PREGNANCY TO ONE YEAR
EFFECT OF FOETAL EXPOSURE TO MATERNAL CHILDHOOD ABUSE AND DEPRESSION ON OFFSPRING BEHAVIOURAL AND PHYSIOLOGICAL REGULATION

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EFFECT OF FOETAL EXPOSURE TO MATERNAL CHILDHOOD ABUSE AND DEPRESSION ON OFFSPRING BEHAVIOURAL AND PHYSIOLOGICAL REGULATION

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Thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

Institute of Psychiatry, Psychology and Neuroscience

King's College London

2014

To Enrica, my beloved grandmother, and to my brother

Abstract

Background: It is well established that adverse experiences during childhood increase the chances of developing emotional psychopathology in adulthood, particularly during vulnerable times, such as pregnancy. Furthermore, research has also demonstrated an association between maternal depression in pregnancy and poorer offspring developmental outcomes. The primary aim of this thesis is to investigate the pathways by which maternal history of abuse during childhood interacts with depression during pregnancy, and how both conditions affect offspring development at six days, eight weeks and one year after birth. The second aim of this study is to explore the alterations in maternal hypothalamic-pituitary-adrenal (HPA) axis functioning during pregnancy among women who are depressed and/or have experienced childhood abuse, and whether these are related to offspring behavioural and physiological regulation as well as to offspring HPA axis response to stress.

Methods: The sample comprises 125 pregnant women recruited in the area of South London. Women were assessed in pregnancy for maternal depression and history of abuse in childhood in a one-to-one clinical interviews (25 weeks gestation), and for maternal HPA axis (25 and 32 weeks gestation) through the collection of salivary samples. Infants were administered the Neonatal Behavioural Assessment Scale (NBAS) at six days, with an assessment of the HPA axis functioning before and after the NBAS. Infant HPA axis response was reassessed at eight weeks and one year before and after routine immunization.

Results: Women who have been abused in their childhood were 7 times more likely to develop depression in pregnancy than non-abused women. Furthermore, women who were depressed in pregnancy and especially those with both childhood abuse and antenatal depression, showed an increase in the evening cortisol levels at 32 weeks gestation compared with the other women. Neonates of depressed women had poorer behavioural regulation at 6 days, with an increase in their HPA axis stress response

following the NBAS compared with neonates of non-depressed mothers, irrespective of maternal history of childhood abuse. At one year, infants of mothers with childhood abuse and depression exhibited greater stress following the immunization compared with infants of non-depressed mothers, but this difference was not seen at 8 weeks.

Conclusions: The effects of exposure to childhood abuse and depression in pregnancy can be seen in the mothers' high level of stress hormone circulating in the evening in the 3rd trimester of pregnancy. Moreover, the effects are also seen in the next generation during the first year of life, as observed in the persistent biological and behavioural changes in the offspring. These findings have implications for clinical practice: doctors and midwives in antenatal clinics should be aware of the importance of asking about women's own childhood histories and their mental health during pregnancy in order to offer support during their transition to motherhood.

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Acknowledgments

I am extremely grateful to all those who made this work possible.

Thank you to my first supervisor, Prof Carmine Pariante, and to my second supervisor, Dr Susan Pawlby, for their professional and personal guidance, and their patience during the hardest times of my PhD.

Thank you my senior colleagues Sue Conroy and Dr Sarah Osborne, for being such a wonderful presence at work and for their incredible support.

Thank you to the colleagues of the PRAM study, some of whom have become very special to me. Thank you also to the amazing families that took part in our study and have been so dedicated, kind and very appreciative of our work.

My immense gratitude goes to all my family and friends all over the world. Thank you for your unconditional love and encouragements and for being at my side throughout the years. Thank you for believing in me, always.

Finally, my special thanks go to Giovanni, Arturo, Ronete, Pina and Vita, the most important people in my life.

List of abbreviations

ACTH	Adrenocorticotrophic hormone
AUC	Area under the curve
AUCg	Area under the curve with respect to ground
AUCi	Area under the curve with respect to increase
BDI	Beck Depression Inventory
CA	Childhood abuse
CAR	Cortisol awakening response
CECA-Q	Childhood Experience of Care and Abuse Questionnaire
CI	Confidential interval
CRF	Corticotrophin Releasing Factor
CRH	Corticotrophin Releasing Hormone
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th Edition
EEG	Electroencephalogram
EPDS	Edinburgh Postnatal Depression Scale
M	Mean
MDD	Major depressive disorder
MDD & CA	Major depression and childhood abuse
NBAS	Neonatal Behavioural Assessment Scale
OR	Odds ratio
PTSD	Post-Traumatic Stress Disorder
SADS	Schedule for Affective Disorders and Schizophrenia
SCID	Structured Clinical Interview for DSM-IV Axis I Disorders
SCS	Salimetric's Children Swabs
SD	Standard deviation
SE	Standard error

1 Introduction

1.1 Overview

Evidence of an association between childhood abuse and heightened levels of depression in adulthood, particularly in vulnerable periods such as pregnancy, is now well established (Benedict et al., 1999) (Bifulco et al., 2002), with further evidence that childhood abuse leads to disruptions in the neurobiology of stress in adulthood (Trickett et al., 2010). Furthermore, a vast body of research has shown evidence of links between maternal depression in pregnancy and infant outcomes. In fact, there is evidence that depression in pregnancy can have detrimental effects on infant development after birth (Field et al., 2006) (Deave et al., 2008) and on infant stress response regulation (Davis et al., 2011).

It is with this in mind that this thesis aims to investigate the clinical and molecular mechanisms by which maternal abuse in childhood interacts with maternal depression in pregnancy and how both conditions impact the baby's development after birth. This is the first study that explores potential pathways of this intergenerational transmission and the interaction between clinical and biological factors shaping this trajectory. This investigation will assess both mothers and infants from pregnancy to one year, using a combination of maternal diagnostic and infant behavioural measures, as well as biological measures with both mothers and their babies.

The purpose of the introductory chapter that follows is to offer an overview of the literature and the work that has been conducted in this field up to now, with a focus on the interaction between the biological system and psychopathology in mothers (childhood abuse and depression in pregnancy). First, a brief overview of childhood abuse, its relevance to the perinatal period and its association with antenatal depression is provided. Next, I review the literature on the functioning of the biological systems in healthy adults and then in women who experienced childhood abuse and/or antenatal

depression and finally I discuss the biological and behavioral alterations in the offspring.

1.2 Childhood abuse

1.2.1 Phenomenology

Childhood abuse is a term that covers a wide spectrum of phenomena. Bifulco identifies 4 categories of abuse – physical, emotional, sexual abuse and neglect. **Physical abuse** is "defined in terms of hitting by parents or other older household members. A range of attacks is reflected by those rated severe are usually repeated attacks where implements such as belts or sticks are used, or punching or kicking occurs with the possibility of causing harm" ((Bifulco et al., 2005), page 567). **Emotional or psychological abuse** is caused by the failure of parents to provide a safe, caring and loving environment for their children. It is concerned with cruelty demonstrated by verbal and nonverbal acts, repeated or singular, intended or not, from a close other in a position of power or responsibility over the child. Such acts have the potential for damaging the social, cognitive, emotional, or physical development of the child and are demonstrated by behaviours which are humiliating or degrading, terrorising, extremely rejecting, depriving of basic needs or valued objects, inflicting marked distress or discomfort, corrupting or exploiting, cognitively disorientating, or emotionally blackmailing (Moran et al., 2002). **Sexual abuse** is defined as an act that "involves physical contact or approach of sexual nature by any adult to the child, but excludes willing sexual contact with peers. Severe sexual abuse (marked or moderate severity) includes all repeated sexual contact with an adult or single incidents of a serious nature, such a rape or sexual contact with a family member" ((Bifulco et al., 2005) page 567). **Neglect** has been defined as the "parents disinterest in material care (feeding and clothing), health, school work and friendships" ((Bifulco et al., 2005), page 567).

1.2.2 Epidemiology

Several studies in the UK report that the rate of maltreatment amongst children and adolescents is unacceptably high (May-Chahal and Cawson, 2005). Recent statistics from the Child Protection Register show that currently in the United Kingdom over 50,000 children are under the child protection services (NSPCC, 2014). These children are known to have been abused or to be at high risk of abuse. The Department of Education (2013) has published data on the prevalence of specific types of childhood abuse in the UK, as shown in Table 1 (NSPCC, 2014). The data show an increase in the total number of reported cases of abuse particularly in the number of children experiencing emotional abuse.

Table 1. Prevalence of different types of abuse in the UK

Category of abuse	**2009**	**2010**	**2011**	**2012**	**2013**
Physical abuse	4,400	5,000	4,800	4,690	4,670
Emotional abuse	9,100	10,800	11,400	12,330	13,640
Sexual abuse	2,000	2,300	2,400	2,200	2,030
Neglect	15,800	17,300	18,600	18,220	17,930
Multiple abuse	2,900	3,700	5,500	5,390	4,870
Total abuse	34,100	39,100	42,700	42,850	43,100

Importantly, the statistics also show that one in four adults have been abused in their own childhood (NSPCC, 2014). The most recent data gathered by the NSPCC on the prevalence of child abuse and neglect among UK families are based on a large study published by Radford and colleagues (Radford et al., 2011). Researchers interviewed more than 2,000 young adults, adolescents and parents of young children. The Juvenile Victimization Questionnaire (Finkelhor et al., 2005) was used to gather information about physical violence, emotional abuse, sexual abuse and neglect by a parent or other adult living in the family. In this study physical violence was defined as "the act of being beaten, kicked, hit or physically hurt by a parent or guarding or physically attacked with or without a weapon but no including smacking" ((Radford et al., 2011) page 44).

Emotional abuse included "being scared or made feel really bad because the caregiver called the child name, said mean things, or said that they did not want the child; breaking or ruining the child's things and threatening him/her with violence" ((Radford et al., 2011) page 45), while sexual abuse included both contact and non-contact abuse by a caregiver. Neglect was defined as "the absence of physical care, lack of health care, educational neglect, poor supervision and monitoring, and a caregiver being unresponsive to the child's emotional needs to such an extent that significant harm is likely to result" ((Radford et al., 2011) page 43). A further category of severe maltreatment was judged on the basis of frequency, level of injury, use of weapon, having different abuse experiences, whether the act would be seen as severe in criminal law (Radford et al., 2011).

Table 2 reports the data regarding the lifetime rate of physical, emotional and sexual abuse and neglect by a parent or a guardian (Radford et al., 2011). As shown, 8.9% of children under the age of 11, 21.9% of adolescents aged 11 to 17 and almost a quarter of young adults aged 18 to 24 have experienced some form of maltreatment in their life (Radford et al., 2011).

Table 2. Lifetime rate of maltreatment by a parent or a guardian in the UK

Maltreatment type	**< 11 years % (N)**	**11-17 years % (N)**	**18-24 years % (N)**
Physical violence	1.3 (34)	6.9 (119)	8.4 (159)
Emotional abuse	3.6 (74)	6.8 (116)	6.9 (131)
Sexual abuse	0.1 (2)	0.1 (2)	1.0 (20)
Neglect	5.0 (130)	13.3 (229)	16.0 (303)
Maltreatment JVQ[a]	7.3 (188)	20.7 (358)	23.0 (436)
All maltreatment	8.9 (229)	21.9 (379)	24.5 (465)

[a] Juvenile Victimization Questionnaire (Finkelhor et al., 2005)

Interestingly, the data show neglect to be the most prevalent type of maltreatment in the family across all age groups. The study also found a very strong association between childhood abuse and poor emotional wellbeing (Radford et al., 2011).

1.3 Childhood abuse and perinatal period

As we have seen the prevalence of childhood abuse in the UK is high with almost a quarter of young adults having reported being abused in their childhood or adolescence. Not only does abuse in the current generation of children constitute a major social problem (Lang et al., 2010) but also the effects of experiencing abuse as a child may have long lasting effects into adulthood. For example, Bifulco and colleagues (1994) showed that neglect and abuse during childhood predicted depression in adulthood, particularly in vulnerable periods. Such effects may become particularly visible when a woman becomes a mother herself (Lang et al., 2010). It is now acknowledged that an experience of childhood abuse may lead to negative consequences on pregnancy outcome, childbirth, parenting and later child development (Leeners et al., 2006), both from a medical and psychological point of view. Moreover, there is evidence to show an association between a history of childhood abuse and heightened levels of postpartum depression (Benedict et al., 1999). For example, physical abuse has been linked to elevated postpartum depressive symptoms (Buist, 1998), and sexual abuse has been linked with heightened anxiety among postpartum women (Grimstad et al., 1999). However, surprisingly few studies have examined the impact of the experience of childhood abuse on maternal depression in pregnancy. Findings from studies that have addressed this question suggest an association with depressive symptomatology during pregnancy (Romano et al., 2006) (Rich-Edwards et al., 2011) (Chung et al., 2008). More recently Plant and colleagues (2013) have demonstrated in a South London sample that mothers who have experienced abuse in their own childhoods are 10 times

more likely than those who were not abused to develop clinically diagnosed depression in pregnancy.

1.4 Antenatal depression

1.4.1 Phenomenology

Antenatal depression is a major depressive disorder (MDD) that occurs in pregnancy. As studies published today use DSM-IV criteria and I use these same DSM-IV criteria for my Thesis, I will refer to these rather than to the more recent DSM-5 criteria, which are in any case virtually identical. According to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR; (American Psychiatric Association, 2000), a diagnosis of MDD is rated if an individual has suffered from at least one core symptom of (i) depressed mood or (ii) diminished interest or pleasure in activities, and at least three of the following symptoms: (iii) significant weight change or change in appetite; (iv) insomnia or hypersomnia; (v) psychomotor agitation or retardation; (vi) fatigue or loss of energy; (vii) feelings of worthlessness or excessive or inappropriate guilt; (viii) diminished ability to think or concentrate or indecisiveness; (ix) recurrent thoughts of death, recurrent suicidal ideation or plans for committing suicide. These symptoms must be present for most of the day, nearly every day, for at least two weeks. Furthermore, the symptoms must cause significant distress or impairment in normal functioning, and should not be attributable to the death of a loved one, a general medical condition or substance use (American Psychiatric Association, 2000). This last criteria (the so called “bereavement exclusion” is no longer valid for DSM-5.

1.4.2 Epidemiology

A meta-analysis was conducted in 2004 into the prevalence of antenatal depression (Bennett et al., 2004). The meta-analysis included studies published since 1980. Twenty-one studies were identified, with seven based on clinical diagnoses of

depression rated through structured clinical interviews (Schedule for Affective Disorders and Schizophrenia; SADS). The remaining studies were based on ratings of depression using self-report measures such as the Edinburgh Postnatal Depression Scale (EPDS) and the Beck Depression Inventory (BDI). The authors reported the point prevalence of depression by trimester, with rates of 7.4% in the first, 12.8% in the second and 12.0% in the third trimester. Prevalence estimated through structured interviews did not differ significantly from rates attained through the EPDS, but were significantly lower that rates attained through the BDI.

Gavin and colleagues (2005), reviewed studies where depression during pregnancy had been rated only through structured clinical interviews (Gavin et al., 2005). They reported a period prevalence rate (conception to birth) for MDD of 12.7%. In both studies there were methodological flaws, such as the exclusion of women with previous psychiatric histories and of low socioeconomic status. Given that both of these factors are well-known risk factors for depression (Gotlib and Hammen, 2008), it is likely that these are conservative estimates.

One of the potential mechanisms that might underlie the association between the experience of childhood abuse, an early-life adversity, and depression in pregnancy is through the long-term alteration of the neuroendocrine system. Specifically, and as reviewed below, childhood abuse (especially in the context of adult depression) leads to persistent activation of the main hormonal stress system, the hypothalamic-pituitary-adrenal (HPA) axis. In turn, pregnancy in itself is also associated with a persistent activation of the HPA axis, and therefore it is plausible that this makes pregnancy a particular “risk period” for the development of depression following childhood abuse.

1.5 Hypothalamus-Pituitary-Adrenal (HPA) axis

1.5.1 HPA axis general functioning in healthy adults

The hypothalamic-pituitary-adrenal (HPA) axis controls reactions to stress and regulates many body processes, including digestion, the immune system, mood and emotions, sexuality and energy storage (Pruessner et al., 2003) (Heim et al., 2002). The activated HPA axis not only regulates body peripheral functions such metabolism and immunity but also has profound effects on the brain (Pariante and Lightman, 2008).

The HPA axis is a complex set of direct influences and feedback interactions among three endocrine glands: the hypothalamus, the pituitary gland (located below the hypothalamus), and the adrenal glands (located on top of the kidneys). It is activated via the hippocampus, resulting in secretion of corticotrophin releasing hormone (CRH) from the hypothalamus. In turn, CRH stimulates secretion of the adrenocorticotrophic hormone (ACTH) from the pituitary gland, which causes secretion of cortisol from the adrenal glands. In addition to its actions to prepare the body to counteract the stressor, cortisol has an inhibitory action on secretion of CRH at the hypothalamic level via the glucocorticoid receptor though the activation of a negative feedback loop (Pruessner et al., 2003) (Heim et al., 2002).

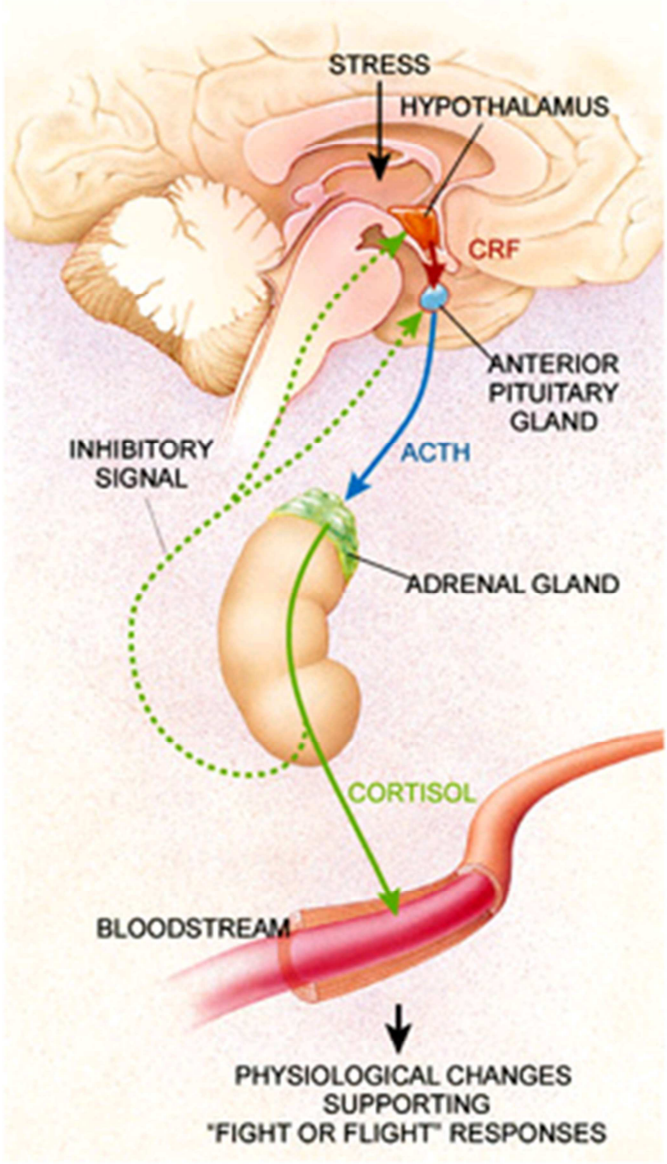


Figure 1. HPA axis functioning in stressed patients

1.5.2 HPA axis in depressed adults

A number of biological mechanisms have been found to be involved in the pathophysiology of maternal depression (Leung and Kaplan, 2009). Specifically, one of the most consistent findings in the biology of depression is the alteration of the HPA axis and the role of cortisol (Belvederi Murri et al., 2014). Although this evidence has been consistently shown in studies over the last 40 years, the mechanisms that lead to this abnormality are still unclear (Pariante and Lightman, 2008).

These neuroendocrine alterations have been confirmed by an analysis of studies testing HPA axis function (by dexamethasone suppression test) in thousands of depressed patients suggested evidence of an increased production of CRH in the brain and impaired negative feedback regulation by glucocorticoids in 44% of the sample (Arana et al., 1985) (Pariante, 2003). Moreover, those depressed patients show increased levels of cortisol in urine, plasma (Sachar et al., 1970) and cerebrospinal fluid (Nemeroff et al., 1984) and increased volume of pituitary (Krishnan et al., 1991) and adrenal and pituitary glands in depression (Rubin et al., 1995) (Nemeroff et al., 1992) (Pariante, 2003).

This biological vulnerability has been tested in a large cohort study on subjects with a history of major depression (Vreeburg et al., 2009). Salivary cortisol was collected at 7 time points throughout the day. Results have shown higher cortisol awakening response in subjects with a current or previous diagnosis of major depression compared to subjects with no mental health diagnosis.

1.5.3 HPA axis in adults abused in childhood

A history of child sexual abuse has been associated with HPA axis dysregulation across a number of studies, although the direction of its effect remains controversial, with studies finding in general HPA hyperactivity in the context of adult depressed subjects with a history of childhood abuse. (Bublitz and Stroud, 2012). Childhood sexual abuse

has been associated with greater cortisol awakening response in a group of patient with first episode of psychosis (Mondelli et al., 2010) and with a blunted cortisol response in a laboratory setting after an experimental stress challenge (Pierrehumbert et al., 2009). A Chinese study investigated the cortisol awakening response (as a measure of the HPA axis) in a group of 30 depressed subjects who were victims of emotional and physical neglect and 30 depressed patients without history of child neglect. Results showed a significant increase in the cortisol awakening response in patients with both depression and neglect compared with those who were only depressed. Interestingly, child neglect was found to have an effect on the HPA axis response but not on the severity of depression. Despite this initial evidence of an association between childhood abuse and HPA axis functioning, the studies cited above have a relatively small sample size and used different instruments to assess childhood abuse. This might further explain the lack of clear direction in the associations found.

1.5.4 HPA axis in pregnancy

The maternal HPA axis undergoes dramatic changes during pregnancy and the postpartum (Duthie and Reynolds, 2013). Specifically, in healthy pregnant women, circulating cortisol increases during pregnancy, with a peak in the third trimester (Jung et al., 2011). This is due to the increased oestrogen stimulation of corticosteroids and the higher placental secretion of CRH into maternal blood during the second and third trimester of pregnancy (Jung et al., 2011). Placental CRH stimulates maternal pituitary glands and, consequently, there is a higher production of cortisol level. As pregnancy progresses, this increased circulation of cortisol down regulates the hypothalamic production of CRH and decreases the HPA axis response (Duthie and Reynolds, 2013). This cortisol pattern is stable across pregnancy.

HPA axis in depressed pregnant women

Evidence suggests that this mechanism is altered in pregnant women who experience depression but the effects of this alteration remain unclear. A study by Dyane and colleagues shows that depression in pregnancy might heighten cortisol levels as well as the placental secretion of corticotrophin releasing factors (CRF), although its role remains unclear (Dayan et al., 2010). A different outcome was found in a longitudinal study of a Swedish pregnant women (Hellgren et al., 2013). Recruited in a scanning clinic of Uppsala, participants were invited to complete a web-based questionnaire regarding their mood at 17 and 32 weeks gestation. 57 women were free from mental health diagnosis, while 38 had depression during the current pregnancy. The women collected saliva samples at awakening, 15, 30 and 45 minutes post-awakening. Surprisingly, results showed no difference in the cortisol levels of depressed pregnant women and healthy pregnant controls (Hellgren et al., 2013). The author suggests that the physiological changes related to pregnancy that lead to higher cortisol levels, might suppress the higher cortisol awakening response seen in non-pregnant subjects (Hellgren et al., 2013)

Two other studies have compared HPA axis measures in pregnant women with an operationally defined diagnosis of major depression and healthy controls. Both found significantly higher cortisol, measured at a single 2nd trimester time-point, in the depressed group (Field et al., 2009b). By measuring both morning and evening cortisol, O'Keane (O'Keane et al., 2011) demonstrated cortisol hyper-secretion especially in the evening, a pattern frequently found also in adults with major depression outside of pregnancy. This study also made fuller assessments of the HPA axis and demonstrated significantly higher plasma CRH in depressed women in the 2nd trimester. Cortisol was measured in another study which assessed antenatal depression and foetal growth restriction; compared with the non-depressed group, depressed women had higher levels of cortisol (Diego et al., 2009).

The difference in methodology adopted by O'Keane and by Hellgren might further explain the difference in the results of the two studies. It is possible to speculate that face-to-face interview, as administered by O'Keane, is more effective in detecting maternal psychopathology compared with the web-based evaluations by Hellgren.

1.5.5 HPA axis in women with a history of childhood abuse and antenatal depression

A study by Bublitz and colleagues, investigated the association between childhood abuse and the HPA axis in a sample of pregnant women (Bublitz and Stroud, 2012). Specifically, the sample comprised 30 women with history of childhood sexual abuse, 58 women with history of non-sexual abuse and 47 healthy controls with no history of abuse. Participants collected salivary cortisol at awakening, +30 minutes and bedtime for three consecutive days during the second and third trimester of their pregnancy. Women with history of childhood sexual abuse displayed higher cortisol levels at awakening compared with women in the other two groups (Bublitz and Stroud, 2012). A study of Canadian pregnant women, investigated the effects of depression and early childhood maltreatment on cortisol awakening response during pregnancy. The sample comprised 33 depressed women and 33 healthy women between week 25 and 33 of gestation. Cortisol response was measured at awakening and at +30 and +60 minutes thereafter. Results showed that cortisol awakening response was not different between women with antenatal depression and those with no diagnosis. However, those with history of childhood maltreatment showed an attenuated cortisol awakening response (Shea et al., 2007). While my Thesis will replicate some of these investigations, by assessing awakening and diurnal cortisol in pregnancy, it will also extend these findings by focusing on history of childhood abuse and by adding measurement of HPA axis in the baby and correlating the HPA axis results with a range of behavioural measures.

1.6 Interim summary

For the research reviewed in this section, we can see that a history of physical, emotional, sexual abuse and neglect in childhood can have detrimental long-lasting effects into adulthood. These effects include a greater risk of developing psychopathology such as anxiety and depression later in life together with a greater predisposition to neuroendocrine alterations, such as the hyper activation of the hypothalamic-pituitary-adrenal (HPA) axis, which leads to an abnormal response to stress. These changes are particularly manifested in vulnerable periods of life, such as when a woman is about to become a mother herself. In fact, women who experienced depressive symptomatology in pregnancy are seen to have a biological vulnerability and alteration of the stress response that, in turn, may contribute to an alteration in the intrauterine environment. These changes in the biological system of the foetus are one of the potential mechanisms that underlie the intergenerational transmission of vulnerability to psychopathology.

This initial review of the literature on childhood abuse and depression shows that there is a strong association between childhood experience of physical, sexual and emotional abuse and neglect with the hyperactivity of the HPA axis, which is also considered to be part of the pathogenesis of depression.

In the next section I will explore the effects of maternal childhood abuse and antenatal depression on the intrauterine environment (foetal programming hypothesis) and the infant behavioural outcomes and biological response to stress in the first year.

1.7 The fetal programming hypothesis

There is increasing evidence that adult vulnerability to diseases is programmed during foetal life and shaped by the environment in utero (Duthie and Reynolds, 2013). Findings from a series of studies on a British cohort that demonstrated that the lower the birth rate the higher the risk from dying of heart disease later in life, led Barker to

hypothesise that coronary heart disease, type 2 diabetes, stroke and hypertension, originate in developmental plasticity, in response to under nutrition during foetal life (Barker et al., 1993). In his pioneer work, he found that foetal exposure to under nutrition in utero leads to changes in the foetus metabolism and organ structure (Barker, 1997). In fact, children of mothers with low weight during pregnancy (under nutrition, less than 45 kilos) were more prone to develop coronary heart disease in adult life compared with children of mothers without nutrition problems (Barker, 1997). Further human and animal studies have corroborated Barker's original findings but the molecular mechanisms have not yet been clarified (Smith et al., 2011).

Studies investigating the Dutch Hunger Famine (1944-1945), explored the effects of the exposure to maternal under nutrition during gestation on the offspring outcomes in later life, between age 50 and 60 (de Rooij et al., 2006). Seven hundred participants, half of whom were exposed to the famine while in utero, were invited to perform a computerized stress test, which comprised a colour-word conflict challenge (Stroop test), a mirror test and a speech test. In the mirror test, participants were asked to trace a star that could only be seen in the mirror; a beep sound would appear when there was a divergence from the circuit of the start and the line of star. In the speech test, people were asked to imagine a scenario where they were falsely accused of pickpocketing and to prepare a 3 minute speech to give in front of a camera to prove their innocence. During this procedure, salivary cortisol was collected at 7 time points between the baseline period and after the completion of the test. Despite an increase in the cortisol levels after the end of the test, results showed no group difference in cortisol response between those who were exposed to famine and those who were not exposed to such condition in utero. This study suggested that exposure to famine in utero does not have an association with an altered neuroendocrine profile in adulthood, although the stress test used in the study might not have been good enough to provoke an activation of the HPA axis.

Further evidence on the magnitude of big phenomena in pregnancy and its long-lasting effects on the offspring, such as the Dutch Famine, has been provided by a longitudinal study that investigates the effects of in utero exposure to a natural disaster. The ice storm study looks at the effects of the natural disaster occurred in Quebec in 1998 on later child outcome and development (King et al., 2012). More than two-hundred pregnant women were recruited in the study and assessed for mental health and HPA axis functioning and children were seen up to age 13. Results show that babies of women who experience high levels of stress during the exposure to natural disaster have slower physical growth rate at birth and impaired cognitive development and language at 5 ½ years, as show in lower IQ levels (Laplante et al., 2008). Furthermore, results from a follow-up at age 12, show that there is a great association between exposure to stress in utero and later immune-deficiencies in those children. In fact, children of mothers experienced high stress levels following the natural disaster had greater chances to develop asthma and chronic use of corticosteroids, but this condition was found only in girls (Turcotte-Tremblay and Lim, 2014). Taken together, the Quebec Ice Storm project supports the foetal programming hypothesis that the antenatal environment programs the infant outcome from a developmental but also an immunological prospective.

1.7.1 HPA axis in offspring of mothers depressed in pregnancy

More recently it has been hypothesized that maternal HPA axis alterations during pregnancy expose the foetus to higher levels of stress and that this might affect the foetal HPA axis (Duthie and Reynolds, 2013). A study by Davis and Sandman (2010) assessed maternal HPA axis in pregnancy by measuring plasma at 15, 19, 25, 31 and 36 weeks gestation and maternal stress, anxiety and depression. Infants' HPA axis was measured 24 hours after birth by collecting a hair sample from the baby. An evaluation of the behavioural response to the hair pull was also observed. They found that the

infant's cortisol response to stress was associated with maternal high levels of stress and higher cortisol in pregnancy (Davis et al., 2011). Moreover, infants of mothers with high stress found it more difficult to regulate their behaviour after the hair was removed, compared with infants of non-stressed mothers (Davis et al., 2011). This research suggests that infant capacity to regulate their stress is indeed influenced by maternal stress response, although no causal relationship has been demonstrated.

There is evidence to suggest that exposure to maternal stress in pregnancy goes far beyond the infancy period as is suggested by the foetal programming hypothesis. Indeed evidence from a study by Ashman et al., (2002) shows that exposure to maternal depression from pregnancy to the first two years of the infant's life predicts infant cortisol response following a stress test at 8 years (Ashman et al., 2002). Women were interviewed retrospectively about their symptoms of depression during pregnancy and the postnatal period when their infants were 14 months old, 24 months old, 3.5 years old, 4.5 years old, 6.5 years old and 8 years old. Children were invited for a laboratory stress task, during which they were administered the fear-potentiated startle paradigm. This procedure allows measuring the reflexes and anxiety levels experienced by the child when s/he anticipates the fear related to an aversive stimulus. Specifically, the threatening stimulus involved an intense noise that the child would perceive as unsafe (Ashman et al., 2002). Infant cortisol levels were measure prior and after the administration of the stress test. The authors concluded that children of depressed mothers had a more reactive hormonal stress system compared with children of healthy mothers. Furthermore there is evidence showing that children of mothers who experienced stress during pregnancy were more likely to develop emotional or cognitive problems, such as attention deficit, hyperactivity, anxiety and language delay compared with children of mothers who were not stressed in gestation (Talge et al., 2007). It has been suggested that this association is accounted for by changes in the foetal environment seen when exposed to maternal stress (Talge et al., 2007).

Further work by Glover and colleagues, offers substantial evidence that maternal stress in pregnancy has permanent effects on the infant neurodevelopmental system (Bergman et al., 2007). In her work, Glover found that women with higher cortisol levels in pregnancy, due to maternal stress, are strongly associated with greater cortisol levels in the amniotic fluid, which is an indicator of the foetal exposure to maternal environment (Sarkar et al., 2007). More than two hundred women were recruited and assessed for stress and depression in pregnancy. Maternal cortisol was assessed through maternal blood, which was drawn immediately before the amniocentesis procedure, during which foetal cortisol was collected (Glover et al., 2009). Results show that there is a very robust association between maternal plasma cortisol and foetal amniotic fluid cortisol among women who experienced high stress in pregnancy, suggesting that the emotional state in pregnancy can not only affect women's biological profile but also the functioning of the placenta, which moderates foetal exposure to maternal environment. This, in turn, increases the potential high risk of compromising the infant's later development (Sarkar et al., 2007). Despite accumulating evidence on the mechanisms that allow the transmission of stress from mother to baby, little is known about which stresses in pregnancy are the most detrimental for the infant and when in pregnancy is the most critical time for the transmission of maternal stress through the placenta (O'Donnell et al., 2009).

A further study showed that the maternal HPA axis during pregnancy had a strong effect on the newborn's regulation of his own HPA axis (Smith et al., 2011), with evidence to suggest that the neonatal HPA axis was associated with maternal HPA axis at the time of delivery (Smith et al., 2011). Two hundred women were recruited on the basis of their current psychiatric diagnosis at 14 weeks gestation: of those, 67.5% met the criteria for depression, while 20% of women had anxiety disorders. The remaining 10% had another diagnosis. Maternal and infant cortisol profile was obtained at birth, with a blood test on the mother and the umbilical cord blood for the infant. After

controlling for delivery methods, results show that maternal cortisol levels in pregnancy are predictive of infant cortisol levels at birth.

These findings further support the foetal programming hypothesis suggesting that there in an intergenerational transmission of the HPA axis functioning and that offspring whose mothers had been stressed in pregnancy are themselves compromised in that they may have difficulties in regulating their own HPA axes making them vulnerable to the adverse consequences of stressful life experiences. Again, my Thesis will extend these findings by investigating how these HPA axis change may be related to maternal history of childhood abuse.

1.7.2 HPA axis in offspring of mothers abused in childhood

The majority of studies focus on the effects of childhood abuse on the adult HPA axis, and the effects of maternal childhood abuse on offspring physiological regulation remain mainly unexplored. A study by Brand et al (2010) recruited 126 women in the postpartum period with a history of major depression. Maternal childhood abuse was assessed using the Childhood Trauma Questionnaire (Bernstein et al., 1994). In order to examine the effects of maternal childhood abuse on the mothers' and children's physiological response, the authors measured salivary cortisol in a laboratory stress paradigm at 6 months postpartum (Brand et al., 2010). During the laboratory stressor the infant was placed in a car seat and the mother was placed behind a screen out of the infant's view; the mother could see her baby on a TV monitor. The stressor was a noise burst and an arm restraint task experienced by the infant. Saliva samples were collected (1) before from mothers and infant at baseline, when they were still together in the same room; (2) after 20 minutes when they were separated; (3) immediately after the stressor; (4) 20 minutes later. Results suggest that infants of mothers abused in their own childhoods had lower cortisol at baseline compared with infants of healthy controls mothers. This might suggest an impaired baseline cortisol response in the offspring of

mothers who had themselves experienced trauma in their own childhoods (King et al., 2001). However, infant cortisol response after the stressor was not associated with a maternal history of childhood abuse but infants whose mothers had both a history of childhood abuse and were depressed had a greater cortisol response after the stressor compared with children of mothers in the healthy group (Brand et al., 2010). Overall, the study suggests a biological vulnerability that exposes women with abuse in early life to develop depressive symptoms and this vulnerability is seen in the child's increased response to stress.

A study on parents who survived the Holocaust and their adult offspring, showed that those exposed to severe traumatic experiences of abuse have an increased rate of post-traumatic stress disorder (PTSD) compared with a healthy control group (Yehuda et al., 2001). Researchers assessed the offspring of those parents with PTSD by measuring their psychopathology and HPA axis functioning in adulthood. Results showed that offspring of parents that were abused in the Holocaust had higher urinary cortisol levels and a higher level of reported PTSD symptoms as adults compared with the offspring in the control group (Yehuda et al., 2001). The authors believe that traumas experienced in the parent might lead to a transmission of altered stress response in the offspring, resulting in a vulnerability to increased psychopathology (Yehuda et al., 2001).

To my knowledge, our research is the first to investigate the impact of early life stresses in the mother's own childhood on her mood in pregnancy and the biological correlates between the maternal and offspring stress response.

1.8 Developmental and behavioral outcomes

1.8.1 Offspring of mothers depressed in pregnancy

The earliest studies on infants of depressed mothers focussed on mother-infant interaction (Field et al., 2006). Most studies show that maternal depression has long-term effects on child's social and emotional development and on the mother-infant

relationship (Tronick and Reck, 2009). Some neurobehavioral studies show that infants of depressed mothers have greater right frontal electroencephalogram (EEG) activation compared with infants of non-depressed mothers, which is associated with negative affect, depression and poor capacity to regulate emotions (Field et al., 2006). Moreover, as depression is associated with an abnormal biological profile (such as elevated cortisol and norepinephrine), we might expect that infants of depressed women who have been exposed to an abnormal prenatal environment might themselves have an abnormal response to stress. In fact, neonates of depressed mothers show a physiological profile that mimics their mother's prenatal profile, including cortisol alterations (Field et al., 2006). In a study by Lundy et al. (1999), cortisol was measured in depressed and non-depressed women at the end of their pregnancy and 24 hours after delivery, together with infant cortisol. The findings suggest that maternal cortisol in pregnancy predicts infant cortisol after birth (Lundy et al., 1999).

Newborn behaviour has been widely assessed using the Neonatal Behavioural Assessment Scale (NBAS) (Brazelton and Nugent, 1995). Field has extensively studied neonatal behaviour in relation to maternal psychopathology, especially depression in pregnancy. Data from a study in 2006 suggests that babies of women who experienced depression during pregnancy showed sub-optimal performance on the NBAS within a few hours of birth, compared with babies of non-depressed women. Neonates of depressed mothers showed higher levels of irritability, lower activity levels, were less robust and less mature compared with neonates of non-depressed mothers (Field et al., 2006). A review by Field (Field et al., 2009a) reported that new-borns of depressed mothers were also less responsive to voices and faces, showed higher arousal levels and were less attentive compared with neonates of non-depressed mothers. These findings have been further confirmed by Hernandez (Hernandez-Reif et al., 2006), who found that babies of depressed mothers had more difficulties in learning facial and vocal expressions compared with babies of healthy women. Furthermore, the authors showed

that neonates of depressed mothers took twice as long as babies of healthy mothers to habituate to their mother's face and voice, finding it harder to distinguish their mother's face and voice from that of a stranger (Hernandez-Reif et al., 2006). Field (Field et al., 2002) also investigated the effects of anger and depression during pregnancy on the baby's behaviour. One hundred and sixty-six women were classified as experiencing low or high anger in pregnancy: the latter also had high levels of antenatal depression. Babies were assessed at 2 days after birth with the NBAS (Brazelton and Nugent, 1995). Results showed that babies of depressed and high anger women had less optimal orientation, motor organization and state regulation capacities compared with babies of non-anger and non-depressed women. Another study by Field and colleagues compared neonates of depressed versus non-depressed women on the NBAS supplementary items (Hernandez-Reif et al., 2006). Interestingly, neonates of depressed mothers had lower scores in their orientation to animate visual and auditory stimuli (face and voice of the examiner) and were less alert than babies of non-depressed women. In addition, neonates of depressed mothers had lower scores in cuddliness and hand to mouth items, suggesting that they were less able to be soothed and to self-soothe than neonates of non-depressed women.

The literature shows that the effects of exposure to maternal depression in utero extend further than the immediate post birth period. Indeed there is now a wealth of studies showing that the effects extend into adolescence. Offspring of mothers depressed in the antenatal period are at greater risk than those whose mothers were well in pregnancy of becoming depressed themselves in adolescence (Pawlby et al., 2009). The same study showed that exposure to maternal antenatal depression also predicts violence and antisocial outcomes in the offspring (Hay et al., 2010). Moreover, exposure to antenatal depression was also found to increase offspring maltreatment (Pawlby et al., 2011).

More evidence on the long-term effects of the exposure to maternal depression/stress in pregnancy is observed in the Avon Longitudinal Study of Parents and Children

(ALSPAC). This study involved pregnant women, partners and their infants living in the area of Avon, in the UK, initially assessed in 1990 and followed-up thereafter.

A cross-cohort study that comprises 3442 participants from the ALSPAC sample and 2280 participants from Generation R study sample, looks at the effects of maternal depression in pregnancy, assessed respectively at 18 and at 20 weeks gestation, on infant emotional regulation at age 4 (Van Batenburg-Eddes et al., 2013). Both studies found a direct association between maternal depressive symptoms during pregnancy and child attention problems at age 4, which was independent of other psychosocial confounders (Van Batenburg-Eddes et al., 2013). These findings further support the idea of foetal programming as a potential pathway for the transmission of stress from mother to baby.

Similarly, a study by O'Connor and colleagues uses the ALSPAC sample, which comprises more than seven thousand women, to explore the effects of maternal anxiety and depression during pregnancy on child outcomes at age 4 (O'Connor et al., 2002). Maternal mental health in pregnancy (anxiety and depression) was assessed at 18 and 32 weeks' gestation, and in the postnatal period at 8 weeks, 8 months, 21 months and 33 months. Infant behavioural and emotional regulation was evaluated at 4 years, with specific focus on conduct problems, emotional problems and hyperactivity/inattention (O'Connor et al., 2002). Findings suggest that antenatal anxiety/stress late in pregnancy has a very strong association with hyperactivity/inattention in children at age 4, even when controlling for antenatal and postnatal anxiety and depression. A follow-up study (O'Connor et al., 2003) showed that maternal high anxiety/stress in the late stage of pregnancy remains associated with infant behavioural and emotional problems in children at 81 months, even when controlling for maternal depression and other psycho-social factors (O'Connor et al., 2003).

In order to cast further light on the potential trajectories that lead to the development of emotional problems in infants exposed to maternal depression in utero, a longitudinal

study by Halligan and colleagues assessed 121 pregnant women and their children up to age 5. Women were assessed for anxiety and depression in pregnancy at 28 and 34 weeks gestation as well as in the postpartum period at 10 days, 4 weeks and 12 weeks infant age, and 12 months, 18 months and 5 years after birth. (Halligan et al., 2013). Infant behavioural regulation was first assessed at 10 days and 4 weeks after birth using the Neonatal Behavioural Assessment Scale (Brazelton and Nugent, 1995). The Bayley Scales of Infant Development, Second Edition (Bayley, 1993) was administered to the infants at 12 and 18 months, while mothers were asked to complete the Behaviour Screening Questionnaire (Richman and Graham, 1971) as a measure of the child behavioural problems. A final assessment was done when the child was five years old, using a novel assessment called "buzz wire", a difficult task in which children are asked to move a hoop along a wire without touching it, otherwise it would buzz and a red light would appear as an indicator that the child would have to restart the task. The examiner, who would make the red light appear four times in each trial, irrespectively of the child performance, controlled this last part of the experiment. This last procedure showed the child's regulation strategies during the frustration period. This study found that the child's behavioural regulatory capacities were stable from the neonatal period to age 5, when children of depressed mothers showed externalizing problems (Halligan et al., 2013). Second, maternal psychological adversities were associated with poorer parenting capacities, which, in turn, led to poorer behavioural regulation for the offspring (Halligan et al., 2013).

1.8.2 Children of mothers abused in childhood

Over the last decade there have been a number of studies that have shown that children of mothers abused in childhood have greater emotional disturbances, overall psychological distress, hyperactivity and behavioural problems compared with children of non-abused mothers (Dubowitz et al., 2001).

The Avon Longitudinal Study of Parents and Children (ALSPAC) recruited 13,000 pregnant women in the 1990s into a prospective longitudinal study of mothers and their children. Among many other measures women were asked about their experiences of childhood abuse. Findings showed that a maternal history of child abuse predicted offspring adjustment problems at age 4 and 7 (Collishaw et al., 2007). Specifically, a dose-response relationship was found, indicating that the more severe the childhood abuse experienced by the mothers, the more severe the adjustment problems experienced by the child (Collishaw et al., 2007).

Similar findings come from a longitudinal study by Pawlby et al (2011) in South London. They investigated the links between the mother's experience of childhood maltreatment, depression in pregnancy and offspring maltreatment (Pawlby et al., 2011). The children were assessed at 11 and 16 years for maltreatment and psychopathology. Results showed that children of mothers who experienced antenatal depression had a greater risk of experiencing maltreatment in childhood (Pawlby et al., 2011). Offspring of mothers who experienced *both* maternal childhood maltreatment *and* antenatal depression were exposed to significantly greater levels of childhood maltreatment and exhibited significantly higher levels of adolescent antisocial behaviour compared with offspring not so exposed. (Plant et al., 2013). Furthermore, maternal childhood maltreatment accounted for a significant proportion of the variance in offspring childhood maltreatment in only those offspring exposed to depression *in utero*.

Further evidence for the intergenerational association between a maternal history of childhood abuse and adverse offspring outcome comes from a 23-year longitudinal study that shows that children of mothers who have been abused in childhood have greater risk of maltreatment compared with children of non-abused mothers (Trickett et al., 2011).

A recent population-based longitudinal study of nurses taking part in the Nurses' Health Study in the USA investigated the effects of maternal childhood abuse on offspring outcomes. There was a dose-response relationship between the severity of abuse and 10-year-old offspring autism (Roberts et al., 2013). Findings showed that a maternal history of combined emotional, physical and sexual abuse was associated with greater risk for autism in the offspring.

1.9 Aims and hypothesis

1.9.1 Aims of the study

The literature reviewed above has shown evidence for an association between maternal experience of abuse in her childhood and the development of depression during adulthood, particularly in vulnerable periods such as during pregnancy. However the majority of studies reported in the existing literature are mainly of correlational design, indicating the existence of associations between variables rather that a causal relationship.

It is also established that depression during pregnancy leads to poor outcome for the offspring. However, no prospective study has yet found evidence of an association between maternal experience of abuse in childhood and poor behavioural and physiological outcome for the infant offspring, nor investigated the potential mechanisms involved in this transmission.

The primary aim of the study is to investigate whether offspring exposure to antenatal maternal depression predicts infant behavioural and physiological regulatory response at 6 days, 8 weeks and one year and whether a mother's experience of childhood abuse cumulatively adds to this effect.

The second aim of this study is to explore the biological associations that are linked with alterations in maternal HPA axis functioning during pregnancy among women who are depressed and/or have experienced childhood abuse. The purpose of this investigation is to gain a better understanding of whether dysregulation in the maternal stress axis accounts for changes in the offspring HPA axis, neonatal behaviour and infant development to one year. These investigations will help clarify the intergenerational transmission of stress.

1.9.2 Hypotheses

Maternal experience of childhood abuse and antenatal depression

Maternal experience of childhood abuse (birth to 17 years) is associated with depression in pregnancy.

HPA axis in pregnancy

Depressed women will have an increased cortisol response in pregnancy (25 and 32 weeks gestation) compared with women who were not depressed.

Depressed women exposed to abuse in childhood will have greater cortisol response (25 and 32 weeks gestation) than depressed women who were not abused. Furthermore, women who were not depressed in pregnancy and were exposed to childhood abuse will have a blunted cortisol response (25 and 32 weeks gestation) compared with those who are healthy and have not been abused.

Neonatal behavioural regulation at 6 days

Babies of women who have been exposed to maternal depression in utero will show behavioural dysregulation at 6 days after birth.

Maternal experience of abuse in her own childhood will interact with maternal antenatal depression and impact on the baby's behavioural dysregulation at 6 days.

Neonatal physiological regulation at 6 days

Babies of women who have been exposed to maternal depression in utero will have a higher cortisol response to the stress of NBAS administration at 6 days

Maternal experience of abuse in her own childhood will interact with maternal antenatal depression and impact on the baby's physiological response to stress at 6 days.

Infant physiological regulation at 8 weeks

Babies of women who have been exposed to maternal depression in utero will have a higher cortisol response to the stress of vaccination at 8 weeks.

Maternal experience of abuse in her own childhood will interact with maternal antenatal depression and impact on the baby's physiological response to stress at 8 weeks.

Infant development at one year

Children of women who have been exposed to maternal depression in utero will have lower scores on the Bayley scales of infant cognitive and socio-emotional development.

Maternal experience of abuse in her own childhood will interact with maternal antenatal depression and impact on the children's cognitive and socio-emotional developmental scores at one year.

Infant physiological regulation at one year

Children of women who have been exposed to maternal depression in utero will have a higher cortisol response to the stress of vaccination at one year.

Maternal experience of abuse in her own childhood will interact with maternal antenatal depression and impact on the child's physiological response to stress at one year.

Antenatal maternal HPA axis and offspring behavioural and physiological functioning

Maternal HPA axis functioning in pregnancy accounts for the differences in offspring behavioural and physiological regulation at 6 days, 8 weeks and one year.

2 Methods

2.1 Design

This thesis is part of the Psychiatry Research And Motherhood (PRAM) study, a prospective, longitudinal, observational study of depressed and non-depressed women and their offspring. The study was set up in 2007, with the purpose of assessing whether maternal alterations of the HPA axis in pregnancy associated with depression are correlated with altered HPA axis in the babies of these pregnancies throughout the first year of life.

2.1.1 Contribution of work by the candidate

I was responsible for recruiting and assessing the pregnant women. I have also conducted follow-up assessments of women in the postnatal period and performed the Neonatal Behavioural Assessment on the babies at 6 days after birth and been responsible for obtaining infant cortisol samples at 6 days, 8 weeks and 12 months. Furthermore, I was responsible for administering the Childhood Experience of Care and Abuse Questionnaire to the women in the study.

Dr Patricia Zunszain (Lecturer at the IoPPN, King's College London) carried out all the salivary cortisol analyses. Dr Sarah Osborne (Visiting Research Associate, IoPPN, KCL) and Sue Conroy (Senior Researcher, IoPPN, KCL) have contributed to the rating of the psychiatric interviews. Trained research assistants helped with some of the interviews. I conducted the statistical analyses under the supervision of Dr Susan Pawlby (Secondary Supervisor, Lecturer at the IoPPN, KCL) and Professor Carmine Pariante (Primary Supervisor, Professor at the IoPPN, KCL).

2.2 Sample

Women recruited into the study were aged between 18 and 50 years, with a singleton pregnancy. A total of 125 women were recruited into the study.

Of those, 67 had a current DSM-IV diagnosis of Major Depressive Disorder of at least moderate severity and 58 were free from psychiatric disorders during their lifetime. Women with obstetric complications, chronic medical conditions (pulmonary, cardiac, autoimmune, endocrine), those who were taking medication (except nutritional supplement) at the time of recruitment, those unable to communicate in English and those with a psychiatric history other than affective or anxiety disorder were excluded from the study.

2.3 Procedure

Ethics

Full Ethical approval for the study was given by King's College Hospital Research Ethics committee (REC ref 07/Q0703/48).

Participant recruitment

Participants were identified and recruited through the Obstetric Ultrasound Department at the Harris Birthright Centre and the Perinatal Liaison Psychiatry Services, both at King's College Hospital in London.

1. King's College Hospital (KCH) Ultrasound Department: Women attending for antenatal ultrasound at approximately 20 weeks gestation were approached by me or a colleague and invited to participate in the study. Names and addresses were taken and women were told that a researcher would contact them by telephone to tell them more about the study.
2. The Perinatal Psychiatry clinical liaison team at KCH: A weekly meeting reviews referrals from midwives (who screen all pregnant women for past and current mental health problems) and general practitioners. PRAM researchers have regularly attended the referrals meeting and potentially suitable women were identified and invited to take part in the study.

Potential participants were then contacted by telephone and screened for past and current mental health as well as physical health. Researchers provided a full description of the study procedure and, to those who fulfilled the study criteria and who expressed an interest in the study, a detailed study information sheet (Appendix A) was sent to their home. The women were informed, in particular, of the longitudinal nature of the study and how, if they agreed to take part, the researchers would like to visit them twice in pregnancy, when the baby was born and then twice thereafter, when the baby was 8-weeks and 12 months old. Those who were willing to take part in the study were booked for the baseline assessment at 25 weeks gestation.

Before commencing the interview, women were given an informed consent form to sign (Appendix B), to guarantee confidentiality and data protection. All clinical interviews were audio-recorded and discussed with the lead clinician of the team, for the agreement of the final diagnosis.

Participants received £10 for each assessment, a total of £40 by the end of the study and thanked for their time and commitment to it.

Table 3. Outline of the study assessments

25 week gestation	32 week gestation	Birth of the baby	6 days postnatal	8 weeks postnatal	1 year postnatal
Maternal socio-demographics	Maternal mood		Neonatal behavior	Maternal mood	Maternal mood
Maternal mood	Maternal IQ		Neonatal saliva cortisol	Infant cortisol	Infant cortisol
Maternal childhood abuse	Maternal saliva cortisol		Obstetric and birth details		Infant development
Maternal cortisol					

Antenatal clinical assessment: 25 and 32 weeks gestation

The baseline assessment was carried out at 25 week gestation and comprised a structured clinical interview. Women were also asked about socio-demographic characteristics and about their own childhood experiences.

Full data on the Childhood Experience of Care and Abuse Questionnaire were collected from 104 women. Specifically, retrospective questions on childhood family arrangements, physical abuse and sexual abuse were collected from a face-to-face interview from 116 women during pregnancy. The remaining 9 women refused to be contacted for the completion of the questionnaire. Additional questions related to psychological abuse and neglect during childhood were administered after the interview in pregnancy. All women in the sample were sent the additional questions by post and information was obtained from 104 participants. Specifically, 27 women were interviewed in person, 42 were asked the questions by phone, 27 returned the questionnaire by post, 8 returned it by email and 21 did not return the questionnaire. Among those 21, 10 women were no longer in the study, 9 were impossible to contact and 2 had moved out of the country.

Eighty-two women were administered the Wechsler Test of Adult Reading (WTAR).

Antenatal cortisol assessment: 25 and 32 weeks gestation

At the end of the clinical interview, women were provided with two sets of six Salivette swabs (Sarstedt, Leicester, UK) and asked to collect saliva samples the day following the baseline assessment and again at 32 weeks gestation.

Women who were living far from King's College Hospital were provided with a stamped envelope and asked to return samples by post, while for those who were local were contacted by a team member who arranged a home sample collection. All saliva samples were then stored in a -20C freezer before being analysed.

6 days after birth

At 6 days after birth, a researcher trained in the administration of Neonatal Behavioural Assessment Scale (NBAS), but unaware of the mother's clinical diagnosis, visited the home to conduct the assessment. Mothers were asked where possible to have fed the baby before the arrival of the researcher. Mothers were informed that the assessment would be looking at the newborn's abilities to interact with the examiner and how we would be looking at the way that the baby responded to being undressed and handled. The mother was asked that if the baby cried she would let the assessor allow him or her to cry for 15 seconds before using a graded set of responses in order to calm the baby. The NBAS was started only once the mother had given her verbal consent. Before starting the NBAS procedure, the researcher took a salivary sample from the baby. A further sample was taken immediately after the NBAS and then again 30 minutes later. If the baby fed at all during any part of the assessment, the researcher made sure that the salivary sample was not taken until 15 minutes had elapsed after the end of the feed, in order to prevent contamination of the sample by the milk.

Obstetric and birth details were taken from the mother's discharge summary from the hospital notes.

8 week postnatal maternal assessment

Maternal clinical assessment

The eight weeks postnatal assessment also comprised a structured clinical interview, as for the antenatal assessment.

Infant neuroendocrine assessment at 8 weeks postpartum

Infant saliva samples were collected at the time of the routine immunisation at 8-weeks post-partum. The researcher arranged to accompany the mother to the clinic and collected one saliva sample from the baby a few minutes before the injection and another exactly 20 minutes after the injection. The researcher sits inside the clinic with the mother, the infant and the nurse to ensure that the time of the injection is properly recorded on the assessment sheet and to note any other important information, such as if

the mother was present, how long the baby cried for. Mothers were asked not to feed the baby after the injection and wait 20 minutes before doing so; in cases where the baby was fed, we waited 15 minutes after the end of the feeding before collecting the second sample of saliva.

One-year postpartum assessment

Maternal clinical assessment at 1 year post-partum

Women were contacted by a researcher a few weeks before the baby's first birthday in order to arrange the one-year visit. Babies were also sent a birthday card, as way of keeping participants engaged in the research study.

At the one year assessment, women were administered the same clinical interview and questionnaires as for the assessment at 8 weeks postpartum.

Infant neuroendocrine assessment at 1 year post-partum

We measured the infant stress using the same procedure as for the 8 weeks postnatal assessment. A researcher accompanied the mother and her baby to the immunisation clinic and saliva was collected before and twenty minutes after the immunisation.

Infant developmental assessment at 1 year post-partum

Participants were contacted again two weeks after the immunisation by a researcher who was unaware of the maternal diagnosis. The researcher visited the participant's home in order to assess the baby's developmental stage.

2.4 Measures

2.4.1 Maternal depression

At 25 weeks gestation, 8 weeks and 1 year postpartum, an operational diagnosis of depression was made using the *Structured Clinical Interview for DSM-IV Axis I Disorders Non-patient Edition* (First et al., 2002) (Appendix C), a widely used semi-structured diagnostic interview to assess DSM-IV Axis I psychiatric disorders. The interview consists of standardized diagnostic questions arranged in modules

corresponding to each Axis I disorder. The mood disorder module of the SCID-I takes about 1 to 2 hours to be completed, depending on the complexity of the psychopathology, on the experience of the assessor and on the patient's capacity to properly describe her situation.

2.4.2 Maternal experience of childhood abuse

Measures of maternal sexual abuse, physical abuse, emotional abuse and neglect were obtained using the Childhood Experience of Care and Abuse questionnaire (CECA-Q) (Bifulco et al., 2005) (Appendix D) and scores were based on experiences occurred in childhood from age 0 to 17. Scores were dichotomized according to the cut-off A points published by Bifulco (Bifulco et al., 2005). This cut-off had the highest reliability with the CECA interview.

Maternal and paternal antipathy scores from 8 to 24 were recoded into 0 (no or minimal maternal/paternal antipathy), while the scores greater than 25 were recoded into 1 (severe maternal/paternal antipathy). After the total scores for maternal and paternal antipathy were calculated, emotional abuse was rated if either maternal or paternal antipathy scored 1 (0= no or minimal emotional abuse; 1 = emotional abuse).

Maternal neglect scores from 8 to 21 were recoded into 0 (no or minimal maternal neglect), while scores greater than 22 were recoded into 1 (severe maternal neglect). In the same way, paternal neglect scores from 8 to 23 were recoded into 0 (no or minimal paternal neglect), while scores greater than 24 were recoded into 1 (severe paternal neglect). If severe neglect was experienced from either parents, neglect was rated as 1 (0 = no or minimal neglect; 1 = neglect).

Physical abuse was rated as a sum of the responses to the four main questions on physical experience before age 17: (i) if the hitting from either parent happened on more than one occasion (1), only once (0); (ii) if hit with a belt or stick (1), if hit with hand or other (0); (iii) if ever injured, black eyes or broken limbs (1), if not (0); (iv) if the person

was so angry that they seemed out of control (1), if not (0). If the child experienced any form of physical abuse (1) from either parent the criteria for physical abuse would be met (0 = no or minimal physical abuse; 1 = physical abuse).

Sexual abuse was rated on the basis of the unwanted sexual experience before age 17. A total score for sexual abuse was made by the sum of the answers to the items: (i) the abuser was someone known (1), not known (0); (ii) the abuser was a relative (1), not a relative (0); (iii) the abuse happened in more than one occasion (1), no (0); (iv) the abuser touched private parts of the body (1), no (0); (v) the abuse involved intercourse (1), no (0). If the child experienced any form of unwanted sexual experience (1), the criteria for sexual abuse would be met (0 = no or minimal sexual abuse; 1 = sexual abuse).

If the child experienced any antipathy, neglect, physical abuse or sexual abuse, the criteria for childhood abuse would be met.

2.4.3 Maternal cognitive assessment

Women's cognitive performance was assessed in pregnancy with the Wechsler Test of Adult Reading (Wechsler, 1939) (WTAR), a neuropsychological assessment tool that provides a measure of the premorbid intelligence and intellectual functioning (Holdnack, 2001). Specifically, the WTAR comprises 50 irregularly spelt words and it takes about 10 minutes to complete. Participants were audio recorded during the assessment and scored to a maximum of 50 by a native English-speaker. Scorings take into account the participant's age and level of education.

2.4.4 Saliva samples analysis

Salivary cortisol was measured through the collection of saliva samples. Participants were asked to collect six saliva samples during one day at 25 weeks gestation, as close as possible to the baseline assessment, and during another day at 32 weeks gestation, following a reminder from one of the researchers. Cortisol was collected at awakening

(ideally before 10 am), +15 minutes, +30 minutes, +60 minutes, at midday and at 8 pm during the same day. Women were asked not to brush their teeth, not to drink coffee or tea and not to eat before the sample collection within the first hour after awakening and at least for the 30 minute preceding the midday and 8 pm collections.

The time of the cortisol collection varied and, following the recommendations of Dr Patricia Zunszain, samples were excluded when the following criteria were not met: interval from awakening actual time to awakening sample collection is equal to or less than 5 minutes; +15 sample collected between 10 and 20 minutes after awakening; +30 sample collection between 20 and 40 minutes after awakening; +60 sample collection between 45 and 75 minutes after awakening. Maternal and infant saliva samples were analysed using a standard commercial enzyme linked immune-sorbent (ELISA; Salimetrics, Newmarket, UK) (appendix I) by Dr Patricia Zunszain. SoftMax Pro 4.8 software was used to calculate the cortisol values, following a 4-parameter fit. The analytical sensitivity was set to 0.33 nmol/l. Inter and intra-assay co-efficients of variations ranged from 8% to 11% and 6% to 10%, respectively, so only results into this range were considered in the analysis. Cortisol results have been screened by senior researchers working on the project (Dr Sarah Osborne and Sue Conroy, with the additional help of Dr Patricia Zunszain) and cortisol data have been entered into the databases and double checked by two researchers.

2.4.5 Maternal socio-demographic characteristics

Maternal ethnicity

Information on maternal ethnicity has been recorded during the baseline interview at 25 week pregnant. A dichotomized variable of “white” versus “black and minorities” was created.

Maternal education

Information on maternal education achievements have been obtained during the baseline interview at 25 weeks antenatal interview. A dichotomized variable of “GCSE or lower” versus “A level or higher” has been created.

Maternal employment status

Information on maternal employment status at baseline has been recorded during the baseline interview at 25 week pregnant. A dichotomized variable of “working outside the home” versus “not working outside the home” was created.

Maternal classification of employment

Information on maternal employment classification has been obtained during the baseline interview at 25 week pregnant. A dichotomized variable of “professional or managerial” versus “not professional or managerial” was created.

Marital status

Information on maternal marital status has been obtained during the baseline interview at 25 week pregnant. A dichotomized variable of “married or cohabiting” versus “single +/- partner” was created.

2.4.6 Neonatal behaviour at 6 days after birth

The Neonatal Behavioural Assessment Scale (NBAS), also known as the Brazelton Neonatal Assessment Scale (Brazelton and Nugent, 1995) , was developed in 1973 by paediatrician Dr. T. Berry Brazelton and his colleagues at Harvard University. Dr Brazelton believed that new-born babies are organized and responsive already shortly after birth. In fact, they are capable of responding to stimuli, of interacting with the environment and the caregiver, and of adapting to challenges. The NBAS was created in order to formulate a comprehensive profile of the baby, including infants’ individual differences and neonatal general functioning, between 3 days and 4 weeks after birth.

The NBAS offers a profile that describes the baby's strengths, adaptive responses and possible vulnerabilities. It has been widely used both in research and in clinical setting,

where it has been used as a therapeutic tool to help parents to get to know their baby more, to develop appropriate caregiving strategies and to promote their earliest relationship with their child. It has also been used to evaluate babies who suffered intrauterine deprivation, exposure to maternal substance abuse in pregnancy, and other perinatal conditions and, in more general terms, to identify concerns about the baby.

The NBAS comprises five main packages, which refer to different areas of baby's functioning, and assesses the infant behavioural repertoire on 28 behavioural items, each scored on a 9 point scale. It also offers an assessment of the baby's neurological status using 18 reflex items, each scored on a 4 point scale.

The assessment starts with an observation of the child's initial state. An important item of the NBAS is the state regulation. Babies go through six main states: state 1, fast asleep with slow and breathing and eyes fully closed, no spontaneous activity; state 2, asleep with faster and irregular respiration and some minimal eye movements observed under closed eyes, random body movements or startles; state 3, drowsy, with eyes at times open, response to sensory stimuli; state 4, the ideal state, baby is alert and responsive, eyes are bright open and the activity is minimum; state 5, fussy state, baby is not quiet but the cry is not robust, eyes are open and body movements are intense; state 6, baby's cry is robust and difficult to calm down, intense body movement.

The social-interactive cluster measures baby's alertness and baby's capacity to respond to inanimate and animate visual and auditory orientations. Specifically, alertness refers to how the infant is aware of the environment and brightens his eyes while looking around. Inanimate visual auditory stimuli refer to the infant's capacity to follow a red ball and turn to the sound of a rattle during the assessment, while animate auditory and visual stimulation is obtained by the examiner's face and voice eliciting a response from the child. This section measures how much babies can fixate their attention on an object (ball or rattle) or a face and to follow it horizontally or vertically with 30, 60 and 90 degrees head and eyes movements. It also measures the quality of the infant response:

how alert is the child when the stimuli are presented, how much he brightens, how the respiration and facial expression changes, and the eyes widen.

The motor system cluster assesses the infant's general tone, the motor maturity, activity levels during the assessment, the pull-to-sit and defensive movements. Specifically, this section assesses the baby's motor response, both spontaneous and elicited; if the movements are smooth and jerky, if the arm movements are 30, 45 or 90 degrees in the upper quadrant of the body, if he is able to bring his head up and the body tone (floppy versus tense).

The state organization scale includes: peak of excitement, the overall amount of activity and arousal shown during the assessment; rapidity of build-up, at which time point during the examination the baby starts crying; irritability, the number of time the child gets upset or frustrated; lability of states, the number of state changes observed during the examination.

The state regulation cluster comprises: cuddliness, the infant's response to being hold while alert; consolability, the manoeuvers the examiner has to put in place in order to bring the child to a quite state; self-quieting, the attempts the child make to calm himself down; hand into mouth, the attempts the child makes to insert the hands into mouth.

The autonomic system cluster items assess the central nervous system of the baby by counting the number of tremors and startles observed, and the lability of skin colour throughout the examination.

Some other supplementary items are evaluated during the NBAS: quality of alertness, which is the overall quality of infant responsiveness during the assessment; cost of attention, how hard it is for the baby to maintain attention and be responsive; examiner facilitation, how hard the examiner has to work in order to elicit from the child a good response; general irritability, infant's general response to mild and moderate aversive stimuli; robustness and endurance, the energy and strengths shown during the

assessment; state regulation, the ability of the infant to regulate his states, from sleeping to crying, and to move from one state to another without signs of disorganization; examiner's emotional response, how the examiner feels about the child.

The NBAS also assesses some basic reflexes: plantar, Babinski, ankle clonus, rooting, sucking, glabella, passive resist arms and legs, palmar hand grasp, placing, standing, walking, crawling, incurvation, tonic deviation of head and eyes, nystagmus, tonic neck reflex and Moro reflex.

The Habituation package of the NBAS is administered when the infant is asleep, ideally in states 1 or 2, and it assesses how the infant habituates to disturbing stimuli, such as the sound of a bell or a rattle, as well as a light on the eyes. This package allows an observation of the infant's capacity to shut out intrusive and negative stimuli.

One key concept that examiners keep in mind while performing the NABS is that with the NBAS we aim to get the best performance out of the baby.

At the end of the examination, we also record the first and second predominant state during the NBAS, which gives an idea of how the child has been throughout the examination; e.g. he spent most time being withdrawn and difficult to engage (state 2-3), or difficult to calm (states 5-6).

Figure 2 and 3. Neonatal Behavioural Assessment Scale administration

Since the items in each cluster of the NBAS were highly correlated, not all items were included in the analyses. Specifically, I have used baby's alertness and animate auditory (social-interactive), irritability (state organization), motor maturity (motor system), cuddliness and consolability (state regulation), tremulousness (autonomic system), and all the supplementary items. I did not use baby's reflexes as the motor maturity is already an indicator of the infant motor system regulation.

At the end of the NBAS, the researcher recorded the baby's gender, gestational age, birth weight, and the mother's parity from the hospital discharge notes.

2.4.7 Infant neuroendocrine assessment at 6 days after birth

Infant cortisol was measured as an indicator of the infant response to stress (HPA axis functioning). Saliva samples were collected from babies before, immediately after and 30 minutes after the administration of the Neonatal Behavioural Assessment Scale. The saliva collection was done using the Salimetric's Children Swabs (SCS, Salimetrics Europe Ltd, Suffolk, UK) (Appendix G) and then stored into the Salivette tubes and kept at -20 degrees. The SCS were placed into the infant's mouth for about 90 seconds, passing it under the tongue and in the cheeks in order to absorb as much saliva as possible and ensure optimal saturation. This procedure is not painful for the infant.

Infant neuroendocrine assessment at 8 weeks

As for 6 days, infant saliva collection was done using the Salimetric's Children Swabs (SCS, Salimetrics Europe Ltd, Suffolk, UK) (Appendix H). The SCS was placed into the infant's mouth for about 90 seconds, passing it under the tongue and in the cheeks in order to absorb as much saliva as possible and ensure optimal saturation. Once collected, samples were stored into -20 degrees freezers.

2.4.8 Infant cognitive and social-emotional development at one year

The Bayley Scales of Infant Development (Bayley, 2005) are a standard measure of infant social, emotional, cognitive, language and motor development. The scale

consists of a series of developmental play tasks and its administration takes between 45-60 minutes. Specifically, the Cognitive, Language, Motor Scales are completed by the examiner, while the Social-Emotional and Adaptive Behaviour Scales are completed by the caregiver.

The Social-Emotional Scale assesses the child mastery of self-regulation and interest in the world, his capacity to communicate and engage with others while establishing relationships, and to use his emotional skills in problem solving (Bayley, 2005). Caregivers are asked to complete questions about their child's attention capacity, how easy is he to calm down and engage, how he responds to other with sounds and how he interacts.

The Adaptive Behaviour Scale assesses the daily functions of a child; measuring what the child actually does and his potential.

It comprises ten subscales that assess different skills: Communication, which looks at the child's speech, language, listening and nonverbal skills; Community Use, his interest in activities outside the home environment and his capacity to recognize familiar places; Functional Pre-Academics, his capacity to recognize letters, to count and draw simple shapes; the Home Living, if the child helps adults with the house tasks and takes care of personal possessions; the Health and Safety; his capacity to recognize danger and keep safe; Leisure, the child's capacity to play, follow rules, and engage in pleasurable activities at home; Self-Care skills, the child's eating, toileting and bathing capacities; Self-Direction, his capacity to self-control, to follow directions and make choices; Social skills, the child capacity to get along with people, to use manners, to assist others, recognize emotions and seek friendships; Motor skills, the child locomotion and manipulation of the environment.

Raw scores of successfully completed items are converted to age-correlated scale scores and the sum of scale scores are converted into composite scores with a standardised

mean of 100 and standard deviation of 15. The composite scores allow for a comparison of the child's performance against typically developing children of that age.

Infant neuroendocrine assessment at 1 year

As for 6 days and 8 weeks postnatal, infant saliva was collected using the Salimetric's Children Swabs (SCS, Salimetrics Europe Ltd, Suffolk, UK) (Appendix H). The SCS was placed into the infant's mouth for about 90 seconds, passing it under the tongue and in the cheeks in order to absorb as much saliva as possible and ensure optimal saturation. Once collected, samples were stored into -20 degrees freezers.

2.5 Data analysis

Data were analyzed using the SPSS Statistical Software, Version 20.0 (IBM Ltd, Portsmouth, UK). Data normality was assessed using Kolmogorov-Smirnov test and Levene's test for homogeneity of variance. I have applied Log Transformation where it was needed to improve the normality of the data. Data that were not normally distributed after the Log Transformation were treated with non-parametric statistics (the non-transformed values were reported in the tables).

Descriptive statistics are shown as mean, standard deviation (SD), and percentages (%). In the graphs, the Standard Error of the mean is also reported (*SE*). Within the univariate analysis, the independent sample T-test was used to compare the means of parametric continuous variables between two groups. ANOVAs were used in group comparisons for parametric data. Mann-Whitney test was applied to non-parametric continuous data (z scores are reported) and Kruskal-Wallis test was used for group comparisons. Pearson's chi-square test (X^2) test for independence was used to analyze categorical data. In situations where the cell count was less than five, Fisher's exact test was applied. Correlations were used to test the association between continuous variables; specifically Person's correlation was applied in parametric data, while

Spearman's correlation was applied in non-parametric data. General Linear Model procedures were used to control for potential confounders.

Generally, statistic values are reported with one decimal place. Exceptions are made when two or more decimal places would reveal important information about the data. P values > .05 are reported at two decimal places, while those <.05 are reported at three. Significant results in the tables are highlighted in **bold** and, in the figures, the asterisk (*) will indicate the significance level. In the tables, maternal depression in pregnancy is presented as "MDD only", maternal childhood abuse as "CA only", while both conditions of maternal depression and childhood abuse as "MDD & CA".

In this Thesis, I will conduct multiple statistical tests. In particular, I will use Analysis of Variance (ANOVA) to compare four groups of women over time (before and after birth) and their babies' outcomes (also in four groups), controlling for potential confounders using Generalized Linear Model procedures. As the data are not normally distributed, Kruscal-Wallis test has been used to compare the four independent samples and the p values have been corrected according to the number of group comparisons made, in order to avoid an inflation of false positive results. When conducting multiple statistical tests with a modest sample size there is a greater risk of obtaining p values less than 0.05 purely by chance, even if the null hypotheses are really true. Using Bonferroni corrections (for parametric data) and pairwise comparisons (for non-parametric data) enables to control the family wise error rate. Hence, instead of setting the p value to the standard 0.05, a lower critical value is used. Specifically, the most common way of controlling for type I errors is to divide the familywise error rate (usually 0.05) by the number of tests performed. Thus, when I conduct 4 statistical tests, the critical value for an individual test would be 0.05/4=0.0125 and only individual tests with a p value <=0.0125 are considered to be significant.

3 Results

3.1 Part 1: Maternal depression and experience of childhood abuse

3.1.1 Overview

The primary aim of the first set of analyses was to investigate the hypothesis that abuse during childhood increases the risk of developing depression during pregnancy. The secondary aim was to test whether depression alters the HPA functioning of women in pregnancy and then whether an experience of abuse in childhood moderates this association.

First, I describe the sample by presenting the socio-demographic characteristics at 25 weeks gestation. Next, I examine the association between maternal childhood abuse and maternal depression in pregnancy. I then compare the impact of exposure to childhood abuse and of current depression on maternal salivary cortisol levels at 25 weeks and 32 weeks gestation.

3.1.2 Descriptive analyses

Sample characteristics at 25 week gestation

One hundred and twenty-five pregnant women participated in the study. Of those, 58 (46.4%) were healthy controls, free from mental health diagnosis and 67 (53.6%) were cases, with a current DSM-IV diagnosis of Major Depressive Disorder (single episode, code 296.2x or recurrent, code 296.3x).

Basic maternal socio-demographic characteristics at 25 weeks gestation are shown in Table 4. The majority of women (65.6%) were of white ethnic origin and their mean age was 31.12 years. Most women (78.4%) pursued a higher education and were employed (62.4%). More than half of the women (65.6%) were married or cohabiting and the majority were primiparous (81.6%).

Table 4. Socio- demographic characteristics of the sample at 25 weeks gestation

Mother's age (years)	
Mean (S.D.)	31.1 (6.0)
Range	18-46
Maternal ethnicity (%)	
White	82 (65.6)
Black and minority	43 (34.4)
Maternal qualifications (%)	
GCSE or lower	27 (21.6)
A level or higher	98 (78.4)
Maternal employment status (%)	
Working outside the home	78 (62.4)
No working outside the home	47 (37.6)
Marital status (%)	
Married or cohabiting	82 (65.6)
Single or with a partner living out	43 (34.4)
Parity (%)	
Primiparous	102 (81.6)
Multiparous	23 (18.4)

Socio-demographic characteristics at 25 week gestation: healthy and depressed women

The socio-demographic characteristics across diagnostic groups are shown in Table 5. Women with depression were significantly more likely to have achieved lower education level, being unemployed, being single, being multiparous and having lower IQ. Indeed, there were significant differences in the educational level of women with and without depression in pregnancy ($X^2(1) = 13.8$; $p<.001$). Specifically, 65.7% of the women with antenatal depression obtained A levels or higher education, compared with 93.1% in the control group. There were also significant differences in the current employment status of women with and without depression in pregnancy ($X^2(1) = 6.4$; $p=.012$). 47.8% of the women with depression were not working, compared with 25.9% in the control group. Similarly, there were also significant differences in marital status between women with and without depression in pregnancy ($X^2(1) = 20.4$; $p<.001$). Specifically, 52.2% of the women with antenatal depression were single, compared with 13.8% of the control group. Furthermore, there were significant differences between women with and without depression in pregnancy in their parity ($X^2(1) = 4.7$; $p=.03$). The proportion with women who were multiparous was higher among the depressed group (25.5%) than the control group (10.3%). There were also differences in the IQ scores as measured by the WTAR ($t(67.53)=2.8$; $p=.007$), with women with depression having significantly lower scores ($M = 109$, $SD = 13.1$) compared with women who were not depressed ($M = 98.8$, $SD = 19.6$).

Table 5. Socio-demographic characteristics in healthy and depressed women at 25 weeks gestation

Variable	**Healthy** N=58	**MDD** N=67	**Statistic**
Mother's age (years)			
Mean (S.D.)	31.7 (4.6)	30.7 (7.0)	t(114.61)=1.0; p=.33
Range	21-40	18-46	
Maternal ethnicity (%)			
White	43 (74.1)	39 (58.2)	$X^2(1)=3.5$; p=.06
Black and minority	15 (25.9)	28 (41.8)	
Maternal qualification (%)			
GCSE or lower	4 (6.9)	23 (34.3)	$X^2(1)=13.8$; **p=<.001**
A level or higher	54 (93.1)	44 (65.7)	
Maternal employment status (%)			
Working outside the home	43 (74.1)	35 (52.2)	$X^2(1)=6.4$; **p=.012**
No working outside the home	15 (25.9)	32 (47.8)	
Marital status (%)			
Married or cohabiting	50 (86.2)	32 (47.8)	$X^2(1)=20.4$; **p<.001**
Single or partner living out	8 (13.8)	35 (52.2)	
Maternal parity (%)			
Primiparous	52 (89.7)	50 (74.6)	$X^2(1)=4.7$; **p=.031**
Multiparous	6 (10.3)	17 (25.4)	
Maternal WTAR[a]			
Mean (S.D.)	109 (13.1)	98.8 (19.6)	t(67.53)=2.8; **p=.007**

[a] For WTAR: Healthy women n=42; Depressed women n=40

Childhood abuse

A history of childhood abuse was obtained from 109 women (87.2%) in the sample. Sixteen women did not complete the Childhood Experience of Care and Abuse Questionnaire. Of those who completed the questionnaire 51 women (46.8%) did not experience childhood abuse, while 58 (53.2%) experienced some form of childhood abuse. Of those who had been abused, 26 (44.8%) women experienced sexual abuse, 20 (34.5%) experienced severe physical abuse from at least one parent, 38 (65.5%) experienced severe neglect from at least one parent and 40 (69.0%) experienced antipathy from at least one parent.

3.1.3 Antenatal depression and maternal exposure to childhood abuse

There was a strong association between antenatal depression and childhood abuse ($X^2_{(1)}$=21.7; p<.001, OR=6.9, 95% CI [2.96, 15.98]). Among women with antenatal depression, 44 (71.3%) women experienced some form of sexual abuse, physical abuse, neglect or antipathy during childhood whereas in the control group only 14 (28.6%) women experienced some form of abuse (Table 6). Compare with women who had not experienced abuse in childhood, women who had been abused were almost 7 times more likely to become depressed in pregnancy.

Table 6. Childhood abuse in healthy and depressed women

Groups	**No childhood abuse**	**Any childhood abuse**	Total
Healthy (%)	35 (71.4)	14 (28.6)	49 (100)
Depressed (%)	16 (26.7)	44 (73.3)	60 (100)
Total (%)	51 (46.8)	58 (53.2)	109 (100)

$X^2_{(1)}$=21.7; p<.001, OR=6.9, 95% CI [2.96, 15.98]

Socio demographic characteristics at 25 week gestation: healthy, depressed and women with childhood abuse

Given the strong association between childhood experience of abuse and depression in pregnancy, further analyses compared the socio-demographic characteristics across the four groups: healthy women, free from mental health diagnosis and child abuse history (HEALTHY, N=35); women with a current diagnosis of major depressive disorder (MDD only, N=16); women abused in childhood but free from any mental health diagnosis during their lifetime (CA only, N=14); women with a current diagnosis of MDD and a history of childhood abuse (MDD & CA, N=44). Findings are presented in Table 7. Women with MDD & CA achieved lower education levels, were single, and had lower IQ scores. Indeed, significant differences were found between the 4 groups of women in the level of educational qualifications obtained by the women ($X^2(3)=20.3$; $p<.001$) and in their marital status ($X^2(3)=25.2$; $p<.001$). 59.1% of the women with MDD & CA had qualifications of A level or above compared with 97.1% (HEALTHY), 87.5% (MDD only) and 92.9% (CA only). 40.9% of the women with MDD & CA were in a married or cohabiting relationship compared with 88.6% of the HEALTHY women, 68.8% MDD only and 92.9% CA only. There was also a significant group difference in the women's IQ scores as measured by the WTAR ($F(3)=3.6$; $p=.018$). Tamhane post-hoc tests shows that MDD & CA women had significantly lower IQ scores ($M = 97.2$, $SD = 20.8$) compared with CA only women ($M = 113.1$, $SD = 12.1$).

Table 7. Socio-demographic characteristics in women with/without depression and/or childhood abuse at 25 weeks gestation

Variables	**Healthy** N=35	**MDD only** N=16	**CA only** N=14	**MDD & CA** N=44	**Statistic**
Mother's age (years)					
Mean (S.D.)	32.5 (4.7)	32.1 (6.1)	31.1 (3.8)	30.3 (7.5)	F(3)=1.0;
Range	21-40	19-42	24-37	18-46	p=.42
Maternal ethnicity (%)					
White	28 (80.0)	12 (75.0)	11 (78.6)	26 (59.1)	X^2(3)=4.9;
Black and minority	7 (20.0)	4 (25.0)	3 (21.4)	18 (40.9)	p=.19
Maternal qualification (%)					
GCSE or lower	1 (2.9)	2 (12.5)	1 (7.1)	18 (40.9)	X^2(3)=20.3;
A level or higher	34 (97.1)	14 (87.5)	13 (92.9)	26 (59.1)	**p<.001**
Maternal employment status (%)					
Working outside the home	25 (71.4)	11 (68.8)	10 (71.4)	22 (50.0)	X^2(3)=4.9; p=.19
Not working outside the home	10 (28.6)	5 (31.2)	4 (28.6)	22 (50.0)	
Marital status(%)					
Married/cohabiting	31 (88.6)	11 (68.8)	13 (92.9)	18 (40.9)	X^2(3)=25.2;
Single or with partner living out	4 (11.4)	5 (31.2)	1 (7.1)	26 (59.1)	**p<.001**
Parity (%)					
Primiparous	31 (88.6)	15(93.8)	13(92.9)	32(72.7)	X^2(3)=6.5;
Multiparous	4 (11.4)	1 (6.2)	1 (7.1)	12 (27.3)	p=.08
Maternal WTAR[a]					
Mean (S.D.)	109.3 (12.7)	104.5 (16.3)	113.1 (12.1)	97.2 (20.8)	F(3)=3.6; **p=.02**

[a] For WTAR: Healthy n=28; MDD only n=8; CA only n=10; MDD & CA n=30

3.1.4 Maternal depression and HPA axis

The HPA axis activity was measured at 25 weeks and 32 weeks gestation, with the aim of further understanding the physiological responses of women with current depression and women who had been abused during childhood.

At 25 weeks gestation, valid cortisol data were obtained from 26-27 healthy women and 46-48 depressed women. Among the participants who collected all cortisol samples at each time point at this stage of pregnancy, data are available for 25 healthy and 42 depressed women. Due to a change in the study protocol, at 32 weeks gestation, a larger number of women have cortisol only at awakening and 8 pm (n=91), than at awakening, 12 pm and 8 pm (n=50).

Descriptive analyses: HPA axis at 25 weeks gestation

The cortisol awakening response (CAR) was indexed through the measurement of the maternal salivary cortisol at the time of awakening (before 10 am), and at 15+, 30+ and 60+ minutes after awakening (Figure 3). Cortisol was also collected at 12 pm and 8 pm in order to index the cortisol levels during the day. Table 8 illustrates the mean cortisol levels throughout the day in healthy and depressed women, while Table 9 illustrates the mean cortisol levels throughout the day in women with/without depression and/or childhood abuse.

As it can be seen from Figure 3, the mean cortisol level upon awakening was 10.3 (SD=4.4) nmol/L in healthy women and 12.2 (SD=11.9) nmol/L in depressed women. Cortisol levels rose during the first 15 minutes after awakening to a mean peak of 11.8 (SD=5.0) nmol/L in healthy women and 14.6 (SD=17.4) nmol/L in depressed women. Cortisol continued increasing in both groups at 30 minutes post-awakening (healthy: M = 12.0 (SD=5.7) nmol/L; depressed: M = 13.7 (SD=13.7) nmol/L and started decreasing at 60 minutes after awakening, where both controls and cases reached a similar mean

cortisol level (healthy: M = 10.7 (SD=3.7) nmol/L; depressed: M = 10.2 (SD=6.0) nmol/L. As can be seen from Figure 5, at 12 pm, cortisol levels in both groups continued dropping, remaining slightly lower in healthy women: M = 6.0 (SD=2.5) nmol/L; depressed: M = 7.7 (SD=6.2) nmol/L. As expected based on the diurnal cycle, at 8 pm, salivary cortisol reaches lower levels compared to that observed during the day, with M = 3.2 (SD=2.0) nmol/L in healthy women and M = 3.7 (SD=3.5) nmol/L in depressed women.

There were no significant differences between healthy and depressed pregnant women in their awakening response or in the cortisol levels at midday or in the evening at 25 weeks gestation.

A similar pattern (no differences) was found in the awakening response, cortisol levels at midday and in the evening at 25 weeks gestation when comparing healthy women, those with MDD only, CA only women and those with MDD & CA. Mean cortisol levels are presented in Table 9, alongside the statistical values.

Table 8. Maternal depression and salivary cortisol at 25 weeks gestation

Cortisol time collection	**Healthy** N=26-27	**MDD only** N=46-48	**Statistic**
Awakening Mean (SD)	9.9 (4.5)	11.8 (11.3)	Z=.1; p=.9
Awakening+15 minutes Mean (SD)	11.6 (5.1)	14.3 (16.7)	Z=-.6; p=.57
Awakening+30 minutes Mean (SD)	11.8 (5.7)	13.6 (13.2)	Z=-.1; p=.89
Awakening+60 minutes Mean (SD)	10.2 (4.0)	10.1 (6.3)	Z=-.7; p=.51
Midday Mean (SD)	6.0 (2.5)	7.5 (5.9)	Z=-.4; p=.66
8 pm Mean (SD)	3.2 (2.0)	3.7 (3.4)	Z=-.1; p=.93

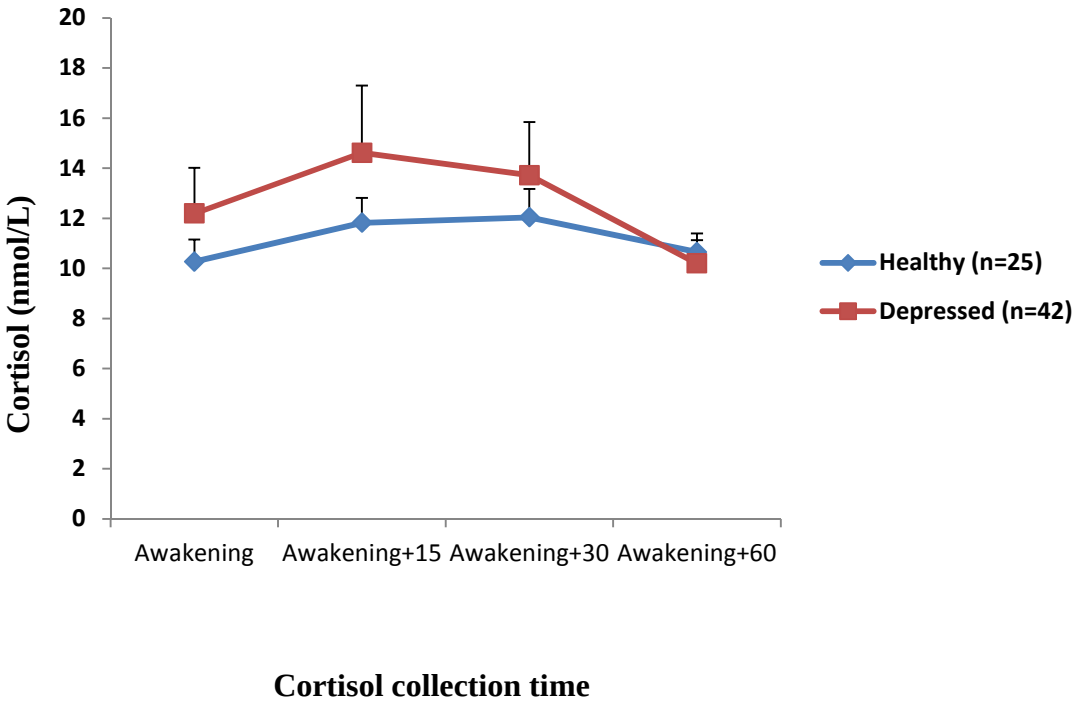


Figure 3. 25 weeks maternal awakening response, with *SE*

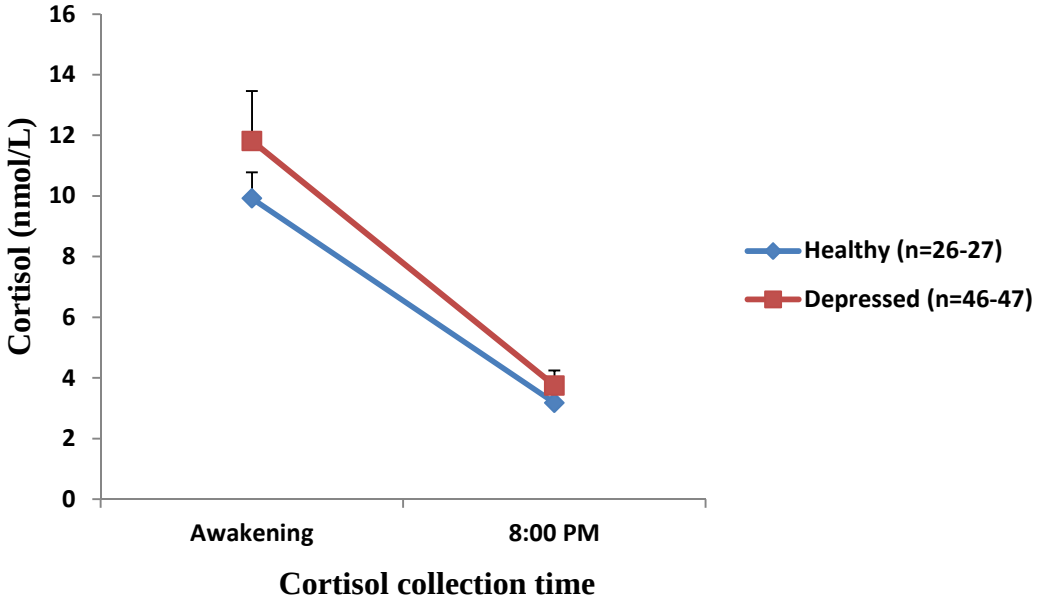


Figure 4. 25 weeks salivary cortisol at awakening and 8 pm in healthy and depressed women, with *SE*

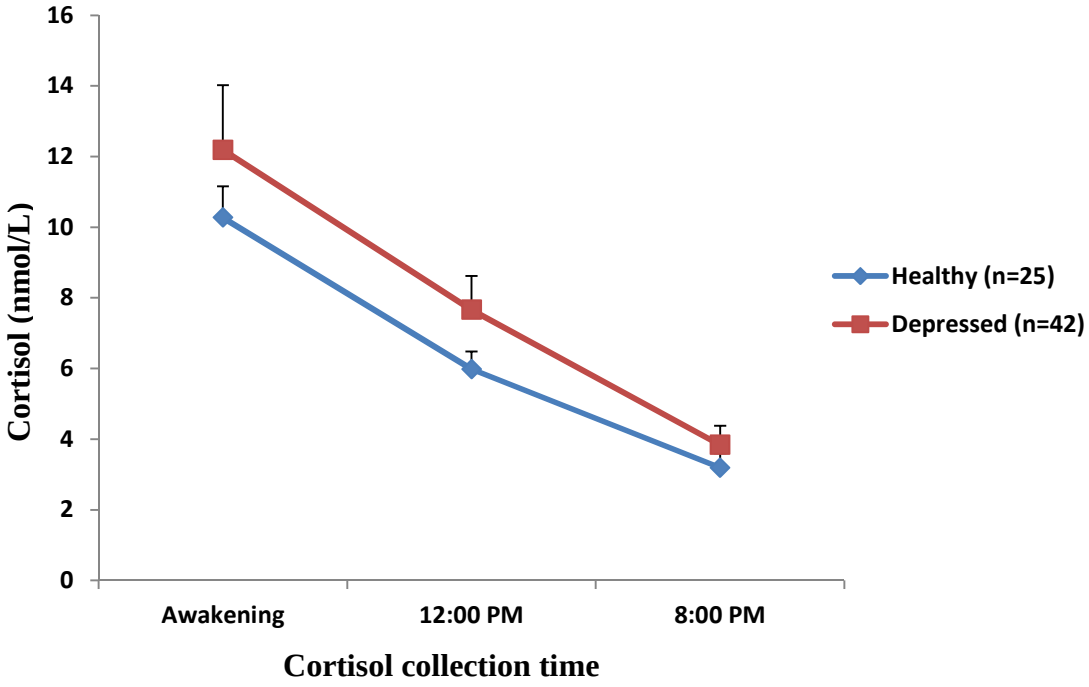


Figure 5. 25 weeks salivary cortisol at awakening, 12 pm and 8 pm in healthy and depressed women, with *SE*

Table 9. Salivary cortisol at 25 weeks gestation in women with/without antenatal depression and/or childhood abuse

Cortisol time collection	**Healthy** N=19-20	**MDD only** N=10-11	**CA only** N=4	**MDD & CA** N=33-35	**Statistic**
Awakening Mean (SD)	9.9 (4.5)	13.7 (4.8)	11.5 (4.4)	11.2 (12.9)	K-$W_{(3)}$=6.2;p=.11
Awakening+15 minutes Mean (SD)	12.2 (4.8)	19.2 (17.3)	11.6 (4.0)	12.6 (17.0)	K-$W_{(3)}$=4.6;p=.21
Awakening+30 minutes Mean (SD)	12.5 (6.1)	12.5 (5.8)	10.7 (3.8)	14.0 (15.2)	K-$W_{(3)}$=.4; p=.94
Awakening+60 minutes Mean (SD)	10.5 (4.2)	12.4 (6.8)	10.8 (2.7)	9.7 (6.1)	K-$W_{(3)}$=2.5; p=.48
Midday Mean (SD)	5.9 (2.7)	9.6 (6.5)	7.1 (2.0)	6.8 (5.8)	K-$W_{(3)}$=5.1;p=.16
8 pm Mean (SD)	3.0 (2.0)	2.9 (1.4)	3.8 (2.7)	3.9 (3.8)	K-$W_{(3)}$=.4; p=.95

Maternal depression and salivary cortisol 32 weeks gestation

At 32 weeks gestation, salivary cortisol was available from 56 healthy women and from 49 depressed women.

As for 25 weeks gestation, the cortisol awakening response (CAR) was indexed through the measurement of the maternal salivary cortisol at the time of awakening (before 10 am), and at 15+, 30+ and 60+ minutes after awakening. Cortisol was also collected at 12 pm and 8 pm in order to index the cortisol levels during the day. The mean cortisol values have been calculated at each time points at 32 weeks gestation and plotted in the graphs below. It is of note that, because of a change in protocol after the initial period of recruitment, a larger number of women have cortisol only at awakening and 8 pm (n=105) than at awakening, 12 noon and 8 pm (n=55) or indeed throughout the awakening response curve (n=55). Results of these samples are presented separately in Figures 6, 7 and 8, but together in Table 11.

Figure 6 illustrates the cortisol awakening response (CAR) in healthy and depressed women, while Figure 7 illustrates the mean cortisol levels at awakening, 12 pm and 8 pm in healthy and depressed women in women who have all the three time points. As can be seen from Figure 6, the mean cortisol level upon awakening was 10.2 (SD=3.8) nmol/L in healthy women and 11.2 (SD=6.0) nmol/L in depressed women. Cortisol levels rose during the first 15 minutes after awakening to a mean of 12.0 (SD=4.6) nmol/L in healthy women and 13.4 (SD=13.8) nmol/L in depressed women. Cortisol increased up to 30 minutes post-awakening, 13.0 (SD=5.4) nmol/L in healthy women and 13.0 (SD=6.3) nmol/L in depressed women, and started dropping at 60 minutes after awakening, where cortisol in both controls and cases reach a very similar level to those observed upon awakening (healthy: 11.6 (SD=5.0); nmol/L; depressed: 11.7 (SD=6.9) nmol/L)

In Figure 7, cortisol levels decreased in both healthy and depressed women through the morning, reaching a mean of 7.8 (SD=2.8) nmol/L in healthy women and a mean of 8.2

(SD=5.6) nmol/L in depressed women at 12 pm. Cortisol levels further decreased through the rest of the day to a mean level of 4.5 (SD=2.8) nmol/L in healthy women, and a mean of 8.0 (SD=14.4) nmol/L in depressed women at 8 pm. In this smaller sample of women who have all the three time-points, the 8 pm cortisol levels is higher in depressed women, but the difference is not statistically significant.

Figure 8 represents the awakening and 8 pm cortisol values in the larger sample (n=105) where there is data only at these two time points. The mean awakening and 8 pm cortisol values were similar in this larger sample compared with the smaller one in Figure 7. Specifically, at awakening, the mean cortisol level in healthy women was 9.2 (SD=3.7) nmol/L and in depressed women 11.1 (SD=5.7) nmol/L. At 8 pm, cortisol levels in healthy women decreased to a mean of 3.6 (SD=3.3) nmol/L while in depressed women they only decreased to a mean of 7.1 (SD=11.8) nmol/L. However, because of the larger power, the difference in the 8 pm salivary cortisol now reached significance, with depressed mothers having higher cortisol levels than control mothers ($t_{(54.49)}$ =-2.0; p=.05).

Means and statistical values of all the data (irrespective of sample size), and the statistics, are also presented in Table 11.

To quantity further the cortisol indexes across groups (healthy, n=24; depressed, n=36), the area under the curve (AUC) has been calculated for each participant across the first hour after awakening, and during the day (at awakening, 12 pm and 8 pm). First, the total AUC was calculated on the basis of the actual time points, the ground (AUCg), and subsequently on the basis of the awakening cortisol levels, with respect to its increase (AUCi). The mean AUCg for cortisol levels across the first hour after awakening was 715.2 (SD=264.8) nmol/L in healthy women and 746.0 (SD=428.6) nmol/L in depressed women. The mean for AUCi in the first hour after awakening was 107.9 (SD=179.3) nmol/L in healthy women and 70.2 (SD=247.7) nmol/L in depressed

women. Overall, these results offer a further index for the CAR, which can be interpreted as the natural response to stress during the day across diagnostic groups.

There was no significant difference in AUCg and AUCi between healthy and depressed women (respectively, t(58)= -.31; p=.76; t(58)= .64; p=.53).

Next, the AUC was also calculated during the day. The mean cortisol secretion during the day among healthy women was 5372.9 (SD=1626), nmol/L while in depressed women it was 6588.1 (SD=5263) nmol/L. There was no significant difference between the two groups (t(54)=-1.1; p=.29).

Socio-demographic confounders

Earlier it was shown that at 25 weeks gestation maternal depression was significantly associated with maternal marital status, parity, maternal qualifications and employment (page 67, Table 5). However, none of these potential confounders was found to be associated with maternal salivary cortisol levels at 25 weeks gestation.

Table 10. Association between maternal socio-demographics and maternal 32 weeks salivary cortisol at 8 pm

Potential confounders	**8 pm maternal cortisol (nmol/L) at 32 weeks**	**Statistics**
Marital status mean (SD)		
Married/cohabiting	5.2 (9.7)	t(103)=-.03; p=.98
Single or Partner living out	5.3 (5.4)	
Parity mean (SD)		
Primiparous	5.2 (9.1)	t(103)=-.1; p=.92
Multiparous	5.4 (5.4)	
Qualifications mean (SD)		
GCSE or lower	5.7 (5.9)	t(103)=.3; p=.77
A level or higher	5.1 (9.1)	
Employment mean (SD)		
Working outside the home	3.9 (3.3)	t(37.1)= -1.6; p=.11
Not working outside the home	7.7 (13.7)	

Table 11. Salivary cortisol at 32 weeks in women with and without depression

Cortisol time collection	**Healthy**	**MDD**	**Statistic**
Awakening[a] Mean (SD)	9.2 (3.7)	11.1 (5.8)	Z=-1.3; p=.2
Awakening+15 minutes[b] Mean (SD)	11.6 (4.6)	12.9 (12.5)	Z=-.4; p=.72
Awakening+30 minutes[c] Mean (SD)	12.4 (5.4)	12.2 (6.71)	Z=-.5; p=.6
Awakening+60 minutes[d] Mean (SD)	11.3 (4.8)	11.9 (6.6)	Z=-.2; p=.87
Midday[e] Mean (SD)	7.5 (3.1)	8.4 (5.4)	Z=-.3; p=.8
8 pm[f] Mean (SD)	3.6 (3.3)	7.1 (11.9)	Z=-3.2; **p=.002**

[a] healthy, n=56 ; depressed, n= 49

[b] healthy, n=25 ; depressed, n= 40

[c] healthy, n=26 ; depressed, n= 44

[d] healthy, n=25 ; depressed, n= 43

[e] healthy, n=25 ; depressed, n= 42

[f] healthy, n=56 ; depressed, n= 49

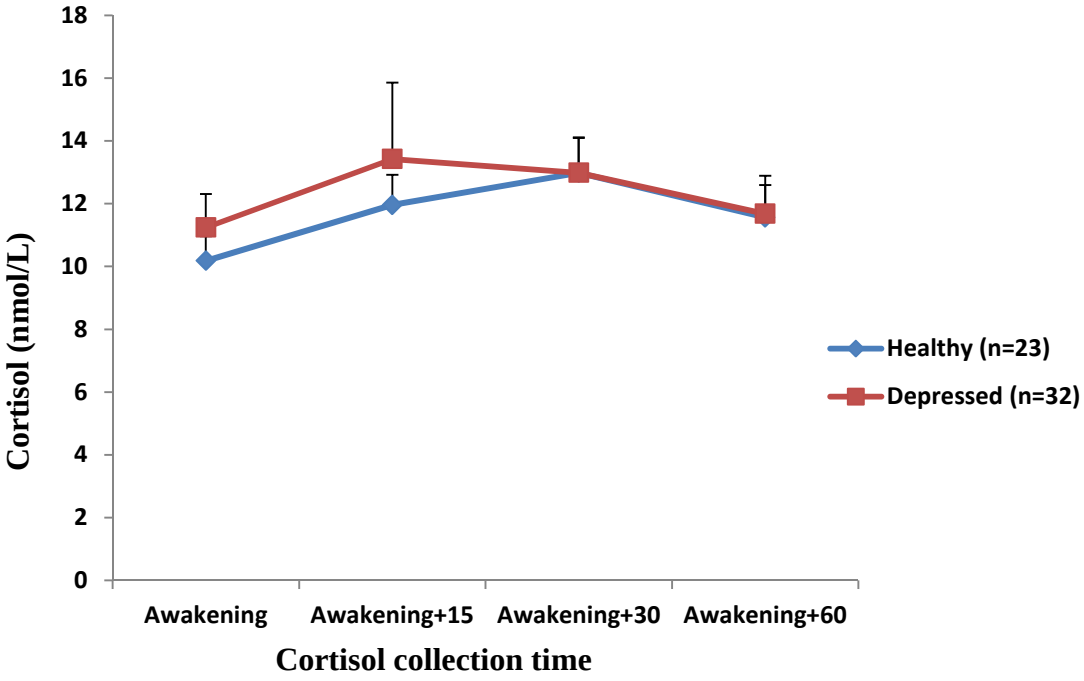


Figure 6. 32 week awakening cortisol response in healthy and depressed women, with *SE*

Note: Numbers represent women who collected cortisol at all times, not the full sample

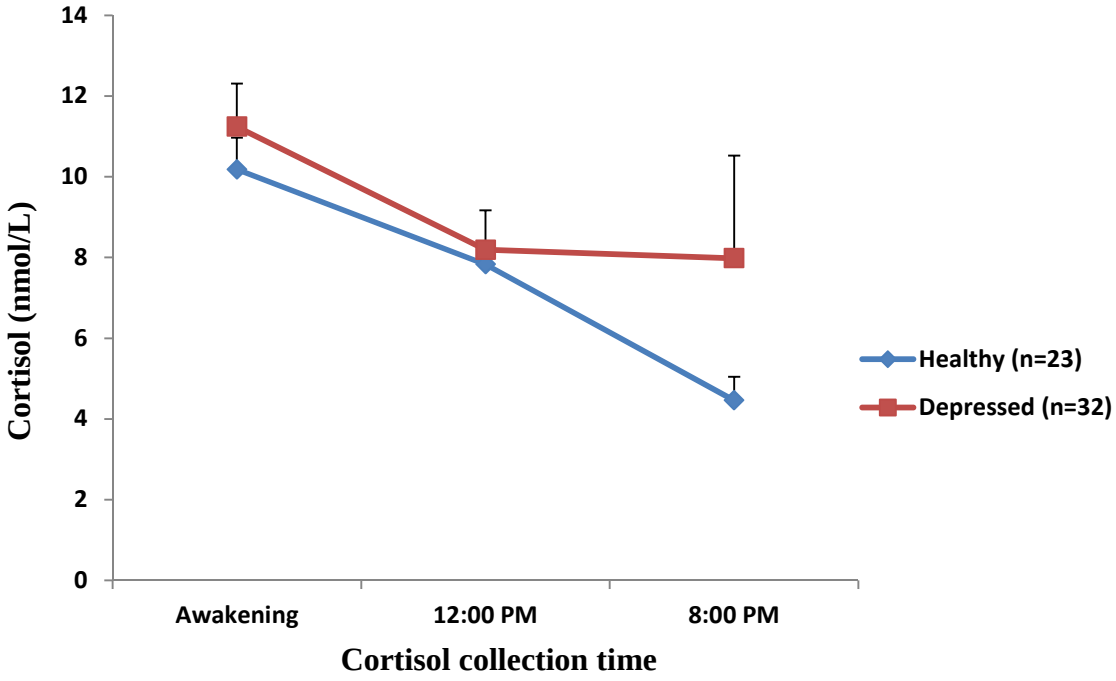
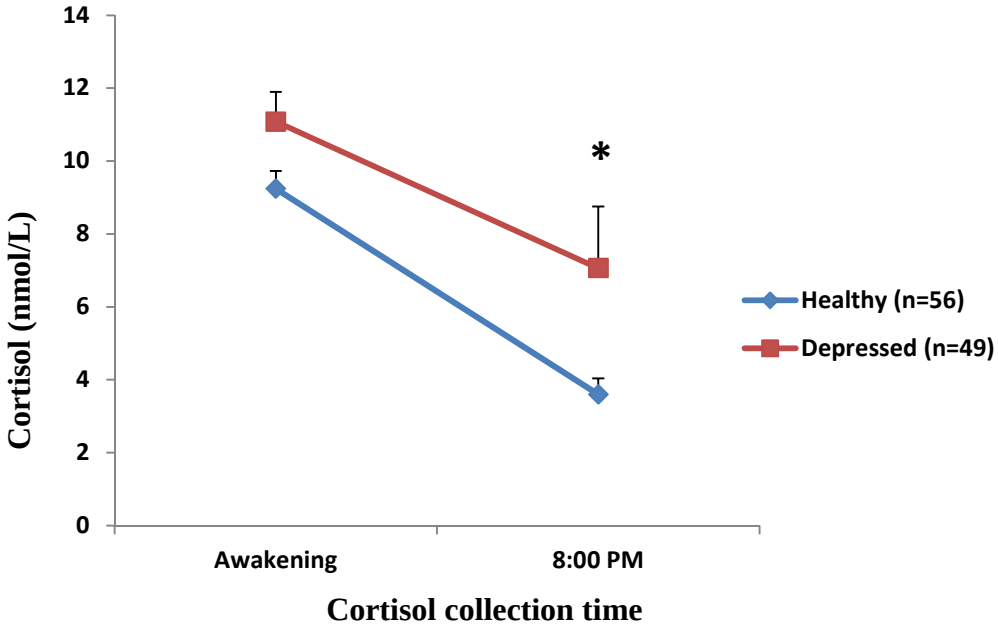


Figure 7. 32 weeks cortisol at awakening, 12 pm and 8 pm in healthy and depressed women, with *SE*

Note: Numbers represent women who collected cortisol at all times, not the full sample



* p=.05

Figure 8. 32 weeks cortisol at awakening and 8 pm in healthy and depressed women, with *SE*

Maternal childhood abuse and antenatal depression and salivary cortisol at 32 weeks gestation

Maternal salivary cortisol during the day was also compared between the group of healthy women, MDD only women, CA only women and the group of MDD & CA women. Mean (and SD) cortisol levels are presented in Table 12, alongside the statistical values.

As for the results described above, a larger number of women have cortisol only at awakening and 8 pm (n=91) than at awakening, 12 noon and 8 pm (n=50) or indeed throughout the awakening response curve (n=50).

Figure 9 shows cortisol levels at awakening in the small sample of women with/without depression and/or childhood abuse. There is an increase in the cortisol levels within the first hour after awakening across all 4 groups. During the first 15 minutes post-awakening, cortisol rose to a mean peak of 12.1 (SD=4.5), nmol/L in healthy women 12.5 (SD=3.6) nmol/L in women with MDD only, 11.9 (SD=4.5) nmol/L in women with CA only, and 12.5 (SD=14.3) nmol/L in women with MDD & CA. Cortisol continued increasing at 30 minutes post awakening to a mean peak of 12.7 (SD=5.1) nmol/L in healthy women, 15.8 (SD=7.7) nmol/L in women with MDD only, 15 (SD=5.6) nmol/L in women with CA only, and 10.5 (SD=5.8) nmol/L in women with MDD & CA. Similarly, at 60 minutes post-awakening, cortisol means reached 11.9 (SD=4.2) nmol/L in healthy women, 14.1 (SD=6.8) nmol/L in women with MDD only, 12.8 (SD=7.0) nmol/L in women with CA only, and 10.6 (SD=6.2) nmol/L in women with MDD & CA. However, at no time was there a difference between the four groups.

Figure 11 represent the awakening and 8 pm cortisol values in the larger sample (n=91) that has only these two time points. The mean awakening and 8 pm cortisol values are similar in this larger sample compared with the smaller one in Figure 10.

Specifically, maternal cortisol at 32 weeks gestation decreases across all groups during the day. Mean cortisol upon awakening was 9.8 (SD=3.9) nmol/L in healthy women, 12.8 (SD=3.6) nmol/L in women with MDD only, 9.4 (SD=2.8) nmol/L among women with CA only and 10.0 (SD=5.6) nmol/L among women with MDD & CA. As observed from the graph, at awakening, cortisol in healthy women was very similar to cortisol in women who had experienced childhood abuse only and both conditions of childhood abuse and antenatal depression, whilst depressed only women have the highest cortisol levels in the groups, although the difference is not statistically significant. At 8 pm, cortisol across all groups drops, reaching a mean of 3.7 (SD=3.4) nmol/L in healthy women, 6.6 (SD=6.0) nmol/L in women with MDD only, 3.8 (SD=3.9) nmol/L among women with CA only and 7.3 (SD=14.6) nmol/L among women with MDD & CA. Mann-Whitney post-hoc test with the significance level adjusted for multiple testing to p=.02, shows that MDD women and women with MDD & CA have significantly higher evening cortisol levels when compared with those of healthy women (respectively, Z=-2.3, p=.02; Z=-2.3, p=.02).

Means and statistical values of all the data (irrespective of sample size), and the statistics, are also presented in Table 12. Figure 10 illustrates cortisol at awakening, 12 noon and 8 pm in the small sample. Cortisol levels decreased in both healthy and depressed women through the morning, reaching a mean of 8.1 nmol/L (SD=2.8) in healthy women and a mean of 6.9 nmol/L (SD=1.9) in depressed women at 12 pm. Cortisol levels further decreased through the rest of the day to a mean level of 4.7 nmol/L (SD=3.2) in healthy women, and a mean of 5.9 nmol/L (SD=7.4) in depressed women at 8 pm. In this smaller sample of women who have all the three time-points, the 8 pm cortisol levels is higher in the MDD & CA group and the MDD group, but the difference is not statistically significant.

Table 12. 32 week salivary cortisol in women with/without depression and/or childhood abuse

Cortisol time collection	**Healthy**	**MDD only**	**CA only**	**MDD & CA**	**Statistic**
Awakening[a] Mean (SD)	9.8 (3.9)	12.8 (3.6)	9.4 (2.8)	10.0 (5.6)	K-$W_{(3)}$=7.2; p=.07
Awakening+15[b] **minutes** Mean (SD)	12.1 (4.5)	12.5 (3.6)	15.0 (5.6)	10.5 (5.8)	K-$W_{(3)}$=2.9; p=.41
Awakening+30[c] **minutes** Mean (SD)	12.7 (5.1)	15.8 (7.7)	15.0 (5.6)	10.5 (5.8)	K-$W_{(3)}$=7.4; p=.06
Awakening+60[d] **minutes** Mean (SD)	11.9 (4.2)	14.1 (6.8)	12.7 (7.0)	8.1 (5.4)	K-$W_{(3)}$=3.1; p=.37
Midday[e] Mean (SD)	7.6 (3.2)	7.9 (3.0)	8.5 (2.6)	8.1 (5.4)	K-$W_{(3)}$=1.2; p=.76
8 pm[f] Mean (SD)	3.7 (3.4)	6.6 (6.0)	3.8 (3.9)	7.3 (14.6)	K-$W_{(3)}$=8.9; **p=.03**

[a] Healthy, n=33 ; MDD only, n=14 ; CA only, n=14; MDD & CA, n=30
[b] Healthy, n=18 ; MDD only, n=8 ; CA only, n=4; MDD & CA, n=30
[c] Healthy, n=19 ; MDD only, n=10 ; CA only, n=4; MDD & CA, n=32
[d] Healthy, n=18 ; MDD only, n=10 ; CA only, n=4; MDD & CA, n=31
[e] Healthy, n=19 ; MDD only, n=10 ; CA only, n=3; MDD & CA, n=30
[f] Healthy, n=33 ; MDD only, n=15 ; CA only, n=14; MDD & CA, n=29

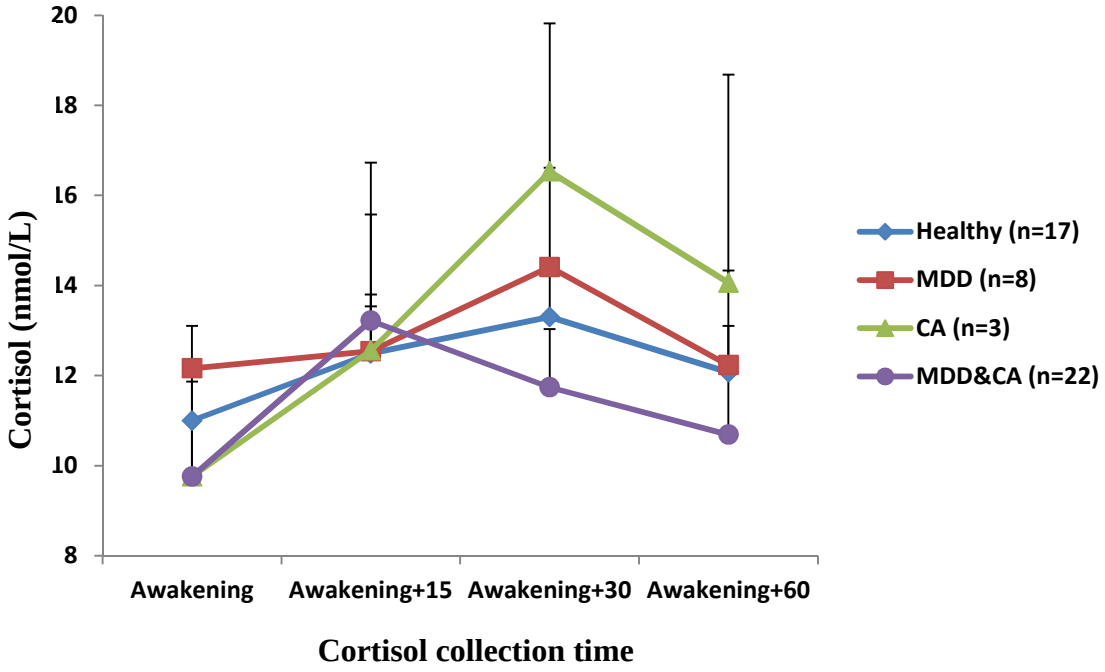


Figure 9. 32 weeks cortisol awakening response in women with/without depression and/or childhood abuse

Note: Numbers represent women who collected cortisol at all times, not the full sample

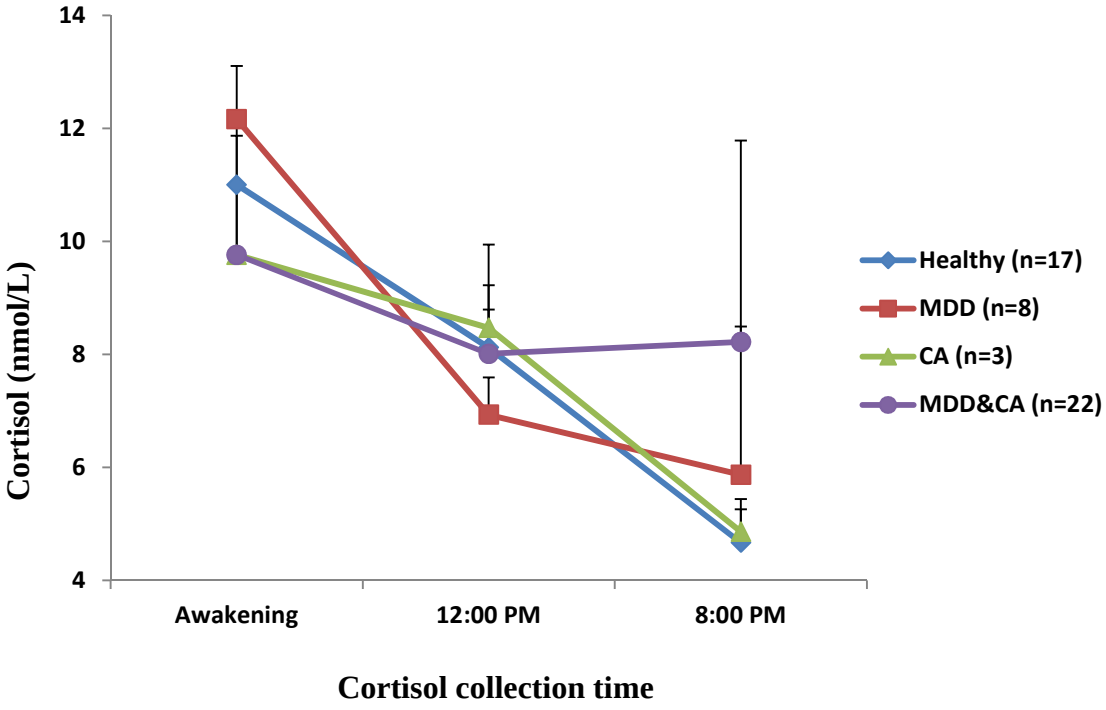


Figure 10. 32 weeks cortisol at awakening, 12 pm and 8 pm in women with/without depression and/or childhood abuse

Note: Numbers represent women who collected cortisol at all times, not the full sample

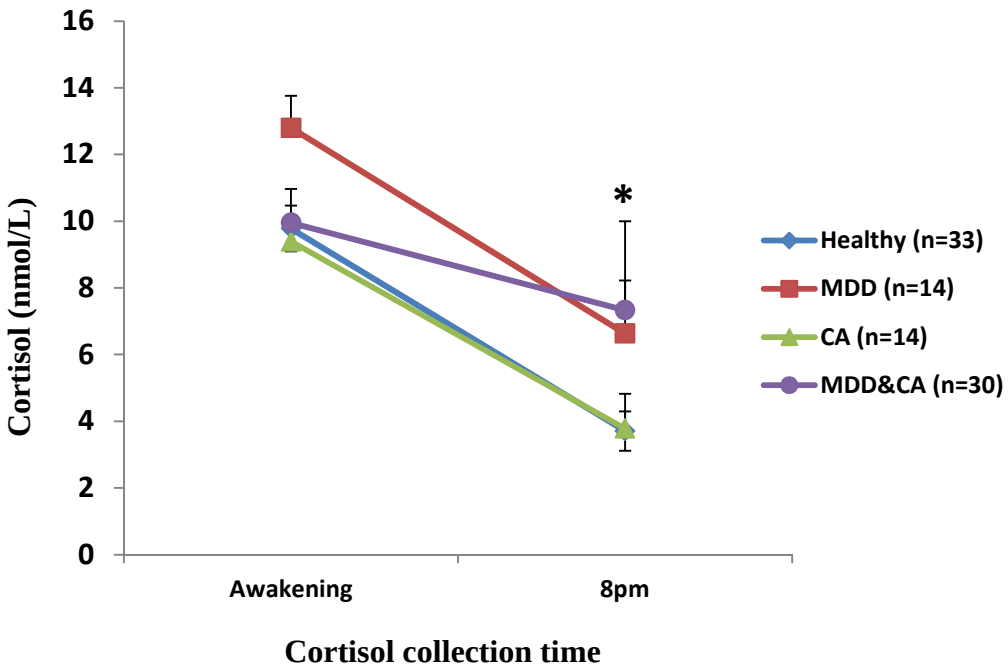


Figure 11. 32 weeks cortisol at awakening and 8 pm in women with/without depression and/or childhood abuse, with *SE*

Note: Numbers represent the full sample of women who collected cortisol

3.2 Part 2: Offspring outcomes at 6 days, 8 weeks and 1 year after birth

Overview

The aim of this section is to investigate the neonatal behavioural and physiological outcomes of babies exposed to maternal depression in utero and then to explore whether a mother's own childhood experiences of abuse moderate these outcomes. First, I evaluate neonatal outcome at birth. Next, I examine the infants' behavioural regulation during the administration of the Neonatal Behavioural Assessment Scale (NBAS) and their stress response (cortisol) following the NBAS. Next, the infant stress response at 8 weeks and one year following the routine immunization is explored in relation to maternal diagnosis and cortisol levels in pregnancy (32 weeks). Finally, infant cognitive and socio-emotional development at one year is examined in relation to maternal diagnosis in pregnancy.

3.2.1 Descriptive analyses

Neonatal outcomes in healthy and depression groups

Information on neonatal outcomes was obtained from the maternal hospital discharge notes and is reported in Table 13. There was a statistically significant difference in gestational age of babies of healthy and depressed mothers ($t(116)=2.1$; $p=.04$). As expected, babies of depressed women had a shorter gestational age (M = 39.6 weeks, S.D. 2.3) compared with healthy women (M = 40.3 weeks, S.D. 1.4). There was also a group difference in the weight of the baby at birth ($t(115)=2.7$; $p=.008$). Specifically, babies of depressed mothers (M = 3.311 kg, S.D. 565 grams) were lighter than those of healthy mothers (M = 3.560 kg, S.D. 424 grams).

Table 13. Infant outcomes at birth in mothers with/without depression and/or childhood abuse

	Healthy	**MDD only**	**CA only**	**MDD & CA**	**Statistic**
Gestational age at birth[a] Mean (S.D.)	40.2 (1.6)	40.3 (1.3)	40.4 (1.2)	39.4 (2.6)	F(3)=1.7; p=.17
Birth weight[b] Mean (S.D.)	3563.0 (440.8)	3629.2 (459.7)	3456.6 (308.5)	3231.5 (584.5)	F(3)=3.8; **p=.012**
Head circumference[c] Mean (S.D.)	356.4 (15.4)	354.7 (11.7)	354.5 (9.0)	351.3 (18.4)	F(3)=.4; p=.74
Mode of delivery[d] %					
Vaginal	25 (71.4)	10 (66.7)	12 (85.7)	35 (87.5)	$X^2_{(3)}$=4.7; p=.197
C-section	10 (28.6)	5 (33.3)	2 (14.3)	5 (12.5)	
Labour onset[e] **%**					
Spontaneous	24 (70.6)	10 (62.5)	10 (83.3)	31 (77.5)	$X^2_{(3)}$=2.1; p=.558
Non-spontaneous	10 (29.4)	6 (37.5)	2 (16.7)	9 (22.5)	
Feeding method[f] **%**					
Breastfeeding	32 (91.4)	11 (73.3)	13 (92.9)	22 (55.0)	$X^2_{(6)}$=18.2 ; **p=.006**
Bottle	0 (0.0)	0 (0.0)	0 (0.0)	4 (10.0)	
Bottle and breast	3 (8.6)	4 (26.7)	1 (7.1)	14 (35.0)	

[a] Healthy, n=35 ; MDD only, n=15 ; CA only, n=14; MDD & CA, n=40
[b] Healthy, n=35 ; MDD only, n=14 ; CA only, n=14; MDD & CA, n=40
[c] Healthy, n=17 ; MDD only, n=10 ; CA only, n=4; MDD & CA, n=37
[d] Healthy, n=35 ; MDD only, n=15 ; CA only, n=14; MDD & CA, n=40
[e] Healthy, n=34 ; MDD only, n=16 ; CA only, n=12; MDD & CA, n=40
[f] Healthy, n=35 ; MDD only, n=15 ; CA only, n=14; MDD & CA, n=40

There was a significant difference in the birth weight of babies of women in the 4 groups – HEALTHY, MDD only, CA only, MDD & CA - ($F(3)=3.8$; $p=.012$). Bonferroni post-hoc test showed that women with who had experienced childhood abuse and were depressed in pregnancy had a significantly lower weight at birth ($M=3231.5$; $SD=584.5$) compared with babies of healthy mothers ($M=3563.0$; $SD=440.8$).

Since gestational age at birth and infant birth weight are significantly different between groups, they have been taken into account as potential confounders. As these two variables were highly correlated (Pearson's $r=.6$; $p<.001$), further analyses have controlled for infant birth weight only. Mode of delivery is also likely to affect newborns' behavior but, as no group difference was found, this variable has no longer been treated as confounder.

Table 14. Infant outcomes at birth in healthy and depressed mothers

	Healthy N=24-58	MDD N=48-62	**Statistic**
Gestational age at birth Mean (S.D.)	40.3 (1.4)	39.6 (2.3)	t(116)=2.1; **p=.04**
Birth weight Mean (S.D.)	3560.3 (423.6)	3311.4 (564.9)	t(115)=2.7; **p=.008**
Head circumference Mean (S.D.)	356.5 (13.7)	352.0 (16.9)	t(70)=1.2; p=.26
Mode of delivery % Vaginal C-section	 46 (79.3) 12 (20.7)	 48 (80.0) 12(20.0)	$X^2_{(1)}$=.009; p=1
Labour onset% Spontaneous Non-spontaneous	 39 (72.2) 15 (27.8)	 46 (74.2) 16 (25.8)	$X^2_{(1)}$=.06; p=81
Feeding method % Breastfeeding Bottle Bottle and breast	 49 (84.5) 0 (0) 9 (15.5)	 37 (61.7) 5 (8.3) 18 (30)	$X^2_{(2)}$=9.6; p=.47

3.2.2 Neonatal Behavioural Assessment Scale at 6 days post-partum

A first step was to explore the baby's performance on the NBAS across the whole sample, which offers a general understanding of the babies, irrespective of maternal diagnosis. Means are presented in Table 15.

As previously explained, because of the high correlation between the items in each NBAS cluster only some of the items from each NBAS cluster were selected for analysis. Although I have log transformed the data, I am presenting the mean values and the range in the tables in order to show the typical values of the data, as consistent with existing literature.

Correlations between the NBAS items in Table 16 showed a strong negative correlation between infant alertness and irritability at 6 days after birth (Spearman's r=-.7, p<.001). Similarly, irritability was also negatively correlated with motor maturity (Spearman's r=-.2, p<.01) and cuddliness (Spearman's r=-.4, p<.001). Alertness was positively correlated with motor maturity (Spearman's r=.4, p<.001) and cuddliness (Spearman's r=.5, p<.001). As expected, motor maturity was negative correlated with tremulousness (Spearman's r=-.2, p<.05). Furthermore, both consolability (higher scores represent the ease with which the infant can be consoled and soothed by the examiner) and animate auditory were positively correlated with alertness (respectively, Spearman's r=.41, p<.001; Spearman's r=.85, p<.01) and cuddliness (respectively, Spearman's r=.41, p<.01; Spearman's r=.45, p<.01) and negatively correlated with irritability (respectively, Spearman's r= -.31, p<.01; Spearman's r= -.62, p<.01).

Obstetric variables that were significantly different between groups have been considered as potential confounders for infant behavioural outcomes, hence baby's birth weight has been controlled for using Generalized Linear Model, as explained later in this chapter.

Table 15. Descriptive characteristics of the NBAS in the whole sample

NBAS items (n=114-118)	Means (SD)	Range
Alertness	6.60 (1.84)	2-9
Animate auditory	7.26 (1.64)	2-9
Irritability	4.36 (2.01)	1-8
Motor maturity	5.67 (1.42)	2-9
Cuddliness	6.42 (1.5)	3-9
Consolability	5.33 (1.95)	2-9
Tremulousness	3.96 (2.56)	1-8
Quality of alertness	6.57 (1.81)	2-9
Cost of attention	6.23 (1.41)	4-9
Examiner facilitation	6.18 (1.77)	3-9
General irritability	5.97 (2.22)	2-9
Robustness & endurance	6.27 (1.75)	3-9
State regulation	7.24 (1.3)	4-9
Examiner's emotional response	6.41 (2.15)	1-9

Table 16. Spearman's correlations between items in the NBAS

NBAS items	1	2	3	4	5	6	7
(1)Alertness	-						
(2)Irritability	-.71***	-					
(3)Motor maturity	.42***	-.24**	-				
(4)Cuddliness	.53***	-.40***	.19*	-			
(5)Tremulousness	-.11	.05	-.19*	-.01	-		
(6) Consolability	.41***	-.31**	.27*	.41**	-.28*	-	
(7) Animate auditory	.85**	-.62**	.37**	.45**	-.10	.40**	-

*p<.05; **p<.01; ***p<.001

Next, infant stress response was examined by taking saliva from the baby before, immediately after, and 30 minutes after the administration of the Neonatal Behavioural Assessment Scale. Figure 12 shows that before the administration of the assessment, infant mean cortisol level was 8.8 nmol/L (n=67, SD=11.2), whilst immediately after the NBAS, it reaches a peak of 10.1 nmol/L (n=69, SD=10.1). Thirty minutes post-assessment, infant cortisol drops again, returning to a similar level observed before the NBAS, to a mean of 8.3 nmol/L (n=70, SD=9.3).

Simple paired t-test between neonatal cortisol before and immediately after the NBAS show that there is no significant increase in the cortisol levels immediately after the NBAS ($t(64) = -1.2$; $p=.241$). Similarly, there is no significant increase in the neonatal cortisol levels 30 minutes after the NBAS ($t(64) = .1$; $p=.911$).

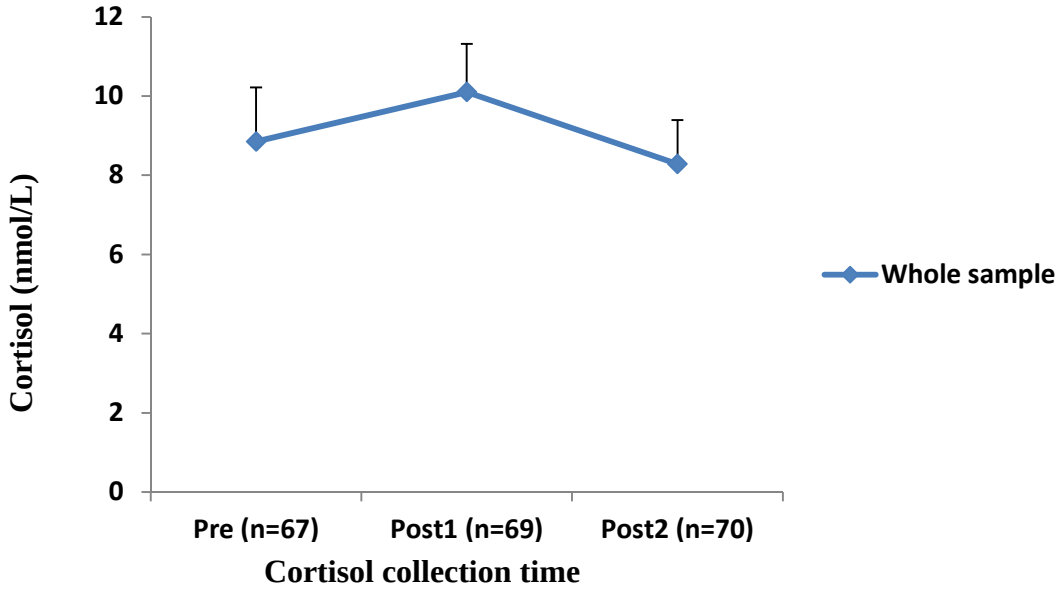


Figure 12. Baby's cortisol before, immediately after and 30 minutes after the administration of the NBAS, with *SE*

Exposure to maternal depression in utero and Neonatal Behavioral Assessment at 6 days

The scores of the babies on the NBAS items are given for babies of healthy mothers and for those exposed to maternal depression in utero (Table 17). Babies of women with depression were more dysregulated in their behavior compared with that of babies of healthy women. In particular, compared with babies of healthy mothers, those of depressed mothers were less alert (U=1038.5; Z=-3.8; p<.001), less capable of responding to auditory stimuli (U=1185; Z=-3.0; p=.003) and more irritable (U=1151.5; Z=-2.7; p=.007). Furthermore, compared with babies of healthy mothers, those of depressed women showed poorer motor maturity (U=1318; Z=-2.4; p=.018), and a higher number of tremors during the assessment (U=1184.5; Z=-3.1; p=.002). Infant of depressed mothers were also more resistant to being cuddled by the examiner during the procedure (U=1196.5; Z=-3.0 p=.003) compared with infants of healthy women. There was no group difference for consolability (Healthy, N=34; MDD, N=45; U=599; Z=-1.7; p=.09).

Babies' scores on the supplementary items of the NBAS were also compared. The scoring is unidirectional, with high score representing the baby's best performance Compared with infants of healthy mothers, the quality of alertness of the infants of depressed mothers was poorer (U=1160; Z=-3.2; p=.001) and they were less able to maintain a state of attention during the assessment (U=1246.5; Z=-2.7; p=.007). Compared with babies of healthy mothers, those of depressed mothers were also more irritable (U=1338; Z=-2.2; p=.027) and were rated as being more difficult to assess by the examiner (U=1279.5; Z=-2.5; p=.012). Furthermore, compared with babies of healthy women, those of depressed women were less robust (U=1214; Z=-2.9; p=.004) and found it more difficult to regulate their state (U=1178.5; Z=-3.1; p=.002).

Finally the scores given by the examiners rating their emotional response to the infant were lower than those given to the babies of healthy women (U=1103; Z=-3.5; p=.001).

The infant's predominant state throughout the duration of the assessment was dichotomized into "optimal" and "non-optimal". In the whole sample, 7 (5.6%) babies had a predominant state of 3 (drowsy), 74 (59.2%) infants had a predominant state of 4 (alertness), 34 (27.2%) infants had a predominant state of 5 (fussy), and 3 (2.4%) infants had a predominant state of 6 (crying). Given the small number of babies in each of the states other than 4 (alert), the "optimal" group includes infants in state 4, while the "non-optimal" includes infants in states 3, 5 and 6, as they are not in the ideal condition to perform the assessment. Although fewer babies of depressed mothers compared with babies of healthy mothers were in the optimal state (55% vs. 70.7%), the difference in the predominant state between babies of healthy and depressed mothers did not reach significance.

When controlling for baby's birth weight, Generalized Linear Model procedures show that the differences in the NBAS scores (alertness and irritability) between babies of healthy and depressed women remained significant, independently of the baby's weight (alertness: $F(1)=8.4$; $p=.005$; irritability: $F(1)=7.3$; $p=.008$).

Table 17. NBAS in babies of healthy and depressed mothers

NBAS item	**Healthy** N=55-58	**MDD** N=59-60	**Statistic**
Alertness Mean (S.D.)	7.2 (1.6)	6.0 (1.9)	Z=-3.8; **p<.001**
Animate auditory Mean (S.D.)	7.6 (1.6)	6.9 (1.6)	Z=-3.0; **p=.003**
Irritability Mean (S.D.)	3.8 (2.0)	4.9 (2.0)	Z=-2.7; **p=.007**
Motor maturity Mean (S.D.)	6.05 (1.3)	5.3 (1.5)	Z=-2.4; **p=.018**
Cuddliness Mean (S.D.)	6.9 (1.3)	6.0 (1.6)	Z=-3.0; **p=.003**
Consolability Mean (S.D.)	5.7 (1.8)	5.1 (2.1)	Z=-1.7; p=.09
Tremulousness Mean (S.D.)	3.2 (2.6)	4.7 (2.3)	Z=-3.1 **p=.002**
Quality of alertness Mean (S.D.)	7.1 (1.7)	6.1 (1.8)	Z=-3.2 **p=.001**
Cost of attention Mean (S.D.)	6.6 (1.3)	5.9 (1.4)	Z=-2.7 **p=.007**
Examiner facilitation Mean (S.D.)	6.6 (1.8)	5.8 (1.7)	Z=-2.5 **p=.012**
General irritability Mean (S.D.)	6.4 (2.1)	5.6 (2.3)	Z=-2.2 **p=.027**
Robustness & endurance Mean (S.D.)	6.7 (1.8)	5.9 (1.6)	Z=-2.9 **p=.004**
State regulation Mean (S.D.)	7.6 (1.3)	6.9 (1.3)	Z=-3.1 **p=.002**
Examiner's emotional response Mean (S.D.)	7.1 (1.9)	5.8 (2.2)	Z=-3.5 **p=.001**
Infant predominant state Optimal (%) Non-optimal (%)	41 (70.7) 17 (29.3)	33 (55.0) 27 (45.0)	$X^2_{(1)}$=3.1 p=.078

Exposure to maternal depression in utero, maternal history of childhood abuse and Neonatal Behavioral Assessment at 6 days

In order to explore further the effects of maternal childhood abuse and maternal depression in pregnancy on the infant behavioral outcomes at 6 days post-partum, comparisons were made between the NBAS scores of the babies of the 4 groups - HEALTHY, MDD only, CA only, MDD &CA. Results are presented in Table 18.

Kruskal-Wallis test shows that there are group differences in alertness ($K\text{-}W_{(3)}$=19.4; p<.001) at 6 days post-partum. Mann-Whitney post-hoc test shows that MDD & CA babies were significantly less alert compared with babies of HEALTHY women (U=327; Z=-4.1; p<.001) and compared with babies of CA only women (U=136; Z=-2.9; p=.004).

More group differences are found for Animate Auditory (K-W(3)=15.3; p=002) at 6 days after birth. Mann-Whitney post-hoc test shows that babies of MDD & CA women were significantly less responsive to the animate auditory stimuli compared with babies of HEALTHY women (U=400; Z=-3.8; p=.001), with babies of MDD only women (U=173.5; Z=-2.5; p=.014) and babies of CA only women (U=145.5; Z=-2.7; p=.006).

Similarly, Kruskal-Wallis test shows that there are group differences in irritability ($K\text{-}W_{(3)}$=11.8; p=.008) at 6 days post-partum. Mann-Whitney post-hoc tests show that babies of MDD & CA women had significantly higher irritability scores compared with babies of HEALTHY women (U=409; Z=-2.7; p=.007) and compared with babies of CA only women (U=126; Z=-2.7; p=.006).

Further groups differences are found in motor maturity (K-W(3)=6.4; p=.09) at 6 days post-partum. Mann-Whitney post-hoc test shows that babies of MDD & CA women had significantly lower motor maturity compared with babies of HEALTHY women (U=481; Z=-2.4; p=.02).

Group differences were also found for infant cuddliness (K-W(3)=19.7; p<.001)**.** Mann-Whitney post-hoc test shows that babies of MDD & CA women were significantly less

cuddly compared with babies of HEALTHY women (U=343; Z=-3.9; p<.001), babies of MDD only women (U=176; Z=-2.4; p=.02), and babies of CA only women (U=116.5; Z=-3.3; p=.001). No group difference was present for consolability.

Tremulousness is also different across groups (K-W(3)=10.9; p=.013). Mann-Whitney post-hoc test shows that babies of MDD & CA women had a significantly higher number of tremors compared with babies of HEALTHY women (U=472.5; Z=-2.5; p=.01), babies of CA only women (U=143; Z=-2.7; p=.006).

Similar results were found for the NBAS supplementary items. There were group differences in quality of alertness (K-W(3)=17.4; p=.001), cost of attention (K-W(3)=12.7; p=.005)**,** examiner facilitation (K-W(3)=10.5; p=.02) general irritability (K-W(3)=8.9; p=.03), robustness and endurance (K-W(3)=12.1; p=.007), state regulation (K-W(3)=12.1; p=.007) and the examiner's emotional response to the baby (K-W(3)=20.9; p<001). Compared with babies of HEALTHY women and babies of CA only women, Mann-Whitney post-hoc tests respectively showed that babies of MDD & CA had a lower quality of alertness (Z=-3.6; p<.001; Z=-2.9; p=.004); were less attentive (Z=-2.6; p=.009; Z=-2.9; p=.004); needed more help from the examiner (Z=-2.7; p=.008; Z=-2.4; p=.02); showed more general irritability (Z=-2.3; p=.02; Z=-2.3; p=.02); were less robust (Z=-3.0; p=.003; Z=-2.3; p=.02) and less able to regulate their state (Z=-3.1; p=.002; Z=-2.3; p=.02); examiners also found them less emotionally engaging (Z=-3.8; p<.001; Z=-3.3; p=.001). Furthermore, compared with babies of MDD only women, those of MDD & CA women were less cuddly (U=176; Z=-2.4; p=.02); showed lower quality of alertness (U=178.5; Z=-2.3; p=.02); and were less emotionally engaging by the examiner (U=166.5; Z=-2.6; p=.01).

When controlling for baby's birth weight, Generalized Linear Model procedures show that the differences in the NBAS scores (alertness and irritability) between babies of HEALTHY, MDD only, CA only and MDD & CA women remained significant,

independently of the baby's weight (alertness: $F(3)=5.4$; $p=.002$; irritability: ($F(3)=4.3$; $p=.007$)).

To summarize, infants of MDD & CA mothers show greater signs of behavioral dysregulation during the Neonatal Behavioral Assessment Scale at six days compared with infants of HEALHTY and CA only mothers. Interestingly, no difference was found between the behavior of babies of HEALTHY and CA only women, while only a few differences were observed in babies of MDD & CA and MDD only mothers.

Table 18. NBAS in women with/without depression and/or childhood abuse

NBAS item	**Healthy** N=33-35	**MDD only** N=15	**CA only** N=13-14	**MDD & CA** N=39-40	**Statistic**
Alertness Mean (S.D.)	7.3 (1.4)	6.9 (1.8)	7.1 (1.9)	5.7 (1.9)	K-W(3)=19.4; **p<.001**
Animate auditory Mean (S.D.)	7.7 (1.5)	7.7 (.9)	7.7 (1.7)	6.5 (1.8)	K-W(3)=15.3; **p=002**
Irritability Mean (S.D.)	4.0 (1.8)	4.5 (1.7)	3.3 (2.3)	5.2 (1.9)	K-W(3)=11.8; **p=.008**
Motor maturity Mean (S.D.)	6.0 (1.2)	5.8 (1.0)	5.9 (1.3)	5.1 (1.6)	K-W(3)=6.4; p=.09
Cuddliness Mean (S.D.)	6.9 (1.3)	6.7 (1.6)	7.1 (1.1)	5.5 (1.5)	K-W(3)=19.7; **p<.001**
Consolability Mean (S.D.)	5.6 (1.8)	5.6 (2.2)	5.5 (1.9)	4.9 (2.1)	K-W(3)=3.5; p=.32
Tremulousness Mean (S.D.)	3.5 (2.7)	3.9 (2.2)	2.9 (2.4)	5.1 (2.2)	K-W(3)=10.9; **p=.013**
Quality of alertness Mean (S.D.)	7.1 (1.4)	6.9 (1.6)	7.1 (2.0)	5.7 (1.8)	K-W(3)=17.4; **p=.001**
Cost of attention Mean (S.D.)	6.4 (1.2)	6.3 (1.5)	7.0 (1.4)	5.6 (1.4)	K-W(3)=12.7; **p=.005**
Examiner facilitation Mean (S.D.)	6.5 (1.6)	6.5 (1.7)	6.7 (1.8)	5.5 (1.7)	K-W(3)=10.5; **p=.02**
General irritability Mean (S.D.)	6.3 (2.0)	6.3 (2.1)	6.7 (2.4)	5.1 (2.2)	K-W(3)=8.9; **p=.03**
Robustness & endurance Mean (S.D.)	6.6 (1.7)	6.6 (1.6)	6.8 (2.0)	5.5 (1.6)	K-W(3)=12.1; **p=.007**
State regulation Mean (S.D.)	7.5 (1.0)	7.4 (1.1)	7.6 (1.7)	6.7 (1.3)	K-W(3)=12.1; **p=.007**
Examiner's emotional response Mean (S.D.)	6.9 (1.7)	6.8 (2.0)	7.4 (2.2)	5.1 (2.1)	K-W(3)=20.9; **p<001**
Infant predominant state Optimal (%) Non-optimal (%)	 24 (68.6) 11 (31.4)	 10 (66.7) 5 (33.3)	 11 (78.6) 3 (21.4)	 19 (47.5) 21 (52.5)	$X^2_{(3)}$=6.0; p=.11

Exposure to maternal depression in utero and cortisol response to the administration of the NBAS at 6 days

Neonatal cortisol was measured in infants before, immediately after and 30 minutes after the administration of the Neonatal Behavioral Assessment Scale at 6 days. As can be observed in Figure 13, infants of depressed mothers have generally higher cortisol levels at each collection time than infants of healthy women. Before the assessment, infants of healthy women have a mean cortisol of 7.4 (SD=5.9) nmol/L. The mean remains similar immediately after the NBAS (M = 7.3, SD=4.0 nmol/L) and at 30 minutes post-NBAS (M = 6.5, SD=3.6 nmol/L). The cortisol response in babies of depressed mothers followed a slightly different pattern. In fact, pre-NBAS the mean cortisol is 9.6 (SD=13.1) nmol/L followed by an increase immediately after the NBAS 11.6 (SD=11.9) nmol/L and a subsequent drop at 30 minutes post-NBAS, with cortisol mean of 9.2 (SD=11.1) nmol/L similar to that observed at pre-NBAS. Babies of depressed mothers have a significantly higher response to stress immediately after the NBAS compared with babies of healthy mothers ($t_{(59.49)}$ =-2.2; p=.03).

A similar pattern can be seen when analyzing the delta cortisol response following the administration of the NBAS.

Table 19. Infant NBAS delta1 and delta2 in babies of healthy and depressed women

	Healthy N=22	**MDD** N=43	**Statistic**
Baby cortisol Δ1 Mean (SD)	-.2 (5.4)	2.2 (11.0)	Z=-.8; p=.45
Baby cortisol Δ2 Mean (SD)	-.1 (6.2)	-1.5 (9.9)	Z=-.2; p=.88

Specifically, Δ1 represents the cortisol level immediately after the NBAS minus the cortisol level pre NBAS, whilst Δ2 represents the cortisol level 30 minutes after the NBAS minus the cortisol level pre NBAS. Mean cortisol Δ1 was -0.2 (SD=5.4) nmol/L in babies of healthy mothers while it rapidly increases in babies of depressed mothers, reaching a mean of 2.2 (SD=11.0) nmol/L, although the difference between groups is not significant. On the other hand, Δ2 has very similar means across babies of healthy and depressed mothers. In fact, Δ2 cortisol mean for babies of healthy women was -.1 (SD=6.2) nmol/L and -.15 (SD=9.9) nmol/L in babies of depressed mothers.

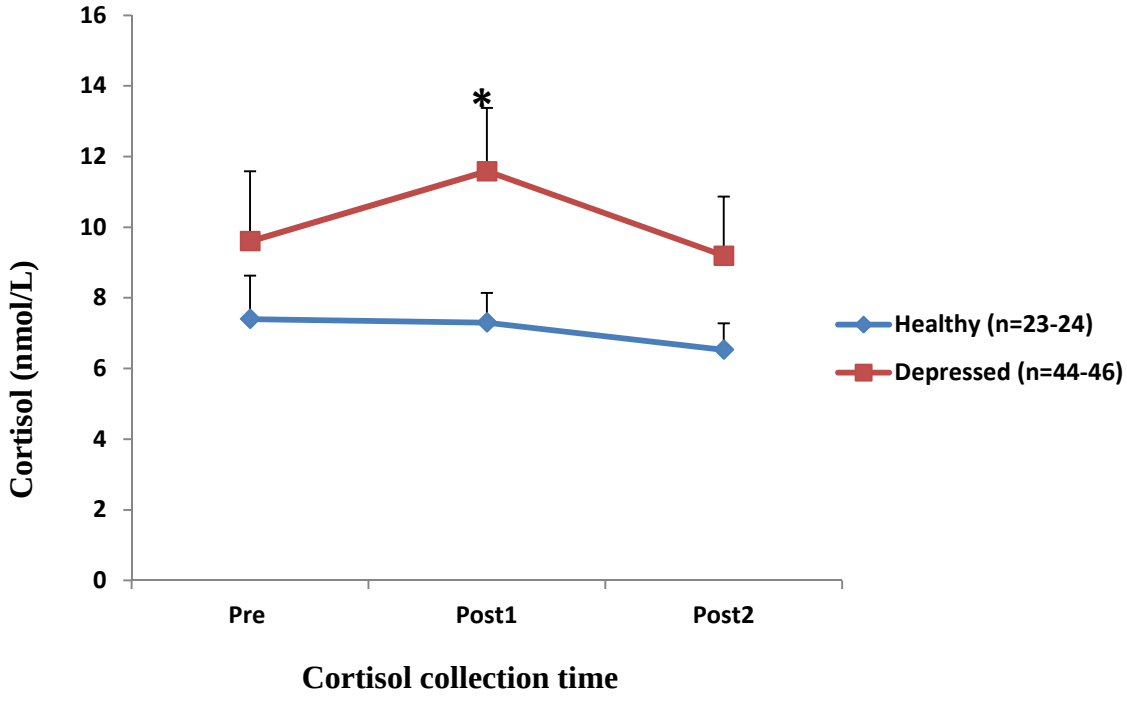


Figure 13. Baby's cortisol before, immediately after and 30 minutes after the administration of the NBAS in babies of healthy and depressed mothers

Exposure to maternal depression in utero and maternal history of childhood abuse and cortisol response to the administration of the NBAS at 6 days

There was no significant difference in neonatal cortisol response to the administration of the NBAS across the 4 groups – HEALTHY, MDD only, CA only, and MDD & CA (pre: $F(3)=.3$; $p=.827$; post1: $F(3)=1.3$; $p=.3$; post2: $F(3)=.3$; $p=.818$).

However it is worth commenting on the levels as presented in Figure 14. The mean cortisol levels immediately after the NBAS remained almost stable in the healthy groups (Pre: M = 8.1, SD = 6.5; post1: M = 8.1, SD = 4.3), increased in the depression only (Pre: M = 7.1, SD = 6.0; post1: M = 8.8, SD = 4.9), and depression with abuse groups (Pre: M = 10.3, SD = 14.6; post1: M = 12.5, SD = 13.4), while decreased in babies of women with childhood abuse only to a mean of 7.0 (SD = 4.0) nmol/L before, 3.8 (SD = 1.0) nmol/L immediately after the NBAS.

Table 20. Infant NBAS cortisol delta 1 and 2 in babies of women with/without depression and/or childhood abuse

	Healthy N=16	**MDD only** N=9-10	**CA abuse** N=3	**MDD & CA** N=33-34	**Statistic**
Baby cortisol delta1 Mean (SD)	-.1 (5.9)	1.7 (5.8)	-3.3 (3.1)	2.4 (12.2)	k-$W_{(3)}$=1.8 p=.61
Baby cortisol delta2 Mean (SD)	-.3 (6.9)	2.7 (9.5)	-.3 (4.9)	-.9 (10.0)	k-$W_{(3)}$=1.1 p=.78

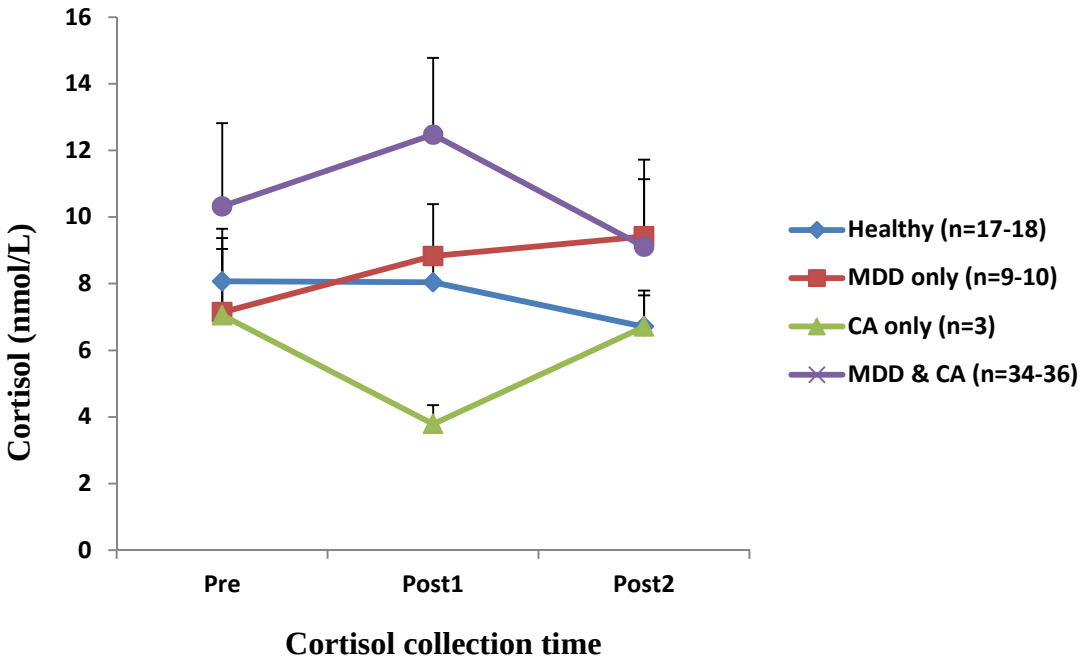


Figure 14. Neonatal cortisol before, immediately after and 30 minutes after the administration of the NBAS in babies of women with/without depression and/or childhood abuse

Associations between infant cortisol and Neonatal Behavioral Assessment Scale items

Correlations (Table 21) are presented between the items of the NBAS at 6 days after birth and the infant cortisol Δ1 (immediately after the NBAS minus pre NBAS). At 6 days, neonatal self-regulatory skills were negatively associated with salivary cortisol Δ1 increase. Further correlations were measured in cortisol Δ1 in babies of healthy and depressed mothers. As can be seen from Table 23, neonatal self-regulatory skills are negatively associated with salivary cortisol Δ1 increase but only in babies of depressed mothers.

Correlations were also examined between items of the NBAS at 6 days after birth and the infant cortisol Δ2 (30 minutes after minus pre NBAS). As can be observed in Table 22, neonatal behavioral regulation at 6 days was not associated with cortisol Δ2. Further comparisons were made between cortisol Δ2 in babies of healthy and depressed mothers (Table 24). Δ2 in babies of healthy women is negatively correlated with motor maturity (r= -.4; p=.05) and tremulousness (r= -.5; p=.02), while in babies of depressed women it is negatively correlated with cuddliness (r= -.3; p=.04), cost of attention (r= -.3; p=.05) and robustness and endurance (r= -.3; p=.04).

Table 21. Spearman's correlations between NBAS items and baby's cortisol delta1 (immediately post minus pre)

NBAS item	**Spearman's correlation N=65**
Alertness	r= -.29; **p=.02**
Animate auditory	r= -.18; p=.15
Irritability	r= .24; p=.06
Motor maturity	r= -.25; **p=.04**
Cuddliness	r= -.13; p=.30
Consolability	r= -.13; p=.40
Tremulousness	r= -.1; p=.40
Quality of alertness	r= -.24; **p=.05**
Cost of attention	r= -.32; **p=.01**
Examiner facilitation	r= -.27; **p=.03**
General irritability	r= -.21; p=.08
Robustness and endurance	r= -.26; **p=.04**
State regulation	r= -.25; **p=.05**
Examiner's emotional response	r= -.24; **p=.05**

Table 22. Correlations between NBAS items and infant delta2 (30 minutes post NBAS minus pre NBAS)

NBAS item	**Spearman's correlation N=65**
Alertness	r= -.13; p=.31
Animate auditory	r= -.03; p=.80
Irritability	r= .16; p=.21
Motor maturity	r= -.08; p=.53
Cuddliness	r= -.16; p=.22
Consolability	r= -.11; p=.50
Tremulousness	r= -.18; p=.15
Quality of alertness	r= -.21; p=.09
Cost of attention	r= -.22; p=.08
Examiner facilitation	r= -.20; p=.12
General irritability	r= -.19; p=.12
Robustness and endurance	r= -.22; p=.08
State regulation	r= -.15; p=.22
Examiner's emotional response	r= -.22; p=.08

Table 23. Correlations between NBAS items and delta1 (immediately after NBAS minus pre NBAS) in offspring of healthy and depressed women

NBAS item	**Healthy** N=22	**MDD** N=43
Alertness	r= -.08; p=.71	r=-.38; **p=.01**
Animate auditory	r= .03; p=.91	r= -.24; p=.12
Irritability	r= .12; p=.62	r=.31; **p=.05**
Motor maturity	r= -.34; p=.13	r=-.2; p=.20
Cuddliness	r= .39; p=.07	r=-.27; p=.09
Consolability	r= .31; p=.31	r= -.17; p=.33
Tremulousness	r= -.28; p=.21	r=.00; p=.98
Quality of alertness	r= .03; p=.91	r=-.28; p=.07
Cost of attention	r= -.04; p=.87	r=-.43; **p=.004**
Examiner facilitation	r= -.00; p=.99	r=-.36; **p=.02**
General irritability	r= .15; p=.49	r=-.30; **p=.05**
Robustness and endurance	r= .00; p=.99	r=-.35; **p=.02**
State regulation	r= .07; p=.76	r=-.33; **p=.03**
Examiner's emotional response	r= -.00; p=.99	r=-.28; p=.07

Table 24. Correlations between NBAS items and cortisol NBAS delta2 in offspring of healthy and depressed women

NBAS item	**Healthy** N=22	**MDD** N=43
Alertness	r= -.24; p=.28	r=-.19; p=.22
Animate auditory	r=-.18; p=.41	r=-.03; p=.87
Irritability	r= .05; p=.84	r=.22; p=.15
Motor maturity	r= -.43; **p=.05**	r=.04; p=.78
Cuddliness	r= .21; p=.35	r=-.31; **p=.04**
Consolability	r=-.03; p=.93	r=-.14; p=.44
Tremulousness	r= -.49; **p=.02**	r=-.02; p=.90
Quality of alertness	r= -.20; p=.37	r=-.29; p=.06
Cost of attention	r= -.20; p=.38	r=-.3; **p=.05**
Examiner facilitation	r= -.20; p=.38	r=-.29; p=.06
General irritability	r= -.01; p=.98	r=-.28; p=.07
Robustness and endurance	r= -.14; p=.53	r=-.32; **p=.04**
State regulation	r= -.10; p=.66	r=-.22; p=.15
Examiner's emotional response	r= -.24; p=.28	r=-.28; p=.07

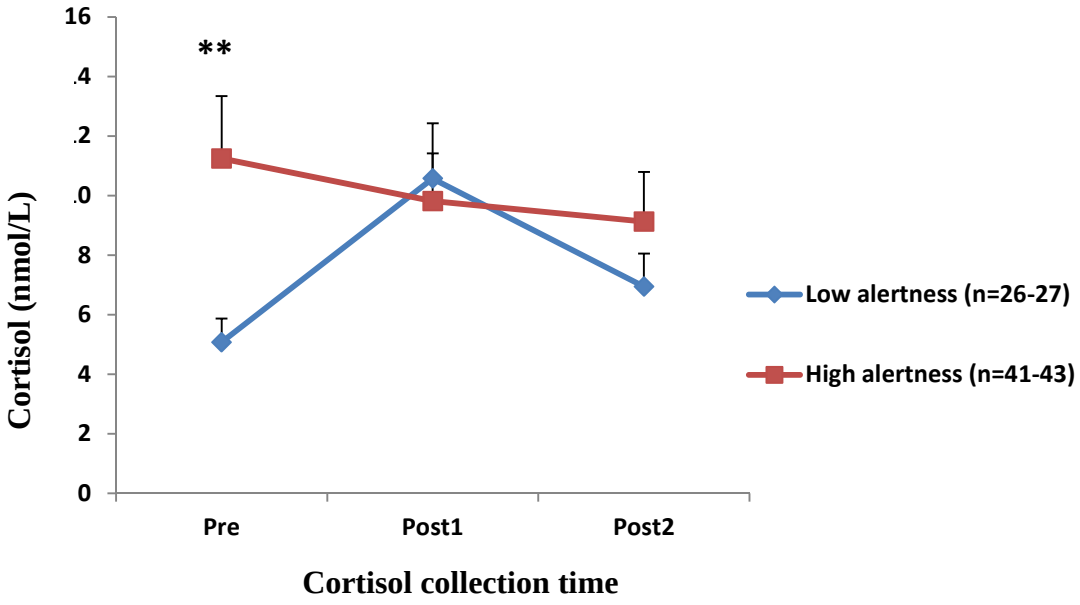
Infant physiological response to the mild stressor of the NBAS administration

In order to demonstrate visually the association between the NBAS scores and the infant response to the minor stressor of the NBAS administration I calculated the median for infant alertness (median = 7) and irritability (median = 4) as these items are representative of infants' behavioural regulation and their ability to engage with the examiner during the administration of the NBAS. Neonates have been divided into high alertness (the median and above) versus low alertness (below the median) and high irritability (the median and above) versus low irritability (below the median). Figure 15 and Figure 16 illustrate respectively the cortisol response before, immediately after and 30 minutes after the administration of the NBAS in babies with high versus low alertness and in those with high versus low irritability.

As can be seen from Figure 15, neonates with low alertness scores (<7) had significantly lower levels of cortisol than neonates with high alertness scores (>=7) before the assessment with the NBAS ($t(50.8)=-2.75$; $p=.008$). Their cortisol levels increased following the NBAS.

Similarly, there was a significant difference between the cortisol delta (immediately after minus pre) in neonates with low alertness scores ($M = 5.9$; $SD = 10.6$) and in those with high alertness scores ($M = -1.4$; $SD = 7.6$) ($t(63) = 3.3$; $p= .002$).

Figure 16 shows that neonates with low irritability scores (<4) had higher levels of cortisol than neonates in the high irritability group (>=4) before the assessment with the NBAS but their cortisol dropped following the NBAS while it increased for babies with high irritability score. However the differences in cortisol levels at the three time points were not significant. There was a significant difference between the cortisol delta (immediately after minus pre) in neonates with low irritability scores ($M = -2.2$; $SD = 7.4$) and in those with high irritability scores ($M = 3.1$; $SD = 10.0$) ($t(63) = -2.1$; $p= .036$).



p=.008

Figure 15. Neonatal cortisol before, immediately after and 30 minutes after the NBAS in babies with high and low alertness levels

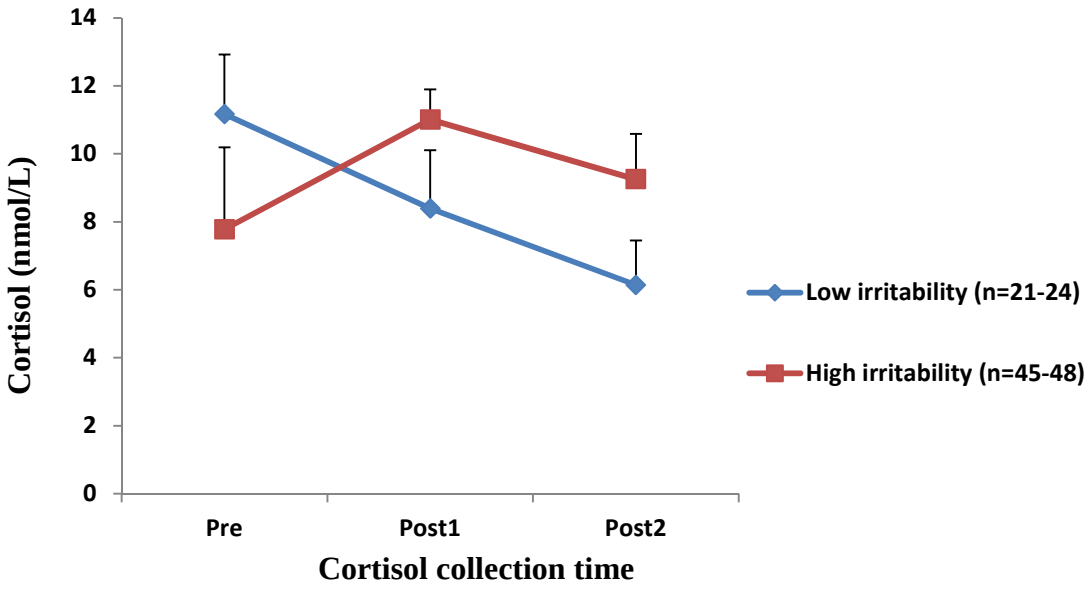


Figure 16. Neonatal cortisol before, immediately after and 30 minutes after the NBAS in babies with high and low irritability levels

Maternal salivary cortisol at 32 weeks and infant behavioural and physiological regulation at 6 days

As it can be seen from Table 25, there is no significant correlation between maternal evening salivary cortisol at 32 weeks gestation and the infant behavioural regulation at 6 days after birth. In the same way, as it can be observed in Table 26, maternal cortisol was not associated with neonatal cortisol secretion before, immediately after and 30 minutes after the administration of the Neonatal Behavioural Assessment Scale.

Table 25. Correlations between maternal evening cortisol at 32 weeks and NBAS items

NBAS item	**Maternal saliva at 32 weeks**
Alertness	r= .02; p=.81
Animate auditory	r= -.03; p=.75
Irritability	r= .06; p=.54
Motor maturity	r= -.002; p=.98
Cuddliness	r= -.15; p=.14
Consolability	r= .11; p=.38
Tremulousness	r= .15; p=.13
Quality of alertness	r= .02; p=.88
Cost of attention	r= -.01; p=.92
Examiner facilitation	r= .01; p=.94
General irritability	r= -.004; p=.97
Robustness and endurance	r= -.01; p=.95
State regulation	r= -.07; p=.52
Examiner's emotional response	r= -.05; p=.62

Table 26. Correlations between maternal evening salivary cortisol at 32 weeks and infant delta1 and delta2 at NBAS

	Baby Δ1	**Baby Δ2**
Maternal cortisol 8 pm	r=-.12; p=.37	r=-.1; p=.47

3.3 Infant HPA axis at 8 weeks postpartum

Infant cortisol response was assessed pre and 20 minutes post the 8-week immunization. Mean cortisol values at each time point were calculated and plotted on two separate graphs: Figure 17 illustrates the mean cortisol levels before and after the immunization across the whole sample, whilst Figure 18 shows the mean cortisol levels before and after the immunization in babies of healthy and depressed women. As can be seen in Figure 17, the mean cortisol levels before the immunization was 8.6 (SD=11.4 nmol/L), followed by a rapid rise 20 minutes after the immunization, reaching a mean of 14.7 (SD=13.5) nmol/L which is significantly higher than the pre-immunization one ($t(96) = 10.7$; $p<.001$).

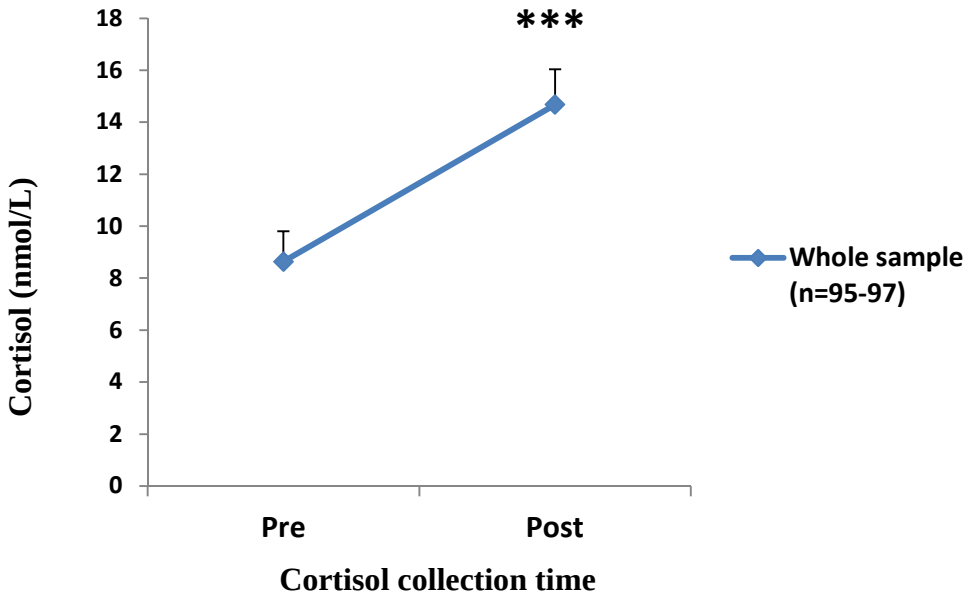
From Figure 18, it can be seen that, pre-injection, the mean cortisol in babies of healthy mothers was 7.6 (SD=5.2) nmol/L, whilst the mean for the babies of depressed mothers was 9.7 (SD=15.2) nmol/L. Twenty minutes after the immunization, cortisol levels increased rapidly in babies of both groups, with those of healthy mothers reaching a mean of 14.1 (SD=10.8) nmol/L and those of depressed women reaching a mean of 15.3 (SD=15.8) nmol/L. There was no significant difference between the two groups.

The difference between infant cortisol after the immunization minus pre-immunization was calculated as a delta. Infant cortisol delta at 8 weeks was analysed in babies of healthy and depressed mothers and in babies of women with/without depression and/or childhood abuse.

Although babies of depressed women have higher cortisol delta than babies of healthy women (Table 27), the difference between the two groups is not significant.

Turning to the cortisol levels before and after the immunization in babies of women with/without depression and/or childhood abuse (Figure 19), cortisol levels increased following the immunization in babies across all groups, with babies of healthy women showing the lowest levels of cortisol in the sample. Specifically, babies of HEALTHY women reached a mean peak of 11.4 (SD=6.7) nmol/L, those of MDD only women

reached a mean peak of 17.2 (SD=15.7) nmol/L, those of CA only women reached a mean peak of 17.9 (SD=15.0) nmol/L and those of MDD & CA women reached a mean of 15.3 (SD=16.8) nmol/L. There was no significant difference in cortisol levels across groups. Similarly, there were no differences in the deltas between the four groups (Table 28). Interestingly, as for infant cortisol at 6 days, no association was observed between maternal evening salivary cortisol at 32 weeks and infant cortisol at 8 weeks postnatal (Spearman's r= -.09; p=.41) (Table 29).



p<.001

Figure 17. Infant 8-week salivary cortisol before and 20 minutes after the immunization

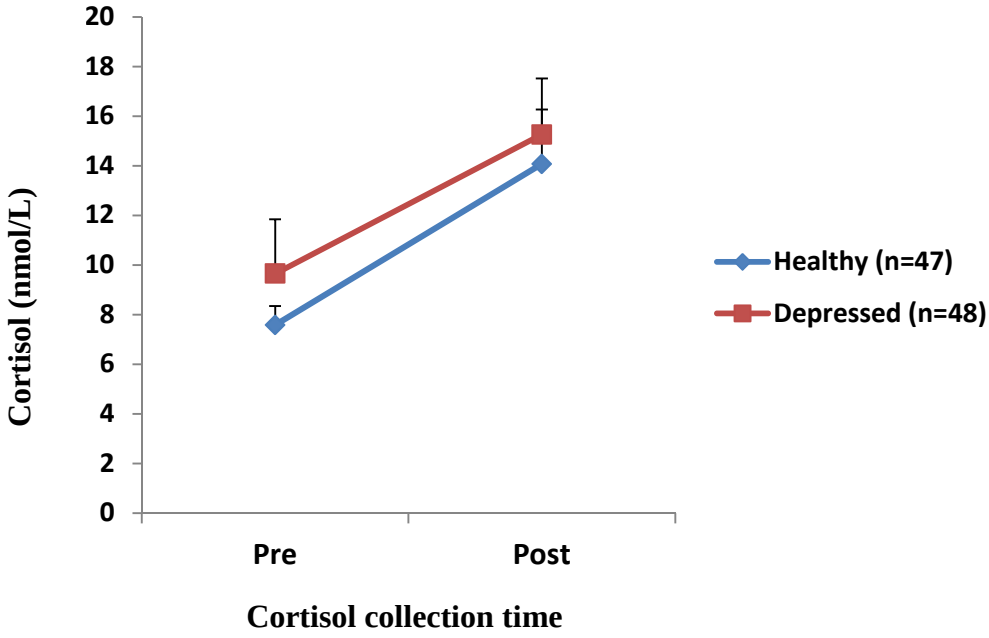


Figure 18. Infant 8-week salivary cortisol before and 20 minutes after the immunization in babies of healthy and depressed mothers

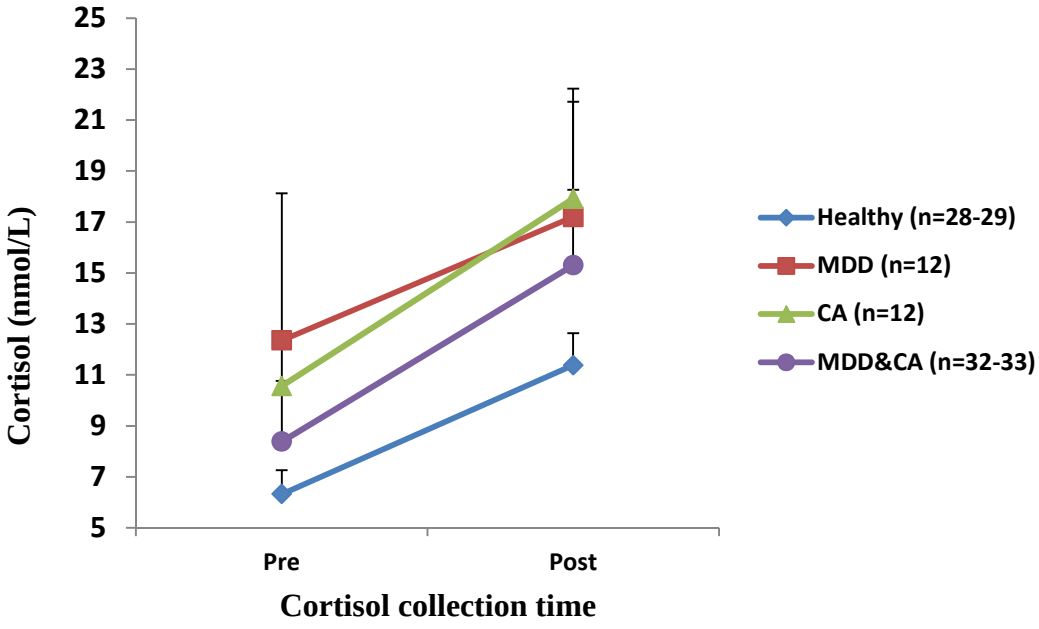


Figure 19. Salivary cortisol at 8 weeks postpartum before and 20 minutes after the immunization in infants of women with/without depression and/or childhood abuse

Table 27. Infant cortisol delta at 8 weeks postpartum in babies of healthy and depressed mothers

	Healthy N=46	**MDD** N=46	**Statistic**
Baby Δ Mean (SD)	6.8 (11.8)	7 (10.5)	Z=-.1; p=.91

Table 28. Infant cortisol delta at 8 weeks postpartum in babies of women with/without depression and/or childhood abuse

	Healthy N=28	**MDD only** N=11	**CA only** N=11	**MDD &** **CA** N=32	**Statistic**
Baby Δ Mean (SD)	5.4 (8.2)	10.2 (10.7)	7.6 (15.9)	6.8 (9.7)	k-$W_{(3)}$=1.4 p=.71

Table 29. Correlations between maternal evening salivary cortisol at 32 weeks and infant cortisol at 8 weeks postpartum

	Spearman correlation with Δ cortisol 8 weeks (post minus pre immunization)	**Spearman correlation with infant cortisol actual values – post immunization**
Maternal cortisol at 8 pm	r=.03; p=.77	r= -.09; p=.41

3.4 Infant HPA axis at 1 year postpartum

Infant cortisol response was assessed before and 20 minutes after the 1 year immunization, as for the 8-week cortisol assessment. Mean cortisol values at each time point are presented in Table 30, which illustrates the mean infant cortisol delta (after minus pre immunization). Babies of depressed mothers had significantly higher cortisol delta at 1 year after birth compared with babies of healthy mothers (Z=-3.1; p=.002). The cortisol values, themselves are shown in Figure 20. Although the difference at Time 2 was not statistically significant, infants of depressed mothers had an increase in the cortisol levels from a mean of 5.9 (SD = 6.4) nmol/L to a mean of 9.8 (SD = 14.1) nmol/L 20 minutes after the immunization compared with infants of healthy mothers from a mean of 6.7 (SD = 12.8) nmol/L to a mean of 6.6 (SD = 13.6) nmol/L 20 minutes after the immunization.

Table 30. Infant cortisol delta at 1 year postpartum in babies of healthy and depressed mothers

	Healthy N=42	**MDD** N=32	**Statistic**
Baby Δ Mean (SD)	-1.6 (2.8)	4.2 (12.4)	Z=-3.1; **p=.002**

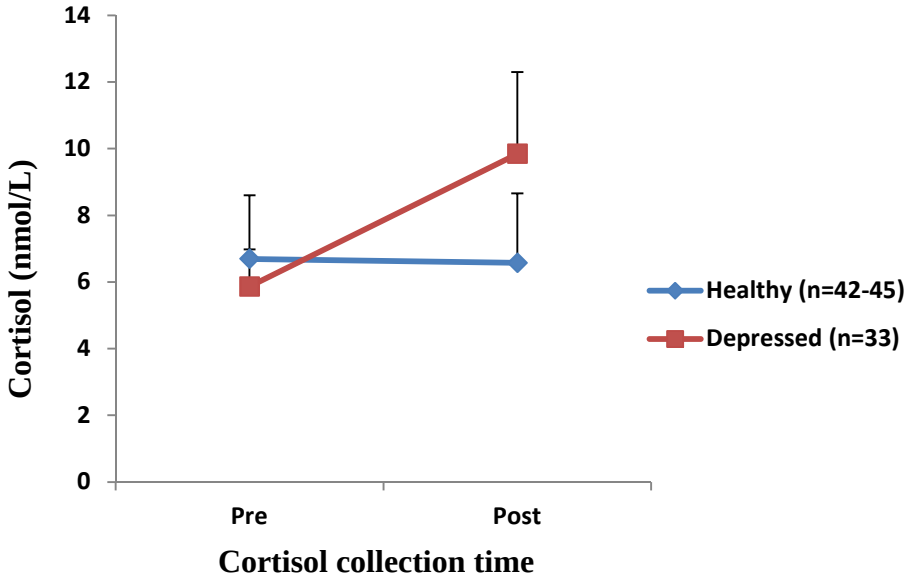


Figure 20. Cortisol at 1-year in infants of healthy and depressed mothers

Infant cortisol delta (after immunization minus before immunization) at 1 year postpartum in babies of women with/without depression and/or childhood abuse

Results analysing the deltas show that there was significant difference in the infant stress response at 1 year, between babies in the four diagnostic groups, as it can be seen from Table 31. Kruskal-Wallis test followed by Mann-Whitney post-hoc test (adjusted for significance) show that babies of women with MDD & CA had a significantly higher response to stress following the one-year immunization compared with babies in the other groups ($K\text{-}W_{(3)}=21.5$; $p<.001$); compared with babies of the HEALTHY women ($U=88$; $Z=-3.5$; $p<.001$), babies of MDD only women ($U=29$; $Z=-3.0$; $p=.002$), and babies of CA only women ($U=21$; $Z=-3.8$; $p<.001$).

Infant salivary cortisol before and 20 minutes after the immunization in babies of women with/without depression and/or childhood abuse is further illustrated in Figure 21. Most importantly, as seen in Table 32, maternal salivary cortisol at 32 weeks gestation was highly correlated with infant cortisol response to the immunization at one year ($r=.4$; $p=.002$).

Table 31. Infant cortisol delta at 1 year in babies of women with/without depression and/or childhood abuse

	Healthy N=25	**MDD only** N=10	**CA only** N=12	**MDD & CA** N=19	**Statistic**
Baby Δ Mean (SD)	.6 (2.9)	-1.6 (5.5)	-1.1 (2.4)	7.9 (14.6)	$K\text{-}W_{(3)}=21.5$; **p<.001**

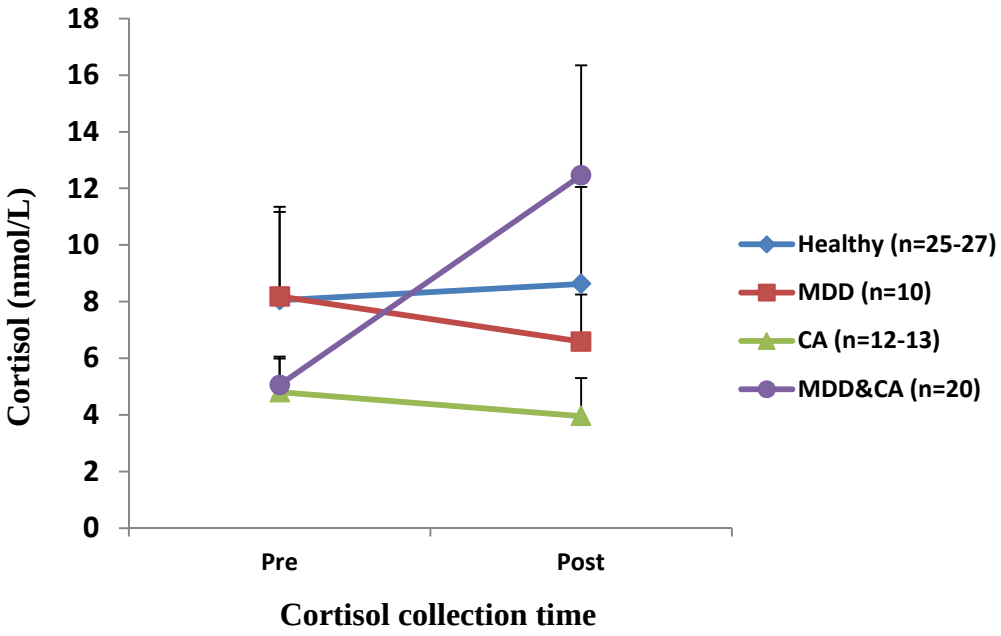


Figure 21. Cortisol at 1 year in babies of women with/without depression and/or childhood abuse

Table 32: Correlations between maternal evening salivary cortisol at 32 weeks and infant cortisol at 1 year postpartum

	Spearman correlation with infant Δ cortisol 1 year (post minus pre-immunization) N= 67	**Spearman correlation with infant cortisol actual values – post immunization** N = 67
Maternal cortisol 32 weeks at 8 pm	r=.37; **p=.002**	r= .41; **p=.001**

3.5 Infant cognitive development at 1 year after birth

Mean composite scores of the Bayley Scale of Infant Development in infants of healthy and depressed women are presented in Table 33, while those of women with/without depression and/or childhood abuse are presented in Table 34. Infants of healthy women have higher composite scores in the cognitive, language, motor and socio-emotional domains compared with infants of depressed mothers, but the group difference is not statistically significant. In the same way, when comparing the Bayley composite scores in infants of mothers with/without depression and/or childhood abuse, no group difference is observed (Table 34).

Because maternal IQ, as measured by the WTAR, was different between the depressed group of mothers and control mothers (see above, Table 5) and was highly correlated with infant cognitive composite scores (Pearson's $r=.4$; $p<.001$) and language composite scores (Pearson's $r=.3$; $p=.03$) at one year, we repeated all the analyses while controlling for maternal IQ: but, again, there were no significant effect of exposure to maternal depression in pregnancy and/or maternal childhood abuse.

Similarly, there was no effect of baby's gender on the Bayley cognitive composite scores ($t(99)=.4$; $p=.703$) and language composite scores ($t(99)= -1.6$; $p=.112$).

Table 33. Bayley composite scores in infants of healthy and depressed mothers

Bayley composite scores	**Healthy** N=38-50	**MDD** N=33-51	**Statistic**
Cognitive composite scores Mean (SD)	113.8 (14.0)	110.4 (12.6)	t(99)=1.3; p=.2
Language composite scores Mean (SD)	101.4 (11.8)	99.1 (12.2)	t(99)=.1; p=.33
Motor composite scores Mean (SD)	101.1 (10.9)	103.8 (13.0)	t(99)=-1.1; p=.27
Socio-emotional composite scores Mean (SD)	103.8 (13.5)	102.4 (11.6)	t(75)=.5; p=.61
General Adaptive GAC composite scores Mean (SD)	96.4 (10.2)	98.9 (12.0)	t(69)=-1.0; p=.35
Practical composite scores Mean (SD)	91.2 (10.9)	93.7 (10.4)	t(71)=-1.0; p=.31

Table 34. Bayley composite scores in infants of women with/without depression and/or childhood abuse

Bayley composite scores	**Healthy** N=23-31	**MDD only** N=11-15	**CA only** N=11-13	**MDD & CA** N=20-32	**Statistic**
Cognitive composite scores Mean (SD)	113.7 (15.1)	110.3 (14.3)	111.9 (12.2)	110 (11.6)	F(3)=.5; p=.72
Language composite scores Mean (SD)	99.7 (12.9)	101.1 (16.5)	102.8 (9.4)	97.6 (10.4)	F(3)=.7; p=.59
Motor composite scores Mean (SD)	102.1 (10.6)	102.3 (13.7)	99.1 (12.5)	103.8 (13.2)	F(3)=.5; p=.72
Socio-emotional composite scores Mean (SD)	107.4 (14.0)	98.2 (9.3)	100.5 (10.8)	105.0 (12.5)	F(3)=1.8; p=.15
General Adaptive GAC composite scores Mean (SD)	98.3 (11.8)	96.5 (9.3)	92.1 (6.6)	100.5 (13.6)	F(3)=1.4; p=.26
Practical composite scores Mean (SD)	91.6 (11.9)	91.0 (9.2)	87.9 (9.2)	94.7 (10.4)	F(3)=1.0; p=.4

4 Discussion

4.1 Overview

This thesis used a prospective longitudinal study of women and their babies in order to investigate the possible associations between maternal history of abuse during childhood and antenatal depression and the interactive effects on the baby's outcomes in the first year after birth. This Discussion comprises a summary of the main findings, a critical appraisal of current literature, strengths and limitation of this study, and suggestions for future research. Potential clinical implications are also discussed.

4.2 Maternal depression and experience of childhood abuse

The findings from this study show that history of abuse during childhood is strongly associated with antenatal depression. Specifically, results suggest that women who have been abused in childhood (age 0-17) are 7 times more likely than those who have not been abused to develop depression during pregnancy. These findings not only support the hypothesis of an association between maternal abuse during childhood and maternal antenatal depression, but also are consistent with previous research demonstrating that experiences of abuse during childhood increase the chances of developing depression during adulthood, especially during vulnerable periods such as pregnancy and adjustment to motherhood. For example they replicate the findings of the South London Child Development study, which assessed a cohort of women in pregnancy in 1986 and followed them up for almost thirty years. This research showed that women who were abused during childhood were ten times more likely than those who were not abused to develop depression in pregnancy (Plant et al., 2013). Both studies, almost 30 years apart, used a prospective design to assess the association between the experience of childhood abuse and depression in pregnancy and we thus feel confident that the association is robust, although genetic and environmental factors have not yet been explored.

A study by Lang and colleagues (Lang et al., 2010) specifically investigated the effects of maternal childhood abuse and psychopathology on parenting capacity, mother-infant interaction and infant temperament at the age of one year. Results show that a maternal experience of childhood abuse is associated with impaired mother-infant interaction; furthermore, women who were emotionally abused, found it more difficult to regulate the distress of their infant, hence their babies showed more irritable temperament at age one than babies of non-abused mothers. Notably, the association between maternal exposure to abuse in childhood and depression in pregnancy was not explored; moreover, the sample size was small and maternal neglect in childhood was not assessed (Lang et al., 2010).

This link between exposure to childhood abuse and the development of depression during pregnancy casts light on the natural development of psychopathology in adulthood, particularly when a woman is about to become mother.

Notably, women have been recruited into this study on the basis of their mental state during pregnancy and not on the basis of their childhood experiences. Hence, the finding that 52% of the women had experienced abuse in childhood reflects the high prevalence among the general population living in South London.

The high prevalence of childhood abuse in the control group (29%) raises the issue of the pathways which lead to the absence of depression during pregnancy. We know that exposure to abusive experiences in childhood is a major risk factor for the later development of mental health problems, so it could be speculated that those who do not develop mental health problems are highly resilient individuals. This account is in line with theoretical knowledge by Pariante et al, which suggests that the pathways leading to psychopathology have to be framed in the context of a bio-psycho-social model (Wertz and Pariante, 2014) and that more than one mechanism is involved in the development of resilience to stress (Charney, 2004). Similarly, several factors could

affect the association observed between childhood abuse and increased risk of adult psychopathology. Genetic predisposition to abuse and psychopathology, as well as other potential environmental or individual factors have not been explored in this Thesis but might contribute to explaining this strong association. In my sample, however, there are significant differences in the socio-demographic characteristics of healthy and depressed women. Employment and a stable married or cohabiting relationship with the baby's father were seen to be protective factors against the development of depression in pregnancy. Similarly maternal IQ was higher in women who were not depressed compared to those who were depressed. Moreover primiparous mothers were less likely to become depressed compared with mothers who already had children. The current findings would suggest that being in employment, in a stable relationship, with a higher IQ would support a woman in being effective in the self-regulation of emotions and functional coping strategies when facing stress or major changes such as a pregnancy. One could further speculate that women who were abused as children and living in very abusive and frightening environments, had to become very aware of what was going on around them and be able to anticipate potentially life-threatening parental behaviours. Hence, from a very early age, these women might have had to use their intellectual abilities as coping strategies in order to survive in a potentially dangerous environment (successful adaptation). This might explain the functionality of the differences in the IQ levels among women with/without depression and/or history of childhood abuse.

Even though some women who have experienced abuse as a child develop coping strategies, the findings presented here show that the majority of women exposed to abuse during childhood do develop depression in pregnancy. It is well established that childhood abuse predisposes women to persistent psycho-biological alterations of the HPA axis in adulthood, although the directions of those alterations is unclear (Bublitz and Stroud, 2012). Specifically, exposure to abuse in early life has been robustly

associated with the development of pathological changes which, in turn, increase vulnerability to stress and mental illness in adult life (Nemeroff, 2004). It has been hypothesised that individuals who are abused in childhood and develop later psychopathology have some pre-existent genetic vulnerability that is triggered by the exposure to early adversities and, in turn, increases the chances of developing mental illnesses later in life (Nemeroff, 2004). Hence, a more holistic approach as suggested in the bio-psycho-social model might offer a clearer picture of how several factors play a significant role in the context of childhood abuse and its association with adult psychopathology.

It is known that the Central Nervous System, which is characterized by plasticity, is highly affected by early personal adverse experiences (Nemeroff, 2004). In fact, as the Central Nervous System is more plastic in young age, it might well be more affected by events occurring in the early stage of life. Evidence from animal studies suggests that when young animals are deprived of maternal care during the early postnatal period some major neurobiological alterations occur that predispose the animal to greater stress in adult life (Nemeroff, 2004).

Hence, a persistent activation of the stress response following traumatic experiences in early life is thought to predispose individuals to greater stress in adulthood, such as anxiety and depression. The main biological system involved in the regulation of the stress response is the hypothalamic-pituitary-adrenal (HPA) axis, which has been largely studied as one possible mediator of the impact of childhood abuse on later mental health. A hyperactivity of the HPA axis has been found to be programmed by early life experiences and, in consequence, its alterations predispose an individual to depression in adulthood (Pariante and Lightman, 2008). In fact, hyperactivity of the HPA axis is one of the most consistent findings in studies on major depression overall the last few decades and it is considered part of the pathogenesis of depression (Pariante

and Lightman, 2008). Hence, in psychobiology it is believed that persistent dysregulation of the HPA axis functioning following the exposure to abuse in childhood is the main biological mechanism leading to adult depression.

4.3 Hypothalamic-pituitary-adrenal axis in women at 25 and 32 weeks gestation

With regards to the maternal cortisol response at 25 weeks gestation, no significant differences were found between healthy and depressed women, nor between women with/without depression and/or childhood abuse.

We know that circulating cortisol increases during pregnancy in healthy subjects, reaching its peak in the third trimester (Jung et al., 2011) and so one possible reason that no difference was detected at 25 weeks could be that the cortisol profile of depressed women has not yet started differentiating from that of non-depressed women. Hence, findings at 25 weeks gestation suggests that maternal endocrine profile at this time of pregnancy is not yet affected by maternal mental state, or by her experience of childhood abuse.

At 32 weeks, the findings present a different picture from that at 25 weeks gestation. First, when comparing cortisol levels in healthy and depressed women only, results show that depressed women have higher cortisol levels throughout the day and that at 8 pm it is significantly higher than healthy women. Furthermore, when comparing women in the healthy with/without abuse and depressed with/without abuse groups, the cortisol levels at 8 pm for women with depression alone and depression plus childhood abuse are significantly higher than in healthy women and women with childhood abuse alone. These findings suggests two important points: (i) that childhood abuse alone does not have an effect on the cortisol response at 32 weeks; (ii) that is maternal depression in pregnancy, with or without the added insult of childhood abuse, that affects the HPA axis regulation at 32 weeks.

These findings are consistent with a study by O'Keane et al, demonstrating elevated cortisol levels in depressed women in the third trimester of pregnancy compared with healthy controls, with no significant group difference in the diurnal salivary cortisol (O'Keane et al., 2011). It is known that pregnancy in general brings major changes to the HPA axis regulation in healthy individuals, with greater levels at awakening, and a decreasing cortisol pattern during the day (Jung et al., 2011). The additional presence of depression during pregnancy further contributes to a dysregulation of the stress response, with changes in the cortisol levels observed in depressed and healthy pregnant women. One reason might be that severe and persistent psychological distress, as seen in major depression, contributes to a further increase of the overall activity of the HPA axis, increasing, in turn, the cortisol levels secreted during the day. Due to an accumulation of stress, the HPA is no longer able to bring the cortisol levels back to normal in the evening due to persistent alterations.

Literature on this topic suggests that women with depression exhibit flatter diurnal cortisol response compared with non-depressed subjects (Jarcho et al., 2013). Specifically, cortisol secretion in depressed patients is characterized by a peak at awakening, followed by a decrease during the day and a slight increase at night (Balardin et al., 2011). This pattern represents a flattening of the cortisol cycle during the day.

4.4 Infant obstetric outcomes at birth

The findings from the present study show that babies of depressed mothers have a significantly lower gestational age at birth (M=39.6, SD=2.3, weeks) and lower birth weight (M=3311.4, SD=564.9, grams) compared with babies of healthy mothers (respectively: M=40.3, SD=1.4, weeks; M=3560.3, SD=423.6, grams). Although the difference is not very great in clinical terms the findings mirror those of O'Keane et al

(Healthy, M=40.23, SD=1.05. weeks; Depressed: M=39.46, SD=1.7, weeks) (O'Keane et al., 2011).

Adverse obstetric outcomes in depressed women are well documented in the literature (Diego et al., 2009) (Duthie and Reynolds, 2013), where stress in pregnancy is thought to be the key factor contributing to the dysregulation of the HPA axis in women which, in turns, leads to poorer obstetric and developmental outcomes for the child later on. Although the exact mechanisms underlying HPA axis alterations remain speculative, these initial findings confirm that the intra-uterine environment plays a key role as a pathway to explain the trajectory of stress in the next generation (Talge et al., 2007).

These findings are further supported by other large studies investigating the perinatal outcomes among women with depression and stress in pregnancy. In a study by Hobel et al, 524 women were assessed at 18-20, 28-30 and 35-36 weeks gestation and at delivery (Hobel et al., 1999). Stress in pregnancy was assessed with the Perceived Stress Scale, and cortisol levels were measured at each time. Results reveal that women who delivered pre-term babies had higher concentration of circulating cortisol compared with those who delivered full term (Hobel et al., 1999). Similarly, a study by Sandman et al, assessed a sample of two hundred pregnant women at 15, 19, 25 and 31 weeks gestation and followed them up at birth (Sandman et al., 2006). At each visit, maternal cortisol and CRH were assessed via a blood test. Results showed that women who gave birth preterm (<37 weeks) had an increase in the CRH levels already at 15 weeks gestation, suggesting that stress stimulates greater production of cortisol and CRH levels which, in turn, induce preterm birth. Furthermore, this study suggests that CRH and cortisol levels at 31 weeks are the best predictor of gestational lengths and pre-term birth (Sandman et al., 2006). High levels of stress in pregnancy trigger early parturition. The present study's findings are in line with those from the two prospective studies that investigated the effects of stress in pregnancy on gestational length.

A recent meta-analysis of 29 studies by Grote et al, also highlighted the role of antenatal depression as a factor predisposing women and their offspring to a greater risk of low birth weight and other obstetric adverse outcome that might affect neonatal survival (Grote et al., 2010). In the meta-analysis, most studies assessed major depression in pregnancy by using short screening tools with no structured interview. The present data add to these findings not only by replicating this robust association between antenatal depression and low birth weight but also by extending their validity as depression is assessed with a structured clinical interview.

4.5 Infant behavioral and physiological outcomes at 6 days after birth

Women's experience of childhood abuse and depression in pregnancy has a significant impact on baby's behavioural and physiological regulation at 6 days after birth. The findings in the current study revealed that infants exposed to both maternal history of childhood abuse and depression in utero were more difficult to engage in the Neonatal Behavioural Assessment Scale, were less responsive to stimulation, less alert and more irritable than neonates not exposed to such conditions.

These findings are consistent with other studies on maternal depression in pregnancy and infant behavioural regulation. In a study by Lundy et al, (1999) women were recruited in pregnancy and assessed for their current mood, while their babies were assessed within one week after birth with the NBAS. Results from this study suggest that babies of depressed mothers have lower scores in the orientation items than babies of healthy mothers, indicating that they find it more difficult to engage in the assessment and respond to facial and auditory stimulation. Furthermore, maternal antenatal cortisol, which was higher in the depressed group compared with healthy group, was associated with neonatal abnormal reflexes (Lundy et al., 1999). The present study replicates Lundy's findings showing an association between maternal depression in pregnancy and neonatal behavioural dysregulation at 6 days, and in addition we show

that depression in pregnancy is associated with the baby's response to the stressor of the administration of the NBAS. However in the present study there was no direct association between the mother's cortisol in pregnancy and the baby's behavioural and biological response to the stressor at 6 days.

The finding that neonatal behavioural regulation is affected by maternal depression in pregnancy is further confirmed in a study by Field et al, 2006. Data suggests that babies of women who experienced depression during pregnancy show sub-optimal performance on the NBAS within a few hours of birth, compared with babies of non-depressed women. Specifically, neonates of depressed mothers showed higher levels of irritability, lower activity levels, were less robust and less mature compared with neonates of non-depressed mothers, although maternal history of childhood abuse was not assessed. In the present study, babies of mothers who were depressed in pregnancy had problems in behavioural regulation and these were exacerbated when maternal depression occurred with the added insult of childhood abuse.

Our findings are also in line with those by Davies et al, 2007, who found an association between elevated maternal stress in pregnancy (anxiety and depression) and infant temperament at two months (Davis et al., 2007). More than two hundred dyads were assessed. Maternal cortisol in pregnancy was assessed at 18-20, 24-26, 30-32 weeks one hour after eating in the early afternoon and maternal psychological state was evaluated using the Centre for Epidemiological Studies Depression Inventory (Santor and Coyne, 1997). Infant temperament was evaluated by the mother at 2 months using the Infant Behaviour Questionnaire (Gartsen and Rothbart, 2003), with a particular focus on infant response to fear (Davis et al., 2007). In this study, elevated maternal cortisol levels at 30-32 weeks gestation were strongly associated with and predictive of high levels of infant negative reactivity. Again, this study confirms the hypothesis of the existence of a link between the antenatal environment and foetal programming, with adverse

consequences for infant development but our findings do not observe a direct association between maternal cortisol at 32 weeks gestation and baby's behavioural regulation at 6 days.

Finding of an association between maternal high cortisol levels in pregnancy and infant impaired emotional regulation, activity and adaptation scores at 7 weeks was also found in a study carried out in the Netherlands by de Weerth et al. where a small sample of pregnant women (n=17) were assessed in pregnancy and followed-up until the baby was 20 weeks old. Maternal cortisol was evaluated in later pregnancy and infant behaviour was video-taped during a bath session at 1, 3, 5, 7, 18 and 20 weeks of infant age (de Weerth et al., 2003). Maternal cortisol was categorized as either high or low, and infant temperament was rated on the basis of the amount of crying, fussing and negative facial expression shown during the bath sessions. In line with the foetal programming hypothesis, babies of high-cortisol women showed more difficult behaviours at each time point. Interestingly, this association between maternal high stress and infant high numbers of crying and fussing episodes and negative facial expressions was stronger at 1-7 weeks than at a later developmental stage (18-20 weeks) (de Weerth et al., 2003). This further illuminates the pathway found in my study, linking maternal stress in pregnancy to infant behavioural regulation shortly after birth. Together the literature supports the association between maternal depression in pregnancy and difficulties in neonatal behavioural and physiological regulation shortly after birth. The current study adds to the literature by providing strong evidence of an association between maternal cumulative stress in pregnancy, as indicated here by exposure to maternal depression in utero and the mother's experience of childhood abuse, on neonatal regulatory capacity after birth.

The results of my study also demonstrate that neonates of depressed mothers had significantly higher levels of circulating cortisol immediately after the administration of

the NBAS compared with babies of healthy women. Specifically, although the group difference was not significant, babies of women with both conditions of childhood abuse and antenatal depression have the greatest cortisol increase after the NBAS, followed by babies of women with depression only. Interestingly, neonates of mothers with childhood abuse alone (n=3) show a blunted cortisol response after the NBAS. This is consistent with research (Nemeroff, 2004) on abused individuals, neonates of women abused in childhood show a blunted cortisol response following the mild stress of the NBAS, although the group difference is not statistically significant. There is some evidence to suggest that maternal childhood abuse per se is associated with hypo-activity of the neonatal HPA axis but when comorbid with antenatal depression the neonates' HPA axis functioning at 6 days is hyperactive.

Several studies have now examined the effects of childhood trauma on adult physiological regulation. First, it is well established that there is an association between childhood trauma and greater stress and psychopathology in adulthood, such as post-traumatic stress disorder. A study by Yehuda et al, examined the cortisol response and the association between exposure to childhood trauma and PTSD in adults offspring of Holocaust survivors by analysing 24-h urine secretion among those subjects. Offspring of Holocaust survivors reported greater levels of emotional abuse and neglect as well as physical neglect and sexual abuse compared to controls. Interestingly, adults who were sexually abused and developed PTSD showed lower cortisol levels than those who were sexually abused only. Moreover, adults who were sexually abused and developed depression in adulthood showed greater cortisol levels than those with depression alone (Yehuda et al., 2001). In this sample, an increase in the cortisol secretion in sexually abused adults was seen only in the presence of depression. Likewise, my results show that women exposed to both childhood abuse and depression show greater cortisol

levels in pregnancy compared with women who were depressed only or exposed to childhood abuse only.

Following this reasoning, these results could be interpreted as a demonstration of the first signs of an intergenerational transmission from mother to infant, where both conditions of maternal childhood abuse and antenatal depression have a detrimental effect on baby's behavioural regulation at 6 days, while it is maternal depression that has an impact on the baby's physiological response to stress at 6 days. According to the psycho-bio-social model of stress, genetic factors might have an impact of the transmission of stress from one generation to the next, as previously discussed, but they have not been explored in the studies reported above, nor in this Thesis.

4.6 Infant HPA axis at 8 weeks postpartum

Maternal childhood abuse and/or antenatal depression does not modulate the infant HPA axis functioning at 8 weeks after birth. Findings suggest that there is an increase in the cortisol levels in babies following the 8 weeks injection, but their stress levels are not associated with maternal diagnosis and/or abuse history. Although infants of depressed mothers have non-significantly higher cortisol levels before and 20 minutes after the immunization compared with those of healthy mothers, no other abnormality in the HPA axis function is observed while comparing the difference between the cortisol collected before and after the injection across groups. Furthermore, there is no association between maternal salivary cortisol at 32 weeks gestation and infant cortisol levels at 8 weeks after birth. It is interesting to note that, contrary to baby's stress response at 6 days after birth, at 8 weeks we could not find a similar effect. There are several possible reasons for this. First, one reason could be that infant HPA axis changes during time. Babies at 6 days could be more sensitive to stress compared with infants at 8 weeks, when they are more adjusted to the new environment and to maternal care. At this stage of the baby's life, protective effects such as mother-infant interaction

might play a key role in the regulation of the child's biological system (Field et al., 2006).

My findings are not in line with other studies that found an association between maternal depression in pregnancy and infant HPA axis dysregulation at 8 weeks after birth. Data from a study in a South India give evidence that maternal depression and high cortisol levels in the third trimester of pregnancy were associated with infant cortisol response following the immunization at two months of age (Fernandes et al., 2014). Infant salivary cortisol was collected 10 minutes prior and 20 minutes after the immunization, as in the current study. Compared with the families living in the area of South London, the socio-economic status of rural Indian families with low income needs to be considered as a potential factor affecting the stress response of those mothers and their infants. Moreover, given the small sample size of Fernandes' study (n=19), one could speculate that the association between maternal depression and infant cortisol response could no longer be present with a bigger sample size.

The nature of the stressor may also account for the fact that we find differences in the babies' response to the stressor at 6 days but not at 8 weeks. The Neonatal Behavioural Assessment can be seen as a mild stressor as it involves being handled by a stranger for about 20 minutes. It demands concentration from the baby and draws on their ability to self-regulate. This could be more stressful than an injection at 8 weeks that is quicker and is administered when the infant is in the caregiver's arms. The infant's perception of pain and the consequent response to stress might explain the difference in the stress response at 6 days and 8 weeks. Davies and colleagues give further support to the idea that infants respond differently to stressors depending on their age. They have demonstrated that the association between the sympathetic branch of the autonomic nervous system and the secretion of salivary alpha-amylase develops between two and

six months of age, suggesting that young babies (0-2 months) are less responsive to painful stressors (Davis and Granger, 2009). This might explain my results at this stage.

4.7 Infant HPA axis at 1 year postpartum

At one year of age the data in the present study shows a marked difference in the response to the immunisation in the babies of mothers who had been depressed and had experienced abuse in their childhood compared with those of healthy women, and women with depression or childhood abuse alone. Babies of women with both depression and history of childhood abuse have a significant increase in the cortisol levels 20 minutes after the 1 year immunization compared with those of healthy mothers, of mothers with depression only and of mothers with experience of childhood abuse only, indicating that the infants of these women are more sensitive to stress. Thus, these data provide support to the hypothesis that the one-year-old babies of women with both conditions of childhood abuse and antenatal depression are particularly vulnerable to stress.

The fact that the association between maternal salivary cortisol at 32 weeks and infant cortisol is present at 1 year but not at 8 weeks might cast further light on the nature of the HPA axis regulation and development in children throughout the first year of life. In fact, it could be speculated that maternal depression in pregnancy might have different effects on the regulation of the infant HPA axis at different ages. This has been shown to be the case in animal studies, which have shown that the HPA axis immediately after birth becomes hyper-sensitive to mild stressors in offspring of anxious mothers who had high cortisol levels in pregnancy (Van den Hove et al., 2005). After these initial hyper concentrations of circulating cortisol in young babies, the HPA axis becomes “desensitized” and leads to a down-regulation of cortisol receptors a few weeks after birth (Elzinga et al., 2008). Another potential explanation is that cortisol levels are

associated with the stress tasks. For example, babies might find the handling during the administration of the Neonatal Behavioural Assessment at 6 days more stressful than an injection at 8 weeks. Hence, we could speculate that babies of women with depression in pregnancy do not respond equally in stressful situations at 6 days, 8 weeks and 1 year after birth.

Other studies have explored the cortisol response in children in the first year of life although, to my knowledge, this is the first study using a prospective longitudinal design to explore cortisol at 8 weeks and 1 year in infants of mothers who were abused in their childhood and developed depression in pregnancy. An association between maternal depression in pregnancy and increased salivary cortisol in the offspring at six month of age has been found in a study by Brennan and colleagues. Specifically, infants were placed in a car seat behind an occlusion seat blocked from the mother view and exposed to an intense sound burst for three times in 90 seconds. Salivary cortisol was collected at three time point during this procedure in order to assess child stress response. This finding suggests that exposure to maternal depression in the antenatal and postnatal period increases the infant cortisol response following a stressor (Brennan et al., 1998). These results are also consistent with another study on infants of healthy women, demonstrating that infant response to stress becomes more consistent between two and six months after birth, showing no difference between premature and full term babies (Grunau et al., 2010). This study suggests that two to six months is window of time offers the maximal cortisol response following an immunization, which will be less at later ages. Overall, despite some methodological and sample differences across the studies, data consistently show cortisol reactivity in infants exposed to a stressor from the third month of life, although the literature lacks studies that specifically look at the stress response following the injection at one year.

In the present study maternal salivary cortisol at 32 weeks gestation is robustly associated with infant cortisol at 1 year, suggesting that there might be an intergenerational transmission of biological alterations from one generation to the next, as suggested by the foetal programming hypothesis (Duthie and Reynolds, 2013).

Evidence of an association between maternal HPA axis in pregnancy and offspring HPA axis is supported by studies on clinical and non-clinical samples. A prospective cohort study on a Swedish population assessed the natural physiologic trajectory of hair cortisol in healthy pregnant women and their babies during pregnancy, at 1, 5, 8 years, findings a significant linear association over time between maternal and infant HPA (Karlen et al., 2013). This study suggests that the physiology of stress has a set point and raises over time, with a high heritability of the cortisol traits from mother to infant. While this study comprises healthy subjects only, my sample suggests that a strong association between maternal cortisol at 32 weeks and infant cortisol at 1 year persists from one generation to the next, both in the healthy mothers and in cases when there are alterations of the stress response due to maternal depression and childhood abuse history.

4.8 Infant cognitive development at 1 year

The finding that there was no group difference in the composite scores of the Bayley Scale of Infant Development do not confirm the hypothesis that infants of women with depression would have an impaired cognitive performance at 12 months compared with infants of non-depressed mothers. Similarly, no association was found between maternal IQ in pregnancy and infant cognitive scores at 12 months of age in babies of healthy and depressed women and babies of women with/without antenatal depression and/or childhood abuse.

One possible reason could be that the administration of the Bayley Scale of Infant Development in infants at 12 months does not yet detect a difference in babies'

cognitive performance. In fact, the literature suggests that several studies have used the Bayley Scales of Infant Development in infants who were 18 months old. A longitudinal study that by Conroy et al, 2012, has examined the effects of comorbid maternal depression and personality disorders in the postpartum period on infant cognitive development at 18 months (Conroy et al., 2012). A sample of two hundred women was recruited in the study following the birth of their baby and was assessed for depression and/or personality disorders two months after delivery, including a control group. Maternal depression and personality disorders were diagnosed with the Structured Clinical Interview for DSM-IV (First et al., 2002), respectively for Axis I and II disorders. Infant emotional and social development was assessed at 18 months with the Infant-Toddles Social and Emotional assessment (Carter and Briggs-Gowan, 2006), while infant cognitive development was evaluated with the Bayley Scales of Infant Development (Bayley, 2005). Findings suggest that infants of mothers who had depression and personality disorders in the postnatal period, had lower cognitive scores in the Bayley Scales of Infant Development compared with those of healthy women. Furthermore, infants of mothers with depression and personality disorder had a higher rate of externalizing and internalizing problems compared with those of healthy women (Conroy et al., 2012).

Interestingly, another study by Zhu et al (Zhu et al., 2014) explored the effects of maternal stress during pregnancy on the offspring cognitive development at 18 months. In this study, women's stressful life events were assessed at 32 weeks gestation using the Prenatal Life Events Checklist (Zhu et al., 2013) that assessed maternal stress related to negative life events happened during pregnancy. This study found that babies exposed to maternal stress in pregnancy had impaired cognitive performance at 18 months compared with those of women who were not stressed in pregnancy. Hence, we might speculate that the baby's age at the time of the assessment might be important in

detecting the difference between babies of healthy and depressed mothers. Another important key factor to consider in the interpretation of these results is that the timing of maternal depression and its effects on infant cognitive outcome is not clear (Evans et al., 2012). A large longitudinal study of parents and infants (ALSPAC study) with more than five thousand dyads taking part, examined the effects of maternal depression both in the antenatal and postnatal period on the offspring development after birth. Results from this study show that maternal depression during pregnancy has detrimental effects on infant cognitive development at 18 months of age, while no effect has been observed for depression in the postnatal period (Evans et al., 2012). It is possible that, in the present study, the age of the baby at the time of the administration of the Bayley Scales and the small sample size could account for the lack of findings.

Evidence from the South London Child Development Study showed that it was the effects of depression in the postnatal period and not the antenatal period that accounted for the cognitive deficits in children exposed to maternal depression. (Hay et al., 2001).

4.9 Methodological considerations

This study has a number of strengths. The prospective longitudinal design from pregnancy to one year allowed for the exploration of the temporal relationship between maternal childhood history and mental state in pregnancy on the infant outcomes at 12 months of age. Second, the use of a community sample has increased the ecological validity of the findings, making them possible to generalize findings beyond clinical samples. Thirdly, the data was obtained in a face-to-face interview or by direct behavioural assessment of the baby, using validated instruments and measures of maternal psychopathology and child development.

Alongside the strengths of this study, there are also some methodological limitations and study considerations that need to be addressed and considered when interpreting the findings of this thesis.

A first limitation is the sample size, which is relatively small, especially in each of the four groups. Some of the unexpected negative findings (such as the lack of a significant association between maternal antenatal cortisol and infant physiological response at 6 days and 8 weeks, as well as the lack of significant difference in the cortisol levels between women with one condition of abuse or depression alone versus those with both depression and childhood abuse in the antenatal and postnatal period) could be explained by this limitation. Similarly, some results related to the condition of maternal depression alone or maternal childhood abuse alone might be due to the small sample size of each group.

Women were not recruited on the basis of their childhood histories but on their mood during pregnancy. Hence, the recruitment of women who experienced abuse during childhood and did not develop depression has been difficult.

Secondly, the fact that the majority of women in the sample were highly educated means that the sample is not representative of the population living in the area of South London. This might affect the generalizability of the findings to the general population. Although there were other socio-demographic differences between depressed and healthy women as found for employment, marital status, parity and maternal IQ, these did not account for the differences observed in the behavioural or physiological regulation of the infants.

Thirdly, it is best practice if the Neonatal Behavioural Assessment Scale is administered on two consecutive days, in order to assess the child's best performance (Brazelton and Nugent, 1995). In the present study, the scale was only administered once, due to time constraints because the assessments were conducted in the participants' homes.

Fourth, after the 8 weeks immunization, women's methods of consoling their baby's distress varied. Between the injection and the collection of the infant saliva sample 20

minutes afterwards, some women fed their baby while others gave them a pacifier, which could potentially bring the infant cortisol levels down. I did not control for this variable in the analysis. Similarly, at 12 months women used different consoling strategies, although at this age infants are easier to distract with a toy or are able to walk around.

Last, alternative possible explanations for the results might be due to environmental effects, genetic and other factors that have not been explored in this work. Hence the associations found do not define a specific trajectory for the data but offer a view on what the transmission of stress across generations might involve.

Suggestions for future research

Taken together, there are long term detrimental effects of child maltreatment on adult heath, child development and the associated economic costs (May-Chahal and Cawson, 2005). It becomes, therefore, of vital importance to investigate further the psychobiological and biological mechanisms linking maternal history of childhood abuse and perinatal outcomes. This would also allow for the targeting of risk factors for optimal child development (Mohler et al., 2008) and the development of clinical interventions to prevent later mother-child psychopathology.

To further explore the present findings, future studies should comprise a bigger sample size, especially for the group of ‘abused only’ women.

Future research will need to explore maternal sensitivity and mother-infant interaction as a potential mediator between maternal history of childhood abuse and antenatal depression on infant developmental and physiological outcomes after birth. The identification of patterns leading to mother-infant wellbeing would provide the opportunity to identify the optimal time for targeted interventions.

Furthermore, it would be interesting to further explore the role of fathers as a protective factor that mediates the association between maternal experience of childhood abuse

and depression in pregnancy with the infant outcomes. Also, given the interesting finding that 29% of women had been exposed to childhood adversities and did not develop depression in adulthood, it would be important to understand further the mechanisms by which childhood experiences did not lead to mental health problems later in life, and look closely at the children of this group of women in a larger sample. Future studies should further investigate resilience processes in people who have been abused in childhood, in order to predict, potentially prevent, and treat stress-related adult psychopathology.

Future studies also need to consider wider biological markers as indicators of physiological abnormal response in mothers and babies, which could potentially clarify further the cortisol response and the HPA axis function in these women. For example maternal cytokines in pregnancy might be another possible mediator between maternal antenatal depression and foetal outcomes. Further investigations on placenta enzymes, maternal cortisol in the amniotic fluid and maternal serotonin levels might offer a better understanding of the mechanisms underlying the transfer of maternal cortisol from mother to foetus. Epigenetics is another area that is becoming more explored as it is involved in the mediation of the stress from mother to baby. Current environmental factors should also be explored as potentially being involved in the development of psychopathology and the alteration of neurobiological responses in an individual's lifetime.

Finally, studies should look at how the brain changes happening in women who suffered abusive childhood experiences and their babies affect the psychological and biological response both in mothers and babies. For example, future studies using neuroimaging, could look at the brain volume differences and brain activation of women who have been abused in childhood and their infants after birth, in order to discover other pathways of this intergenerational transmission.

4.10 Clinical implications

This research has far-reaching implications in the context of identifying factors that affect infant development in early life and could be targeted in preventive or therapeutic interventions in women and their babies. Several initiatives have been launched by the government and by national and local charities in order to ensure support to children and families since the early postnatal period. One of those is Sure Start, which aimed to improve childcare, health and family support in specific areas in need. Furthermore, several programs, such as Family Nurse Partnership, supports very young parents in their transition into parenthood by offering home-based consultation on request. In a similar way, the National Childbirth Trust offers courses to families in the antenatal period as well as consultations after birth about breastfeeding and other issues that families might face in their adjustment to the new-born. With these premises, it is known that some of the people at great risk of depression are either in isolation, so they do not reach the services available, or live in deprived areas of the city, where services might lack or they might not be aware of the existence of these sources of support. Hence, professionals dealing with women in pregnancy and the postnatal period (such as nurses and health visitors that often see women in their homes) should all undertake specialist trainings that enable them to detect difficulties in mother-infant bonding and/or in the infant behaviour. Trainings in Neonatal Behavioural Observation would provide professionals with a new perspective to think about mothers and infants that would enable them to detect sign of difficulties in the bonding process and then refer those mothers to seek advice before the difficulties become pervasive.

Furthermore, these findings cast light on the importance of the childhood histories of women in pregnancy. Training of professionals involved in the care of pregnant women could include developing awareness of the consequences of unresolved childhood traumas, screening for early childhood experiences and depression in pregnancy, and

close monitoring of the symptomatology after birth, together with further support during the transition to parenthood, by offering not only prioritized access to psychological therapy to pregnant women with mental health problems, but also parenting and child assessments and follow-up after birth, to ensure the wellbeing of the mother and the dyad.

My findings also demonstrate that neglect, physical, sexual and emotional abuse during childhood increases the chances of developing mental illness in adulthood and then potentially in the next generation. This is a serious health problem, with incredibly high costs both in terms of health care and its social implications. By intervening at a very early stage, even before conception (e.g. during family planning visits), and tackling the unresolved trauma that often follows adverse experiences, it might be possible to reduce and prevent the impact of later psychopathology.

4.11 Conclusions

In conclusion, several new findings appear as a result of this thesis. Women who suffered physical and sexual abuse, neglect, and felt unwanted and unloved during childhood (0-17 years) are seven times more likely to develop depression during pregnancy compared to women who did not suffer such abuses. Furthermore, women who have been abused and developed antenatal depression have high levels of circulating evening cortisol in the 3rd trimester of pregnancy. Effects of exposure to maternal childhood abuse and depression in pregnancy can be seen in the next generation during the first year of life, as observed in the biological and behavioural changes in the offspring. Maternal HPA axis dysregulation in pregnancy could be one of the pathways leading to this transmission. Overall, these findings show an association between a maternal childhood history of abuse and psychopathology in pregnancy and infant behaviour and physiological response to stress as early as 6 days that is also evident at one year.

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Appendices

Appendix A: Participant Information Sheet

King's College Hospital NHS
NHS Trust

Participant Information Sheet

Study title
Does the maternal stress system during pregnancy modify stress responses in babies following birth?

Invitation paragraph
We would like to invite you to take part in a research study. Before you decide, you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish.
Part 1 tells you the purpose of this study and what will happen to you if you take part. Part 2 gives you more detailed information about the conduct of the study. Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

PART 1

What is the purpose of the study?

We are studying the response to stress of babies born to mothers who have been depressed during pregnancy compared with babies whose mothers have not been depressed during pregnancy. It is commonly believed that pregnancy is a time of good mental health; in fact, research suggests that depression during pregnancy is relatively common, occurring in up to 10% of pregnant women and its occurrence may also have an impact on baby outcome; it is therefore an important area for further research.

We are particularly interested in the endocrine system known as the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is sometimes known as the "stress system" because it is activated by stress; we can measure the level of hormones from this system in the body. Cortisol is a major hormone from this "stress system"; during normal pregnancy, levels of cortisol become very high and are linked to the timing of birth. Of particular importance is that abnormally high levels of cortisol in pregnancy may be associated with premature birth and lower birth-weight babies. Depression is also associated with high levels of cortisol. Our previous research has suggested that pregnant women who are also depressed tend to have higher levels of cortisol & related

hormones than those who are not depressed; also that their babies may have higher levels of cortisol and have a different hormone response to stress than the babies of women who have not been depressed in pregnancy. We wish to study this further, and look at other hormones related to this stress system and the way that genetic material (DNA) might influence the "stress system".

Why have I been invited?
You have been invited to participate because you are pregnant and routine screening at your initial meeting with your midwife either has not identified you as someone who is suffering from depression or has identified you as someone who may be at risk of developing, or actually suffering from, depression. At Kings College Hospital, pregnancy services are linked closely with a team of specialists concerned with the mental health of pregnant women and new mothers. In total, we will include 204 pregnant women; 62 with depression and 142 who are not depressed; we will also include their babies after they are born.

Do I have to take part?
It is up to you to decide. We will describe the study and go through this information sheet, which we will then give to you. We will then ask you to sign a consent form to show you have agreed to take part. You are free to withdraw at any time, without giving a reason; this would not affect the standard of care you receive.

What will happen to me and my baby if I take part?
Your participation will be for up to 18 months, the study will go on for 3 years in total. There will be up to 5 study visits, each visit lasting from 30 minutes to 4 hours:

Visit 1 occurs when you are about 25 weeks pregnant. You will be seen by a clinical researcher who will ask you some background questions such as age, number of children, employment and ethnic origin, life events and childhood experiences. You will also complete some brief questionnaires, ***a cognitive assessment,*** and be asked about any symptoms of depression/anxiety. You will have a blood test to look at hormone levels and DNA for genetic studies (30mls blood - about 2 tablespoons). The researcher may also obtain background information from your medical notes. You will be asked to provide 6 specimens of your saliva on one day, during the week after this visit. You will be shown how to do this at visit 1. We are looking at cortisol ("stress hormone") levels in saliva samples.

When you are about 32 weeks pregnant we will ask you to repeat the saliva samples and complete some brief questionnaires and post them back to us. There is no need for a visit at this stage.

Following the delivery of your baby, the midwives will take a small section of the umbilical cord or some blood from the umbilical cord after it has been removed from your baby. We will use this to look at the baby's DNA for genetic studies. A study visit

is not required at this stage; the sample will be collected by the researcher at a later point.

Visit *2* occurs 6 days after your baby is born. A clinical researcher will visit you at home to assess your baby's behaviour; they will use a standardised rating scale to make this assessment, which takes about 30 minutes. The researcher will collect a specimen of your baby's saliva shortly before and after the assessment, to look at levels of the stress hormone - cortisol, cotinine (a marker of exposure to tobacco) and DNA, and ask you to complete some brief questionnaires.

Visit *3* occurs the day before your baby is due for routine immunizations, 8 weeks after birth. At this time, as for visit 1, we will evaluate any symptoms of depression/anxiety. We will also look at the interactions between you and your baby; to do this we will make a 3-5 minute video recording at your home, you will be asked to play and talk to your baby as you normally would. The video data will be analysed using existing, validated observational scales by a trained observer.

We will also obtain saliva samples from your baby, to look at "stress hormone" levels, cotinine and DNA. The clinical researcher will meet with you and your baby when you attend for the baby's routine vaccinations, and show you how to obtain the sample by inserting a cotton swab between your baby's upper lip & gum prior to & 20 minutes after the immunization. We would then ask you to repeat this procedure twice on the following day, 12 hours apart and in between feeds. You will also be asked to provide 6 of your own saliva samples on the day after your baby's vaccination.

Visit *4* occurs the day before your baby is due for routine immunizations at one year of age. At this time, as for visit 3, we will evaluate any symptoms of depression/anxiety and observe interactions between you and your baby. We will also make an assessment of your child's development at that stage, using a standardised rating scale. We will obtain saliva samples from you and your baby as for visit 3.

The study assessments are over and above those involved in standard care; normal treatment will not be withheld during the study and will continue as needed after this. All video recordings are treated as confidential, will not be used for commercial purposes and will be destroyed when the study is completed.

Expenses and payments.
You will be reimbursed for travel expenses you incur in attending for study visits and as a token of our appreciation you will receive a £20 gift voucher at the end of the study.

What will I have to do?
If you wish to take part in the study, you will be asked to sign the consent form at the end of this document; you will be given a copy to keep. You should be prepared to undertake the 4 study visits, as detailed above, either in your own home or at the hospital. Please also consider that in agreeing to participate, you are also providing

consent on behalf of the baby you are expecting. If you have recent or current participation in other research studies please consider whether you should also participate in this study.

What are the possible disadvantages and risks of taking part?
You may experience some discomfort and/or bruising from the blood test. Although it is not painful, your baby may experience some distress on collection of saliva samples.
You may find the study visits/procedures inconvenient, particularly after your baby is born, as this is often a busy period for new mothers.

During the study, it is possible that other conditions are discovered of which you were unaware, which may have implications for your future health, or otherwise impacts on your interests. If anything is identified, your GP or hospital consultant will be informed, with your agreement.

What are the possible benefits of taking part?
There are no direct benefits to you of taking part in the study; however the knowledge gained from this study may be of help to other people in the future.

What if there is a problem?
Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed; detailed information on this is given in Part 2.

Will my taking part in the study be kept confidential?
Yes. We will follow ethical and legal practice and all information about you will be handled in confidence; details are included in Part 2.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

PART 2

What if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions (tel. 020 7848 5009). If you remain unhappy & wish to complain formally, you can do this through the NHS Complaints Procedure; details can be obtained from the hospital.

In the event that something does go wrong & you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against King's College Hospital Foundation NHS Trust or the study

sponsor, King's College London, but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Will my taking part in this study be kept confidential?

Yes, your confidentiality will be safeguarded during and after the study, which is conducted in accordance with the Data Protection Act 1998.

An identification code will be allocated to you and later to your baby. The information we collect will be recorded and put into electronic databases using this code rather than your name. Paper and electronic records are stored securely at the Institute of Psychiatry; the custodian of all study materials is Dr Carmine Pariante (Chief Investigator).

The researcher will have access to your clinical notes, and those of your baby, and by signing the consent from you will be giving consent for the researcher to examine your notes and those of your baby.

Study data will be analysed and results will be submitted for publication; your identity will not be revealed. Study data will be retained and may be used in future studies, if this happens, further Research Ethics Committee approval will be sought.

Authorised persons such as researchers, sponsors, regulatory authorities and Research and Development audit will have access to view identifiable data, for monitoring of the quality of the research.

Study data will be retained for 10 years after completion of the study; and will be disposed of securely.

You have the right to check the accuracy of data held about you and correct any errors according to local law and procedures.

Involvement of the General Practitioner/Family doctor (GP)

If you consent, we will write to your GP to inform them of your participation, and provide a brief study outline.

What will happen to any samples I give?

All samples from you and your baby will be processed then stored prior to analysis using the identification code already described. The researchers and laboratory scientists will have access to the samples; the researcher will be able to link your other study data to data from the analysis of your sample by the identification code. All samples will be destroyed once the study is completed.

Will any genetic tests be done?

Yes, we will look at genetic material (DNA) which might be relevant to the development of stress and depression.

What will happen to the results of the research study?

The data and results from this study may be published in medical journals or used in scientific reports and may be communicated to the regulatory authorities. You will not be identified by name. Once the study has been completed, a report of the findings will be prepared for participants; you can request a copy using the contact details below.

Who is organising and funding the research?
The Chief Investigator, Dr Carmine M. Pariante is organising the research, which is sponsored by the Institute of Psychiatry, King's College London. Funding is being sought from medical research charities.

Who has reviewed the study?
All research in the NHS is looked at by independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing & dignity. This study has been reviewed & given favourable opinion by The Kings College Hospital Research Ethics Committee.

Further information and contact details.
Chief Investigator:

Dr Carmine M, Pariante
Head of the Joint Sections of Perinatal Psychiatry & Stress, Psychiatry and Immunology Institute of Psychiatry
Reader, MRC Clinician Scientist Fellow
Division of Psychological Medicine and Psychiatry
Centre for the Cellular Basis of Behaviour,
Room 2-055
The James Black Centre
125 Coldharbour Lane
London SE5 9NU

Tel. 020 7848 5009

You will receive a copy of the information leaflet and signed consent form to keep.

Thank you for reading this information sheet.

Appendix B: Participant consent form

King's College Hospital NHS
NHS Trust

Consent Form

Title:	**Does the maternal stress system during pregnancy modify stress responses in babies following birth?**

Participant Identification Number: __ __ __ __ - __ __ __		Please initial each box
I confirm that I have read & understood the participant information sheet dated ***03.10.11 (version 4.1)*** for the above study. I have had the opportunity to consider the information, ask questions & have had these answered satisfactorily.		
I understand that my participation is voluntary & that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.		
I understand that relevant sections of my medical notes & data collected during the study may be looked at by individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.		
I agree to my GP being informed of my participation in the study.		
I agree that my GP or hospital consultant will be informed if, during the study, other conditions are discovered of which I was unaware.		
I agree to give samples of blood, saliva or hair for the above study. I understand how the samples will be collected and that giving the sample is voluntary.		
I understand that the samples I give will be used (hormone and genetic analysis) for		

research rather than clinical purposes, and that these results will have no implications for me personally.		
I agree that I may be contacted in the future regarding the study, should the research be extended, but I am under no obligation to participate. I understand that information held by the NHS and records maintained by the General Register Office may be used to keep in touch with me.		
I agree to take part in the above study, and that my baby will be included after birth.		

Name of Participant:_____

Signature of Participant: _____ Date: _____

Name of Investigator: _____

Signature of Investigator: _____ Date: _____

When completed, 1 copy for participant; 1 copy for researcher site file; 1 copy filed in medical notes

Appendix C: Structured Clinical Interview for DSM-IV Axis I Disorders, Clinician Version – Mood Disorder section

Raters initials:
Date:

STRUCTURED CLINICAL INTERVIEWFOR DSM-IV AXIS 1 DISORDERS
Baseline
OVERVIEW

I'm going to be asking you about problems or difficulties you may have had, and I'll be making some notes as we go along. Do you have any questions before we begin?

OCCUPATIONAL HISTORY

IF CURRENTLY WORKING: How long have you been in your current job? IF < 6 MONTHS: Why did you leave your last job? Have you always done that kind of work? IF NOT CURRENTLY WORKING: What kind of work have you done before? How are you supporting yourself now?	
IF UNKNOWN: Has there ever been a period of time when you were unable to work or go to school/ college? IF YES: When? Why was that?	

STATUS OF CURRENT TREATMENT (PATIENTS ONLY)

IF UNKNOWN: Have you been in any kind of treatment in the past month?	*Treatment setting: (Circle one)* 1 - Current inpatient (including residential treatment) 2 - Current outpatient 3 - Other (e.g. psychotherapy) 4 - No current treatment

IF INPATIENT: When did you come into hospital? IF OUTPATIENT: When did you start attending the (hospital, clinic?)	*Date:*

CHIEF COMPLAINT AND DESCRIPTION OF PROBLEM (PATIENTS ONLY)

What led to your going there (this time)? (What is the major problem you are having trouble with?) IF DOES NOT GIVE DETAILS OF PRESENTING PROBLEM: Tell me more about that. (What do you mean by . . .?)	

ONSET OF PRESENT ILLNESS OR EXACERBATION (PATIENTS ONLY)

When did this begin? (When did you first notice that something was wrong?) When were you last feeling OK (your usual self)?	

NEW SYMPTOMS OR RECURRENCE (PATIENTS ONLY)

Is this something new or a return of something you had before? (What made you come for help now?)	

ENVIRONMENTAL CONTEXT AND POSSIBLE PRECIPITANTS (PATIENTS ONLY)
(USE FOR REPORTING AXIS IV.)

Did anything happen or change just before this all started? (Do you think this had anything to do with your [PRESENT ILLNESS]?) What other kinds of problems were you having when this began?	

COURSE OF PRESENT ILLNESS OR EXACERBATION (PATIENTS)

After it started, what happened next? (Did other things start to bother you?)	
Since this began, when have you felt the worst? IF MORE THAN A YEAR AGO: In the last year, when have you felt the worst?	

TREATMENT HISTORY

PATIENTS: When was the first time you saw someone for emotional or psychiatric problems? (What was that for? What treatment(s) did you get? What medications?) **NON-PATIENTS:** Have you ever seen anybody for emotional or psychiatric problems? IF YES: What was that for? (What treatment(s) did you get? Any medications?) IF NO: Was there ever a time when you, or someone else, thought you should see someone because of the way you were feeling or acting?	

What about treatment for drugs or alcohol? (THE LIFE CHART ON NEXT PAGE MAY BE USED TO DOCUMENT A COMPLICATED HISTORY OF PSYCHOPATHOLOGY AND TREATMENT)	
Have you ever been a patient in a psychiatric hospital? IF YES: What was that for? (How many times?) IF GIVES AN INADEQUATE ANSWER, CHALLENGE GENTLY: e.g. Wasn't there something else? People don't usually go to psychiatric hospitals just because they are (TIRED / NERVOUS / OWN WORDS)	
Have you ever been a patient in a hospital for treatment of a medical problem? IF YES: What was that for?	

OTHER CURRENT PROBLEMS

PATIENTS: Have you had any other problems in the past month? **NON-PATIENTS:** How have things been in the last month?	
What has your mood been like?	
How has your physical health been? (Have you had any medical problems?)	

CURRENT SOCIAL FUNCTIONING

How have you been spending your free time? Whom do you spend time with?	

OVERVIEW DIAGNOSES

MOST LIKELY CURRENT DIAGNOSIS: _____

LIFE CHART

Age (or date)	Description (symptoms, triggering events)	Treatment
_____	_____	_____
_____	_____	_____
_____	_____	_____

A. MOOD EPISODES

CURRENT MAJOR DEPRESSIVE EPISODE CRITERIA

Now I am going to ask you some more questions about your mood.	(A) Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure.	
A1 In the past month . . has there been a period of time when you were feeling depressed or down most of the day nearly every day? (What was that like?) IF YES: How long did it last? (As long as 2 weeks?)	(1) depressed mood most of the day, nearly every day as indicated by either subjective report (e.g., feels sad or empty) or observation made by others (e.g. appears tearful).	**A1 current ? - +**
A2 . . . what about losing interest or pleasure in things you usually enjoyed? IF YES: Was it nearly every day? How long did it last? (As long as 2 weeks?)	(2) markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation made by others).	**A2 current ? - +**

If **neither A1 nor A2** is "+" during the current month, go to **A1a** *(Past Major Depressive Episode In This pregnancy)*

FOR THE FOLLOWING QUESTIONS, FOCUS ON THE WORST TWO WEEKS OF THE LAST MONTH (OR PAST TWO WEEKS IF EQUALLY DEPRESSED FOR THE ENTIRE MONTH)

During [2-WEEK PERIOD] … From: …../…../…… To: …./…../…... *(insert dates)*

A3 . . .did you lose or gain any weight? (How much? Were you trying to lose weight?) IF NO: How was your appetite? (What about compared with your usual appetite?) Did you have to force yourself to eat? Eat [less/more] than usual? Was that nearly every day?)	(3) significant weight loss when not dieting or weight gain (e.g. a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day.	**A3** **? - +**
A4 . . how were you sleeping? (Trouble falling, waking frequently, trouble staying asleep, waking too early, OR sleeping too much? How many hours a night compared with usual? Was that nearly every night?)	(4) insomnia or hypersomnia nearly every day	**A4** **? - +**

A5 . . were you so fidgety or restless that you were unable to sit still? (Was it so bad that other people noticed it? What did they notice? Was that nearly every day?) IF NO: What about the opposite - talking or moving more slowly than is normal for you? (Was it so bad that other people noticed it? What did they notice? Was that nearly every day?)	(5) psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down) *NOTE:* ALSO CONSIDER BEHAVIOUR DURING THE INTERVIEW	**A5** **? - +**
A6 . . what was your energy like? (Tired all the time? Nearly every day?)	(6) fatigue or loss of energy nearly every day	**A6** **? - +**
A7 . . how did you feel about yourself? (Worthless? Nearly every day?) IF NO: What about feeling guilty about things you had done or not done? (Nearly every day?)	(7) feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick) *NOTE:* CODE "-" IF ONLY LOW SELF-ESTEEM	**A7** **? - +**

A8 . . did you have trouble thinking or concentrating? (What kinds of things did it interfere with? Nearly every day?) IF NO: Was it hard to make decisions about everyday things?	(8) diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others)	**A8** **? - +**
A9 . . were things so bad that you were thinking a lot about death or that you would be better off dead? What about thinking of hurting yourself? IF YES: Did you do anything to hurt yourself?	(9) recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide	**A9** **? - +**

A10 **AT LEAST FIVE OF A1 TO A9 ARE "+" AND AT LEAST ONE OF THESE IS ITEM A1 OR A2**	**A10** **? - +**

If **A10** above is "-" (i.e. fewer than five are "+") go to **A1.a**

A11 IF UNCLEAR: Has (the depression/OWN WORDS) made it hard for you to do your work, take care of things at home, or get along with other people?	C. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.	**A11** **? - +**

If **A11** above is "-" (i.e. symptoms do not cause clinically significant distress or impairment) go to **A1.a**

A12 Just before this began, were you physically ill? Just before this began, were you taking any medications?	D. The symptoms are not due to the direct effects of a substance (e.g. a drug of abuse, medication) or to a general medical condition. <u>Etiological general medical conditions include</u>: degenerative neurological illnesses (e.g.,	**A12** **? - +**

IF YES: Any change in the amount you were taking? Just before this began, were you drinking or using any street drugs?	Parkinson's disease), cerebrovascular disease (e.g., stroke), metabolic conditions (e.g., Vitamin B-12 deficiency), endocrine conditions (e.g., hyper- and hypothyroidism, hyper- and hypoadreno-corticism); viral or other infections (e.g., hepatitis, mononucleosis, HIV), and certain cancers (e.g., carcinoma of the pancreas). <u>Etiological substances include:</u> alcohol, amphetamines, cocaine, hallucinogens, inhalants, opioids, phencyclidine, sedatives, hypnotics, anxiolytics. Medications include antihypertensives, oral contraceptives, corticosteroids, anabolic steroids, anticancer agents, analgesics, anticholinergics, cardiac medications.	

If **A12** above is "-" (i.e. mood <u>is</u> due to substance or general medical condition), is "-" go to **A1.a**

If there is any indication that the depression may be secondary (i.e., a direct physiological consequence of a general medical condition or substance), go to A61 and return here to make a rating of "-" or "+"

A13 IF UNKNOWN: Did this begin soon after someone close to you died?	E. The symptoms are not better accounted for by Bereavement, i.e., after the loss [death] of a loved one, the symptoms persist for longer than 2 months or are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation.	**A13** **? - +**

If **A13** above is "-" (i.e. the depressed mood <u>is</u> better accounted for by Bereavement), go to **A1.a**

CRITERIA A, C, D & E ARE "+" **CURRENT MAJOR DEPRESSIVE EPISODE**	**A14** **? - +**

Go to **A1.b** *(Past Major Depressive Episode Before This Pregnancy)*

PAST MAJOR DEPRESSIVE EPISODE IN THIS PREGNANCY CRITERIA

A1.a During this pregnancy, has there .. been a period of time when you were feeling depressed or down most of the day nearly every day? (What was that like?) IF YES: How long did it last? (As long as 2 weeks?)	1) depressed mood most of the day, nearly every day as indicated by either subjective report (e.g., feels sad or empty) or observation made by others (e.g. appears tearful).	**AI.a** **? - +** past - in this pregnancy

A3.a . . .did you lose or gain any weight? (How much? Were you trying to lose weight?) IF NO: How was your appetite? (What about compared with your usual appetite?) Did you have to force yourself to eat? Eat [less/more] than usual? Was that nearly every day?)	(3) significant weight loss when not dieting or weight gain (e.g. a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day.	**A3.a** **? - +** past - in this pregnancy
A2.a . . . what about losing interest or pleasure in things youusually enjoyed? IF YES: Was it nearly every day? How long did it last? (As long as 2 weeks?)	(2) markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation made by others).	**A2.a** **? - +** past - in this pregnancy

A4.a . . how were you sleeping? (Trouble falling, waking frequently, trouble staying asleep, waking too early, OR sleeping too much? How many hours a night compared with usual? Was that nearly every night?)	(4) insomnia or hypersomnia nearly every day	**A4.a** **? - +** past - in this pregnancy
A5.a . . were you so fidgety or restless that you were unable to sit still? (Was it so bad that other people noticed it? What did they notice? Was that nearly every day?) IF NO: What about the opposite - talking or moving more slowly than is normal for you? (Was it so bad that other people noticed it? What did they notice? Was that nearly every day?)	(5) psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down)	**A5.a** **? - +** past - in this pregnancy
A6.a . . what was your energy like? (Tired all the time? Nearly every day?)	(6) fatigue or loss of energy nearly every day	**A6.a** **? - +** past - in this pregnancy

A7.a . . how did you feel about yourself? (Worthless? Nearly every day?) IF NO: What about feeling guilty about things you had done or not done? (Nearly every day?)	(7) feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick) *NOTE:* CODE "-" IF ONLY LOW SELF-ESTEEM	**A7.a** **? - +** past - in this pregnancy
A8.a . . did you have trouble thinking or concentrating? (What kinds of things did it interfere with? Nearly every day?) IF NO: Was it hard to make decisions about everyday things?	(8) diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others)	**A8.a** **? - +** past - in this pregnancy
A9.a . . were things so bad that you were thinking a lot about death or that you would be better off dead? What about thinking of hurting yourself? IF YES: Did you do anything to hurt yourself?	(9) recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide	**A9.a** **? - +** past - in this pregnancy

FOR THE FOLLOWING QUESTIONS, FOCUS ON THE WORST TWO WEEKS OF THE PAST MAJOR DEPRESSIVE IN THIS PREGNANCY

During [2-WEEK PERIOD] … From: …../…../…… To: …./…../….. *(insert dates)*

A10.a **AT LEAST FIVE OF A1 TO A9 ARE "+" AND AT LEAST ONE OF THESE IS ITEM A1 OR A2**	**A10.a** **? - +** past - this pregnanc

	y

A11.a IF UNCLEAR: Did (the depression/OWN WORDS) made it hard for you to do your work, take care of things at home, or get along with other people?	C. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.	**A11.a** **? - +** past - this pregnanc y

If **A11.a** above is "-" (i.e. symptoms not clinically significant) go to **A1.b**

A12.a Just before this began, were you physically ill? Just before this began, were you taking any medications? IF YES: Any change in the amount you were taking? Just before this began, were you drinking or using any street drugs? If there is any indication that the depression may be secondary (i.e., a direct physiological consequence of a general medical condition or substance), go to A61 and return here to make a rating of "-" or "+"	D. The symptoms are not due to the direct effects of a substance (e.g. a drug of abuse, medication) or to a general medical condition. <u>Etiological general medical conditions include</u>: degenerative neurological illnesses (e.g., Parkinson's disease), cerebrovascular disease (e.g., stroke), metabolic conditions (e.g., Vitamin B-12 deficiency), endocrine conditions (e.g., hyper- and hypothyroidism, hyper- and hypoadreno-corticism); viral or other infections (e.g., hepatitis, mononucleosis, HIV), and certain cancers (e.g., carcinoma of the pancreas). <u>Etiological substances include:</u> alcohol, amphetamines, cocaine, hallucinogens, inhalants, opioids, phencyclidine, sedatives, hypnotics, anxiolytics. Medications include antihypertensives, oral contraceptives, corticosteroids, anabolic steroids, anticancer agents, analgesics, anticholinergics, cardiac medications.	**A12.a** **? - +** past - this pregnanc y

If **A12.a** above is "-" (i.e. mood <u>is</u> due to substance or general medical condition), go to **A1.b**

A13.a IF UNKNOWN: Did this begin soon after someone close to you died?	E. The symptoms are not better accounted for by Bereavement, i.e., after the loss [death] of a loved one, the symptoms persist for longer than 2 months or are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation.	**A13.a** **? - +** past - this pregnanc y

If **A13.a** above is "-" (i.e. depressed mood is better accounted for by Bereavement), go to **A1.b**

CRITERIA A, C, D & E ARE "+" **PAST MAJOR DEPRESSIVE EPISODE IN THIS PREGNANCY**	**A14.a** **? - +**

PAST MAJOR DEPRESSIVE EPISODE BEFORE THIS PREGNANCY CRITERIA

A1.b Has there ever ... .. been a period of time when you were feeling depressed or down most of the day nearly every day? (What was that like?) IF YES: How long did it last? (As long as 2 weeks?)	1) depressed mood most of the day, nearly every day as indicated by either subjective report (e.g., feels sad or empty) or observation made by others (e.g. appears tearful).	**AI.b** **? - +** past - ever
A2.b . . . what about losing interest or pleasure in things you usually enjoyed? IF YES: Was it nearly every day? How long did it last? (As long as 2 weeks?)	(2) markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation made by others).	**A2.b** **? - +** past - ever

IF AT LEAST ONE PAST DEPRESSED PERIOD: Have you had more than one time like that? Which one was the worst?

FOR THE FOLLOWING QUESTIONS, FOCUS ON THE WORST TWO WEEKS OF THE PAST MAJOR DEPRESSIVE EPISODE

During [2-WEEK PERIOD] … From: …../…../…… To: …./…../….. *(insert dates)*

A3.b . . .did you lose or gain any weight? (How much? Were you trying to lose weight?) IF NO: How was your appetite? (What about compared with your usual appetite?) Did you have to force yourself to eat? Eat [less/more] than usual? Was that nearly every day?)	(3) significant weight loss when not dieting or weight gain (e.g. a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day.	**A3.b** **? - +** past - before pregnancy
A4.b . . how were you sleeping? (Trouble falling, waking frequently, trouble staying asleep, waking too early, OR sleeping too much? How many hours a night compared with usual? Was that nearly every night?)	(4) insomnia or hypersomnia nearly every day	**A4.b** **? - +** past - before pregnancy
A5.b . . were you so fidgety or restless that you were unable to sit still? (Was it so bad that other people noticed it? What did they notice? Was that nearly every day?) IF NO: What about the opposite - talking or moving more slowly than is normal for you? (Was it so bad that other people noticed it? What did they notice? Was that nearly every day?)	(5) psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down)	**A5.b** **? - +** past - before pregnancy

A6.b . . what was your energy like? (Tired all the time? Nearly every day?)	(6) fatigue or loss of energy nearly every day	**A6.b** **? - +** past - before pregnancy
A7.b . . how did you feel about yourself? (Worthless? Nearly every day?) IF NO: What about feeling guilty about things you had done or not done? (Nearly every day?)	(7) feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick) *NOTE:* CODE "-" IF ONLY LOW SELF-ESTEEM	**A7.b** **? - +** past - before pregnancy
A8.b . . did you have trouble thinking or concentrating? (What kinds of things did it interfere with? Nearly every day?) IF NO: Was it hard to make decisions about everyday things?	(8) diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others)	**A8.b** **? - +** past - before pregnancy
A9.b . . were things so bad that you were thinking a lot about death or that you would be better off dead? What about thinking of hurting yourself? IF YES: Did you do anything to hurt yourself?	(9) recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide	**A9.b** **? - +** past - before pregnancy
A10.b **AT LEAST FIVE OF A1 TO A9 ARE "+" AND AT LEAST ONE OF THESE IS ITEM A1 OR A2**		**A10.b** **? - +** past - before pregnancy

IF "-" go to **A16** (*Manic Episode*)

A11.b IF UNCLEAR: Did (the depression/OWN	C. The symptoms caused clinically significant distress or impairment in social, occupational,	**A11.b** **? - +**

WORDS) made it hard for you to do your work, take care of things at home, or get along with other people?	or other important areas of functioning.	past - before pregnancy

IF "-" go to **A16** (*Manic Episode*)

A12.b Just before this began, were you physically ill? Just before this began, were you taking any medications? IF YES: Any change in the amount you were taking? Just before this began, were you drinking or using any street drugs?	D. The symptoms are not due to the direct effects of a substance (e.g. a drug of abuse, medication) or to a general medical condition. <u>Etiological general medical conditions include</u>: degenerative neurological illnesses (e.g., Parkinson's disease), cerebrovascular disease (e.g., stroke), metabolic conditions (e.g., Vitamin B-12 deficiency), endocrine conditions (e.g., hyper- and hypothyroidism, hyper- and hypoadreno-corticism); viral or other infections (e.g., hepatitis, mononucleosis, HIV), and certain cancers (e.g., carcinoma of the pancreas). <u>Etiological substances include:</u> alcohol, amphetamines, cocaine, hallucinogens, inhalants, opioids, phencyclidine, sedatives, hypnotics, anxiolytics. Medications include antihypertensives, oral contraceptives, corticosteroids, anabolic steroids, anticancer agents, analgesics, anticholinergics, cardiac medications.	**A12.b** **? - +** past - before pregnancy

If there is any indication that the depression may be secondary (i.e., a direct physiological consequence of a general medical condition or

If "-" go to **A16** (*Manic Episode*)

A13.b IF UNKNOWN: Did this begin soon after someone close to you died?	E. The symptoms are not better accounted for by Bereavement, i.e., after the loss [death] of a loved one, the symptoms persist for longer than 2 months or are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation.	**A13.b** **? - +** past - this pregnanc y

If "-" go to **A16** (*Manic Episode*)

CRITERIA A, C, D & E ARE "+" **PAST MAJOR DEPRESSIVE EPISODE BEFORE THIS PREGNANCY**		**A14.b** **? - +**
How many separate times have you been (depressed/ OWN WORDS) nearly every day for at least 2 weeks and had several of the symptoms that you described, such as (SYMPTOMS)?	Total number of Major Depressive Episodes including current. (CODE 99 if too numerous or indistinct to count). _____	**A15**

Appendix D: Childhood History of Care and Abuse Questionnaire

CECA-Q

I'm going to ask you some questions about your childhood experiences. If you prefer not to answer any of the questions, that's fine – just say you'd rather not answer.

Who brought you up before age 17?

Instructions to researcher: *Write below the PARENT FIGURES who brought participant up in childhood. List each family arrangement with different types of parent figures which* ***lasted a year or longer****. Consider natural parents, step parents (including parents' live in partners), aunts, friends of the family, adoptive parents, foster parents, etc.*

If participant has only lived in one arrangement, then fill in the first family arrangement and leave the other boxes blank. For example, if this was with their biological parents, tick 'natural mother' and 'natural father' and write in age '0'.

If they have lived in other arrangements that lasted a year or longer, such as with mother alone or mother and step-father, then list them a second/third etc family arrangement together with age they were when the arrangement began.

a) First Family Arrangement *(all)*

i) Mother figure:
1 = Natural mother
2 = Step-mother/ father's live-in partner
3 = Other relative e.g. aunt, grandmother
4 = Other non-relative e.g. foster/adoptive/godmother
5 = Other
6 = No mother figure

ii) Father figure:
1 = Natural father
2 = Step-father/ mother's live-in partner
3 = Other relative e.g. uncle, grandfather
4 = Other non-relative e.g. foster/adoptive/godfather
5 = Other
6 = No father figure

iii) Your age at start: _____ years iv) Your age at finish: _____ years

b) Second Family Arrangement *(if applicable)*

i) Mother figure:
1 = Natural mother
2 = Step-mother/ father's live-in partner
3 = Other relative e.g. aunt, grandmother
4 = Other non-relative e.g. foster/adoptive/godmother
5 = Other
6 = No mother figure

ii) Father figure:
1 = Natural father
2 = Step-father/ mother's live-in partner
3 = Other relative e.g. uncle, grandfather
4 = Other non-relative e.g. foster/adoptive/godfather
5 = Other
6 = No father figure

iii) Your age at start: _____ years iv) Your age at finish: _____ years

c) Third Family Arrangement *(if applicable)*

i) Mother figure:
1 = Natural mother
2 = Step-mother/ father's live-in partner
3 = Other relative e.g. aunt, grandmother
4 = Other non-relative e.g. foster/adoptive/godmother
5 = Other
6 = No mother figure

ii) Father figure:
1 = Natural father
2 = Step-father/ mother's live-in partner
3 = Other relative e.g. uncle, grandfather
4 = Other non-relative e.g. foster/adoptive/godfather
5 = Other
6 = No father figure

iii) Your age at start: . _____ years iv) Your age at finish: _____ years

d) Fourth Family Arrangement *(if applicable)*

i) Mother figure:
1 = Natural mother
2 = Step-mother/ father's live-in partner
3 = Other relative e.g. aunt, grandmother
4 = Other non-relative e.g. foster/adoptive/godmother
5 = Other
6 = No mother figure

ii) Father figure:

1 = Natural father
2 = Step-father/ mother's live-in partner
3 = Other relative e.g. uncle, grandfather
4 = Other non-relative e.g. foster/adoptive/godfather
5 = Other
6 = No father figure

iii) Your age at start: _____ years iv) Your age at finish: _____ years

e) Fifth Family Arrangement *(if applicable)*

i) Mother figure:

1 = Natural mother
2 = Step-mother/ father's live-in partner
3 = Other relative e.g. aunt, grandmother
4 = Other non-relative e.g. foster/adoptive/godmother
5 = Other
6 = No mother figure

ii) Father figure:

1 = Natural father
2 = Step-father/ mother's live-in partner
3 = Other relative e.g. uncle, grandfather
4 = Other non-relative e.g. foster/adoptive/godfather
5 = Other
6 = No father figure
3 = Other relative e.g. uncle, grandfather 6 = No father figure

iii) Your age at start: _____ years iv) Your age at finish: _____ years

g) Were you ever in a children's home or institution prior to age 17?

i) 0 = No 1 = Yes

ii) If YES, type of institution:

1 = Local authority care	i) Age entered: _____ years	ii) Age left: _____
2 = Hospital	ii) Age entered: _____ years	ii) Age left: _____
3 = Boarding school	iii) Age entered: _____ years	ii) Age left: _____
4 = Other	iv) Age entered: _____ years	ii) Age left: _____

5 = Unknown vi) Age entered: _____ years ii) Age left: _____

Parental Loss and Separation

a) Did either parent die before you were aged 17?

i) Mother: 0 = No 1 = Yes

ii) If YES, your age: _____ years

iii) Father: 0 = No 1 = Yes

iv) If YES, your age: _____ years

b) Have you ever been separated from either parent for 6 months or more before 17?

1) Mother: 0 = No 1 = Yes

ii) If YES, your age at first separation: _____ years

iii) Number of years of separation: _____ years _____ months

iv) Reason for separation from mother:

1 = Parental illness
2 = Parental divorce, separation
3 = Abandoned by parent or never knew parent
4 = Other (please specify below)

Please describe your experience *(not for data entry)*

_____
_____

2) Father: 0 = No 1 = Yes

i) If YES, your age at first separation: _____ years

ii) Number of years of separation: _____ years _____ months

iii) Reason for separation from father:

1 = Parental illness

2 = Parental divorce, separation
3 = Abandoned by parent or never knew parent
4 = Other (please specify below)

Please describe your experience *(not for data entry)*

---

3. Parent figures

Please circle the appropriate numbers to describe your Mother Figure, as you remember her in your first 17 years. If you had more than one, choose the one you were with the longest, or the one you found most difficult to live with.

3a. Which mother figure are you describing below?

1. Natural mother
2. Step-mother/father's live-in partner
3. Other relative e.g. aunt, grandmother
4. Other non-relative e.g. foster mother, godmother
5. Other (describe)……

	Yes, definitely		**Unsure**		**No, not at all**
3b She was very difficult to please	1	2	3	4	5
3c She was concerned about my worries	1	2	3	4	5
3d She was interested in how I did at school	1	2	3	4	5
3e She made me feel unwanted	1	2	3	4	5
3f She tried to make me feel better when I was upset	1	2	3	4	5
3g She was very critical of me	1	2	3	4	5
3h She would leave me unsupervised before I was 10 years old	1	2	3	4	5
3i She would usually have time to talk to me	1	2	3	4	5
3j She would hit me	1	2	3	4	5
3k At times she made me feel I was a	1	2	3	4	5

nuisance					
3l She often picked on me unfairly	1	2	3	4	5
3m She was there if I needed her	1	2	3	4	5
3n She was interested in who my friends were	1	2	3	4	5
3o She was concerned about my whereabouts	1	2	3	4	5
3p She cared for me when I was ill	1	2	3	4	5
3q She neglected my basic needs (e.g. food and clothes)	1	2	3	4	5
3r She did not like me as much as my brothers and sisters (leave blank if no siblings)	1	2	3	4	5

3s. Do you want to add anything about your mother?……..

4. Please circle the appropriate numbers to describe your Father Figure, as you remember him in your first 17 years. If you had more than one, choose the one you were with the longest, or the one you found most difficult to live with.

4a. Which father figure are you describing below?

1. Natural father
2. Step-father/mother's live-in partner
3. Other relative e.g uncle, grandfather
4. Other non-relative e.g. foster father, adoptive father
5. Other (describe)……

	Yes, definitely		**Unsure**		**No, not at all**
4b He was very difficult to please	1	2	3	4	5
4c He was concerned about my worries	1	2	3	4	5
4d He was interested in how I did at school	1	2	3	4	5
4e He made me feel unwanted	1	2	3	4	5
4f He tried to make me feel better when I was upset	1	2	3	4	5
4g He was very critical of me	1	2	3	4	5
4h He would leave me	1	2	3	4	5

unsupervised before I was 10 years old					
4i He would usually have time to talk to me	1	2	3	4	5
4j He would hit me	1	2	3	4	5
4k At times he made me feel I was a nuisance	1	2	3	4	5
4l He often picked on me unfairly	1	2	3	4	5
4m He was there if I needed him	1	2	3	4	5
4n He was interested in who my friends were	1	2	3	4	5
4o He was concerned about my whereabouts	1	2	3	4	5
4p He cared for me when I was ill	1	2	3	4	5
4q He neglected my basic needs (e.g. food and clothes)	1	2	3	4	5
4r He did not like me as much as my brothers and sisters (leave blank if no siblings)	1	2	3	4	5

4s. Do you want to add anything about your father?……

……..

5. **CLOSE RELATIONSHIPS IN CHILDHOOD**

(Please circle as appropriate – if you circle NO to any question, SKIP the rest of that section and go on to the next one)

5a When you were a child or teenager, were there any ADULTS you could go to with your problems or to discuss your feelings?

0 = No 1 = Yes

5b If YES: Who was that? (circle more than one if relevant)

1. mother / mother figure
2. father / father figure
3. other relative
4. family friend
5. teacher, vicar etc
6. other (describe) …….

5d Do you want to note anything about the relationship(s)? ……

5e Were there other CHILDREN OR TEENAGERS of your age that you could discuss your problems and feelings with? 0 = No 1 = Yes

5f If YES: Who was that? (circle more than one if relevant)

1. sister
2. brother
3. other relative
4. close friend
5. other less close friend(s)
6. other person (describe)……..

5h Do you want to note anything about the relationship(s)?……

5i Who would you describe as the TWO CLOSEST people to you as a child or teenager?
(circle up to two)

1. mother / mother figure
2. father / father figure
3. sister or brother
4. other relative
5. family friend (adult)
6. friend your age
7. other (describe) …….

5k Do you want to note anything about the relationship(s)?……

Physical Punishment Before the Age of 17 by a Parent Figure or Other Household Member

a)When you were a child or a teenager were you ever hit repeatedly with an implement (such as a belt or stick) or punched, kicked or burnt by someone in the household?

0 = No 1 = Yes

b) How old were you when it began?

i) Mother figure - your age: _____ years

ii) Father figure - your age: _____ years

c) Did the hitting happen on more than one occasion?

i) Mother figure 0 = No 1 = Yes

ii) Father figure 0 = No 1 = Yes

d) How were you hit by your mother?

1 = Belt or stick 2 = Punched or kicked No 3 = Hit with hand 4 = Other

e) How were you hit by your father?

1 = Belt or stick 2 = Punched or kicked No 3 = Hit with hand 4 = Other

f) Were you ever injured, e.g. bruises, black eyes, broken limbs?

i) Mother figure 0 = No 1 = Yes

ii) Father figure 0 = No 1 = Yes

g) Was this person ever so angry they seemed out of control?

i) Mother figure 0 = No 1 = Yes

ii) Father figure 0 = No 1 = Yes

Please describe your experience *(not for data entry)*

_____

_____

h) Did you experience this from anyone else in the household?

Sibling	0 = No	1 = Yes
Grandparent	0 = No	1 = Yes
Uncle/aunt	0 = No	1 = Yes
Other	0 = No	1 = Yes

Please describe your experience *(not for data entry)*

_____

_____

Unwanted Sexual Experiences Before Age 17

a) When you were a child or teenager did you ever have any unwanted sexual experiences?

0 = No 1 = Yes

b) Did anyone force you or persuade you to have sexual intercourse against your wishes before age 17?

0 = No 1 = Yes

c) Can you think of any upsetting sexual experiences before age 17 with a related adult or someone in authority, e.g. teacher?

0 = No 1 = Yes

(If YES or UNSURE to any of the above then continue)

d) 1st Experience:

i.) How old were you when it began? Age: _____ years

ii) Was the other person someone you knew? 0 = No 1 = Yes

iii) Was the other person a relative? 0 = No 1 = Yes

iv) Did this person do it on more than one occasion? 0 = No 1 = Yes

v) Did it involve touching private parts of your body? 0 = No 1 = Yes

vi) Did it involve sexual intercourse? 0 = No 1 = Yes

Please describe your experience *(not for data entry)*

_____

_____

e.) 2nd Experience:

ii.) How old were you when it began? Age: _____ years

ii) Was the other person someone you knew? 0 = No 1 = Yes

iii) Was the other person a relative? 0 = No 1 = Yes

iv) Did this person do it on more than one occasion? 0 = No 1 = Yes

v) Did it involve touching private parts of your body? 0 = No 1 = Yes

vi) Did it involve sexual intercourse? 0 = No 1 = Yes

Please describe your experience *(not for data entry)*

_____

Appendix E: Neonatal Behavioural Assessment Scale

NBAS Scoring Form

Baby's first (given) name.....

Sex..... dob..... Gestational age..... Birth weight..... Height.....HC..... Mode of delivery..... Length of Labour.....Apgar scores..... ...Parity.....Type of feeding.....

Is this information collected from the hospital discharge record (Y/N)

Infant Behavioural Assessment

Social-Interactive	**9**	**8**	**7**	**6**	**5**	**4**	**3**	**2**	**1**	**Comments**
Animate visual										
Animate visual & auditory										
Inanimate visual										
Inanimate visual & auditory										
Inanimate auditory										
Animate auditory										
Alertness										

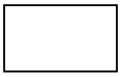
Motor System	**9**	**8**	**7**	**6**	**5**	**4**	**3**	**2**	**1**	**Comments**
General tone										

Motor maturity										
Pull- to-sit										
Defensive										
Activity level										

State Organisation	**9**	**8**	**7**	**6**	**5**	**4**	**3**	**2**	**1**	**Comments**
Peak of excitement										
Rapidity of build- up										
Irritability										
Lability of states										

State Regulation	**9**	**8**	**7**	**6**	**5**	**4**	**3**	**2**	**1**	**Comments**
Cuddliness										
Consolability										
Self-quieting										
Hand-to-mouth										

Autonomic System	**9**	**8**	**7**	**6**	**5**	**4**	**3**	**2**	**1**	**Comments**
Tremulousness										
Startles										
Lability of skin colour										

Smiles 

Supplementary Items	**9**	**8**	**7**	**6**	**5**	**4**	**3**	**2**	**1**	**Comments**
Quality of alertness										
Cost of attention										
Examiner facilitation										
General irritability										
Robustness & endurance										
State regulation										
E's emotional response										

Reflexes	**0**	**1**	**2**	**3**	**Asym**	**Comments**
Plantar						
Babinski						
Ankle clonus						
Rooting						
Sucking						
Glabella						
Passive resist - legs						
Passive resist - arms						
Palmar (hand grasp)						
Placing						
Standing						
Walking						
Crawling						
Incurvation						
Tonic dev. – head & eyes						
Nystagmus						
TNR						
Moro						

1st Predominant state: _____

2nd Predominant state: _____

Predominant state between “Post 1” and “Post 2” saliva samples: _____

SUMMARY : INFANT	
Strengths	Concerns

SUMMARY : PARENT(S)	
Strengths	Concerns

Appendix F: Maternal saliva sampling collection record and instructions

MATERNAL SALIVA SAMPLE COLLECTION RECORD

Subject ID (no.-initials) | | | | | - | | | |

Researcher's Initials | | | |

Baseline 32 wks gestation MRI 8 weeks postnatal 1 year postnatal

---

DATE OF COLLECTION (DD-MM-YY) | | | - | | | - | | |

IMPORTANT NOTES:

- Please **do not** eat, drink, smoke or brush your teeth between samples at 0, 15, 30 and 60 minutes.
- Please **do not** eat, drink, smoke or brush your teeth 30 minutes before samples at Midday and 8 pm.
- You may drink water if you need to, but only immediately **after** you have taken a sample.
- Collect the saliva as on the instruction diagram. Place the swab **under your tongue** and leave it there for 1-2 minutes, then place it as shown in the correct tube. Then close the tube firmly and store in the fridge in the bag supplied.
- Please use the **timer** provided to ensure accurate timing.

- Try to sit down and relax for the next hour after waking up.

If you have any questions about the process, please call **the research team on 020 7848 5009.**

Complete	**When?**	**Record the Time:**
BOX 1 (Tube 0)	When you wake up	
BOX 2 (Tube 15)	15 minutes after you wake up	
BOX 3 (Tube 30)	30 minutes after you wake up	
BOX 4 (Tube 60)	60 minutes after you wake up	
BOX 5 (Tube 12pm)	Midday (12:00)	
BOX 6 (Tube 8pm)	8 o'clock pm (20:00pm)	

BOX 1: When you wake up **USE TUBE 0**

- What time is it now?
  _____
- Did you accidentally brush your teeth, smoke or have anything to eat or drink before taking the sample? If yes, please describe it here and record the time:
  _____
- Did you have any difficult or tense situation, unpleasant thoughts or any kind of pain before taking this sample? If yes, please describe it here:
  _____

BOX 2: 15 minutes after waking up **USE TUBE 15**

- What time is it now?

  _____

- What were you doing before giving the sample?

  _____

- Did you accidentally brush your teeth, smoke or have anything to eat or drink before taking the sample? If yes, please describe it here and record the time:

  _____

- Did you have any difficult or tense situation, unpleasant thoughts or any kind of pain before taking this sample? If yes, please describe it here:

  _____

BOX 3: 30 minutes after waking up **USE TUBE 30**

- What time is it now?

  _____

- What were you doing before giving the sample?

  _____

- Did you accidentally brush your teeth, smoke or have anything to eat or drink before taking the sample? If yes, please describe it here and record the time:

  _____

- Did you have any difficult or tense situation, unpleasant thoughts or any kind of pain before taking this sample? If yes, please describe it here:

  _____

BOX 4: 60 minutes after waking up **USE TUBE 60**

- What time is it now?

  _____

- What were you doing before giving the sample?

  _____

- Did you accidentally brush your teeth, smoke or have anything to eat or drink before taking the sample? If yes, please describe it here and record the time_____

- Did you have any difficult or tense situation, unpleasant thoughts or any kind of pain before taking this sample? If yes, please describe it here:

  _____

*****<u>YOU CAN NOW EAT, DRINK, SMOKE AND BRUSH YOUR TEETH</u>! *****

Please **do not eat, drink, smoke or brush your teeth** for **30 minutes** before collecting the **sample at Midday**

<u>BOX 5</u>: Midday (12:00pm) before lunch **USE TUBE 12pm**

- What time is it now?

  _____

- What were you doing before giving the sample?

  _____

- Did you accidentally brush your teeth, smoke or have anything to eat or drink before taking the sample? If yes, please describe it here and record the time:

  _____

- Did you have any difficult or tense situation, unpleasant thoughts or any kind of pain before taking this sample? If yes, please describe it here:

  _____

*******<u>YOU CAN NOW EAT, DRINK, SMOKE AND BRUSH YOUR TEETH AGAIN</u>! *******

Please **do not eat, drink, smoke or brush your teeth** for **30 minutes** before collecting the **sample at 8 o'clock pm**

<u>BOX 6</u>: At 8 o'clock pm (20:00pm)	**USE TUBE 8pm**

- What time is it now?

  _____

- What were you doing before giving the sample?

  _____

Did you accidentally brush your teeth, smoke or have anything to eat or drink before taking the sample? If yes, please describe it here and record the time:

_____

- Did you have any difficult or tense situation, unpleasant thoughts or any kind of pain before taking this sample? If yes, please describe it here:

  _____

Please note the name and time of **any medication you have taken <u>today</u>** (including the contraceptive pill):

_____

Do you have any medical problems? If so, please list them here

_____

*******<u>THANK YOU FOR COMPLETING THIS SALIVA COLLECTION</u>*******

<u>Returning the samples:</u>

Your researcher will tell you if they will collect your samples from you or if you should post them. Please remember to place all your samples in the fridge.

Posting: Please post your samples back to us the following Monday or Tuesday, and leave your samples in your fridge until posting.

Place all the samples in the plastic bag provided and seal it carefully. Place the bag and the collection record into the stamped addressed envelope provided. Please remember to make a note of the date you will post the specimens on the record form before sealing the envelope.

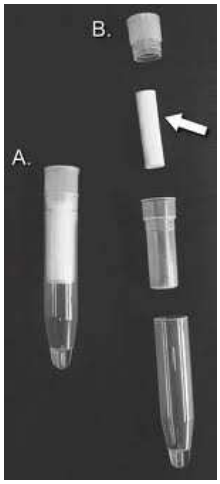
Date posted: __ / __ / __

Office use only:

[Date of sample receipt: __ / __ / Date of sample storage: __ / __ / __

How to collect the saliva samples:

Salivette with swab



Lid

Swab

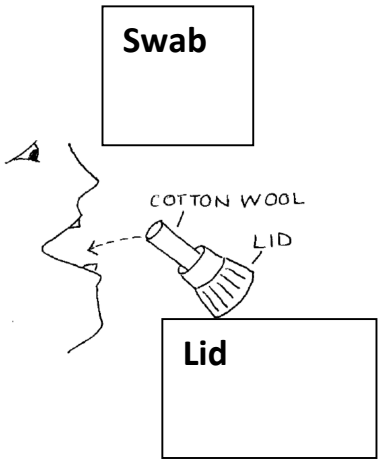
Inner tube

Outer tube

Take care to find the salivette tube marked with the appropriate time.

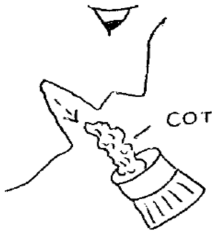
Carefully remove the lid (the part on the end with ridges on).

Tip the swab into the lid and use this to place the swab under the front of your tongue. Do not touch the swab with your fingers.



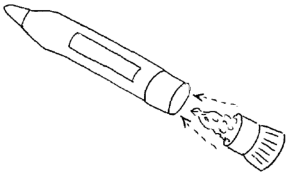
Keep the swab in place for 1-2 minutes to ensure that it is saturated.

Take the swab out of your mouth with the help of the lid (so you are not touching the swab with your fingers).



Swab with saliva

Carefully tip the swab into the salivette tube without touching it with your fingers.



Replace the lid firmly.

Store the samples in your fridge. **THANK YOU.**

Appendix G: Neonatal Behavioral Assessment form and infant saliva – 6 days postpartum

<u>ORDER OF ASSESSMENTS</u>:

1. **Collect 1st saliva sample ("PRE") with SCS/Salivette before starting NBAS**
2. **NBAS**
3. **Collect 2nd saliva sample ("POST 1") with Sorbette/Salivette immediately on completion of NBAS**
4. **Ask the participant to complete "PRAM 6 day postnatal (NBAS) self-report booklet"**
5. **Collect 3rd saliva sample ("POST 2") with Sorbette/Salivette 30 minutes after completion of NBAS**
6. **Collect saliva sample with Oragene DNA kit**
7. **Measure neonate head circumference**

N.B. Feeding should be avoided during the visit if possible.

_____

<u>Saliva Oragene DNA sample</u>:

Record the following:

Was the baby fed in the 30 minutes before taking the sample (Y/N)

If yes, when and for how long: Start: ____ Finish: ____ Sample time: _____

How many sponges were used: _____

<u>Neonate head circumference</u> measured on the day of NBAS: ____ mm

BABY SALIVA SAMPLE COLLECTION RECORD FOR NBAS

N.B. Ideally the samples should not be taken within the 15 minutes after a feed; please inform the mother of this before starting the NBAS.

What time was the baby's most recent nap? Start: _____Finish: _____

What time was the baby's most recent feed? Start: _____Finish: _____

Before the baby's NBAS assessment collect the baby's saliva as on the instruction diagram in the instructions document. Take the **tube marked "PRE."** Place the SCS ***under the baby's tongue*** and leave them there for a total of 60-90 seconds (the SCS can be placed under the tongue for 15 to 30 seconds at a time and reintroduced as needed), then place it in the tube, cut to length, close the tube firmly and store in the bag supplied.

EXACT TIME OF SAMPLING: _____

TIME OF NBAS ASSESSMENT: Start _____ Finish _____

Immediately after the NBAS assessment collect the baby's saliva in the **tube marked "POST-1"** as for the 1st sample. Then close the tube firmly and store in the bag supplied.

- What time is it now?
  _____
- Was the baby fed? If yes, when and for how long: Start: _____ Finish: _____
- Did the baby sleep? If yes, when and for how long: Start: ____Finish: _____

30 minutes after the end of the NBAS assessment collect the baby's saliva in the **tube marked "POST-2"** as for the 1st sample. Then close the tube firmly and store in the bag supplied.

- What time is it now?
  _____
- Was the baby fed? If yes, when and for how long: Start: _____Finish: _____

- Did the baby sleep? If yes, when and for how long: Start: _____ Finish: _____

Please note the name and time of any medication the baby is taking _____

Does the baby have any medical problems? If so, please list them here

---

Appendix H: Infant saliva sampling collection record and instruction at the time of the 8 weeks and 12 months immunization

BABY SALIVA SAMPLE COLLECTION RECORD

Subject ID (no.-initials) | **b** | | | | | - | | | |

Researcher's Initials | | | |

8 weeks postnatal 1 year postnatal (please delete)

DAY 1 (to be completed by researcher and brought back to the IOP with the saliva samples)

DATE OF COLLECTION (DD-MM-YY) | | | - | | | - | | |

N.B. Ideally the samples should not be taken for at least 15 minutes after a feed.

What time was baby's most recent nap? Start: _____ Finish: _____
What time was baby's most recent feed? Start: _____ Finish: _____

Before the immunization collect baby's saliva with the help of the researcher (as on the instructions for the researcher). Take the **tube marked "PRE."** Place the Salimetrics children's swab ***under child's tongue*** and leave it there for 60 to 90 seconds (the swab can be placed under the tongue for a few seconds at a time and reintroduced as needed), then place the wet end in the salivette tube, the researcher will fold the swab and close the tube firmly.
EXACT TIME OF SAMPLING: _____

TIME OF Immunization: _____
Comments e.g. Was mother present? Did baby cry much?

Exactly 20 minutes after the immunization collect baby's saliva in the **tube marked "POST"** as for the 1st sample. Then close the tube firmly and store in the bag supplied.

- What time is it now?
  _____
- Was baby fed? (Y/N) If yes, when and for how long: Start: ____Finish:_____
- Did baby fall asleep? (Y/N) If yes, when: Start: _____ Finish: _____

Please note the name and time of any medication baby is taking _____

Does baby have any medical problems? If so, please list them here

---

Office use only: [Date of sample receipt: __ / __ / __
Date of sample storage: __ / __ / __

Appendix I: Assay procedure for salivary cortisol

On arrival to the laboratory, the samples were frozen at -20°C. After thawing, saliva samples were centrifuged at 3000 rev/min for 15 minutes at room temperature, which resulted in a clear supernatant of low viscosity. Determination of cortisol levels was done using the High Sensitivity Salivary Cortisol ELISA KIT from Salimetrics, following the recommended procedure. Briefly, 25 µl of saliva and standards were assayed in duplicates, by incubation on a microtitre plate coated with monoclonal antibodies against cortisol. Cortisol linked to horseradish peroxidase was then added, to compete with cortisol in the standards and unknowns for the antibody binding sites. After incubation, unbound components were washed away and bound cortisol peroxidase measured by reaction of the peroxidase enzyme on the substrate tetramethylbenzidine. The amount of cortisol peroxidase detected, as measured by the intensity of colour developed, is inversely proportional to the amount of cortisol present. Optical density was read at 450 nm with correction at 620 nm, using a Beckman Coulter DTX 880 plate reader, with Multimode Detection Software 2.0.0.12. Values of cortisol were calculated using SoftMax Pro 4.8 software, following a 4-parameter fit.