attention. Interpretation of the available findings is complicated by the lack of definition of baseline considered earlier, and the various conditions under which both baseline and withdrawal vagal tone have been studied. The relatively few studies that have examined the relationship are reviewed here. Calkins (1997) found modest correlations between vagal tone during baseline RSA (recorded with the children watching the same cartoon of neutral content mentioned earlier) and RSA withdrawal during a positive and a delay episode. No correlations were found between baseline and change of vagal tone during a negative task. Another set of exploratory analyses were conducted on two groups of

children. The first group (*N*=19) consisted of the individuals who consistently suppressed vagal tone during all three conditions following baseline, while the second group (*N*=20) included the children who did not suppress across tasks, or who suppressed inconsistently across conditions (i.e. only suppressed to one or two, but not all three phases of the experiment). Results showed that children who consistently suppressed vagal tone had higher baseline vagal tone than the ones who did not suppress at all or suppressed inconsistently.

In an earlier study which involved 24 normal and 11 regulatory disordered infants (exhibiting symptoms of extreme fussiness, intolerance to change and hyperarousal) aged 8 to 11 months, DeGangi, DiPietro, Greenspan, and Porges (1991) found that normal infants with high baseline RSA (baseline RSA in this study was measured with the infant sat on the mother's lap and not engaged in any activities) were able to suppress RSA during a sensory functioning task and a mental test, and baseline RSA was correlated with RSA withdrawal during the task (*r*=.63). On the other hand, the regulatory disordered infants showed a tendency for high vagal tone, but did not show consistent suppression, or did not suppress vagal tone at all in the tasks. Their baseline RSA scores did not correlate with the RSA withdrawal scores. Because of the very small sample, the results in this study should be interpreted with caution.

In a more recent study involving 62 newborns, Arditi et al. (2006) found a moderate correlation (*r*=.55) between baseline RSA (averaged from two conditions: quiet sleep and being held while not interacting) and RSA withdrawal during a painful heel-prick procedure. Also, infants with high baseline RSA (above the median scores) showed larger decreases during the procedure than their low RSA counterparts.

Taken together, these findings from small samples suggest some positive associations between baseline RSA and RSA withdrawal and that the presence or absence of a significant association may be related to the child's reactivity and regulation capacities, i.e. significant and positive associations are found more in samples of children with high reactivity and better regulation. High baseline vagal tone infants, found to be more reactive behaviourally and emotionally, may be better able to regulate themselves because they can allocate resources more readily during challenges or tasks, as seen when applying the vagal brake (Beauchaine, 2001).

1.3.9 Developmental Aspects of Vagal Tone

Vagal tone has been included as a measure of physiological reactivity and regulation in studies spanning from birth to adulthood and has inevitably been subjected to the question of whether or not it shows stability in time. If stability could be identified, it would suggest that RSA may provide a useful measure of developmental mediators. Low stability could have several implications, such as that the link between RSA and underlying mechanisms changes over time, or that measurements – particularly of vagal withdrawal – are not sufficiently adapted to different developmental periods. Equally, low stability may indicate lack of stability of the underlying mechanisms. Moreover, when studying phenomena from a developmental perspective, Borstein and Suess (2000a) stress the necessity of reporting results both in terms of continuity (i.e. unchanged absolute values as measured in time) and stability (i.e. unchanged rankings of individual scores in a group in time) noting that these two concepts, although interrelated, are different.

In a small sample of full-term babies, Harper et al. (1978) found steady increases in baseline vagal tone from the first month of life to 6 months. In this study, RSA was measured in three states (i.e. awake, quiet sleep, and active sleep) and vagal tone was higher in quiet sleep than in the other two states. However, results during the first month of life were generally inconsistent. Fox (1989) speculates that the first few weeks after birth may be a period of intense neurophysiological change and therefore not as a reliable period to collect data with predictive value.

Richards and Cameron (1989) recorded baseline RSA longitudinally at 14, 20, and 26 weeks and found that it significantly increased over the 3-month period. In another study, Stifter and Fox (1990) examined stability of vagal tone from age 2 days to 5 months and found no significant association between the two measurements. Also, RSA decreased from the first to the second assessment, which is in opposition to what other studies have found, but this might be because RSA in this study was measured in different states at different ages (i.e. during active sleep in the neonatal period versus quiet alertness at 5 months). Fracasso, Porges, Lamb, and Rosenberg (1994) also did not find developmental increases in vagal tone, but found stability in both baseline, and post-stress values across several 3-month periods of time within ages 5-13 months. Also, Izard, Porges, Simons, Haynes, & Cohen (1991) made five vagal tone measurements from 3 to 13 months and found good stability from 3 and 4 ½ months to 6, 9, and 13 months. Porter, Brian, and Hsu (1995) recorded baseline vagal tone on a small sample of infants aged 1-6 months and found no association between vagal tone at 1 month and the 3-month or 6-month values, although 3-month data predicted vagal tone 3 months later. Finally, moderate stability (r=.55), as well as a developmental increase in baseline vagal tone from 2 to 5 months were reported by Bornstein and Suess (2000b).

Overall, these studies suggest that after the neonatal period, findings on stability and developmental shifts in infancy tend to lead to more convergent results, namely developmental increase in vagal tone, with correlations ranging between r=.28 and r=.67 among measurements recorded a few months apart.

Studies also tried to address the issue of vagal tone stability from infancy to preschool age, some with encouraging results. Porges et al. (1994) included measures of baseline and withdrawal vagal tone in a study that examined physiological correlates of infant difficultness at 9 months in relation to outcomes at 3 years. Moderate to high correlations between RSA at 9 months and 3 years were found in both baseline (r=.55), and testing (r=.69) conditions. Consistent with previous research, baseline RSA increased longitudinally, perhaps due to increasing maturity of the nervous system during the first years of life. Similarly, Bornstein and Suess (2000a) examined continuity and stability of baseline RSA and RSA withdrawal in response to visual attention tasks in a longitudinal study from 2 months to 5 years and found a developmental increase, too. Calkins and Keane (2004) tested stability from 2 to 4 ½ years in a sample of 122 children. They found a developmental increase in the baseline vagal tone and some stability of suppression of vagal tone from certain tasks at age 2 to other tasks at age 4 ½. However, it is difficult to find a plausible interpretation for this final finding. Same tasks were used at both ages but with small adjustments to make them more age-appropriate (modifications of tasks were mainly made with regard to length of administration). It may be that even with these minor adaptations the tasks were still not age-appropriate, or their content was changed to the extent to which they did not measure the same construct anymore. In another investigation that involved older children, Doussard-Roosevelt, Montgomery, and Porges (2003) studied short-term stability of baseline and change RSA in preschoolers aged 5 to 6 years. They made three recordings at 2-week intervals and found moderate stability

(*r*=.58) across the three testings for the baseline values, but no stability was found for RSA withdrawal. This finding, together with similar results from the above-mentioned studies, suggests a greater stability of baseline vagal tone, as opposed to vagal suppression. The explanation may be in the higher complexity of vagal withdrawal, which may be subject to more situational influences than the baseline value.

Overall, studies examining stability and developmental shifts in vagal tone point toward a neonatal period characterised by high instability and poor RSA predictive values, which may be due to the difficult transition from the womb to the new environment, and possibly also due to central nervous system's immaturity (e.g. unmyelinated fibers), followed by a steady increase in stability during infancy and early childhood, with some speculation that baseline vagal tone may reach a plateau by age 5 years (Bornstein and Suess, 2000a). In contrast, vagal suppression to various challenges shows little stability across infancy or preschool-age, pointing toward some more complex higher level mechanisms which may be involved in physiological self-regulation in the context of a rapidly changing environment.

1.3.10 Methodological Considerations With Regard to Creation of Groups in Vagal Tone Research

In the absence of normative vagal tone data, investigations that have opted for group analyses in vagal tone had to decide on the method for assigning participants into groups based on their baseline and withdrawal RSA. The studies reviewed in this thesis have employed three types of methods. The first one was dividing the sample based on the median split of all scores, thus yielding high versus low RSA groups (see e.g. Arditi et al., 2006). A second approach was to use the mean split which produced the same high

versus low RSA groups (see e.g. Huffman et al., 1998). Finally, a few studies (e.g. Bazhenova et al., 2001; Moore and Calkins, 2004) have assigned individuals in two groups based on whether or not the pattern of their RSA scores matched the pattern of the mean RSA for the whole sample. For example, in Moore and Calkins the whole group pattern was that mean RSA during the second episode of the Still Face procedure (i.e. when mother becomes unresponsive) decreased from the first episode of social interaction and then increased in the second social interaction phase. Consequently, all infants whose RSA followed a similar trajectory (i.e. decreased from initial social engagement to the still face followed by an increase in the last social interaction episode) were assigned to the group of RSA suppressors, with the rest of the infants counting as RSA non-supressors.

Thus the grouping methods that have been used seem heterogeneous and somewhat arbitrary, and some of the results based on group comparisons should be approached with caution, particularly where the sample sizes were small.

1.3.11 Current Level of Understanding and Future Questions to Be Addressed in Vagal Tone Research

Previous sections have described the concept of vagal tone as proposed by Porges (e.g., 1995, 2007) in the polyvagal theory. His view is that the myelinated vagus has evolved in mammals to provide metabolic support for the increasingly complex processes of social interaction and emotional regulation. However vagal tone cannot be measured directly and most of the investigations had to rely on indirect measures, mostly in the form of RSA. The roles of baseline vagal tone and of vagal withdrawal as presented in the theory were then described. The proposed view is that baseline vagal tone is thought to reflect

homeostasis and support growth and restoration in the body in the absence of environmental demand; while vagal withdrawal is believed to be adaptive in the presence of challenges and help mobilising metabolic resources to allow the individual to face up to the stressor. Evidence was then provided regarding links with behaviours, some indicating that different processes may be linked with different behaviours (i.e. baseline vagal tone may be more linked with reactivity and temperament characteristics, while vagal withdrawal may reflect more regulation of behaviour). The limited evidence on influences on vagal tone was also presented.

A number of methodological issues were highlighted including difficulties in specifying the conditions of a baseline, and hence of withdrawal. A possible approach for overcoming these problems might be the use of a composite measure of RSA based on several measurements, which may reflect better the typical RSA level than when using one recording under conditions with little known impact on the individual's state. Research would be needed for testing the "goodness of the fit" of such a multidimensional model of RSA measurement. A second methodological issue concerns the lower stability (at least for vagal withdrawal) than what might be expected for a biologically based process with long term implications for development. Future studies should seek an agreement in the measurement of RSA and investigate RSA stability in different conditions.

Finally, there is also a critique of the polyvagal theory. One of the most critical views with regard to Porges's theory was presented by Grossman and Taylor (2007) and refers to both theoretical and methodological considerations. For example, Grossman and Taylor argue that RSA may not be a sole reflection of the activity of nucleus ambiguus, but also of that in the dorsal motor nucleus. Also, they disagree with Porges's evolutionary view

that the smart vagus (i.e. the vagal efferent fibers that originate in nucleus ambiguus) evolved in mammals only, while Berntson, Cacioppo, and Grossman (2007) emphasise the speculative nature of the view regarding the existence of separate neural pathways for cardiac vagal control. Regarding methodology, the main issue raised by Grossman and Taylor is the need to control for respiration and physical movement when measuring RSA. However, Denver, Reed, and Porges (2007) have found little evidence that respiration would be a confounding variable for RSA, while the evidence on the potential influence of movement seems less clear. To conclude, although the polyvagal theory does not lack criticism, it has become influential in the study of the link between the autonomic system and behaviour.

1.3.12 Why Study Vagal Tone?

As early as over one hundred years ago, scientists have promoted the idea of a direct link between emotional states and the activity of the internal organs. For example, James and Lange's theory of emotion, which emphasised the role played by the viscera and particularly the cardiovascular system, became influential at the beginning of the 20th century. Although their theory was later dismissed mainly because of its simplistic approach on causation of emotion, its original view on embodiment of emotions is currently receiving an interest greater than ever (Dalgleish, 2004).

The ANS may act as a window into the study of emotion because it controls the internal organs which regulate the mobilization of metabolic resources under stress. The heart is the main visceral organ that regulates metabolic output and is under sympathetic and parasympathetic control. Thus, neural regulation of the heart is likely to have an impact on the regulation of the emotional processes.

Vagal tone, which is a non-invasive measure of cardiac parasympathetic activity, is thought to have links with emotions and behaviours, although it is not clear what the nature of the links is. One possibility is that vagal tone is an underlying physiological system that supports emotional reactivity and regulation. Still face studies may be one of the best examples in support of this view, with findings which are somewhat consistent in that vagal tone decreases from the social engagement episode to the stressful still face episode and is followed by recovery during the resuming of the social interaction (see, e.g. Moore & Calkins, 2004; Weinberg & Tronick, 1996). Moreover, these studies in which vagal tone decreased and then increased to comparable levels as before the stressor also found an increase in negative emotions during still face followed by their decrease during the social reunion, which may suggest that withdrawal of vagal tone was a coping mechanism. Interestingly, some studies in which vagal tone did not recover (i.e. RSA remained at a low level) during the resuming of the social interaction also reported a carry-over effect of negative state from the still face (see, e.g. Moore, 2010), which again suggests that vagal withdrawal may index a prolonged reaction of coping for the duration of the negative emotions.

Another possibility is that vagal tone precedes behaviour. Evidence for this may come from a study conducted by Porges et al. (1994) in which vagal tone at 9 months was found to be a predictor for difficultness at age 3 which suggests that vagal tone in infancy may be a physiological marker for later behaviour.

And yet another view might be that vagal tone reflects something different from behaviour, but equally important, as suggested, for instance, by a study in which children who had either high or low vagal withdrawal during a series of tasks had more symptoms

of psychopathology than those who decreased vagal tone moderately (Calkins et al., 2007).

Such possible interpretations of the nature of the vagal tone construct in relation to emotion and behaviour suggest that the processes involved with vagal tone may be complex and not well understood. Nevertheless, evidence exists in support of each of these understandings. Conducting longitudinal studies of vagal tone and behaviours that include the possibility of testing of prediction and mediation may elucidate some of these issues.

1.3.13 Why Study Prenatal Prediction to Vagal Tone?

The work on fetal origins of adult disease (presented in Section 1.2.2) has found reliable links between low birth weight and cardiovascular disease at adult age (Barker, 1995). The fetal adaptations to intrauterine adverse conditions include altered levels of catecholamines (Westgren et al., 1997) which are involved in regulating the autonomic reactions to stress. There is evidence from both animal and human studies (see Schaffer et al., 2008) suggesting links between indicators of autonomic reactivity (such as heart rate and heart rate variability) and consequences of prenatal stress (e.g. low birth weight), although some have found an effect only in women (e.g. Ward et al., 2004). Moreover, disturbances in the activity of the sympatho-adrenal system are associated with hypertension (de Champlain et al., 1989), while the role of the parasympathetic system has been less studied (El-Wazir et al., 2008). Therefore, it is plausible to hypothesise possible programming effects of the sub-optimal prenatal environment on the parasympathetic control on the heart since the cardiac sympathetic and parasympathetic actions are usually coupled.

There is evidence suggesting concerted dysregulation in several neurobiological systems, vagal tone included, following prenatal stress. For example, Field et al. (2004) found lower vagal tone, together with greater frontal EEG asymmetry, higher cortisol and lower dopamine and serotonin levels in the neonates of depressed mothers (a review of the studies that examined prenatal stress in association with vagal tone in the offspring is presented in Section 1.3.14 below). Reactions to stress are likely to involve changes in several regulatory systems and vagal tone should be considered one of the main focuses in the study of stress reactivity (Gunnar et al., 1995).

Finally, clear evidence suggests links between maternal prenatal stress and infant temperament (see Section 1.2.3.2). Vagal tone, on the other hand, was consistently found to be linked with several temperamental characteristics in infancy and childhood (see Section 1.3.5.3). Mechanisms for the link between prenatal stress and offspring temperament may involve dysregulation in the activity of the HPA axis (Davis et al., 2007), although this does not eliminate the possibility of other concurrent pathways being involved (Van den Bergh & Marcoen, 2004). This provides support for the current investigation of an association between maternal stress in pregnancy and vagal tone adaptation in infancy.

1.3.14 Review of Studies That Examined Prenatal Stress in Relation to Vagal Tone

Very little is known about influences on vagal tone. There have been no genetic studies, and a small number has examined prenatal stress. All but one of the studies of prenatal stress have assessed vagal tone in the neonatal period and none has included estimates of vagal withdrawal. This means that no previous study is directly comparable with the one

reported in this thesis. Nevertheless they are highly relevant to the general topic of prenatal stress and vagal tone and each is reviewed in some detail here.

Jones, Field, Fox, Lundy, and Hart (1998) examined 63 pregnant mothers from a larger sample (details on the method of selection was performed are not provided) with 35 being classed as depressed and 28 as non-depressed in the third trimester of pregnancy based on scores at the Center for Epidemiological Studies of Depression Scale (CES-D). No information on the cut-offs used for the CES-D was presented and no demographic characteristics of the sample were shown. Results were that 1-week-olds of prenatally depressed mothers had lower baseline vagal tone than those of non-depressed mothers. The analyses did not appear to control for postnatal maternal mood.

Field et al. (2003) studied 132 predominantly low risk mothers who completed the Trait Anxiety measure of the Spielberger's State-Trait Anxiety Scale (STAI) at 20 weeks gestation. Half of them were classed as highly anxious using the median split. Results showed that newborns of mothers with high anxiety had lower baseline vagal tone. Although measures of maternal depression and anger were administered both pre- and postnatally, the analyses did not comprise *t* tests comparing mean scores. Analyses controlling for possible confounding variables were not presented.

Field et al. (2004) examined maternal mood and biochemical (e.g. cortisol levels from first-morning urine samples) indicators in140 mothers of lower to middle socioeconomic status at 20 weeks gestation, and then tested their neonates using several behavioural and biological measures. Method of recruitment was as follows: the first 70 mothers who scored at the cut-off of 16 or higher on the CES-D were assigned to the depressed group and the first 70 mothers scoring at or below 12 formed the non-depressed group

(although the description of procedure is not very clear, one can interpret that the mothers with scores of 13 to 15 were excluded). Of the 140 women, 119 (*N*=58 depressed) participated at the neonatal phase also. Results showed that newborns of mothers with high depression had lower baseline vagal tone. Also, prenatal cortisol levels predicted infant vagal tone. These results should be viewed with caution because of the possible selection bias.

In a small study conducted by Ponirakis, Susman, and Stifter (1998), 27 primiparous adolescents completed maternal mood questionnaires and a personality inventory at 16 weeks gestation and then again in the third trimester of pregnancy (i.e. 32-34 weeks), and had their infants assessed for baseline vagal tone at 3-4 weeks of age. To reduce the number of analyses, negative trait and state emotionality factors were generated. The negative trait factor comprised the NEO-AC Personality Inventory subscales of depression, anxiety and hostility, the STAI trait scale, and the Trait measure of the State-Trait Anger Scale (STAS). The negative state emotionality factor consisted of the STAI and STAS state scales and Beck Depression Inventory (BDI). The hypotheses were that both the trait and state factors at each of the two time points in pregnancy would be associated with vagal tone. Results showed that only one of the four hypothesised links was significant, that the Trait factor at 16 weeks prenatal predicted baseline vagal tone in the expected direction. Moreover, social support was found to mediate the association. The small size of the study makes interpretation of findings difficult and in particular non-significant associations may have arisen from Type II errors.

The only study of prenatal stress and vagal tone that seems to have reported findings at an age other than the neonatal period was conducted by DiPietro et al. (2006) and involved 84 2-year-olds. One baseline vagal tone measurement was collected with the

children sitting on the mothers' lap and being entertained with books and toys. There were several associations between prenatal stress and lower vagal tone although none was statistically significant after controlling for infant sex. The inconsistencies with the findings reported in the newborn studies may be explained by differences in the methodology that was used, or the age of the participants.

Overall, the investigations on the link between prenatal stress and vagal tone found some evidence in support of the hypothesis that negative emotions in the mother measured in pregnancy may be linked with infant vagal tone in baseline condition. Only one study (i.e. DiPietro et al., 2006), which was conducted with young children, reported a lack of associations. However, the designs of these studies have generally been less strong, reflected in small sample sizes or measurement biases and a preference for group-based analyses while using debatable cut-offs. Besides, the outcome was usually assessed in single measurements, which together suggest that more research with stronger designs and larger samples is needed to address the question.

1.4 Sex Differences and Psychopathology in the Context of Prenatal Stress

1.4.1 Sex Differences and Psychopathology – General Considerations

Sex differences in timing and incidence of child and adolescent psychopathology are well documented. Externalising disorders are more frequent in boys and are usually characterised by an early onset (Hill, 2002), while internalising problems, which are more common in girls, have a later onset, usually in adolescence (Angold & Costello, 2001). These could arise in two distinct ways. Firstly there may be sex differences in rates of vulnerability but not in mechanisms, or there could be different mechanisms.

1.4.2 Evidence That Sex Differences Arise From Differences in Rates of Vulnerabilities but Not in Mechanisms

The causes of the difference are not well understood. In relation to early onset conduct problems, evidence from the Dunedin Multidisciplinary Health and Development study, a general population, longitudinal study from early childhood to adult life, suggests that males have higher levels of the vulnerabilities than girls (Moffitt, Caspi, Rutter, & Silva, 2001). Males have higher rates of undercontrolled temperament and neuro-cognitive deficits, and more hyperactivity and more peer problems than females. Moffitt et al. examined their data in detail for sex differences in mechanisms and did not identify any.

In considering the relevant mechanisms it is essential to keep in mind the complexity of the processes. In relation to childhood aggression, Hay (2007) concludes that three principles must be kept in mind when trying to understand the origins of sex differences. Firstly, the earlier biological maturation of girls may exert control over their aggressive

tendencies thus acting as a protective factor against developing later aggression. Second, boys' increased emotional and cognitive deficits linked to later aggression are translated into a small number of highly aggressive boys that raises the overall level of male aggression. Third, because children engage in sex-segregated social activities from an early age and because conflicts are dealt with differently in groups of boys and girls it is plausible to say that boys engage more often in overt aggression, while girls' violent behaviours may be expressed in an indirect manner, such as verbal disputes.

Similar considerations need to be applied to depression. There are multiple causal processes and the condition is probably heterogenous (Jaffee et al., 2002). Sex differences may in part arise from sex hormone effects. The increase in incidence of depression at puberty appears to be accounted for substantially by increases in estrogens in females (Angold, Costello, & Worthman, 1999). This is consistent with the proposal that females have an increased level of vulnerability, but there are not sex differences in mechanisms.

1.4.3 Candidates for Sex Differences in Mechanisms in the Context of Prenatal Exposure to Stress

In spite of the evidence reviewed in the previous section, studies of perinatal stressors and development provide indications of sex differences in at least three ways, sex differences in psychopathology related to early stressors, sex differences in stress related biological mechanisms found in animal studies, and sex differences in the physiological consequences of early stressors.

1.4.3.1 Sex Differences in Mechanisms for Psychopathology Following Prenatal Stress

Costello et al. (2007) set out to test the fetal origins hypothesis in a large general population representative sample, the Great Smoky Mountains Study. They examined whether low birth weight was an independent predictor of adolescent depression, and whether there was evidence of stress reactivity over development whereby low birth weight was associated with an elevated effect of childhood adversities. Low birth weight was associated with adolescent depression in females, and it appeared to potentiate childhood adversities such as neglect, physical and sexual abuse. Thus low birth weight created a vulnerability to depression which markedly increased the risk for depression in the presence of childhood adversities. Crucially, for this study, this effect was seen only in females and was entirely absent in males (interaction odds ratio, 0.015 [95% CI, 0.001-0.172]; $p<.001$). Although the Smoky Mountains findings are in many ways compelling the study had the major limitation that measurement started at age 9 years. This meant not only that low birth weight was based on maternal report, but also that the many other possible prenatal and immediate postnatal risks could not be measured.

Prospective studies from pregnancy, however, have also provided some support for sex differences in mechanisms. Van den Bergh et al. (2008) examined prenatal effects of maternal anxiety in 58 boys and girls aged 14-15. They found an interaction with sex of child in the association between anxiety measured at 12-22 weeks in pregnancy and adolescent depressive symptoms ($p=.022$) which held at p level of .05 after controlling for confounders. Increasing maternal anxiety scores were associated with increasing depressive symptoms in girls ($p=.0027$), while in boys depression remained constant ($p=.85$). However, due to the small sample size these results would need replicating in larger studies.

Hay, Pawlby, Waters, and Sharp (2008) conducted a prospective study of 121 mothers starting in pregnancy with interview based assessments of DSM diagnoses. Prenatal depression predicted adolescent depression after controlling for postnatal depression but only in girls. The sex of child by prenatal depression interaction was significant. This study was notable for documenting the high rate of recurrence of depression over the children's childhoods in women with prenatal and postnatal depression. When the prenatal depression by sex interaction was examined jointly with depression after the perinatal period, it no longer made a significant prediction to adolescent depression. However, the odds ratio for the interaction remained moderately high (i.e. 5.36) and there were no indications that there was an interaction between later maternal depression and sex of child. Thus, although firm conclusions cannot be reached, this study suggests that there may be a sex by maternal depression interaction in relation to prenatal but not subsequent depression.

In the ALSPAC study (e.g. O'Connor et al. 2002, 2003), which assessed around 7000 dyads from pregnancy to adolescence, associations were found between maternal anxiety in late pregnancy and total problem scores in both girls and boys at 47 and 81 months. There were no significant sex of child by prenatal stress interactions. However there were some non-significant findings consistent with sex differences. The strength of prediction slightly decreased from 47 to 81 months in boys but stayed at the same level in girls. Furthermore, for girls, links were found between maternal anxiety at 18 weeks gestation and behavioural/emotional problems (*OR*=1.77) at age 81 months after controlling for anxiety at multiple points in time, both antenatal and postnatal, while the same prediction was not significant in boys (*OR*=.72).

De Bruijn, van Bakel, and van Baar (2009) examined associations between reports of maternal and paternal stress at three times in the prenatal period, and internalising, externalising and total problems in a sample of 444 boys and girls aged 14-54 months, while controlling for confounders. Only 5 predictions of a total of 36 were significant, with maternal negative emotions at 12 weeks gestation being linked with internalising and total problems in boys, while associations were found between reported stress in the last trimester (i.e. 36 weeks) and internalising, externalising and total problems in girls. Although the number of significant predictions is only slightly higher from what would be expected to occur by chance, this study suggests that there may be sex differences in predicting problems in early childhood, particularly with regard to timing of stress in pregnancy.

In spite of these promising findings, it is important to emphasise that sex by prenatal stressor effects in relation to psychopathology are not well established. This is in part because studies of externalising problems commonly only include males, and of depression only females, and because sex of child by risk interactions are often not examined. For example, Costello et al. (2007), commenting on the inconsistent literature on low birth weight, point out that there were only two other studies up to 2007 that examined for sex differences. Both found an effect only in girls (see Patton, Coffey, Carlin, Olsson, & Morley, 2004; and, Frost, Reinherz, Pakiz-Camras, Giaconia, & Lefkowitz, 1999).

1.4.3.2 Prenatal Stress and Consequences for the Offspring in Animal Studies – Evidence of Sex Differences

Animal studies have reported large amounts of evidence with regard to sex differences in biological regulatory systems and behaviours in the postnatal life of the offspring of prenatally stressed mothers. Although most of these studies have been conducted in rodents, sex differences in behaviours following stress in pregnancy have been reported in other animals, such as monkeys (Schneider, 1992). Weinstock (2007) reviewed the rodent literature and concluded that these differences might be widespread, even if also variable, depending on type and intensity of stressor, timing of stressor, outcome measured and species. A variety of behaviours were found to differ in males and females following exposure to prenatal stress, but the most striking, yet sometimes contradictory, differences were found in reactions of fear associated with novel situations, depression-like behaviours and spatial learning and memory. On the other hand, reactivity of HPA axis has been the most widely studied biological system potentially altered by stress in pregnancy.

Focusing on examination of anxiety-like behaviours, Zagron and Weinstock (2006) found that females of control (i.e. prenatally non-stressed) rats spent more time in the open arms of a maze than males, which indicates that in normal conditions females experience less novelty-induced fear. However, a different pattern was noted in the prenatally stressed offspring. When a mild form of stress (i.e. one 30-minute restraint daily) was applied during days 14-21 gestation, a significant decrease of time spent in the maze (indicative of raised anxiety) was seen in females, while no significant change occurred in males. Interestingly, these effects were totally abolished by maternal adrenalectomy and replacement with maintenance levels of corticosterone, which suggests that offspring

behaviours were in direct connection with dysregulation of maternal HPA axis. The fact that females became more anxious than males following moderate prenatal stress suggests that prenatally stressed females may be more sensitive to anxiety than males.

Zuena et al. (2008) also found sex differences in the anxiogenic behaviours of prenatally stressed pups, although the direction of effects was in opposite direction to that reported in Zagron and Weinstock (2006) and other studies. In Zuena et al., prenatally stressed females were found to have decreased fearful behaviours inside the maze compared to the males. It may be that differences in the intensity and duration of the stressor accounted for this discrepancy, as it has been demonstrated that more severe stress was associated with increased anxiety in both males and females (Weinstock, 2007). However, explanations may be more complex and may go beyond the characteristics of the stressful stimuli that were applied to the mothers in pregnancy. This is because in Zuena et al. the biological and behavioural measures in the PS offspring were not coupled. More specifically, in prenatally stressed females who showed decreased anxiety it was noted activation, and not inhibition of specific glucocorticoid receptors in the hippocampus thought to have a role in the expression of anxious behaviours. The opposite mechanism (i.e. decreased function of the glucocorticoid receptors) was observed in the prenatally stressed males, suggesting that prenatal stress may have a decoupling effect in the interdependency of biological and behavioural parameters.

In a related line of research, Welberg, Thrivikraman, and Plotsky (2006) studied the effects of environmental enrichment on the regulation of the HPA axis and found that the offspring of the mothers who benefited from improved housing conditions in the perinatal period displayed different patterns of reactivity of the HPA axis in chronic and acute stress than the offspring of controls, and that these patterns also differed by sex.

Specifically, the chronically-stressed females of the environmental enrichment mothers showed a higher level of secretion of ACTH to acute stress than the chronically-stressed females of the controls, while no differences were noticed in males. This suggests that chronic stress desensitised the ACTH response to subsequent stress only in females of mothers who did not benefit from environment enrichment, and that enrichment in early life seemed to buffer female offspring from showing hyporesponsivity in the HPA axis response to acute stress following a period of chronic stress.

Differences in maternal care in the early postnatal period may also have a programming effect on the HPA axis, as maturation of the brain extends in the postnatal life both in humans, and non-humans. Desbonnet, Garrett, Daly, McDermott, and Dinan (2008) showed that maternal separation was associated with sex-specific effects in secretion of hormones (e.g. CRH) regulating the activity of the HPA axis. CRH is crucially involved in the production and release of ACTH which secretes cortisol (corticosterone in rodents) from the adrenal cortex. Subjects in the study were exposed to maternal separation for a few hours a day in the neonatal period. In adulthood, the level of corticotrophin-releasing factor was measured following stress (i.e. forced swim test). Results showed that the maternal separation females had higher CRH levels in comparison to their male counterparts, which shows that neuroendocrinological stress system in females may be more reactive following early maternal separation stress.

Finally, limited but highly relevant evidence exists on sex differences in cardiovascular response to restraint stress in prenatally stressed rats. In a study conducted by Igosheva et al. (2004) male and female offspring were subjected to acute stress in a restraint procedure and had their systolic and diastolic blood pressure, heart rate and blood pressure variability (i.e. extent in variation of blood pressure within short intervals of time

and indexed by its standard deviation, thus the higher the standard deviation the greater the blood pressure variability) measured during the restraint procedure and shortly after returning to their cages. Results showed sex differences within the prenatal stress condition group in systolic blood pressure and blood pressure variability in the recovery period. Specifically, prenatally stressed females had higher systolic blood pressure and higher blood pressure variability while recovering from the stressor than the males.

In summary, animal studies provide support for a programming effect of prenatal stress with different consequences for females and males in behaviour and hormonal stress response. Although there is still some contradiction with regard to direction of effects which is likely to arise, at least in part, from differences in the characteristics of the stressful stimuli applied in pregnancy, these studies generally suggest that prenatally stressed females have a greater vulnerability for developing anxiety-like behaviours and alterations in the activity of the HPA axis than males.

1.4.3.3 Prenatal Stress and Consequences for Cardiovascular Activity in Humans – Evidence of Sex Differences

Human research into the medical consequences of prenatal stress may also be relevant in elucidating links with autonomic reactivity. An increasing body of evidence suggests that prenatal stress, as evidenced by preterm birth or low birth weight, is associated with altered autonomic functioning under psychosocial stress, and in the majority of studies, these associations are modified by sex (for a review, see Kajantie & Raikkonen, 2009). It is generally proposed that women of low birth weight respond predominantly with increased blood pressure and elevated heart rate to stressful situations, which is likely to result from large vagal withdrawal and strong sympathetic control on the heart. This is

consistent with the findings reported in rodents in Igosheva et al. (2004) on cardiovascular reactivity following acute stress. Low birth weight men, on the other hand, are more likely to react with increased HPA axis response to stressors in everyday life. However, the evidence concerning women can be somewhat conflicting because of the potentially crucial role played by age and menstrual cycle. Specifically, in childhood and at postmenopause age women seem to be at a higher risk for dysregulated autonomic reactions than during young and middle adulthood (Phillips & Jones, 2006) due to lower secretion of estrogens, which seems to be involved in the regulation of the autonomic system responsiveness. Also, there is evidence suggesting that prenatally stressed women show attenuated autonomic reactions to stress during the luteal phase.

Mechanisms for the suggested link between prenatal stress and autonomic reactivity and regulation are unknown, but it is possible that they involve transplacental passage of maternal stress hormones such as cortisol, with alterations in the structure and functioning of the amygdala, which is highly involved in the autonomic responses to stress, and possibly in other cortical and subcortical areas. However, human studies have not yet examined possible pathways and evidence on the autonomic system mediation coming from animal work is very limited as most of the work has focused on the HPA axis.

Taken together, these findings suggest that prenatal stress may have programming effects on the ANS which are associated with different patterns of sex-related responsiveness to psychosocial stress and that age and sex hormones in females seem to play vital roles in understanding these differences.

1.5 The Current Study – Aim and Hypotheses

1.5.1 Rationale for Predictions Regarding Sex Differences in Vagal Tone Following Prenatal Stress

The general case has been made that vagal tone provides an index of physiological regulation that contributes to, but is not the same as, emotionality, and similarly is relevant to, but does not map directly on to, emotional and behavioural symptoms in childhood (see Section 1.3.12). In generating the hypotheses, while studies of sex differences in prenatal stress and emotionality and psychopathology were informative, they could not provide a straightforward guide to the direction of effects.

There were three main starting points in hypothesising direction of effects in sexes. First, elevated vagal tone and withdrawal can be protective, as in the case of vagal withdrawal buffering the impact of marital conflict on child behavioural symptoms (El-Sheikh & Whitson, 2006). Second, there may be differential associations of vagal tone to emotionality over time. For example, in the small study by Porges et al (1994) presented in Section 1.3.5.3, high vagal tone was associated with increased emotionality at 9 months but decreased at three years. The authors speculated that high vagal tone and increased emotionality infancy led to greater parental engagement and hence developmental gains. Third, high, or, perhaps, excessive vagal tone or withdrawal, can contribute to sustained increased emotionality and to internalising symptoms (Calkins et al., 2007).

An extensive literature on sex differences in emotional expression and social problem solving indicates that females are more likely to respond to stress or challenges with emotion associated social problem solving, while males are more likely to attempt less

emotion laden and instrumental solutions (Vigil, 2009). Consistent with this view, Booth, Granger and Shirtcliff (2008) have proposed that in conditions of stress, males may be more inclined to adopt fight/flight reactions, while females seem to be rather predisposed to "tend and befriend". This appears to be a universal difference, and writers from Darwin onwards have speculated as to its evolutionary function, implying a biological underpinning (Darwin, 1872). Against this background it was plausible to propose that there may be biological mechanisms whereby females exposed to risk are likely to intensify their attempts at adaptation, especially within social contexts, by increasing vagal and emotional reactivity with developmental gains of the kind described by Porges et al. (1994). Increased vagal tone has been discussed in connection to a greater capacity for social engagement (see Sections 1.3.2 and 1.3.5.5) and vagal withdrawal has been linked to more advanced social skills (Stifter & Corey, 2001; Graziano et al., 2007). However, this adaptive activation of stress-response systems, when intense or repeated, may under some conditions and at later ages contribute to eroding health by compromising the immune system (Booth et al., 2008). Similar mechanisms, perhaps linked to intense and frequent episodes of negative emotionality, may underpin sex differences in associations between prenatal stress and depression (Costello et al., 2007). Thus it was hypothesised that the prenatal risks of depression and anxiety would be associated with increasing vagal tone and withdrawal in females, both because of the potential for increasing adaptation under some conditions, and conferring vulnerability to internalising disorders under others.

1.5.2 Aim

The aim of the project is to examine links between maternal stress during pregnancy and infant vagal tone and vagal withdrawal, neurophysiological indices of emotion reactivity and regulation, in the context of the fetal origins hypothesis.

1.5.3 Hypotheses

Hypothesis 1: There will be main effects and interactions with infant sex of maternal depression and anxiety at 20 and 32 weeks gestation on vagal tone in the infant at age 29 weeks.

Hypothesis 2: There will be main effects and interactions with infant sex of maternal depression and anxiety at 20 and 32 weeks gestation on vagal withdrawal in the infant at age 29 weeks.

Chapter 2

Method

2.1 Overview of the Chapter

The Method section contains information on all methodological aspects that were employed in the current research. First, it presents an outline of the study design, followed by an explanation of the statistical analyses that were used and reasons for choosing certain types of analyses. The Sample section presents information on participants' recruitment protocol, detailing the assessment waves, and also the sample characteristics. The Measures section describes the instruments that were used throughout the study, together with a rationale on why these measures were used and how do they fit within the aims of study. Finally, information on the ethical approval of the study is presented.

2.2 Study Design

2.2.1 Sample Overview

The sample for this study was derived from a consecutive sample of first time mothers recruited in the antenatal clinic of a large hospital serving a defined geographical area. The aim of recruitment to this consecutive, *extensive*, sample was to establish a representative sample for epidemiological study. Subjects for this study were identified from the extensive sample on the basis of psychological abuse reported in their current partner relationship, using a standard measure, and formed the *intensive* sample. The aim of recruitment to the intensive sample was to provide a well defined group of mothers and babies, with elevated psychosocial risk, with which experimental and therefore labour intensive procedures could be used.

2.2.2 Power Analysis

Power was explored using the Stata procedure powerreg. Using a sample size of 250, which is typical of the analyses presented here, 80% power is retained to detect the effects of a covariate that explains just 3% of the variance of a continuous response variable in a bivariate regression using a 2-tailed test and p=0.05. For multivariate regressions 80% power is retained for examining the effects of each of 5 predictors in which the predictor adds a further 4% to the explained variance from a base of 25%.

Interactions have been examined where the study has power to do so and interactions with sex of infant are the prime example. With an equal sex distribution power for interactions is maximised, and it is further increased where the main effects are small, which several times in this study appeared to be the case. Under such circumstances 80% power is retained for interactions that contribute as little as 4.3% to the explained variance when testing main effects and interaction (2 tailed p=0.05).

2.2.3 Measurement Overview

The extensive sample was recruited at the 20 weeks ultrasound scan, and participants completed a range of questionnaire and brief interview measures covering current mental health and psychosocial stressors. The intensive sample was assessed using questionnaires, interviews, experimental procedures and cortisol estimations at around 32 weeks gestation. Intensive and extensive sample measures were taken when the infants were 5 weeks old. Further observational and experimental measures were used with the mothers and infants in the intensive sample when the children were 29 weeks old. Information was gathered from medical records of all participants about pregnancy and

birth, using a standard protocol. In this thesis findings are presented using assessments conducted at 20 and 32 weeks gestation, and at 5 and 29 weeks after the birth.

2.3 Approach to Statistical Analyses

2.3.1 General Description of Approach to Variables

2.3.1.1 Variables

Distributions of all scores were examined for skewness, and where required, appropriate transformations were used. Logarithmic transformation is an effective way of reducing skewness because it pulls outlying data from the tails of the distribution to its center. Where simple log transformations were not effective, the approach was to use modified log transformations according to the model ln(x + score), where x represents the mean of the values that added to the corresponding scores led to zero skewness. The values used to calculate the mean x were identified by employing the zero-skewness command in Stata. These mean values were revealed as +7.4 for depression and -15.87 for the state anxiety scores. Values of skewness less than twice the standard error of skewness were accepted for parametric analyses.

2.3.1.2 The Analysis of Vagal Tone Data

Two contrasting approaches were used. In the first, the emphasis was on the difference between mean scores for each procedure, examined either using repeated measures analysis of variance, or using difference scores in multiple linear regression. In the second, the possibility of an underlying latent vagal tone construct was examined using principal component analysis. This is a justifiable data reduction method when several measurements that correlate highly are available (Field, 2009).

2.3.2 General Approach to Analysis of Longitudinal Data

Several approaches were used in relation to the analyses of longitudinal data.

First, correlation analyses were employed to assess the strength of the associations of scores across the time points. With mood data this approach is a necessary first step given that associations usually remain strong.

A second approach is to assess changes in scores across time which is done using repeated measures analysis of variance. Significant drops or increases can help identifying sensitive periods and thus interpret associations easier.

A third approach was to test whether later outcomes (e.g. infant vagal tone on its own or in interaction with sex of infant) were specifically associated with maternal stress at a certain point in time, thus testing for timing. This was done in multivariate analyses of variance (MANOVA) but in a rather unusual way by entering the later measures as independent factors and the four maternal mood scores as dependent variables.

2.3.3 Approach to Possible Confounders

Including potential confounders in testing associations between predictors and outcomes is essential in order to identify whether a genuine link exists between the studied variables. Confounders are variables that are associated with both the predictor and the outcome. In the current study, possible confounders were selected from risk factors that have been identified in the literature as being linked with both the predictors and outcomes. The first step in identifying confounders was to examine associations with

predictors using *t* tests or correlations. The variables where significant associations were found were then entered in linear regression models to test for links with outcomes.

2.3.4 Approach on Association Between Predictors and Outcomes While Accounting for Confounders

Only the predictors most strongly linked with outcome measures were considered for further analyses. This was done because less strongly linked variables are likely to explain same variances in the outcome as with the more strongly linked scores, which would make the association with the outcome less robust. Separate analyses were performed for different mood measures to avoid colinearity which is widely reported. Further investigations used regression analyses because the predictors were preferred to be used as continuous variables; using them as categorical variables would have yielded small numbers in the symptomatic groups which would have made it very difficult testing for interactions. In the first step the maternal mood scores together with confounders and current mood were entered. Confounders in interaction with infant sex were entered in the second step and in the last block the hypothesised maternal mood by sex interaction was entered. Individual contributions of predictors and interaction terms were assessed by examining the improvement in the overall fit of the model and magnitude and directions of effects were assessed by looking at the beta scores and the associated *p* values.

2.3.5 Approach to Interpretation of Analyses

Level of significance was set at a level of .05 throughout the analyses. It was considered that a more stringent approach was not appropriate in the current study because of

several reasons. First, the number of tested hypotheses was limited and specific, which reduced the chances for occurrence of Type I errors. Second, testing for associations between maternal mood at all four points in time and infant measures in multivariate models reduced the number of analyses which again prevented from falsely rejecting null hypotheses. Third, data reduction in RSA analyses and use of one RSA measure instead of five contributed to keeping the number of analyses low.

The issue of statistical power can be regarded under two different aspects. First, sizes of risk groups for some of the categorical confounders were quite small which made useful calculation of effect sizes. Second, examining such variables in interaction further reduces the size of groups which may make results difficult to interpret. In the case of the variables that can be analyzed both continuously and categorically, this possible power limitation contributed to the decision of using the variables as scores.

2.3.6 Statistical Software

All statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS) version 15.0 for Windows, except for the power analyses (presented in Section 2.2.2) and the transformation formulae for the maternal depression and anxiety data (explained in Section 2.3.1.1) where the Stata software was used.

2.4 Sample

2.4.1 Sample Selection

2.4.1.1 The Extensive Sample

The extensive sample was recruited from the sole maternity clinic serving a well-defined geographical area with a wide demographic mix, ranging from inner-city deprivation in Birkenhead to the leafy suburbs of Hoylake. The socio-economic status of the sample is described in Section 2.4.3.1 and reveals that this sample was somewhat more deprived than the UK population generally. Also, figures for 2007 published by the Office for National Statistics (ONS, 2008) on the ethnicity of inhabitants of the Wirral showed that the region was under-represented in terms of ethnic minorities, with only 3% of its total population being of non-White origin, compared to the average of 11.3% in England and Wales. It was expected that the sample for the current study would show reduced non-White ethnic representation compared with figures at national level.

2.4.1.1.1 Procedure for Recruitment to the Extensive Sample

At the 12-week scan the clinic midwives in the hospital asked all eligible pregnant women whether they would be willing to hear more about a study on pregnancy and child development at their next hospital appointment. Information sheets were given to the future mothers to take home, and posters were put in the waiting area. Mothers' verbal agreement with regard to being approached again at the 20-week scan were recorded by the hospital midwives in a computer file which was made available to the research team

The research team for the 20-week recruitment was formed of three research midwives based in the antenatal clinic. Two rooms located nearby the clinic reception were made available to the study and served as location for consenting the participants and storing the files. All future mothers who agreed to hear more about the study at 12 weeks, together with those who were missed by the clinic midwives were approached individually at the 20-week scan by the research midwives who explained the extensive study and gave the mothers information sheets to read. All women were told that all first time mothers on the Wirral giving birth during 2007-2008 would be invited to take part in the study in order to find out more about children's health and development and what influences might stress and events in pregnancy have on them, and that participation involved questionnaires that day, and again when the babies were 8 weeks and 12 months. Partners, if present, were also approached with regard to taking part in the study and were provided with information sheets. The women and partners who agreed to take part were invited separately in a room and were given separate consent forms to sign. The consent forms included issues regarding confidentiality of data, sharing the information with other researchers, as well as permission for tracking down the participant via General Practitioner (GP) or another member of the family had all means of trying to contact the participant directly failed. Also, permission was requested from the mother to contact her again at the beginning of the third trimester of pregnancy to arrange an appointment to describe the intensive part of the study and gain informed consent to take part if her name was selected for follow-up. Copies of the information sheet and consent form for the mother for the 20 weeks gestation phase, together with consent of tracking her down via the GP are provided in Appendix A (sections A1, A2 and A3). After being consented, the future mothers and partners were given separately a different set of questionnaires to complete. The mother's pack asked about demographic information, maternal mood (i.e. The Edinburgh Postnatal Depression Scale, EPDS, and

the State Anxiety Scale of The State-Trait Anxiety Inventory, STAI) and psychological abuse in current relationship (The Dunedin Relationships Scale) which formed the basis for identifying the intensive sample (see Sections 2.5.1.1 to 2.5.1.3 for description of these measures). The women who declined participation were asked whether they would agree to provide with their age and postcode in order to be able to compare them with the women who accepted being in the study, which most of them did.

2.4.1.1.2 Inclusion Criteria for the Extensive Sample

All expectant primiparous mothers of singletons who had been booked in for the 12-week scan at the antenatal clinic of the Wirral Arrowe Park University Hospital between 12 February 2007 and 26 September 2008 and were of age 18 and over at the appointment day were invited to take part in the study. Mothers below age 18 were not selected because of the legal and ethical issues regarding children's disclosure of potentially sensitive information. Mothers who had premature or low birth weight babies were kept in the study because these conditions may be linked to prenatal stress, which is one of the project's main aims.

2.4.1.2 The Intensive Sample

The intensive sample in the study was designed to be over-representative in terms of risk for later behaviour problems in children by selecting a high-risk group of future mothers who report highly on a partner psychological abuse scale together with a smaller random sample reporting low levels of abuse. Psychological abuse is a major source of stress in pregnancy and a risk indicator for mental health problems which are associated with later psychopathology in the offspring, including behaviour problems. On the other hand,

many women who engage in abusive relationships have a history of childhood behaviour problems, therefore psychological abuse might provide with a window into investigating mechanisms for intergenerational transmission of conduct problems. The recruitment of a predominantly high-risk sample to be studied more intensely also provided with the opportunity of exploring other potential mechanisms such as partner violence which is associated with very serious and pervasive dysfunctions in the individual (e.g. personality disorder). However, these aspects have not been a focus of this thesis and are not reported here.

2.4.1.2.1 Procedure for Recruitment to the Intensive Sample

All women who were consented into the extensive study and scored above the threshold on the psychological abuse scale, together with a random selection of the control women, were approached to also become part in the intensive study. A team of several research assistants were responsible for contacting the mothers around the beginning of the third trimester of pregnancy (i.e. at 29 weeks gestation) and a database was set up to trigger the IDs of the participants a couple of weeks before the date they were due to be invited. The researchers were blind to the mother's risk status. Contact was made with each mother by a designated researcher predominantly via the phone or using the alternative routes agreed upon at the previous contact with the research midwife if they could not be contacted directly. All mothers were told that they were selected by computer to take part in the more intensive study and were given the opportunity to ask questions about the study. Those who were unsure were given time to take the decision and were contacted again after a week, while those who declined participation were asked for reason of refusal and were reminded that they still remained in the extensive study and would be sent questionnaires as agreed at 20 weeks gestation. For mothers who accepted to

become part in the intensive study arrangements were made for a first appointment which took place in private either at the research base or in the mother's home and when the study was fully explained, information sheets were given to read and written informed consent was taken. The consent form mentioned, among other aspects, about assuring confidentiality of data, sharing the information with other researchers and asking for permission for audio recording of the interviews (copies of the information sheet and consent form for the 32 weeks gestation phase are provided in Appendix A, sections A4 and A5). The mother was also asked to complete an updated contact information sheet. At the end the researcher made a new appointment with the mother for carrying out the assessments at 32 weeks gestation, which consisted of collecting demographic information, maternal mood (e.g. EPDS, STAI) and physical abuse questionnaires, standardised interviews covering interpersonal functioning, personality disorders and other mental health problems, a brief intellectual ability test, emotion recognition tasks and recording of life events. Saliva samples were also collected several times on two occasions from the mother to measure level of cortisol. Typically, all measures were completed in two sessions of several hours each. However, with some mothers it was needed 3-5 sessions to complete the assessments.

2.4.1.2.2 Inclusion Criteria for the Intensive Study

All the mothers who were recruited in the extensive sample completed at 20 weeks gestation the Dunedin Relationships Scale which asked about psychological abuse in current relationship (see Section 2.5.1.3 for a description of the scale). The scale was used to identify mothers at risk for psychological abuse who would form the high-risk group in the intensive sample projected to be around 200 women, with another 100 women chosen randomly of those scoring low on the scale.

Data provided by Professor Moffitt from the Dunedin Multidisciplinary Health and Development Study based on assessments carried out at age 26 years indicated that approximately 16% of the sample would score above a cut-off of 3-4 on the scale, and that this would yield the group size estimated above assuming the screen operates in a similar manner and recruitment in the intensive study is high. Although the number of women reporting psychological abuse above the threshold was similar (212 of 1286, 16.48%) to what was predicted, there were fewer mothers who consented to take part in the intensive study than what was expected (see Section 2.4.3.2 for details on rate of recruitment to the intensive study). Consequently, the 3-4 threshold was lowered to 2-3 after approximately 11 months of recruitment, and because that still did not yield the originally planned numbers of high risk mothers, every available future mother was invited to participate in the intensive study in the last 4 months of recruitment irrespective of their reported abuse score, but provided they were first-time mothers of singletons and aged 18 and over.

2.4.2 Recruitment and Follow-Up

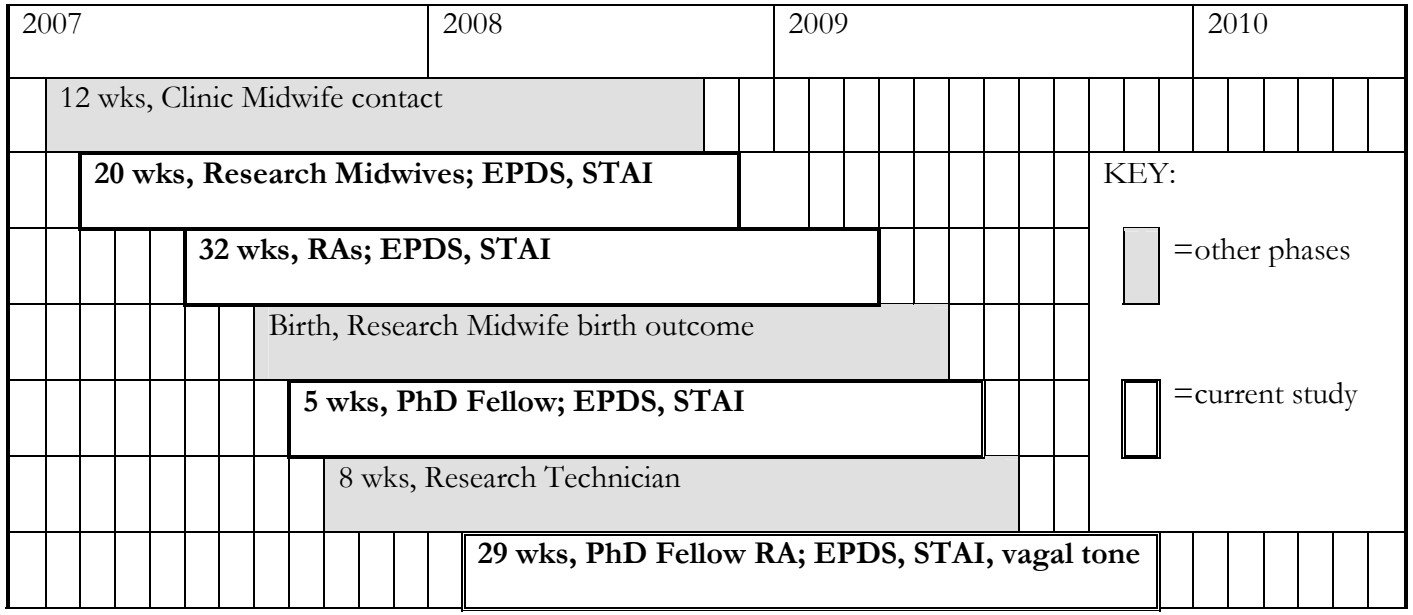
2.4.2.1 Timescale of Recruitment and Assessment Waves

Recruitment and follow-up are summarised in Table 2.1 showing the lengths of data collection waves as well as who conducted the assessments. Also, the four assessment points at which the data for the current study were collected are shown in the non-shaded boxes in bold, while the shaded boxes represent other stages in the original study.

Each phase in the study had duration of approximately 21 months and data collection went smoothly throughout despite the considerable overlap in the assessment waves.

Rate of testing and interviewing participants for the intensive study at the research base increased steadily in 2007 and peaked during most of year 2008 and beginning of 2009 when there were still pregnant mothers to be interviewed and infants were assessed at 5 and 29 weeks. Despite the fact that during the most intensive periods up to 30 families were coming at the research base each week, confidentiality was maintained by the fact that soundproofed purpose-built rooms had been designated for each phase, therefore the entire research protocol from explaining the session to obtaining informed consent and collecting the data was always conducted at highest standards of privacy.

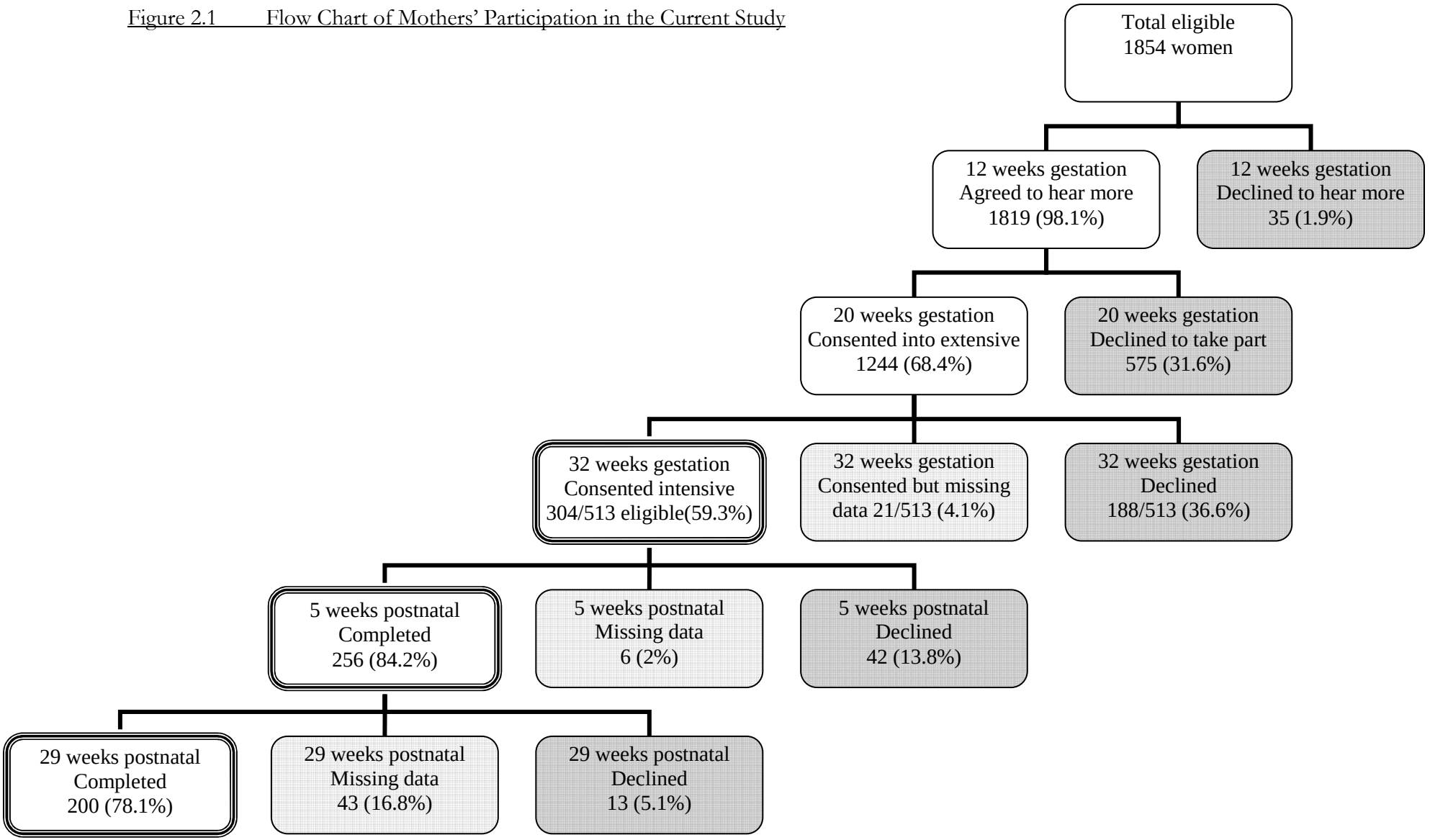
Table 2.1 Timetable for Recruitment and Assessment Waves in the Original Study



2.4.2.2 Generation of Sample for the Present Study

The current study includes the first 200 mothers and babies recruited in the intensive sample with complete data at all four assessments (i.e. 20 and 32 weeks gestation, and 5 and 29 weeks postnatal). The sample size was decided based on two reasons: first, because a relatively large sample is needed in order to confer greater power on statistical tests (e.g. when examining interaction effects) and, second, because of practicality reason. To identify the pool of extensive participants from which the sample originated, the strategy was first to establish the date at which the 200th woman had been booked in for her 12-week scan. The date was identified as 26 September 2008, approximately 5 weeks before the last participant was consented into the larger original study. Once this cut-off date was identified, the strategy was then to work backwards and determine all the eligible women who came to the clinic for the 12-week scan from the start date of recruitment and until the cut-off date. The total number of women from which the current sample was drawn was established as 1854. The last step involved accounting for attrition at each phase (either due to refusal of participation or because of missing data). Numbers and percentages for the total number of participants at each phase from 12 weeks gestation to 29 weeks postnatal are presented in Figure 2.1 and explained for each stage in the next section.

Figure 2.1 Flow Chart of Mothers' Participation in the Current Study



2.4.3 Sample Characteristics

2.4.3.1 Sample Characteristics at 20 Weeks Gestation

As presented in Figure 2.1, 1244 women (68.4%) of the ones approached by the research midwives at 20 weeks accepted to take part in the extensive study and completed questionnaires (mean gestation age was 20.53 weeks, *SD* 1.94). Before that, at the 12 week scan, 98.1% of the total number of 1854 eligible women had said to the clinic midwives that they would be willing to hear more about the study at the next hospital appointment (i.e. at 20 weeks)[1].

A comparison between the women who agreed to take part in the extensive study and those who declined participation revealed that the non-participants were younger (mean age was 25.2 years, *SD* 5.9) than those who consented (mean age was 26.7, *SD* 5.9), $t(1927) = -5.3$, $p < .001$. Also, more of those who declined participation (304/640, 47.5%) were in the most deprived quintile compared to the participants (533/1286, 41.3%), $\chi^2(1)=6.6$, $p<.01$, $OR=1.28$[2].

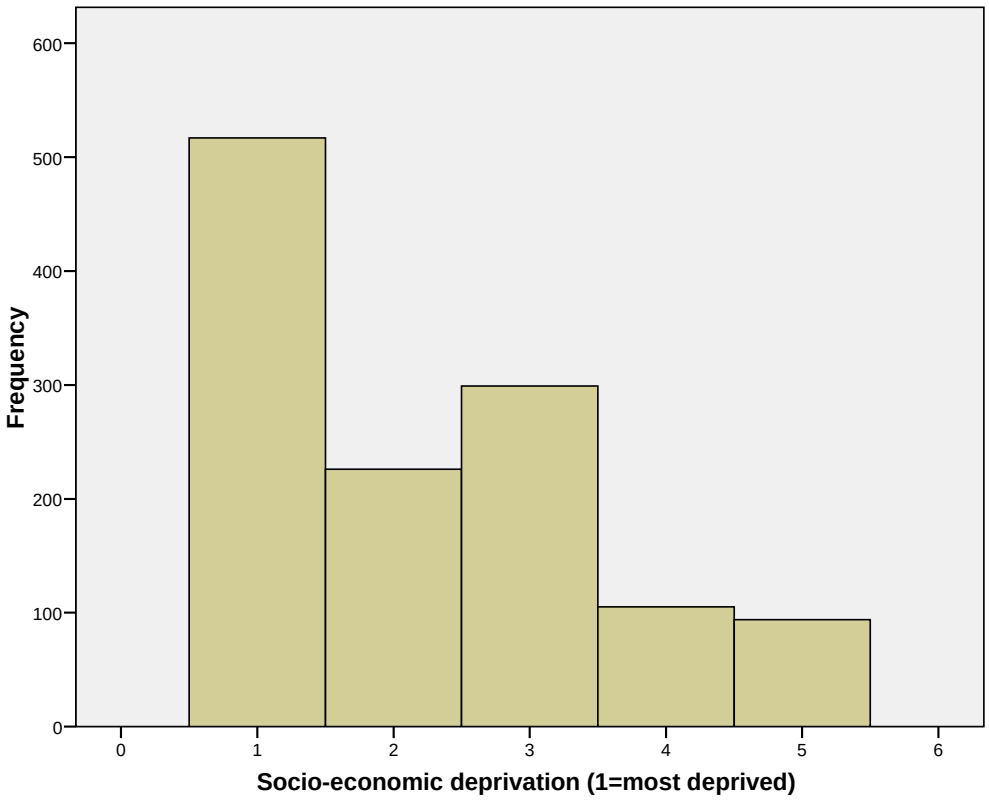
Analyses of the demographic data for the women who agreed to participate in the extensive study showed that the sample was more deprived than the national average. Figure 2.2 showing the quintile distribution for the socio-economic deprivation index (see Section 2.5.1.4 in Measures for a description of the deprivation measure) suggests

[1] The total numbers of eligible women and those who were approached by the clinic midwives at the 12 week scan and agreed to hear more about the study were estimated based on percentages available from the main study.

[2] Tests comparing the non-participants in the extensive study with those who participated were made based on the full sample of 1286 from the main study, which is slightly larger than the extensive sample of 1244 in the current study.

that more than 40% of the mothers in the current study live in the 20% most deprived areas in England.

Figure 2.2 Distribution of Deprivation Scores in the Extensive Sample (*N*=1241)



The analysis of the ethnicity data showed that 1195 (96.1%) women of the total of 1244 who accepted to take part in the extensive sample were White British, which means that the presence of the people with a non-White background in the study is strikingly low. Although much lower than at national level, the small representation of ethnic minorities is in line with reported rates for the Wirral (see Section 2.4.1.1). Therefore further analyses involving ethnicity were not performed due to small numbers.

2.4.3.2. Sample Characteristics at 32 Weeks Gestation

As shown in Figure 2.1, 63.4% of women recruited to the extensive sample and eligible for this study were recruited to the intensive sample and provided with at least partial data, raising the question of the representativeness of the intensive sample. Participants (N=325) and non-participants (N=188) were compared on maternal age, socio-economic deprivation, and depression and state anxiety scores assessed at 20 weeks. The analyses revealed that the non-participants were younger, mean age 24.85 years, *SD* 5.26 vs. 27.38 years, *SD* 6.10, $t(510)=4.75$, $p<.001$, and more were in the lowest deprivation quintile, $\chi^2(1)=7.3$, $p=.007$, $OR=1.65$, compared with those who agreed to participate. However, there were no differences between the women who gave consent and those who declined participation on the level of depression, mean EPDS 8.79, *SD* 5.10 vs. mean EPDS 8.80, *SD* 4.96, $t(507)=-.08$, $p=.94$, or state anxiety, mean STAI score 33.52, *SD* 11.79 vs. 33.51, *SD* 10.11, $t(508)=-.54$, $p=.59$[3].

Mean gestation age at time of assessment for the mothers who consented to take part in the intensive study was 32.52 weeks, *SD* 2.00.

2.4.3.3. Sample Characteristics at 5 Weeks Postnatal

Of the 304 mothers who were consented in the intensive sample and had complete data at 32 weeks gestation, 256 (84.2%) attended for the 5-week assessment, which included a neonatal behavioural exam and questionnaires for the mothers. Eleven of these mothers who came first for the assessment did not complete the mood questionnaires at this

[3] Means and standard deviations for maternal age and depression and state anxiety scores are reported on untransformed scores. *T* test statistics are reported using transformed data.

phase because these measures were introduced later in the study, but their data became available from the 8-week extensive phase. Six others who came for the assessment did not complete the mood measures at 5 or 8 weeks and were excluded.

The group of mothers completing prenatal measures but declining the assessment at 5 weeks (*N*=42) were compared with those providing with complete data at 5 weeks (*N*=256) on age, level of deprivation, and depression and anxiety measured at 20 weeks gestation. The only significant difference between the two groups was regarding age, with the mothers who did not come for the assessment being younger than those who attended, mean age 25.62 years, *SD* 5.10 vs. 27.80 years, *SD* 6.19, $t(296)=-2.09$, $p=.038$[4].

Mean infant age at time of assessment at this wave was 5.32 weeks *SD* 1.22.

2.4.3.4. Sample Characteristics at 29 Weeks Postnatal

Of the 256 who attended for the 5 weeks assessment and provided complete data, 243 also attended for the 29 weeks assessment. The majority of the 43 not included in the final sample of 200 were lost because of problems with the measurement of RSA (vagal tone). In 9 the quality of the recording was too poor for analysis, and in a further 20 the infant did not complete all the five manoeuvres during which RSA was assessed (these are described in Sections 2.5.2.2 to 2.5.2.4). A further 11 were excluded because one of the manoeuvres, the still face, had been conducted with the administrator rather than the mother. These were the first 11 assessments conducted. Three further mothers did not provide completed questionnaires for depression and anxiety at 29 weeks.

[4] Means and standard deviations for maternal age are reported on untransformed scores. *T* test statistics are reported using transformed data.

Participants with complete data (*N*=200) were compared with those with missing data (*N*=43) on maternal age, socio-economic deprivation, maternal relationship status, and maternal depression and anxiety at 20 weeks gestation. The only difference that emerged was for the mother's relationship status. Mothers who did not have a partner at 20 weeks gestation were more likely to have incomplete data at 29 weeks postnatal than those who were in a relationship, $\chi^2(1)=4.48$, $p=.034$, $OR=2.14$.

Mean infant testing age for this assessment wave was 28.96 weeks, *SD* 3.03.

2.4.3.5 Summary of Maternal and Infant Characteristics

Table 2.2 below shows descriptive statistics for the relevant maternal and infant characteristics based on the final sample of 200 dyads.

Table 2.2 Maternal and Infant Characteristics (*N* = 200)

Characteristic	**Data**
1. Mothers	
Age (years)	27.9 (6.2)
Socio-economic deprivation, % (N)[5]	
Q1 (most deprived)	36 (72)
Q2	20.5 (41)
Q3	27 (54)
Q4	7.5 (15)
Q5 (least deprived)	9 (18)

[5] Numbers and percentages for quintiles are based on IMD figures published at national level and described in Section 2.5.1.4.

Table 3.16 Maternal and Infant Characteristics (*N* = 200) - Continuation

Relationship status, % (N)	
Married	42.5 (85)
Cohabiting	37.5 (75)
Single	10 (20)
Partner living elsewhere	10 (20)
Educational level, % (N)	
Left education before 19 years	55.5 (111)
Finished education at 19 years or after	41 (82)
Still in education	3.5 (7)
Smoking during pregnancy, % (N)	
Smoked between 10 and 20 cigarettes a day	3 (6)
Smoked less than 10 cigarettes a day	11.5 (23)
Did not smoke	85.5 (171)
Mother's risk for psychological abuse status, % (N)	
High risk	52 (104)
Low risk	48 (96)
2. Infants	
Birth weight (g)	3437.6 (518.3)
Low birth weight (<2,500 g), % (N)	2.5 (5)
Gestational age (weeks)	40.2 (1.5)
Premature births (<37 weeks gestational age), % (N)	2.5 (5)
Sex, % (N)	
Males	47 (94)
Females	53 (106)

Continuous variables presented as mean (standard deviation).

2.4.4 Rationale for Choice of Measures

2.4.4.1 Maternal Mood Questionnaires

In the current study self-report questionnaires were used to measure maternal depression and state anxiety throughout the assessments. Their use has two major advantages. First, it provides comparability with many other studies that investigated biological indicators and risks in populations of infants and older children. Indeed, large studies (e.g. The ALSPAC Study) that examined prenatal maternal stress in relation to HPA functioning have used self-reports and predicted outcomes over long periods of time (i.e. from pregnancy to school-age). Second, questionnaires are a good choice of measures in studies that aim to measure timing effects during a relatively short period of interest (e.g. timing of stress in pregnancy) because they are quick to complete and easy to administer.

Moreover, a search was performed on the studies in the infant vagal tone literature that examined links with maternal pre- and postnatal stress and yielded that all have used self-reports which provides strong support for their use in the present study. However, self-report questionnaires also have their limitations, which are referred to in the Discussion (Section 5.3.4).

2.5 Measures

2.5.1 Measures Used at 20 and 32 Weeks Gestation

2.5.1.1 The Edinburgh Postnatal Depression Scale (EPDS; Cox, Holden, & Sagovsky, 1987) is a widely-used self-report questionnaire for identifying perinatal depression. It contains 10 items scored 0-3 with higher scores indicating increased severity of symptomatology, and usually takes 5 minutes to complete. Seven items receive reverse scorings. The minimum score that one can obtain is 0 (no apparent signs of depression) while the maximum is 30 (extremely high level of depression). A copy of the measure is presented as Appendix B1.

Self-report measures of maternal depression in the pre- and postpartum periods have been used extensively (e.g. Laplante et al., 2004; O'Connor et al., 2002; Davis et al., 2007). Because they are easy and quick to administer, they can be used with great effectiveness, especially in studies where several assessment waves in relatively short periods of time are planned. Besides, the measured outcome in questionnaires with established cut-off scores in identifying highly-symptomatic participants can be used both as a score, and as categorical variables, which confer flexibility in choosing the statistical design.

2.5.1.2 The State Anxiety Scale of the State-Trait Anxiety Inventory (STAI; Spielberger et al., 1970) is a self-report questionnaire used for measuring state anxiety, which is defined as "a temporal cross-section in the emotional stream-of-life of a person, consisting of subjective feelings of tension, apprehension, nervousness, and worry, and activation or arousal of the autonomic nervous system" (Spielberger, 1985, p. 10). All 20 items describing current anxiety are rated on a four-point Likert scale from 1 (*not anxious*) to 4

(*highly anxious*), yielding a total score in the range 20 to 80 (higher score indicates more anxiety). Ten items receive reverse scorings. The instrument is the most widely-used tool for detecting anxiety during pre- and postnatal periods (see, Van den Bergh et al., 2005; and, Glasheen, Richardson, & Fabio, 2009). In a review of all studies that used the STAI between 1990 and 2000, Barnes et al. (2002) report a mean Cronbach's *alpha* of .91 and a test-retest reliability score of .7 on the state anxiety scale. A copy of the measure is presented as Appendix B2.

Studies investigating influence of prenatal stress/anxiety on children's development have predominantly made use of self-report measures of anxiety rather than clinical interviews (Talge et al., 2007). They have been used in studies with large cohorts of pregnant women in which anxiety predicted outcomes in preschool and preadolescent children (e.g. O'Connor et al., 2002; 2005). Therefore self-administered questionnaires are convenient and valid tools for measuring aspects of stress.

2.5.1.3 The Dunedin Relationship Scale (Moffitt et al., 1997) is a 20 item self-report measure consists of 2 items from the Conflict Tactics Scales (Strauss, 1990) and 18 items devised by Moffitt and colleagues to capture controlling, demeaning and other psychologically abusive behaviours within relationships. Each question is read aloud and participants respond *yes* or *no*, recording their answer on a separate answer sheet, in order to allow privacy whilst overcoming literacy problems. The measure has good internal reliability for the participant's own abusive behaviour towards their partner and their partner's behaviour toward them. High scores on this Dunedin Relationship scale correlated highly with actual physical violence assessed using the Conflict Tactic Scales (r = .78; Form *R*). The questions are embedded amongst items which ask about positive aspects of relationship functioning so that the range of questions is broad in focus and

has face validity to those completing it. They follow a number of graduated questions concerning positive relationship functioning and modes of resolution of every day difficulty. A copy of the measure is presented as Appendix B3.

2.5.1.4 Level of Socio-Economic Deprivation was measured at 20 gestation using the revised English Index of Multiple Deprivation (IMD; Noble et al., 2004) based on data collected from the UK Census in 2001. According to this system, postcode areas in England are ranked from most deprived (i.e. IMD of 1) to least deprived (i.e. IMD of 32,482) based on deprivation in seven domains: income, employment, health, education and training, barriers to housing and services, living environment and crime. All mothers were given IMD ranks according to the postcode of the area where they lived and quintile distributions were examined.

2.5.1.5 Self-Reported Smoking was measured at 20 weeks gestation using one item that asked whether the mother smoked during pregnancy. The options were 0 (*None*), 1 (*Less than 10 cigarettes per day*), 2 (*Between 10 and 20*) and 3 (*More than 20*).

2.5.2 Measures Used at 29 Weeks Postnatal

2.5.2.1 Vagal Tone

Vagal tone is thought to represent the parasympathetic influence on the heart's sino-atrial node exerted through the vagus nerve (see Section 1.3.2 in the Background). Because the activity of the vagus on the heart cannot be measured directly for obvious reasons, indirect methods had to be considered in assessing cardiac vagal input. One such measure is heart rate variability (HRV), which represents spontaneous changes in beat-to-beat intervals. Although variation in heart rate occurs not only as a result of the parasympathetic activity, but also due to other influences such as the sympathetic nervous system activity or the baroreceptor reflex, inter-beat variations occurring in phase with the respiratory cycle are believed to reflect solely the activity of the vagus. This measure of HRV taking place at respiratory pace is called RSA and Porges (1995) refers to it as vagal tone. The actual RSA scores in the current study were calculated by using the Cardioedit software (recommended and provided by the US trainer) according to a procedure developed by Porges (1985) that consists of a sequence of steps. First, the R-R (i.e. inter-beat) intervals are timed to the nearest millisecond which results in a time series of consecutive heart periods (HP). An algorithm is applied to the sequential inter-beat intervals data that uses a 3rd order 21-point moving polynomial filter (Porges & Bohrer, 1990) which detrends periodicities in HP slower than RSA. Then, a bandpass filter extracts the variance of HP within the frequency band of spontaneous respiration in infants (i.e. 0.24 –1.04 Hz). Finally, RSA is derived by calculating the natural log of this variance and is reported in units of ln(msec)^2.

RSA was assessed during five standardised procedures. The first two were procedures designed to induce a quiet alert state of predominantly neutral emotional content in the infant, thought to be reflective of a basal level of vagal functioning. The last three episodes formed the Still Face paradigm, which provides the infant with social engagement followed by a mild social challenge that would elicit withdrawal of vagal tone as an adaptive response. Although limited understanding seems to exist on what kind of state or behaviour would be most indicative of a baseline condition (see Section 1.3.5.1), according to Porges (1995) baseline RSA is the level of cardiac vagal input in the absence of a task or challenge of any kind and reflects a first functional role of the vagus which is maintaining homeostasis in the organism. In contrast to that, a mild stressor or task requires active coping through mobilising of metabolic resources that would trigger a lowering of the parasympathetic input to the heart and indexed by a decrease in RSA. This regulatory mechanism is thought to reflect a second role of the parasympathetic nervous system through the vagus nerve in which the drop in cardiac vagal input prepares the individual to face up to the challenge by providing with the necessary energies. The five procedures that were used in the current study were designed to offer conditions for both baseline, and withdrawal of vagal tone to be measured.

The experimental sequence was close to the set-up used in other vagal tone studies with infants (e.g. Bazhenova et al., 2001) and potentially allowed us to investigate both baseline, and withdrawal of vagal tone in developmentally-appropriate conditions with participants of similar ages. Moreover, it was fluent, looked interesting enough to be enjoyed by infants and mothers, and did not take too much time to administer. Descriptions of the procedures are presented below.

2.5.2.2 The Helper-Hinderer (Hamlin, Wynn, & Bloom, 2007) is an experimental paradigm developed in recent years by researchers from Yale University's department of psychology, and is aimed at assessing the early preference for pro-social versus anti-social contexts. The infant is seated on the mother's lap and views a large display (3x5 feet) situated in front of him/her approximately 6 feet away in which coloured shapes (square, circle, triangle) with googly eyes are shown either helping each other up a slope or hindering each others' progress up the slope. After several trials over which the infant has shown a predetermined level of habituation, he/she is asked to choose which shape he/she prefers. Infants of 6 months have been reported to reliably favour shapes that have been helpers. The duration of the procedure is not standard but varies depending on how quickly the infant habituates to the presentation of the stimuli. In the current study the mean duration was 6.41 minutes, *SD* 1.85, minimum 1.05 minutes, maximum 13.35 minutes. There was only one infant (0.5%) who habituated in less than 2 minutes, 17 (8.5%) with whom the procedure lasted for less than 4 minutes, while almost a quarter of all the infants (*N*=44; 22%) watched the stimuli for 8 minutes or more before they habituated and looked away.

2.5.2.3 The Novel Toy Exploration Procedure is a 2-minute episode in which the infant is presented at a table with a 4-facet triangular pyramid-shaped toy to explore for two minutes while sitting on mother's knees. There have been debates in the literature as to whether or not a toy exploration episode is close to what is thought to represent baseline condition, as focusing of attention may elicit changes in vagal tone in infants (e.g. Huffman et al., 1998). However, several studies (e.g. Calkins & Dedmon, 2000) have made use of procedures in which attention was involved in order to be able to keep small children entertained. From the experience of running infant assessments in the present study it became apparent that 6-month-olds tended to become agitated or upset if they

were prevented from interacting with toys or people while sat in a high chair, which might have an impact on the level of vagal tone due to negative emotional states, whereas providing the infants with a toy to play with was thought not to differ a lot from a quiet exploration of the surroundings.

2.5.2.4 The Still Face Paradigm (SFP) was first developed by Tronick and colleagues (Tronick et al., 1978) who introduced the idea that infants contributed actively to social communication and that they should not be seen as passive recipients in the mother-infant dyad. Although there has been some variation in the design of the SFP, it is generally presented as a three-phase face-to-face interaction with an adult that lasts for a few minutes and in which: (1) the adult engages with the infant in a natural social manner, then, (2) stops reacting and maintains a blank non-expressive face while still in front of the infant, and, (3) re-engages with the infant in the same manner as in the first episode. What is now known as the still-face effect and which has been proved to be reliable in samples of 3-6 month olds of various characteristics comprises a constellation of reactions in the infant that include communication initiation and adult response awaiting, gaze aversion, increased negative and decreased positive emotionality. Tronick and his team later developed their view on explaining the effect into the formulation of the Mutual Regulation Model (e.g. Tronick & Gianino, 1986) in which both infant and adult contribute to the regulation of their interaction based on complex feedback of emotional nature. The SFP has been used primarily to assess infant physiological, affective and behavioural reactivity and regulation following a mild social stressor.

In the current study the SFP involved the infant in interaction with the mother, and each of the three episodes lasted for 2 minutes. Mothers were instructed not to touch their infants during the still face, but touching was allowed in the other two phases of the

procedure. Also, use of toys was not permitted throughout. The procedure was performed with the infant placed in a padded wooden high chair. If the infant became very upset during the procedure, the phases were shortened, or even stopped if the infant continued to be in inconsolable crying for more than 30 seconds.

2.6 Procedure at 29 Weeks Postnatal

2.6.1 Assessment Protocol

Upon mother's arrival at the study base, the researchers introduced themselves and accommodated the mother and the infant in one of the available rooms. Whenever possible, the mother was greeted by one of the researchers whom the mother had already met at a previous phase in the study. The session was then explained to the mother and the information sheet was given to her to read. The researcher who was taking consent provided additional details and explanations to the mothers who had questions and then written consent was taken (copies of the information sheet and consent form for the 29 weeks postnatal phase are provided in Appendix A, sections A6 and A7).

After consent was obtained, three Biopac (Biopac Systems, Inc., USA) paediatric self-adhesive disposable ECG electrodes were placed on the infant's back in a triangle pattern. The electrodes were padded and pre-gelled to ensure comfort to back-lying and optimal conductance of the heart signal. Then the mother and infant were led to the adjacent assessment room where all infant procedures were run. The 12x7-feet room was equipped with four remotely movable cameras to ensure close and accurate video recording. With the infant sat on the mother's knees in the assessment room, three cables linked to a Biopac Student MP35 preamplifier were attached to the electrodes on the baby's back. The cardiac measurements were performed using the 3.9.1 version of the AcqKnowledge data recording software installed on a Windows XP laptop computer. The output in the form of an ECG with a sampling rate of 1000 Hz was transmitted live to the computer and stored for later off-line extraction of heart period data. All assessments were recorded on DVDs using a split-screen procedure, with 3 video

outputs from the cameras and one from the AcqKnowledge software showing the ECG being combined on a monitor. An electronic timer was also shown on the screen to allow coding of timings for procedures. Figure 2.3 shows a still image of the split-screen view during the second episode of the Still Face procedure. Written consent was obtained from the mother for using her and her baby's images for research purposes only.

Figure 2.3 Video Output Obtained Through a Split-Screen View Showing Close-Ups of the Mother and Infant, a Full View of the Assessment Room and Live ECG Recording During the Still Face Procedure

Where possible, all five procedures were administered one immediately after the other to prevent the infant from getting bored or upset due to long time spent in the assessment room. Total time for running the whole sequence was typically around 20 minutes.

Infants who needed to be fed or changed or who needed a break because of getting tired or upset were disconnected from the vagal tone equipment and were invited outside the room to spend a few minutes with the mother on a couch. Assessments were resumed as soon as possible thereafter.

I and another researcher (KM) conducted jointly most of the infant assessments (a third researcher, KM, joined us during the last 4 months of the data collection). Each of us had set roles in the assessments in order to minimise potential biases associated with the presence of one researcher or the other in the proximity of the mother and baby. I conducted the work of editing vagal tone files and deriving the final RSA values for all the infants in the study.

2.6.2 Editing and Computing of Vagal Tone Data

Prior to manipulation of vagal tone data the DVDs containing the assessments were watched in order to match the start and end times of the procedures with the corresponding ECG segments. Editing and computation of vagal tone data took place off-line and started with electronic detection of inter-beat interval (IBI) data from the ECG files by running the Cardioedit software provided by the University of Chicago's Brain Body Research Center. The next step was editing the raw data which required visual inspection and identification of outliers and which was performed using the same software. Typically errors occur because of R peaks being missed and T peaks being misinterpreted as R peaks in the conversion process. The first type of error results in longer heart periods, while the second error produces shorter intervals. The editing process corrects the longer intervals by dividing them by integers into equal shorter intervals which are comparable in duration to the nearby values, while shorter intervals

are added-up to "reconstruct" real heart period data points. For points that need editing and are more than 5 times higher than the "real" values, the software does not allow integer division in order to prevent missing too many real values and artificially lowering heart rate variability; instead, a more advanced editing technique was available in which the actual ECG trace was superimposed on the IBI data so that real IBI points were manually inserted as indicated by the positioning of the R peaks, or reconstructed from adding up smaller values. Figure 2.4 presents a segment of approximately 4 seconds of raw IBI data, while figure 2.5 shows the same sequence after the editing process.

Figure 2.4 Video Image of Raw Inter-Beat Interval Data (i.e. Before Editing)

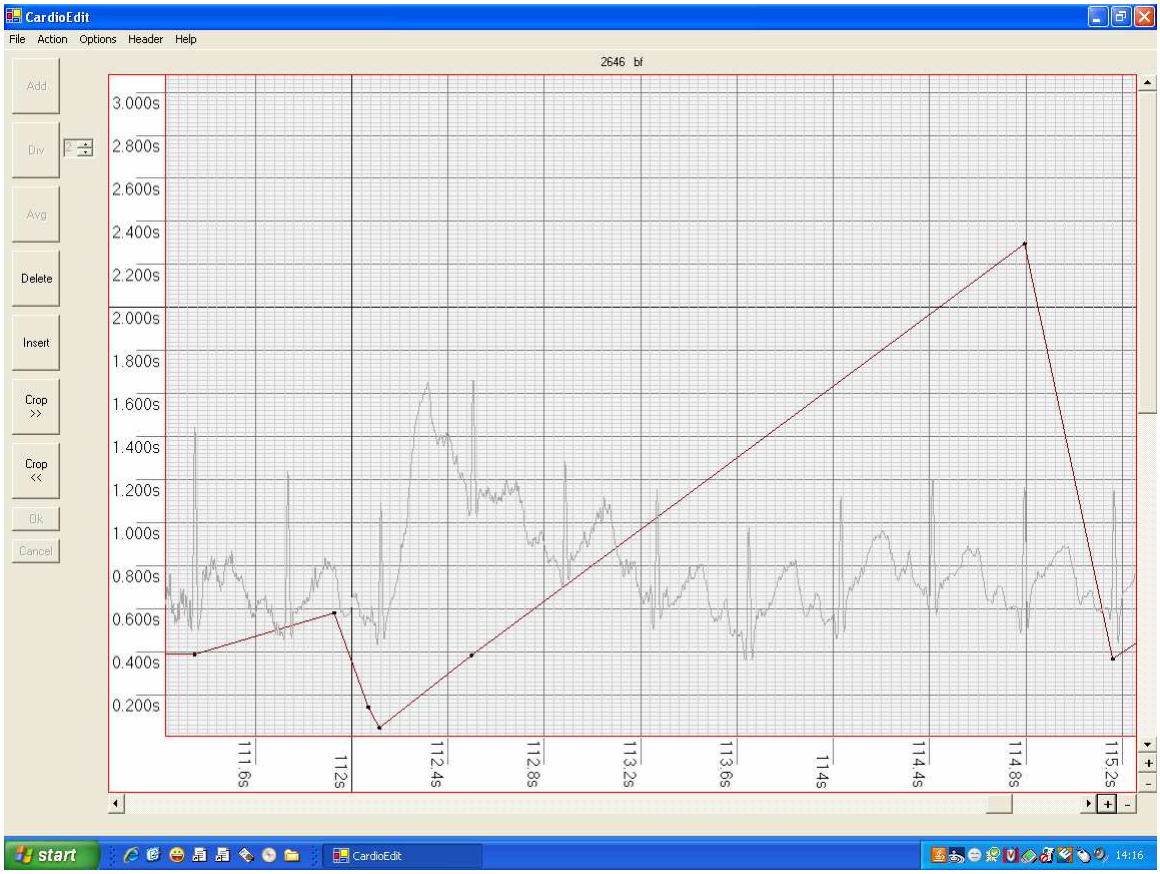
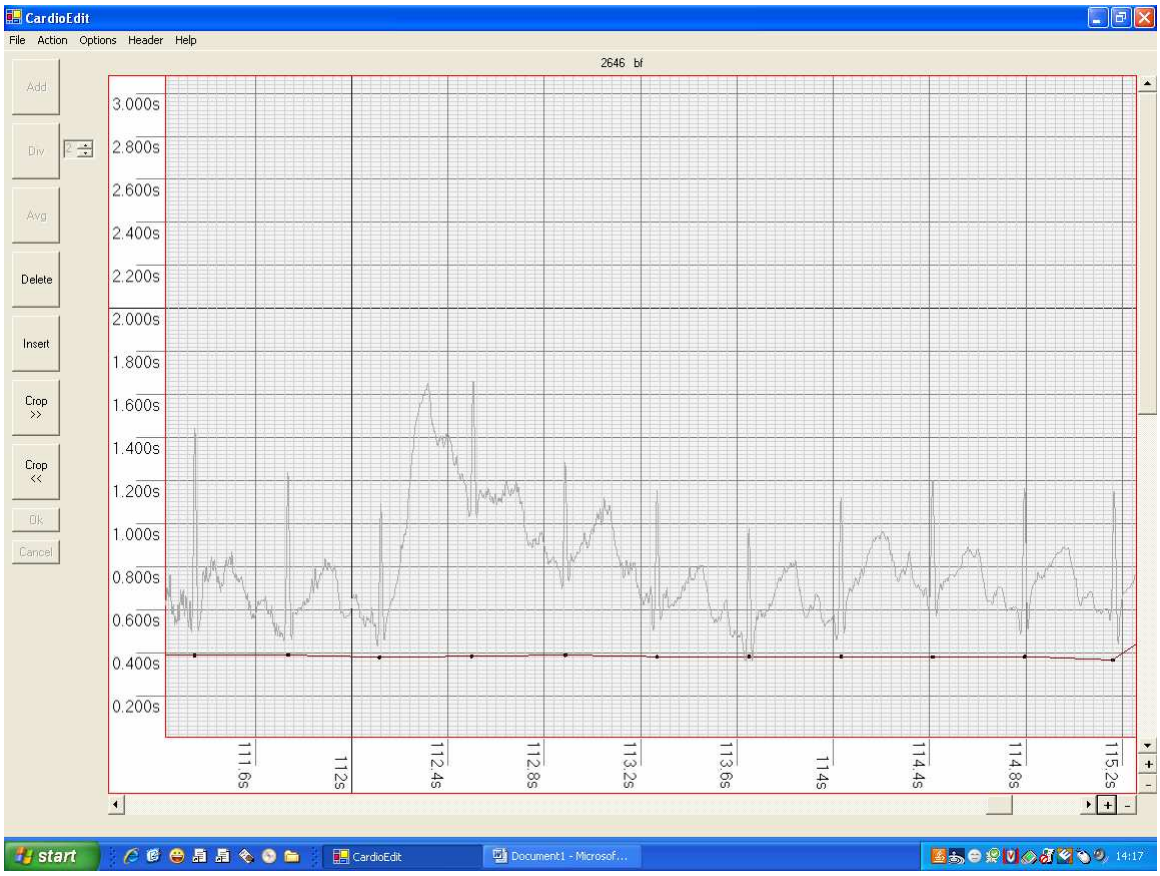


Figure 2.5 Video Image of Inter-Beat Interval Data After Editing



Files that needed more than 5% of editing (typically more than 15 IBIs in a 2-minute recording in infants) required the use of the more advanced editing method. After the files were edited the RSA values were computed by running the CardioBatch software (also recommended and provided by the trainer) on the edited files, which produced Excel outputs. The CardioBatch calculates RSA according to the Porges (1985) method.

2.6.3 Training and Reliability in the Editing of Vagal Tone Data

I underwent 3-day training from an expert based at the Brain Body Center of the University of Chicago at Illinois in the USA. The training was followed by the editing of 30 practice files in which feedback was provided to me mainly for building up my confidence in taking decisions whether certain types or sequence patterns of inter-beat intervals needed editing or were reflecting true respiration-based heart rate variability. When I felt comfortable with the technique I was given to edit 20 reliability files for which guidance was not permitted. I was certified as reliable after each of the edited 20 files had RSA values within .1 in absolute value to those derived by the trainer. After certification and throughout the editing work I maintained contact periodically with the US trainer to ensure all arising matters were dealt with appropriately under supervision. Reliability between me and the US trainer was verified again towards the end of the whole editing process (approximately 2 years after the training) on a random sample of 20 files collected from the infants in the current study. Intraclass correlations were very high on each of the five procedures ranging from .89 to .99.

2.7 Ethical Approval

The current study is part of the larger study called The Wirral Child Health and Development Study which is run jointly by the Universities of Manchester and Liverpool between 2007 and 2010 and had been approved by the Cheshire North and West Research Ethics Committee on 27 June 2006, Reference no 05/Q1506/107. Amendments to the study were approved by the Cheshire Ethics Research Committee on 20 July 2007. Copies of the approval forms are shown in Appendix C.

Chapter 3

Results I

Preliminary Analyses of Maternal Depression and Anxiety and Infant Vagal Tone Scores

3.1 Maternal Depression

3.1.1 Distribution of Maternal Depressed Mood Scores

Visual inspection of the distributions of the depression scores at each time point indicated that they were skewed towards the left, and this was supported by skewness statistics, *S*=.72, Kolmogorov-Smirnov (*K-S*) test *D*=.08 at 20 weeks gestation, *S*=.67 and *D*=.10 at 32 weeks gestation, *S*=.84 and *D*=.13 at 5 weeks postnatal and *S*=1.11 and *D*=.14 at 29 weeks postnatal, for *K-S* tests all *p*'s<.005 and all *df*'s=200. Log transformation (as detailed in the Approach to Statistical Analyses Section 2.3.1.1 in the Method section) led to considerable improvement and in each case the skewness of the distributions approached the value of 0. Therefore parametric tests with transformed scores were used in all further analyses unless stated otherwise.

3.1.2 Summary Statistics of Maternal Depressed Mood

The means, standard deviations and range of untransformed scores for the Edinburgh Postnatal Depression Scale (EPDS) at each time point are presented in Table 3.1. Figures D1, D3, D5 and D7 in Appendix D show the four histograms with distributions of untransformed maternal depression scores at the four time points, while distributions of the transformed maternal depression scores are presented in Figures D2, D4, D6 and D8.

Table 3.1 Maternal Depression Scores at Each of the Four Assessment Points (*N*=200)

Time of assessment	Mean	*SD*	Minimum	Maximum
20 weeks gestation	8.51	5.27	0	27
32 weeks gestation	8.26	4.63	0	26
5 weeks postnatal	5.79	4.42	0	19
29 weeks postnatal	5.13	4.42	0	24

The reduction in mean EPDS scores from the pre- to postnatal period was striking. A repeated measures ANOVA with the four transformed depression scores as the within-subjects variables was employed to test for differences in EPDS scores across the time points. Mauchly's test indicated that the assumption of sphericity had been violated, $\chi^2(5)=20.90$, $p=.001$, therefore results are reported using Greenhouse-Geisser corrections. The model was highly significant, $F(2.81, 558.59) = 61.02$, $p<.001$. Post hoc paired *t* tests showed that both postnatal depression scores were significantly lower than both of the prenatal scores, with mean differences of untransformed scores ranging from 2.48, 95% confidence interval [CI; 1.59 – 3.36], to 3.38, 95% CI [2.46 – 4.29], all *p*'s<.001.

3.1.3 Associations Between Depression (EPDS) Scores Over the Four Time Points

As shown in Table 3.2, there were moderate to high correlations between EPDS scores at all time points and, in spite of the falling mean scores, no evidence that the correlations diminished with greater time spans.

Table 3.2 Associations Between Transformed Depression (EPDS) Scores Across the Four Time Points (Pearson's *r*)

	20 weeks gestation	32 weeks gestation	5 weeks postnatal	29 weeks postnatal
20 weeks gestation	1			
32 weeks gestation	.65(**)	1		
5 weeks postnatal	.43(**)	.46(**)	1	
29 weeks postnatal	.53(**)	.52(**)	.53(**)	1

** $p<.01$ (2-tailed)

3.1.4 Is Maternal Depression Associated With Other Possible Indices of Maternal Stress?

Given that maternal risks are commonly associated (Lyons-Ruth, Connell, Grunebaum, & Botein, 1990) associations with maternal depression are shown in detail with tabulation either where previous studies have shown strong evidence of links with depression (e.g. socio-economic deprivation), or where prenatal biological effects on vagal tone might be expected (e.g. smoking). Maternal age, socio-economic deprivation, smoking in pregnancy, relationship status and level of education were included in the analyses as maternal risks potentially associated with depression. Also, analyses with gestational age at birth and birth weight were conducted because there is evidence linking these infant risks to depression in pregnancy (Kelly et al., 2002). The tests used were independent-samples *t* tests for comparisons of means and correlations for associations between scores.

3.1.4.1 Maternal Age and Maternal Depression

Distribution of maternal age showed that it was skewed towards the left. The natural logarithmic transformation ln(age of mother) was used, which reduced skewness to close to zero. All further analyses in the study involving maternal age were performed using the transformed variable unless stated otherwise. Associations between maternal age and the four EPDS scores were calculated using Pearson's *r*. The results, shown in Table 3.3, revealed that age was strongly associated with maternal depression at 20 weeks and 32 weeks gestation and moderately at 29 weeks postnatal. Younger mothers were more depressed than older mothers at all time points except in the neonatal period.

Table 3.3 Associations Between Maternal Age and Maternal Depression Scores (*N*=200)

	Maternal depression	*r*	*p*
Maternal age	20 weeks gestation	-.28	<.001
	32 weeks gestation	-.24	.001
	5 weeks postnatal	-.02	.74
	29 weeks postnatal	-.18	.01

3.1.4.2 Socio-Economic Disadvantage and Maternal Depression

The association between maternal depression and social and economic disadvantage, using the deprivation score described in Section 2.5.1.4 was examined by comparing those falling in the lowest national quintile (*N*= 72; 36 % in this sample) with the remainder. None of the associations was statistically significant (Table 3.4).

Table 3.4 Associations Between Maternal Social and Economic Deprivation and Maternal Depression (*N*=200)

	Mean EPDS (*SD*)		Mean difference [95% CI]	*t*	*p*
	Less deprived	Most deprived			
20 weeks gestation	8.41 (5.20)	8.68 (5.43)	-.27 [-1.81 – 1.26]	-.25	.81
32 weeks gestation	8.45 (4.73)	7.93 (4.47)	.52 [-.83 – 1.86]	-.75	.45
5 weeks postnatal	5.73 (4.41)	5.89 (4.48)	-.16 [-1.45 – 1.13]	-.18	.86
29 weeks postnatal	4.98 (4.67)	5.39 (3.97)	-.41 [-1.69 - 0.88]	-.95	.34

Means, standard deviations, mean differences and confidence intervals in Table 3.4 are shown with the untransformed EPDS scores. The values of *t* and *p* were derived from comparisons of transformed depression scores.

3.1.4.3 Smoking in Pregnancy and Maternal Depression

There were 29 women (14.5%) who reported smoking in pregnancy in the sample of 200. Twenty-three of them said that they smoked less than 10 cigarettes per day, while the other 6 smoked between 10 and 20. The two groups of smokers (i.e. light and heavy) were collapsed into one group because of their small numbers. As shown in Table 3.5, smoking in pregnancy was strongly associated with elevated depression scores at 20 weeks and 32 weeks gestation, and 29 weeks postnatal, while at 5 weeks postnatal the difference in EPDS scores approached significance.

Table 3.5 Associations Between Smoking in Pregnancy and Maternal Depression (*N*=200)

	Mean EPDS (*SD*)		Mean difference [95% CI]	*t*	Cohen's *d*	*p*
	Non-smokers	Smokers				
20 weeks gestation	7.89 (5.19)	12.10 (4.24)	-4.21 [-6.21 – 2.20]	-4.26	.98	<.001
32 weeks gestation	7.87 (4.70)	10.59 (3.46)	-2.72 [-4.52 – .92]	-3.23	.73	.001
5 weeks postnatal	5.54 (4.31)	7.21 (4.87)	-1.67 [-3.40 – .08]	-1.86	.37	.064
29 weeks postnatal	4.66 (4.31)	7.90 (4.12)	-3.24 [-4.93 – 1.54]	-4.00	.83	<.001

Means, standard deviations, mean differences and confidence intervals in Table 3.5 are shown with the untransformed EPDS scores. The values of *t* and *p* were derived from comparisons of transformed depression scores.

3.1.4.4 Maternal Relationship Status and Maternal Depression

The mother's relationship status was recorded at the start of the study at 20 weeks gestation. There were 40 women (20%) of the total of 200 who did not have a partner at that time (they were either single, widowed, divorced, separated, or their partners were living elsewhere) while the other 160 were married or cohabiting. Maternal relationship status was strongly associated with maternal depression at 20 weeks and 32 weeks gestation, while the association with 29 weeks postnatal EPDS, although significant, was

somewhat weaker. The mothers who did not have a partner generally had higher EPDS scores than the mothers who were in a relationship, as shown in Table 3.6.

Table 3.6 Associations Between Maternal Relationship Status at 20 Weeks Gestation and Maternal Depression at the Four Time Points (*N*=200)

	Mean EPDS (*SD*)		Mean difference [95% CI]	*t*	Cohen's *d*	*p*
	With partner	No partner				
20 weeks gestation	7.51 (4.59)	12.50 (5.94)	-4.99 [-6.70 – 3.29]	-5.24	.92	<.001
32 weeks gestation	7.74 (4.36)	10.33 (5.15)	-2.59 [-4.16 – 1.00]	-2.97	.50	.003
5 weeks postnatal	5.63 (4.20)	6.40 (5.23)	-.77 [-2.31 – .77]	-.74	.18	.46
29 weeks postnatal	4.73 (4.18)	6.73 (5.04)	-2.00 [-3.51 – .47]	-2.61	.46	.01

Means, standard deviations, mean differences and confidence intervals in Table 3.6 are shown with the untransformed EPDS scores. The values of *t* and *p* were derived from comparisons of transformed depression scores.

3.1.4.5 Maternal Level of Education and Maternal Depression

Mothers who had left education at age 18 or before (*N*= 111; 58%) were more depressed at all time points than the mothers who had remained in education beyond age 18 (see Table 3.7). Only the difference at 20 weeks gestation was statistically significant at $p<.05$.

Table 3.7 Associations Between Maternal Level of Education and Maternal EPDS Scores at the Four Time Points (*N*=193)

	Mean EPDS (*SD*)		Mean difference [95% CI]	*t*	Cohen's *d*	*p*
	In education beyond 18	Left education at or before 18				
20 weeks gestation	7.30 (4.80)	9.22 (5.50)	-1.92 [-3.41 – .41]	-2.41	.34	.017
32 weeks gestation	7.59 (4.29)	8.65 (4.88)	-1.06 [-2.40 – .27]	-1.40	.20	.16
5 weeks postnatal	5.50 (3.89)	6.02 (4.89)	-.52 [-1.81 – .77]	-.47	.09	.64
29 weeks postnatal	4.34 (3.70)	5.62 (4.80)	-1.28 [-2.53 – -.03]	-1.79	.25	.076

Means, standard deviations, mean differences and confidence intervals in Table 3.7 are shown with the untransformed EPDS scores. The values of *t* and *p* were derived from comparisons of transformed depression scores.

3.1.4.6 Gestational Age at Birth and Maternal Depression

The distribution of gestational ages was skewed towards the right. Data transformation did not reduce skewness at an acceptable level, and consequently all analyses that involved gestational age were performed with the untransformed values using non-parametric tests. Associations between gestational age and the four EPDS scores were calculated using Spearman's *rho*. The results showed that gestational age was not

associated with maternal depression at any point in time, with all rank correlation coefficients ranging between .01 and .08, all *p*'s ns.

3.1.4.7 Birth Weight and Maternal Depression

Maternal depression was not associated with the infant's birth weight at any point in time, with Pearson's *r* at a level between -.11 and .06, all *p*'s ns.

3.1.4.8 Summary of Associations Between Maternal Depression and Potential Risk Factors

Neither gestational age at birth, nor birth weight showed significant associations with maternal depression, and these variables were not considered in subsequent analyses. Maternal age, smoking in pregnancy and maternal relationship status were consistently found to be associated with maternal depression. Therefore these three variables were retained in analyses of associations between maternal depression and vagal tone. Associations among these three maternal risk variables are presented in Section 3.1.4.9.

3.1.4.9 Associations Between Maternal Age, Smoking in Pregnancy and Maternal Relationship Status

There was a significant association between smoking in pregnancy and maternal relationship status, $\chi^2(1)=6.82$, $p=.009$, $OR=2.99$, 95% CI [1.28 – 7.00]. Mothers who did not have a partner were three times more likely to have smoked during pregnancy than the mothers who were in a relationship at 20 weeks gestation.

Maternal age was strongly associated with relationship status, $t=5.17$, mean difference 4.95, 95% CI [2.89 – 7.00][6], $p<.001$. Mothers who did not have a partner were younger than the mothers who were in a relationship at 20 weeks gestation.

Finally, maternal age was strongly associated with smoking during pregnancy, $t=3.60$, mean difference 4.04, 95% CI [1.64 – 6.44][7], $p=.001$. Mothers who smoked during pregnancy were also younger than those who reported that they did not smoke.

[6] Mean difference and confidence interval are reported with untransformed maternal age.
[7] Mean difference and confidence interval are reported with untransformed maternal age.

3.2 Maternal Anxiety

3.2.1 Distribution of Maternal State Anxiety Scores

Visual inspection of the distributions of the state anxiety scores at each time point indicated that they were skewed towards the left, and this was supported by skewness statistics, *S*=1.36 and *K-S*'s *D*=.16 at 20 weeks gestation, *S*=1.36 and *D*=.11 at 32 weeks gestation, *S*=1.04 and *D*=.15 at 5 weeks postnatal and *S*=1.23 and *D*=.13 at 29 weeks postnatal, for all *K-S* tests *p*'s<.005 and *df*'s=200. Log transformation (as detailed in the Approach to Statistical Analyses Section 2.3.1.1. in the Method section) largely improved the aspect of the distributions and skewness approached zero in value. Therefore parametric tests were used in all further analyses unless stated otherwise.

3.2.2 Summary Statistics of Maternal State Anxiety

The means, standard deviations and range of scores for the state anxiety scale of the State-Trait Anxiety Inventory (STAI) untransformed scores at all time points are presented in Table 3.8. Figures E1, E3, E5 and E7 in Appendix E show the histograms with distributions of untransformed maternal state anxiety scores at the four time points, while distributions of the transformed maternal state anxiety scores are presented in Figures E2, E4, E6 and E8.

Table 3.8 Maternal State Anxiety Scores at Each of the Four Assessment Points (*N*=200)

Time of assessment	Mean	*SD*	Minimum	Maximum
20 weeks gestation	32.78	11.94	20	80
32 weeks gestation	33.45	10.30	20	76
5 weeks postnatal	29.23	8.67	20	57
29 weeks postnatal	29.84	8.63	20	65

The mean STAI scores decreased moderately from the prenatal to the postnatal period. A repeated measures ANOVA with the four transformed state anxiety scores as the within-subjects variables was performed to test for differences in STAI scores across the time points. Mauchly's test indicated that the assumption of sphericity had been violated, $\chi^2(5)=27.60$, $p<.001$, therefore results are reported using Greenhouse-Geisser corrections. The model had high significance, $F(2.73, 543.44) = 19.37$, $p<.001$. Post hoc paired *t* tests showed that both postnatal state anxiety scores were significantly lower than both of the prenatal scores, with mean differences of untransformed scores ranging from 2.94, 95% CI [0.76 – 5.12], to 4.22, 95% CI [2.46 – 5.97], all *p*'s<.001.

3.2.3 Associations Between State Anxiety (STAI) Scores Over the Four Time Points

As presented in Table 3.9 there were moderate to high correlations between STAI scores at all time points and they stayed at high level in time despite the fact that the mean scores decreased from pre- to postnatal period.

Table 3.9 Associations Between Transformed State Anxiety (STAI) Scores Across the Four Time Points (Pearson's *r*) (*N*=200)

	20 weeks gestation	32 weeks gestation	5 weeks postnatal	29 weeks postnatal
20 weeks gestation	1			
32 weeks gestation	.63(**)	1		
5 weeks postnatal	.50(**)	.53(**)	1	
29 weeks postnatal	.40(**)	.43(**)	.59(**)	1

** $p<.01$ (2-tailed)

3.2.4 Is Maternal State Anxiety Associated With Other Possible Indices of Maternal Stress?

The maternal and infant risk factors considered as possibly linked with depression (see Section 3.1.4) were also examined in association with state anxiety. The tests were independent-samples *t* tests for comparisons of means and correlations for associations between scores.

3.2.4.1 Maternal Age and Maternal State Anxiety

Associations between maternal age and the four STAI scores were calculated using Pearson's *r*. The results, shown in Table 3.10, revealed that maternal age was weakly associated with state anxiety at 29 weeks postnatal. Younger mothers were more anxious than older mothers 29 weeks after birth, but not before.

Table 3.10 Associations Between Maternal Age and Maternal State Anxiety Scores (*N*=200)

	Maternal state anxiety	*r*	*p*
Maternal age	20 weeks gestation	-.08	.26
	32 weeks gestation	-.10	.17
	5 weeks postnatal	-.10	.19
	29 weeks postnatal	-.16	.02

3.2.4.2 Socio-Economic Disadvantage and Maternal State Anxiety

The association between maternal state anxiety scores and social and economic disadvantage, using the deprivation score described in Section 2.5.1.4 was examined by comparing those falling in the lowest national quintile (*N*= 72; 36 % in this sample) with the remainder. None of the associations was statistically significant (Table 3.11).

Table 3.11 Associations Between Maternal Social and Economic Deprivation and Maternal State Anxiety (*N*=200)

	Mean STAI (*SD*)		Mean difference [95% CI]	*t*	*p*
	Less deprived	Most deprived			
20 weeks gestation	33.34 (12.10)	31.76 (11.67)	1.58 [-1.89 – 5.05]	1.21	.23
32 weeks gestation	34.13 (10.57)	32.22 (9.75)	1.91 [-1.08 – 4.90]	1.27	.21
5 weeks postnatal	28.91 (8.44)	29.79 (9.10)	-.88 [-3.40 – 1.65]	-.74	.46
29 weeks postnatal	29.49 (8.76)	30.44 (8.42)	-.95 [-3.46 - 1.56]	-.91	.36

Means, standard deviations, mean differences and confidence intervals in Table 3.11 are shown with the untransformed STAI scores. The values of *t* and *p* were derived from comparisons of transformed anxiety scores.

3.2.4.3 Smoking in Pregnancy and Maternal State Anxiety

There were 29 women who reported smoking in pregnancy. As shown in Table 3.12, smoking in pregnancy was moderately associated with maternal state anxiety scores at 5 weeks and 29 weeks postnatal.

Table 3.12 Associations Between Smoking in Pregnancy and Maternal State Anxiety (*N*=200)

	Mean STAI (*SD*)		Mean difference [95% CI]	*t*	Cohen's *d*	*p*
	Non-smokers	Smokers				
20 weeks gestation	32.46 (12.07)	34.66 (11.17)	-2.20 [-6.93 – 2.53]	-1.22	.25	.23
32 weeks gestation	33.06 (9.99)	35.72 (11.92)	-2.66 [-6.74 – 1.41]	-1.00	.19	.32
5 weeks postnatal	28.59 (8.27)	33.00 (10.12)	-4.41 [-7.80 – -1.02]	-2.44	.48	.016
29 weeks postnatal	29.12 (7.96)	34.03 (11.09)	-4.91 [-8.27 – -1.55]	-2.62	.50	.009

Means, standard deviations, mean differences and confidence intervals in Table 3.12 are shown with the untransformed STAI scores. The values of *t* and *p* were derived from comparisons of transformed anxiety scores.

3.2.4.4 Maternal Relationship Status and Maternal State Anxiety

There were 40 women of the total of 200 who did not have a partner at 20 weeks gestation. The analyses showed that maternal relationship status was strongly associated with maternal state anxiety at 20 weeks gestation. Also, at all the other three points in time the associations were at significance level or marginally significant. The mothers

who did not have a partner had higher STAI scores than the mothers who were in a relationship, with the larger difference occurring at 20 weeks gestation (see Table 3.13).

Table 3.13 Associations Between Maternal Relationship Status at 20 Weeks Gestation and Maternal State Anxiety at the Four Time Points (*N*=200)

	Mean STAI (*SD*)		Mean difference [95% CI]	*t*	Cohen's *d*	*p*
	With partner	No partner				
20 weeks gestation	31.13 (10.33)	39.38 (15.38)	-8.25 [-12.26 - -4.24]	-3.61	.63	<.001
32 weeks gestation	32.48 (9.36)	37.33 (12.84)	-4.85 [-9.19 - -0.51]	-2.20	.38	.029
5 weeks postnatal	28.73 (8.47)	31.23 (9.31)	-2.50 [-5.51 – 0.52]	-1.78	.31	.077
29 weeks postnatal	29.14 (7.96)	32.60 (10.58)	-3.46 [-6.43 - -0.48]	-1.92	.32	.056

Means, standard deviations, mean differences and confidence intervals in Table 3.13 are shown with the untransformed STAI scores. The values of *t* and *p* were derived from comparisons of transformed anxiety scores.

3.2.4.5 Maternal Level of Education and Maternal State Anxiety

Mothers who had left education at age 18 or before (*N*= 111; 58%) were more anxious at all time points than the mothers who had remained in education beyond age 18 (see Table 3.14). However, none of the comparisons was statistically significant at $p < .05$.

Table 3.14 Associations Between Maternal Level of Education and Maternal STAI Scores at the Four Time Points (*N*=193)

	Mean STAI (*SD*)		Mean difference [95% CI]	*t*	Cohen's *d*	*p*
	In education beyond 18	Left education at or before 18				
20 weeks gestation	31.37 (11.54)	33.86 (12.17)	-2.49 [-5.92 – 0.92]	-1.42	.21	.16
32 weeks gestation	32.83 (10.13)	34.00 (10.47)	-1.17 [-4.14 – 1.80]	-.79	.10	.43
5 weeks postnatal	27.96 (7.56)	30.21 (9.48)	-2.25 [-4.75 – 0.26]	-1.64	.23	.10
29 weeks postnatal	28.45 (7.33)	30.68 (9.32)	-2.23 [-4.68 – 0.23]	-1.60	.22	.11

Means, standard deviations, mean differences and confidence intervals in Table 3.14 are shown with the untransformed STAI scores. The values of *t* and *p* were derived from comparisons of transformed anxiety scores.

3.2.4.6 Gestational Age at Birth and Maternal State Anxiety

Associations between gestational age and the four STAI scores were calculated using Spearman's *rho*. The results showed that gestational age was not associated with maternal state anxiety at any point in time, with all correlation coefficients ranging between -.04 and .08, all *p*'s ns.

3.2.4.7 Birth Weight and Maternal State Anxiety

Maternal state anxiety was not associated with the infant's birth weight at any point in time, with Pearson's *r* at a level between -.04 and .02, all *p*'s ns.

3.2.4.8 Summary of Associations Between Maternal State Anxiety and Potential Risk Factors

The pattern of associations between potential confounding variables and maternal state anxiety was similar to that for maternal depression, and did not point to a need to retain any additional variables in subsequent analyses. Therefore maternal age, smoking in pregnancy and relationship status were included.

3.3 Associations Between Maternal Depression (EPDS) and Maternal State Anxiety (STAI) Across the Four Time Points

Associations between depression and state anxiety across the four assessment points were investigated with transformed scores using Pearson's *r*. Correlations in cross section and over time were all of medium to high levels and are shown in Table 3.15. As outlined in Approach to Statistical Analyses Section 2.3.2, depression and anxiety scores at all four time points were examined in two separate MANOVAs with vagal tone as the predictor, in order to identify the strongest associations, followed by regressions of vagal tone and vagal withdrawal on to the identified depression and anxiety scores.

Table 3.15 Associations Between Transformed Maternal Depression (EPDS) and Maternal State Anxiety (STAI) Scores Across the Four Time Points (Pearson's *r*) (*N*=200)

	20 weeks gestation STAI	32 weeks gestation STAI	5 weeks postnatal STAI	29 weeks postnatal STAI
20 weeks gestation EPDS	.68(**)	.46(**)	.46(**)	.38(**)
32 weeks gestation EPDS	.48(**)	.59(**)	.41(**)	.38(**)
5 weeks postnatal EPDS	.35(**)	.38(**)	.68(**)	.51(**)
29 weeks postnatal EPDS	.35(**)	.33(**)	.45(**)	.57(**)

** $p<.01$ (2-tailed)

3.4 Vagal Tone

3.4.1 Distributions and Summary Statistics of Vagal Tone Scores

Visual inspection of vagal tone (i.e. RSA) scores indicated that throughout the experiment they were normally distributed. Skewness was small with values ranging from -.17 to .17 and z scores ranging from -.98 to .96, all p's ns. Therefore parametric tests were conducted in all analyses. Means, standard deviations and range of scores of the RSA values across the five procedures are presented in Table 3.16.

Table 3.16 Descriptive Statistics for RSA Values Across the Five Experimental Procedures (N=200)

Procedure	Mean	*SD*	Minimum	Maximum
Helper-Hinderer	3.34	0.86	.95	5.76
Novel Toy	2.97	0.82	.39	5.75
Social Engagement	3.33	0.84	.61	6.04
Still Face	2.85	0.79	.56	5.03
Social Reunion	3.40	0.98	.55	5.89

Figures F1 - F5 in Appendix F show the distributions of RSA scores during the five procedures.

3.4.2 Comparability of Vagal Tone Scores With Other Relevant Infant Studies

The mean RSA scores in the current investigation were comparable to what others have found at similar or close ages, as described in Table 3.17. Presented studies were selected based on similar ages, sample sizes and characteristics (other studies with smaller samples are not included) and inclusion in the study design of a challenge or task which shows RSA withdrawal.

Table 3.17 Comparability of RSA Values With Other Infancy Studies

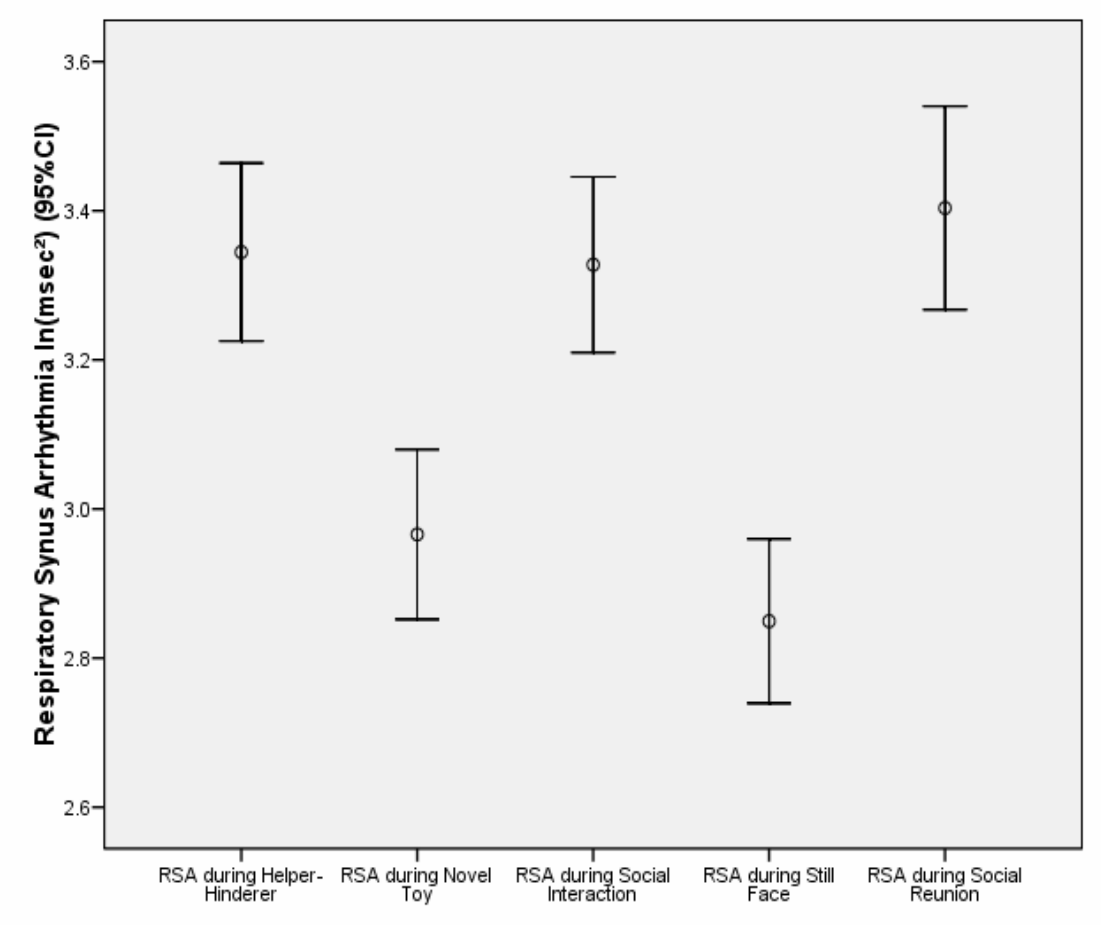
Study	Sample characteristics	Age	Mean RSA prior to challenge or task (*SD*)	Mean RSA challenge or task (*SD*)	Nature of challenge or task
Fracasso et al., 1994	*N*=52 (low risk community)	7 mo	3.25 (0.75)	2.97 (0.86)	Visual stimuli
Weinberg & Tronick, 1996	*N*=50 (low risk community)	6 mo	3.17 (*SD* not reported)	3.03 (*SD* not reported)	Still face
Bazhenova et al., 2001	*N*=40 (low risk community)	5 mo	3.73 (1)	3.28 (0.8)	Still face
Moore & Calkins, 2004	*N*=73 (depressed and non-depressed)	3 mo	2.84 (0.76)	2.64 (0.71)	Still face
Alkon et al., 2006	*N*=150 (low income)	6 mo	3.3 (0.9)	3.2 (0.7)	Bayley test
Moore et al., 2009	*N*=152 (racially diverse urban)	6 mo	3.50 (0.93)	3.38 (0.96)	Still face
Moore, 2010	*N*=48 (urban predominantly low-risk)	6 mo	3.68 (1.08)	3.41 (0.96)	Still face

In general, the means and the standard deviations of the RSA values in the above studies were similar to the values in the current study. The study by Alkon et al. (2006) is perhaps the most similar in size and sample characteristics, and their mean RSA during the resting state of 3.3 (*SD* 0.9) was very close to that during the helper-hinderer and social engagement phase of the still face procedure. The study by Moore and Calkins (2004), which is of particular significance to the current study due to similarities in protocol and objectives, shows somewhat lower mean RSA values compared to all other studies, including the current one. However, this is in line with research (e.g. Richards & Cameron, 1989; Bornstein & Suess, 2000a) suggesting developmental increases in RSA from birth to school age.

3.4.3 Changes in Mean Vagal Tone Scores Across the Five Experimental Procedures

Changes in RSA mean scores across the five manoeuvres were compared using repeated measures ANOVA. Mauchly's test revealed that the assumption of sphericity had been violated, $\chi^2(9)=47.89$, $p<.001$, therefore results are reported using Greenhouse-Geisser corrections. The model was highly significant, $F(3.57, 710.45) = 46.24$, $p<.001$. In post hoc paired *t* tests there were differences in mean RSA, significant at $p<.001$, in 6 of the 10 comparisons. Of particular relevance to the question of vagal withdrawal (see Section 1.3.6 in the Background, and Section 5.4.1 in the Discussion) RSA decreased from helper-hinderer to the novel toy episode (mean difference .38, $p<.001$) to be followed by an increase in the social engagement episode (mean difference .36, $p<.001$). RSA decreased from the engagement in face to face play with mother to the still face (mean difference 0.48, $p<.001$), and increased from the still face to the social repair phase (mean difference 0.55, $p<.001$). Figure 3.1 presents mean RSA scores and confidence intervals across the five procedures.

Figure 3.1 Error Bar Plot of Mean RSA Scores Across the Five Procedures



3.4.4 Associations Between Vagal Tone Scores Across the Five Procedures

Correlations amongst RSA values across the five episodes of vagal tone recording are shown below in Table 3.18. Coefficients were between medium and high, suggesting consistency in RSA scores throughout the 30-minute experimental paradigm.

Table 3.18 Pearson Correlation Coefficients Amongst the RSA Values

	RSA helper-hinderer	RSA novel toy	RSA engaging	RSA still face	RSA reunion
RSA helper-hinderer	1				
RSA novel toy	.70(**)	1			
RSA engaging	. 60(**)	. 68(**)	1		
RSA still face	. 62(**)	. 61(**)	. 65(**)	1	
RSA reunion	. 62(**)	. 52(**)	. 67(**)	. 66(**)	1

** p<.01 (2-tailed)

3.4.5 Principal Component Analysis of the Vagal Tone Scores

As discussed in Section 1.3.8 of the Background, correlations between RSA scores in contrasting conditions have been reported, but the question of whether there is an underlying RSA factor or latent variable has not previously been addressed. Reasons for taking such an approach are reviewed in Section 1.3.11 of the Background.

All five RSA scores were entered as variables into the factorial model. The default Principal Component method of extraction was chosen and no rotation solution was

opted for because no theoretical assumptions were made regarding the possible relatedness of the factors. *Eigenvalue* was set at the default Kaiser's 1 although the decision regarding the number of factors retained was taken primarily by examining the scree plot. As presented in Table 3.19, the analysis yielded one factor with an Eigenvalue of 3.54 which explained 70.73% of the total variance. The component matrix is shown in Table 3.20, suggesting that the five RSA scores loaded highly and equally on to the factor. This provided strong support for an underlying RSA construct, which was evident despite the variability of RSA scores across the experiments.

Table 3.19 RSA Factor and Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %
1	3.54	70.73	70.73	3.54	70.73	70.73
2	0.52	10.30	81.03			
3	0.39	7.69	88.72			
4	0.35	6.91	95.63			
5	0.22	4.37	100.00			

Table 3.20 Factor Loadings of the Five RSA Scores

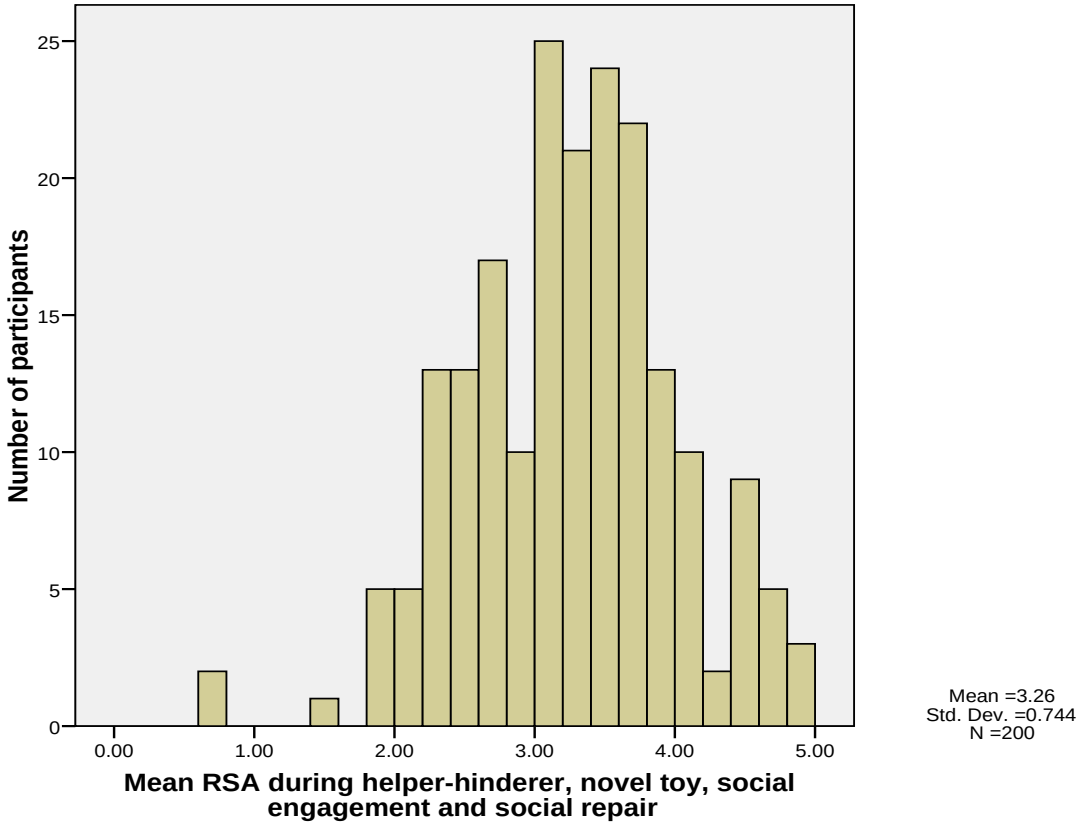
	Component 1
RSA during helper-hinderer	.86
RSA during novel toy	.84
RSA during social engagement	.84
RSA during still face	.84
RSA during social reunion	.82

3.4.6 Procedure for Calculation of Vagal Tone and Vagal Withdrawal Scores in the Current Study

3.4.6.1 The Vagal Tone Measure

In the light of the lack of consistency in the literature with regard to establishing the initial RSA value from which RSA during the challenge or task is subtracted in order to assess vagal withdrawal (see Sections 1.3.5.1 and 1.3.6.1 in the Background) vagal tone in the current study was assessed using a novel method. RSA during the still face was subtracted from the mean of the RSA scores during the other four episodes (i.e. helper-hinderer, novel toy, social engagement and social reunion). The rationale for this approach was justified by the principal component analysis which showed high and equal loadings from all RSA scores onto the factor. Also, mean RSA of the four procedures was preferred to the factor score as the overall measure of vagal tone for reasons of comparability with RSA levels reported in the literature. Figure 3.2 shows the distribution of the vagal tone measure (i.e. mean RSA of the four procedures).

Figure 3.2 Distribution of Vagal Tone Scores (i.e. Mean RSA of the Four Procedures)



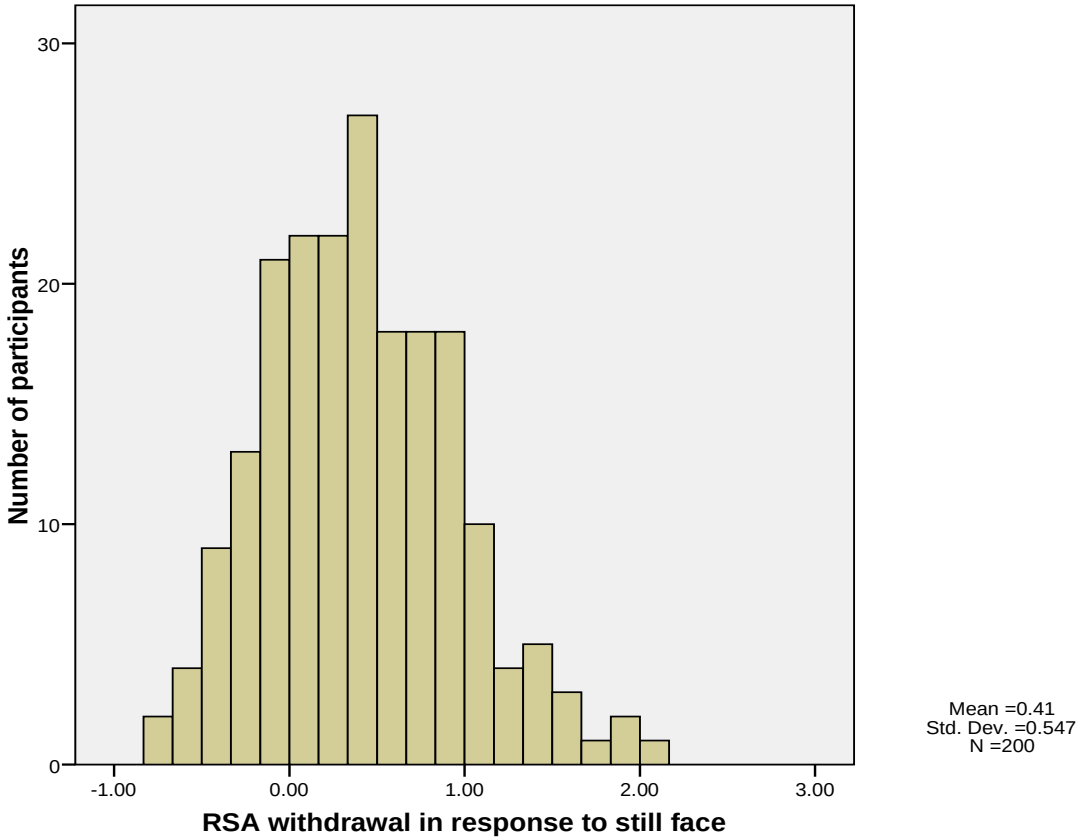
Mean RSA of the four procedures was normally distributed and parametric tests were used in all further analyses.

3.4.6.2 The Vagal Withdrawal Measure

Vagal withdrawal in response to the social stressor was assessed by subtracting the RSA during still face from the mean RSA of the other four procedures. Paired-samples *t* test showed that RSA during still face was significantly lower than the mean RSA during the

other four procedures, $t(199)=10.64$, mean difference 0.41, 95% CI [0.33 – 0.49], $p<.001$. Figure 3.3 shows the distribution of scores for RSA withdrawal.

Figure 3.3 Distribution of Vagal Withdrawal Scores (i.e. RSA Withdrawal)



RSA withdrawal was normally distributed and parametric tests were used in all further analyses.

3.5 Summary of the Chapter

Maternal depression (EPDS) and state anxiety (STAI) at the four points in time were examined visually and tested formally for normality of distributions. Transformation of the EPDS and STAI scores was necessary and performed as described in Section 2.3.1.1. EPDS and STAI scores decreased from the prenatal to postnatal period and correlations amongst them were of moderate to high levels. Maternal age, relationship status and smoking during pregnancy were identified as possible confounders for depression and anxiety and were considered for future analyses of associations between maternal mood and vagal tone.

Vagal tone scores in the five experimental procedures were normally distributed and comparable to those reported in other infant studies of interest. There were high correlations amongst the five sets of scores despite the high variability of the mean scores across the procedures. Principal component analysis revealed one factor with high and equal loadings from all five RSA scores which provided strong support for the idea that a vagal tone score which is reflective of not just one, but several RSA measurements would be a more appropriate measure of the infant's characteristic level of RSA. The overall vagal tone measure was assessed as the mean RSA in all procedures except for the still face, and vagal withdrawal was calculated by subtracting the RSA during still face from the mean RSA in the other four procedures.

Chapter 4

Results II

Predictions of Vagal Tone and Vagal Withdrawal From Maternal Depression and Anxiety and Confounders, and Their Associations With Sex of Infant

4.1 Infant Vagal Tone, Maternal Risks and Sex of Infant in Association With Maternal Depressed Mood

4.1.1 Overall Vagal Tone as Estimated by RSA and Maternal Depression

Associations between RSA and maternal depression scores were examined with the mean RSA score identified previously (see Section 3.4.6.1) as the overall measure of vagal tone, and with transformed EPDS scores. This was conducted in MANOVA in order to determine whether RSA was associated specifically with depression assessed at one or more of the four time points. In MANOVA with the four depression scores as dependent variables there were no significant associations between RSA and any of the depression scores (see Table 4.1).

Table 4.1 Summary of the Results of Multiple Analysis of Variance Examining Associations Between RSA and Prenatal and Postnatal Maternal Depression (EPDS) Scores

Independent variable		Mean RSA Score		
Dependent variables		*F*	*df*	*p*
Prenatal EPDS	20 Weeks	1.00	1,198	.32
	32 Weeks	0.03	1,198	.87
Postnatal EPDS	5 Weeks	1.33	1,198	.25
	29 Weeks	0.82	1,198	.37
Multivariate		1.31	4,195	.27

The MANOVA was conducted using the transformation ln (EPDS score + 7.4).

4.1.2 Examining for Sex Differences in the Link Between Maternal Depression and Vagal Tone

In order to examine the role of sex of infant in associations between maternal depressed mood and vagal tone, the MANOVA was repeated with the addition of sex as a between-subjects factor and sex by mean RSA interaction term. As shown in Table 4.2, in the multivariate model there was a significant main effect of sex and a significant sex by mean RSA interaction, and the associations with transformed EPDS scores at 20 weeks and 32 weeks were statistically significant for sex and sex by mean RSA interaction. By contrast the associations with postnatal depression scores were very small, and did not approach significance.

Table 4.2 Summary of the Results of MANOVA Examining the Main Effect of Sex and the Interaction Between Sex of Infant and RSA in Relation to Prenatal and Postnatal Maternal Depression (EPDS) Scores

Independent variables		Mean RSA Score			Sex of Infant			Mean RSA Score by Sex Interaction		
Dependent variables		*F*	*df*	*p*	*F*	*df*	*p*	*F*	*df*	*p*
Prenatal EPDS	20 Weeks	0.34	1,196	.56	9.43	1,196	.002	10.30	1,196	.002
	32 Weeks	0.02	1,196	.89	3.94	1,196	.048	5.08	1,196	.025
Postnatal EPDS	5 Weeks	1.45	1,196	.23	0.17	1,196	.68	0.26	1,196	.61
	29 Weeks	0.89	1,196	.35	0.04	1,196	.84	0.06	1,196	.81
Multivariate		0.96	4,193	.43	3.71	4,193	.006	3.56	4,193	.008

The MANOVA was conducted using the transformation ln (EPDS score + 7.4)

As the effect of sex of infant by mean RSA interaction was somewhat stronger for 20 weeks depression than for 32 weeks depression, subsequent analyses focused on 20 weeks. It was not, however, assumed that the results provided strong evidence in favour of 20 weeks.

4.1.3 The Role of Sex of Infant in the Association Between Maternal Depression at 20 Weeks Gestation and Vagal Tone

Multiple linear regression was used to test the association between sex of infant and the interaction of sex by 20 weeks gestation depression and infant vagal tone. Depression and sex of infant were entered in step 1 and the depression by sex interaction term was entered in step 2. The results, presented in Table 4.3, show a significant association between the 20 weeks depression by sex interaction term and mean RSA score.

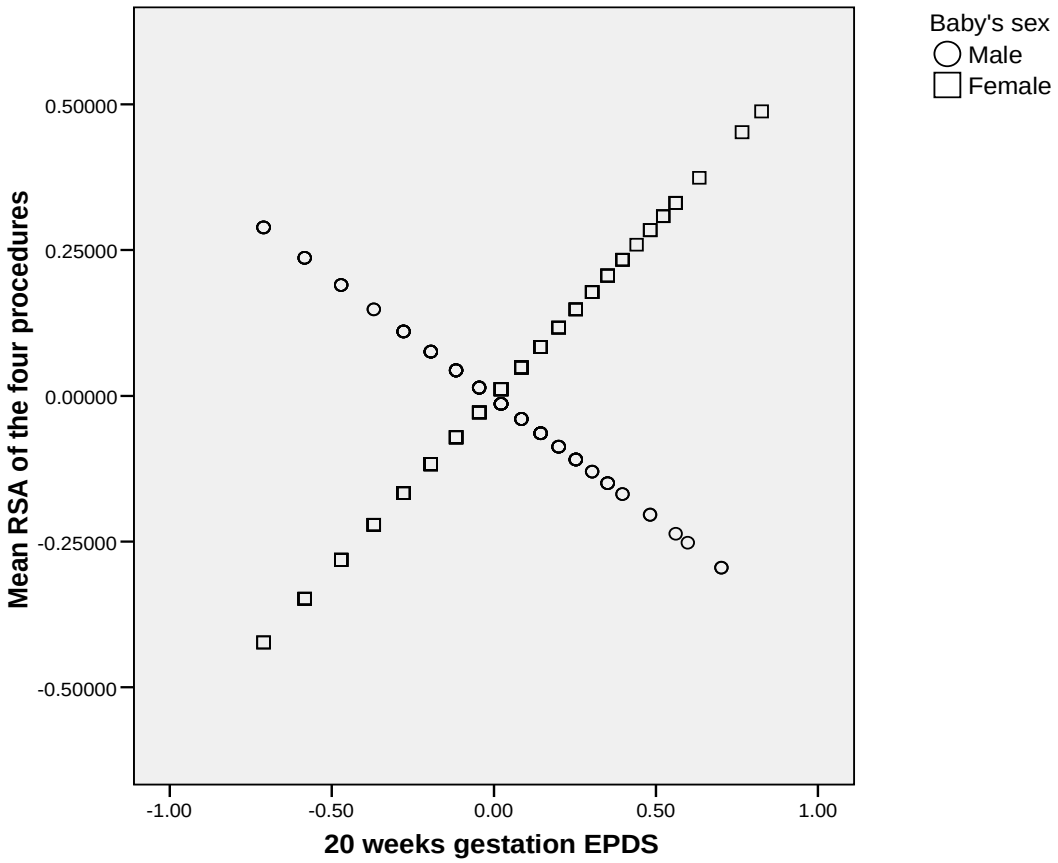
Table 4.3 Summary of Multiple Linear Regression of the Mean RSA Score on Maternal Depression at 20 Weeks Gestation, Sex of Infant and Depression by Sex Interaction

Step	ΔR^2	ΔF	*df*	*p*	Variables	β	*p*
1	.005	.50	2,197	.61	20 weeks EPDS Sex of infant	.071 -.001	.32 .99
2	.050	10.29	1,196	.002	20 weeks EPDS Sex of infant 20 weeks EPDS X Sex of infant	.265 -.002 -.296	.004 .98 .002

The regression was conducted with the EPDS score transformed (according to the same logarithmic formula presented in Section 2.3.1.1) and centred.

Predicted values of mean RSA score from the regression are shown in Figure 4.1. In females increasing maternal depression was associated with increasing vagal tone. whereas in males increasing maternal depression was associated with decreasing vagal tone.

Figure 4.1 Predicted Values of Mean RSA from the Regression Model Showing the Sex of Infant by Prenatal Depression Interaction



4.1.4 Examination of Possible Confounders in the Interaction Between Infant Sex and Maternal Depression in Predicting Vagal Tone.

Three possible confounders for maternal depression were identified following the analyses conducted in Section 3.1.4. Maternal age was correlated with maternal depression at 20 weeks gestation as well as at two more points in time (see Section 3.1.4.1). Smoking during pregnancy was strongly associated with depression both pre- and postnatally (see Section 3.1.4.3). And, links were found between maternal relationship status and depression at three assessment points (see Section 3.1.4.4). Therefore links between each of these three risks and vagal tone were explored.

Three separate multiple hierarchical regression analyses were conducted, each including one of the identified maternal risk factors (i.e. maternal age, maternal relationship status and smoking in pregnancy), sex of infant and their interaction.

4.1.4.1 Maternal Age, Sex of Infant and Maternal Age by Sex of Infant Interaction Predicting Vagal Tone

Multiple hierarchical regression analyses included maternal age and sex in the first block and the interaction term in the second block. The results are presented in Table 4.4 and show a modest contribution of the maternal age by sex of infant interaction to the prediction of mean RSA score.

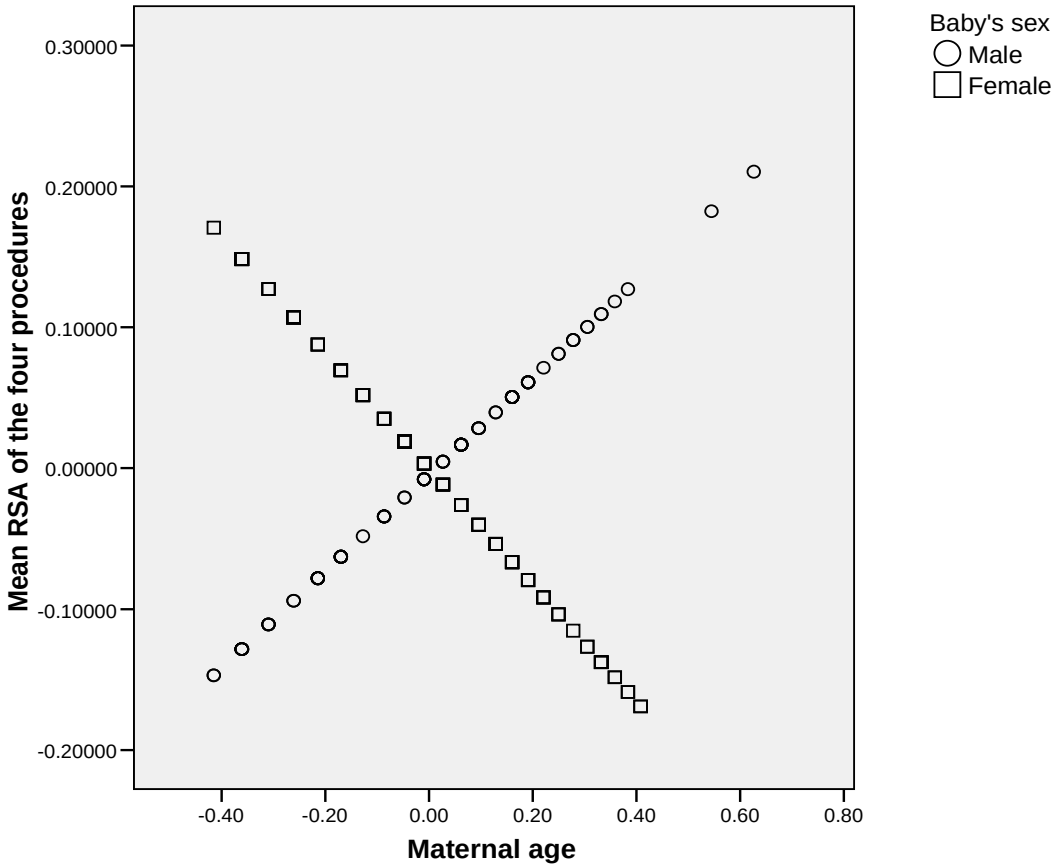
Table 4.4 Summary of Multiple Linear Regression of the Mean RSA Score on Maternal Age, Sex of Infant, Including the Maternal Age by Sex Interaction

Step	ΔR^2	ΔF	*df*	*p*	Variables	β	*p*
1	<.001	.03	2,197	.98	Maternal age	-.016	.82
					Sex of infant	-.002	.98
2	.013	2.48	1,196	.117	Maternal age	-.122	.21
					Sex of infant	-.003	.97
					Maternal age X Sex	.154	.12

The regression was conducted with maternal age transformed according to the formula ln (maternal age) and centred.

Predicted values of mean RSA score from the regression are shown in Figure 4.2. In females increasing maternal age was associated with decreasing vagal tone, while in males increasing age was associated with increasing vagal tone.

Figure 4.2 Predicted Values of Mean RSA from the Regression Model Showing the Sex of Infant by Maternal Age Interaction



Although the effect of the interaction term was non-significant, in view of the moderate size of the standardised beta coefficient, a conservative approach was taken and maternal age was included in subsequent analyses.

4.1.4.2 Maternal Relationship Status, Sex of Infant and Maternal Relationship Status by Sex Interaction Predicting Vagal Tone

At the first assessment wave of the study (i.e. 20 weeks gestation) there were 40 mothers who were not in a relationship and 160 mothers who had a partner.

Multiple hierarchical regression analyses were conducted with maternal relationship status and sex of infant in the first block and their interaction term in the second block. Results (shown in Table 4.5) revealed that none of the predictors was associated with mean RSA, and the effect of the sex by relationship status interaction was small. Therefore maternal relationship status and the interaction with sex of infant were not included in further analyses predicting vagal tone.

Table 4.5 Summary of Multiple Linear Regression of Mean RSA Score From Maternal Relationship Status, Sex of Infant and Maternal Relationship Status by Sex Interaction

Step	ΔR^2	ΔF	*df*	*p*	Variables	β	*p*
1	.012	1.21	2,197	.30	Relationship status	.11	.122
					Sex of infant	-.003	.96
2	.001	.17	1,196	.69	Relationship status	.137	.16
					Sex of infant	.011	.89
					Relationship status X Sex	-.042	.69

4.1.4.3 Smoking in Pregnancy, Sex of Infant and Smoking by Sex Interaction Predicting Vagal Tone

Twenty-nine women in the study of the total of 200 reported that they smoked during pregnancy.

In multiple linear regression there was a non-significant trend for an association between the mean RSA score and the sex of infant by smoking interaction (see Table 4.6). Smoking and sex of infant were entered in the first step of the regression model and the smoking by sex interaction term was entered in the second block.

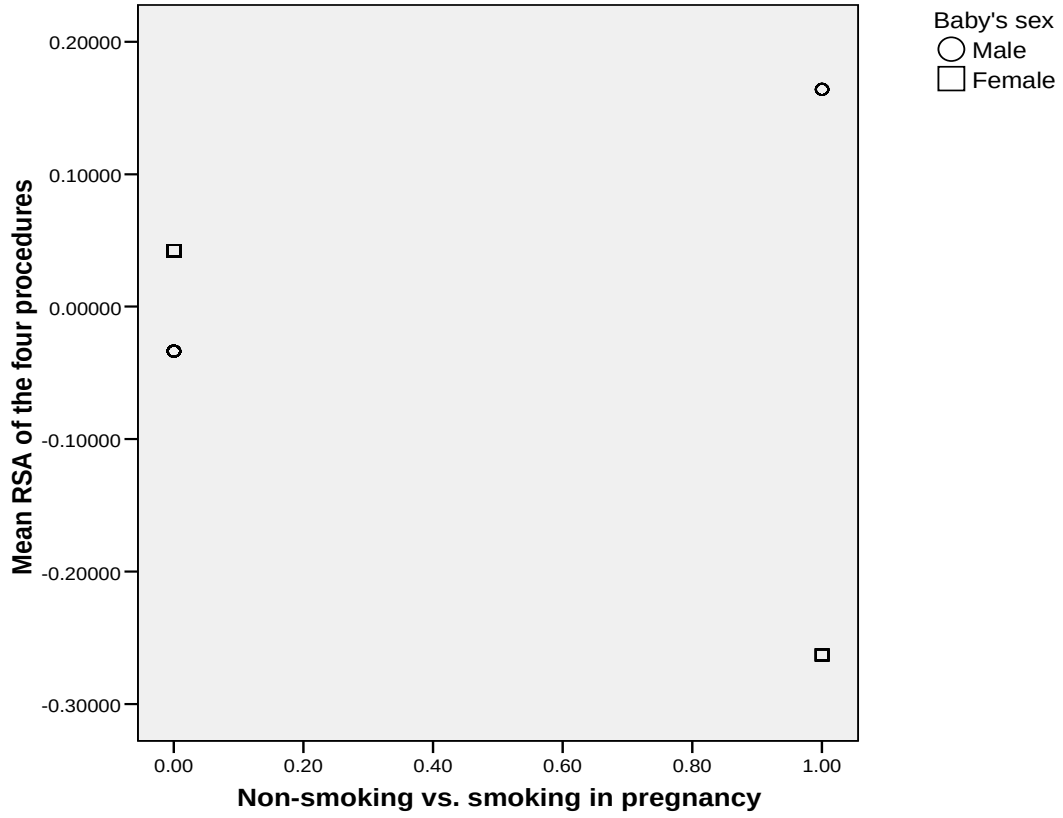
Table 4.6 Summary of Findings of the Mean RSA Score Regressed on to Smoking in Pregnancy, Sex of Infant and the Smoking by Sex Interaction

Step	ΔR^2	ΔF	*df*	*p*	Variables	β	*p*
1	.001	.05	2,197	.95	Smoking	-.023	.74
					Sex of infant	-.002	.98
2	.014	2.83	1,196	.094	Smoking	-.145	.16
					Sex of infant	-.051	.51
					Smoking X Sex	.178	.094

Predicted values of mean RSA score from the regression are shown in Figure 4.3. In females maternal smoking during pregnancy was associated with lower vagal tone whereas in males maternal smoking was associated with higher vagal tone. If it is assumed that both maternal depression and smoking during pregnancy are risks, the interaction shown in the figure is in the opposite direction than that for maternal depression.

Females have elevated vagal tone in the presence of one risk (maternal depression), but decreased in the presence of the other (maternal smoking), and males *vice versa.*

Figure 4.3 Predicted Values of Mean RSA From the Regression Model Showing the Sex of Infant by Maternal Smoking During Pregnancy Interaction



Although the effect of the interaction term was non-significant, in view of the moderate size of the standardised beta, a conservative approach was taken and smoking was included in subsequent analyses.

4.1.5 Does Maternal Depressed Mood, in Interaction With Sex, Predict Infant Vagal Tone After Accounting for Age of Mother, Smoking in Pregnancy and Current Depressed Mood?

Given the evidence outlined in Sections 4.1.4.1 and 4.1.4.3 of sex of infant interactions with both age of mother and smoking in pregnancy, both interactions were included in regression in a test of whether maternal depressed mood made an independent contribution to vagal tone in 29 weeks old infants.

A 3-step hierarchical regression analysis was used to test the association between maternal prenatal depression at 20 weeks, possible confounders, and the interactions with sex of infant and the overall measure of vagal tone in the infant (i.e. mean RSA score). In the first step the confounders were entered (i.e. maternal age and smoking) together with sex of infant and transformed 29 weeks postnatal and 20 weeks gestation EPDS scores. The interactions between each of the two confounders with sex of infant were entered in the second step. Finally, the interaction term of the 20 weeks gestation maternal depression score and sex was entered. Results are shown in Table 4.7.

Table 4.7 Summary of Multiple Linear Regression Predicting Vagal Tone (Mean RSA Score) From Maternal Depression in Interaction With Sex of Infant, After Accounting for Maternal Age, Smoking in Pregnancy and Current Depressed Mood

Step	ΔR^2	ΔF	*df*	*p*	Variables	β	*p*
1	.021	.82	4,195	.54	Maternal age	-.006	.94
					Smoking	-.030	.70
					Sex of infant	-.018	.81
					29 weeks postnatal EPDS	-.140	.105
					20 weeks gestation EPDS	.151	.083
2	.038	3.93	2,193	.021	Maternal age	-.127	.21
					Smoking	-.194	.063
					Sex of infant	-.095	.23
					29 weeks postnatal EPDS	-.158	.066
					20 weeks gestation EPDS	.164	.058
					Maternal age X Sex	.197	.051
					Smoking X Sex	.265	.016
3	.059	12.88	1,192	<.001	Maternal age	-.071	.47
					Smoking	-.259	.012
					Sex of infant	-.121	.113
					29 weeks postnatal EPDS	-.158	.059
					20 weeks gestation EPDS	.395	<.001
					Maternal age X Sex	.119	.24
					Smoking X Sex	.358	.001
					20 weeks gestation EPDS X Sex	-.346	<.001

Overall, the model explained almost 12% of the total variance in the mean RSA score. Consistent with the opposite directions of association between maternal depression and vagal tone, and smoking and vagal tone, the contributions of each in interaction with sex of infant were strengthened somewhat when they were examined jointly.

4.1.6 Sex-Specific Contributions of 20 Weeks Gestation Maternal Depression on to Predicting Vagal Tone After Accounting for Maternal Age and Smoking in Pregnancy

Follow-up analyses of the interactions were conducted with separate linear regression analyses of females and males. In order to assess the contribution of depression after accounting for the possible confounds, maternal age and smoking during pregnancy, these variables were entered first, followed by maternal depression at 20 weeks gestation. Results for both boys and girls are presented in Table 4.8.

Table 4.8 Vagal Tone Regressed on to Maternal Age, Smoking and Maternal Depression at 20 Weeks Gestation Presented by Sex of Infant

	Step	ΔR^2	ΔF	*df*	*p*	Variables	β	*p*
Females	1	.039	2.08	2,103	.13	Maternal age	-.147	.14
						Smoking	-.161	.104
	2	.087	10.12	1,102	.002	Maternal age	-.073	.46
						Smoking	-.243	.014
						20 weeks gestation EPDS	.318	.002
Males	1	.032	1.50	2,91	.23	Maternal age	.155	.16
						Smoking	.151	.17
	2	.039	3.74	1,90	.056	Maternal age	.112	.31
						Smoking	.197	.077
						20 weeks gestation EPDS	-.209	.056

The separate regressions revealed that the sex of infant by depression interaction was accounted for by a strong positive effect in females and a somewhat weaker effect in the opposite direction in males. The pattern was similar, but in the reverse direction for the sex of infant by smoking in pregnancy interaction.

4.1.7 Summary of Findings Regarding Maternal Depression and Vagal Tone

To summarise, maternal depression in interaction with sex was associated with the overall measure of vagal tone (i.e. mean RSA score) in the prenatal period, but not postnatally. The link was stronger for the level of depression reported earlier in pregnancy and therefore further analyses were conducted with the 20 weeks EPDS score. Maternal depression at 20 weeks gestation in interaction with sex of infant made a strong independent contribution to vagal tone scores (explaining nearly 6% of the variance) after accounting for maternal age, smoking and current depression. Males of mothers who were more depressed at 20 weeks gestation had lower mean RSA, while females of depressed mothers had higher RSA. The contribution of depression in predicting vagal tone was somewhat stronger for females than males after accounting for maternal age and smoking. The association between the interaction of smoking with sex of infant and vagal tone was in direct opposition to that of maternal depression, with the males of mothers who smoked during pregnancy showing higher vagal tone than the males of mothers who did not smoke.

4.2 Infant Vagal Tone, Maternal Risks and Sex of Infant in Association With Maternal State Anxiety

4.2.1 Overall Vagal Tone as Estimated by RSA and Maternal State Anxiety

Associations between RSA and maternal state anxiety were examined with the mean RSA score as the overall measure of vagal tone, and with transformed STAI scores. This was conducted in MANOVA in order to ascertain whether RSA was associated specifically with state anxiety at one or more of the four points in time. In MANOVA with the four state anxiety scores as dependent variables there were no significant associations between mean RSA and any of the state anxiety scores (see Table 4.9).

Table 4.9 Summary of the Results of MANOVA Examining Associations Between RSA and Prenatal and Postnatal Maternal State Anxiety (STAI) Scores

Independent variable		Mean RSA Score		
Dependent variables		*F*	*df*	*p*
Prenatal STAI	20 Weeks	0.00	1,198	.99
	32 Weeks	0.93	1,198	.34
Postnatal STAI	5 Weeks	0.54	1,198	.47
	29 Weeks	0.15	1,198	.70
Multivariate		0.74	4,195	.57

The MANOVA was conducted using the transformation ln (STAI score – 15.87).

4.2.2 Examining for Sex Differences in the Link Between Maternal State Anxiety and Vagal Tone

In order to examine the role of sex differences in associations between maternal state anxiety and vagal tone, the MANOVA was repeated with adding sex of infant as a between subjects factor and sex by mean RSA interaction term. As presented in Table 4.10, in the multivariate model there was a significant main effect of sex and a significant sex by mean RSA interaction, and the associations with transformed STAI scores at 20 weeks and 32 weeks were statistically significant. On the other hand, the associations with postnatal state anxiety scores were very small, and did not approach significance.

Table 4.10 Summary of the Results of MANOVA Examining the Main Effect of Sex and the Interaction Between Sex of Infant and RSA in Relation to Prenatal and Postnatal Maternal State Anxiety (STAI) Scores

Independent variables		Mean RSA Score			Sex of Infant			Mean RSA Score by Sex Interaction		
Dependent variables		*F*	*df*	*p*	*F*	*df*	*p*	*F*	*df*	*p*
Prenatal STAI	20 Weeks	0.17	1,196	.69	6.84	1,196	.01	9.10	1,196	.003
	32 Weeks	1.74	1,196	.19	6.16	1,196	.01	6.86	1,196	.01
Postnatal STAI	5 Weeks	0.50	1,196	.48	0.04	1,196	.83	0.01	1,196	.92
	29 Weeks	0.14	1,196	.71	0.00	1,196	.99	0.003	1,196	.96
Multivariate		0.85	4,193	.50	3.40	4,193	.01	4.01	4,193	.004

The MANOVA was conducted using the transformation ln (STAI score – 15.87).

As the infant sex by mean RSA interaction was stronger for the state anxiety scores reported at 20 weeks than for 32 weeks, subsequent analyses focused on 20 weeks. It was not however assumed that the results provided strong evidence in favour of 20 weeks.

4.2.3 The Role of Infant Sex in the Link Between Maternal State Anxiety at 20 Weeks Gestation and Vagal Tone

Multiple linear regression was used to assess the association between sex of infant and the interaction of sex by 20 weeks gestation state anxiety and infant vagal tone. State anxiety and sex were entered in block 1 and the state anxiety by sex interaction term was entered in block 2. The results are shown in Table 4.11 and indicated a significant association between the state anxiety by sex interaction term and mean RSA.

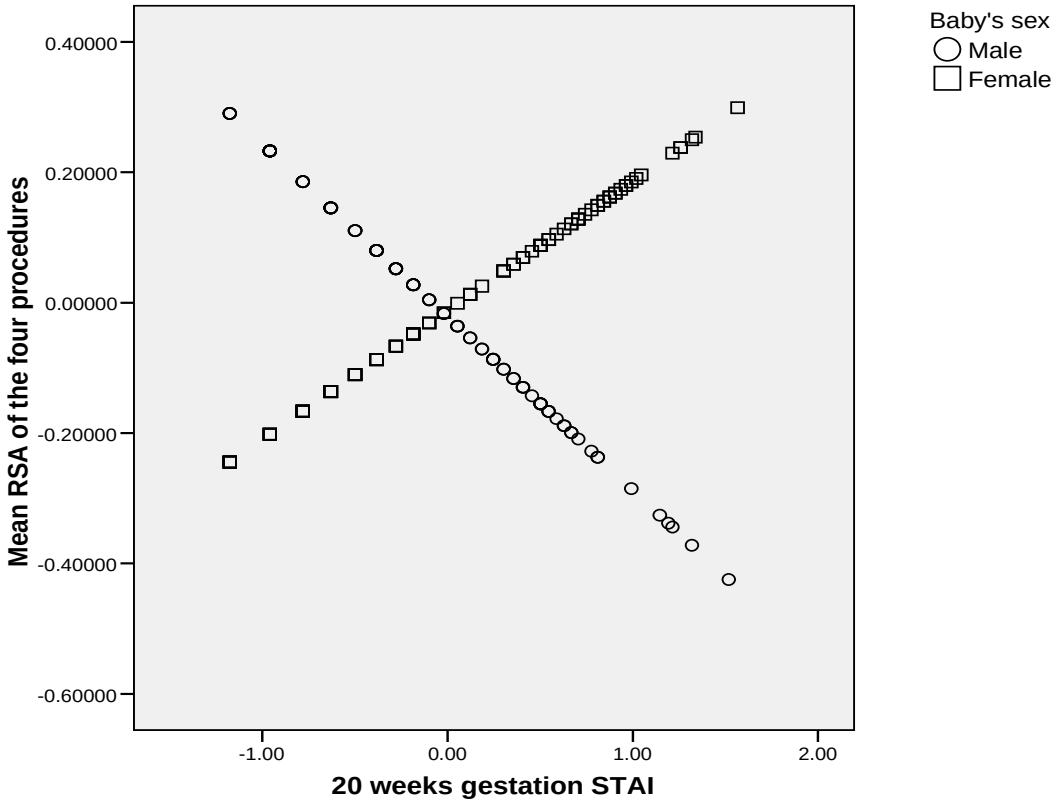
Table 4.11 Summary of Multiple Linear Regression of the Mean RSA Score on Maternal State Anxiety at 20 Weeks Gestation, Sex of Infant and State Anxiety by Sex Interaction

Step	ΔR^2	ΔF	*df*	*p*	Variables	β	*p*
1	<.001	.001	2,197	.99	20 weeks STAI Sex of infant	<.001 -.003	.99 .97
2	.044	9.13	1,196	.003	20 weeks STAI Sex of infant 20 weeks STAI X Sex of infant	.183 -.007 -.280	.05 .92 .003

The regression was conducted with the STAI score transformed (according to the same logarithmic formula presented in Section 2.3.1.1) and centred.

Predicted values of mean RSA score from the regression are presented in Figure 4.4. Increasing maternal state anxiety was associated in girls with increasing vagal tone while in males increasing maternal state anxiety was associated with decreasing vagal tone.

Figure 4.4 Predicted Values of Mean RSA From the Regression Showing the Sex of Infant by Prenatal State Anxiety Interaction



4.2.4 Examination of Possible Confounders in the Interaction Between Infant Sex and Maternal State Anxiety in Predicting Vagal Tone.

Three possible confounders of maternal state anxiety were identified following the analyses conducted in Section 3.2.4. Maternal age was moderately correlated with maternal state anxiety at 29 weeks postnatal (see Section 3.2.4.1). Maternal relationship status was found to be associated with state anxiety at 20 weeks gestation (see Section 3.2.4.4). And, there were weak links between smoking in pregnancy and maternal state anxiety at both times in the postnatal period (see Section 3.2.4.3).

The same three confounders were found to be associated with maternal depression at various points in time (see Sections 3.1.4.1, 3.1.4.3 and 3.1.4.4). Moreover, the regression analyses conducted in Sections 4.1.4.1 and 4.1.4.3 revealed weak associations between the maternal age by sex of infant and smoking by sex interactions and mean RSA score (the maternal relationship status by sex of infant interaction did not approach significance and therefore relationship status was excluded from further analyses).

The examination of the patterns of associations between maternal state anxiety at 20 weeks gestation (see Figure 4.4), age (see Figure 4.2) and smoking (see Figure 4.3) in interaction with sex revealed that the association between smoking by sex of infant interaction and vagal tone was in the opposite direction to that of state anxiety by sex interaction. However, the link between maternal age by sex of infant interaction and vagal tone followed the same direction as in the association of state anxiety by sex interaction and vagal tone.

In summary, because both maternal age and smoking were found to be linked with maternal anxiety and because the associations between vagal tone and the two maternal risks in interaction with sex were marginally significant, further analyses investigating links between maternal state anxiety and vagal tone will include controlling for maternal age, smoking and their interactions with sex of infant.

4.2.5 Does Maternal State Anxiety, in Interaction With Sex, Predict Infant Vagal Tone After Accounting for Maternal Age, Smoking in Pregnancy and Current State Anxiety?

In light of the evidence outlined in Sections 4.1.4.1 and 4.1.4.3 of sex of infant interactions with maternal age and smoking in pregnancy, both interactions were included in regression to test whether maternal state anxiety made an independent contribution to vagal tone measured at 29 weeks in infants.

A 3-step hierarchical regression analysis was used to test the link between maternal state anxiety at 20 weeks gestation, possible confounders, current state anxiety and interactions with sex and the overall measure of vagal tone in the infant (i.e. mean RSA score). In the first block of the regression the confounders were entered (i.e. maternal age and smoking) together with sex of infant and transformed maternal state anxiety scores at 29 weeks postnatal and 20 weeks gestation. The interactions between each of the two confounders with sex of infant were entered in the second block. Lastly, the interaction term of the 20 weeks maternal state anxiety and sex was entered. Table 4.12 shows the main regression coefficients.

Table 4.12 Summary of Multiple Linear Regression Predicting Vagal Tone (Mean RSA) From 20 Weeks Gestation State Anxiety in Interaction With Sex of Infant, After Accounting for Maternal Age, Smoking in Pregnancy and Current State Anxiety

Step	ΔR^2	ΔF	*df*	*p*	Variables	β	*p*
1	.002	.08	4,195	.99	Maternal age	-.019	.80
					Smoking	-.033	.66
					Sex of infant	-.002	.98
					29 weeks postnatal STAI	.035	.66
					20 weeks gestation STAI	-.012	.88
2	.035	3.46	2,193	.033	Maternal age	-.148	.14
					Smoking	-.180	.086
					Sex of infant	-.066	.39
					29 weeks postnatal STAI	.021	.79
					20 weeks gestation STAI	.015	.85
					Maternal age X Sex	.207	.043
					Smoking X Sex	.238	.034
3	.047	9.77	1,192	.002	Maternal age	-.140	.16
					Smoking	-.228	.029
					Sex of infant	-.074	.33
					29 weeks postnatal STAI	-.004	.96
					20 weeks gestation STAI	.223	.029
					Maternal age X Sex	.186	.064
					Smoking X Sex	.259	.019
					20 weeks gestation STAI X Sex	-.294	.002

Wholly, the model explained over 8% of the total variance in the mean RSA score. Consistent with the opposite directions of association between maternal state anxiety and vagal tone, and smoking and vagal tone, the contributions of each in interaction with sex of infant were strengthened somewhat when they were in joint examination.

4.2.6 Sex-Specific Contributions of 20 Weeks Gestation Maternal State Anxiety on to Predicting Vagal Tone After Accounting for Maternal Age and Smoking in Pregnancy

Follow-up analyses of the interactions were conducted with separate linear regression analyses of females and males. In order to assess the contribution of maternal state anxiety after accounting for the possible confounders, maternal age and smoking in pregnancy, these variables were entered first, followed by maternal state anxiety at 20 weeks gestation. Table 4.13 presents the results for both males and females.

Table 4.13 Vagal Tone Regressed on to Maternal Age, Smoking and Maternal State Anxiety at 20 Weeks Gestation Shown by Sex of Infant

	Step	ΔR^2	ΔF	*df*	*p*	Variables	β	*p*
Females	1	.039	2.08	2,103	.13	Maternal age Smoking	-.147 -.161	.14 .104
	2	.046	5.11	1,102	.026	Maternal age Smoking 20 weeks gestation STAI	-.134 -.212 .221	.17 .034 .026
Males	1	.032	1.50	2,91	.23	Maternal age Smoking	.155 .151	.16 .17
	2	.050	4.93	1,90	.029	Maternal age Smoking 20 weeks gestation STAI	.136 .127 -.226	.21 .24 .029

The separate regressions revealed that the sex of infant by state anxiety interaction was accounted for by a moderately strong positive effect in females and a similarly strong effect in the opposite direction in males. For the sex of infant by smoking in pregnancy interaction the pattern was similar, but in the reverse direction and significant in females only.

4.2.7 Summary of Findings Regarding Maternal State Anxiety and Vagal Tone

Summing up, maternal state anxiety in interaction with sex of infant was associated with the overall measure of vagal tone (i.e. mean RSA score) in the prenatal period but not at any of the two postnatal time points. The association was stronger for the state anxiety at 20 weeks and therefore further analyses were conducted with the 20 weeks STAI score. Maternal state anxiety at 20 weeks gestation in interaction with sex of infant made a strong independent contribution to vagal tone scores explaining nearly 5% of the variance, after accounting for maternal age, smoking and current state anxiety. The direction of the association between state anxiety and infant sex interaction and vagal tone was that males of mothers who had increasing STAI scores at 20 weeks gestation had decreasing mean RSA scores, while females had increasing mean RSA. The contribution of state anxiety in predicting vagal tone was equally strong for females and males after accounting for maternal age and smoking. The association between the interaction of smoking with sex of infant and vagal tone was in the opposite direction to that of maternal state anxiety, with the females of mothers who smoked during pregnancy showing decreased mean RSA scores compared to the females of mothers who did not smoke.

In view of the moderately high correlations amongst maternal depression and state anxiety across all four points in time (see Section 3.3), they were not examined jointly in the regressions in order to prevent them cancelling each other out (see Section 2.3.4). However, joint analyses with both depression and anxiety at the two prenatal time points were still conducted and these findings are presented in Appendix G.

4.3 Infant Vagal Withdrawal, Maternal Risks and Sex of Infant in Association With Maternal Depressed Mood

4.3.1 Vagal Withdrawal as Estimated by RSA Withdrawal and Maternal Depression

RSA withdrawal was computed by subtracting the RSA during the still face episode from the mean RSA of the other four procedures (see Section 3.4.6.2). Associations between vagal withdrawal and maternal depression scores were examined with the RSA withdrawal score and with transformed EPDS scores. This was conducted in MANOVA in order to determine whether RSA withdrawal was associated specifically with depression assessed at one or more of the four time points. In MANOVA with the four depression scores as dependent variables there were no significant associations between RSA withdrawal and any of the EPDS scores (see Table 4.14).

Table 4.14 Summary of the Results of MANOVA Examining Associations Between RSA Withdrawal and Prenatal and Postnatal EPDS Scores

Independent variable		RSA withdrawal		
Dependent variables		*F*	*df*	*p*
Prenatal EPDS	20 Weeks	0.01	1,198	.93
	32 Weeks	0.003	1,198	.96
Postnatal EPDS	5 Weeks	0.10	1,198	.75
	29 Weeks	0.12	1,198	.73
Multivariate		0.11	4,195	.98

The MANOVA was conducted using the transformation ln (EPDS score + 7.4).

4.3.2 Examining for Sex Differences in the Link Between Maternal Depression and Vagal Withdrawal

In order to examine the role of sex differences in associations between maternal depressed mood and vagal withdrawal, the MANOVA was repeated with the addition of sex of infant as a between-subjects factor and the sex by RSA withdrawal interaction term. As shown in Table 4.15, in the multivariate model there was a trend of an association approaching significance for the sex by RSA withdrawal interaction, and the links with transformed EPDS scores at 20 weeks gestation and 29 weeks postnatal were statistically significant.

Table 4.15 Summary of the Results of MANOVA Examining the Main Effect of Sex and the Interaction Between Sex of Infant and RSA Withdrawal in Relation to Prenatal and Postnatal Maternal Depression Scores

Independent variables		RSA Withdrawal			Sex of Infant			RSA Withdrawal by Sex Interaction		
Dependent variables		*F*	*df*	*p*	*F*	*df*	*p*	*F*	*df*	*p*
Prenatal EPDS	20 Weeks	0.00	1,196	.96	1.39	1,196	.24	5.26	1,196	.023
	32 Weeks	0.00	1,196	.99	0.13	1,196	.72	3.48	1,196	.064
Postnatal EPDS	5 Weeks	0.11	1,196	.74	0.04	1,196	.84	0.64	1,196	.42
	29 Weeks	0.15	1,196	.70	0.04	1,196	.84	5.31	1,196	.022
Multivariate		0.14	4,193	.97	0.59	4,193	.67	1.88	4,193	.115

The MANOVA was conducted using the transformation ln (EPDS score + 7.4).

The link between prenatal depression at 20 weeks in association with sex of infant and vagal withdrawal was further explored to test the hypothesis of prenatal prediction to vagal withdrawal after accounting for the confounders. However, in view of the significant association between the 29 weeks postnatal depression by sex interaction and vagal withdrawal, a more stringent approach was adopted and the test of prenatal influence included accounting for the effect of postnatal depression at 29 weeks in interaction with sex of infant.

4.3.3 The Role of Sex of Infant in the Association Between Maternal Depression at 20 Weeks Gestation and Vagal Withdrawal

Multiple linear regression was used to test the association between the interaction of 20 weeks gestation depression and sex of infant and vagal withdrawal. Depression and sex of infant were entered in step 1 and the depression by sex interaction term was entered in step 2. The results, presented in Table 4.16, showed a significant association between the depression by sex interaction term and RSA withdrawal.

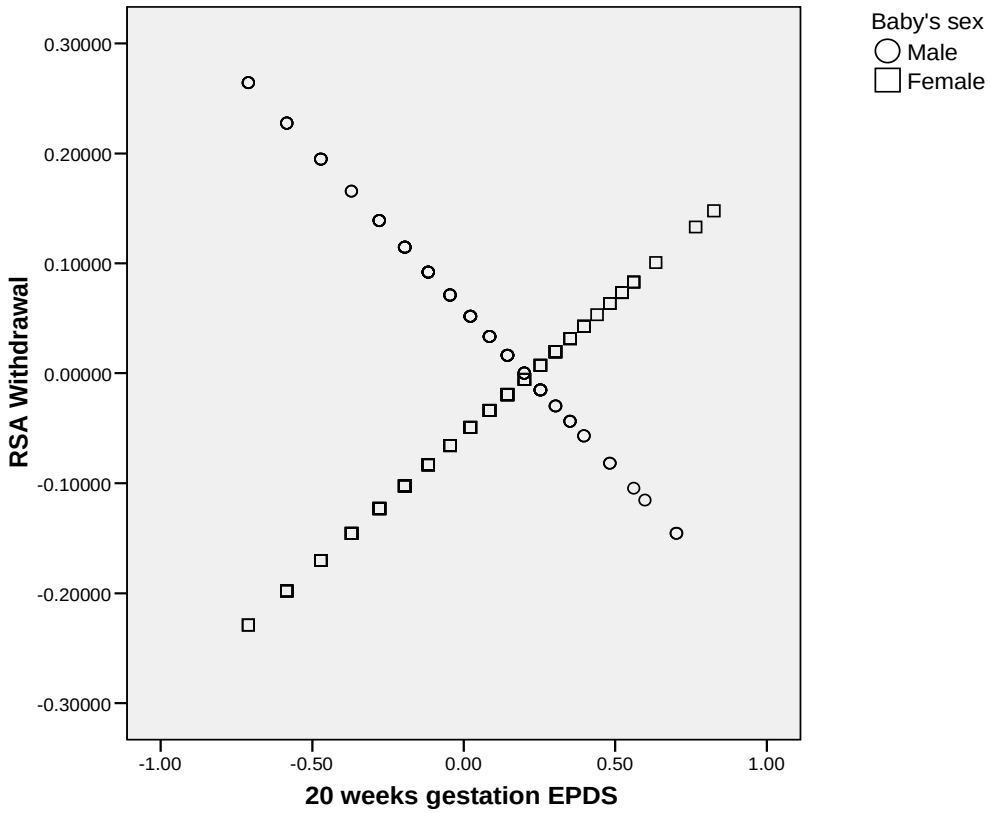
Table 4.16 Summary of Multiple Linear Regression of RSA Withdrawal on Maternal Depression at 20 Weeks Gestation, Sex of Infant and Depression by Sex Interaction

Step	ΔR^2	ΔF	*df*	*p*	Variables	β	*p*
1	.011	1.08	2,197	.34	20 weeks EPDS Sex of infant	.009 .104	.90 .15
2	.026	5.29	1,196	.022	20 weeks EPDS Sex of infant 20 weeks EPDS X Sex	.149 .103 -.214	.11 .14 .022

The regression was conducted with the EPDS score transformed (according to the same logarithmic formula presented in Section 2.3.1.1) and centred.

Predicted values of RSA withdrawal score from the regression are shown in Figure 4.5. In females increasing maternal depression was associated with increasing vagal withdrawal, whereas in males increasing maternal depression was associated with decreasing vagal withdrawal.

Figure 4.5 Predicted Values of RSA Withdrawal Score From the Regression Model Showing the Sex of Infant by Prenatal Depression Interaction



4.3.4. Examination of Possible Confounders in the Interaction Between Infant Sex and Maternal Depression in Predicting Vagal Withdrawal

Three possible confounders of maternal depression were previously identified, as shown in Sections 3.1.4.1, 3.1.4.3, and 3.1.4.4. They are maternal age, maternal relationship status and smoking in pregnancy. Each of these three maternal risk factors was examined in interaction with sex of infant in order to establish possible links with vagal withdrawal.

Three separate multiple hierarchical regression analyses were conducted, each including one of the identified risks, sex and their interaction.

4.3.4.1 Maternal Age, Sex of Infant and Maternal Age by Sex of Infant Interaction Predicting Vagal Withdrawal

Multiple hierarchical regression analyses included maternal age and infant sex in the first block and the interaction term in the second block. The results are presented in Table 4.17 and showed no contribution of the maternal age by sex of infant interaction to the prediction of RSA withdrawal. Therefore, the follow-up analyses linking prenatal maternal depression to vagal withdrawal did not include the maternal age.

Table 4.17 Summary of Multiple Linear Regression of RSA Withdrawal on Maternal Age, Sex of Infant, and Maternal Age by Sex Interaction

Step	ΔR^2	ΔF	*df*	*p*	Variables	β	*p*
1	.01	1.21	2,197	.30	Maternal age	-.04	.60
					Sex of infant	.11	.14
2	.01	1.08	1,196	.30	Maternal age	-.11	.27
					Sex of infant	.11	.14
					Maternal age X Sex	.10	.30

The regression was conducted with maternal age transformed according to the formula ln (maternal age) and centred.

4.3.4.2 Maternal Relationship Status, Sex of Infant and Maternal Relationship Status by Sex Interaction Predicting Vagal Withdrawal

Multiple hierarchical regression analyses were conducted with maternal relationship status and sex in the first block and their interaction term in the second block. Results are shown in Table 4.18 and indicated that relationship status, but not the interaction with sex of infant, was marginally associated with the RSA withdrawal score.

Table 4.18 Summary of RSA Withdrawal Regressed on to Maternal Relationship Status, Sex of Infant and Maternal Relationship Status by Sex Interaction

Step	ΔR^2	ΔF	*df*	*p*	Variables	β	*p*
1	.027	2.77	2,197	.065	Maternal relationship status	.129	.068
					Sex of infant	.103	.14
2	.003	.60	1,196	.44	Maternal relationship status	.181	.064
					Sex of infant	.130	.10
					Maternal relationship X Sex	-.080	.44

Although the main effect of the relationship status marginally significant and the effect of the interaction with sex of infant was small, in view of the moderate size of the standardised beta coefficient, a conservative approach was taken and maternal relationship status was included in subsequent analyses.

4.3.4.3 Smoking in Pregnancy, Sex of Infant and Smoking by Sex Interaction Predicting Vagal Withdrawal

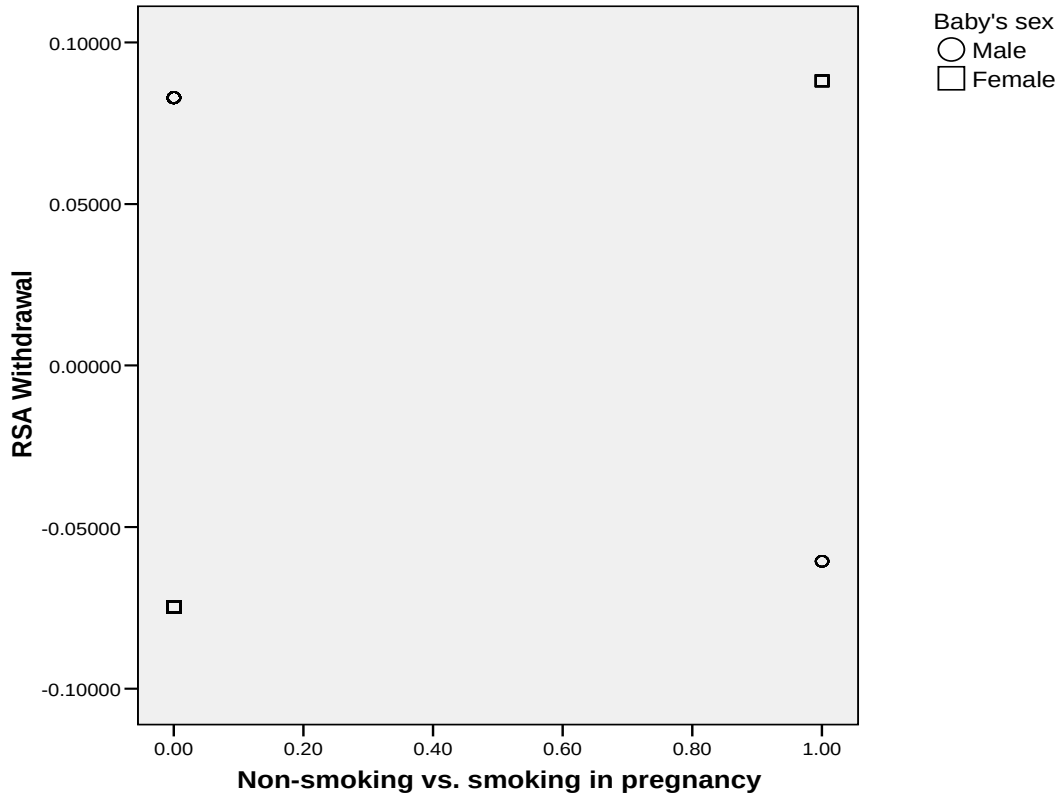
In multiple linear regression smoking and sex of infant were entered in the first step of the regression model and the smoking by sex interaction term was entered in the second block (see Table 4.19).

Table 4.19 Summary of Findings of the RSA Withdrawal Regressed on to Smoking in Pregnancy, Sex of Infant and the Smoking by Sex Interaction

Step	ΔR^2	ΔF	*df*	*p*	Variables	β	*p*
1	.011	1.07	2,197	.35	Smoking	.004	.95
					Sex of infant	.103	.15
2	.010	1.95	1,196	.16	Smoking	.105	.30
					Sex of infant	.144	.06
					Smoking X Sex	-.148	.16

Predicted values of RSA withdrawal score from the regression are shown in Figure 4.6. Male infants of mothers who smoked had lower vagal withdrawal than the males of mothers who did not smoke, while females of mothers who smoked had higher vagal withdrawal than of those who did not smoke. If it is assumed that both maternal depression and smoking during pregnancy are risks, the interaction shown in the figure is in the same direction as that for maternal depression. Females have elevated and males decreased vagal withdrawal in the presence of both risks. Compared to the prediction to vagal tone, the direction of effect of smoking in predicting vagal withdrawal in males and females showed a reverse pattern. In the presence of the same risk (i.e. smoking during pregnancy), females show decreased vagal tone but increased vagal withdrawal, and males *vice versa.*

Figure 4.6 Predicted Values of RSA Withdrawal From the Regression Model Showing the Sex of Infant by Maternal Smoking Interaction



Although the effect of the interaction term was not significant, in view of the moderate size of the standardised beta, a conservative approach was taken and smoking in pregnancy was included in subsequent analyses.

4.3.5 Does Maternal Depressed Mood at 20 Weeks Gestation, in Interaction With Sex, Predict Vagal Withdrawal After Accounting for Maternal Relationship Status, Smoking in Pregnancy and Current Depression in Interaction With Sex?

Given the evidence outlined in Sections 4.3.4.2 and 4.3.4.3 of the marginal effect of maternal relationship status and the somewhat weak sex interaction with smoking in predicting vagal withdrawal, relationship status and the smoking by sex interaction were included in regression in a test of whether maternal depressed mood measured in pregnancy at 20 weeks made an independent contribution to vagal withdrawal in 29 weeks old infants. Besides, in the regression it also accounted for the sex interaction with 29 weeks postnatal depression, as explained in Section 4.3.2.

A 3-step hierarchical regression analysis was used to test the association between maternal depression at 20 weeks gestation, the confounders and the depression by sex interaction, and vagal withdrawal in the infant (i.e. RSA withdrawal). In the first step, the confounders were entered (i.e. maternal relationship status and smoking) together with sex of infant and transformed maternal depression scores at 29 weeks postnatal and 20 weeks gestation. The sex interactions with smoking and 29 weeks postnatal depression were entered in the second step. The interaction term of the 20 weeks gestation maternal depression and sex was entered in the last step. Results are shown in Table 4.20.

Table 4.20 Summary of Multiple Linear Regression Predicting Vagal Withdrawal From 20 Weeks Gestation Depression in Interaction With Sex, After Accounting for Maternal Relationship Status, Smoking and Current Depression in Interaction With Sex

Step	ΔR^2	ΔF	*df*	*p*	Variables	β	*p*
1	.031	1.24	5,194	.29	Maternal relationship status	.145	.057
					Smoking	-.018	.81
					Sex of infant	.110	.13
					29 weeks postnatal EPDS	.053	.54
					20 weeks gestation EPDS	-.064	.47
2	.031	3.20	2,192	.043	Maternal relationship status	.155	.046
					Smoking	.097	.35
					Sex of infant	.149	.06
					29 weeks postnatal EPDS	.181	.09
					20 weeks gestation EPDS	-.081	.36
					Smoking X Sex	-.147	.20
					29 weeks postnatal EPDS X Sex	-.168	.10
3	.007	1.49	1,191	.22	Maternal relationship status	.157	.043
					Smoking	.076	.47
					Sex of infant	.145	.067
					29 weeks postnatal EPDS	.137	.22
					20 weeks gestation EPDS	.007	.95
					Smoking X Sex	-.127	.27
					29 weeks postnatal EPDS X Sex	-.097	.41
					20 weeks gestation EPDS X Sex	-.136	.22

Overall, the model explained 7% of the total variance in RSA withdrawal. The contribution of the interaction between depression at 20 weeks gestation and sex of infant was small, of less than 1% in the variance, after stringently controlling for maternal relationship status, smoking during pregnancy by sex interaction and postnatal depression at 29 weeks in interaction with sex of infant. Prenatal depression at 20 weeks in association with sex of infant did not predict vagal withdrawal in the infant at 29 weeks, after controlling for maternal risks and postnatal depression in interaction with sex of infant.

4.3.6 Summary of Findings Linking Maternal Depression and Vagal Withdrawal

To sum up, in MANOVA, maternal depression in interaction with sex of infant was associated with vagal withdrawal (i.e. RSA withdrawal score) at two points in time – one prenatal at 20 weeks and one postnatal at 29 weeks. Further regression analyses were conducted to test prediction from maternal prenatal depression to infant vagal withdrawal in association with sex. After stringently controlling for maternal risks and postnatal depression at 29 weeks in interaction with sex of infant, the independent contribution of 20 weeks prenatal depression in interaction with sex of infant was small and statistically non-significant.

4.4 Infant Vagal Withdrawal, Maternal Risks and Sex of Infant in Association With Maternal State Anxiety

4.4.1 Vagal Withdrawal as Estimated by RSA Withdrawal and Maternal State Anxiety

Associations between RSA withdrawal and maternal state anxiety were examined with the RSA withdrawal calculated as the difference between the mean RSA of the four procedures and the RSA during the still face, using transformed STAI scores. This was conducted in MANOVA in order to determine whether RSA withdrawal was associated specifically with state anxiety at one or more points in time. In MANOVA with the four STAI scores as dependent variables there were no significant associations between RSA withdrawal and any of the STAI scores (see Table 4.21).

Table 4.21 Summary of the Results of MANOVA Examining Associations Between RSA Withdrawal and Prenatal and Postnatal STAI Scores

Independent variable		RSA Withdrawal		
Dependent variables		*F*	*df*	*p*
Prenatal STAI	20 Weeks	0.003	1,198	.96
	32 Weeks	0.83	1,198	.36
Postnatal STAI	5 Weeks	0.14	1,198	.71
	29 Weeks	0.56	1,198	.46
Multivariate		0.80	4,195	.53

The MANOVA was conducted using the transformation ln (STAI score – 15.87).

4.4.2 Examining for Sex Differences in the Link Between Maternal State Anxiety and Vagal Withdrawal

In order to examine the role of sex differences in associations between maternal state anxiety and vagal withdrawal, the MANOVA was repeated with adding sex of infant as a between-subjects factor and the sex by RSA withdrawal interaction term. As presented in Table 4.22, in the multivariate model there was a highly significant sex of infant by RSA withdrawal interaction, and the association with transformed STAI scores at 32 weeks was very strong, while for 20 weeks the link was weaker, yet still significant. On the other hand, the association with 29 weeks postnatal state anxiety in interaction with sex was also strong and close to the level with 32 weeks gestation, while for the 5 weeks postnatal anxiety the link was weak and somewhat approaching significance level.

Table 4.22 Summary of the Results of MANOVA Examining the Main Effect of Sex and the Interaction Between Sex of Infant and RSA Withdrawal in Relation to Prenatal and Postnatal Maternal State Anxiety (STAI) Scores

Independent variables		RSA Withdrawal			Sex of Infant			RSA Withdrawal by Sex Interaction		
Dependent variables		*F*	*df*	*p*	*F*	*df*	*p*	*F*	*df*	*p*
Prenatal STAI	20 Weeks	0.00	1,196	.97	0.04	1,196	.84	5.08	1,196	.025
	32 Weeks	1.40	1,196	.24	4.06	1,196	.05	13.07	1,196	<.001
Postnatal STAI	5 Weeks	0.19	1,196	.66	0.30	1,196	.59	2.15	1,196	.15
	29 Weeks	0.33	1,196	.57	2.06	1,196	.15	7.27	1,196	.008
Multivariate		0.86	4,193	.49	1.66	4,193	.16	4.14	4,193	.003

The MANOVA was conducted using the transformation ln (STAI score – 15.87).

As the infant sex by RSA withdrawal interaction was stronger for the state anxiety scores reported at 32 weeks gestation than at 20 weeks gestation, subsequent analyses focused on 32 weeks. It was not however assumed that the results provided strong evidence in favour of 32 weeks.

4.4.3 The Role of Infant Sex in the Link Between Maternal State Anxiety at 32 Weeks Gestation and Vagal Withdrawal

Multiple linear regression was used to assess the association between the interaction of sex by 32 weeks gestation state anxiety and infant vagal withdrawal. State anxiety and sex were entered in block 1 and the state anxiety by sex interaction term was entered in block 2. The results are shown in Table 4.23 and indicated a strong association between the state anxiety by sex interaction term and RSA withdrawal.

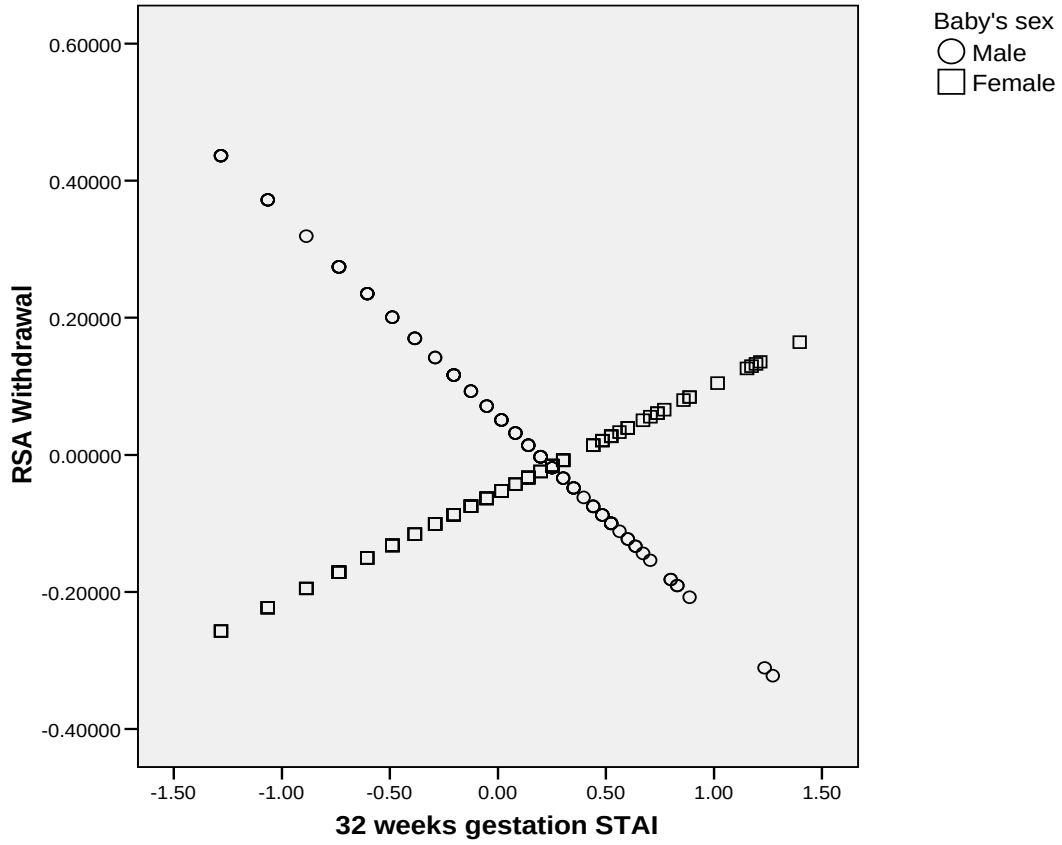
Table 4.23 Summary of Multiple Linear Regression of RSA Withdrawal on Maternal State Anxiety at 32 Weeks Gestation, Sex of Infant and State Anxiety by Sex Interaction

Step	ΔR^2	ΔF	*df*	*p*	Variables	β	*p*
1	.015	1.46	2,197	.24	32 weeks STAI Sex of infant	-.062 .102	.38 .15
2	.061	12.88	1,196	<.001	32 weeks STAI Sex of infant 32 weeks STAI X Sex	.171 .102 -.339	.072 .14 <.001

The regression was conducted with the STAI score transformed (according to the same logarithmic formula presented in Section 2.3.1.1) and centred.

Predicted values of RSA withdrawal score from the regression are presented in Figure 4.7. In females, increasing maternal state anxiety was associated with increasing vagal withdrawal, while in males increasing maternal state anxiety was associated with decreasing vagal withdrawal.

Figure 4.7 Predicted Values of Vagal Withdrawal From the Regression Showing the Sex of Infant by Prenatal State Anxiety Interaction



4.4.4 Examination of Possible Confounders in the Interaction Between Infant Sex and Maternal State Anxiety in Predicting Vagal Withdrawal

Three possible confounders of maternal state anxiety were identified, as shown in Sections 3.2.4.1, 3.2.4.3, and 3.2.4.4. They are: maternal age, maternal relationship status and smoking in pregnancy. Each of these three maternal risk factors was examined in interaction with sex of infant in order to establish possible links with vagal withdrawal.

The regression analyses conducted in Sections 4.3.4.2 and 4.3.4.3 revealed weak associations between maternal relationship status and the sex of infant interaction with smoking and RSA withdrawal score (the effects of maternal age and in interaction with sex of infant did not approach significance and therefore age was excluded from further analyses).

Because both maternal relationship status and smoking were found to be linked with maternal anxiety and because the associations between vagal withdrawal and relationship status on one hand, and smoking in interaction with sex on the other hand, somewhat approached significance, further analyses investigating prediction from maternal prenatal state anxiety and vagal withdrawal will include controlling for maternal relationship status as a main effect and smoking in interaction with sex of infant.

4.4.5 Does 32 Weeks Gestation State Anxiety, in Interaction With Sex, Predict Infant Vagal Withdrawal After Accounting for Maternal Relationship Status, Smoking and Current State Anxiety in Interaction With Sex of Infant?

Given the evidence outlined in Sections 4.3.4.2 and 4.3.4.3 on the marginal effect of maternal relationship status and the somewhat weak sex of infant interaction with smoking in predicting vagal withdrawal, relationship status and smoking by sex interaction were included in regression in a test of whether maternal state anxiety at 32 weeks gestation made an independent contribution to changes in vagal withdrawal scores in 29 weeks old infants. Besides, in view of the strong association between vagal withdrawal and the 29 weeks postnatal state anxiety by sex interaction, a more stringent approach was taken, which was to also control for the effect of the postnatal state anxiety by sex of infant interaction.

A 3-step hierarchical regression analysis was employed to test the association, with the confounders (i.e. relationship status and smoking), sex of infant and transformed scores of state anxiety at 29 weeks postnatal and 32 weeks gestation entered in the first step. The sex interactions with smoking and 29 weeks postnatal state anxiety were entered in the second step. The final block contained the interaction term of the 32 weeks gestation state anxiety score and sex of infant. Results are shown in Table 4.24.

Table 4.24 Summary of Multiple Linear Regression Predicting Vagal Withdrawal From 32 Weeks Gestation Anxiety in Interaction With Sex, After Accounting for Maternal Relationship Status, Smoking and Sex by Current State Anxiety Interaction

Step	ΔR^2	ΔF	*df*	*p*	Variables	β	*p*
1	.041	1.67	5,194	.14	Maternal relationship status	.141	.054
					Smoking in pregnancy	-.030	.68
					Sex of infant	.103	.15
					29 weeks postnatal STAI	-.121	.12
					32 weeks gestation STAI	.092	.25
2	.045	4.73	2,192	.010	Maternal relationship status	.162	.026
					Smoking in pregnancy	.069	.49
					Sex of infant	.139	.07
					29 weeks postnatal STAI	-.132	.09
					32 weeks gestation STAI	.232	.016
					Smoking X Sex	-.137	.20
					29 weeks postnatal STAI X Sex	-.224	.013
3	.030	6.45	1,191	.012	Maternal relationship status	.158	.027
					Smoking in pregnancy	.073	.46
					Sex of infant	.139	.062
					29 weeks postnatal STAI	.065	.55
					32 weeks gestation STAI	.141	.16
					Smoking X Sex	-.138	.19
					29 weeks postnatal STAI X Sex	-.127	.19
					32 weeks gestation STAI X Sex	-.263	.012

Overall, the model explained over 11% of the variance in RSA withdrawal. The contribution of the 32 weeks prenatal state anxiety by sex interaction term in the last step of the regression was moderately strong, adding a further 3% to the model, and was statistically significant. Prenatal maternal state anxiety at 32 weeks in association with sex of infant predicted infant vagal withdrawal at 29 weeks after stringently controlling for postnatal influences and their interactions with sex of infant.

4.4.6 Sex-Specific Contributions of 32 Weeks Gestation Maternal State Anxiety on to Predicting Vagal Withdrawal After Accounting for Maternal Relationship Status, Smoking and Current Depression

Follow-up analyses of the interactions were conducted with separate linear regression analyses of females and males. In order to assess the contribution of prenatal state anxiety after accounting for the possible confounds, maternal relationship status and smoking in pregnancy, and postnatal state anxiety at 29 weeks, these variables were entered first, followed by maternal state anxiety at 32 weeks gestation. Results for both boys and girls are presented in Table 4.25.

Table 4.25 Vagal Withdrawal Regressed on to Maternal Relationship Status, Smoking and Maternal State Anxiety at 29 Weeks Postnatal and 32 Weeks Gestation Presented by Sex of Infant

	Step	ΔR^2	ΔF	*df*	*p*	Variables	β	*p*
Females	1	.075	2.74	3,102	.047	Relationship status	.156	.11
						Smoking	.068	.48
						29 weeks postnatal STAI	184	.061
	2	.003	0.35	1,101	.56	Relationship status	.149	.13
						Smoking	.070	.47
						29 weeks postnatal STAI	.153	.17
						32 weeks gestation STAI	.065	.56
Males	1	.048	1.50	3,90	.22	Relationship status	.139	.21
						Smoking	-.113	.32
						29 weeks postnatal STAI	-.162	.13
	2	.093	9.59	1,89	.003	Relationship status	.172	.11
						Smoking	-.121	.26
						29 weeks postnatal STAI	-.058	.59
						32 weeks gestation STAI	-.324	.003

The separate regressions revealed that the sex of infant by prenatal state anxiety interaction was accounted for by a strong negative effect in males and a weak effect in the opposite direction in females.

4.4.7 Summary of Findings Regarding Maternal State Anxiety and Vagal Withdrawal

In summary, maternal state anxiety in interaction with sex was strongly associated with vagal withdrawal (i.e. RSA withdrawal) at three points in time – two prenatal and one postnatal. The link was stronger for the level of state anxiety reported in the later stage in pregnancy and therefore further analyses were conducted with the 32 weeks STAI scores. Maternal state anxiety at 32 weeks gestation in interaction with sex of infant made a significant independent contribution to vagal withdrawal scores (explaining 3% of the variance) after accounting for maternal relationship status, smoking in pregnancy and the interaction between sex of infant and state anxiety at 29 weeks postnatal. Males of mothers with increasing state anxiety at 32 weeks gestation had decreasing RSA withdrawal scores, while the females of mothers with increasing state anxiety had increasing RSA withdrawal. The contribution of 32 weeks gestation state anxiety in predicting vagal withdrawal after accounting for the confounders and current maternal state anxiety was strong in boys and very weak in girls.

In view of the moderately high correlations amongst maternal depression and state anxiety across all four points in time (see Section 3.3), they were not examined jointly in the regressions in order to prevent them cancelling each other out (see Section 2.3.4). However, joint analyses with both depression and anxiety at the two prenatal time points were still conducted and these findings are presented in Appendix G.

4.5 Summary of the Chapter

Associations were tested between prenatal and postnatal maternal depression and state anxiety, sex of infant and vagal tone (mean RSA of the four procedures) and vagal withdrawal (RSA withdrawal). Depression at 20 weeks gestation in interaction with sex of infant was most strongly associated with vagal tone, while the link with vagal withdrawal was strongest for the state anxiety at 32 weeks gestation in interaction with sex of infant. Postnatal influences of maternal mood in interaction with sex of infant on vagal tone were very weak, while for vagal withdrawal the interactions between sex of infant and both depression and anxiety at 29 weeks postnatal were moderately strong. In the two tests of prenatal prediction (one for depression and one for anxiety) to vagal tone after accounting for maternal risks, the effects of the sex of infant interaction with 20 weeks gestation depression and 20 weeks gestation state anxiety, respectively, remained strong. However, after controlling for maternal risks and current depression in interaction with sex of infant, prenatal depression at 20 weeks in interaction with sex of infant did not continue to predict vagal withdrawal. By contrast, in the same stringent test of prenatal prediction of vagal withdrawal from state anxiety at 32 weeks in interaction with sex of infant after controlling for maternal risks and postnatal state anxiety in interaction with sex, the association held as moderately strong. With regard to the sex-specific directions of effects, in males, increasing maternal depression and anxiety scores were linked with decreasing vagal tone and vagal withdrawal, while in females the opposite pattern was found, i.e. the females of mothers with increasing levels of symptoms had increasing vagal tone and vagal withdrawal. By contrast, the effect of smoking during pregnancy did not show consistency and varied for females and males, sometimes in the similar direction to that for maternal risks, and other times in the opposite one.

Chapter 5

Discussion and Conclusions

5.1 Overview of Findings

There were three main elements in the background to this study. First, there is a growing body of evidence in favour of effects of prenatal stress on neurodevelopment, behaviours and psychopathology in infancy and childhood. Second, many forms of psychopathology entail limitations in emotion regulation which may be evident in infancy. Autonomic activity, and in particular RSA, taken as an indicator of vagal tone, assessed in infancy, may provide an early index of emotion regulatory capabilities, and provide new insights into early developmental pathways in psychopathology. Thirdly, recent reviews have pointed to sex differences in the effects of prenatal stress on behaviour and on autonomic regulation, which is of great potential interest in the light of sex differences in the timing of onset and nature of psychopathology in childhood and adolescence.

The main aim of the current study was to investigate links between pre- and postnatal maternal depression and anxiety and vagal tone and vagal withdrawal in 6-month-old infants. It was hypothesised that prenatal depression and anxiety would predict vagal tone and vagal withdrawal, and that this association would remain after controlling for postnatal symptomatology. It was proposed that the sex of infant would moderate this association in that increasing maternal symptomatology would be associated with decreased vagal tone and vagal withdrawal in boys, but increases in girls.

There was no evidence that prenatal depression and anxiety were associated with vagal tone or with vagal withdrawal, taking the sample as a whole. However, there were associations moderated by infant sex. There were interactions between sex and maternal depression and anxiety at 20 weeks gestation in the prediction of vagal tone explained by decreasing vagal tone associated with increasing maternal symptomatology in males, and

increasing vagal tone in females. Vagal withdrawal was predicted by 32 weeks maternal anxiety moderated in similar fashion by sex. These associations were not substantially altered after accounting for confounders.

5.2 Methodological Issues

5.2.1 Vagal Tone Measurement

As outlined in Section 3.4.2, the field of vagal tone studies is relatively new and most studies have utilised samples of less than 100, with considerable variations in behavioural procedures for eliciting changes in vagal tone. For example, only two infant studies (i.e. Alkon et al. 2006; Moore et al., 2009) with more than 100 participants have been identified in which both baseline vagal tone and vagal withdrawal were assessed. Commonly, studies attempt to identify a baseline condition during which to assess vagal tone and an arousing or threatening condition during which to assess vagal withdrawal. However, as several studies have commented, there is probably no true baseline condition in the awake child because if no stimuli are presented infants generally seek stimulation by exploring equipment, pointing to features of the room or trying to make contact with accompanying adults. In this study five standard procedures were used each with different characteristics, designed to be engaging for the child, but in different ways. No assumption was made that any represented a baseline condition.

As highlighted in Section 1.3.8, relatively few studies have examined vagal tone in more than one condition in infants. Consequently, little is known about the relationship between different contexts and how vagal tone changes across conditions. Generally where studies have assessed baseline condition, this has been interpreted as an index of the child's characteristic level of physiological state and reflecting homeostasis (Calkins, 1997). However, studies have not yet explored the possibility of an underlying process or competence where several measurements exist across different contexts and that a mean level of RSA might be a better estimate for the overall level of vagal input on the heart in

the absence of a threat or task than a single context to which the child might engage with varying motivation, attention or affect. In this study preliminary analyses found that vagal tone varied considerably across the experimental sequences, which is in line with the existing literature and at the same time there were moderate to high RSA correlations across all episodes. Furthermore, principal component analysis identified one clear factor which gives support for the innovative approach of using an RSA score derived from several measurements.

The issue of the method for calculation of vagal withdrawal requires special consideration. Moore et al. (2009) argues that the best approach for computation of vagal reactivity during a task (e.g. still face) is to subtract the RSA during the still face from the baseline RSA, while in Moore (2010) subtraction from the episode immediately preceding the still face is recommended, which is usually the initial social interaction. Therefore, consensus does not exist regarding the most appropriate way of calculating RSA withdrawal, and this is reflected in the heterogeneity of the methods employed. For example, in Moore et al. and Moore and Calkins (2004) the reference measure from which RSA during still face was subtracted was baseline RSA, while in Moore, and Weinberg and Tronick (1996) it was the RSA during the social interaction. To add even more to the lack of agreement, in Bazhenova et al. (2001) it was RSA during a toy attention task which served as the basis for the subtraction, and this was because the experimental design in Bazhenova et al. did not include an initial play with the mothers, infants being presented with the still face straight after a task in which they were looking at objects.

The inconsistencies that exist in the literature with regard to how baseline RSA is assessed and which is the most appropriate method for measuring RSA withdrawal were

addressed in the current study by exploring the possibility of an underlying vagal tone construct that would reflect more than one measurement. Principal component analysis clearly revealed one factor to which all RSA scores contributed highly and equally, thus the mean RSA across the four episodes of non-challenging nature was taken as the basis from which the RSA during still face was subtracted. The mean RSA of the four procedures can be regarded as an overall measure of vagal tone functioning and it averages RSA scores during conditions reflecting engagement with the non-threatening environment. By contrast, RSA withdrawal is the measure of change from the characteristic level of autonomic functioning (i.e. mean RSA of the four procedures) to that during the social challenge (RSA during still face).

5.3 Strengths and Limitations of the Current Study

Several strengths and limitations should be acknowledged in the present research. They are mainly related to the sample and design of the study, and also the use of the questionnaire measures.

5.3.1 Sample

The present study includes a community-based consecutive sample of first-time mothers recruited from the sole maternity clinic serving a well-defined geographical area in the UK. Such a recruitment design aiming to create a sample which is representative of the general population reduces the biases associated with samples of clinically referred participants or volunteers. Recruitment in the current study, as presented in Figure 2.1 at Section 2.4.2.2, was relatively high in the antenatal clinic (i.e. the extensive sample), but the refusal rate for participation in the intensive sample was higher than in the extensive sample. However, once recruitment in the intensive study was completed, the attrition rates at the subsequent assessment waves were very low.

The questionnaires collected on the extensive subjects allowed me to compare the participants in the intensive sample with the non-participants. Compared to non-participants, participants were significantly less deprived and they were older. Nevertheless both groups were more deprived than the UK general population. Also, representation of ethnic minorities in the present sample, although comparable to figures published for the Wirral, is lower than the current rates of non-white British people that live in the UK (see Section 2.4.1.1). This may have implications in both directions: on

one hand, the findings in the current study cannot be generalised to other cultural groups, but the advantage of it may be the removal of a potential source of heterogeneity.

5.3.2 Longitudinal Design

This was a longitudinal design with measurement at two time points during pregnancy and two following the birth of the child. Following participants longitudinally in investigations of psychopathology offers some clear advantages in comparison to conducting studies in cross-section (Rutter, 1994). First, causality implies a temporal sequence which can be identified with longitudinal methods. Equally, if there is a temporal association it does not prove causality. Second, measures of maternal psychopathology at any one time point are generally strongly associated with measures at other time points, so that any one association may be a proxy for another. Having several measurements over time allows one to take account of this. Third, although levels of psychopathology are generally correlated over time, there are also fluctuations, so there may be specificity in timing which only longitudinal studies can assess. Fourth, timing effects make genetic explanations less likely.

5.3.3 Sample Size

The present research includes 200 mothers and infants with complete data at all assessment waves making this the largest sample in published studies that examined infant vagal tone and started prenatally.

5.3.4 Maternal Questionnaire Measures

Despite their advantages (see Section 2.4.4.1) self-report measures also have limitations and they should be acknowledged. First, they rely on the mother's personal interpretation of items and, therefore, responses may sometimes reflect psychological characteristics of the respondent rather than evidence for the actual phenomenon which is conveyed (Hoyt, 2000). However, because in the current study the outcome measure (i.e. vagal tone) is assessed independently of maternal report, the rater bias is less likely to pose a major problem (see Davis et al., 2007). Besides, findings of differential associations between predictors and outcomes (e.g. between pre- but not postnatal maternal depression and anxiety and vagal tone and vagal withdrawal) minimise the risk of presence of respondent bias (see O'Connor et al., 2002). Another disadvantage of using self-report measures is the commonly associated missing data due to non-responding. However, the amount of missing data was very low in the current study, with very few mothers not responding to one or two items per measure. The approach in this case was to replace the missing values with the median scores of the respective items for the whole sample, as suggested by Field (2009). Finally, the use of questionnaires for assessing mental health conditions implies caution in using such terms as "depression", which would be more appropriate in studies that make use of clinical diagnostic tools.

5.3.5 Controlling for Order Effects in the Infant Procedures

Another limitation in the current study is the absence of controls for order effects in the administration of the 3 procedures (i.e. helper-hinderer, novel toy and still face). It may be that the infant's level of RSA during certain procedures is influenced by reactions or parameters that are linked to the preceding condition (e.g. the level of attention or

emotional arousal). Counterbalancing the order of presentation for the three procedures in the current study might have added to the understanding of change in RSA levels across the tasks.

5.4 Interpretations of Findings

This section presents possible interpretations of results in the context of previous studies. First, changes in mean vagal tone across the five procedures are discussed, and then the vagal tone results in relation to maternal symptomatology and sex of infant are examined.

5.4.1 The Relationship Between Vagal Tone and Experimental Procedure

In the current study vagal tone was measured across a sequence of five procedures with different contents expected to elicit some changes in the level of RSA. As shown in Section 3.4.3, mean RSA scores varied significantly across the five procedures. Each of the first 2 episodes (i.e. Helper-Hinderer and Novel Toy) could be considered as possible baseline procedures. As was discussed in Section 1.3.5.1, there is little consensus on what constitutes baseline. Although these two procedures had apparently similar contents, mean RSA decreased significantly from the helper-hinderer to the toy exploration episode. One possible explanation for the difference between the two scores might be that both procedures involve some degree of attention engagement and it is not clear whether heightened attentiveness elicits low or high vagal tone. DiPietro, Porges, and Uhly (1992) found that mean RSA increased from baseline to a surprise stimulus episode in a small sample of term and pre-term 8-month-olds. On the other hand, Calkins and Keane (2004) reported vagal withdrawal from baseline to an attention task at both 2 and 4 years. However, in both studies baseline RSA was recorded during situations in which children's attention was already engaged with other toys to facilitate collecting the ECG data so it is not clear whether shifting the focus from a toy to other visual stimuli did in fact lead to significant changes in the level of attention. Another possible explanation for the RSA difference in these two tasks is that this may have arisen due to the different

nature of the attentional processes that were involved during the procedures. Ruff and Rothbart (1996) propose that attention directed outward (e.g. as in attending to novel stimuli) is associated with decreased heart rate (which may be linked with increased RSA), while focusing attention inward (as in cognitive tasks) makes the heart beat faster (which is potentially seen as RSA withdrawal) to provide metabolic resources to support the costly cognitive demands. It may be that infants perceived watching the helper-hinderer more like a pleasantly surprising event and reacted with increased vagal tone as suggested by DiPietro et al. with their attention engaged on stimuli in the outer environment, while the toy exploration was seen more like a task in which they had to shift attention inward in order to solve it. Also, it may be that the difference between the two RSA means has to do with the difference in duration of tasks. The Helper-Hinderer procedure was significantly longer (mean duration 6.41 minutes) than the 2-minute novel toy episode and although the literature does not seem to have studied how length of focusing attention might impact on the RSA level, it may be that supporting metabolic demands for longer periods of time is associated with higher vagal tone. And yet another possible explanation is that infants might have processed the informational contents in the two procedures differently so that the helper-hinderer was given a social meaning, while the pyramid-shaped toy did not qualify for anything more than an object. Indeed, research using the helper-hinderer paradigm suggests that 6-month-old infants can make social evaluations (Hamlin et al., 2007) and give social meanings to the characters involved in the procedure (all characters have googly eyes and are manipulated at a pace which is comparable to that of humans). These possible human-like characteristics may have increased infants' RSA while engaging to the characters. Thus, it may be that the two procedures offered different contents to which the infants engaged their attention differently, which may explain the difference in the RSA levels.

In the still face paradigm, there was a significant decrease in RSA from the initial social engagement to the still face, followed by a sharp increase in RSA during the social reunion episode to a value which was similar to that in the initial play with the mother. This is consistent with the proposal that the perturbation which occurred in the still face triggered infants' physiological reaction of mobilisation of internal resources to help coping with the disruption, followed by regulation of vagal tone once the mother become available to interact again. This pattern of RSA change during the still face paradigm adds to only a handful of studies which measured vagal response in Tronick's procedure and found the same variations in RSA. More exactly, only five known published studies measured RSA in infants during the three phases of the SFP (i.e. Weinberg & Tronick, 1996; Bazhenova et al., 2001; Moore & Calkins, 2004; Moore et al., 2009; and Moore, 2010). A sixth study conducted by Ham and Tronick (2006) is of less interest because of the very small sample of 12 infants and because it does not report the actual RSA values. Also, it is worth mentioning that in the Bazhenova et al. the still face was done with a stranger and not with the mother. In all these five studies vagal withdrawal was found during the still face, making it one of the most robust findings in psychophysiology in infancy. On the other hand, only three of the above mentioned studies found recovery of vagal tone following the still face, with the other two studies reporting non-significant trends of increases in RSA during the social reunion episode, which still makes vagal regulation a reliable response. The current findings regarding infants' vagal reactivity and regulation during the SFP are important in several respects. First, this is the first study that investigated reactivity and regulation of vagal tone in a sample recruited and assessed during pregnancy. Previous investigations that started in pregnancy (e.g. Field et al., 2003, 2004) measured vagal tone in one baseline condition. Second, the current research replicates in a large sample recruited antenatally what other still face studies have found in samples smaller of size and of generally low risk. This is a highly informative finding

because it extends previous results of the characteristic V-shaped vagal tone pattern to a large high risk consecutive community sample thus building a strong case for generalizability of results regarding vagal reactivity and regulation in response to the still face. By contrast, one of the most frequently cited studies on vagal tone measured during the still face (i.e. Bazhenova et al., 2001) recruited its sample of 41 infants from a commercial mailing list of 500. Third, the design of the study and the large sample size allow addressing more complex questions which other studies could not explore, such as the issue of pre- versus postnatal maternal influences and that of the moderating role of sex of infant.

5.4.2 Review of Findings in Relation to Studies That Examined Prenatal Stress and Vagal Tone and Early Development in the Offspring

5.4.2.1 Studies in Relation to Vagal Tone

The results of the current study suggest that there are associations between stress in the mother during pregnancy and vagal tone and vagal withdrawal in infants and that these associations are moderated by sex. As reviewed in Section 1.3.14, previous research, mainly conducted by the Tiffany Field group, have found links between maternal depression and anxiety at various time points in pregnancy and vagal tone (measured in one baseline condition) and these links suggest that the infants of symptomatic mothers have lower vagal tone than the non-stressed mothers. The results in the current study suggesting that there were no main effects of depression or anxiety on the overall level of vagal tone or on vagal withdrawal during the still face are not consistent with the findings in the Field studies. Several possible explanations can be formulated as to why these results differ compared to previous investigations. First, it may be that the age of the

infants has an impact on the proposed association. In the studies reporting links the infants were assessed mainly during the neonatal period. The first few weeks in life are characterised by processes of physiological adaptation following birth with rapid maturational changes occurring in the functioning of the regulatory systems of the newborn. Vagal tone is one of the systems requiring a certain level of organismic stability in order to be able to be measured reliably, possibly up to 6 months after birth (Porges, personal communication, 2008). Second, the generally small size of samples in studies that report links and the fact that measurement of vagal tone was taken in one baseline condition suggest that results should be approached with care. Finally, the presence of less established methods of classing future mothers as symptomatic, such as the use of median split for questionnaire scores as in Field et al. (2003) or the use of extreme scorers (see Field et al., 2004) may explain the differences between what the current study has found and findings from published research.

5.4.2.2 Studies of Prenatal Stress in Relation to Other Relevant Aspects of Development – Positive and Negative Findings and How Do We Understand Them?

Studies that examined effects of prenatal stress on offspring behaviour report relatively few links with outcomes measured from the neonatal period into adolescence (see Section 1.2.3.2). Although most convincing findings come from the studies of medical consequences of stress (e.g. prematurity, low birth weight) some evidence regarding behaviour, cognitive outcomes and neurodevelopmental deficits appears to be present. For example, most of the infant studies have focused on and found links between maternal stress and temperament scales and cognitive performance. Some (e.g. ALSPAC, Davis et al., 2007) have used large samples and strong designs with repeated measures of stress in pregnancy while controlling for postnatal mood and thus giving support for the

hypothesis of fetal origins of behaviour, while other studies used samples of less than 100 and less thorough measurements. Despite the evidence, some important issues need to be considered when assessing the reliability of these findings. First, the range of outcomes receiving focus has been relatively small and rarely assessed through direct observation. For instance, fearfulness has been one of the main temperament scales that was studied, while very little is known on possible links with other relevant domains like activity, anger or soothability. Also, the predominant use of reports of behaviours rather than standardised observations cannot exclude the maternal bias, as symptomatic mothers tend to describe the behaviours of their infants as more negative. Second, some of the outcome measures that were used were too global and predict poorly on future outcomes. It is the case of the studies focusing on mental development indices such as Bayley scores, which have not proved as being relevant for psychopathology or future development, including later cognitive performance. Third, the focus has mainly been towards examination of behaviour in the developing infant, rather than biological outcomes. Reported or even observed behaviour at such an early stage in life may represent only a thin surface of what prenatal stress might be associated with in the infant. It may be that shifting the focus to measures of development likely to underpin behaviour, such as cortisol reactivity or functional brain imaging, would elucidate more of the mechanisms involved. Finally and more importantly, little is actually known on what is the proportion of positive findings in the link between stress in pregnancy and offspring outcome, as studies may not always report their negative findings. In a review by Van den Bergh et al. (2005), 6 of the total of 17 infant and childhood studies were found to report negative results or results in the opposite direction to what was predicted. These results were diverse in both the type and timing of maternal symptoms and the type of offspring behaviour and the age at what were they recorded, which suggests that the fetal origins hypothesis has received varying degrees of support.

5.4.3 Sex Differences in the Associations Between Prenatal Stress and Vagal Tone and Vagal Withdrawal – How Do We Interpret the Findings?

5.4.3.1 General Considerations

In the current investigation the sex of infant was found to moderate the associations between maternal prenatal depression and anxiety and infants' vagal functioning across procedures. However, although the results for the interactions with sex generally showed consistency and supported the hypothesis of prenatal prediction of maternal mood to infant physiology, some of the associations between maternal depression and anxiety in interaction with sex of infant and vagal tone were different compared to vagal withdrawal. First, in the link between vagal tone and maternal depression and anxiety in interaction with sex of infant, there was no indication of postnatal mood influence and the strongest associations were for maternal depression and anxiety during early pregnancy i.e. at 20 weeks (see Table 4.2 in Section 4.1.2 and Table 4.10 in Section 4.2.2). By contrast, for vagal withdrawal, there was some indication of postnatal influence, particularly at 29 weeks. The strongest prenatal link with vagal withdrawal was found for the sex of infant interactions with anxiety in late pregnancy i.e. at 32 weeks. A possible interpretation of the association between vagal withdrawal and postnatal anxiety in interaction with sex is that maternal postnatal behaviour may have an impact on the infant's capacity for vagal withdrawal, but not on vagal tone. Indeed, vagal withdrawal as measured in the current study is likely to reflect more the infant's physiological reaction of coping with the stress of maternal unresponsiveness during the still face (Weinberg & Tronick, 1996), whereas overall vagal tone, computed in the present study as the mean RSA from two object-related and two person-related episodes, may rather reflect the infant's more general capacity to engage with the environment (Porges, 1995).

Another issue that may provide with room for speculation concerns the difference in timing of maternal depression and anxiety in pregnancy in relation to vagal tone and vagal withdrawal. Specifically, links between vagal tone and prenatal depression and anxiety in interaction with sex were stronger at 20 weeks gestation, whereas for vagal withdrawal the association was stronger at 32 weeks gestation. It may be that differences in timing of stress may impact in different ways on the infant's vagal functioning. It is thought that vagal tone and vagal withdrawal may play different roles for the individual (see Section 1.3.4), and there is some evidence on differences in timing of stress in relation to human behaviour (e.g. O'Connor et al., 2003; see, also, the review by Van den Bergh et al., 2005). However, these interpretations in relation to vagal tone can only remain at a speculative level, as there are no previous studies that have addressed the issue of timing of stress in pregnancy in relation to vagal tone.

This is the first study to report sex-specific associations between maternal stress in pregnancy and infant vagal tone and vagal withdrawal, therefore further interpretations are made in the context of findings from existing animal and human studies which focused on physiological measures and behaviours thought to be linked with vagal tone. Animal studies that looked at mothers' observed stress during pregnancy and offspring response and found sex differences focused mainly on examining behaviours indicative of negative emotions such as depression and anxiety. On the other hand, a few human studies were found to report sex-specific links between mothers' perceived stress in pregnancy and behaviour and psychopathology in childhood and adolescence, which are of interest to the current research because of the links in the literature between vagal tone and later psychopathology. Moreover, a potentially useful literature that examined associations with sex between medical consequences of stress in pregnancy (e.g. prematurity and low birth weight) and autonomic reactions to stress is explored to gather

evidence on possible long-term sex-specific adaptation of autonomic responses with roots in pregnancy.

5.4.3.2 Animal Studies of Prenatal Stress and Offspring Outcomes

As mentioned in Section 1.4.3.2, sex differences are a common finding in animal research of effects of prenatal stress. One of the studies with results in similar fashion to the sex interaction findings in the current study is that conducted by Zagron and Weinstock (2006) in which a mild form of prenatal stress was associated with increased anxiety to novelty in females and unchanged behaviour in males. On the other hand, there is human research (see Beauchaine, 2001) suggesting that a high level of vagal withdrawal may be a biological marker for anxiety disorders. If high vagal withdrawal underpins anxiety-like behaviours, the findings in Zagron and Weinstock match the pattern of association between 32 weeks prenatal anxiety and sex interaction and vagal withdrawal found in females in the current study.

However, not all animal studies found sex differences in anxiety or depression-like behaviours in the same direction as in Zagron and Weinstock (2006), as presented in Section 1.4.3.2 in the Background. It is difficult to interpret why changes in anxiety behaviours of both sexes are not consistent across studies. It may that different investigations have used different criteria or scales of measurement of behaviours. Or, it may be that different rodent species were used in experiments and that cross-species differences in behaviours exist following exposure to stress. Another possibility is that varying types and degrees of stress were used across these studies, which may result in changes in the sex differences patterns in the anxiety-like response. This latter possible answer is supported by another set of findings reported by Zagron and Weinstock, who

found that a more severe form of prenatal stress resulted in elevated anxiety in both females and males.

And yet another explanation for the contradictory findings in sex-related behaviour of prenatally stressed rats may the decoupling of the behaviour and HPA axis response reported in Zuena et al. (2008). The fact that decreased anxiety was accompanied by activation instead of normally expected inhibition of the glucocorticoid receptors in the hippocampus of the female offspring suggests that behavioural reactions may reflect other processes hence the need for studying behaviour in conjunction with physiology.

The elevated parasympathetic response to the social stressor exhibited by the females in the current study also parallels the increased and prolonged female reactivity of their cardiovascular system during the recovery phase after experiencing the stressor shown in the rodent study by Igosheva et al. (2004). It is possible that both responses involve similar underlying processes, since the vagus nerve is involved in cardiovascular regulation.

However, animal studies need to be interpreted cautiously in relation to humans. There are marked species differences within animal models, and processes in humans are likely to be either more complex or at least in some instances different.

5.4.3.3 Studies of Medical Consequences of Stress in Pregnancy and Autonomic Response to Stress in Humans

Research into cardiovascular reactions to psychosocial stress in human adults constitutes another quite substantial body of evidence as to how men likely to have been exposed to stress in their intra-uterine life may differ from their female counterparts. However, interpretation of findings coming from this literature is not straightforward as age seems to play a crucial role particularly for women into how experimental stress associates with vasocardiac effects. As commented in Section 1.4.3.3, there is evidence suggesting that young women show lower increases in blood pressure and heart rate during stressful conditions or tasks than pre-adolescent girls and postmenopausal women. Higher secretion of oestrogen may have buffering effects for women at younger ages and may partly explain why adult men are more at risk for cardiac problems and show increased autonomic stress response. Although the gap between sexes in propensity to exhibit costly vasocardiac reactions in response to stress seems to close in late adulthood, there is enough evidence suggesting that during most of the adulthood there are sex-specific patterns of physiological reactions to psychosocial stress in men and women of low birth weight, which is commonly understood as an indicator of stress during fetal period.

5.4.3.4 Human Studies of Sex Differences in the Link Between Prenatal Stress and Later Psychopathology

The human literature on sex differences in the link between prenatal stress and behaviours and psychopathology is much less established than the evidence coming from the animal studies or the cardiovascular research presented before. Human research has rarely addressed the issue of sex differences, although it is difficult to estimate the extent of negative findings that are not reported.

The current study's findings of maternal prenatal depression and anxiety by sex interactions predicting infants' vagal tone and vagal withdrawal seem to parallel results from previous investigations linking prenatal stress with psychopathology. For example, Hay et al. (2008) found that more girls of antenatally depressed mothers were diagnosed with emotional disorders in adolescence compared with those of mothers who were well in pregnancy. Although the prediction to offspring emotional problems was subsequently explained by mother's exposure to depression after the postpartum period, the sex interaction with prenatal depression still showed a similar trend. This suggests that antenatal depression may be an early contributor to risk for late onset problems specifically in females.

Moreover, the findings reported in Van den Bergh et al. (2008) indicating that trait anxiety in the prenatal period was associated with increased depressive symptomatology in adolescent females only and that this association was mediated by a high flattened pattern of diurnal cortisol suggests the implication of at least one biological mechanism in the link between stress in early pregnancy and offspring psychopathology. Human studies have not yet explored other biological indices as potential mechanisms, but the consistent

findings in the current study of vagal tone and vagal withdrawal being predicted by elevated maternal symptoms in interaction with offspring sex together with the evidence suggesting links between vagal tone and later problems in children (Porges et al., 1996) makes vagal tone a possible mediation candidate for the association. Alternatively, it is possible that the impaired HPA axis alters autonomic reactivity through modifications at the level of higher structures involved in regulation of cardiovascular response in conditions of stress (e.g. brainstem, hippocampus) (Igosheva et al., 2004). However, this area of study is at its beginnings and more research is needed in elucidating how other biological pathways (e.g. sex hormones) may be seen as plausible.

The pattern of sex-related findings in the present study is also consistent with a general population study conducted by Costello et al. (2007). They found that risk for pre-adolescent depression increased sharply only in females of low birth weight but this effect was not seen in boys. Thus there may be sex specific prenatal programming mechanisms.

The fact that there may be differences between males and females in specific forms of psychopathology following stress in pregnancy is discussed in O'Connor et al. (2003) based on findings from the ALSPAC study. The main set of results giving rise to this discussion is that maternal anxiety in early pregnancy predicts behavioural and emotional problems at 81 months in girls but not in boys. A second set of findings that prompted the commenting was that differences emerged in the predictions from late antenatal anxiety to subscales of problems in the sense that associations were noted for boys with hyperactivity and emotional problems, but no links were found with any of the subscales for girls. The authors acknowledge the presence of sex differences, but do not speculate on their possible causes suggesting that the differences in patterns of associations for types of problems in males and females is not clear and that more research is needed.

5.4.3.5 Sex Differences, Prenatal Stress, Vagal Tone and Psychopathology

The most striking aspect of the findings was that there were no effects of prenatal maternal anxiety or depression unless account was taken of the heterogeneity associated with the sex of the infant. In relation to vagal tone this was accounted for by roughly equal but opposite effects whereby elevated anxiety or depression was associated with increasing vagal tone in girls, but decreasing in boys. In relation to vagal withdrawal, the effect arose mainly because of a marked reduction, in boys, of vagal withdrawal associated with prenatal anxiety, with a small positive effect in girls.

As discussed in Section 1.5.1, sex differences in the association of prenatal stress with developmental outcomes could be predicted on the basis of the animal literature, and a small number of human studies of cardiovascular functioning and of psychopathology. This provided a solid basis for predicting sex differences in the association between prenatal anxiety and depression and vagal tone or vagal withdrawal, but a less certain basis for predicting the direction of effect. The prediction that prenatal anxiety and depression would be associated with increased vagal tone and withdrawal in females was based on the way the fetal origins hypothesis is formulated together with the literature on sex differences in emotional and interpersonal functioning. The fetal origins hypothesis proposes that vulnerability to ill health later in life arises from changes in fetal physiology that have an adaptive function in some environments, but not in others. A broad literature on sex differences suggests that females are likely to attend to "trustworthiness" cues in social contexts, in contrast to males' preference for displays of "capacity" and dominance (Vigil, 2009). These cues include intense emotional involvement in dealing with challenges, increased empathy and more comforting behaviours towards others. If, as it has been argued, these sex differences have served an evolutionary function, then

they may, at least to some degree, represent the characteristic adaptive strategies of females and males. Given that vagal tone is thought to promote emotion regulation and social engagement, the "female strategy", drawing on the fetal origins hypothesis, the prediction was made that females exposed to anxiety or depression would have elevated vagal tone or withdrawal. Consistent with the fetal origins hypothesis that differences may be adaptive under some circumstances, but not others, increased vagal tone may confer advantages under certain conditions (e.g. establishing strong bonds with peers and children), but confer vulnerability under others (e.g. threatening or abusive environments leading to frequent and intense autonomic and emotional reactivity) to psychopathology.

This is consistent with recent evidence suggesting that both excessive and diminished vagal tone and vagal withdrawal may underpin symptoms of psychopathology in childhood and later. As discussed in Section 1.3.7, the study conducted by Calkins et al. (2007) may represent a turning point in our understanding on how different RSA levels may reflect different behaviour. Up until recently the predominant view has been that high RSA and RSA withdrawal are associated with good outcomes and that low RSA and RSA withdrawal are associated with non-optimal behaviour. On the contrary, the findings presented in Calkins et al. suggest that both low and high RSA withdrawal may equally be associated with psychopathology, just that in different ways: low RSA may be a marker for externalising behaviour (which is consistent with research suggesting links between low autonomic response and antisocial behaviour), while high vagal withdrawal, thought to reflect large reductions in the parasympathetic input to the heart and possibly freezing behaviour, may be more characteristic in children who score highly on internalising problems. By contrast, the children who exhibited least problems had consistently shown moderate vagal withdrawal throughout the experimental procedures.

Applied to girls in this study, the programming hypothesis would predict that exposure to prenatal depression or anxiety leads to an adaptive increase in vagal tone and withdrawal, which will underpin emotion regulation and interpersonal functioning that serves a protective function. This would lead to the prediction that girls exposed to prenatal anxiety or depression will be protected from the effects of later environmental adversities, via the buffering effects of elevated vagal tone (El-Sheikh, Harger, & Whitson, 2001). However, in the light of evidence reviewed earlier, these girls may be more vulnerable under two kinds of condition. First, they may be vulnerable to internalising difficulties if vagal tone or withdrawal becomes "too high" contributing to high levels of physiological and emotional reactivity, consistent with the Calkins et al (2007) findings. Second, elevated vagal tone, even in the mid-range, may create vulnerability in the face of severe environmental adversities.

In relation to boys, the question arises as to whether reduced vagal tone and vagal withdrawal also may have an adaptive function. It is likely that there are some, perhaps highly threatening, environments in which reduced physiological reactivity may be adaptive, in effect helping the child to turn away from social and emotional engagement. Equally, the fetal origins hypothesis is silent as to whether there may be sex differences in the extent to which it applies. In other words, it may be that only girls generate an adaptive response to prenatal anxiety or depression. A pure risk model for the association of prenatal anxiety with low vagal withdrawal in boys seems more straightforward. Failure to reduce vagal tone in the face of a stressor will be associated with a lack of increase in heart rate, consistent with the widely replicated finding of low pulse rate in antisocial children and adults. Furthermore, it may represent a more general lack of physiological reactivity to stress, with parallels in cortisol reactivity, which is reduced in antisocial children, especially those with low levels of anxiety. This is consistent also with the

Calkins et al (2007) finding that children with externalising problems, in the absence of internalising problems, had reduced vagal withdrawal.

In view of these points and considering the findings in the current study, we can speculate that altered physiology in both the males and females of prenatally stressed mothers may render the infants vulnerable in terms of expression and regulation of emotionality and may confer risk for later mental health problems, only in different ways and possibly at different stages in life. It may the that boys prenatally exposed to stress and who showed low vagal tone and vagal withdrawal will be at increased risk for externalising symptoms early in life, while their female counterparts, with high vagal tone and vagal withdrawal, may start developing problems of internalising nature with a later onset (e.g. at puberty). Of course, this is not to be understood that every infant with high or low vagal tone may carry risk for later psychopathology. Actually, it is quite likely that some of these infants will not grow into the troubled children or adolescents simply because their postnatal environment may not continue to be adverse as before birth and may even contribute to reversing some of the consequences of prenatal stress, as suggested by some recent animal research (Morley-Flecher et al., 2003), or they may have other intrinsic sources of resilience. Conversely, not everybody showing moderate vagal tone and vagal withdrawal responses should be automatically regarded as being at low risk for later problems. Harsh parenting, life events or other inner vulnerabilities may contribute to the development of psychopathology despite early sources of resilience in the child. Prospective investigations into childhood and adolescence are needed to test such hypotheses linking prenatal stress and vagal tone in infancy to later psychopathology.

5.5 Future Directions of Research

The main findings in the current study with regard to the role played by the sex of infant in the link between maternal prenatal stress and vagal tone in infants pose some new questions regarding early neurophysiology and its importance for psychopathology. However, there may be considerable challenges in addressing these questions.

First, a major requirement is to establish whether these findings can be replicated, preferably in studies with more frequent prenatal measurement of anxiety and depression and relevant physiological measures, for example of maternal cortisol. Further heterogeneity may be introduced by genetic variations, leading to gene by environment interactions. With regard to timing, although the current findings suggest stronger sex-specific links of vagal tone and vagal withdrawal with prenatal maternal mood, the current design did not permit to investigate thoroughly at what stage in pregnancy may stress pose a higher risk. The current results do provide with some indication that early stress pregnancy may be linked with sex-specific patterns of overall vagal tone functioning, whereas later maternal psychological difficulties may reflect variations in vagal withdrawal during stressful situations. However, only two snapshots of maternal mood may not be sufficient for providing with an accurate image of the dynamics of negative feelings during pregnancy, therefore several such investigations might be able to assess at a finer-grain level possible consequences for the baby associated with timing.

Second, longitudinal follow up of the children reported in this study is required to examine the developmental outcomes of variations in vagal tone and withdrawal, and the role of subsequent experiences. For example, it would be interesting to investigate to what extent the sex-specific associations between maternal depression and anxiety and

vagal tone and vagal withdrawal reflect stable patterns of physiological adaptation and how may they be influenced by the exposure to the postnatal environment. Previous research with samples recruited postnatally indicates good vagal tone stability but less stability in vagal withdrawal scores from infancy to preschool age (see Section 1.3.9) and it would be interesting to assess whether changes occur in time in prenatally stressed children. Another useful aspect would be to include concurrent observations of emotion reactivity and regulation to test hypothesised links between vagal tone and emotion. To the extent to which vagal tone may underpin emotion-related processes (Porges, et al., 1996) it is important to bring more evidence for this proposed link, particularly from longitudinal investigations and samples with varying degrees of risks.

Third, testing the predictions outlined in the previous section will require either large samples, or ones stratified by the variables of interest. For example, examining the different conditions under which elevated vagal tone confers resilience or vulnerability requires sufficient numbers across the range of vagal tone, sufficient numbers with variations in later environmental conditions, and sufficient numbers with externalising and internalising symptoms.

Finally, future studies might find it useful to include multiple measures of autonomic reactivity in order to obtain a more complete picture of the underlying physiological processes of adaptation. For example, recent evidence published by El-Sheikh et al. (2009) found that coupled parasympathetic and sympathetic responses, rather than the action of only one system alone, may buffer school-age children of discordant families from developing externalising problems, therefore concurrent use of cardiac sympathetic (e.g. pre-ejection period) and parasympathetic measures may be able to reveal further adaptations.

5.6 Conclusions

The current study's main focus was to investigate the associations between prenatal maternal stress and neurophysiological adaptation in the infant measured via an index of vagal tone (i.e. RSA) in a community-based predominantly high risk sample. The findings provide support for the fetal origins hypothesis, but only in relation to sex of infant, in that the males and females of mothers with high scores on depression and anxiety showed low and high, respectively, RSA levels of reactivity in response to object-related and person-related behavioural procedures. These patterns of RSA reactivity may index early non-optimal physiological responses to stress and may underpin disturbances at emotional level, with possible consequences for later mental health problems. There is evidence suggesting that both high and low neural regulation of the heart can be associated with psychiatric outcomes of different nature (e.g. Calkins et al., 2007), but no previous investigations have studied this in a sample recruited in pregnancy. Clinicians should become aware of possible prenatal origins of behavioural and emotional problems and policy-makers should develop early programs of referral and monitoring for mothers who report emotional burden in pregnancy and their children.

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Appendices

Appendix A

Parent Information Sheets and Consent Forms for Participation in the Study

Study Base:
The Lauries Centre, 142 Claughton Road,
Birkenhead, Wirral, CH41 6EY
Freephone: 0800 051 7597
(from a mobile) 800 051 7597
Text: 07956 297412

Parent Information Sheet (Mother)– Study 1500
Title of study: The Wirral Child Health and Development Study

Investigators: Jonathan Hill, Helen Sharp, Andrew Pickles, Gill Lancaster

Research Staff: Karen Lunt, Carol Bedwell, Belinda Thompson, Julie Carlisle, Kate Marks, Kate Marshall,Liz Green, Florin Tibu, Jo Roberts, Jenny Lee, Nichaela Broyden, Carol Sadler, Jeanette Appleton

You are being invited to take part in a research study. Before you decide whether you want to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part. Thank you for reading this.

What is the study about?
We would like to invite you to participate in a new study of children's early development from birth to their first birthdays. This study is based at the Universities of Liverpool and Manchester. It is part of a programme of research into how children learn how to behave with other people, and why some children have difficulties controlling their behaviours. In order to fully understand this we need to measure the early development of children in many different ways. The aim of the study is to find out about the effects of many different forms of stress on parents and babies during the antenatal period and in the first months after birth. We know that for some parents and children the effects are quite long lasting, and others find ways of coping. We want to understand these processes better so that services to support families experiencing stress can be improved.

Who is being invited to take part?
We are approaching all first time mothers and their partners who are booked into the antenatal clinic at Arrowe Park Hospital over a two year period. It is important that we have participants in the study with low, medium and high levels of stress. If you have agreed to take this letter home a research midwife will contact you at your 20 week appointment or slightly after, to tell you more about the study, answer any questions you have and to invite you to take part.

Do I have to take part?
It will be up to you to decide whether or not you would like to take part. If you agree, and change your mind later, you can withdraw from the study. This will not affect the care you receive.

How often will I be contacted?
We will contact you again six weeks after the birth of your baby, and when your baby is 8 months old.
We would also like to contact some mothers more often up to the first birthdays of their children, so that we can ask them more about their lives, and understand better their ways of coping, and assess their babies' health and development in more detail. If you decide to take part, the computer will tell us who to invite for the additional contacts after we have entered the information you provide now. If
your name does come up we hope very much that you will be able to help us, but at this stage we are only asking you to participate now and at 6 weeks and eight months.

What will I be asked to do at each time point?
During your pregnancy we will interview you and ask you to complete some questionnaires about your current health and relationships, and about your expectations of the baby and being a mother. This can be done here at the antenatal clinic or at another clinic on the Wirral or at the study base in the Lauries Centre. It should take about 25 minutes.

We will also ask you for consent for us to have access to your medical records for the pregnancy, the birth, and your new born infant following the birth.

When your baby is 6 weeks old we will send you some short questionnaires about your health, your relationships, and about your baby by post, and ask you to 'Freepost' them back to us.

When your baby is 8 months old we will send you more questionnaires about your health and about your baby, and ask you to return them 'Freepost' to us or return them to your health visitor when you attend for your baby's routine 8 month developmental check-up. We will also ask your health visitor for the results of their 9-12 month assessment of your baby's development.

If you give written consent to take part in this study and you are selected by the computer to be invited for additional contacts, one of the research team named on the front of this information sheet will contact you at home, using the contact details you give to the research midwife. They will only contact you if you agree to it.

How will this information be used?
All information that we receive from you will be treated as strictly confidential, under the guidelines of the Universities of Liverpool and Manchester, the UK Medical Research Council, and the Data Protection Act. Information that we enter on the computer will be identified only by a number. We will report general findings about parents and children, but you or your child will never be identified. The only reason we might have to share information from the study with other people is if there are concerns about you or a child being at risk of serious harm. If that happens we will talk with you first to decide on the best way forward. Concerns like this would be addressed by seeking appropriate forms of help for you and/or following Trust Child Protection Guidelines.

Who is organising and funding the research study?
The study is being run by Professor Jonathan Hill of the University of Manchester and Dr Helen Sharp of the University of Liverpool. The research is funded by the Medical Research Council.

Are there any benefits in taking part in this study?
There are no benefits to you or your child's health in taking part in this study. However we hope that you will feel you are contributing to medical research in a way that will help children and families in the future.

What if something goes wrong?
If you feel you or your child have been harmed by taking part in this research and that the researchers have been negligent or at fault, then you may be able to make a legal claim for compensation to their employer. You might have to pay the legal costs of doing this. However, if you are harmed and the researchers are not at fault, there is no facility for you to make a claim. If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, normal University or National Health Service complaints procedures should be available to you.

Are there any risks to myself or my child taking part in this study?
No, there are no known or likely risks.

Who has reviewed and approved the study?
A team of international experts on child development has reviewed this study for the Medical Research Council. The study has been reviewed and approved by the Research & Development committees of Wirral Hospitals NHS Trust, Wirral PCT and the Cheshire Local Research Ethics Committee.

Can I ask further questions?
When the research midwife meets you, at or after your 20 week scan appointment, she will be very happy to answer any questions you might have. In the meantime, if you would like any more information, please do not hesitate to contact Professor Jonathan Hill, Dr Helen Sharp, or Liz Green on the freephone number shown on the front page.

Study Base:
The Lauries Centre, 142 Claughton Road,
Birkenhead, Wirral, CH41 6EY
Freephone: 0800 051 7597
(from a mobile) 800 051 7597
Text: 07956 297412

RESEARCH CONSENT FORM

Title of study: **Wirral Child Health and Development Study**
Names of researchers: **Jonathan Hill, Helen Sharp, Andrew Pickles, Gill Lancaster**

1. I confirm that I have read and understand the information sheet dated March 2007 for the above study. I have had an opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my care or legal rights being affected. ☐
3. I agree for the research team to have access to my medical records to obtain information about my pregnancy, delivery and my baby's birth record ☐
4. I agree to my health visitor releasing a copy of my baby's 9-12 month routine development assessment in paper form and in the red book recorded in the Child Development Centre ☐
5. I agree to my GP being notified that I am taking part in this study ☐
6. I understand that any concerns about a child being in potential danger, will be addressed in line with the Trust Child Protection Guidelines. ☐
7. I agree to take part in the above study. ☐

8. I agree that one of the research team named on the front of the information sheet can contact me ☐

Name of Participant	Date	Signature
Name of person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

1 for participant;1 for researcher; 1 for NHS notes (if applicable)

Study Base:
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Birkenhead, Wirral, CH41 6EY
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RESEARCH CONSENT FORM
to follow-up via GP records.

Title of study: **Wirral Child Health and Development Study**
Names of researchers: **Jonathan Hill, Helen Sharp, Andrew Pickles, Gill Lancaster**

For participants in the intensive study only:

1. In the event that I have moved home in the future and cannot be reached with the Study team. I confirm that I consent to the research team using my NHS number to identify and contact my new GP to request my new address and phone number. ☐

2. I consent to my new GP giving the research team my contact details without checking with me first. ☐

3. I understand that giving consent to contact me via my GP does not affect my right to withdraw from the study at any time if I wish. ☐

Name of Participant	Date	Signature
Name of person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

1 for participant;1 for researcher; 1 for NHS notes (if applicable)

APPENDIX A4 – Parent Information Sheet at
32 Weeks Gestation and 5 Weeks Postnatal

Study Base:
The Lauries Centre, 142 Claughton Road
Birkenhead, Wirral, CH41 6EY
Freephone: 0800 051 7597
(from a mobile) 800 051 7597
Text: 07956 297412

Parent Information Sheet – Study 300
Title of study: The Wirral Child Health and Development Study
Investigators: Jonathan Hill, Helen Sharp, Andrew Pickles, Gill Lancaster

Research Staff: Karen Lunt, Carol Bedwell, Belinda Thompson, Julie Carlisle, Kate Marks, Liz Green, Florin Tibu, Carol Sadler, Joanne Roberts, Jenny Lee, Nichaela Broyden

A few weeks ago you kindly agreed to help with a study that we are conducting designed to understand better how stress affects mothers to be, their partners and their babies, and how good experiences and support can make a difference. We are following 1500 women up to the first birthday of their babies mainly using questionnaires. In addition, we are asking 300 to be interviewed in more detail and to agree to us filming their baby's development during the first year of their life. We would like to invite you to be one of the 300. Before you decide whether you want to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part. Thank you for reading this.

What is the study about?
The aim of the study is to find out about the effects of different forms of stress on mothers and babies during the antenatal period and in the first months after birth. We plan to measure each baby's development and how they interact with their mother in some detail. We believe that for some parents and children the effects of some stresses are quite long lasting, and others find ways of coping. We want to understand these processes better so that services to support families experiencing stress can be improved. We are focussing on mothers for this detailed part of the study because most babies spend most time with their mother.

Who is being invited to take part?
The computer chooses the names of women who we approach based on the information they have given about how much stress they are facing. Because we particularly want to understand about stress in pregnancy the computer will pick more women who are experiencing stress. Your name has been chosen either because you have indicated that you may be dealing with quite a lot of stress or because you have said you are not facing a lot.

Do I have to take part?
It will be up to you to decide whether or not you would like to take part. If you agree, and change your mind later, you can withdraw from the study. This will not affect the care you receive.

How often will I be contacted?
We would like to meet with you four times in all. We would like to

- Talk with you before your baby is born.
- Invite you and your baby to come to our study centre or visit you with your baby soon after he or she is born.
- Invite you and your baby to come to our study centre for about half a day when your baby is 6 months old.
- Invite you and your baby to come to our study centre for about half a day when your baby is one year old.

We will provide all the transport for all the visits, which can be arranged at a time to suit you and your family.

What will I have to do at each time point?

- During your pregnancy we will ask you to complete some more questionnaires and we will interview you about yourself, about your relationships, your physical and mental health over the past years, and your feelings about the pregnancy. We will ask you about aspects of your life now that you feel are a source of support and what you see as sources of stress. We will ask about your expectations of motherhood and your future baby. Also, we'll ask you to watch some video clips of babies and answer some questions about them. At any point, if you are not happy with a question you will be able to let the interviewer know and she will move on to the next topic.
- The whole session will take between two to four hours depending on how much there is to talk about. It can be done in one sitting (with comfort breaks) or can be divided up if that is more convenient. It can be done at any time that is convenient for you and wherever is most convenient for you. We have private interview rooms at our base on the Wirral, or we can come to your home.
- During your pregnancy we also want to see how much your body produces the kinds of hormones that help people to deal with challenging or stressful situations. To do this, all we have to do is ask you to wipe some cotton swabs in your mouth each time. This is completely safe. It will allow us to collect a sample of your saliva, which can then be analysed to measure the hormones. We would like to ask you to do this eight times, six times yourself during two days at home, and twice with the researcher before and after viewing short video clips of different babies.
- During the first two to four weeks after your baby is born we would like you and your baby to visit our study base at a time that is convenient to you. A trained child researcher will assess your baby's different reactions and watch and video

him/her carefully for about 30 minutes, to find out about his or her 'personality'. For example, does your baby seem happier when left to be quiet or does he or she like to hear sounds or look at things? They will also put two patches on your baby's chest that will record your baby's heart rate. We will measure how much your baby's heart rate changes**.** This may help us to understand more about each baby's emotional responses to everyday events as well as to stress. **You will of course be with your baby at all times.**

- When your baby is 6 months old and again at the time of his/her first birthday we will ask you to come with your baby to the study centre. We will talk with you about your feelings and experiences since the last visit, ask you about your baby's usual behaviour, watch you playing with the baby and observe how your baby responds to everyday events such as having to sit in a car seat. We will record your baby's development. When your baby is one year old we will also ask for your agreement to take a sample of his/her saliva for DNA analysis. This would only be to help us understand babies' behavioural development in more detail. We will give you more detailed information about the 6 month and one year visits nearer the time. We will also ask for your written consent for those visits separately and for the DNA sample.

How will this information be used?

- We would like to make an audio recording of the interview so that the interviewer can go over what you have said in detail afterwards. We will also video you and your child together at times. The audio and video recordings will be identified only by a number, so that information on it cannot be traced to you. The recording will be kept secure at the university base for up to ten years.

- All information that we receive from you will be treated as strictly confidential, under the guidelines of the Universities of Liverpool and Manchester, the UK Medical Research Council, and the Data Protection Act.

- All the information from the assessments will be stored on computer but will only be identified by a number. A list of names and addresses of participants and their case numbers will be kept separately and securely at the university base.

- We will report general findings about parents and children, and you or your child will never be identified. Reports will only be based on the ratings that we make from the interview and none of what you say will be reported.

- The only reason we might have to share information from the study with other people is if there are concerns about you or a child being at risk of serious harm. If that happens we will talk with you first to decide on the best way forward. Concerns like this would be addressed by seeking appropriate forms of help for you and following Trust Child Protection Guidelines.

Who is organising and funding the research study?

The study is being run by Professor Jonathan Hill of the University of Manchester and Dr Helen Sharp of the University of Liverpool. The research is funded by the Medical Research Council.

Are there any benefits in taking part in this study?
There are no benefits to you or your child's health in taking part in this study. However we hope that you will feel you are contributing to medical research in a way that will help children and families in the future.

Will my expenses be paid?
We will be pleased to organise transport to the interview, or to pay for your transport. We are able to pay up to £30 in gift vouchers to compensate you for time taken from home or from work or any other expenses incurred from taking part in the study.

What if something goes wrong?
If you feel you or your child have been harmed by taking part in this research and that the researchers have been negligent or at fault, then you may be able to make a legal claim for compensation to their employer. You might have to pay the legal costs of doing this. However, if you are harmed and the researchers are not at fault, there is no facility for you to make a claim. If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, normal University or National Health Service complaints procedures should be available to you.

Are there any risks to myself or my child taking part in this study?
No, there are no known or likely risks.

Who has reviewed and approved the study?
A team of international experts on child development has reviewed this study for the Medical Research Council. The study has been reviewed and approved by the Research & Development committees of Wirral Hospitals NHS Trust, Wirral Primary Care Trust and the Cheshire Local Research Ethics Committee.

Can I ask further questions?
When the researcher meets you they will be very happy to answer any questions you might have. In the meantime, if you would like any more information, please do not hesitate to contact Professor Jonathan Hill, Dr Helen Sharp or Liz Green on the freephone number shown on the front page.

MANCHESTER 1824

The University of Manchester

Wirral University Teaching Hospital NHS
NHS Foundation Trust

UNIVERSITY OF LIVERPOOL

Study Base:
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Freephone: 0800 051 7597
(from a mobile) 800 051 7597
Text: 07956 297412

RESEARCH CONSENT FORM

Title of study: **Wirral Child Health and Development Study**
Names of researchers: **Jonathan Hill, Helen Sharp, Andrew Pickles, Gill Lancaster**

1. I confirm that I have read and understand the information sheet dated ……. for the above study. I have had an opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my care or legal rights being affected. ☐
3. I agree to my GP being notified that I am taking part in this study. ☐
4. I agree to an audio recording of the antenatal interview being made ☐
5. I agree to being contacted so my baby can be observed and filmed at the study base shortly after birth ☐
6. I understand that any concerns about a child being in potential danger, will be addressed in line with the Trust Child Protection Guidelines. ☐
7. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Name of person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

1 for participant;1 for researcher; 1 for NHS notes (if applicable)

Study Base:
The Lauries Centre, 142 Claughton Road,
Birkenhead, Wirral, CH41 6EY
Freephone: 0800 051 7597
(from a mobile) 800 051 7597
Text: 07956 297412

Parent Information Sheet – Study 300
Title of study: The Wirral Child Health and Development Study

Investigators: Jonathan Hill, Helen Sharp, Andrew Pickles, Gill Lancaster

Research Staff: Karen Lunt, Carol Bedwell, Belinda Thompson, Julie Carlisle, Kate Marks, Nichaela Broyden, Kate Marshall, Florin Tibu, Carol Sadler, Jeanette Appleton, Jo Roberts, Jenny Lee, Liz Green

When you were pregnant, and again just after your baby was born you kindly helped us with a study that we are conducting designed to understand better how stress affects mothers to be, their partners and their babies, and how good experiences and support can make a difference. We are following 1500 women up to the first birthday of their babies mainly using questionnaires. In addition we are asking 300 to take part in interviews and to agree to us filming their babies during the first year of their life. You are one of the 300 that we would like to see again now that your baby is nearly 6 months old. Before you decide whether you want to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part. Thank you for reading this.

What is the study about?

The aim of the study is to find out about the effects of stress on parents and children during the antenatal period and in the first months after birth. We plan to measure each baby's development and how they interact with their mother in some detail. We believe that for some parents and children the effects are quite long lasting, and others find ways of coping. We want to understand these processes better so that services to support

families experiencing stress can be improved. We are focussing on mothers for this detailed part of the study because most babies spend most time with their mother.

Who is being invited to take part?
The computer chooses the names of women who we approach based on the information they have given about how much stress they may be experiencing. Because we particularly want to understand about stress in pregnancy the computer is picking more women who are experiencing stress. Your name has been chosen either because you have indicated that you are dealing with quite a lot of stress or because you have said you are not facing a lot.

Do I have to take part?
It will be up to you to decide whether or not you would like to take part. If you agree, and change your mind later, you can withdraw from the study. This will not affect the care you receive.

How often will I be contacted?
Now that your baby is around 6 months old we would like you and your baby to come to our study centre for a full morning or afternoon, We will ask to see you again close to your baby's first birthday.

What will we have to do?

- We would like to see you and your baby at the Study Centre. You will be with your baby at all times.
- We will talk with you about your feelings and experiences since the last visit, and ask you about your baby's usual behaviour. We will audio tape part of this talk. We may ask to visit you at home to complete these assessments if it becomes easier to do so.
- We would like to make a short video (about 20 minutes) of your baby playing with you .
- We will also make a video of how your baby responds to everyday events such as watching new things, the researcher talking and playing with them, hearing a loud noise or not being allowed to play with a toy for a short time.
- We will put three patches on your baby's back or chest to record your baby's heart while we are watching your baby.
- We will gather two saliva samples from your baby by wiping a cotton swab in his/her mouth at the start of the visit to the Study Centre and once again at the end. This is completely safe and will be used to measure your baby's stress hormones.

Will my expenses be paid?
We will be pleased to organise transport to the interview, or to pay for your transport. We are able to pay up to £30 in vouchers to compensate you for time lost from home or work or any other expenses incurred from taking part in the study.

How will this information be used?

- We would like to make a video recording of your baby and you so that we go over what has happened in detail afterwards. The recording will be identified only by a number, so that information on it cannot be traced to you. The recording will be kept secure at the university base for up to ten years.

- All information that we receive from you will be treated as strictly confidential, under the guidelines of the Universities of Liverpool and Manchester, the UK Medical Research Council, and the Data Protection Act.
- Information on audio and video recordings, on paper records, and that we enter on to the computer will be identified only by a number. A list of names and addresses of participants and their case numbers will be kept separately and securely in the university base.
- We will report general findings about parents and children, and you or your child will never be identified. Reports will only be based on the ratings that we make from the interview and none of what you say will be reported.
- The only reason we might have to share information from the study with other people is if there are concerns about you or a child being at risk of serious harm. If that happens we will talk with you first to decide on the best way forward. Concerns like this would be addressed by seeking appropriate forms of help for you and following Trust Child Protection Guidelines.

Who is organising and funding the research study?
The study is being run by Professor Jonathan Hill of the University of Manchester and Dr Helen Sharp of the University of Liverpool. The research is funded by the Medical Research Council.

Are there any benefits in taking part in this study?
There are no benefits to your or your child's health in taking part in this study. However we hope that you will feel you are contributing to medical research in a way that will help children and families in the future.

What if something goes wrong?
If you feel you or your child have been harmed by taking part in this research and that the researchers have been negligent or at fault, then you may be able to make a legal claim for compensation to their employer. You might have to pay the legal costs of doing this. However, if you are harmed and the researchers are not at fault, there is no facility for you to make a claim. If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, normal University or National Health Service complaints procedures should be available to you.

Are there any risks to myself or my child taking part in this study?
No, there are no known or likely risks.

Who has reviewed and approved the study?
A team of international experts on child development has reviewed this study for the Medical Research Council. The study has been reviewed and approved by the Research & Development committees of Wirral University Teaching Hospital NHS Foundation Trust, Wirral Primary Care Trust and the Cheshire Local Research Ethics Committee.

Can I ask further questions?
When the researcher meets you they will be very happy to answer any questions you might have. In the meantime, if you would like any more information, please do not hesitate to contact Professor Jonathan Hill, Dr Helen Sharp, or Liz Green on the freephone number shown on the front page.

Study Base:
The Lauries Centre, 142 Claughton Road,
Birkenhead, Wirral, CH41 6EY
Freephone: 0800 051 7597
(from a mobile) 800 051 7597
Text: 07956 297412

RESEARCH CONSENT FORM

Title of study: **Wirral Child Health and Development Study**
Names of researchers: **Jonathan Hill, Helen Sharp, Andrew Pickles, Gill Lancaster**

1. I confirm that I have read and understand the information sheet dated March 2008 for the above study. I have had an opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my care or legal rights being affected. ☐

3. I agree to my GP being notified that I am taking part in this study. ☐

4. I agree to a video recording being made of my baby and me, and an audio recording of my interview. ☐

5. I consent to a saliva sample being taken from my baby ☐

6. I consent to my baby's heart rate being monitored. ☐

7 I understand that any concerns about a child being in potential danger, will be addressed in line with the Trust Child Protection Guidelines. ☐

8 I agree to take part in the above study. ☐

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature

1 for participant;1 for researcher; 1 for NHS notes (if applicable)

Appendix B

Copies of the Questionnaire Measures

APPENDIX B1 – The Edinburgh Postnatal Depression Scale (EPDS; Cox et al., 1987)

Wirral Child Health and Development Study

We are very grateful to you for helping us with our research. The questions we ask are not a test, so there are no right or wrong answers.

How have you been feeling in the past week …

Please **underline** the answer which comes closest to how you have felt **IN THE PAST WEEK**, not just how you feel today.

Here is an example, already completed: I have felt happy:

Yes, all the time
Yes, most of the time
No, not very often
No, not at all

This would mean "I have felt happy most of the time" during the past week. Please complete the other questions in the same way.

In the past seven days:

1. **I have been able to laugh and see the funny side of things**
   As much as I always could
   Not quite as much now
   Definitely not as much now
   Not at all

2. **I have looked forward with enjoyment to things**
   As much as I ever did
   Rather less than I used to
   Definitely less than I used to
   Hardly at all

3. **I have blamed myself unnecessarily when things went wrong:**
   Yes, most of the time
   Yes, some of the time
   Not very often
   No, never

4. **I have been anxious or worried for good reason**
   No, not at all
   Hardly ever
   Yes, sometimes
   Yes, very often

5. **I have felt scared or panicky for no very good reason:**
   Yes, quite a lot
   Yes, sometimes
   No, not much
   No, never

6. **Things have been getting on top of me:**
   Yes, most of the time I haven't been able to cope at all
   Yes, sometimes I haven't been coping as well as usual
   No, most of the time I have coped quite well
   No, I have been coping as well as ever

7. **I have been so unhappy that I have had difficulty sleeping**
   Yes, most of the time
   Yes, sometimes
   Not very often
   No, not at all

8. **I have felt sad or miserable:**
   Yes, most of the time
   Yes, quite often
   Not very often
   No, not at all

9. **I have been so unhappy that I have no been crying**
   Yes, most of the time
   Yes, quite often
   Only occasionally
   No, never

10. **I have thought of harming myself:**
    Yes, quite often
    Sometimes
    Hardly ever
    Never

APPENDIX B2 – The State Anxiety Scale of the State-Trait Anxiety Inventory (STAI)

Your mood and feelings now?

AGAIN, a number of statements which people have used to describe themselves are given below. Read each statement and then circle the most appropriate number to the right of the statement to INDICATE HOW YOU FEEL RIGHT NOW, AT THIS MOMENT. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

	HOW DO YOU FEEL RIGHT NOW..?	**Not at all**	**Somewhat**	**Moderately so**	**Very much so**
1	I feel calm	1	2	3	4
2	I feel secure	1	2	3	4
3	I feel tense	1	2	3	4
4	I am strained	1	2	3	4
5	I feel at ease	1	2	3	4
6	I am upset	1	2	3	4
7	I am presently worrying over possible misfortunes	1	2	3	4
8	I feel satisfied	1	2	3	4
9	I feel frightened	1	2	3	4
10	I feel comfortable	1	2	3	4
11	I feel self-confident	1	2	3	4
12	I feel nervous	1	2	3	4
13	I feel jittery	1	2	3	4
14	I feel indecisive	1	2	3	4
15	I am relaxed	1	2	3	4
16	I feel content	1	2	3	4
17	I am worried	1	2	3	4
18	I feel confused	1	2	3	4
19	I feel steady	1	2	3	4
20	I feel pleasant	1	2	3	4

APPENDIX B3 - The Dunedin Relationship Scale

In the past year have YOU ever?

1	Been supportive of your partner at a difficult time	Yes	No
2	Just kept your opinions quietly to yourself	Yes	No
3.	Damaged a household item or some part of the home out of anger towards your partner	Yes	No
4	Given your partner more affection to make up after an argument	Yes	No
5.	Deliberately disposed of or hid an important item of your partner's	Yes	No
6.	Got very upset if dinner, housework or home repair work was not done when you thought it should be	Yes	No
7	Initiated a discussion to air (talk about) all points of view	Yes	No
8.	Purposely damaged or destroyed your partner's clothes, car or other personal possessions	Yes	No
9.	Insulted or shamed your partner in front of others	Yes	No
10	Listened carefully to your partner	Yes	No
11.	Locked your partner out of the house	Yes	No
12.	Told your partner that he/she could not work or study	Yes	No
13	Stated your position clearly	Yes	No
14.	Tried to stop your partner from seeing or talking to family or friends	Yes	No
15	Been flexible about how you handle differences of opinion	Yes	No
16	Repeated a point to make sure it was understood	Yes	No
17.	Restricted your partner's use of the car or telephone	Yes	No
18.	Made threats to leave the relationship	Yes	No
19	Cooled down through physical work or activity	Yes	No
20.	Tried to turn family, friends, or children against your partner	Yes	No
21	Given your partner helpful advice to solve a problem	Yes	No
22.	Ordered your partner around	Yes	No
23	Tried new ways of dealing with problems together	Yes	No
24	Admitted your faults or taken responsibility for a problem	Yes	No
25.	Frightened your partner	Yes	No
26.	Treated your partner like he/she was stupid	Yes	No
27	Come up with helpful ideas for your partner	Yes	No
28.	Given in to your partner but planned revenge	Yes	No
29	Brought in, or tried to bring in someone to help settle things	Yes	No
30.	Ridiculed your partner	Yes	No
31	Expressed regret about something you did or said to your partner	Yes	No
32.	Threatened to hit your partner or throw something at him/her in anger	Yes	No
33.	Told your partner he/she was ugly or unattractive	Yes	No

34	Been able to agree to a compromise over a problem	Yes	No
35.	Become abusive after using drugs or drinking alcohol	Yes	No
36.	Thrown, smashed, hit or kicked something in a disagreement	Yes	No
37	Said sorry after an argument	Yes	No
38	Agreed to disagree with your partner to settle an argument	Yes	No
39	Agreed to go along with what your partner wanted	Yes	No

In the past year has YOUR PARTNER ever?

1	Been supportive of you at a difficult time	Yes	No
2	Just kept his/her opinions quiet	Yes	No
3.	Damaged a household item or some part of the home out of anger towards you	Yes	No
4	Given you more affection to make up after an argument	Yes	No
5.	Deliberately disposed of or hid an important item of yours	Yes	No
6.	Got very upset if dinner, housework or home repair work was not done when he/she thought it should be	Yes	No
7	Initiated a discussion to air (talk about) all points of view	Yes	No
8.	Purposely damaged or destroyed your clothes, car or other personal possessions	Yes	No
9.	Insulted or shamed you in front of others	Yes	No
10	Listened carefully to you	Yes	No
11.	Locked you out of the house	Yes	No
12.	Told you that you could not work or study	Yes	No
13	Stated his/her position clearly	Yes	No
14.	Tried to stop you from seeing or talking to family or friends	Yes	No
15	Been flexible about how he/she handles differences of opinion	Yes	No
16	Repeated a point to make sure it was understood	Yes	No
17.	Restricted your use of the car or telephone	Yes	No
18.	Made threats to leave	Yes	No
19	Cooled down through physical work or activity	Yes	No
20.	Tried to turn family, friends, or children against you	Yes	No
21	Given you helpful advice to solve a problem	Yes	No
22.	Ordered you around	Yes	No
23	Tried new ways of dealing with problems together	Yes	No
24	Admitted his/her faults or taken responsibility for a problem	Yes	No
25.	Frightened you	Yes	No
26.	Treated you like you are stupid	Yes	No
27	Come up with helpful ideas for you	Yes	No
28.	Given in to you but planned revenge	Yes	No
29	Brought in, or tried to bring in someone to help settle things	Yes	No

30.	Ridiculed you	Yes	No
31	Expressed regret about something he/she did or said to you	Yes	No
32.	Threatened to hit you or throw something at you in anger	Yes	No
33.	Told you that you are ugly or unattractive	Yes	No
34	Been able to agree to a compromise over a problem	Yes	No
35.	Become abusive after using drugs or drinking alcohol	Yes	No
36.	Thrown, smashed, hit or kicked something in a disagreement	Yes	No
37	Said sorry after an argument	Yes	No
38	Agreed to disagree with you to settle an argument	Yes	No
39	Agreed to go along with what you wanted	Yes	No

APPENDIX C – Ethical Approval for the Study and Amendments to the Study

Cheshire North & West Research Ethics Committee
Cheshire West PCT
1829 Building
Countess of Chester Health Park
Liverpool Road
Chester
CH2 1HJ

Telephone: 01244 650 334
Facsimile: 01244 650 333

27 June 2006

Professor Jonathan Hill
Professor of Child and Developmental Psychiatry
University of Liverpool, Alder Hey Hospital
Mulberry House, Alder Hey Hospital
Eaton Road
L12 2AP

Dear Professor Hill

Full title of study: **The Wirral Child Health and Development Study**
REC reference number: **05/Q1506/107**

Thank you for your letter of 19 May 2006, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Vice-Chairman.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Application		09 January 2006
Investigator CV		
Protocol	1	09 January 2006
Covering Letter		09 January 2006
Summary/Synopsis	1	09 January 2006
Response to Request for Further Information		19 May 2006
Father Information Sheet, Study 1500 - Phases 1, 3, 5 & 7	2	01 May 2006
Study 300 Parent Information Sheet, one year - Phase 8	2	01 May 2006
Study 300 Parent Information Sheet, 6 months - Phase 6	2	01 May 2006

Study 300 Parent Information Sheet, Antenatal Phases 2 & 4	2	01 May 2006
Mother Information Sheet, Study 1500 - Phases 1, 3, 5, & 7	2	01 May 2006
Letter confirming funding - MRC		09 March 2005
Supporting letter from Mr Doyle, Wirral Hospitals NHS Trust		09 December 2005
Supporting letter from Ms Sheila Hillhouse, Birkenhead & Wallasey PCT		09 December 2005
Phase 8: Study 300 12 month mother and baby postnatal assessments	1	09 January 2006
GP Letter Study 1500	1	01 January 2006
GP Letter Study 300		01 January 2006
Parent Consent, Study 1500 - Phases 1, 3, 5 & 7	1	09 January 2006
Consent to contact a relative - Study 1500	1	09 January 2006
Parent Consent, Fathers, - Study 1500 - Phases 1, 3, 5 & 7	1	09 January 2006
Parent Consent - Study 300 Antenatal, perinatal - (Phases 2 & 4)	1	09 January 2006
Study 300 Parent Information Sheet 6 months (Phase 6)	1	09 January 2006
Parent Consent - Study 300, first birthday (Phase 8)	1	09 January 2006
Parent Consent - Study 300, DNA First Birthday (Phase 8)	1	09 January 2006
Phase 1: Study 1500 mother antenatal screen	1	09 January 2006
Phase 1: Study 1500 father antenatal screen	1	09 January 2006
Phase 2: Study 300 mother antenatal interview	1	09 January 2006
Phase 3: Study 1500 pregnancy/obstetric/birth outcomes	1	09 January 2006
Phase 4: Study 300 perinatal baby assessment	1	09 January 2006
Phase 5; Study 1500 6-8 week questionnaire mother	1	09 January 2006
Phase 6: Study 300 6 month postnatal assessments mother and baby	1	09 January 2006
Phase 7: Study 1500 8 month questionnaire and routine health visitor developmental check (mother)	1	09 January 2006
Phase 7: Study 1500 8 month questionnaire (father)	1	09 January 2006

Research governance approval

The study should not commence at any NHS site until the local Principal Investigator has obtained final research governance approval from the R&D Department for the relevant NHS care organisation.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

05/Q1506/107 Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely

Mr Peter Ward
Vice-Chairman

Email: julia.thomas@cwpct.nhs.uk

Enclosures:
Standard approval conditions

NHS

National Research Ethics Service

Cheshire Research Ethics Committee
Western Cheshire PCT
1829 Building
Countess of Chester Health Park
Liverpool Road
Chester
CH2 1HJ

Tel: 01244 650334
Fax: 01244 650333

20 July 2007

Professor Jonathan Hill
Professor of Child and Developmental Psychiatry
Mulberry House, Alder Hey Hospital
Eaton Road
LIVERPOOL
L12 2AP

Dear Professor Hill

Study title: **The Wirral Child Health and Development Study**
REC reference: **05/Q1506/107**
Amendment number: **1**
Amendment date: **31 May 07**

The above amendment was reviewed at the meeting of the Sub-Committee of the REC held on 18 July 2007.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Notice of Substantial Amendment (non-CTIMPs)	1	31 May 2007

Membership of the Committee

The members of the Committee who were present at the meeting are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

05/Q1506/107:	**Please quote this number on all correspondence**

Yours sincerely

Mr Robert Emmett
Committee Co-ordinator

E-mail: rob.emmett@wcheshirepct.nhs.uk

Enclosures List of names and professions of members who were present at the meeting and those who submitted written comments

Appendix D

Histograms With Untransformed and Transformed Scores of the Edinburgh Postnatal Depression Scale (EPDS)

Figure D1 Distribution of 20 Weeks Gestation Maternal Depression (EPDS) Untransformed Scores

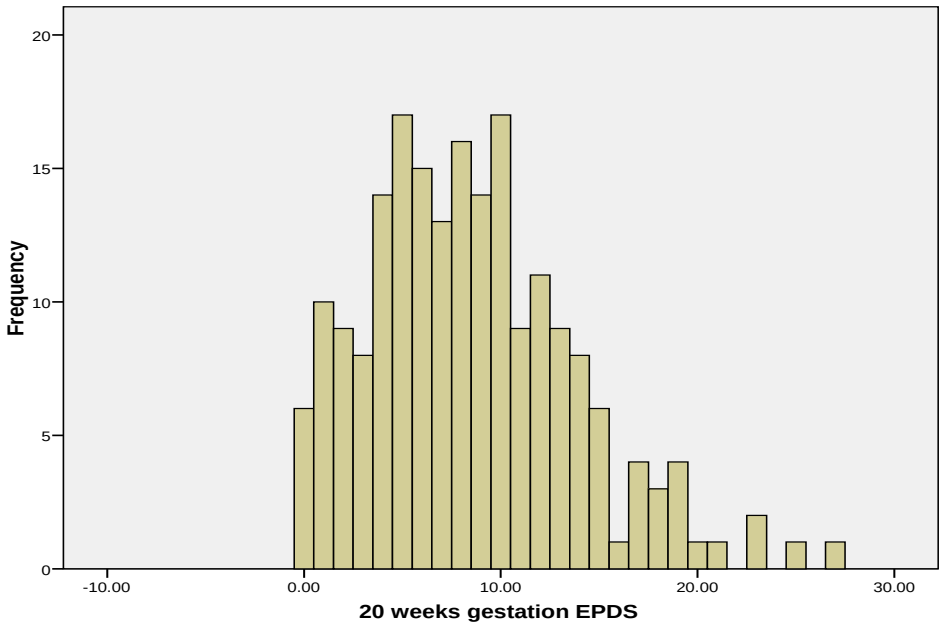


Figure D2 Distribution of 20 Weeks Gestation Maternal Depression (EPDS) Transformed Scores According to Formula ln(EPDS + 7.4)

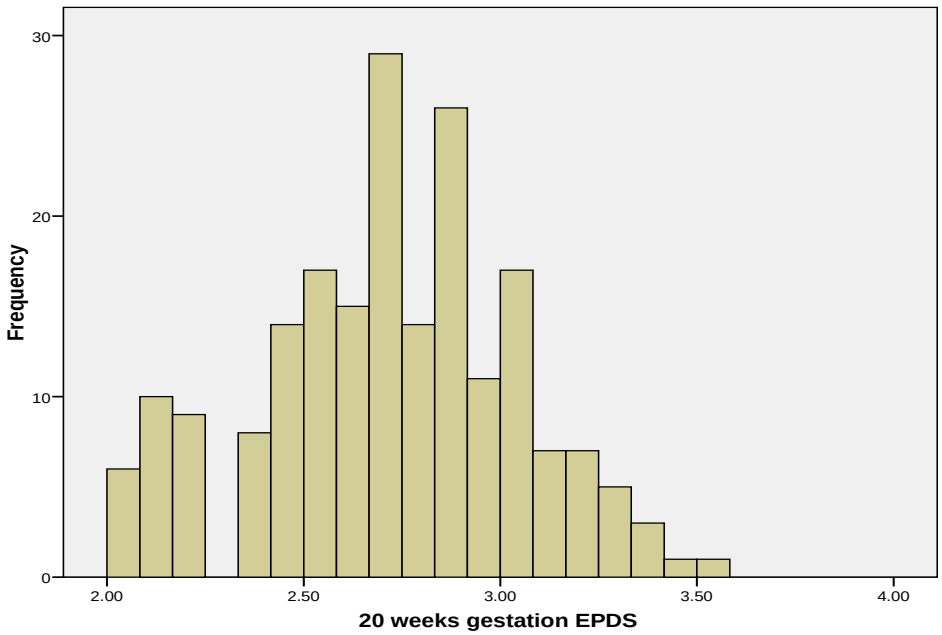


Figure D3 Distribution of 32 Weeks Gestation Maternal Depression (EPDS) Untransformed Scores

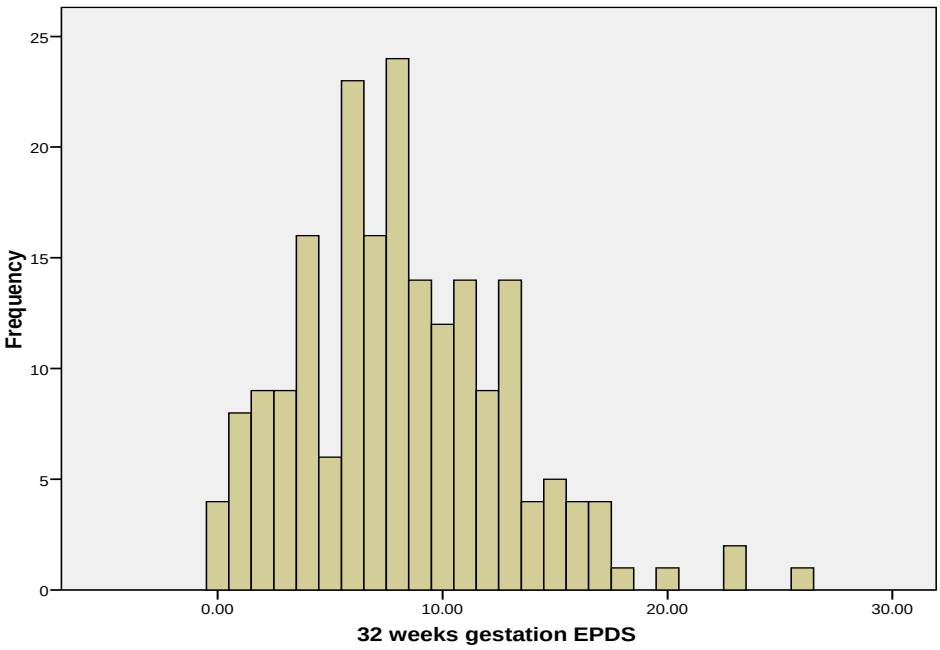


Figure D4 Distribution of 32 Weeks Gestation Maternal Depression (EPDS) Transformed Scores According to Formula ln(EPDS + 7.4)

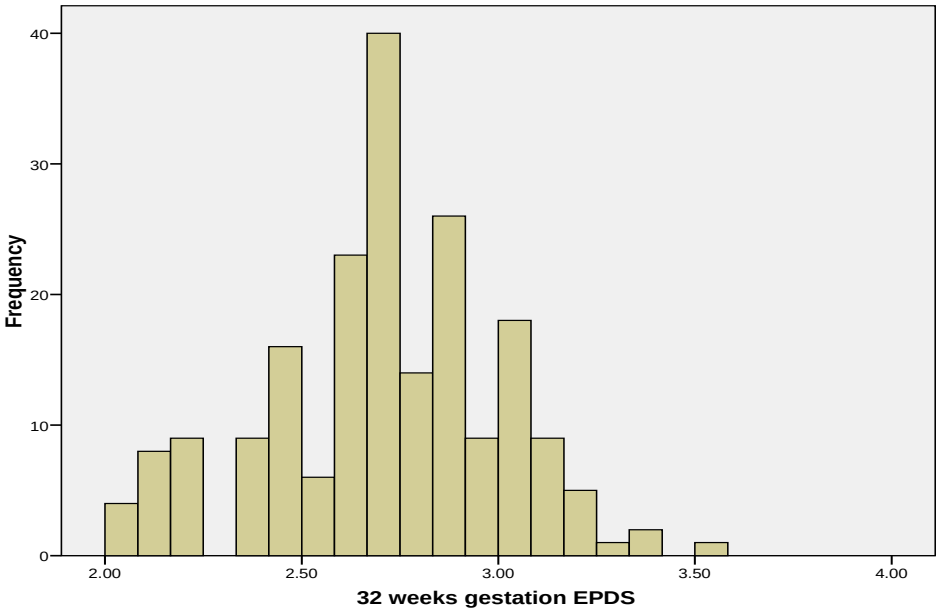


Figure D5 Distribution of 5 Weeks Postnatal Maternal Depression (EPDS) Untransformed Scores

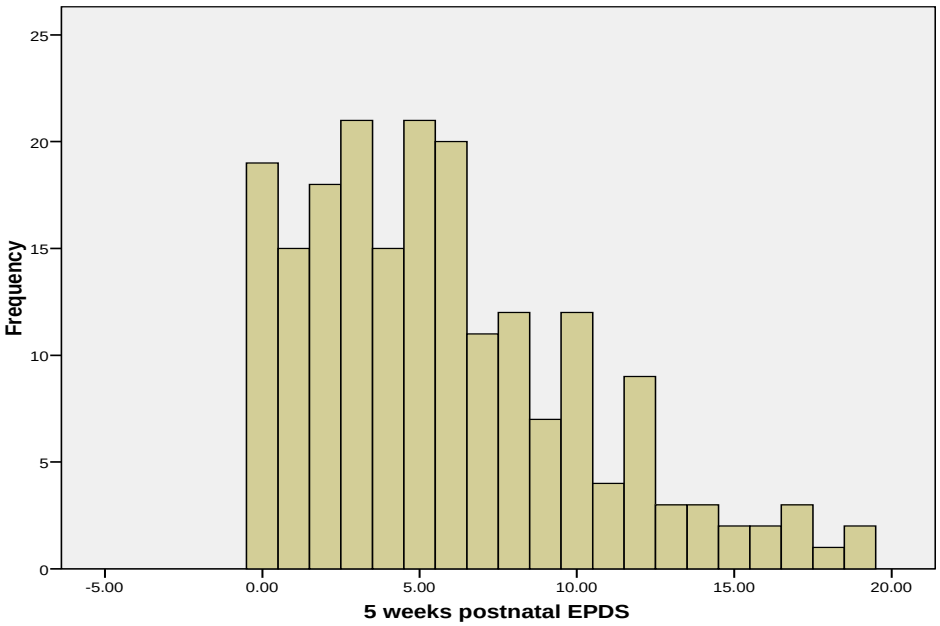


Figure D6 Distribution of 5 Weeks Postnatal Maternal Depression (EPDS) Transformed Scores According to Formula ln(EPDS + 7.4)

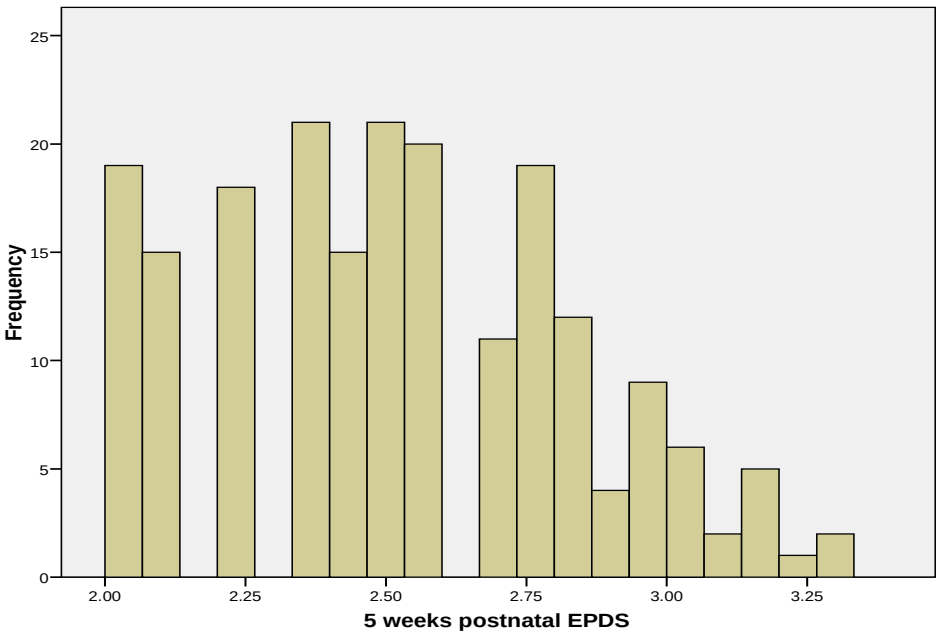


Figure D7 Distribution of 29 Weeks Postnatal Maternal Depression (EPDS) Untransformed Scores

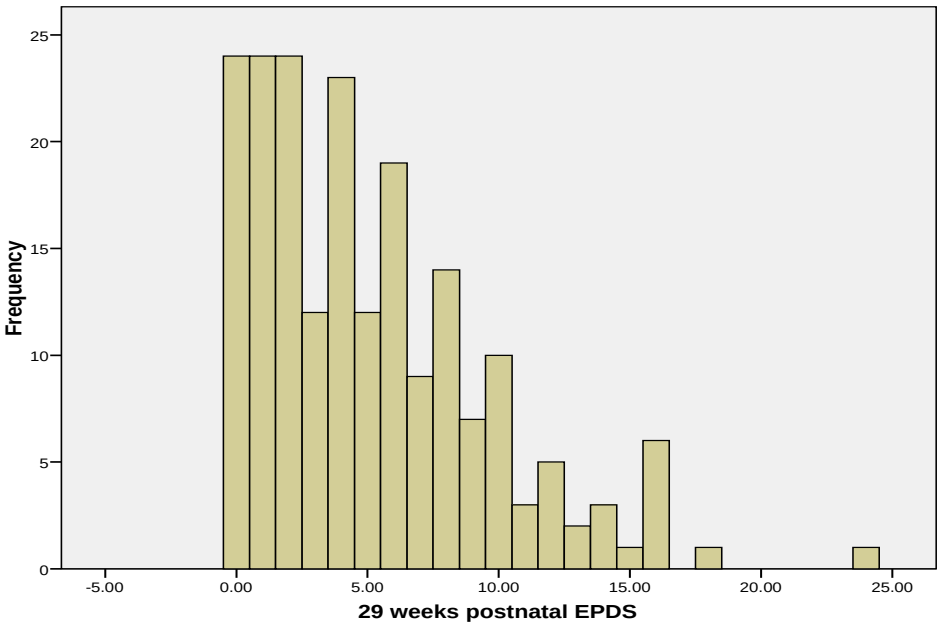
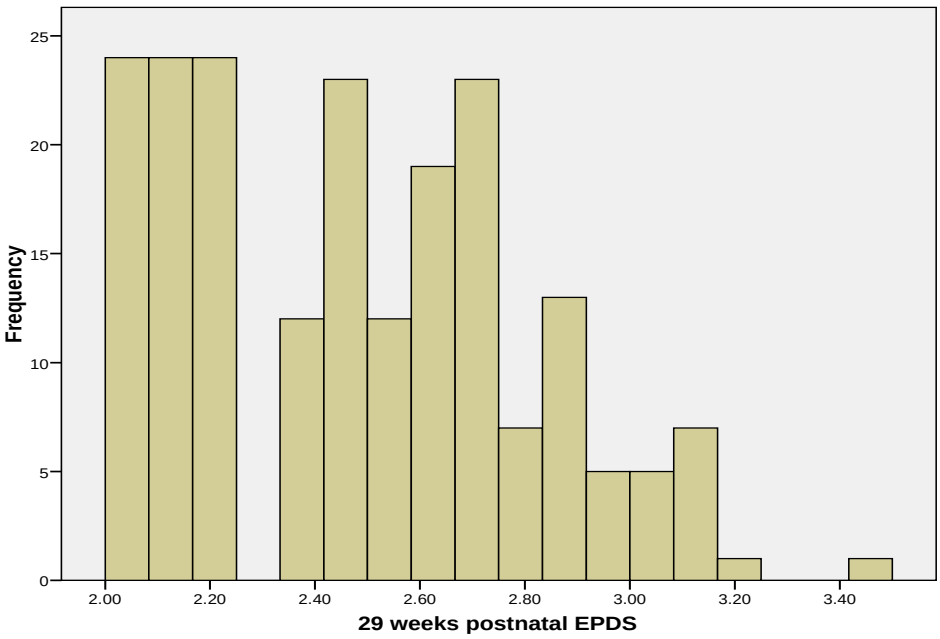


Figure D8 Distribution of 29 Weeks Postnatal Maternal Depression (EPDS) Transformed Scores According to Formula ln(EPDS + 7.4)



Appendix E

Histograms With Untransformed and Transformed Scores of the State Anxiety Scale of the State-Trait Anxiety Inventory (STAI)

Figure E1 Distribution of 20 Weeks Gestation Maternal State Anxiety (STAI) Untransformed Scores

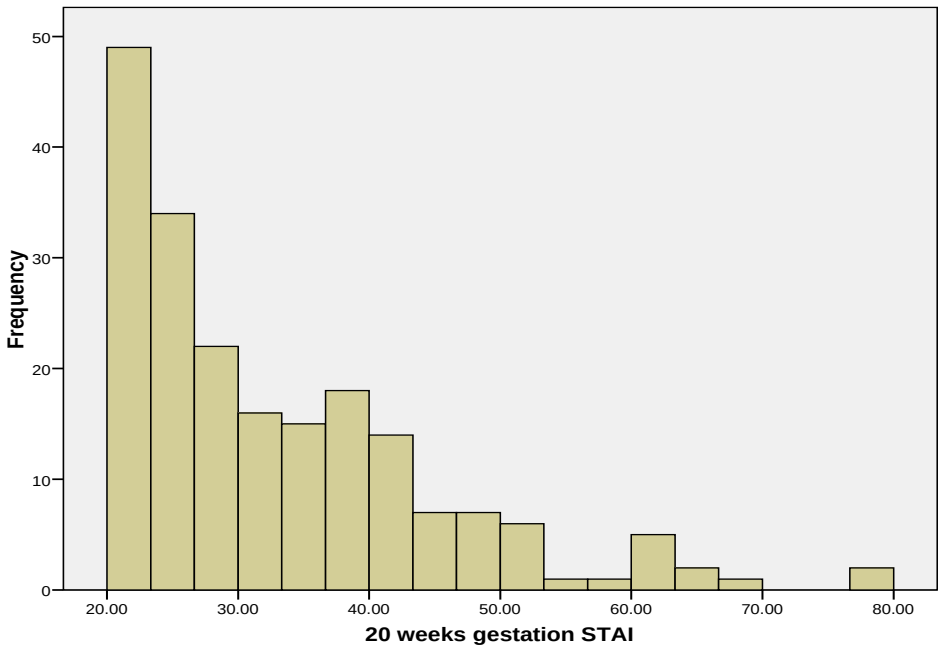


Figure E2 Distribution of 20 Weeks Gestation Maternal State Anxiety (STAI) Transformed Scores According to Formula ln(STAI – 15.87)

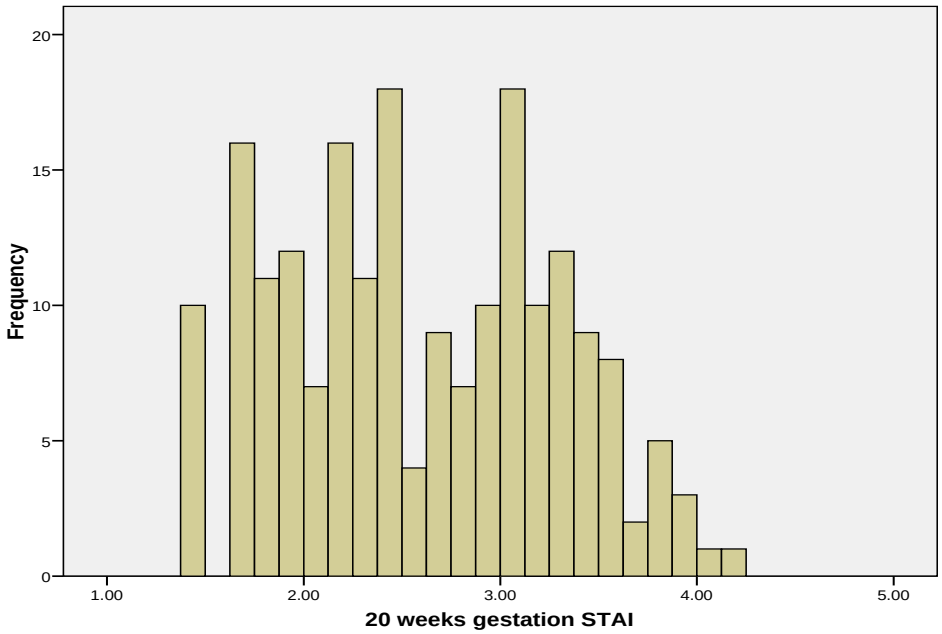


Figure E3 Distribution of 32 Weeks Gestation Maternal State Anxiety (STAI) Untransformed Scores

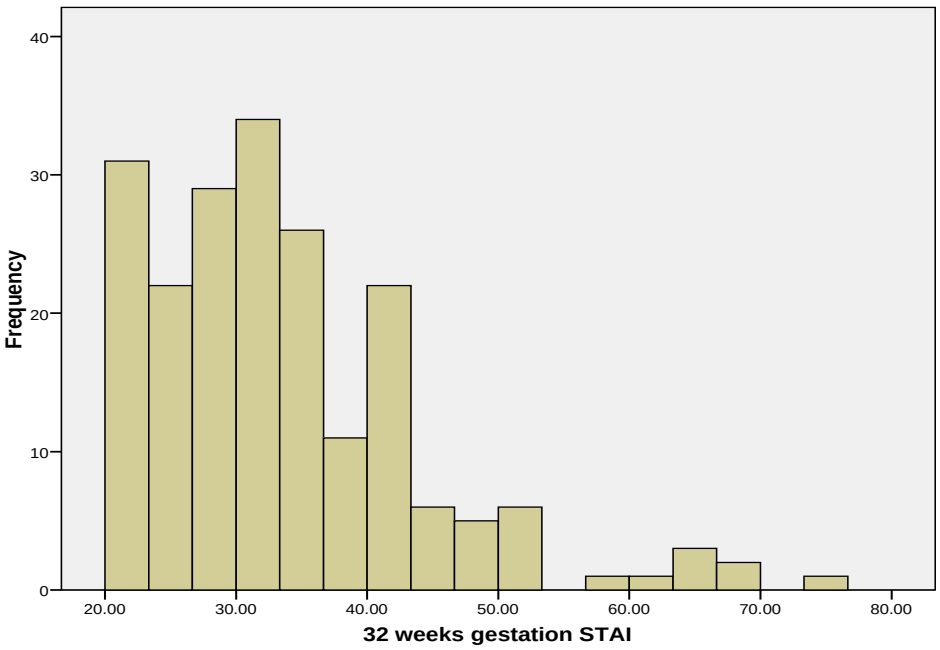


Figure E4 Distribution of 32 Weeks Gestation Maternal State Anxiety (STAI) Transformed Scores According to Formula ln(STAI – 15.87)

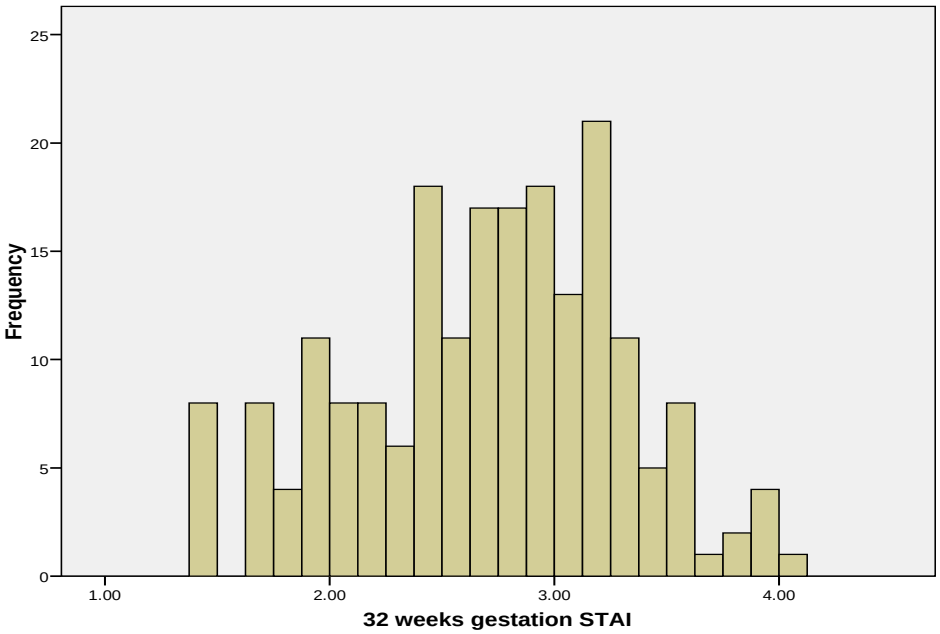


Figure E5 Distribution of 5 Weeks Postnatal Maternal State Anxiety (STAI) Untransformed Scores

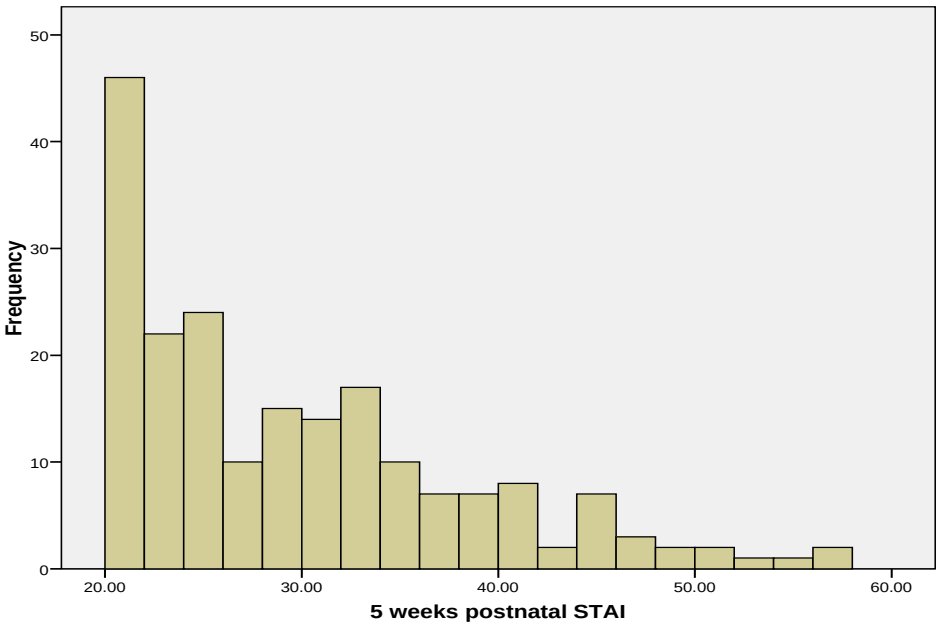


Figure E6 Distribution of 5 Weeks Postnatal Maternal State Anxiety (STAI) Transformed Scores According to Formula ln(STAI - 15.87)

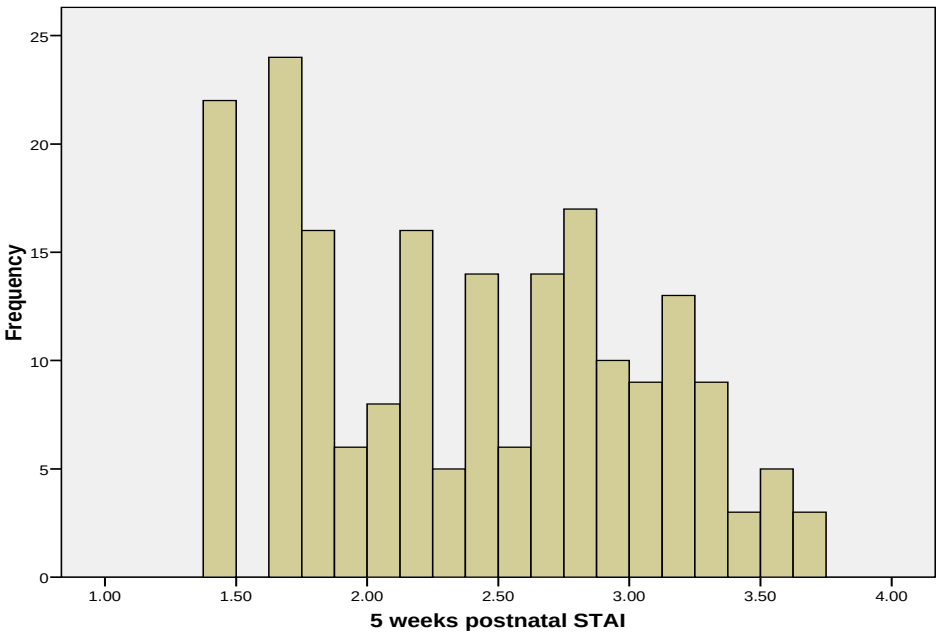


Figure E7 Distribution of 29 Weeks Postnatal Maternal State Anxiety (STAI) Untransformed Scores

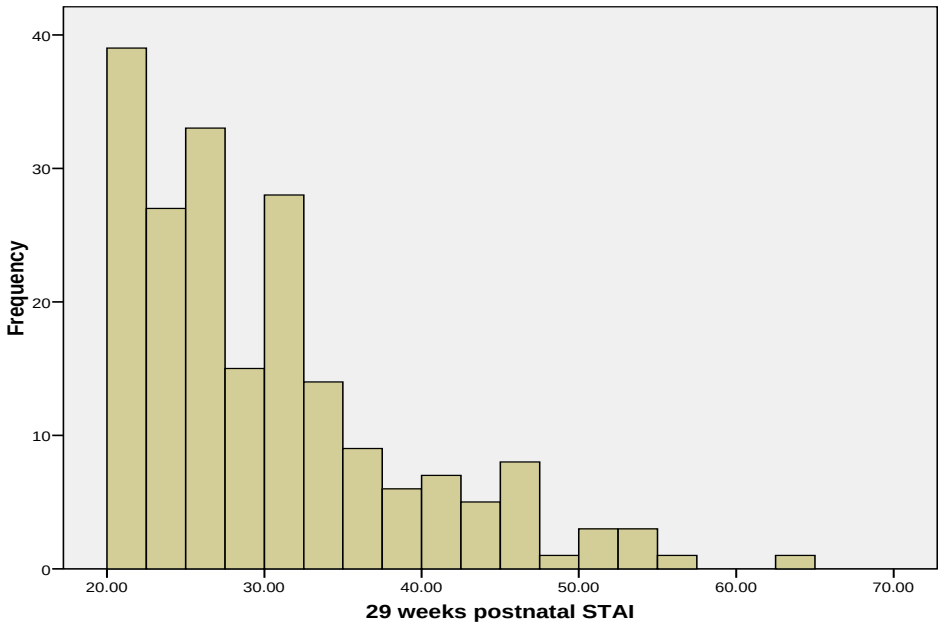
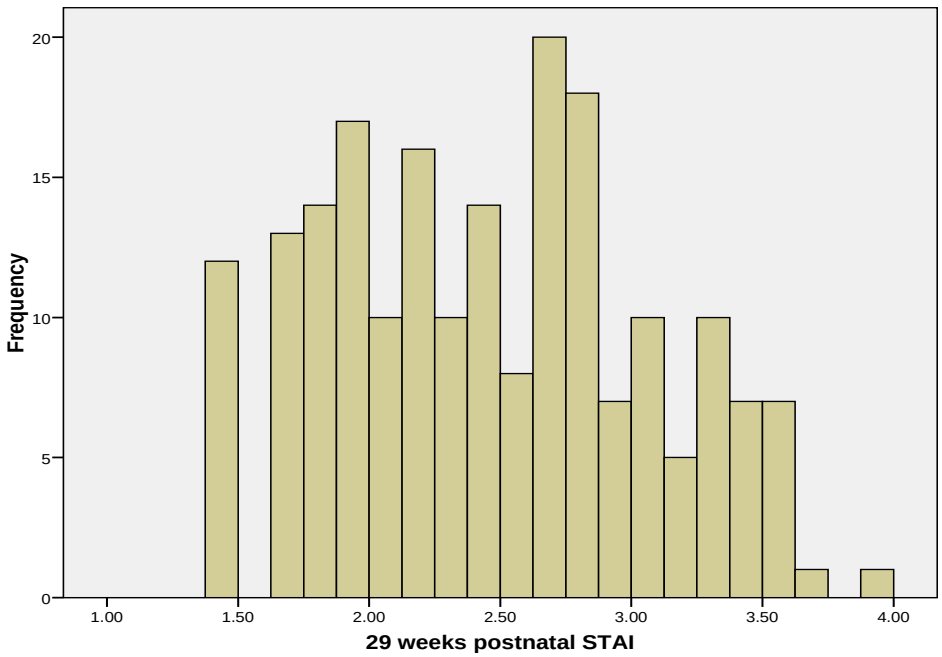


Figure E8 Distribution of 29 Weeks Postnatal Maternal State Anxiety (STAI) Transformed Scores According to Formula ln(STAI – 15.87)



Appendix F

Histograms With RSA (Vagal Tone) Scores in the Five Experimental Procedures

Figure F1 Distribution of RSA Scores in the Helper-Hinderer

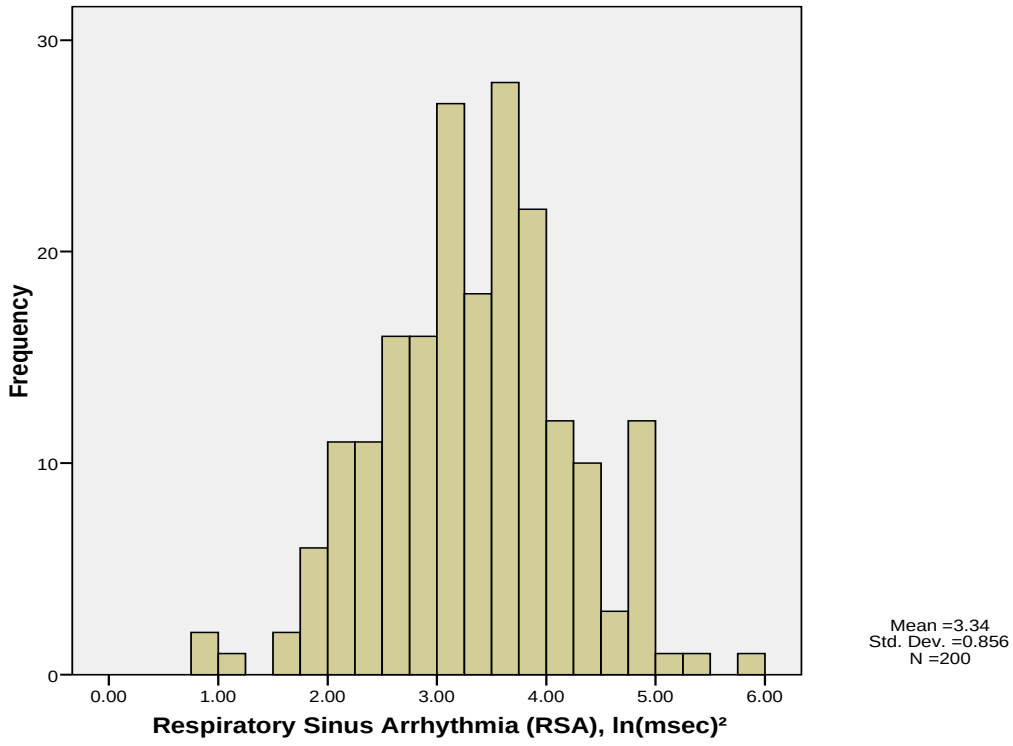


Figure F2 Distribution of RSA Scores in the Novel Toy Exploration

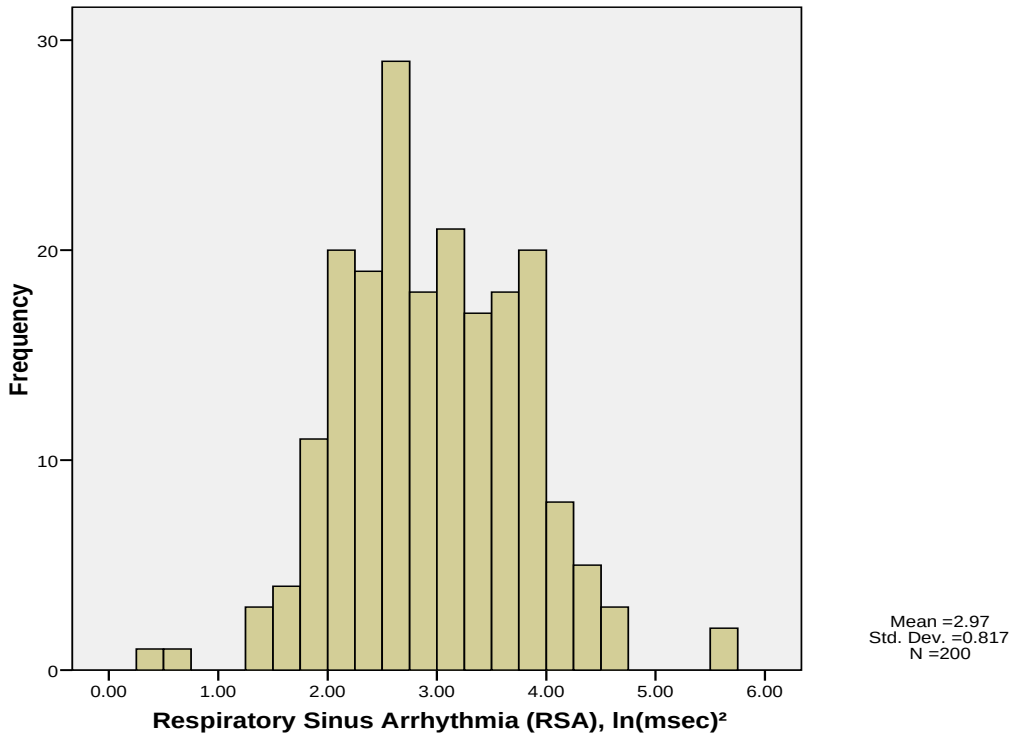


Figure F3 Distribution of RSA Scores in the Social Engagement Episode

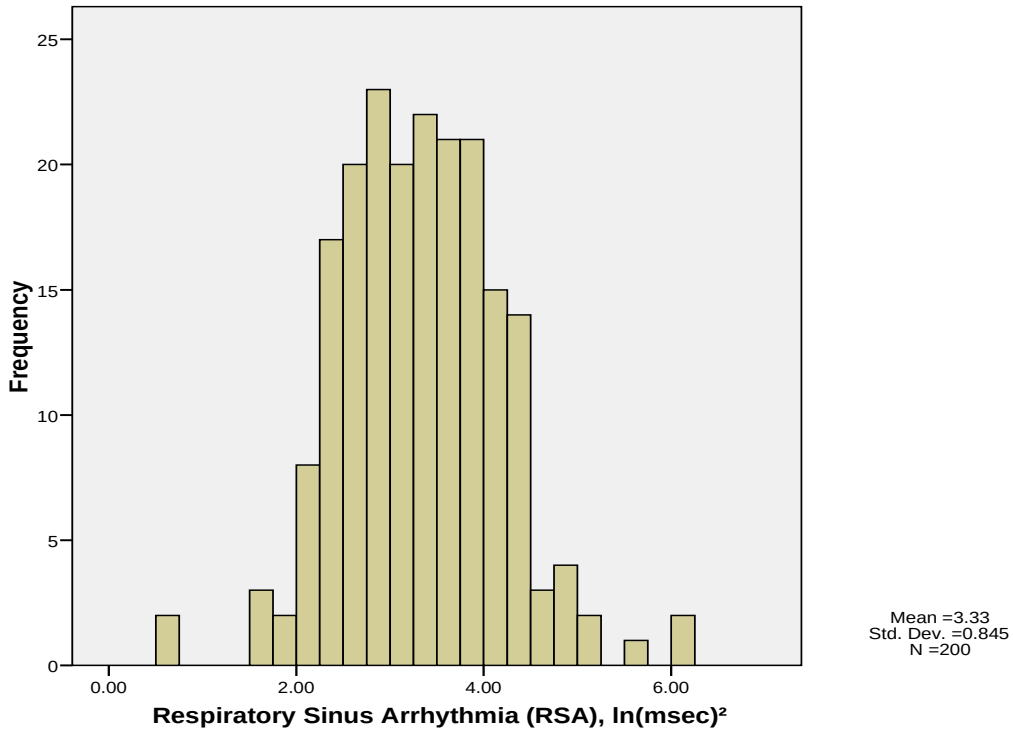


Figure F4 Distribution of RSA Scores in the Still Face Episode

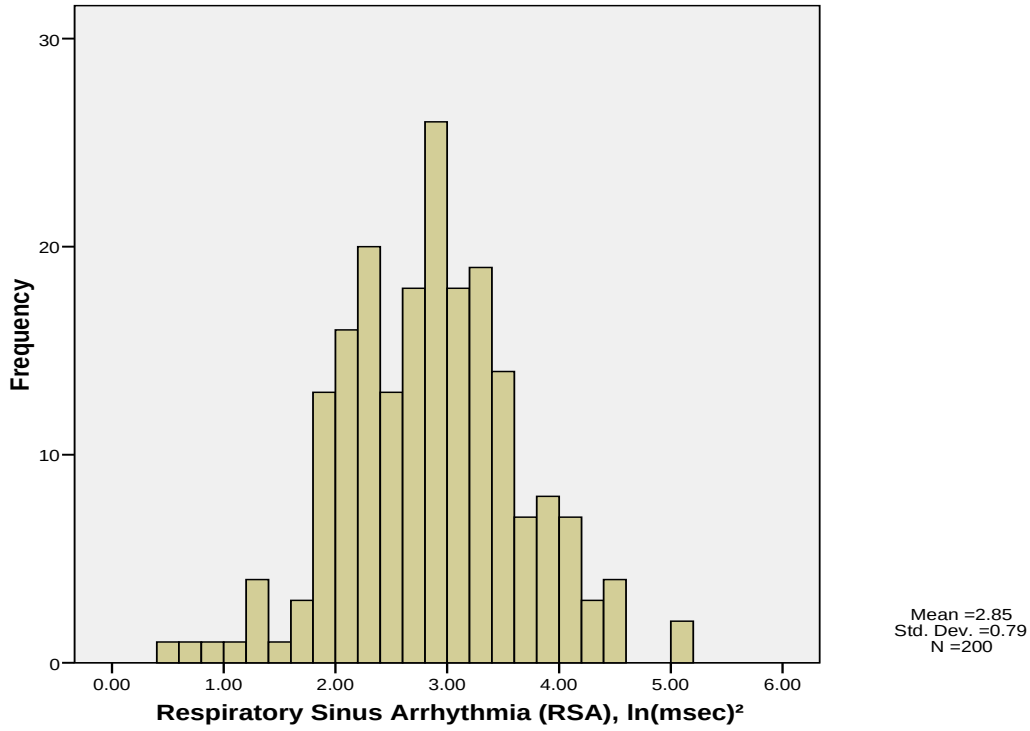
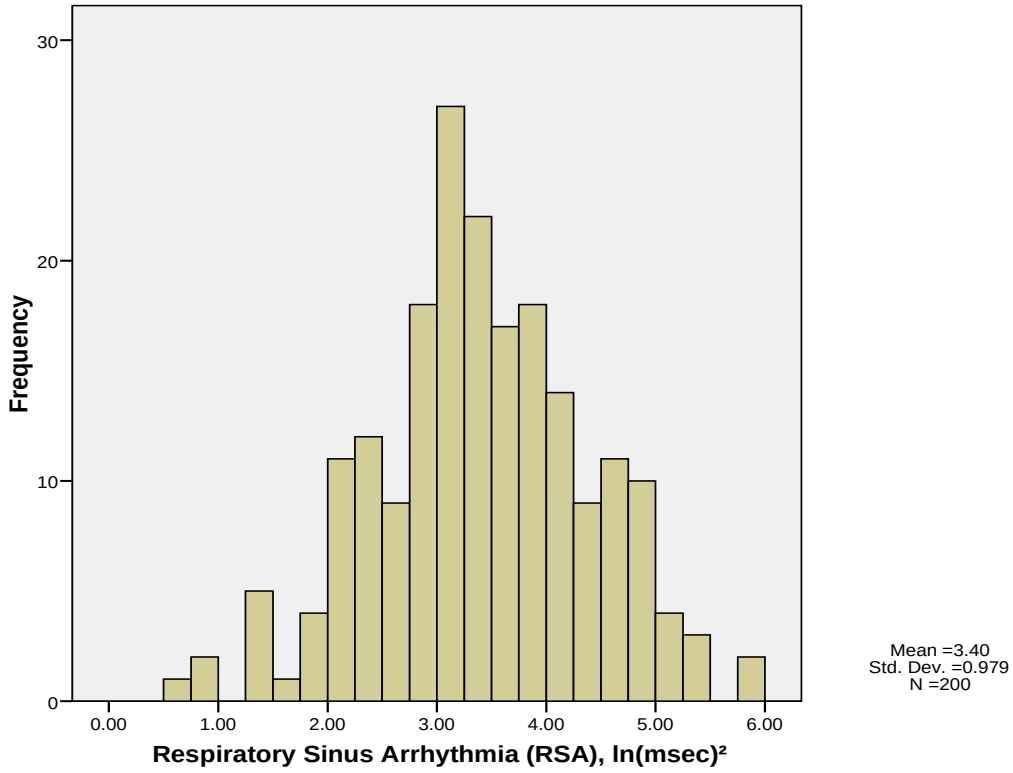


Figure F5 Distribution of RSA Scores in the Social Reunion Episode



Appendix G

Joint Examination of Prenatal Depression and State Anxiety in Association With Sex in Predicting Vagal Tone and Vagal Withdrawal

1. Predictions in Relation to Vagal Tone

1.1 Overall Vagal Tone as Estimated by RSA and Prenatal Maternal Depression and State Anxiety

Associations between RSA and prenatal maternal depression and state anxiety scores were examined with the mean RSA score identified previously (see Section 3.4.6.1) as the overall measure of vagal tone, and with transformed EPDS and STAI scores. This was conducted in MANOVA in order to determine whether RSA was associated specifically with depression and anxiety assessed at one or both of the two time points. In MANOVA with the four maternal mood scores as dependent variables there were no significant associations between RSA and any of the mood scores (see Table G1).

Table G1 Summary of the Results of MANOVA Examining Links Between RSA and Prenatal Maternal Depression (EPDS) and State Anxiety (STAI) Scores

Independent variable		Mean RSA Score		
Dependent variables		*F*	*df*	*p*
Prenatal EPDS	20 Weeks	1.00	1,198	.32
	32 Weeks	0.03	1,198	.87
Prenatal STAI	20 Weeks	1.33	1,198	.25
	32 Weeks	0.82	1,198	.37
Multivariate		1.31	4,195	.27

The MANOVA was conducted using the transformations ln (EPDS score + 7.4) and ln (STAI score – 15.87).

1.2 Examining for Sex Differences in the Link Between Prenatal Maternal Depression and State Anxiety and Vagal Tone

In order to examine the role of sex of infant in associations between maternal depressed and anxious mood and vagal tone, the MANOVA was repeated with the addition of sex as a between-subjects factor and sex by mean RSA interaction term. As shown in Table G2, in the multivariate model there was a significant main effect of sex and a significant sex by mean RSA interaction, and the associations with all four transformed EPDS and STAI scores at 20 weeks and 32 weeks were statistically significant for sex and sex by mean RSA interaction.

Table G2 Summary of the Results of MANOVA Examining the Main Effect of Sex and the Interaction Between Sex of Infant and RSA in Relation to Prenatal Maternal Depression (EPDS) and State Anxiety (STAI) Scores

Independent variables		Mean RSA Score			Sex of Infant			Mean RSA Score by Sex Interaction		
Dependent variables		*F*	*df*	*p*	*F*	*df*	*p*	*F*	*df*	*p*
Prenatal EPDS	20 Weeks	0.34	1,196	.56	9.43	1,196	.002	10.30	1,196	.002
	32 Weeks	0.02	1,196	.89	3.94	1,196	.048	5.08	1,196	.025
Prenatal STAI	20 Weeks	0.17	1,196	.69	6.84	1,196	.01	9.10	1,196	.003
	32 Weeks	1.74	1,196	.19	6.16	1,196	.01	6.86	1,196	.01
Multivariate		0.90	4,193	.46	2.78	4,193	.028	3.11	4,193	.017

The MANOVA was conducted using the transformations ln (EPDS score + 7.4) for depression and ln (STAI score – 15.87) for anxiety.

As the effect of sex of infant by mean RSA interaction was somewhat stronger for 20 weeks depression than for 20 weeks state anxiety, subsequent analyses examined the prediction from 20 weeks depression in interaction with sex while controlling for the effect of 20 weeks anxiety in interaction with sex. It was not, however, assumed that the results provided strong evidence in favour of depression.

1.3 Does Prenatal Maternal Depressed Mood, in Interaction With Sex, Predict Infant Vagal Tone After Accounting for Age of Mother, Smoking in Pregnancy, Current Depressed Mood and Prenatal Maternal Anxiety?

A 3-step hierarchical regression analysis was used to test whether the association between maternal prenatal depression at 20 weeks and infant sex continued to predict infant vagal tone after accounting for the sex interaction with 20 weeks anxiety as well as the other confounders. In the first step the confounders were entered (i.e. maternal age and smoking) together with sex of infant and transformed 29 weeks postnatal and 20 weeks gestation EPDS and 20 weeks gestation state anxiety scores. The interactions between each of the two confounders plus the prenatal anxiety with sex of infant were entered in the second step. Finally, the interaction term of the 20 weeks gestation maternal depression score and sex was entered. Results are shown in Table G3.

Table G3 Summary of Multiple Linear Regression Predicting Vagal Tone (Mean RSA Score) From Maternal Depression in Interaction With Sex of Infant, After Accounting for Maternal Age, Smoking in Pregnancy, Current Depressed Mood and Prenatal Maternal State Anxiety

Step	ΔR^2	ΔF	*df*	*p*	Variables	β	*p*
1	.026	.87	6,193	.52	Maternal age	.005	.95
					Smoking	-.040	.60
					Sex of infant	-.028	.70
					29 weeks postnatal EPDS	-.142	.10
					20 weeks gestation STAI	-.108	.281
					20 weeks gestation EPDS	.232	.044
2	.080	5.70	3,190	.001	Maternal age	-.108	.28
					Smoking	-.239	.021
					Sex of infant	-.103	.18
					29 weeks postnatal EPDS	-.150	.074
					20 weeks gestation STAI	.129	.27
					20 weeks gestation EPDS	.207	.063
					Maternal age X Sex	.173	.081
					Smoking X Sex	.268	.015
					20 weeks gestation STAI X Sex	-.289	.002
3	.017	3.77	1,189	.054	Maternal age	-.072	.47
					Smoking	-.259	.012
					Sex of infant	-.119	.12
					29 weeks postnatal EPDS	-.154	.065
					20 weeks gestation STAI	.011	.93
					20 weeks gestation EPDS	.386	.008
					Maternal age X Sex	.127	.21
					Smoking X Sex	.328	.004
					20 weeks gestation STAI X Sex	-.114	.38
					20 weeks gestation EPDS X Sex	-.263	.054

Overall, the model explained over 12% of the total variance in the mean RSA score. The interactions between confounders and prenatal state anxiety and infant sex in the second step accounted for 8% of the variance. Nevertheless, the interaction entered in the final step between 20 weeks gestation depression and sex continued to predict marginally vagal tone beyond the large effect accounted for by the interaction between prenatal anxiety and infant sex.

2. Predictions in Relation to Vagal Withdrawal

2.1 Vagal Withdrawal as Estimated by RSA Withdrawal and Prenatal Maternal Depression and State Anxiety

Associations between RSA withdrawal and prenatal maternal depression and anxiety were examined with the RSA withdrawal calculated as the difference between the mean RSA of the four procedures and the RSA during the still face, using transformed EPDS and STAI scores. This was conducted in MANOVA in order to determine whether RSA withdrawal was associated specifically with depression and anxiety assessed at one or both of the two time points. In MANOVA with the four maternal mood scores as dependent variables there were no significant associations between RSA withdrawal and any of the mood scores (see Table G4).

Table G4 Summary of the Results of MANOVA Examining Links Between RSA Withdrawal and Prenatal Maternal Depression (EPDS) and State Anxiety (STAI) Scores

Independent variable		RSA Withdrawal		
Dependent variables		*F*	*df*	*p*
Prenatal EPDS	20 Weeks	0.01	1,198	.93
	32 Weeks	0.003	1,198	.96
Prenatal STAI	20 Weeks	0.003	1,198	.96
	32 Weeks	0.83	1,198	.36
Multivariate		0.40	4,195	.81

The MANOVA was conducted using the transformations ln (EPDS score + 7.4) and ln (STAI score – 15.87).

2.2 Examining for Sex Differences in the Link Between Prenatal Maternal Depression and State Anxiety and Vagal Withdrawal

In order to examine the role of sex of infant in associations between maternal depressed and anxious mood and vagal withdrawal, the MANOVA was repeated with the addition of sex as a between-subjects factor and sex by RSA withdrawal interaction term. As shown in Table G5, in the multivariate model there was a significant sex by RSA withdrawal interaction, and the associations with the transformed STAI scores at 20 weeks and 32 weeks and the transformed EPDS score at 20 weeks were statistically significant for sex by RSA withdrawal interaction.

Table G5 Summary of the Results of MANOVA Examining the Main Effect of Sex and the Interaction Between Sex of Infant and RSA Withdrawal in Relation to Prenatal Maternal Depression (EPDS) and State Anxiety (STAI) Scores

Independent variables		RSA Withdrawal			Sex of Infant			RSA Withdrawal by Sex Interaction		
Dependent variables		*F*	*df*	*p*	*F*	*df*	*p*	*F*	*df*	*p*
Prenatal EPDS	20 Weeks	0.003	1,196	.96	1.39	1,196	.24	5.26	1,196	.023
	32 Weeks	0.001	1,196	.99	0.13	1,196	.72	3.48	1,196	.064
Prenatal STAI	20 Weeks	0.001	1,196	.97	0.04	1,196	.84	5.08	1,196	.025
	32 Weeks	1.40	1,196	.24	4.06	1,196	.045	13.07	1,196	<.001
Multivariate		0.64	4,193	.63	2.17	4,193	.073	3.53	4,193	.008

The MANOVA was conducted using the transformations ln (EPDS score + 7.4) for depression and ln (STAI score – 15.87) for anxiety.

As the effect of sex of infant by mean RSA interaction was somewhat stronger for 32 weeks state anxiety than for 20 weeks depression and state anxiety, subsequent analyses examined the prediction from 32 weeks anxiety in interaction with sex while controlling for the effect of 20 weeks depression in interaction with sex. It was not, however, assumed that the results provided strong evidence in favour of anxiety.

2.3 Does Prenatal Maternal State Anxiety, in Interaction With Sex, Predict Infant Vagal Withdrawal After Accounting for Maternal Relationship Status, Smoking in Pregnancy, Current Anxiety and Prenatal Maternal Depression?

A 3-step hierarchical regression analysis was used to test whether the association between maternal prenatal state anxiety at 32 weeks and infant sex continued to predict vagal withdrawal after accounting for the sex interaction with 20 weeks depression as well as the other confounders. In the first step the confounders were entered (i.e. maternal relationship status and smoking) together with sex of infant and transformed 29 weeks postnatal and 32 weeks gestation STAI and 20 weeks gestation depression scores. The interactions between smoking and prenatal depression with sex of infant were entered in the second step. Finally, the interaction term of the 32 weeks gestation maternal state anxiety and sex was entered. Results are shown in Table G6.

Table G6 Summary of Multiple Linear Regression Predicting Vagal Withdrawal (RSA Withdrawal Score) From 32 Weeks Gestation State Anxiety in Interaction With Sex of Infant, After Accounting for Maternal Relationship Status, Smoking in Pregnancy, Current State Anxiety and Prenatal Maternal Depression at 20 weeks

Step	ΔR^2	ΔF	*df*	*p*	Variables	β	*p*
1	.04	1.34	6,193	.24	Maternal relationship status	.144	.059
					Smoking	-.024	.75
					Sex of infant	.105	.14
					29 weeks postnatal STAI	.090	.27
					20 weeks gestation EPDS	-.028	.76
					32 weeks gestation STAI	-.105	.21
2	.032	3.32	2,191	.038	Maternal relationship status	.164	.033
					Smoking	.079	.45
					Sex of infant	.144	.06
					29 weeks postnatal STAI	.071	.38
					20 weeks gestation EPDS	.070	.52
					32 weeks gestation STAI	-.083	.32
					Smoking X Sex	-.145	.19
					20 weeks gestation EPDS X Sex	-.170	.085
3	.034	7.29	1,190	.008	Maternal relationship status	.160	.034
					Smoking	.093	.37
					Sex of infant	.149	.048
					29 weeks postnatal STAI	.051	.52
					20 weeks gestation EPDS	.001	.99
					32 weeks gestation STAI	.109	.32
					Smoking X Sex	-.165	.13
					20 weeks gestation EPDS X Sex	-.032	.77
					32 weeks gestation STAI X Sex	-.293	.008

Overall, the model explained nearly 12% of the total variance in the RSA withdrawal score. In the last step of the regression the interaction between 32 weeks gestation state anxiety and infant sex predicted moderately vagal withdrawal beyond the effect accounted for by the sex interactions with smoking and 20 weeks gestation depression.

3. Interpretation of Additional Findings and Implications for Future Research

The results from these additional analyses showed that maternal depression at 20 weeks gestation in interaction with sex of infant predicted marginally vagal tone beyond the effect accounted for by state anxiety at 20 weeks gestation in association with sex. Similarly, state anxiety at 32 weeks gestation in interaction with sex made a significant contribution on to predicting vagal withdrawal after controlling for the effect of 20 weeks depression in interaction with sex. These findings suggest that specific manifestations of stress during pregnancy may be associated with specific effects on early neurophysiology in males and females. Specifically, depression may rather have an impact on the infant's more general capacity of attending to the environment, while anxiety may have effects which translate into the infant's ability of coping with a social stressor.

Previous investigations of prenatal influences on vagal tone have found few links between aspects of maternal stress in pregnancy and baseline vagal tone in the infant. The findings from the current investigation presented in the main body of the thesis together with the ones derived from these additional analyses provide some support for the possibility that specific mechanisms may operate linking specific negative emotions as reported by the mother to specific processes of vagal tone in the infant. Future research should explore further the possibility of specific prenatal pathways to altered infant neurophysiology together with the issue of timing in pregnancy.