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Altered Neurocognitive Functioning in Infants and Young Children Prenatally Exposed to Maternal Anxiety and Mindfulness

Marion I. van den Heuvel

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Altered Neurocognitive Functioning in Infants and Young Children Prenatally Exposed to Maternal Anxiety and Mindfulness

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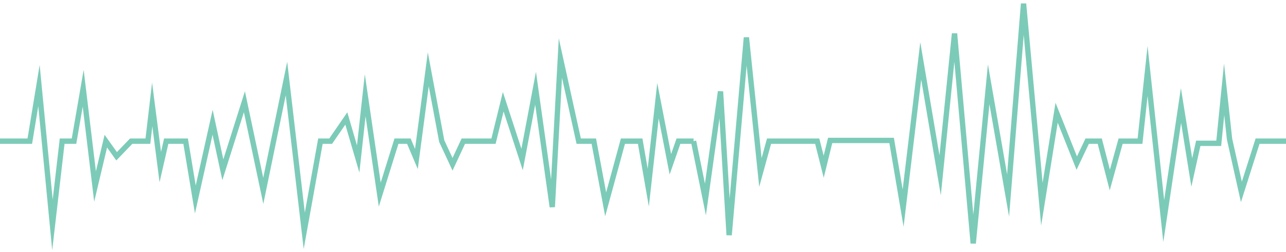
Chapter 1:

General Introduction



“I’m in the 1st trimester and worry at least once a day about something.”

-Anonymous, Pregnancy Forum

“I am having trouble sleeping and eating, but I wonder how much of it is due to anxiety and worry? I’m glad to see that I’m not alone.”

-Anonymous, Pregnancy Forum

1.1. Maternal anxiety during pregnancy

Pregnancy is often viewed as a period of happiness and joy. Mothers are expected to be ‘glowing’ and grateful for the opportunity to bring new life into the world. For a high percentage of women, however, this is not the case. Loomans *et al.* (2013), for instance, investigated maternal psychosocial stress during pregnancy in a large multi-ethnic community-based cohort study in the Netherlands and reported that about 30% of pregnant women experience some form of stress during pregnancy (i.e., anxiety, depression, job strain). In another large cohort study from Norway, researchers reported that over 7,5% of pregnant women experience fear of childbirth in the third trimester of pregnancy (Adams *et al.*, 2012). In addition, in a recent cohort study in the USA, about 14.4% of pregnant white women reported the use of psychiatric medication during pregnancy (Huybrechts *et al.*, 2013).

Although those rates already seem high, they are likely to be an underestimation caused by the tendency to ignore or suppress negative feelings, worries and anxiety during pregnancy or to regard them as ‘just being hormonal’. Research seems to confirm this notion as it has been found that the majority of pregnant women meeting the criteria for psychiatric disorders were undiagnosed and untreated (Andersson *et al.*, 2003; Glover, 2014). In one study, a strikingly low percentage of only 5.5 % of women at need for treatment actually received it (Andersson *et al.*, 2003). Care for the emotional well-being of pregnant women clearly seems to be a neglected part of obstetric medicine (Glover, 2014).

1.2. Developmental Origins of Health and Disease (DOHaD)

Besides causing an emotional burden on pregnant women, accumulating research has shown that maternal anxiety during pregnancy can also affect the unborn child (Van den Bergh, 2011; Graignic-Philippe *et al.*, 2014). The core aim of research in the field of “*Developmental Origins of Health and Disease (DOHaD)*” is to examine the short and long-term consequences of conditions of the prenatal environment for the offspring’s health and disease risks (Barker, 2004). Over the past years, research in this field has suggested that prenatal exposure to maternal anxiety can cause alterations in offspring’s phenotype, a process that has been referred to as modulation of “*developmental programming*” (de Kloet *et al.*, 2005; Gluckman *et al.*, 2008; Laplante *et al.*, 2008).

Although the underlying mechanisms are not fully understood, it has frequently been proposed that maternal anxiety leads to increased production of maternal stress hormones (i.e., cortisol and noradrenalin) and to down-regulation of the placental 11β-HSD2 enzyme that metabolizes maternal cortisol into inactive cortisone (O'Donnell *et al.*, 2012). As a result, cortisol in the fetal circulation increases which, in turn, may elicit long-term changes in structure and function of the developing brain via epigenetic pathways (Van den Bergh, 2011; Monk *et al.*, 2012).

In this way, cues in the intrauterine environment (e.g., elevated cortisol levels) may guide adaptation of the offspring phenotype to the expected environment in order to increase subsequent chances of survival ("*developmental plasticity*") (Bateson *et al.*, 2004; Godfrey *et al.*, 2007; Del Giudice, 2014). However, a 'mismatch' with the postnatal environment can occur when the cues predicted a different environment than the one encountered, resulting in a maladaptive phenotype predisposing the child for behavioral and emotional problems later in life ("*mismatch hypothesis*") (Gluckman and Hanson, 2004, 2006; Frankenhuis and Del Giudice, 2012). In line with this notion, prenatal exposure to maternal anxiety has been associated with a higher risk for a broad range of behavioral and emotional problems in the offspring, such as attention deficit hyperactivity disorder (ADHD), autism, schizophrenia, and affective disorders (e.g., Van den Bergh and Marcoen, 2004; Malaspina *et al.*, 2008; Van den Bergh *et al.*, 2008; Walder *et al.*, 2014). Taken together, it may be clear that maternal anxiety during pregnancy is a major problem for both mother and fetus and warrants further investigation.

1.3. Prenatal exposure to maternal anxiety and the developing brain and behavior: DOBHaD research

The fetal brain may be particular sensitive to developmental programming since the brain is subject to dramatic developmental processes during the prenatal life period (Andersen, 2003; Van den Bergh, 2011; Bock *et al.*, 2015). Animal research has demonstrated that prenatal exposure to maternal stress affects the offspring's brain structure and/or function (Charil *et al.*, 2010; Bock *et al.*, 2015). Recently, research in humans has also found associations between maternal anxiety/cortisol during pregnancy and alterations in the offspring's brain (Buss *et al.*, 2010; Buss *et al.*, 2012; Qiu *et al.*, 2015; Rifkin-Graboi *et al.*, 2015). These brain alterations in early development may result in behavioral problems and altered neurocognitive functioning later in life (Meredith, 2015).

To integrate the early brain and behavioral development into the existing DOHaD hypothesis, Van den Bergh (2011) proposed to extend the DOHaD hypothesis to the ´Developmental Origins of *Behavior*, Health and Disease' (DOBHaD) hypothesis. This extension emphasizes the importance of the prenatal period for later neurocognitive functioning and argues that alterations in the offspring's brain mediate the link between early life influences and behavioral outcomes during childhood. The aim of this dissertation is to contribute to the DOBHaD hypothesis by examining the effects of maternal anxiety during pregnancy on developmental programming of neurocognitive functioning in infants and young children – a topic that only few studies have examined so far. To this end, we focus on the following key aspects of neurocognitive functioning: sensory processing, socio-emotional functioning, and affective processing.

Because brain regions are highly interconnected and a specific region's functioning is dependent on its connectivity to other regions, altered brain-connectivity induced by maternal anxiety during pregnancy might contribute to changes in infant neurocognitive functioning. Yet, the effects of maternal stress during pregnancy on functional brain network connectivity are not known. This dissertation, therefore, also includes an investigation of the effects of prenatal exposure to maternal anxiety on infants' functional brain network configuration.

1.4. Event-related brain potentials (ERPs) in the study of neurocognitive functioning in infants and children

Most previous studies investigating the impact of maternal anxiety during pregnancy on child outcomes used parent-report questionnaires or behavioral measures (e.g., performance on a task). Although these measures can be used to make inferences about underlying alterations in brain functioning, they do not directly measure actual brain functioning (Mennes *et al.*, 2009). A useful tool to directly study neurocognitive functioning in infants and young children is the event-related brain potentials method (ERPs) (de Haan, 2006). Event-related brain potentials are electric brain responses to sensory, cognitive, and/or motor events and they are typically extracted from the electroencephalographic (EEG) signals by averaging single-trial recordings of the repeated target event (Luck, 2005).

Monitoring brain processes in real time requires a temporal resolution of millisecond accuracy in order to follow the typical timing and spectral properties of neural activity. In contrast to other brain imaging techniques, such as functional magnetic resonance imaging (fMRI), EEG/ERP provides such high time resolution (Banaschewski and Brandeis, 2007). In addition, EEG/ERP is non-invasive, often does not require a behavioral response and can be conducted without the subject paying attention to a specific set of stimuli, which is very convenient for infant and child research (de Haan, 2006). In spite of the clear benefits of ERPs for the DOBHaD field, only few studies investigated neurocognitive functioning in the offspring prenatally exposed to maternal anxiety by means of ERPs (Mennes *et al.*, 2009; Hunter *et al.*, 2012; Otte *et al.*, 2015). In the majority of the studies presented in this dissertation EEG and ERPs were used to investigate neurocognitive functioning.

1.5. Possible protective effects of positive maternal traits and states

The majority of the research in the DOBHaD field has focused almost exclusively on effects of exposure to early adverse conditions. Prenatal exposure to maternal well-being is far less investigated, while it might also alter offspring phenotype and, more importantly, some maternal traits and states may protect the fetus from the possible deleterious effects of maternal anxiety. Previous studies investigating positive maternal factors during pregnancy, such as partner support and positive life events, highlight the relevance of positive maternal experiences during pregnancy and their possible beneficial effects for both the mother and the child (Pluess *et al.*, 2012; Stapleton *et al.*, 2012). No study to date, however, has investigated the impact of positive maternal traits and states on the unborn child. To cover the full scope of developmental programming, the effects of maternal well-being during pregnancy on child outcomes should therefore also be investigated.

This dissertation focuses on mindfulness as such a positive factor. Being mindful refers to a state of mind consisting of two key elements: (1) An alert mode of perceiving all mental contents (i.e. perceptions, sensations, cognitions, and emotions) and (2) a friendly, accepting, and non-judgmental attitude towards those mental contents (Kohls *et al.*, 2009). Mindfulness seems the perfect candidate for a positive factor during pregnancy as many studies to date have reported associations between self-reported mindfulness (as a trait) and psychological health (For ar review, see Keng *et al.*, 2011), better work-family balance among working parents (Allen and Kiburz, 2012), better emotion-regulation (Goodall *et al.*, 2012), and lower cortisol levels (Brown *et al.*, 2012). These factors could potentially contribute to a more healthy pregnancy, which, in turn, may provide a better environment for the fetus. To date, mindfulness interventions are being developed and offered to pregnant women, while the effects of mindfulness during pregnancy on child outcomes is largely unknown. To the best of our knowledge, only one study (Sriboonpimsuay *et al.*, 2011) reported effects of mindfulness intervention during pregnancy on birth outcomes (i.e. lower preterm birth weight in the intervention group) and no studies have been published on child outcomes. This dissertation therefore aimed to increase knowledge on the effects of maternal mindfulness on child outcomes.

1.6. Aims and research questions

This dissertation has two primary aims: (1) to examine the effect of exposure to maternal anxiety during pregnancy on neurocognitive functioning in infants and young children, and (2) to examine the effect of maternal mindfulness during pregnancy on neurocognitive functioning.

To address the first aim of this dissertation, the effect of prenatal exposure to maternal anxiety was examined for three key developmental aspects in early life: auditory attention (chapter 3), socio-emotional development and temperament (chapter 4), and affective processing (chapter 5). In addition, the effect of prenatal exposure to maternal anxiety on infants' functional brain network was investigated (chapter 6). To address the second aim, maternal mindfulness was included as a predictor in the studies presented in this dissertation (Chapter 3-6). The aims of the dissertation are discussed by answering the following research questions:

- Are maternal anxiety and mindfulness during pregnancy related to infants' auditory attention? (Chapter 3)
- Are maternal anxiety and mindfulness during pregnancy related to infants' temperament and socio-emotional functioning? (Chapter 4)
- Are maternal anxiety and mindfulness during pregnancy related to affective processing in children? (Chapter 5)

- Are maternal anxiety and mindfulness during pregnancy related to infants' functional brain network configuration? (Chapter 6)

In chapter 3 and 5 we use event-related potentials and in chapter 6 EEG-based network connectivity analysis to provide neurophysiological evidence for altered neurocognitive functioning in infants and children prenatally exposed to maternal anxiety and mindfulness, whereas in chapter 4 we used maternal report questionnaires regarding infant behavior (i.e., temperament) for this purpose. Chapter 5 was submitted without maternal mindfulness during pregnancy as a predictor. We therefore included an addendum at the end of the chapter to add the results for maternal mindfulness.

1.7. Thesis Outline

This dissertation consists of 7 chapters. This present chapter gives an overall introduction of the dissertation, followed by a general description of the participants and methods used for this dissertation in chapter 2. In chapter 3-6 the above stated research questions will be addressed. Finally, chapter 7 presents a general discussion of the findings and overall conclusion.

In addition, the appendix of the thesis includes, a first-author article that was published on data using the same auditory oddball paradigm as was used for this dissertation (in chapter 3), but did not report on early life influences of maternal anxiety or mindfulness during pregnancy. Instead, the paper investigated typical development of auditory attention by investigating maturation of auditory ERPs between 2 and 4 months of age in the same cohort as was used for answering the above mentioned research questions.

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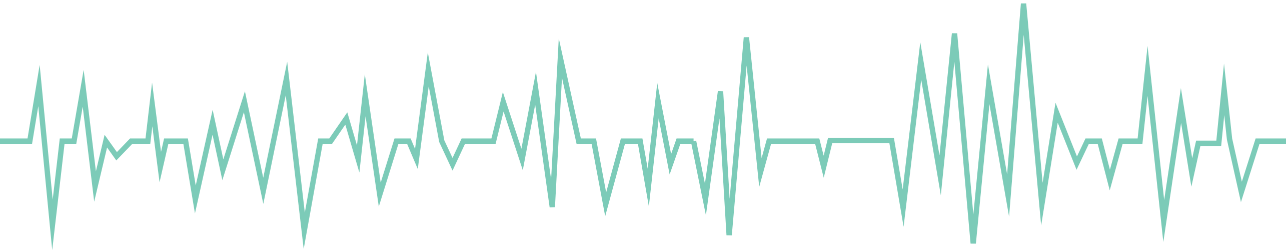
Chapter 2:

Participants & Methods



2.1. Prenatal Early Life Stress (PELS) study and BrainAGE

The data used in this dissertation consists of the first six waves of a prospective cohort study, following pregnant women and their offspring from the first trimester of pregnancy onwards. The first five waves (T1-T5) of this cohort study were conducted as part of the *Prenatal Early Life Stress (PELS)* study, a collaborative study running in three countries participating in the EuroSTRESS program of the European Science Foundation (ESF). Prof. Van den Bergh, the project leader, designed the study in collaboration with the other PELS partners and is principle investigator at Tilburg University, The Netherlands. Other partners were Prof. Glover, principal investigator from Imperial College London (UK), Prof. Claes, principal investigator from KU Leuven (Belgium), and Prof. Rodriguez, associated partner from Uppsala University (Sweden). For this dissertation, only data from the Dutch branch of the PELS study was used. The sixth wave (T6) of the cohort study was conducted as part of the *BrainAGE project* (www.brain-age.eu), a large international research project committed to healthy brain ageing. The BrainAGE project is financed by the European Commission Seventh Framework Program. The principal investigator at Tilburg University (The Netherlands) (as well as at KU Leuven, Belgium) is Prof. Van den Bergh. The project leader is Prof. Schwab from Jena University Hospital (Germany).

2.2. Participants

Between April 2009 and September 2010 a total of 178 women were recruited in the 15th week of pregnancy and 12 women between the 16th and 23nd week of pregnancy. Recruitment took place at the St. Elizabeth Hospital and four midwife practices (“Eva”, “De Zon, “Lente”, and “Isis”) in Tilburg, The Netherlands. If a pregnant woman was interested in the study she could give permission to pass her contact information to the researchers. Subsequently, the researchers made an appointment with the pregnant woman and explained the purpose and procedure of the study in detail. If the pregnant woman and her partner (if involved) agreed to participate in the study, an appointment for the first measurement wave was made. The participating mothers gave birth to 192 infants (including two pairs of twins) between September 2009 and March 2011. Characteristics of the participating mothers and their children can be found in Table 2.1.

Table 2.1. *Characteristics of the mothers (N = 190) and their infants (N = 192, two twins)*

Mothers		**N**	**%**	**Mean (SD)**
Age (years)				31.56 (4.42)
Nationality	Dutch	179	94.2	
	German	2	1.1	
	French	1	.5	
	Russian	1	.5	
	Thai	1	.5	
	Mixed	6	3.2	
Marital status	Married	96	50.5	
	Cohabiting	89	46.8	
	Single	4	2.1	
	Living with parent	1	.5	
Currently has job	Yes	176	92.6	
	No	14	7.4	
Family income (monthly, in €)	<2100	9	4.7	
	2200-3600	38	20.0	
	>3600	131	68.9	
	Don't want to disclose	10	5.3	
Educational level	Primary or secondary	19	10.0	
	General vocational training	49	25.8	
	Higher vocational training	73	38.4	
	University degree or higher	49	25.8	
Primagravida		74	38.9	
Has ever miscarried*		47	24.7	
Assisted pregnancy	None	153	80.6	
	In vitro fertilization	15	7.9	
	Intracytoplastmatic Sperm Injection	8	4.2	
	Hormonal Therapy	7	3.7	
	Intra-uterine Insemination	4	2.1	
	Other	3	1.6	
Smoker	Yes	9	4.7	
	No	161	84.7	
	No information	20	10.5	

Infants		N	%	Mean (SD)
Sex	Girl	96	49.7	
	Boy	92	48.2	
	No information***	4	2.1	
Birth weight (grams)				3444 (519)
Gestational age (weeks)				39.69 (1.6)
Miscarriage/Baby died		2	1.0	
Prematurity (<37 weeks)	Yes	9**	4.7	
	No	183	95.3	

Notes. * Before 12 weeks gestation; **8 infants were born between 32 and 37 weeks of gestation; ***Mothers dropped out before end of pregnancy.

All participating parents provided written informed consent at the start of the cohort and again when the children were 4 years of age. The Medical Ethical Committee of the St. Elizabeth Hospital in Tilburg, The Netherlands approved the study. The study was conducted in full compliance with the Helsinki declaration.

2.3. Procedure

The first three waves were conducted during pregnancy (T1, T2, T3; once in each trimester). The measurement sessions took place at the participant's home, unless the participant preferred meeting somewhere else (e.g., at the university, at work). In addition, mothers individually collected salivary cortisol at home without a researcher present. Both mothers and fathers completed questionnaires during pregnancy for each wave. Participants could choose whether they preferred paper and pencil questionnaire or an electronic version.

After birth, the mothers and their children were invited to the Tilburg University Babylab for postnatal observations at two to four months (T4), at nine months (T5), and once again at four years of age (T6). During the visits, we recorded event-related potentials from the child while they performed tasks measuring neurocognitive development. We also conducted behavioral tasks to measure child temperament, intelligence, and executive functions. In addition, some neuroendocrine and genetic data was collected from both the mother and the child during the visits. Again, mothers and fathers completed questionnaires for each wave. For the fourth and fifth wave they could still choose between a paper and pencil questionnaire or an electronic version. The questionnaires for the sixth wave were all electronic (most participants were in the possession of a computer with internet or IPad at the time).

An overview of the full data collection at the first six waves can be found in table 2.2. In Table 2.3., an overview of data that was used for each chapter of this dissertation is shown. Only the procedure and description for those measures used in this dissertation are discussed in the section below.

2.4. Maternal measures

Maternal anxiety

Maternal anxiety was assessed over all six waves with multiple questionnaires. Maternal self-reported state anxiety was measured using the 10-items anxiety subscale of the Symptom Checklist-90 (SCL-90) developed by Arrindel and Ettema (1981), scored from 1 to 4. Cronbach's alpha for this subscale ranged between .730-.920 (see chapters 3-6). The anxiety subscale of the SCL-90 measures both the somatic (e.g., vegetative arousal, bodily symptoms) and the psychological symptoms of anxiety (e.g., fearful thoughts), while the other anxiety questionnaires often only the psychological anxiety symptoms (Bech, 2011).

Maternal mindfulness

Maternal mindfulness was assessed during the second trimester of pregnancy (T2) using a Dutch translation of the Freiburg Mindfulness Inventory – Short Form (Walach *et al.*, 2006). Mothers rated 14 items (e.g., "*I am open to the experience of the moment*" and "*I observe my mistakes and difficulties without judging myself*") on a four point Likert scale (*1 = rarely to 4 = almost always*). A higher score on the FMI-s reflected higher levels of mindfulness. Cronbach's alpha for the FMI-s ranged between .860-.880 (see chapters 3-6).

Maternal mindfulness was measured at T2 and T6. The studies examining the effects of prenatal exposure to both maternal anxiety and mindfulness (chapter 3-6) used only the questionnaire data obtained at T2 as predictor.

Table 2.2. *Overview of data collection during the first six waves of the current study.*

	T1	T2	T3	T4	T5	T6
Mother data						
Self-report mental health questionnaires*	•	•	•	•	•	•
Self-report mindfulness questionnaire		•				•
Cortisol from saliva samples	•	•	•	•	•	
Psychiatric interview (MINI)		•				•
Cortisol from hair samples	•		•	•	•	
Hearth rate variability (stress & relaxation task)	•		•			
Hearth rate variability (24-hours)	•	•	•			
Hearth rate variability (baseline)					•	
Data on delivery				•		
Father data						
Self-report mental health questionnaires*	•	•	•	•	•	•
Self-report mindfulness questionnaire		•				•
Child data						
Data on birth outcomes				•		
Parental questionnaires**				•	•	•
Hearth rate variability				•	•	•
Cortisol from saliva samples				•	•	
Cortisol from hair samples				•	•	
Bayley Scales of Infant Development				•	•	
Laboratory Temperament Assessment***					•	
Auditory oddball paradigm (ERP)				•	•	
Audio-visual paradigm (ERP)					•	
Affective Pictures paradigm (ERP)						•
Dimensional Change Card Sort (ERP)						•
Resting state – eyes open (EEG)						•
Resting state – eyes closed (EEG)						•
Inhibition measurement (Bear & Tiger task)						•
Child intelligence (SON-r)						•
Buccal cells (epigenetics)						•

Notes. ERP = Event-related potential; EEG = electroencephalography; *Including questionnaires about anxiety, pregnancy-related anxiety, depression, work stress, and life events; **Filled out by both parents; ***Later added to protocol, not all infants were measured.

Table 2.3. *Overview of data used in each chapter of this dissertation*

	Predictor		Outcome	
	Description	**Wave(s)**	**Description**	**Wave(s)**
Chapter 3	Maternal Anxiety & Mindfulness	T2	Infant Auditory Attention	T5
Chapter 4	Maternal Anxiety & Mindfulness	T2	Infant Socio-emotional Functioning & Temperament	T5
Chapter 5	Maternal Anxiety & Mindfulness	T2	Child Affective Picture Processing	T6
Chapter 6	Maternal Anxiety & Mindfulness	T2	Infant Brain Network Measures	T5

2.5. Infant and Child Outcome Measures

Auditory Attention

Infants were presented with an auditory oddball paradigm at 2 or 4 months (T4; Appendix) and at 9 months of age (T5; chapter 3). Electrical brain activity was measured using EEG. The paradigm consisted of four types of sound events: a complex tone of 500 Hz base frequency presented with .7 probability following and an inter-stimulus interval (ISI) of 300 ms (the "standard" tone); the same tone following an ISI of 100 ms (.1 probability; the "ISI deviant"); a white noise segment (.1 probability; 300 ms ISI; "white noise"); and 150 unique environmental sounds (.1 probability; 300 ms ISI; "novel"). All stimuli had durations of 200 ms and were presented at an intensity of 75 dB SPL. In total, 1500 stimuli were delivered, divided into five stimulus blocks, each containing 300 stimuli. Figure 2.1 shows a graphical representation of the auditory oddball paradigm.

Socio-emotional functioning

Around 10 months of age, the social-emotional development of the infants was reported by the mother by filling the 'Ages and Stages Questionnaires: Social-Emotional' for infants aged 9 to 14 months (ASQ:SE; Squires *et al.*, 2001). The questionnaire consists of 22 items with questions about socio-emotional development of the infant (e.g. "*Does your baby laugh or smile at*

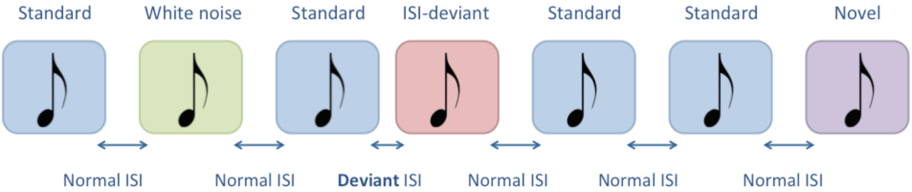


Figure 2.1. *Graphical representation of the auditory oddball paradigm with four types of stimuli: a frequently occurring standard sound ("Standard"; 70%), the same sound with a shorter inter-stimulus interval ("ISI-deviant"; 10%), a white noise segment ("White noise", 10%), and unique environmental sounds ("Novel", 10%).*

you and other family members?" and "*Is your baby interested in things around her, such as people, toys and food?*"). Items were rated on a three point Likert scale (i.e. 0 = *most of the time,* 5 = *sometimes,* 10 = *rarely or never*). Mothers could also indicate if the behaviour covered in the item concerned her. If this was the case, an additional 5 points were added to the total score. The ASQ:SE 12 is subdivided into the following five subscales: self-regulation problems (6 items), communication problems (4 items), adaptive functioning problems (5 items), affect problems (3 items), and interaction problems (5 items). Higher scores on the subscales indicate more problems within the respective dimension of socio-emotional development. In chapter 4, reliability analysis of the total scale revealed a sufficient internal consistency (Cronbach's α = .67).

Infant temperament

At about 10 months of age, temperament of the infants was reported by the mother with the short version of the revised Infant Behaviour Questionnaire (IBQ-R; Gartstein and Rothbart, 2003). The short version of the IBQ-R measures infant temperament using 37 items about the frequency of certain behaviours in specific situations (playing, bathing, etc.) of the previous week (e.g. '*When put into the bath water, how often did the baby smile?*'). Items are scored on a seven point Likert scale (1 = *never*, 7 = *always*). A second order factor analysis on the subscales scores of the original IBQ-R (long version) resulted in the following three broad dimensions that are also used for the short version of the IBQ-R (Gartstein and Rothbart, 2003): surgency (13 items), negative affectivity (12 items), and effortful control (13 items). Surgency is defined as a combination of low shyness and high approach and has been linked to the personality characteristics 'agreeableness' and 'extraversion'. Negative affectivity is defined as the tendency for a child to experience negative feelings and has been associated with neuroticism and negative emotionality in adults. Finally, effortful control is defined as the tendency of a child to consistently plan action and stay focussed and persistent and has been linked to the personality characteristic 'conscientiousness'. In our sample (see chapter 4), the IBQ-R subscales showed a good internal consistency on all scales (Cronbach's α = between .70 and .90).

Affective processing

At the age of four years, affective processing was measured in children by recording ERPs while they were viewing pictures of different emotional valence. Stimulus selection and procedure were based on the study of Solomon *et al.* (2012) in 5-7 years old, with few alterations to match the younger age of the sample. The stimuli consisted of 90 developmentally appropriate pictures of scenes, animals and objects: 30 neutral pictures, 30 pleasant pictures, and 30 unpleasant, taken from the International Affective Picture System (IAPS; Lang *et al.*, 2008). The pictures used in our study were previously rated on arousal and valance in 5-8 year old children by Hajcak and Dennis (2009). An example of the three types of pictures is presented in Figure 2.2.

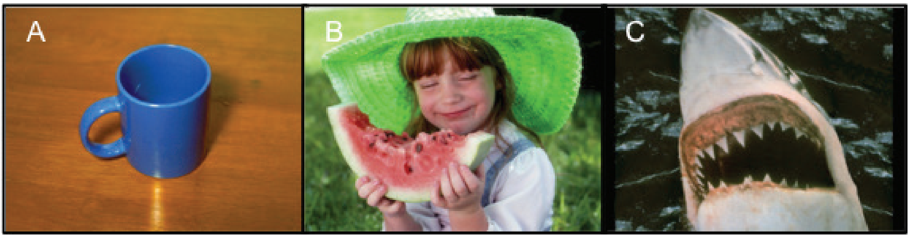


Figure 2.2. *Example of the three picture types: neutral (A), pleasant (B), and unpleasant (C).*

Infant Functional Brain Networks

Functional brain network metrics were obtained by reanalyzing the EEG-signals recorded during the above described auditory oddball paradigm in 9-month-olds. Functional connectivity measures in the infant alpha band (6-9 Hz) and in broad band (1-30 Hz) were computed using the Phase Lag Index (PLI; Stam *et al.*, 2007). Subsequently, functional brain network organization was characterized using minimum spanning tree (MST) indices – a graph-theoretical approach that avoids biases (Tewarie *et al.*, 2015). The following topological characteristics of the MST were calculated: MST degree, MST eccentricity (Ecc), MST betweenness centrality (BC), MST diameter (Diam), MST leaf fraction (Leaf), and MST hierarchy (T_h). More information about these metrics can be found in chapter 6.

2.6. Covariates

To account for potential confounding effects, several covariates were selected and statistically controlled within the studies reported in this dissertation. In all studies, statistical analyses were controlled for gestational age at birth and birth weight, because previous studies showed effects of these factors on cognitive functioning (Shenkin *et al.*, 2004; Espel *et al.*, 2014) and brain development (Ment and Vohr, 2008; Davis *et al.*, 2011; Thomason *et al.*, 2014) in infants and young children. In addition, we examined the effects of sex in chapter 4 and 6, since several studies have found different effects of exposure to prenatal maternal stress on the behavior (Mueller and Bale, 2008; Henrichs *et al.*, 2009; Loomans *et al.*, 2011) and brain development of boys and girls (Weinstock, 2007; Buss *et al.*, 2012). Moreover, all studies were controlled for possible effects of postnatal maternal anxiety and, where possible, for postnatal maternal mindfulness.

2.7. Data analyses

All statistical analyses were performed using IBM SPSS 19.0 for Windows. Instead of dichotomizing the data on maternal anxiety and mindfulness, continuous values were used, because dichotomizing might lead to loss of information, effect size, power, risks missing nonlinear effects, and may cause problems in comparing and aggregating findings across studies (e.g., MacCallum *et al.*, 2002).

In chapters 3 and 5, we used repeated measures ANCOVA with maternal anxiety and mindfulness during pregnancy as continuous predictors to investigate their influence on infant/child neurocognitive functioning. In chapter 4, we used the recently developed and highly powerful bootstrap method (Preacher *et al.*, 2007; Preacher and Hayes, 2008) to conduct mediation analysis. Lastly, to investigate the association between prenatal exposure to maternal anxiety and mindfulness on infant brain network connectivity (chapter 6), we used ANCOVAs with graph theoretical measures as dependent variables and maternal anxiety and mindfulness as independent variables.

Greenhouse-Geisser correction was applied to the repeated measures ANCOVAs when the assumption of sphericity was violated (ε correction factors are reported). All significant results are reported together with the partial η^2 effect size values; $\alpha = .05$.

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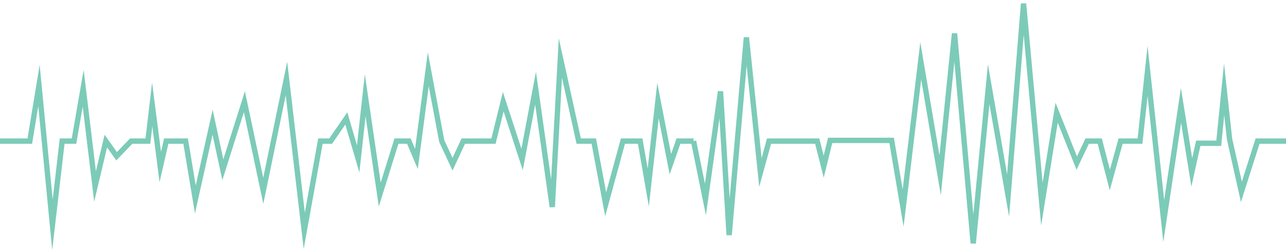
Chapter 3:

Effects of prenatal exposure to maternal anxiety and mindfulness on infant's auditory attention



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Maternal mindfulness and anxiety during pregnancy affect infants' neural responses to sounds

Social Cognitive and Affective Neuroscience, 10(3), 453-460.

Abstract

Maternal anxiety during pregnancy has been consistently shown to negatively affect offspring neurodevelopmental outcomes. However, little is known about the impact of positive maternal traits/states during pregnancy on the offspring. The present study was aimed at investigating the effects of the mother's mindfulness and anxiety during pregnancy on the infant's neurocognitive functioning at 9 month of age. Mothers reported mindfulness using the Freiburg Mindfulness Inventory and anxiety using the Symptom Checklist at ±20.7 weeks of gestation. Event-related brain potentials (ERPs) were measured from 79 infants in an auditory oddball paradigm designed to measure auditory attention - a key aspect of early neurocognitive functioning. For the ERP-responses elicited by standard sounds, higher maternal mindfulness was associated with *lower* N250 amplitudes ($p<.01$, $\eta^2=.097$), whereas higher maternal anxiety was associated with *higher* N250 amplitudes ($p<.05$, $\eta^2=.057$). Maternal mindfulness was also positively associated with the P150 amplitude ($p<.01$, $\eta^2=.130$). These results suggest that infants prenatally exposed to higher levels of maternal mindfulness devote *fewer* attentional resources to frequently occurring irrelevant sounds. The results show that positive traits and experiences of the mother during pregnancy may also affect the unborn child. Emphasizing the beneficial effects of a positive psychological state during pregnancy may promote healthy behavior in pregnant women.

3.1. Introduction

Research under the "prenatal programming hypothesis" examines the short and long-term consequences of the conditions of the prenatal environment for the offspring's health and disease risk (Barker, 2004). To date, accumulating evidence has demonstrated that prenatal exposure to maternal psychological distress affects neurocognitive functioning in the child. This effect probably results from induced alterations to fetal brain development and physiology (Van den Bergh *et al.*, 2005; Räikkönen *et al.*, 2011; Van den Bergh, 2011). Although the majority of the research in this field has focused almost exclusively on effects of exposure to early adverse conditions, prenatal exposure to maternal well-being might also "program" infant development and health. For example, Sriboonpimsuay and colleagues (2011) showed that the incidence of preterm birth was reduced in mothers who received meditation intervention during pregnancy as compared to the control group (i.e. those who underwent routine prenatal care). These results highlight the relevance of positive maternal experiences during pregnancy and their possible beneficial effects for both the mother and the child. Therefore, to cover the full scope of prenatal programming, one should also study the effects of positive experiences and traits during pregnancy on the offspring.

A good candidate for such a positive trait might be mindfulness, as many studies to date have reported associations between self-reported mindfulness (as a trait) and psychological health (for a review, see Keng *et al.*, 2011). Being mindful refers to a state of mind consisting of two key elements: (1) An alert mode of perceiving all mental contents (i.e. perceptions, sensations, cognitions, and emotions) and (2) a friendly, accepting, and non-judgmental attitude towards those mental contents (Kohls *et al.*, 2009). Being more mindful has been associated with better work-family balance among working parents (Allen and Kiburz, 2012) and better emotion-regulation (Goodall *et al.*, 2012). These two factors could contribute to a more healthy pregnancy, which, in turn, may provide a better environment for the fetus. Mindfulness interventions have been developed recently for pregnant women and their effects on psychological stress experience during pregnancy have been evaluated. The initial trials suggested beneficial effects of mindfulness interventions for normal populations of pregnant women as they reduced anxiety and depressive symptoms in mothers both pre- and postnatally (Vieten and Astin, 2008; Dunn *et al.*, 2012). A recent randomized controlled trial also found reduced maternal anxiety after mindfulness intervention in pregnant women with elevated anxiety levels (Guardino *et al.*, 2013). To our knowledge, only the study conducted by Sriboonpimsuay and colleagues (2011) reported effects of mindfulness intervention during pregnancy on birth outcomes (i.e. lower preterm birth weight in the intervention group) and no studies have been published on child developmental and health outcomes. Hence, the current study investigated the effects of dispositional maternal mindfulness during pregnancy and its effects on infant neurocognitive functioning.

A key aspect of early neurocognitive functioning is auditory attention. Auditory attention is an essential building block for developmental milestones, such as speech and language acquisition (Molfese, 2000; Benasich *et al.*, 2002; Benasich *et al.*, 2006; Kushnerenko *et al.*, 2013). It is important for infants to learn to organize the auditory input by rapidly extracting higher-level relationships and regularities from the sensory environment while becoming less responsive to variance in primary sensory features (Kushnerenko *et al.*, 2007). Auditory event-related potentials (ERPs) have long been employed to study these processes in infants and young children. The ERP method is suitable for testing this target group as ERPs can be recorded without the need for a behavioral response and in the absence of focused attention. Further, neurophysiological measures, such as ERPs, can be linked to the neurobiological processes involved in information processing. Despite these advantages, few human studies examining prenatal programming have incorporated neurophysiological measures (Mennes *et al.*, 2009; Buss *et al.*, 2010; Hunter *et al.*, 2012). In the current study, we measured ERPs from nine month old infants in a passive auditory oddball paradigm designed to measure the processing of frequent and rare sound events. Infants were presented with a repetitious train of “standard” sounds, which was occasionally interspersed with acoustically (i.e. white noise sounds and novel sounds) or temporally deviant sounds.

Although the auditory ERP components observed in infants are often not easily comparable to those obtained in adults (de Haan, 2006), the results of a longitudinal study by Kushnerenko et al. (2002) have shown similarities between some of the infantile ERP components and those observed in children and adults. The authors have suggested that the P150 component found in infants may be the precursor of the P100 response observed in children, which then develops into the adult P1 response. Functionally, this component is typically interpreted as an indicator of preferential attention to the auditory input and suppression of unattended information (Key *et al.*, 2005). Kushnerenko et al. (2002) also argued that the infant N250 recorded at 12 months of age could be considered similar to the N250 in children and the adult N2. The adult N2 has been associated with the orienting response and target selection (Key *et al.*, 2005). Finally, development of the infantile large positive component (PC) leads to the emergence of the P3a response in children and adults (Kushnerenko et al., 2002). The P3a has been proposed to reflect stimulus driven attention switching (Knight, 1996; Escera *et al.*, 2000; Polich, 2007). In nine month old infants, the ERP response elicited by standard and temporal deviant sounds typically carry both the P150 and the N250; the responses elicited by rare environmental (novel) sounds usually only show a PC response; white noise sounds typically elicit an ERP-response that comprises all three of the above components (see Kushnerenko *et al.*, 2013).

ERP-studies in adults have associated individual differences in dispositional mindfulness with differences in information processing (e.g. Brown *et al.*, 2013). Here we examine the possible effects of dispositional mindfulness during pregnancy on neurocognitive functioning in the offspring. Support for the notion that maternal traits/states during pregnancy may affect neurocognitive functioning of the offspring comes from ERP-studies examining the effects of prenatal and perinatal exposure to other maternal traits/states, such as prenatal maternal anxiety. These studies consistently associated prenatal (Mennes *et al.*, 2009; Hunter *et al.*, 2012; Van den Bergh *et al.*, 2012) and perinatal exposure (Harvison *et al.*, 2009) to elevated maternal anxiety with altered neurocognitive functioning in the offspring. For example, Mennes and colleagues (2009) found altered ERP patterns in response to an endogenous cognitive control task in 17 year old boys exposed to high maternal anxiety during pregnancy. Because mindfulness and anxiety are negatively correlated (e.g. Brown and Ryan, 2003; Walsh *et al.*, 2009), we hypothesized that prenatal exposure to maternal mindfulness and anxiety would affect the offspring in opposite ways.

3.2. Methods

Participants

The current study was part of the Prenatal Early Life Stress (PELS) project, an ongoing prospective cohort study in Tilburg, The Netherlands, following pregnant women and their offspring from the first trimester of pregnancy onwards. All participating parents provided written informed consent. The Medical Ethical Committee of St. Elizabeth Hospital, Tilburg, The Netherlands, approved the study, which was conducted in full compliance with the Helsinki declaration.

A total of 178 women had been recruited in the 15th week and 12 women between the 16th and 22nd week of pregnancy from a general hospital and four midwife practices. Tests were administered to them three times during pregnancy (T1, T2, T3; once in each trimester) and they were invited with their infant for postnatal observations either at two or four (T4) and once again at nine months after birth (T5). Here, we analyzed the data of those mother-infant dyads of which both maternal mindfulness and anxiety data at T2 and ERP data at nine months were available. From the 128 infants that were brought to the ERP-measurement, two were excluded due to missing mindfulness and anxiety data, two due to technical problems, thirty-five due to excessive movements/artifacts and or excessive crying/fussiness, and four infants because of premature birth (i.e., before week 36 of gestation and/or a birth weight below 2500 grams). We also excluded six infants who fell asleep during the experiment, because previous studies suggested that the state of alertness affects the auditory ERPs (Friederici *et al.*, 2002; Otte *et al.*, 2013). For a full overview of the inclusion and exclusion of mother-infant dyads, see the flowchart in Figure 3.1.

The final sample consisted of 78 mothers and their 79 nine-month-olds (42 girls, one pair of twins). The infants had a mean age of 43.90 weeks (SD = 1.84) and a mean gestational age at birth of 39.98 weeks (SD = 1.26). The mothers had a mean age of 32.09 years (SD =5.55) at the time of the ERP measurement. All infants were healthy and had passed a screening test for hearing impairment performed by a nurse from the infant health care clinic between the fourth and seventh day after birth. The screening test was a simple, non-invasive test utilizing otoacoustic emissions for detecting hearing deficits.

Measurements

Table 3.1 describes the sample of the mothers and their infants including demographic characteristics and scores on the mindfulness and anxiety questionnaires.

Mindfulness. Maternal mindfulness was measured using the Dutch short version of the Freiburg Mindfulness Inventory (FMIs-14; Walach *et al.*, 2006).

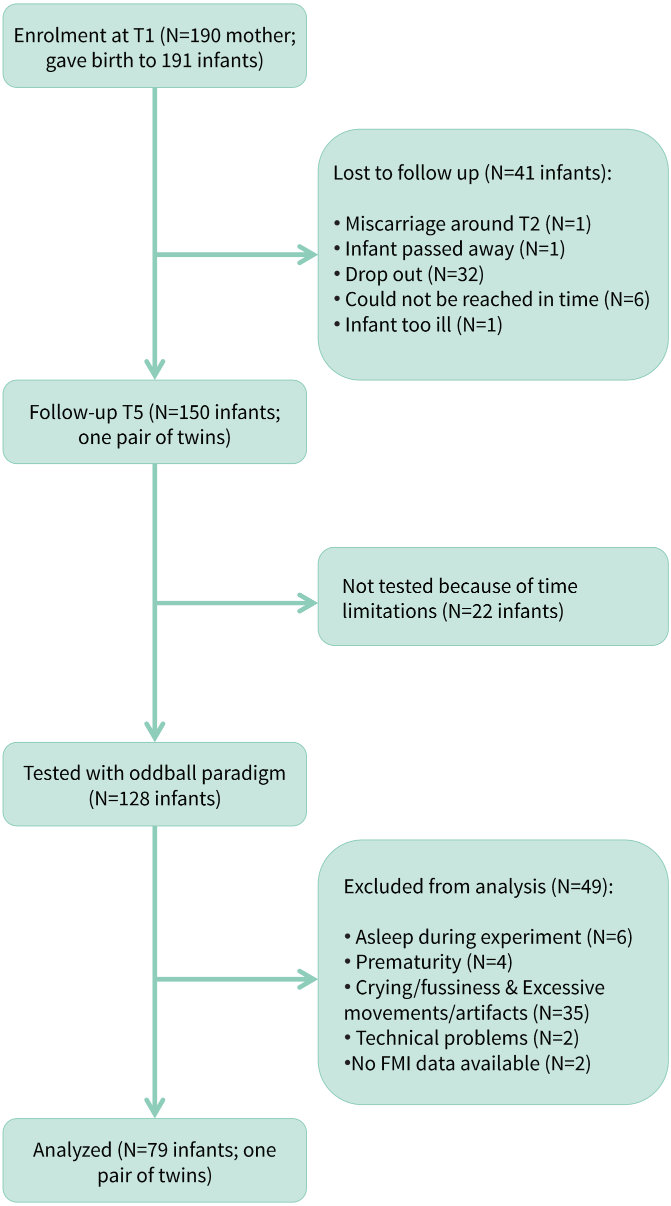

Figure 3.1. *Flowchart of inclusion and exclusion of the participating mothers–infant dyads.*

The original FMI is a self-report questionnaire developed in line with the concept of Buddhist psychology, but requires no knowledge of Buddhism or meditation to complete (Walach *et al.*, 2006). The shorter FMIs-14 consists of 14

Table 3.1. *Characteristics of the participating mother-infant dyad sample*

Infants (N=79)	**N**	**%**	**M (SD)**
Age at EEG-measurement (weeks)	79		43.9 (1.84)
Sex			
Girl	42	53.2	
Boy	37	46.8	
Birth weight (grams)	79		3495.25 (487.26)
Gestational age at birth (weeks)	77		39.98 (1.26)
Mothers (N=78)	**N**	**%**	**M (SD)**
Age (years)	78		32.09 (5.55)
FMI sum at T2	78		40.66 (6.30)
SCL-90 sum at T2	78		13.13 (3.39)
SCL-90 sum at T5	57*		13.26 (5.02)
Education			
Primary or secondary	6	7.7	
General vocational training	22	28.2	
Higher vocational training	34	43.6	
University degree or higher	16	20.5	
Primagravida	29	37.2	

Note FMI = Freiburg Mindfulness Inventory; SCL-90 = anxiety subscale of the Symptom Checklist; T2 = during second trimester; *N=21 mothers included in the study did not complete the postnatal questionnaire.

items with four point Likert scales ranging from 1 (*rarely*) to 4 (*almost always*). Higher aggregate scores indicate higher mindfulness. The FMIs-14 was shown to measure a single dimension and shows good internal consistency (α=.86; Walach *et al.*, 2006).

Anxiety. Maternal anxiety was measured using the Dutch version of the anxiety subscale of the Symptom Checklist (SCL-90; Arrindel and Ettema, 1981). This SCL-90 anxiety subscale is a self-report measure of anxiety symptoms, consisting of 10 items with five point Likert scales ranging from 0 (*not at all*) to 4 (*extremely*). Higher aggregate scores indicate higher anxiety. The scale has good convergent and divergent validity and has good internal consistency (α=.88 for the anxiety subscale; Arrindell and Ettema, 2003).

ERP paradigm. ERPs were measured in a passive auditory oddball paradigm. The stimulus sequences consisted of four different types of sound events: A frequent standard sound (probability of .7) and three types of infrequent deviant events (each with a probability of .1). The standard was a complex tone with 500 Hz base frequency presented following an inter-stimulus-interval (ISI; offset-to-onset) of 300 ms. Standard tones were constructed from the three lowest partials, with the intensity of the second and third partials set 6 and 12 dB lower, respectively, than that of the base harmonic. The deviant sounds were (1) the same tone as the standard but following a shorter ISI of 100 ms ('ISI-deviant'); (2) a white noise segment ('white noise', 300 ms ISI); and (3) 150 unique environmental sounds such as a slamming door, a barking dog, etc. ('novel sound', 300 ms ISI). All stimuli had durations of 200 ms and were presented at an intensity of 75 dB SPL. In total, 1500 stimuli were delivered. They were divided into five stimulus blocks, each containing 300 stimuli. The stimuli were presented in a semi-random order with the restriction that novel/white noise sounds were always preceded by at least two standard tones, and consecutive ISI-deviants were always separated by at least two sounds with a regular ISI (standard, novel, or white noise).

Procedure

Mindfulness and anxiety questionnaires were administered to mothers at the beginning of the second trimester (T2: mean gestation [SD] = 20.72 [2.06] weeks) in their home. To control for postnatal anxiety, the same anxiety questionnaire was also administered to most mothers (N=57) ca. 10 months after birth (T5: mean [SD] = 44.09 [1.84] weeks). The ERP-measurement took place ca. 9 months after birth in a dimly lit and sound-attenuated room at the Developmental Psychology Laboratory of the university. The complete procedure took approximately 60 minutes including electrode placement and removal. During the EEG recording, infants sat on their parents' lap with two loudspeakers placed at a distance of 80 cm from the infant's head, one on each side. The whole experimental procedure was recorded with two cameras of which the first one was placed behind and the other facing the infant and the parent. The camera recordings were used to detect episodes when the baby was crying or moving; these episodes were then excluded from the analyses.

ERP measurement and data processing

EEG was recorded with BioSemi ActiveTwo amplifiers (www.biosemi.com) with a sampling rate of 512 Hz, using caps with 64-electrode locations placed according to the extended International 10-20 system. We analyzed data from the following nine electrode sites: F3, Fz, F4, C3, Cz, C4, P3, Pz and P4. The standard BioSemi reference (CMS-DRL) was used (see www.biosemi.com/faq/cms&drl.htm for details) and two additional electrodes were placed on the left and right mastoids, respectively and mathematically combined off-line to produce an average mastoids reference derivation. All electrophysiological analyses were conducted using the BrainVision Analyzer 2 software package (Brain Products, Munich, Germany). Off-line filter settings consisted of a 50 Hz notch filter and a zero-phase Butterworth bandpass 1.0 – 30 Hz (slope 24 dB) filter. Subsequently, the data were segmented into epochs of 600 ms duration including a 100 ms pre-stimulus period. Epochs with a voltage change exceeding 150 μV within a sliding window of 200 ms duration or with changes exceeding the speed of 80 μV/ms at any of the nine electrodes were rejected from further analysis. Trials that preceded the ISI deviant were removed from the analysis because late responses to these sounds overlapped the early responses elicited by the ISI deviant. The average number of remaining trials included in the analyses of the four stimulus types were as follows, standard: 730; ISI-deviant: 118; white noise: 102; novel: 105. ERPs were averaged separately for the four different stimulus types (standard, ISI-deviant, white noise, novel) and baseline-corrected to the average voltage in the 100 ms pre-stimulus period.

Time windows for measuring the various ERP components were selected on the basis of the grand average ERPs measured from the 9 electrode locations, separately for the standard and the three oddball stimuli (see Figure 3.2). Mean amplitudes were measured from the following time windows/stimuli: a window from 100 to 200 ms for the standard, the ISI-deviant, and the white noise sound to capture the P150-waveform; for the N250, the window was set from 200 to 300 ms for the standard tone and the ISI-deviant and from 150 to 250 ms for the white noise sound in the response to which this component peaked earlier; the window for the PC component was set between 250 and 400 ms for the white noise and novel sounds (the only ones eliciting this component).

Statistical analysis

Firstly, using Pearson's correlation, we checked whether the correlation between mindfulness and anxiety measured at T2 was negative, as was expected on the basis of previous results (e.g. Brown and Ryan, 2003; Walsh *et al.*, 2009). Three series of repeated-measures ANCOVAs were then conducted to test the effects of maternal mindfulness and anxiety on the infant's ERP amplitudes: One with "Mindfulness", one with prenatal (T2) "Anxiety", and one with postnatal (T5) anxiety as the continuous predictor. The latter was introduced for comparing the effects of pre- and postnatal anxiety on the ERP responses. Instead of dichotomizing the variables, continuous predictors were used, because dichotomizing might lead to loss of information, effect size, power, risks missing nonlinear effects, and may cause problems in comparing and aggregating findings across studies (e.g. MacCallum *et al.*, 2002). In each ANCOVA, two within-subject factors "Frontal-Central-Parietal" X "Left-Middle-Right" were also included for assessing effects of the scalp topography of these components. Separate ANCOVAs were performed per stimulus type (standard, ISI-deviant, white noise, and novel sounds) and peak (P150, N250, and PC, where applicable). For significant interactions between the target variables (mindfulness and anxiety) and the "Frontal-Central-Parietal" factor, post hoc tests were conducted by separate ANCOVAs for the frontal (F3, Fz, F4), central (C3, Cz, C4), and parietal arrays of electrodes (P3, Pz, P4). Except for the ANCOVAs with T5 anxiety, gestational age and birth weight of the infants were selected as covariates, because previous studies showed effects of gestational age and birth weight on cognitive functioning (e.g. Fellman *et al.*, 2004; Shenkin *et al.*, 2004) and brain development (e.g. Poston, 2012; van den Heuvel *et al.*, 2015). Postnatal anxiety at T5 was also selected as a covariate to control for possible postnatal effects of anxiety. The covariates were first correlated with the AERP measures using Pearson's correlation and only added to the ANCOVAs if significant correlations were found. All statistical analyses were performed using IBM SPSS 19.0 for Windows. All significant results are reported together with the partial η^2 effect size values; $\alpha = .05$.

3.3. Results

Maternal mindfulness and anxiety during pregnancy (T2) were negatively correlated (r=-.270; p<.05). Maternal anxiety measured during pregnancy (T2) and maternal anxiety measured ca. 10 months after birth (T5) were positively correlated (r=.308; p<.05).

Figure 3.2 shows that all major components are clearly fronto-centrally distributed. The ERP effects of maternal mindfulness and anxiety at T2 on the infants' ERP response to the standard sound is illustrated in Figure 3.3A and

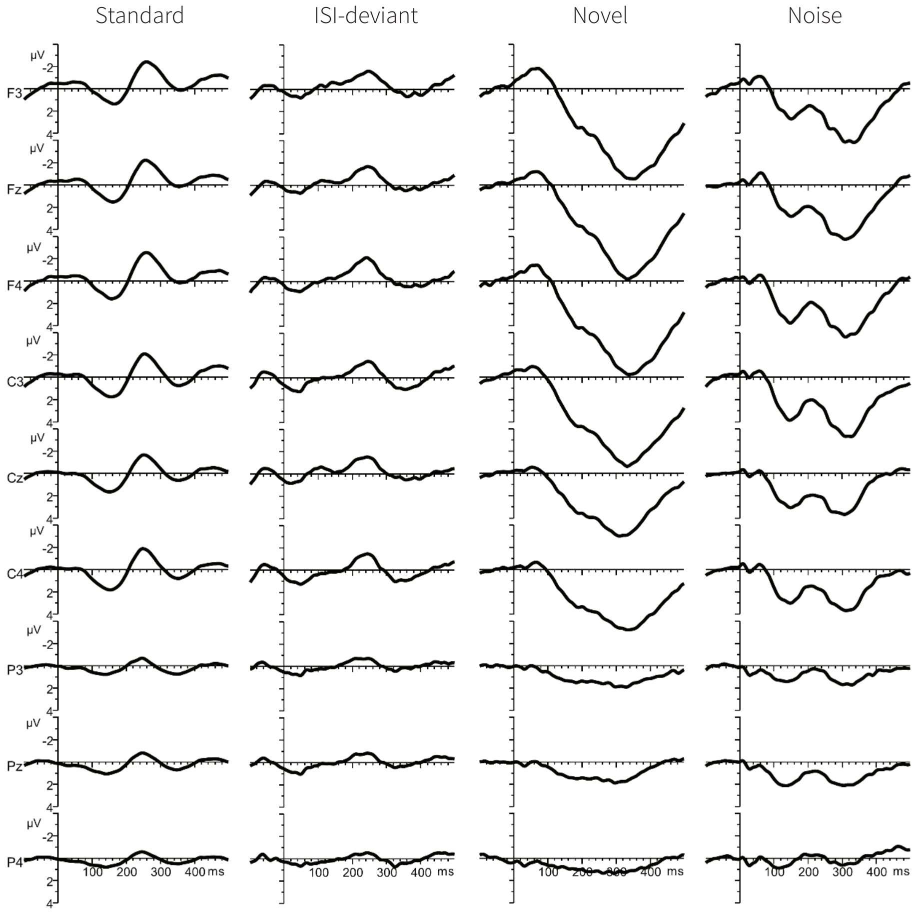

Figure 3.2. *Group-average (N=79) ERP-responses to standard tones, ISI-deviants, white noise segments, and novel sounds (columns) at electrodes F3, Fz, F4, C3, Cz, C4, P3, Pz, P4 (rows).*

B. For illustration purposes only, the infants were divided into low and high maternal mindfulness/anxiety groups and the responses separately averaged for these groups. A mean cut-off was used for the mindfulness measure; the cut-off score (15) for the SCL-90 anxiety subscale was taken from the average for the normal population (Derogatis *et al.*, 1974). Note that in the statistical analyses both of these measures were included as continuous predictors. Because we found no significant association ($p > .05$) between the P150 and N250 amplitudes and the covariates (i.e. gestational age, birth weight, and maternal anxiety at T5), no covariates were entered for the analysis of the results reported below.

For the ERPs elicited by standard tones, a significant positive association was obtained between maternal mindfulness and the P150 amplitude (*F*(1,77) = 10.476, $p = .002$, $\eta^2 = .120$; Figure 3.3C) and a significant negative association between maternal mindfulness and the N250 amplitude (*F*(1,77) = 8.504, $p = .005$, $\eta^2 = .099$; Figure 3.3D). For the N250 amplitude, also a significant interaction between mindfulness and the "Frontal-Central-Parietal" factor was found (*F*(2,77)= 14.743, p=.009, η^2=.066). Follow-up tests showed that the effect of mindfulness was significant at frontal and central scalp locations (*F*(1,77)= 8.515, p=.005, η^2=.100, and *F*(1,77)= 8.841, p=.004, η^2=.103, respectively), but not at parietal sites. For the rare deviant stimuli (i.e. ISI-deviant, white noise, and novel sound) no significant associations were found between maternal mindfulness and any of the ERP amplitudes.

Higher prenatal (T2) maternal anxiety was significantly associated with larger N250 amplitudes for the standard sound (*F*(1,77) = 8.177, $p = .005$, $\eta^2 = .096$; Figure 3.3E)[1]. Scalp distribution factors did not yield significant main effects or interactions with other factors. For the rare deviant stimuli (i.e. ISI-deviant, white noise and novel), no significant associations were found between maternal anxiety and any of the ERP amplitudes. Finally, postnatal (T5) maternal anxiety was not significantly associated with the measured ERP amplitudes.

3.4. Discussion

The aim of the present study was to examine the effects of the mother's mindfulness and anxiety during pregnancy on neurocognitive functioning in the offspring. We found significant opposite effects of maternal mindfulness and anxiety during pregnancy on how infants processed repetitive sounds. In contrast, none of the ERPs elicited by rare auditory events were significantly affected by the independent variables. Whereas effects of maternal anxiety during pregnancy on offspring neurocognitive functioning have already been previously reported (Mennes *et al.*, 2009; Buss *et al.*, 2010; Van den Bergh *et al.*, 2012), our results show that maternal mindfulness may also affect neurocognitive functioning in the offspring.

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[1] The association remained significant (F(1,76)= 6.319, p=.014, η2=.077) even after removing the extreme case (SCL-score = 28; see Figure 3.3E) from the analysis.

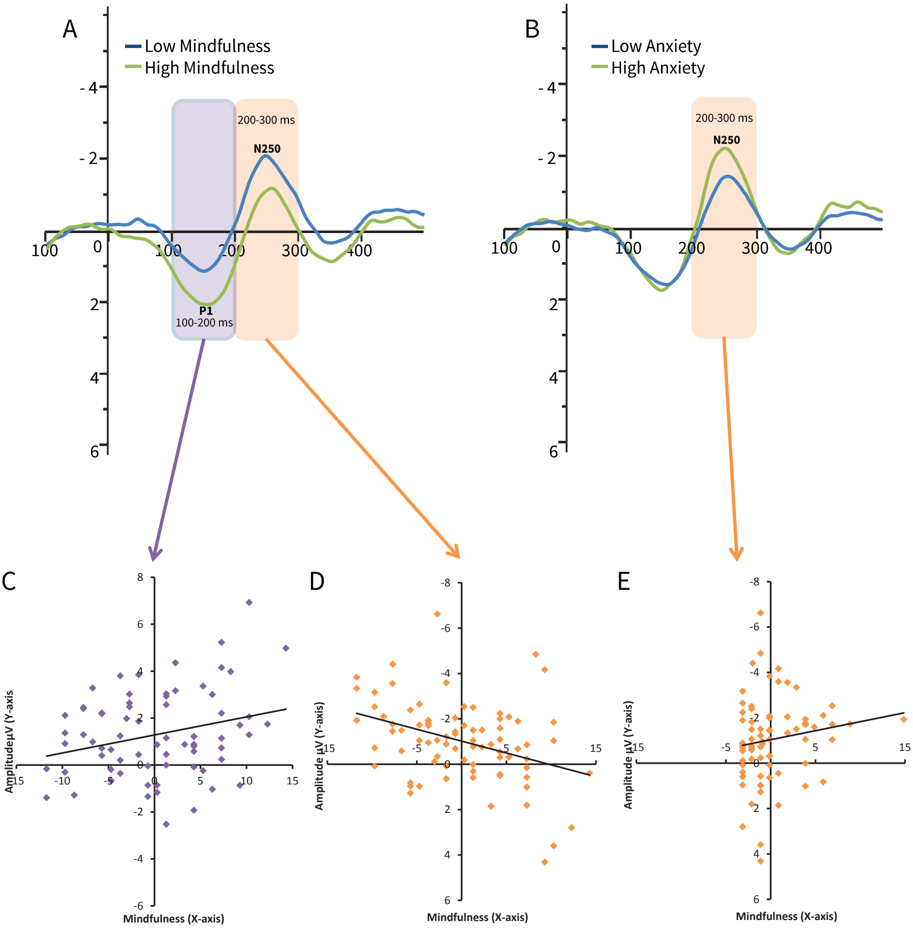

Figure 3.3. *Group-average (N=79) central (Cz) ERP-response to the standard sound of infants of mothers with low (blue line) and high (green line) mindfulness (**A**) and anxiety (**B**). The scatterplots shows the correlation between maternal mindfulness and the amplitude of (**C**) the first positive-going wave 'P150' (measured from the 100-200 ms post-stimulus interval) and (**D**) the first negative-going wave 'N250' (200-320 ms). Panel (**E**) shows the scatterplot for the correlation between maternal anxiety and the amplitude of the 'N250' component. **Notes**: The statistical analyses were performed with mindfulness and anxiety as continuous predictors (**C, D** and **E**). Panels **A** and **B** are for illustration purposes, only.*

Associations between maternal mindfulness and anxiety during pregnancy and their effects on the infants' auditory ERP responses

As was expected from results of previous studies (Brown and Ryan, 2003; Walsh *et al.*, 2009), a negative correlation was observed between maternal mindfulness and anxiety in the current group of pregnant women and, in line with our hypothesis, we found opposite effects of maternal mindfulness and anxiety on the ERPs elicited by the frequent standard tones: Higher maternal mindfulness was associated with *lower* N250 amplitudes, whereas higher maternal anxiety was associated with *higher* N250 amplitudes. Higher maternal mindfulness was also associated with higher P150 amplitudes. P1, the putative adult analogue of P150 is regarded as reflecting early preattentive processes extracting sound features (e.g. Näätänen and Winkler, 1999; Picton, 2010). Thus higher P150 amplitudes may reflect more thorough/elaborate feature extraction in babies of mothers with higher mindfulness scores. In adults, the various subcomponents of N2 (N2a, b, and c - see Pritchard *et al.*, 1991), the putative analogue of the infantile N250, have been associated with various cognitive processes (e.g. selective attention, stimulus identification) evaluating the incoming sound. The lower N250 amplitude found for infants exposed to higher levels of maternal mindfulness during pregnancy might be a consequence of these infants having formed more accurate preattentive representations (i.e. higher P1) and, therefore not needing to process the repetitious standard sound more elaborately. In sum, infants prenatally exposed to higher levels of maternal mindfulness may devote *less* processing to the uninformative repetitious sounds, whereas infants prenatally exposed to higher levels of maternal anxiety may process such sounds *more* extensively. These results suggest that prenatal exposure to maternal mindfulness and anxiety affects neurocognitive functioning in the offspring in at least partly opposite ways.

Significant effects were only observed for the frequent standard sound but not for the rare deviant sounds. This suggests that the infants' deviance detection mechanism was not affected by exposure to maternal mindfulness (or anxiety) during pregnancy. The fact that both mindfulness and anxiety only exerted influence on the responses elicited by the standard sound could mean that these maternal states/traits affect processes involving habituation. Neural habituation has been defined as a process by which the neural response decreases over time during repeated stimulation (Thompson and Spencer, 1966). This is more likely to take place for the high-probability standard sound than for the low-probability deviant sounds. Because extensive and continuous processing of the oft-repeated standard is largely unnecessary, habituation to this stimulus could be considered as adaptive. Building on this line of reasoning, the lower amplitude of the N250 for infants prenatally exposed to higher mindfulness could indicate stronger habituation processes in these infants, which might be a sign of more adaptive brain functioning. In contrast, the fact that higher N250 amplitude was associated with higher maternal anxiety could be interpreted as reflecting weaker habituation processes in these infants, possibly indicating less adaptive brain functioning. The latter suggestions is compatible with the results of Turner et al. (2005), who reported that children of anxious parents were less likely to habituate to fear-relevant auditory and visual stimuli. More research is necessary, however, to test whether infants prenatally exposed to higher levels of maternal mindfulness possess stronger habituation processes.

The non-significant correlations for the ERP responses elicited by the deviant sounds may be possibly attributed to relatively higher noise levels in the deviant-sound responses due to the lower average number of trials for the deviant sounds compared to that for the standard tones. However, at least the novel sounds and the white noise segment used in our study have been shown to elicit ERP responses of higher amplitudes than the standard tone. Therefore, the signal-to-noise ratio for equal numbers of trials is better for these sounds than for the tone stimuli, such as the standard (Kushnerenko et al., 2007) and thus fewer number of trials are needed to achieve the same S/N ratio. Further, the average trial numbers for all three deviants was relatively high and the study includes a large group of infants compared with most studies recording auditory ERPs in infants. Taken these together, the lack of significant correlations between maternal mindfulness/anxiety during pregnancy and the ERP responses elicited by the deviant sounds is likely a robust finding of this study. Future research could focus on exploring the mechanisms selectively affecting the processing of repetitive sounds but leaving the deviance detection mechanism intact.

Possible mechanisms

The fact that mindfulness and anxiety exert influence on the same type of information processing might point to shared underlying mechanisms. However, the mechanisms by which the psychological traits/states of the mother “program” the infants’ brain during pregnancy are not yet known. One often proposed mechanism is the exposure to excessive levels of cortisol, the so-called "stress hormone". The human fetus is relatively protected against direct exposure to high cortisol concentrations as in the placenta 50–90% of maternal cortisol is converted to biologically inactive cortisone by the enzyme 11β-hydroxysteroid-dehydrogenase (11β-HSD-2) (Mulder *et al.*, 2002). However, high maternal anxiety in pregnancy may lead to down-regulation of placental 11β-HSD2 (O'Donnell *et al.*, 2012). Although cortisol is crucial for fetal tissue proliferation and differentiation, exposure to excessive levels of this hormone due to maternal stress can have negative consequences for birth outcome and development, such as e.g. increased risk for premature birth (Sandman *et al.*, 2006) and impaired cognitive performance (Davis and Sandman, 2010). Since dispositional mindfulness has been linked to reduced cortisol levels (Brown *et al.*, 2012) and anxiety is associated with enhanced cortisol levels (Pluess *et al.*, 2010), the suggestion of cortisol secretion as one of the underlying mechanisms appears to be reasonable. Being mindful during pregnancy could make the mother less vulnerable to stressful events. Indeed mindfulness has been linked to psychological health (Keng *et al.*, 2011), better work-family balance (Allen and Kiburz, 2012) and better emotion-regulation (Goodall *et al.*, 2012). In sum, mindfulness could prevent the negative consequences of prenatal exposure to stress on the fetus whereas anxiety during pregnancy might enhance vulnerability to stress in the fetus.

Although ERPs were measured early in development, postnatal influences could have also affected the neurocognitive functioning of the infants. Maternal anxiety and mindfulness during pregnancy may be related to attachment, parenting styles, and ways of taking care of the infant. These factors may, therefore be partly responsible for the observed association between maternal prenatal anxiety/mindfulness and some of the ERP responses. On the other hand, we have found no significant association between the mothers' postnatal anxiety at nine months after birth and the P150 and N250 amplitudes. Thus the effects found cannot be attributed to the influences of anxiety on the mother-child interaction between birth and 9 months of age. Unfortunately the mothers' postnatal mindfulness was not measured. More mindful mothers could have, for instance, (also) affected their infants' brain through more mindful parenting or less postnatal stress. Possible genetic factors cannot be disregarded either; mindful mothers might give birth to babies more disposed towards mindfulness. Nevertheless, human studies evaluating the consequences of stressful life events on development, such as natural disasters (Laplante et al., 2004), suggest that the effects of prenatal stress cannot be explained by genetic predispositions alone. To isolate the effects of prenatal maternal mindfulness from possible postnatal effects and to eliminate genetic influences, future randomized controlled trials are needed with mindfulness interventions for pregnant women.

Clinical applications

The current results could help to suggest directions to nurses, midwives, general practitioners, and gynecologists in providing pregnant women with information about potentially positive prenatal programming effects and in this way contribute to healthier pregnancy. Highly anxious mothers may feel guilty in response to the message that being anxious during pregnancy negatively affects their baby and might in turn experience increased anxiety levels instead of decreased levels. By stressing the potential of mindfulness instead, a positive message can be provided to them. Further, the current results emphasize the possible strengths of a mindfulness intervention for pregnant women. Such an intervention for pregnant women suffering from anxiety may be a very desirable alternative to pharmacological interventions, since several studies have described the detrimental effects of psychopharmacological treatment during pregnancy on the fetus (e.g. Mulder *et al.*, 2011). However, more research into the effects of maternal mindfulness during pregnancy is necessary before firm conclusions about the potential benefits for the infants' neurocognitive functioning can be drawn.

3.5. Conclusion

The results of the current study indicate that (1) infants' processing of the standard but not the deviant sounds (ISI-deviant, white noise, novel) is affected by prenatal exposure to maternal mindfulness and anxiety and (2) mindfulness and anxiety exert, at least partly, opposite effects on the infant's ERP responses to repetitive sounds. We suggest that infants prenatally exposed to higher levels of maternal mindfulness devote *less* in-depth processing to frequently occurring, irrelevant stimuli and/or they habituate *faster* to these stimuli. In contrast, infants prenatally exposed to higher levels of maternal anxiety process such uninformative sounds *more* extensively and/or they habituate *slower* to these stimuli. This difference might stem from infants prenatally exposed to higher maternal mindfulness pre-attentively forming more accurate perceptual representations. The current study contributes to the field of prenatal programming by showing that negative traits and experiences of the mother during pregnancy are not the only ones to have an effect on the child, as is often emphasized in the literature. Positive traits of the mother during pregnancy may also “program” the infant.

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Chapter 4:

Effects of prenatal exposure to maternal anxiety and mindfulness on infant's socio-emotional development and temperament

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Maternal mindfulness during pregnancy and infant socio-emotional development and temperament: The mediating role of maternal anxiety

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Abstract

Background. Accumulating evidence shows that maternal anxiety during pregnancy adversely affects child outcomes. The positive effects of maternal psychosocial factors during pregnancy on child outcomes are not yet studied. This prospective study addresses the association between maternal mindfulness during pregnancy and socio-emotional development and temperament in 10 months-old infants. We also investigated whether this association was mediated by maternal anxiety. **Method**. Mothers (N = 90) provided information about mindfulness and anxiety at the beginning of the second trimester of pregnancy. Infant socio-emotional development (Ages and Stages Questionnaire: Social Emotional; ASQ:SE) and temperament (Infant Behavior Questionnaire – Revised; IBQ-R) was assessed at age 10 months. **Results.** Higher maternal mindfulness during pregnancy was associated with less infant self-regulation problems and less infant negative affectivity. Mediation analysis showed that maternal anxiety mediated the association between infant self-regulation problems and maternal mindfulness. **Conclusion**. These results suggest that maternal mindfulness during pregnancy may have positive effects on infant development. This association may be mediated by reduced anxiety symptoms in pregnant women who score high on mindfulness. Additional replication studies are needed using objective measures of infant behavioural/emotional outcomes and mindfulness of the mother during child development.

4.1. Introduction

Accumulating evidence shows that maternal anxiety during pregnancy is linked to adverse birth outcomes and alterations in early socio-emotional, behavioural, and (neuro)cognitive development and even mental health problems in adolescence and early adulthood (Mulder *et al.*, 2002; Van den Bergh, 2011; Graignic-Philippe *et al.*, 2014). The Developmental Origins of Behaviour Health and Disease (DOBHaD) hypothesis studies short- and long-term effects of an individual's experience during the perinatal period on subsequent phenotypic variations in health and disease (Van den Bergh, 2011). The magnitude of these effects is clinically relevant, since the attributable risk of childhood emotional and behavioural problems caused by prenatal anxiety is estimated to be about 10-15% (Glover, 2014). Considering these substantial negative consequences of prenatal exposure to maternal anxiety (Glover, 2014), anxious women and their infants may benefit from factors promoting maternal wellbeing during pregnancy. Unfortunately, emotional care seems to be an often neglected part of obstetric medicine (Glover, 2014). Research examining factors promoting pregnant women's emotional wellbeing is therefore highly needed.

Only few studies have examined the effect of such promoting factors during pregnancy in relation to pregnancy and child outcomes. An example of such a factor is partner support during pregnancy. Stapleton *et al.* (2012), for instance, found that mothers experiencing higher levels of partner support during pregnancy reported less emotional distress postpartum and less infant distress to novelty. Several studies also observed associations of higher partner support with higher levels of maternal-fetal attachment (for a review, see Van den Bergh and Simons, 2009). In addition, Pluess *et al.* (2012) studied the impact of positive and negative life events during pregnancy on maternal stress hormone levels (i.e. cortisol) and found that positive life events predicted lower maternal cortisol levels, whereas negative life events were unrelated to maternal cortisol level. Elevated levels of maternal cortisol early in gestation are associated with adverse infant outcomes, such as slower development rate of the infant (Davis and Sandman, 2010). The above suggests that positive factors during pregnancy, like negative factors, are also likely to influence maternal and infant developmental outcomes.

The present study addresses the possible positive impact of maternal mindfulness during pregnancy on the offspring, as being mindful has been associated with many factors of psychological health, including increased life satisfaction, optimism and feelings of competence (for a review, see Keng *et al.*, 2011). Kohls *et al.* (2009) present a two-component model of mindfulness consisting of: (a) self-regulation of attention (i.e. the capability to focus one's thoughts and feelings at the present moment) and (b) specific state of mind (i.e. the capability of preserving a non-judgemental state of mind regarding their own experience). Being mindful has been associated with better emotion-regulation (Goodall *et al.*, 2012) and better work-family balance (Allen and Kiburz, 2012). Moreover, several studies found that mindfulness-based interventions significantly reduced anxiety symptoms in women during pregnancy (e.g., Duncan and Bardacke, 2010; Guardino *et al.*, 2014). Considering these desirable outcomes, mindfulness seems to be a useful skill, associated with many advantages for pregnant women. Recently, van den Heuvel *et al.* (2015) showed that infants prenatally exposed to higher maternal mindfulness devote less in-depth processing of irrelevant, frequently occurring sounds as measured with event-related brain potentials. While this study suggests that the advantages of mindfulness for the mother translate into advantages for the developing child in terms of auditory attention, little is known about other key aspects of child development.

An important prerequisite for later healthy psychological functioning is appropriate socio-emotional development of the child, since research indicates that early emerging socio-emotional problems often persist into childhood and preadolescence (Campbell, 1995). In addition, infant temperament is also highly relevant for subsequent healthy psychological functioning, as several studies identified continuities between infant and toddler "difficult" temperament and later psychosocial problems in late childhood (Sanson *et al.*, 1998). Socio-emotional development and temperament are two closely related constructs (Calkins *et al.*, 1996). Self-regulation, for instance, is in some theories seen as a dimension of temperament (e.g., Calkins *et al.*, 1996) but in other theories as a dimension of socio-emotional functioning (Squires *et al.*, 2001). Since we use the theory underlying the 'Ages and Stages Questionnaire: Social-Emotional', self-regulation is viewed as part of socio-emotional development in the current study. Research has shown that maternal anxiety during pregnancy is associated with both infant socio-emotional problems, e.g. deficits in emotion regulation (Bolten *et al.*, 2013), sleep problems in infancy (Gérardin *et al.*, 2011) and excessive infant crying (van der Wal *et al.*, 2007), and "difficult" infant temperament (e.g., Van den Bergh, 1990; Austin *et al.*, 2005; Davis *et al.*, 2007; Henrichs *et al.*, 2009). To the best of our knowledge, no previous study investigated the influence of dispositional mindfulness of the mother during pregnancy on infant socio-emotional development and temperament.

In the current study, we examine whether maternal mindfulness during pregnancy is positively associated with infant social-emotional functioning and temperament at age 10 months. Maternal anxiety and negative affect are associated with adverse outcomes in children (Van den Bergh, 2011) and these psychological factors are inversely related to mindfulness (Keng *et al.*, 2011). We therefore examined whether the association between maternal mindfulness during pregnancy and child outcomes is mediated by maternal anxiety. Furthermore, we explored whether sex of the child moderates this association, as several studies have found different effects of exposure to prenatal maternal stress for boys versus girls (e.g., Henrichs *et al.*, 2009; Loomans *et al.*, 2012). To this aim, the current study examined the following two hypotheses: (a) maternal mindfulness during pregnancy is negatively associated with social-emotional problems and "difficult" temperament (hypothesis 1), (b) anxiety during pregnancy mediates the association between maternal mindfulness and socio-emotional problems (hypothesis 2.1) and temperament characteristics (hypothesis 2.2).

4.2. Method

Study Design and Participants

Data were collected in an ongoing prospective cohort study following pregnant women and their offspring from the beginning of pregnancy onwards. All participating parents provided written informed consent. The Medical Ethical Committee of a local hospital approved the study.

Participants were recruited in the 15th week (*N* = 178) and between the 16th and 22nd week of pregnancy (*N* = 12) from a local hospital and four midwife practices. For the purpose of the current study, we analyzed data of those mother-infant dyads for whom information on maternal mindfulness and anxiety at 20.5 weeks of gestation (*SD* = 1.7) and on infant social-emotional development and infant temperament at 10 months after delivery (M_{age} = 9.7 months (*SD* = 1.3)) was available. Mothers provided this information via postal or digital questionnaires.

The final sample consisted of 90 mother-infant dyads. From the total sample of 190 mothers-infant dyads, one mother had a miscarriage and one infant passed away after birth, excluding two mother-infant dyads. Furthermore, we excluded dyads with extreme low birth weight of the infant (<2500 grams; *N*=16) because behavioural and emotional problems might be associated with low birth weight (McCormick *et al.*, 1996). Additionally, 82 mother-infant dyads were excluded due to missing data on maternal anxiety and mindfulness during pregnancy and/or infant outcome (Table 4.1).

Questionnaires

Maternal mindfulness. Maternal mindfulness was assessed using a Dutch translation of the Freiburg Mindfulness Inventory – Short Form (Walach *et al.*, 2006). Mothers rated 14 items (e.g., "*I am open to the experience of the moment*" and "*I observe my mistakes and difficulties without judging myself*") on a four point Likert scale (*1 = rarely to 4 = almost always*). A higher score on the FMI-s reflected higher levels of mindfulness. The FMI-s has good internal (Cronbach's α = .86; Walach *et al.*, 2006).

Maternal anxiety. Maternal anxiety during pregnancy was assessed with the anxiety subscale of the Symptom Checklist-90 (SCL-90; Arrindell and Ettema, 2003). This subscale of the SCL-90 mainly measures somatic anxiety symptoms (e.g., vegetative arousal) instead of merely psychological anxiety symptoms (e.g., anxious thoughts). Participants rated the scale, which consists of 10 items, on a five point Likert scale (*1= not at all, 2= somewhat, 3= quite, 4= quite a lot and 5= extremely*). A higher score indicates a higher level of experienced anxiety. The scale has good convergent and divergent validity and good internal consistency (Cronbach's α = .88; Arrindell and Ettema, 2003).

Infant socio-emotional development. The social-emotional development of the infants was reported by the mother with the 'Ages and Stages Questionnaires: Social-Emotional' for infants aged 9 to 14 months (ASQ:SE 12; Squires *et al.*, 2001). The questionnaire consists of 22 items with questions about socio-emotional development of the infant (e.g. "*Does your baby laugh or smile at you and other family members*?" and "*Is your baby interested in things around her, such as people, toys and food?*"). Items were rated on a three point Likert scale (i.e. 0 = *most of the time,* 5 = *sometimes,* 10 = *rarely or never*). Mothers could also indicate if the behaviour covered in the item concerned her. If this was the case, an additional 5 points were added to the total score. The ASQ:SE 12 is subdivided into the following five subscales: self-regulation problems (6 items), communication problems (4 items), adaptive functioning problems (5 items), affect problems (3 items) and interaction problems (5

Table 4.1. *Characteristics of the Participating Mother-infant Dyad Sample*

Infants (N=90)	**N**	**%**	**M (SD)**
Sex			
Girl	44	48.9	
Boy	46	51.1	
Birth weight (grams)			3495.25 (487.26)
Gestational age at birth (weeks)			39.98 (1.26)
Self-Regulation (ASQ:SE 12)	90		4.94 (6.02)
Negative Affectivity (IBQ-r)	90		37.01 (10.79)
Mothers (N=90)	**N**	**%**	**M (SD)**
Age (years)	90		32.13 (3.61)
FMI sum (prenatal)	90		40.06 (6.50)
SCL-90 sum (prenatal)	90		13.48 (3.87)
SCL-90 sum (postnatal)	90		13.27 (4.98)
Education			
Low/Medium	31	34.4	
High	59	65.6	
Smoking (during pregnancy)	0	0	
Alcoholic intake (during pregnancy)	6	6.7	
Beer	2	2.2	
Wine	4	4.4	
Liquor	1	1.1	

Note ASQ:SE = Ages and Stages Questionnaire: Social Emotional; IBQ-r = Infant Behavior Questionnaire – revised; FMI = Freiburg Mindfulness Inventory; SCL-90 = anxiety subscale of the Symptom Checklist

items). Higher scores on the subscales indicate more problems within the respective dimension of socio-emotional development. Reliability analysis of the total scale revealed a sufficient internal consistency (Cronbach's α = .67; Squires *et al.*, 2001).

Infant Temperament. Temperament of the infants was reported by the mother with the short version of the revised Infant Behaviour Questionnaire (IBQ-R; Gartstein and Rothbart, 2003). The short version of the IBQ-R measures infant temperament using 37 items, scored on a seven point Likert scale (1 = *never*, 7 = *always*), about the frequency of certain behaviours in specific situations (playing, bathing, etc.) of the previous week (e.g. '*When put into the bath water, how often did the baby smile?*'). The questionnaire consists of three subscales: surgency (13 items), negative affectivity (12 items), and effortful control (13 items). The IBQ-R has shown a good internal consistency for mothers on all scales (Cronbach's $\alpha = .88$; Gartstein and Rothbart, 2003; Parade and Leerkes, 2008).

Covariates. Based on previous research (O'Connor *et al.*, 2002; Henrichs *et al.*, 2009) on the association between maternal psychological functioning during pregnancy and infant socio-emotional and temperamental outcome we considered a number of possible confounders, including: maternal age, smoking and alcohol use during pregnancy, maternal education level, low birth weight, gestational age, infant sex and maternal postnatal anxiety. Infants with extreme low birth weight (<2500 grams) or premature birth (<36 weeks of gestation) were excluded from the study (see Participant section). Mothers reported their age, education level, and smoking and drinking behaviour during pregnancy: educational level (coded as: 0 = "low/medium" = lower vocational training or less, 1 = "high" = at least higher vocational training or a bachelor's degree) and drinking (coded as: 0 = no drinking, 1 = drinking during pregnancy). Because no mother smoked during pregnancy, controlling for this was not necessary. Information on birth weight, gestation age and infant sex was obtained from community midwife and hospital registries at birth.

Statistical analysis

Firstly, we examined which subscales of infant's socio-emotional development and temperament were associated with maternal mindfulness and anxiety during pregnancy using Pearson correlation analysis. Analysis were continued with those outcomes that showed significant (p<.05) correlations with maternal mindfulness and anxiety. Using linear regression analyses we also tested whether these associations were different for boys and girls by adding interactions between infant sex and maternal mindfulness and anxiety to the model. Subsequently, we examined whether maternal anxiety during pregnancy mediated the association between maternal mindfulness during pregnancy and infant outcomes.

For mediation analyses, we used the recently developed and highly powerful bootstrap method by using a macro for the indirect effect (INDIRECT) developed by Preacher and Hayes (2008), with 5,000 resamples and 95% confidence intervals. To account for the relatively small sample size, we used the bias-corrected bootstrap confidence interval (*CI*) because it tends to be more reliable than other methods when applied to smaller samples (Hayes and Scharkow, 2013). Next, to explore whether the mediation effect of maternal anxiety on the association of maternal mindfulness during pregnancy with infant outcomes is different for boys and girls, we tested moderated mediation models including sex as a moderator using the MODMED implementation for SPSS developed by Preacher *et al.* (2007). If moderation by sex was found significant, we computed the conditional effect for boys and girls separately using the bootstrap function in the MODMED implementation, with 5,000 resamples and 95% bias-corrected *CIs*.

To check for confounding variables we added the considered potential confounders one-by-one to the unadjusted models. Covariates were selected and included in the analyses based on the 10 % change-in-estimate criterion of Mickey and Greenland (1989), which implies that confounders were only selected if the effect size estimates of maternal mindfulness changed with 10 % or more. The retained covariates included infant sex and maternal postnatal anxiety.

All statistical analyses were performed using IBM SPSS 19.0 for Windows. *P* values lower than .05 were considered as statistical significant.

4.3. Results

Table 4.2 shows the correlations between maternal mindfulness and anxiety during pregnancy, infant socio-emotional development and the three dimensions of infant temperament. Maternal mindfulness was significantly negatively correlated with maternal anxiety (r=-.284, p<.01).

Higher maternal mindfulness during pregnancy was associated with lower scores on the 'self-regulation problems' subscale of the ASQ:SE and the 'negative affectivity' subscale of the IBQ. In contrast, higher maternal anxiety was associated with higher scores on those subscales. Moreover, higher maternal mindfulness was associated with higher scores on 'effortful control', and maternal anxiety was positively associated with 'surgency' (hypothesis 1). We did not find an interaction effect of infant sex for the association between maternal mindfulness or maternal anxiety on self-regulation problems or negative affectivity. Since maternal mindfulness and anxiety both influenced infant self-regulation and negative affectivity, the mediation analyses were only conducted for those two subscales.

Table 4.2. *Correlation Between Predictors and Outcome Variables*

Variable	2	3	4	5	6	7	8	9	10
1. FMIs	-.284**	-.273**	-.127	.068	-.034	.087	.056	-.213*	.228*
2. SCL-90		.346**	-.088	.024	.056	-.022	.247*	.419**	-.75
3. ASQ:SE: Self-Regulation problems			.277**	.004	.249*	.225*	.047	.331**	-.292**
4. ASQ:SE: Communication problems				.149	.134	.235*	-.201	-.119	-.185
5. ASQ:SE: Adaptive Functioning problems						.141	-.041	.013	.236*
6. ASQ:SE: Affect problems						.245*	-.058	.046	-.257*
7. ASQ:SE: Interaction problems							-.210	-.072	-.141
8. IBQ-r: Surgency								.236*	.199
9. IBQ-r: Negative Affectivity									-.240*
10. IBQ-r: Effortful Control									

Notes. *$p<.05$ ** $p<.01$; FMIs = Freiburg Mindfulness Inventory – Short Form; SCL-90 = Symptom Check List 90; ASQ:SE = Ages and Stages Questionnaire: Social Emotional; IBQ-r = Infant Behavior Questionnaire – revised.

The bootstrap method (Preacher and Hayes, 2008) revealed a significant mediating effect of maternal anxiety during pregnancy concerning the relation between maternal mindfulness during pregnancy and infant self-regulation problems (95 % *CI*: [-.187, -.017]). The significant association between maternal mindfulness and infant self-regulation problems (β = -.253, *p*<.01) was completely attenuated and no longer significant when adding maternal anxiety during pregnancy to the model (β = -.176, *p*>.05). After controlling for the above mentioned confounders the mediation was still significant (95 % *CI*: [-.164, -.002]) (hypothesis 2.1). Figure 4.1A illustrates the examined model.

Maternal anxiety during pregnancy also exerted a significant mediation effect (95 % *CI*: [-.307, -.008]) on the association between maternal mindfulness and infant negative affectivity. The significant association between maternal mindfulness and infant negative affectivity (β = -.360, *p*<.05) was no longer significant after adding maternal anxiety to the model (β = -.217, *p*>.05). However, after adjustment for confounders the mediation was no longer significant (95 % *CI*: [-.279, .003]). This was the case because the association between maternal mindfulness during pregnancy and infant negative affectivity was no longer significant after controlling for infant sex and maternal postnatal anxiety (hypothesis 2.2). Figure 4.1B illustrates the examined model.

Furthermore, we found a borderline significant moderating effect of infant sex (b = -.517, SE_{boot} = -.303, p = .093) on the indirect association of maternal mindfulness on self-regulation problems via maternal anxiety (i.e., moderated mediation). Post-hoc testing with the bias-corrected bootstrap *CIs* for the conditional effect of boys and girls separately showed that the indirect effect of maternal mindfulness on infant self-regulation problems via maternal anxiety was only significant for boys (95 % *CI*: [-.220, -.012]) and not for girls (95 % *CI*: [-.174, .001]). After controlling for postnatal anxiety this moderated mediation effect was still borderline significant, with a significant effect for boys (95 % *CI*: [-.195, -.010]) but not for girls (95 % *CI*: [-.153, .025]). Infant sex was found to be a non-significant moderator in the mediation model with negative affectivity.

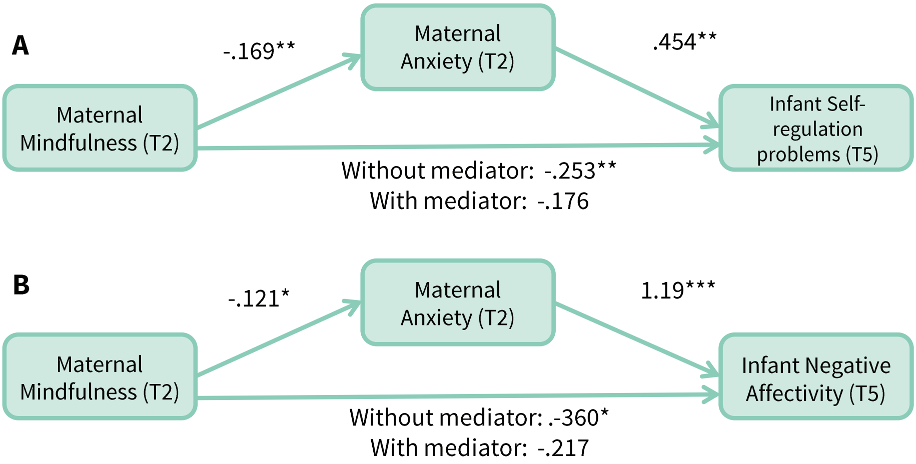

Figure 4.1. *Graphical representation of the hypotheses: (A) social-emotional problems and temperament are associated with maternal mindfulness and anxiety during pregnancy, and (B) socio-emotional problems and temperament are affected by maternal mindfulness via maternal anxiety reduction. Unstandardized path coefficients are presented. Significant coefficients are indicated by * for p < .05, ** for p < .01 and *** for p < .001.*

4.4. Discussion

The current prospective study investigated associations of maternal mindfulness during pregnancy with infant's socio-emotional development and temperament and whether maternal anxiety during pregnancy mediated these associations in a sex specific way. In line with the first hypothesis, maternal mindfulness during pregnancy was associated with less self-regulation problems, less negative affectivity and more effortful control. In contrast, maternal anxiety during pregnancy was related to more self-regulation problems, more negative affectivity, and more surgency problems. This is in line with previous research also indicating negative effects of maternal anxiety during pregnancy on offspring socio-emotional development (van der Wal *et al.*, 2007; Gérardin *et al.*, 2011; Bolten *et al.*, 2013) and temperament (Austin *et al.*, 2005; Davis *et al.*, 2007; Henrichs *et al.*, 2009). This study is the first to show that maternal mindfulness during pregnancy is associated with less mother-reported infant self-regulatory problems and less "difficult" temperament at age 10 months. When controlling for maternal postnatal anxiety and infant sex only the association between maternal mindfulness during pregnancy and infant negative affectivity became non-significant.

Mediation by maternal anxiety

Next, in line with our second hypothesis, the current study demonstrated a mediating role of maternal anxiety during pregnancy. That is, maternal anxiety during pregnancy mediated the association between maternal mindfulness and infant's self-regulation problems. These findings suggest that maternal mindfulness has a positive effect on infant self-regulation via reducing maternal anxiety during pregnancy. This notion is strengthened by previous research showing that dispositional mindfulness is associated with lower levels of anxiety (Keng *et al.*, 2011) and that mindfulness interventions during pregnancy reduce maternal anxiety and negative affect in pregnant women (Guardino *et al.*, 2014). For the association between maternal mindfulness during pregnancy and infant negative affectivity we also found a mediation effect of maternal anxiety, but this effect was non-significant after controlling for confounders (i.e., infant sex and maternal postnatal anxiety).

Moderation by infant sex

We found a moderating effect of infant sex on the indirect association of maternal mindfulness on self-regulation problems via maternal anxiety. The effect of maternal mindfulness on self-regulation problems was only mediated by maternal anxiety during pregnancy for boys, and not for girls. This finding suggests that maternal mindfulness during pregnancy influences self-regulation in infant boys via different pathways than self-regulation in infant girls. Animal research has shown that HPA-axis alterations induced by maternal stress during pregnancy may vary according to sex (e.g., Darnaudéry and Maccari, 2008). In human research, more research is required for a better understanding of the gender-specific underlying mechanisms of prenatal exposure to maternal psychosocial functioning.

Possible mechanisms

Several possible mechanisms may explain the positive effect of maternal mindfulness during pregnancy on child outcomes and its mediation via maternal anxiety. First of all, maternal mindfulness could reduce the anxiety symptoms of the mother which in turn protect the fetus against the adverse effects of in utero exposure to maternal stress (for a recent review, see Graignic-Philippe *et al.*, 2014). Although the results of the current mediation analysis seem to support the idea that maternal mindfulness reduces anxiety symptoms in the mother in a mediation model, causality should be confirmed by future intervention studies.

Furthermore, how maternal anxiety during pregnancy in its turn alters child outcome is still unclear. A yet unknown interplay of complex biological mechanisms is probably underlying this association (Van den Bergh, 2011). An often-proposed mechanism is altered HPA-axis functioning of the mother during pregnancy due to stress, resulting in fetal over-exposure to cortisol. Although cortisol is important for fetal growth, prenatal exposure to extreme levels of cortisol due to maternal anxiety can have negative consequences for early child development (e.g., Davis and Sandman, 2010). Furthermore, insights from the field of epigenetics are recently proposed as possible mechanism. Epigenetic mechanisms of gene regulation (i.e., molecular mechanisms altering the activity of genes without changing their DNA sequence) may underlie alterations in neurodevelopment seen in offspring of highly anxious pregnant women (Van den Bergh, 2011).

Another possibility may be that the reduction of maternal anxiety by maternal mindfulness during pregnancy extents into the postnatal period. As a consequence, the infant is protected from adverse environmental factors associated with postnatal maternal anxiety, such as maternal anxiety induced negative mother-infant interactions (Nicol-Harper *et al.*, 2007), lower quality of care-giving (Field, 2011), and negative family functioning (Henrichs *et al.*, 2009). Finally, genetic influences may partly explain our findings; babies of mindful mothers could have inherited this dispositional tendency and, therefore, be less vulnerable for self-regulation problems and difficult temperament.

Future research is necessary to unravel the mechanisms by which maternal mindfulness positively effects offspring's development and how maternal anxiety mediates this relation. To eliminate possible postnatal effects and genetic influences, future randomized controlled trials evaluating the efficacy of a mindfulness intervention for pregnant women are warranted.

Limitations

Finally, a number of important limitations need to be considered. First of all, maternal self-report was used to assess maternal mindfulness and anxiety during pregnancy and infant outcome. Therefore, we cannot rule out that reporter bias influenced our results since a similar distortion of maternal perception influencing her self-report of mindfulness and anxiety may influence her report of child socio-emotional development and temperament. Previous research emphasized this possibility. Nevertheless, maternal report of infant socio-emotional development and temperament may be more accurate than observers' assessments because parents know their children best and see a wide range of behaviours in different contexts (Rothbart and Bates, 1998). To eliminate maternal reporter bias, more objective measurements of infant self-regulation problems and infant temperament should be used in future research, such as behavioural observations.

Second, the sample size of the study is relatively small, which decreases the power to find small effects. Yet, the use of the bias-corrected bootstrap CI in our study might have partly overcome this issue, since it is more likely to lead to the correct decision (i.e., significant versus non-significant) than any other statistical method regardless of sample size (Hayes and Scharkow, 2013). Nevertheless, the generalizability of our results is limited due to the small sample size of the current study. Third, although we controlled for the effect of postnatal maternal anxiety, we were not able to control for the effect of postnatal maternal mindfulness since this was not measured in the current study. Finally, the results and design of the current study do not allow to completely rule out the possibility that maternal anxiety during pregnancy may concern a confounder instead of a mediator, especially since maternal mindfulness and anxiety during pregnancy were measured at the same assessment time point. Nevertheless, theoretically it seems legitimate to assume that maternal anxiety is a mediator in the association between maternal mindfulness during pregnancy and child outcomes. Previous research has shown that anxiety is related with both the predictor (mindfulness, Keng *et al.*, 2011) and the outcome (child temperament/social-emotional functioning, e.g., Mulder *et al.*, 2002; Van den Bergh, 2011; Graignic-Philippe *et al.*, 2014) and research has shown that mindfulness interventions during pregnancy reduce maternal anxiety in pregnant women (Guardino *et al.*, 2014). Moreover, when a variable meets the criteria for mediation, like maternal anxiety during pregnancy does in the current study, it cannot be considered as a confounder (Rothman *et al.*, 2008). Despite the drawbacks of the study, the results contribute to a relatively unexamined area in the DOHBaD field and might induce replication studies investigating the role of maternal mindfulness during pregnancy in the development of the infant.

Clinical implications

The results of the study, although requiring replication with more objective measures of infant outcomes and a larger sample, emphasize the potential for mindfulness interventions in both clinical and non-clinical pregnant populations. Such interventions might give pregnant women diagnosed with anxiety disorders a desired alternative to pharmacological interventions, since psychopharmacological treatments have been associated with detrimental effects for the fetus, such as abnormal sleeping patterns and increased motor activity (Mulder *et al.*, 2011) and reduced fetal head growth (El Marroun *et al.*, 2012). Moreover, stressing the beneficial effect of being mindful during pregnancy provides a more positive and possibly more effective message to pregnant women than stressing the potential dangers of being anxious. However, more research is needed investigating the effects of mindfulness during pregnancy on child development before concrete clinical recommendations can be made.

4.5. Conclusion

In conclusion, the results of the current study show that maternal mindfulness is associated with less mother-reported infant self-regulation problems and less “difficult” temperament. In addition, maternal anxiety during pregnancy mediated the relation between maternal mindfulness and infant self-regulation, but not the relation between maternal mindfulness and infant negative affectivity. Moreover, the effect of maternal mindfulness on self-regulation problems was only mediated by anxiety for boys, and not for girls. These findings stress the benefits of mindfulness during pregnancy, not only for the well-being of the mother but also and importantly for the developing child. Future randomized controlled trails with mindfulness interventions for pregnant women are needed to rule out the possible influences of postnatal and genetic factors on maternal and offspring outcome.

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Chapter 5:

Effects of prenatal exposure to maternal anxiety and mindfulness on the child's affective processing

This chapter is submitted as:

van den Heuvel, M.I., Henrichs, J., Donkers, F.C.L., & Van den Bergh, B.R.H. (under review).

Children prenatally exposed to maternal anxiety devote more attentional resources to neutral pictures

Abstract

Objective. Maternal anxiety during pregnancy can negatively affect fetal neurodevelopment, predisposing the offspring to a higher risk of behavioral and emotional problems later in life. The current study investigates the association between maternal anxiety during pregnancy and child affective picture processing using event-related brain potentials (ERPs). **Method.** Mothers reported anxiety during the second trimester using the anxiety subscale of the Symptom Checklist (SCL-90). At age 4 years, child affective picture processing (N = 86) was measured by recording ERPs during viewing of neutral, pleasant, and unpleasant pictures selected from the International Affective Pictures System. The late positive potential (LPP) – an ERP component reflecting individual differences in emotion processing – was used as child outcome. **Results.** The expected positive association between maternal anxiety and LPP amplitude for unpleasant pictures was not found. Nevertheless, we did find a positive association between maternal anxiety during pregnancy and LPP amplitudes for neutral pictures in the middle and late time window at central and anterior locations (all p<.05). These associations remained significant after adjusting for maternal postnatal anxiety and gestational age at birth. **Conclusions.** Our study provides neurophysiological evidence that children prenatally exposed to higher maternal anxiety devote more attentional resources to neutral pictures, but not to unpleasant pictures. Possibly, these children show higher vigilance for threat when viewing neutral pictures. Although useful in dangerous environments, this hypervigilance may predispose children prenatally exposed to higher maternal anxiety to developing behavioral and/or emotional problems later in life.

5.1. Introduction

Accumulating evidence shows that children prenatally exposed to maternal anxiety (PREMA) have a higher risk of developing behavioral and emotional problems later in life (Jörg Bock, Rether, Gröger, Xie, & Braun, 2014; Bolten et al., 2013; Van den Bergh, 2011). Experimental animal studies and natural experiments in humans suggest that prenatal exposure to maternal anxiety can elicit modulation of "*developmental programming*" in the offspring (Bock, Wainstock, Braun, & Segal, 2015; Daskalakis, Bagot, Parker, Vinkers, & de Kloet, 2013; de Kloet, Joels, & Holsboer, 2005; Laplante, Brunet, Schmitz, Ciampi, & King, 2008). Although potentially underlying mechanisms are not fully understood, it has frequently been proposed that maternal anxiety leads to increased production of maternal stress hormones (e.g., cortisol, noradrenaline) and to down-regulation of the placental 11β-HSD2 enzyme that metabolizes maternal cortisol into inactive cortisone (O'Donnell et al., 2012; Rakers et al., in press). This results in increased levels of cortisol in the fetal circulation which may elicit long-term changes in structure and function of the developing brain via epigenetic pathways (Bock et al., 2015; Monk, Spicer, & Champagne, 2012; Van den Bergh, 2011).

In this way, cues in the intrauterine environment (e.g., elevated cortisol levels) may guide adaptation of the offspring phenotype to the expected environment in order to increase subsequent chances of survival ("*developmental plasticity*") (Del Giudice, 2014; Godfrey, Lillycrop, Burdge, Gluckman, & Hanson, 2007). A 'mismatch' with the postnatal environment can occur, however, when the cues were incorrect or the environment changes quickly, resulting in a maladaptive phenotype predisposing the child for later onset of behavioral and emotional problems ("*mismatch hypothesis*") (Frankenhuis & Del Giudice, 2012; Gluckman & Hanson, 2006).

The fetal brain may be especially sensitive to environmental influences, since it develops very rapidly during pregnancy (Bock et al., 2015; Fox, Levitt, & Nelson, 2010; Knudsen, 2004). Animal research has clearly demonstrated that prenatal exposure to maternal stress affects the offspring's brain, with most prominent effects shown in the limbic system (e.g., hippocampus, amygdala, corpus callosum, and hypothalamus) and prefrontal cortex (for a review, see Charil, Laplante, Vaillancourt, & King, 2010). Recently, structural alterations in the brain of human offspring prenatally exposed to higher levels of maternal cortisol and/or anxiety are also being examined (Buss, Davis, Muftuler, Head, & Sandman, 2010; Buss et al., 2012). In line with animal studies, the affected brain regions are mostly involved in emotion processing (i.e., amygdala, hippocampus, rostral anterior cingulate cortex). Recently, diffusion tension imaging (DTI) in PREMA newborns, also showed changes is neuronal microstructure in brain pathways important for emotional functioning (Qiu, Tuan, et al., 2015; Rifkin-Graboi et al., 2015).

In the context of developmental plasticity, these alterations in brain structure can be seen as adaptations to prepare the offspring for the predicted dangerous environment (Del Giudice, 2014; Glover, 2011; Gluckman & Hanson, 2007). Changes in the function and structure of the limbic system may, for instance, lead to increased attention allocation to threatening/fearful stimuli in order to facilitate faster fight or flight response of the child, increasing its survival in dangerous environments. Studies demonstrating increased behavioral and physiological reactivity in PREMA children seem in line with this notion (Braeken et al., 2013; de Weerth, Buitelaar, & Beijers, 2013; Henrichs et al., 2009; Monk et al., 2004). Studies that directly examined processing of threatening/fearful stimuli in PREMA children are very sparse, however.

To our knowledge, our group was the first to investigate this (Otte, Donkers, Braeken, & Van den Bergh, 2015). For this purpose, infants' processing of emotional (fearful and happy) face/voice compounds was measured in an event-related potential (ERP) study at nine months of age. The results showed that maternal anxiety during pregnancy was associated with altered infants' brain responses to fearful vocalizations, irrespective of the emotion in the visual prime (fearful or happy). In line with the theory of increased allocation to threatening/fearful stimuli, these results suggest that PREMA infants process threat-related (fearful) auditory stimuli more extensively. Nevertheless, Otte et al. (2015) found no association between maternal anxiety during pregnancy and infants' brain responses to fearful *visual* stimuli, indicating that PREMA infants may not display more threat-related responses when observing visual stimuli than infants not exposed to higher maternal anxiety. Possibly, visual effects may only be found in older children since the auditory cortex matures more rapidly during early development than visual cortex (Anderson & Thomason, 2013). In addition, newborn infants have relatively good auditory acuity (Lary, Briassoulis, de Vries, Dubowitz, & Dubowitz, 1985; Starr, Amlie, Martin, & Sanders, 1977) as well as several months of prenatal auditory experience (Lecanuet & Schaal, 1996), while visual acuity is poor at birth and prenatal exposure to visual stimuli very limited (Volpe, 2001). In addition, it is unclear whether more extensive processing of threatening/fearful stimuli in association with PREMA is also present in early childhood. In the current study, we therefore investigated the response to emotional stimuli in the visual domain in PREMA children at the age of four.

To examine affective processing in these children we adapted the paradigm described by Solomon, DeCicco, and Dennis (2012), focussing on the late positive potential (LPP) – a commonly-used event-related potential (ERP) component to study emotional processing of visual stimuli (Moran, Jendrusina, & Moser, 2013). The LPP is a slow positive waveform that develops approximately 300–400 ms post-stimulus and increases in amplitude in response to more salient pictures, such as emotional pictures (Kujawa, Klein, & Proudfit, 2013). In addition, Solomon et al. (2012) found that larger LPP amplitude differences between unpleasant and neutral stimuli were associated with greater observed fear in 5-7 year old children. The LPP has a good internal consistency (Moran et al., 2013), is stable over development (Kujawa et al., 2013), and is detectable in children as young as four years of age (Hua et al., 2014).

The current study examined the association between prenatal exposure to maternal anxiety and child affective picture processing using event-related brain potentials in four-year-olds. Based on Solomon et al. (2012) and previous PREMA research (Braeken et al., 2013; de Weerth et al., 2013; Henrichs et al., 2009; Catherine Monk et al., 2004) we hypothesized that prenatal exposure to higher levels of maternal anxiety was associated with higher LPP amplitude differences between unpleasant pictures and neutral pictures.

5.2. Methods and Materials

Participants and study design

The present study was a follow-up study of the Prenatal Early Life Stress (PELS) study – a prospective cohort study examining the effect of maternal prenatal stress on child development, following pregnant women and their children from the first trimester of pregnancy onward. All participating mothers and partners provided informed consent. The study was approved by the medical ethical committee of the St. Elisabeth Hospital in Tilburg, The Netherlands, and was conducted in full compliance with the Helsinki declaration.

We recruited a total of 190 pregnant women during early to mid-pregnancy from four midwife practices and a general hospital in the area of Tilburg, The Netherlands. For the current study, we analyzed the data of those mother-child dyads that had complete maternal questionnaire data during mid-pregnancy and child ERP data during the follow-up measurement at age 4 years. From the 103 children aged 4 years that underwent the EEG-measurement, 17 children were excluded due to either: missing maternal questionnaire data (n = 4), technical problems (n = 4), fussiness/tiredness/boredom (n = 5), low number of artifact free trials (<20 trials) (n = 3), and due to cortical visual impairment (n = 1).

The 86 children (44 girls) included in the current study had a mean age of 48.0 ± 0.8 months. On average, their mothers were aged 31.9 years ± 3.7 during the prenatal assessment. Table 5.1 shows the baseline characteristics of mothers and children included in the current study.

Table 5.1. *Characteristics of the participating mother-child dyad sample*

Children (N=86)	**N**	**%**	**M ±SD**
Age at EEG-measurement (months)	86		48.0 ±0.8
Gender			
Girl	44	51.2	
Boy	42	48.8	
Mothers (N=85)	**N**	**%**	**M ±SD**
Age (years)	86		31.9 ±3.7
Anxiety - Prenatal	86		12.6 ±2.8[b]
Anxiety - Postnatal	82[a]		13.3 ±4.7[b]

Note [a] N = 4 mothers included in the study did not complete the postnatal questionnaire; [b] In a Dutch population sample, scores between 12 and 14 are considered 'mean anxiety', scores between 15 and 22 are considered "above average and high anxiety" and scores of 22 and higher as "extremely high anxiety" (47).

Measures

Maternal anxiety. Maternal anxiety was measured at 21.1 ± 1.9 weeks of pregnancy and at age 4 years using the Dutch version of the anxiety subscale of the Symptom Checklist (SCL-90 (Arrindell & Ettema, 2003)) – a self-report measure of anxiety symptoms, consisting of 10 items with five point Likert scales ranging from 0 (*not at all*) to 4 (*extremely*). Higher sum scores indicate higher anxiety. The scale has good convergent and divergent validity and has good internal consistency (α=.88 for the anxiety subscale (Arrindell & Ettema, 2003)). In the current study, the internal consistency also proved to be good (prenatal: α=.73; at age 4 years: α=.92).

Affective picture processing. At age 4 years, child emotion processing was measured by recording ERPs during viewing of emotional pictures. Stimulus selection and procedure were based on the study of Solomon et al. (2012) with few alterations due to the younger age of the current sample[2]. The stimuli consisted of 90 developmentally appropriate pictures all taken from the International Affective Picture System (IAPS; Lang, Bradley, & Cuthbert, 2008): 30 neutral pictures[3] depicting pictures such as household objects or nature scenes, 30 pleasant pictures[4] depicting pictures such as candy and happy scenes, and 30 unpleasant pictures[5] depicting pictures of accidents and scary animals.

The emotion processing task was administered using E-Prime software version 2.0.8.74 (Psychology Software Tools, Pittsburgh, PA). The pictures were all presented full screen (1280 by 1024 display resolution) in color on a 19 inch (48.26 cm) CRT monitor. The assessment of this task was embedded in a 2 hour follow-up assessment at age 4 years (including a break and electrode placement) that took place in a dimly lit and sound-attenuated room in the laboratory. Children were seated approximately 75 cm from the computer screen and were motivated to participate by giving them a sticker after each completed measure and a small toy of choice after the experiment was over. During the emotion processing task the experimenter was always sitting next to the child. The pictures were randomly selected and presented on the screen for 2000 ms with a 500 ms inter stimulus interval. The experiment was videotaped with two cameras (one in front and one behind the child). The videos were used to score whether the child was looking at the pictures and was attentive (e.g., not fussy, bored or too tired). Scoring of the video was

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[2] The following changes to the selection of pictures of Solomon et al. (2012) were made: we replaced picture #5970 (a tornado) with picture #9600 (sinking boat), because Dutch children are not familiar with tornados like American children, and picture #9490 (burned corpse) with picture #8485 (fire accident, without corpse), because the corpse was not deemed age-appropriate for our younger sample.

[3] The IAPS numbers for neutral pictures were 5220, 5711, 5740, 5750, 5800, 5820, 7000, 7002, 7004, 7006, 7009, 7010, 7025, 7031, 7035, 7041, 7050, 7080, 7090, 7100, 7140, 7150, 7175, 7190, 7224, 7233, 7235, 7236, 7595 and 7950.

[4] The IAPS numbers for pleasant pictures were 1460, 1463, 1601, 1610, 1710, 1750, 1811, 1920, 1999, 2070, 2091, 2165, 2224, 2311, 2340, 2345, 2791, 4603, 5831, 7325, 7330, 7400, 7502, 8031, 8330, 8380, 8461, 8490, 8496, and 8620.

[5] The IAPS numbers for unpleasant pictures were 1050, 1120, 1201, 1300, 1321, 1930, 2120, 2130, 2688, 2780, 2810, 2900, 3022, 3230, 3280, 5970, 6190, 6300, 6370, 7380, 9050, 9250, 9421, 9470, 9480, 9490, 9582, 9594, 9600, and 9611.

done afterwards by trained research assistants.

ERP measurement and data processing

EEG was recorded with BioSemi ActiveTwo amplifiers (www.biosemi.com) with a sampling rate of 512 Hz. We used 64-electrode caps placed according to the extended International 10-20 system. The standard BioSemi reference (CMS-DRL) was used for online recording (see www.biosemi.com/faq/cms&drl.htm for details) and two additional electrodes were placed on the left and right mastoids, respectively and mathematically combined off-line to produce an average mastoids reference derivation. Electrooculogram (EOG) was recorded using four electrodes: horizontal EOG was recorded from one electrode placed ±1 cm next to the left and another next to the right eye. Vertical EOG was recorded from one electrode placed ±1 cm above and one below the left eye. BrainVision Analyzer 2 (Brain Products, Munich, Germany) and MATLAB (version R2012b, The Mathworks, Inc.) based EEGLAB (version 13.0.1 (Delorme & Makeig, 2004)) software packages were used to analyze the EEG data. Data were processed in accordance with Solomon et al. (2012) except for artifact rejection criteria – due to our younger sample we used more liberal settings for artifact rejection. In addition, due to extensive artifacts in our EOG data (i.e., many four-year-olds touched the EOG electrodes or even pulled them off during recording), the EEG signal was corrected for blinks and eye movements using independent component analysis (ICA) as implemented in EEGLAB. The data were filtered offline with a zero-phase Butterworth bandpass 0.1 – 30 Hz (slope 24 dB) filter. Subsequently, the data were segmented into epochs of 2400 ms duration including a 400 ms pre-stimulus period. Epochs with a voltage change exceeding 200 μV within a sliding time window of 200 ms duration, with changes exceeding the speed of 75 μV/ms or with activity lower than 0.2 μV per 100 ms were excluded from analysis. Children with less than 20 artifact free trials were excluded from analysis. The average number of remaining trials included in the analyses for the three stimulus types were as follows: neutral: 25; pleasant: 26; unpleasant: 25.

ERPs were constructed by averaging the signal for each stimulus type (neutral, pleasant, unpleasant) and baseline-corrected to the average voltage in the 400 ms pre-stimulus period. Subsequently, mean LPP amplitudes were exported to SPSS for three time windows (cf. Solomon et al., 2012): early (300–700 ms), middle (700–1200 ms) and late (1200–2000 ms). We averaged the LPP over three regions: posterior (PO4, PO8, O2, Oz, POz, PO3, PO7, and O1), central

(C4, C6, CP6, Cz, CPz, C3, C5, and CP5), and anterior (FC4, F4, F6, Fpz, AFz, FC3, F3, and F5).

Statistical analysis

To assess the validity of our main outcome measure, we first examined whether we could replicate previous studies using an almost identical child emotional processing paradigm (Hajcak & Dennis, 2009; Hua et al., 2014; Solomon et al., 2012). More specifically, we examined whether the LPP amplitude for emotional pictures (pleasant and unpleasant) was larger than the LPP for neutral pictures. In correspondence with Solomon et al. (2012), analyses were conducted separately for each region using a 3 (picture type: unpleasant, pleasant, neutral) × 3 (window: early, middle, late) × 2 (gender) repeated measures ANOVA. Greenhouse-Geisser correction was applied where appropriate (ε correction factors reported). Post-hoc tests of main effects were adjusted for multiple testing using Bonferoni correction.

To examine the association of maternal anxiety during pregnancy with child LPP amplitude difference (neutral-unpleasant and neutral-pleasant) we first checked whether the LPP response to neutral pictures was not affected by our predictor (maternal anxiety during pregnancy). If this was the case, we planned to conduct Pearson's correlations between the LPP differences scores and our predictors. If the LPP amplitude to neutral pictures was found to be affected by our predictor, no differences scores would be computed. In this case, we planned to compute correlations between the LPP amplitudes and our predictors separately for the three types of stimuli (neutral, unpleasant, pleasant).

Next, we statistically controlled the significant correlations for maternal anxiety at age 4 years using multiple regression analysis. This was done to control for possible maternal psychosocial influences during early childhood. Gestational age at birth was also entered as a covariate, because previous studies showed effects of gestational age on cognitive functioning (Espel, Glynn, Sandman, & Davis, 2014) and brain development (Davis et al., 2011) in young children.

The LPP and maternal reported psychological ratings were statistically evaluated using IBM SPSS 19.0 for Windows. All significant results are reported together with the partial η^2 effect size values; $\alpha = .05$.

5.3. Results

Emotional processing in four-year-olds

Figure 5.1 presents the stimulus-locked ERPs in response to the three types of pictures at posterior, central and anterior recording sites. Our results replicated previous studies (Hajcak & Dennis, 2009; Hua et al., 2014; Solomon et al., 2012), indicating that the task used in our study is a valid measure to examine differences in emotional processing in four-year-olds. To ease interpretation, the results are reported separately for each region.

Posterior region. For the posterior electrodes sites, the LPP varied by window, *F*(2,168) = 95.153, p = .000, η^2 = .531, ε = .662, and picture type, *F*(2,168) = 23.581, p = .000, η^2 = .219. Interactions were found between window and picture type, *F*(4,336) = 6.868, p = .000, η^2 = .076, ε = .738 and between window and gender, *F*(2,168) = 3.861, p = .040, η^2 = .044, ε = .662. Post-hoc tests for the main effect of window showed that the LPP amplitude was smaller (i.e., less positive) in the late window than in the middle and early window and smaller in the middle than in the early window, early>middle>late, all $p < .001$, correction for multiple testing. Post-hoc tests for the main effect of picture type revealed that both emotional pictures elicited higher LPP amplitudes than the neutral pictures, both $p < .001$, correction for multiple testing. No significant difference in LPP amplitude was found between the pleasant and unpleasant pictures. Post-hoc analysis for the interaction between window and picture type showed that in the early window the LPP elicited by pleasant pictures was significantly larger than for neutral pictures ($p = .041$), while this was not the case for the middle and late window. However, after correction for multiple testing the difference was no longer significant. Post-hoc tests for the interaction effect between window and gender did not reveal any gender differences for the LPP amplitude as a function of time window. This might indicate that the interaction effect is merely a false positive (type 1 error). No significant main effect of gender was found.

Central region. For the central electrode sites, the LPP amplitude varied by window, *F*(2,168) = 635.601, p = .000, η^2 = .882, ε = .727. We also found a significant interaction between window and picture type, *F*(4,336) = 5.747, p = .001, η^2 = .064, ε = .737. Post-hoc tests for the main effect of window showed that the LPP amplitude was smaller (i.e., less negative) in the late window than in the middle and early window and smaller in the middle than in the early window, early>middle>late, all $p < .001$, correction for multiple testing. Post-hoc tests for the interaction between window and picture type showed

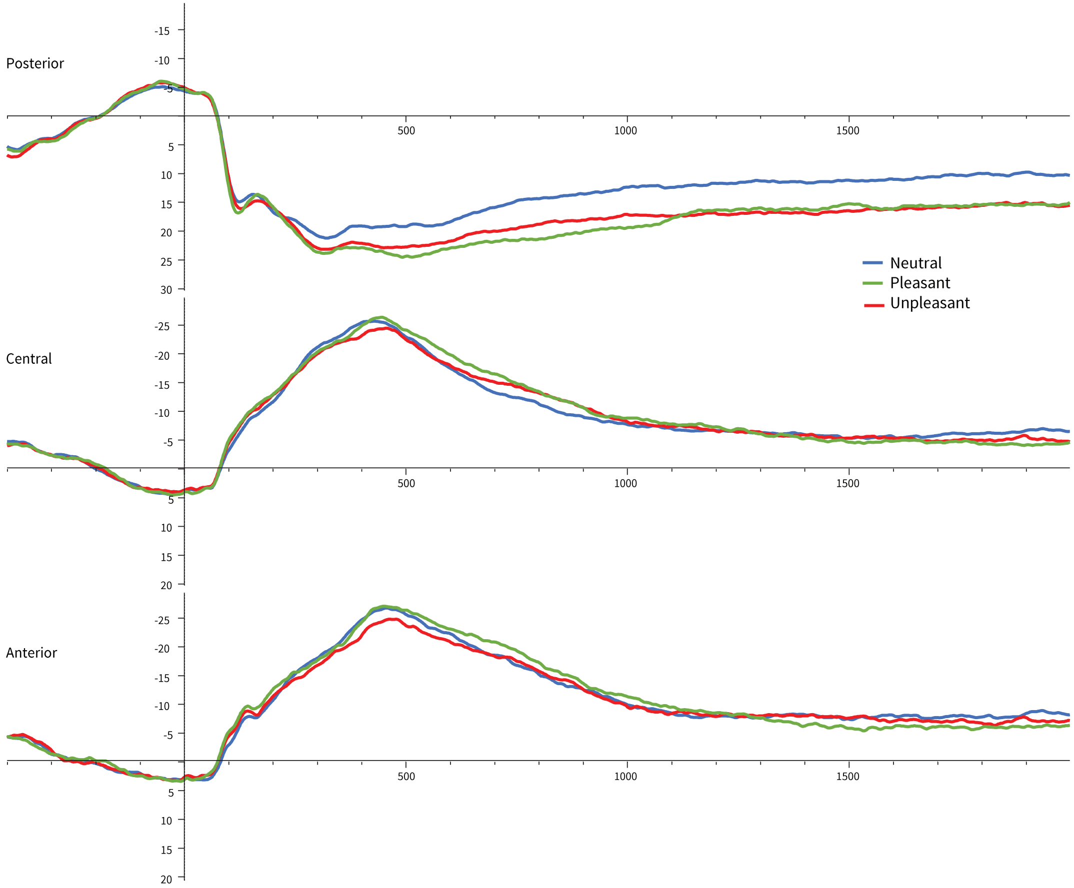

Figure 5.1. *Group-average (N=86) LPP amplitudes elicited by pleasant (green line), unpleasant (red line) and neutral (blue line) pictures.*

that in the middle window the LPP amplitude elicited by pleasant pictures was significantly larger than for neutral pictures (*p* = .034). However, after correction for multiple testing the difference was no longer significant. No significant main effects of picture type and gender were found.

Anterior region. For the anterior electrode sites, the LPP amplitude again varied by window, *F*(2,168) = 408.663, p = .000, η^2 = .829, ε = .717. The interactions between window and picture type, *F*(4, 336) = 5.352, p = .001, η^2 = .060, ε = .828, and window and gender, *F*(2,168) = 5.308, p = .013, η^2 = .059, ε = .717 also reached significance. Post-hoc testing revealed that the LPP amplitude was smaller (i.e., less negative) in the late window than in the middle and early window and smaller in the middle than in the early window, early>middle>late, all *p* < .001, correction for multiple testing. Post-hoc analysis showed that in the early window the LPP elicited by pleasant pictures was larger than for unpleasant pictures on a trend level, *p* = .071. However, after correction for multiple testing the difference was no longer significant. Post-hoc analysis for the interaction effect of window and gender showed that in the early window girls showed a higher LPP amplitude to pleasant pictures than to neutral, *p* = .024, and unpleasant, *p* = .011, pictures. After correction for multiple testing only the higher LPP amplitude for girls to pleasant versus unpleasant pictures remained significant. There were no significant main effects of gender or picture type.

Associations between maternal anxiety during pregnancy and child LPP amplitudes

Since we did observe significant correlations between the LPP amplitudes in response to the neutral stimuli and maternal anxiety (see below) we conducted our analysis with the LPP amplitudes separately for the three types of stimuli, instead of using difference scores. The correlations of maternal anxiety during pregnancy and child LPP amplitudes for the posterior, central and anterior region are presented in Table 5.2.

To ease interpretation, the results are reported separately for each region.

Posterior region. Maternal anxiety during pregnancy was not associated with child LPP amplitudes in the posterior region.

Central region. Maternal anxiety during pregnancy was not associated with child LPP amplitudes in response to unpleasant pictures in the central region. We found a negative association between maternal anxiety during

Table 5.2. *Pearson's correlations between LPP for posterior, central, and anterior region and prenatal maternal anxiety.*

Picture type	Window	Prenatal maternal anxiety		
		Posterior region	Central region	Anterior region
Pleasant	Early	-.076	-.206	-.127
	Middle	-.023	-.193	-.108
	Late	.014	-.062	.042
Unpleasant	Early	-.034	-.092	-.066
	Middle	.034	-.086	-.117
	Late	.004	-.002	.040
Neutral	Early	-.005	-.084	-.064
	Middle	-.044	-.242*	-.243*
	Late	-.103	-.272*	-.272*

Notes. **p*<.05*

pregnancy and the LPP elicited by the neutral pictures in the middle and late time window, indicating that higher maternal anxiety during pregnancy was associated with higher LPP amplitudes (i.e., more negative). The association remained significant after controlling for gestational age and maternal anxiety at age 4 years. A graphical representation of the correlations is presented in Figure 5.2A. To illustrate the difference in child LPP amplitude elicited by the neutral pictures for high and low maternal anxiety, we used a cutoff score for high maternal anxiety (sum score = 15), taken from the average of the normal Dutch population (Arrindell & Ettema, 2003).

Anterior region. Maternal anxiety during pregnancy was not associated with child LPP amplitudes in response to unpleasant pictures in the anterior region. Similar to the results in the central region, we observed negative associations between maternal anxiety during pregnancy and the LPP amplitude elicited by the neutral pictures in the middle and late time window. These associations indicate that higher maternal anxiety during pregnancy was associated with higher LPP amplitudes (i.e., more negative) for the neutral pictures. Both associations remained significant after controlling for gestational and maternal anxiety at age 4 years. A graphical representation of the correlations is presented in Figure 5.2B.

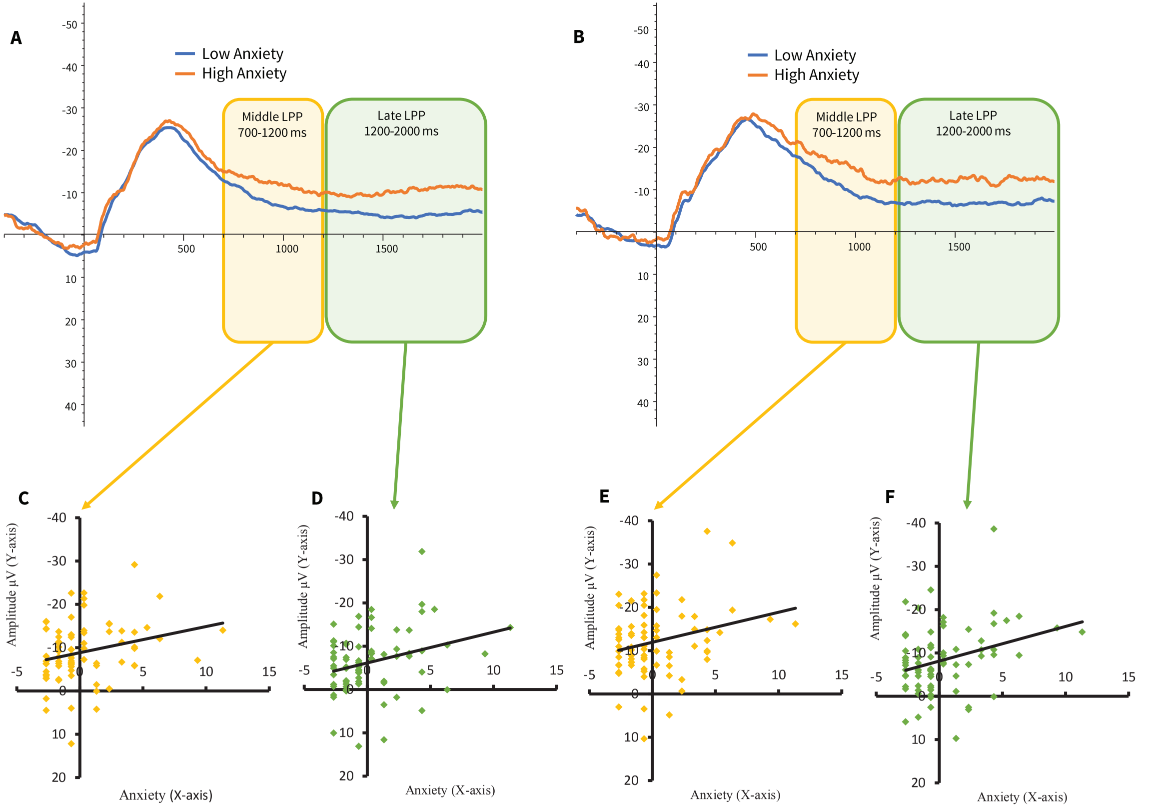

Figure 5.2. *Group-average (N=86) LPP amplitudes to the neutral pictures of children exposed low (blue line) and high (orange line) anxiety, for the central electrode sides (**A**) and the anterior electrode sides (**B**). The scatterplots shows the correlation between maternal anxiety and the LPP amplitudes for the middle and late windows, for the central electrode sides (**C** and **D**, respectively) and the anterior electrode sides (**E** and **F**, respectively). **Notes**: The statistical analyses were performed with anxiety as continuous predictors (**C**, **D**, **E** and **F**). Panels **A** and **B** are for illustration purposes, only.*

5.4. Discussion

The current study investigated the association between maternal anxiety during pregnancy and child emotion processing using event-related brain potentials (ERPs) during exposure to visual stimuli. In contrast to what we expected, maternal anxiety during pregnancy was not associated with LPP amplitudes to unpleasant pictures. We did find significant associations between maternal anxiety and LPP amplitudes in response to the neutral pictures, however. Specifically, we found that higher maternal anxiety during pregnancy was associated with higher LPP amplitudes to neutral pictures in the middle and late time window at the central and anterior electrode locations. Importantly, the results remained significant after controlling for gestational age at birth and maternal anxiety when the child was 4 years old. Taken together, our study provides neurophysiological evidence that children prenatally exposed to maternal anxiety seem to devote more attentional resources to neutral pictures, but not to unpleasant pictures.

Our findings are in line with our previous studies following the current birth cohort, showing that PREMA infants aged 9 months displayed a higher ERP response to neutral sounds in an oddball paradigm (van den Heuvel, Donkers, Winkler, Otte, & Van den Bergh, 2015) and reporting no association between maternal anxiety during pregnancy and infant ERP amplitude to unpleasant pictures (fearful faces) in infants (Otte et al., 2015). Nevertheless, Otte et al. (2015) also reported associations between prenatal exposure to higher levels of maternal anxiety in infants and higher ERP responses to fearful sounds. An explanation for this inconsistency might be that the differences in threat level of the stimuli used in the studies resulted in different outcomes. Previous research on threat processing has demonstrated that when threat exceeds a certain threshold, it captures attention in everyone regardless of anxiety level of the participant (Mogg & Bradley, 1998; Wilson & MacLeod, 2003). It might therefore be that the unpleasant pictures used in the current study and the fearful faces used in (Otte et al., 2015) exceeded the threshold for threat and therefore captured high attention in all infants/children, while the fearful sounds used in Otte et al. (2015) did not the exceed this threshold and hence were experienced as only mildly threatening. However, future studies using ambiguous or mild versus high threat stimuli are needed to test this hypothesis.

Taking the previous and current findings together, it may be the case that the heightened reactivity reported in PREMA offspring (Braeken et al., 2013;

de Weerth et al., 2013; Henrichs et al., 2009; Monk et al., 2004) does not result in more attention allocation to threatening visual stimuli, as was first expected, but instead, may result in more attention allocation to neutral (or ambiguous) visual stimuli. This could be due to PREMA offspring being more vigilant, resulting in a lower threshold for (unconscious) threat appraisal when viewing neutral pictures (Kimble et al., 2014; Weymar, Keil, & Hamm, 2014). Interestingly, the brain regions identified as key structures for (hyper)vigilance and threat perception, i.e., the amygdala, hippocampus, and prefrontal cortex (Dejean et al., 2015; Fox, Oler, Tromp, Fudge, & Kalin, 2015; Lipka, Miltner, & Straube, 2011; Whalen, 1998) are also reported to be affected by prenatal exposure to maternal anxiety (Anne Rifkin-Graboi et al., 2015), maternal depression (Qiu, Anh, et al., 2015; Rifkin-Graboi et al., 2013), and early life stress (Grant et al., 2015; Malter Cohen et al., 2014; Thomason et al., 2015). In a dangerous environment, the benefits of detecting real threat in a seemingly neutral situation may outweigh the costs of misinterpreting a neutral situation as threatening. Although potentially beneficial in certain environments, hypervigilance may ultimately lead to higher risk for developing behavioral and emotional disorders later in life, especially when the postnatal environment does not match the expected (Del Giudice, 2014; Frankenhuis & Del Giudice, 2012; Glover, 2011). Interestingly, previous research found that vulnerability to anxiety primarily stems from a lower threshold for appraising threat, rather than an attention bias to threatening stimuli (Mogg et al., 2000). Building on this, it is tempting to speculate that evaluating relatively innocuous stimuli as having higher subjective threat may place PREMA children at a higher risk for developing internalizing problems later in life (e.g., (Van den Bergh & Marcoen, 2004; Van den Bergh, Van Calster, Smits, Van Huffel, & Lagae, 2007)).

Strengths and limitations

The choice of our stimuli, pictures of scenes, constitutes both strengths and limitations for the current study. An important strength is our inclusion of neutral pictures, as previous work focusing on neurophysiological markers of affective processing in PREMA offspring only included fearful and happy stimuli (Otte et al., 2015). The results of our study emphasize the importance of the inclusion of neutral stimuli for future research. A possible drawback of the choice for unpleasant scenes as ‘fearful stimuli’ might be the diversity of the scenes depicted; some show scary animals while others show sad, angry or scared children. Some of the pictures may not elicit a bias to threatening stimuli. In addition, although previous studies validated the emotional and neutral pictures used in this study in terms of valance and arousal levels (Hajcak & Dennis, 2009), the lack of information on subjective ratings from the current cohort is a limitation. This information would have allowed us to test whether, e.g., PREMA children reported higher arousal for neutral pictures than non-PREMA children.

The design of the current study does not allow for ruling out that shared genetic factors may partly explain the association between maternal anxiety during pregnancy and child neurophysiological responses to neutral pictures. Yet, human studies evaluating the consequences of stressful life events on development, such as natural disasters, suggest that the effects of prenatal stress cannot be explained by genetic predispositions alone (Laplante et al., 2004). Although we controlled for postnatal maternal anxiety, other postnatal factors (e.g., impaired parent-child interactions) may also account for the association between maternal anxiety during pregnancy and child outcome. Future randomized controlled trials testing the efficacy of stress-reduction interventions on maternal well-being and child outcome are needed to show whether maternal anxiety during pregnancy indeed exerts intrauterine effects on offspring development independent of postnatal environmental factors and genetic influences.

5.5. Conclusion

Our study provides neurophysiological evidence that children prenatally exposed to higher maternal anxiety devote more attentional resources to neutral pictures, but not to unpleasant pictures (as was initially expected). These results provide new insights into the functional alterations in PREMA children and emphasize the need to include neutral stimuli, in addition to negative/fearful stimuli, in the study of the effects of prenatal programming on offspring (neuro)behavioral outcome. Our results may indicate that PREMA children display higher vigilance, possibly resulting in a lower threshold for appraising threat in seemingly neutral stimuli. Future research using different stimuli (i.e., ambiguous versus neutral, mild versus high threat, auditory versus visual) should validate this tentative conclusion. Although useful in dangerous environments, hypervigilance may predispose PREMA children to higher risk for developing behavioral and/or emotional problems later in life.

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Addendum

For the purpose of the second aim of this dissertation, we performed additional analyses to examine the effect of maternal mindfulness during pregnancy on affective processing in children. To this aim, maternal mindfulness was measured at 21.1 ± 1.9 weeks of pregnancy and at age 4 years using the Dutch short version of the Freiburg Mindfulness Inventory (FMIs-14; Walach *et al.*, 2006). The FMIs-14 consists of 14 items with four point Likert scales ranging from 1 (*rarely*) to 4 (*almost always*). Higher sum scores indicate higher mindfulness. The FMIs-14 shows good internal consistency (α=.86; Walach *et al.*, 2006). In the current study, the internal consistency also proved to be good (prenatal: α=.83; at age 4 years: α=.88).

We found negative correlations between maternal mindfulness during pregnancy and LPP amplitudes for the pleasant and unpleasant pictures in the posterior window for all windows, $p < .05$ and for the neutral pictures for the early window, $p < .05$. When controlling for maternal mindfulness at age 4 years and gestational age, however, maternal mindfulness during pregnancy was no longer associated with child LPP amplitudes in the posterior region. Maternal anxiety during pregnancy was not associated with child LPP amplitudes in the posterior region. Maternal mindfulness during pregnancy was not associated with child LPP amplitudes in the central and anterior regions.

Contrary to previous research addressing the effect of maternal mindfulness during pregnancy on neurophysiological functioning in infancy (van den Heuvel *et al.*, 2015), we found that maternal mindfulness during pregnancy was not independently associated with child LPP amplitudes at age 4 years. That is, associations between maternal mindfulness during pregnancy and smaller child LPP amplitudes were no longer significant after controlling for postnatal maternal mindfulness. This may indicate that maternal mindfulness has no specific prenatal effects, but exerts its influence (also) after birth.

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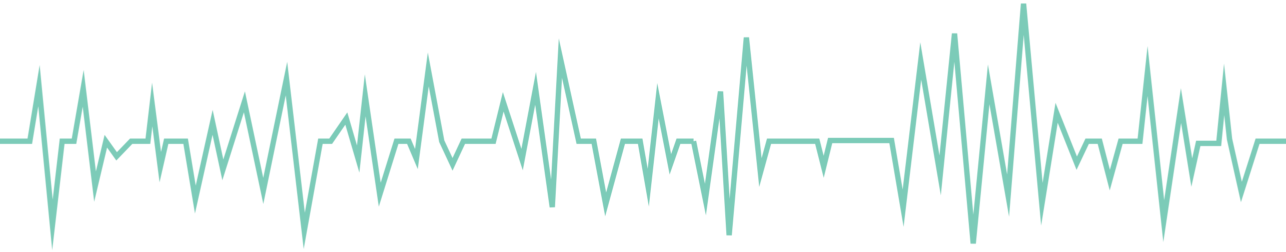
Chapter 6:

Effects of prenatal exposure to maternal anxiety and mindfulness on infant functional brain network connectivity



van den Heuvel, M.I., Donkers, F.C., Hillebrand, A., & Van den Bergh, B.R.H. (in preperation).

Abstract

Background. Maternal anxiety during pregnancy can have negative effects on offspring neurodevelopmental functioning. How functional brain networks are related to these neurodevelopmental changes is not well understood. We hypothesized that the brain network of infants prenatally exposed to higher levels of maternal anxiety would show a less integrated network configuration and explored sex differences. **Method.** EEG-recordings from 59 infants (32 girls) were obtained during a passive auditory oddball paradigm. Functional connectivity measures in the infant alpha band (6-9 Hz) and in broad band (1-30 Hz) were computed using the Phase Lag Index (PLI). Brain network organization was characterized using minimum spanning tree (MST) indices – a graph theoretical approach that avoids biases. Mothers reported anxiety using the Symptom Checklist and mindfulness using the Freiburg Mindfulness Inventory at a mean gestational age of 21 week. **Results.** No significant associations were found between maternal anxiety and mindfulness and infant MST indices. **Conclusion.** We found some indications for an effect of prenatal exposure to maternal anxiety on infant brain network topology, but all significant findings became non-significant after statistically controlling for maternal postnatal anxiety. This might indicate that prenatal exposure to maternal anxiety and mindfulness has no effect on the infants' brain network configuration measured, but methodological issues may have decreased our power to find genuine effects. Future research should replicate our study including more infants and a better paradigm to measure child brain functional network configuration.

6.1. Introduction

During the prenatal life period the brain is subject to dramatic developmental processes. As a result, this period represents a phase of high susceptibility to both positive and negative environmental influences (Andersen, 2003; Van den Bergh, 2011; Anderson and Thomason, 2013; Bock *et al.*, 2015). Altered maternal environmental input during this sensitive time period may have lasting effects on the brain and impact later neurocognitive development. Preclinical research has consistently shown that maternal glucocorticoids pass the placenta and alter the development of the fetal brain and the hypothalamic-pituitary-adrenal (HPA) axis and produce permanent changes in the immune, metabolic and behavioral systems (Coe and Lubach, 2008; Harris and Seckl, 2011; de Kloet *et al.*, 2014). These changes likely reflect the effect of epigenetic changes to the fetal genome (Monk *et al.*, 2012). In humans, high maternal anxiety during pregnancy has been associated with altered offspring neurodevelopmental outcomes, such as altered endogenous cognitive control (Mennes *et al.*, 2009), altered auditory attention (Hunter *et al.*, 2012; van den Heuvel *et al.*, 2015a), and altered auditory processing of emotional vocalization (Otte *et al.*, 2015).

The impact of positive influences during pregnancy on offspring neurocognitive development has been less investigated. Recently, van den Heuvel *et al.* (2015a) showed that positive influences, such as maternal mindfulness during pregnancy, affect infants' neural responses to sound in a positive way: infants exposed to higher levels of maternal mindfulness devoted less attentional resources to frequently occurring irrelevant sounds, whereas infants exposed to higher levels of maternal anxiety devoted more attentional resources to these stimuli. Maternal mindfulness during pregnancy might therefore, like anxiety, also modulate programming of' the infant brain.

In addition to alterations in functional brain development, Buss *et al.* (2010) found structural changes in the offspring's brains in relation to maternal anxiety during pregnancy. They reported decreased gray matter volume in prefrontal cortex in 6-9-year-old children exposed to high maternal anxiety early in pregnancy. In a subsequent study, they found that increased maternal cortisol exposure during pregnancy was associated with increased right amygdala volume in girls (only), and that amygdala volume mediated the association between maternal cortisol and girls' emotional problems (Buss *et al.*, 2012). This later result might indicate that the observed altered neurocognitive functioning is the result of changes in brain structure caused by an altered intrauterine environment, and might be sex specific.

However, brain regions are highly interconnected and a specific region's functioning is dependent on its connectivity to other regions. Altered brain-connectivity induced by maternal stress during pregnancy might therefore (also) underlie changes in infant neurocognitive functioning. Sarkar *et al.* (2014) investigated the effect of maternal stress during pregnancy on microstructural connectivity in human offspring by looking at limbic-prefrontal white matter microstructural organization in 6-9 year old children. Prenatally stressed children showed increased microstructural connectivity, probably reflecting hypermyelination. However, the sample was relatively small (N=22), and only the limbic-prefrontal connection was investigated, not the structural network as a whole. More recently, Rifkin-Graboi *et al.* (2015) investigated the effect of prenatal exposure to maternal anxiety on total microstructural organization of the offspring's brain in a relatively large group of newborns. Prenatally exposed infants showed alterations in regions important to cognitive-emotional responses to stress (i.e., the right insula and dorsolateral prefrontal cortex), sensory processing (e.g., right middle occipital), and socio-emotional function (e.g., the right angular gyrus, uncinate fasciculus, posterior cingulate, and parahippocampus). Yet, the effect of maternal anxiety and stress during pregnancy on functional, instead of structural, brain networks and the effect of positive maternal influences were not investigated. In the current study we examined the effects of both maternal anxiety and maternal mindfulness during pregnancy on infants' functional brain networks using Electroencephalography (EEG),

The topological properties of brain networks can be determined using graph theory (van Straaten and Stam, 2012; Stam *et al.*, 2014; van Diessen *et al.*, 2014). For the graph-theoretical analysis the brain is viewed as a collection of 'nodes' and 'edges'. Nodes usually represent the recording sites (electrodes or brain regions) and edges the functional (in case of e.g. Electroencephalography [EEG], Magnetoencephalography [MEG], or functional Magnetic Resonance Imaging [fMRI]) or structural connectivity (in case of e.g. Diffusion Tensor Imaging [DTI]) between the nodes. Which metric to use to quantify the functional interactions is an important choice (van Diessen *et al.*, 2014) since, especially in the case of EEG and MEG, many of these metrics are biased by the effects of volume conduction and field spread, leading to spurious estimates of functional interactions. To overcome this, the current study uses the phase lag index (PLI), which is relatively insensitive to the effects of volume conduction and field spread (Stam *et al.*, 2007; Hillebrand *et al.*, 2012).

Once all nodes and edges have been constructed, the characteristics of the so-formed network can be determined (for a review of network measures, see Rubinov and Sporns, 2010). Many studies have shown that healthy, adult brains form so-called 'small-world networks' characterized by a combination of high clustering coefficient and short path length (Watts and Strogatz, 1998). This combination forms a balance between local specialization and global integration, which is crucial for efficient information processing (Rubinov and Sporns, 2010). Another characteristic of a healthy brain is the presence of 'modules' (i.e., groups of highly interconnected nodes) and 'hubs' (i.e., central nodes in a network that are highly connected to other nodes) (Meunier *et al.*, 2010; van den Heuvel and Sporns, 2013). (van den Heuvel and Sporns, 2011) also demonstrated that brain hubs tend to form so-called 'rich-clubs' (i.e., subsets of highly interconnected hubs).

Developmental studies have shown that the developing brain network largely resembles that of the adult brain. For instance, Fair *et al.* (2009) showed that the child brain already shows 'small-world'-like properties and Ball *et al.* (2014) found that functional brain networks in new-borns already show rich-club organization. Furthermore, both the existence of hubs and a modular architecture were revealed in the infant brain (Gao *et al.*, 2009; Fransson *et al.*, 2011). Recent research has shown that even the fetal brain has a modular organization and that these modules overlap with the functional systems observed postnatally (Thomason *et al.*, 2014).

Although network analysis has been very useful in developmental studies, network comparison between groups of subjects remains a critical issue as characterization of brain network topology can be biased by network density (i.e. number of edges; for unweighted network analysis[6]) or edge weights (for weighted network analysis[7]). In addition, a range of arbitrary choices, such as the value of a threshold and the use of weighted versus unweighted graphs can have a large influence on the network measures (Stam, 2014). Recently, computation of the minimum spanning tree (MST) of a network (Stam, 2014; Tewarie *et al.*, 2015) has been proposed as a solution to these issues. The MST is a unique[8] connected subgraph that contains only the strongest connections

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[6] In an unweighted network, only the existence or absence of connectivity between two nodes is taken into account. Such a (binary) network is obtained by setting a threshold for the connectivity, above which connectivity is considered to be present (edge = 1) and below to be considered as not present (edge = 0).

[7] In a weighted network, the strength of the connectivity between two nodes is taken into account (edge = weight).

[8] Under the assumption that all weights are unique

of the original graph without forming cycles. In this way, the number of edges (equal to the number of nodes - 1) is fixed in a principled way. Moreover, a recent simulation study has shown that MST characteristics are equally sensitive to alterations in network topology as conventional network measures, and that MST characteristics are strongly associated with conventional network metrics (Tewarie *et al.*, 2015). Importantly, if the original network can be interpreted as a kind of transport network, and if edge weights in the original graph possess strong fluctuations, also called the strong disorder limit, all transport in the original graph flows over the MST, forming the critical backbone of the original graph (Van Mieghem and Magdalena, 2005; Van Mieghem and van Langen, 2005; Wang *et al.*, 2008).

Two extreme topologies of MSTs can be characterized: a star-like or centralized tree and a line-like or decentralized tree (Stam, 2014; Tewarie *et al.*, 2015). The star-like network has one node that is central and connected with all other (leaf) nodes, making it well integrated, while in the line-like tree all nodes are connected in a line, making it less-integrated (Boersma *et al.*, 2013; Stam, 2014). A more line-like tree topology has been associated with several neurological diseases, such as multiple sclerosis (Tewarie *et al.*, 2014), Parkinson's disease (Olde Dubbelink *et al.*, 2014), and epilepsy (Fraschini *et al.*, 2014; van Dellen *et al.*, 2014).

This study investigated the association between maternal anxiety and mindfulness during pregnancy and brain network configuration in 9 month old infants using the MST approach. We hypothesized that the brain of infants prenatally exposed to higher levels of maternal anxiety would show a more line-like, less integrated configuration. Since anxiety is inversely related to mindfulness (Walsh *et al.*, 2009), and our prior work (van den Heuvel *et al.*, 2015a) has indicated potentially opposite neurodevelopmental correlates of maternal anxiety versus mindfulness in early development, we also conducted exploratory analyses to evaluate whether maternal mindfulness during pregnancy is associated with a better neural network integration compared to infants whose mothers scored lower on mindfulness (i.e., a more star-like configuration).We also examined whether the effect of maternal anxiety during pregnancy on infant brain network properties is different for boys and girls, as several studies have found different effects of exposure to prenatal maternal stress for boys and girls (e.g., Henrichs *et al.*, 2009; Buss *et al.*, 2010; Loomans *et al.*, 2011) as well as gender differences in children's brain networks (Boersma *et al.*, 2011; Gong *et al.*, 2011; Boersma *et al.*, 2013).

6.2. Method

Participants

For the current study, we used data that were previously collected as part of the Prenatal Early Life Stress project (PELS-project), an ongoing prospective cohort study in Tilburg, The Netherlands, following pregnant women and their infants from the first trimester of pregnancy onward. All participating parents provided written informed consent. The medical ethical committee of the St. Elizabeth hospital in Tilburg, The Netherlands approved the study, which was conducted in full compliance with the Helsinki declaration.

A total of 128 infants were originally included at age 9 months. From this group, 4 infants were excluded because of premature birth (i.e. before week 36 of gestation and/or a birth weight <2500 grams), 2 infants due to technical problems during recording, 6 infants because they fell asleep during the experiment, and 34 because of crying/fussiness during testing. We also excluded 18 infants from whom we could not extract enough artifact free epochs (< 6 epochs) and data from an additional 5 infants because one or more of the selected EEG channels for the network analysis was/were bad. The resulting dataset therefore contained 59 infant-mother pairs (32 girls).

Measurements

Anxiety. Maternal anxiety was measured using the Dutch version of the anxiety subscale of the Symptom Checklist (SCL-90; Arrindell and Ettema, 2003) in the second trimester of pregnancy. This SCL-90 anxiety subscale is a self-report measure of anxiety symptoms, consisting of 10 items with five point Likert scales ranging from 1 (not at all) to 4 (extremely). Higher sum scores indicate higher anxiety. The scale has good convergent and divergent validity and has good internal consistency (α=.88 for the anxiety subscale; Arrindell and Ettema, 2003). For the current study, we found a good internal consistency for both the prenatal and postnatal questionnaire (prenatal: α=.79; postnatal: α=.83).

Mindfulness. Maternal mindfulness was measured using the Dutch short version of the Freiburg Mindfulness Inventory (FMIs-14; Walach *et al.*, 2006) in the second trimester of pregnancy. The original FMI is a self-report questionnaire developed in line with the concept of Buddhist psychology, but requires no knowledge of Buddhism or meditation to complete. The short version consists of 14 items with four point Likert scales ranging from 1 (*rarely*) to 4 (*almost always*). Higher sum scores indicate higher mindfulness. The FMIs-14 has good internal consistency (α=.86; Walach *et al.*, 2006). For the current study, an alpha of α=.86 was found, indicating a good internal consistency.

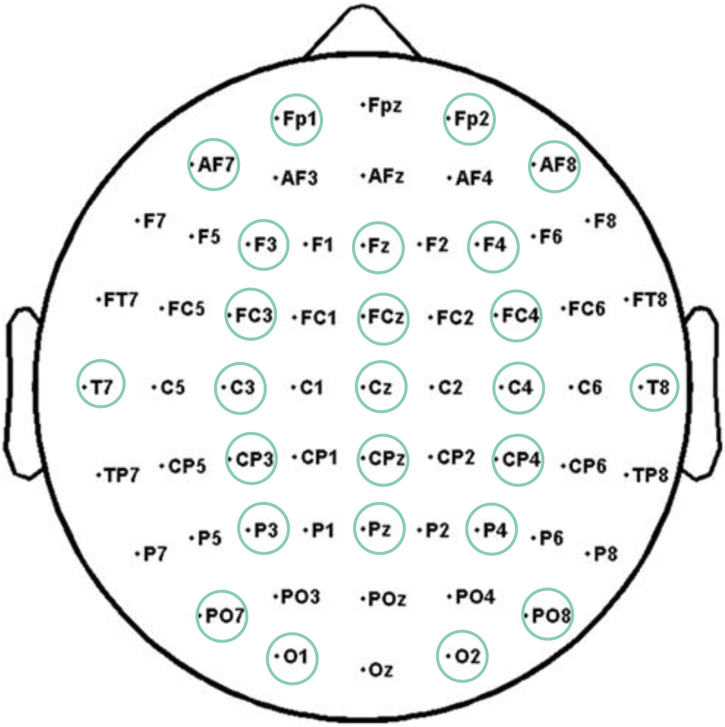
Covariates. All analyses were controlled for gestational age and birth weight, since previous research has shown that both gestational age and birth weight have an effect on the development of infant brain networks (Grieve *et al.*, 2008; Doria *et al.*, 2010; Thomason *et al.*, 2014). Information on gestational age and birth weight was gathered from hospital files.

EEG recordings

We measured EEG activity from the infants while being exposed to a passive auditory oddball task with a frequent (standard) tone and three rare deviant auditory events: 1) a standard tone preceded by a shorter inter-stimulus interval ("ISI-deviant"), 2) a white noise sound, or 3) environmental ("novel") sounds. A more detailed description of the passive auditory paradigm can be found elsewhere (Otte *et al.*, 2013; van den Heuvel *et al.*, 2015a; van den Heuvel *et al.*, 2015b). EEG data were recorded with Biosemi ActiveTwo amplifiers (www.biosemi.com) with a sampling rate of 512 Hz from 64 Ag/AgCl electrodes that were placed on the infant's scalp according to the International 10-20 system.

The data were filtered offline with a 50 Hz notch filter and a zero-phase Butterworth bandpass 1.0 – 30 Hz (slope 24 dB) filter using the BrainVision Analyzer 2 software package (Brain Products, Munich, Germany). Subsequently, the data were exported to MATLAB (version R2012b, The Mathworks, Inc.) and average referenced using the EEGLAB toolbox (version 13.0.1, Delorme & Makeig, 2004). We included 25 channels (midline: Pz, CPz, Fz, FCz, Cz; frontal: Fp1, Fp2, AF7, AF8, F3, F4; frontocentral: FC3, FC4; central: C3, C4; centroparietal: CP3, CP4; parietal: P3, P4; temporal: T7, T8; parieto-occipital: PO7, PO8; occipital: O1, O2) for the network analysis (see Figure 6.1). The non-selected electrodes were excluded because many infants showed a large number of artefacts on these electrodes. The selected channels were used for computing the average reference. The average reference has previously been suggested to overcome the confounding effects of brain activity picked up by common reference channels (Nunez *et al.*, 1997; van Diessen *et al.*, 2014). The resulting data were subsequently converted to ASCII files and exported to the BrainWave software version 0.9.133.1 (http://home.kpn.nl/stam7883/brainwave.html) for further processing. For each subject, in order to obtain stable estimates, we averaged network properties (see below) over six artefact free epochs of 4 seconds (2048 samples) that were selected after visual inspection. Artifacts were caused mainly by head and body movements, crying or drowsiness. For all selected epochs infants had their eyes open.

Figure 6.1. *Graphical representation of the 25 selected electrodes for network analysis.*



Functional connectivity

Functional connectivity was assessed in the “infant” alpha band and in broad band. To obtain the infant alpha band, the broad band filtered epochs (1 -30 Hz) were filtered with a 6-9 Hz bandpass brick-wall Fast Fourier Transform (FFT) filter as implemented in BrainWave. Infant EEG contains more low frequency activity than the adult EEG, resulting in a shift of the characteristic adult frequency bands. The 6-9 Hz frequency range is considered to correspond to the adult alpha band (8-13 Hz) and has emerged as a standard in infant EEG studies (Marshall *et al.*, 2002).

Subsequently, the frequency-band specific PLI was computed between each pair of electrodes for every epoch. The PLI is defined as (Stam *et al.*, 2007):

$$PLI = |<\mathrm{sign}[\sin(\Delta\phi(t_k))]>|,$$

where the phase difference is defined in the interval [-π, π] and <> denotes the mean value. $\Delta\phi(t_{k)}$ denotes a time series of phase differences, and || denotes the absolute value. The PLI ranges between 0 (completely symmetric phase distribution, i.e. no phase synchrony, or coupling with a phase difference centered around zero (modulus π)) and 1 (completely asymmetric phase distribution, i.e. a consistent, non-zero phase difference). As field spread and volume conduction causes a zero phase lag (modulus π) between two time-series, this hardly influences the PLI (Stam *et al.*, 2007).

Minimum spanning tree

The MST was constructed based on the weighted networks with Kruskal's algorithm (Kruskal, 1956). For the construction of the minimum spanning tree the edge weight is defined as 1/(functional connectivity estimate), e.g. 1/PLI, since we are interested in the strongest connections in the network. The algorithm first orders the link weights in an ascending order and starts the construction of the MST with the weakest link weight and adds the following weakest link weight until all nodes N are connected in an acyclic sub-network that consists of M = N -1 links. If adding a link results in formation of a cycle, this link is skipped. In this study, trees with 25 nodes and 24 edges were constructed.

Subsequently, several topological characteristics of the MST were calculated for every epoch and both frequency bands: MST degree, MST eccentricity (Ecc), MST betweenness centrality (BC), MST diameter (Diam), MST leaf fraction (Leaf), and MST hierarchy (T_h). The indices were calculated per epoch and subsequently averaged per subject.

The degree of a node is the number of edges that are connected to it. For this study, we looked at the normalized maximum degree, which was computed by dividing the maximum degree in the network by the total number of edges. Eccentricity of a node was defined as the longest distance (in number of edges) between a node and any other node in the tree, which is small if a node is central in the tree. Here, we report the eccentricity averaged over all nodes. The betweenness centrality of a node is the fraction of the number of shortest paths between any pair of nodes that are running through that node and the total number of paths between that pair of nodes. It can have values ranging from 0 to 1, with higher BC indicating a more central role of that node in the network. Degree, eccentricity and BC are measures that quantify the relative importance of a node in a network (Stam, 2014). The diameter of a graph is the longest path (in number of edges), and the leaf number the number of edges with degree = 1. The diameter was normalized by the total number of edges, and the leaf number was normalized by the maximum possible leaf number (N – 1, with N the number of nodes).

There are two extreme cases of MST network topologies (see Figure 6.2): a star-like and a line-like network. In a star-like network (Figure 6.2A) there is one central node (degree = N – 1; BC = 1) with all other nodes being leaf nodes (degree = 1; BC = 0). A star-like network is very well integrated and facilitates efficient communication, but also has a high risk of overload of the central node. A line-like network (Figure 6.2C) on the other hand, consists of only two leaf nodes, with all other nodes in between (forming a line). In a line-like network the nodes have lower BC and are therefore prevented from overload, but this network is not well integrated. The tree hierarchy quantifies the balance between the integration of the network (i.e. small diameter) and overload of central nodes (i.e. high BC) (Boersma *et al.*, 2013), and is computed as:

$$T_h = L/(2mBC_{max}),$$

where L is leaf number, m is number of edges (N -1), and BC_{max} is the maximum BC in the tree. To assure T_h ranges between 0 and 1, the denominator is multiplied by 2. Hierarchy ranges between 0 and 1: line-like topologies have a hierarchy close to 0, whereas star-like topologies have a hierarchy close to 0.5. In MST networks with leaf numbers in between these two extremes, hierarchy can have higher values. More integrated, star-like networks are characterized by a large leaf number, small diameter, and low eccentricity, whereas less integrated, line-like networks are characterized by a small leaf number, large diameter, and high eccentricity.

Statistical analysis

All analyses were performed using IBM SPSS 19.0 for Windows. We first explored sex differences in brain network connectivity using independent-samples t-tests. Next, to examine the association between maternal anxiety and mindfulness during pregnancy and the functional network characteristics of the infant, we used multiple ANCOVAs with graph theoretical measures as dependent variables and maternal anxiety and mindfulness as independent variables. Four ANOVAs were performed: per frequency band (broad band and alpha band) and per independent variable (maternal anxiety and maternal mindfulness). Both maternal anxiety and mindfulness were included as continuous variables. Sex was included into the model as covariate and as interaction ('sex X maternal anxiety' & 'sex X maternal mindfulness') to examine sex differences for the effect of maternal anxiety and mindfulness during pregnancy on the network parameters. Significant sex interactions were followed up with post-hoc ANCOVAs separately for boys and girls.

Finally, we statistically controlled significant results for postnatal maternal anxiety (when the infants was 9 months of age) using multiple regression analysis. This was done to control for possible maternal psychosocial influences during early childhood. We also controlled for gestational age and birth weight of the infants, since previous research has shown that both gestational age and birth weight have an effect on the development of infant brain networks (Grieve *et al.*, 2008; Doria *et al.*, 2010; Thomason *et al.*, 2014).

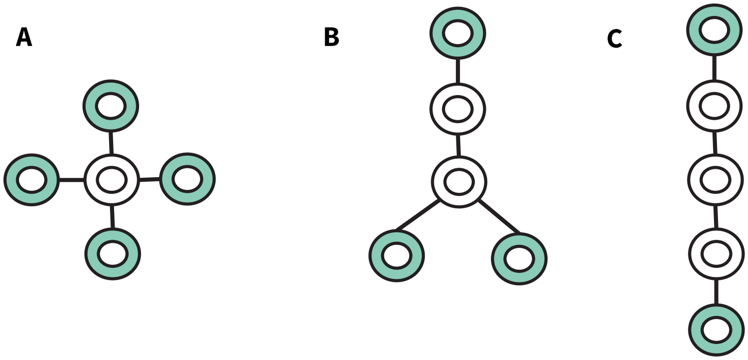


Figure 6.2. *Illustration of the two extreme cases of MST network topologies: a star-like network (**A**) and a line-like network (**C**). **B** shows an example of a MST topology in between the two extremes. The blue nodes represent leaf nodes.*

6.3. Results

Descriptive characteristics of the participants are presented in Table 6.1. Table 6.2 provides an overview of the average PLI and MST measures for the total group and for boys and girls separately.

Table 6.1. *Characteristics of the participating mother-infant dyad sample*

Infants (N=79)	N	%	M (SD)
Age at EEG-measurement (weeks)	59		43.05 (1.90)
Sex			
Girl	32	54.2	
Boy	27	45.8	
Birth weight (grams)	59		3562.63 (510.64)
Gestational age at birth (weeks)	57		39.93 (1.27)
Mothers (N=78)	N	%	M (SD)
Age (years)	58		33.08 (3.82)
FMI sum	58		40.52 (6.75)
SCL-90 sum: prenatal	59		13.27 (3.72)
SCL-90 sum: postnatal	43*		12.74 (4.16)
Education	59		
Primary or secondary	5	8.5	
General vocational training	15	25.4	
Higher vocational training	29	49.2	
University degree or higher	10	17.0	
Primagravida	20	33.9	

Note * N = 16 mothers included in the study did not complete the postnatal questionnaire; FMI = Freiburg Mindfulness Inventory; SCL-90 = anxiety subscale of the Symptom Checklist.

Sex differences

In the alpha band, we found several sex differences: boys showed a significantly higher MST eccentricity ($t = 2.542$, $p = .014$) and MST diameter ($t = 2.536$, $p = .014$) than girls. No significant sex differences were found in the broad band.

Effect of maternal anxiety and mindfulness

Alpha band (6-9 Hz). We found a significant interaction between sex and

Table 6.2. *Descriptives of average PLI and MST parameters, Mean (SD)*

Variable	Total (N=59)	Boys (N=27)	Girls (N=32)	t-value
Broad band				
PLI	.141 (.014)	.138 (.015)	.141 (.013)	-.893
Degree	.264 (.032)	.260 (.034)	.268 (.030)	-.876
Ecc	.301 (.020)	.303 (.021)	.300 (.018)	.679
BC	.716 (.034)	.714 (.033)	.719 (.035)	-.592
Diam	.381 (.025)	.384 (.029)	.380 (.023)	.768
Leaf	.550 (.024)	.544 (.035)	.553 (.028)	-.964
Hierarchy	.386 (.022)	.383 (.022)	.387 (.025)	-.576
Alpha band				
PLI	.202 (.016)	.202 (.013)	.203 (.018)	-.320
Degree	.237 (.024)	.236 (.027)	.236 (.022)	-.382
Ecc	.323 (.022)	.331 (.025)	.317 (.018)	**2.542***
BC	.700 (.021)	.696 (.021)	.703 (.021)	-1.383
Diam	.410 (.031)	.421 (.035)	.401 (.025)	**2.536***
Leaf	.521 (.039)	.519 (.044)	.522 (.034)	-.349
Hierarchy	.375 (.028)	.375 (.029)	.374 (.027)	.078

Notes. * $p<.05$; PLI = Phase Lag Index; Ecc = eccentricity; BC = betweenness centrality; Diam = diameter; Leaf = leaf fraction

mindfulness for MST degree ($F(1,55) = 4.326$, $p = .042$, η2 = .074). Post hoc tests showed that the effect was only significant for girls: higher maternal mindfulness during pregnancy was associated with higher MST degree (more line-like network configuration). However, after correction for the above mentioned confounders, the interaction became non-significant. No effects of maternal anxiety during pregnancy on the graph theoretical measures were found in the alpha band.

Broad band (1-30 Hz). For the effect of maternal anxiety during pregnancy on the graph theoretical measures we did find several sex differences. We found significant interactions between sex and anxiety for MST degree ($F(1,55) = 4.658$, $p = .035$, $\eta^2 = .078$), MST diameter ($F(1,55) = 5.702$, $p = .020$, $\eta^2 = .094$), and MST leaf fraction ($F(1,55) = 6.007$, $p = .017$, $\eta^2 = .098$). Post hoc tests showed that the effect of prenatal exposure to maternal anxiety is opposite for boys and girls: in boys higher maternal anxiety during pregnancy is related to increased MST degree, decreased MST diameter and increased MST leaf number (more star-like network configuration), while in girls higher maternal anxiety during pregnancy is related to decreased MST degree, increased MST diameter and decreased MST leaf number (more line-like network configuration). Nevertheless, all three interactions became non-significant after controlling for maternal postnatal anxiety. No effects of maternal mindfulness during pregnancy on the graph theoretical measures were found in the broad band.

6.4. Discussion

The current study is the first to examine the effect of maternal anxiety during pregnancy on infants' brain functional network configuration at the age of 9 months. We hypothesized that the brain of infants prenatally exposed to higher levels of maternal anxiety would show a more line-like configuration. Despite the fact that we found several interaction effects between and maternal anxiety and sex for the brain network measures, these results did not survive correction for postnatal maternal anxiety (at age 9 months). Based on this we have to conclude we did found no evidence for our hypothesis. We also hypothesized that maternal mindfulness during pregnancy would exert opposite effects on infant's brain network configuration compared to maternal anxiety (e.g., a more star-like configuration), but, similar to maternal anxiety, after controlling for confounders (i.e., gestation age, birth weight, and maternal postnatal anxiety) we did not find any significant effects of maternal mindfulness during pregnancy on infants' brain network configuration.

These results might indicate that prenatal exposure to maternal anxiety and mindfulness has no specific prenatal effect on infants' brain network configuration, but may influence the development of brain network configuration (partly) after birth. The lack of evidence for an effect of prenatal exposure to maternal anxiety on infant's brain functional network configuration seems in contrast to previous studies that reported structural alterations in offspring's brain connectivity (Sarkar *et al.*, 2014; Rifkin-Graboi *et al.*, 2015), but they could also indicate that these structural changes do not produce altered functional brain connectivity. Another reason for our lack of findings could be that our measurement with electroencephalography (EEG) was not able to pick up alterations in connectivity of deeper brain structures, such as the limbic system and hippocampus, since EEG is not able to measure beyond the cortical layer of the brain (de Haan, 2006). Since our analysis was limited to the alpha band and broad band, it could be that effects can be found in other frequency bands. Future research should examine this. Another possibility could be the lack of power to find genuine effects of maternal mindfulness during pregnancy. Although a relatively large group of infants was tested with a relatively large set of electrodes compared to other EEG-studies, for finding correlations the group was still small. Schönbrodt and Perugini (2013) recommend a sample size of n>250 for stable estimates of correlations.

We did find some sex differences in infant brain functional network configuration. More specifically, we found that boys had higher MST eccentricity and MST diameter than girls. A higher eccentricity and diameter both point to a more line-like network in boys compared to girls (Boersma *et al.*, 2013; Tewarie *et al.*, 2015). These results may contribute to sparse knowledge of the development of brain functional network configuration. Only one study has examined maturation of the brain network using MST measures, in 5 to 7 year olds (Boersma *et al.*, 2013).

Boersma *et al.* (2013) reported no significant sex differences in the alpha band, which may indicate that the sex differences we found in the current study may disappear over development. Future longitudinal studies should elucidate the development of brain functional network configurations in children as to date large gaps in this knowledge exist.

The current study used state-of-the-art measures and approaches for network analysis. Firstly, the use of the Phase Lag Index (PLI) increases the reliability of our findings, since this connectivity measure is less sensitive to the effects of volume conduction/field spread than most other connectivity measures (van Diessen *et al.*, 2014). Although the use of the PLI increased the reliability of our measurements, it is also a conservative measure that might miss real connectivity (Cohen, 2015). Another strength is the MST approach, because it provides an unbiased estimate of network topology. Existing research in child populations is often contradicting or hard to compare because different graph methods and/or connectivity measures have been used. Using the MST approach overcomes the problem of network comparison, but only for MSTs with the same number of nodes (i.e. electrodes) (van Diessen *et al.*, 2014). Moreover, for reliable comparison between studies not only identical electrodes should be used, also the same synchronization measure to construct the MSTs. In the future, attempts should be made to standardize measurement of functional brain network configurations in infants and children to enhance comparison quality.

Besides the methodological strengths of our study, an important concern should also be mentioned: the ‘resting-state’ we used to study network configuration in the infants. Resting state can be defined as “the state in which a subject is awake and not performing an explicit mental or physical task" (van Diessen *et al.*, 2014, p.3.). In adults and older children, the resting-state is often measured when subjects have their eyes closed without falling asleep. This procedure cannot be applied to infants and young children, since they cannot be instructed to close their eyes without falling asleep. Measuring resting-state in developmental research is therefore not straightforward and many different procedures have been used. Examples during which the 'resting-state' was assessed in infants and young children are: 1) while infants are watching stimuli or a movie (with or without sound) on a computer screen (e.g., Bathelt *et al.*, 2013), 2) while infant are sleeping (e.g., Fransson *et al.*, 2011; González *et al.*, 2011), or 3) while infants were sedated (e.g., Pandit *et al.*, 2014). Unfortunately, many studies do not explicitly describe their procedure in the method section. As for adults, infants are likely to have heterogeneous experiences during the 'resting-state' which can potentially confound the results (Diaz *et al.*, 2013). In line with the recommendations of van van Diessen *et al.* (2014), we therefore conducted the experiment in the same order for all subjects with a priori defined instructions to the mother to reduce possible differences in their infants' cognitive state during the 'resting-state'. In addition, providing the infants with a passive task, as in the current study, might be superior to providing the infants with no task at all, since it might make the experience more uniform. Nevertheless, no data comparing different 'resting-state' procedures in infants and young children is available to date to support this claim. Similar to the necessity of standardizing measurements of brain network analysis, there is a pressing need for standardization of 'resting-state' procedures for infants and young children.

6.5. Conclusion

In sum, we found no evidence for an effect of prenatal exposure to maternal anxiety on infant brain networks. This might indicate that prenatal exposure to maternal anxiety and mindfulness has no effect on infants' functional brain network configuration. Nevertheless, several methodological issues might have influenced the results, such as low power to detect genuine effects and the use of an auditory oddball paradigm as 'resting-state'. Future research should replicate our study including more infants and a better paradigm to measure child brain functional network configuration.

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Chapter 7:

General discussion & Conclusion

7.1. Introduction

This dissertation aimed at investigating the effects of exposure to maternal anxiety and mindfulness during pregnancy on neurocognitive functioning in infants and young children. All studies included in this dissertation were embedded in a prospective cohort study conducted at Tilburg University, Tilburg, The Netherlands. The study followed pregnant women and their offspring from the first trimester of pregnancy onwards.

In the first section of this general discussion the main findings of the four studies will be summarized. Next, the result will be related to the core aim of the dissertation and a new working hypothesis is proposed to explain the findings. In the section after, we will discuss whether mindfulness can be seen as a positive factor in the modulation of developmental programming. Subsequently, we will describe potential underlying mechanisms of the Developmental Origins of Behavior, Health and Disease (DOBHaD, see chapter 1) that may explain the association between maternal anxiety and mindfulness during pregnancy and offspring outcomes. We will then discuss alternative explanations for our results. This will be followed by ideas for future research directions and implications for public health policy. We will wrap up the dissertation with general conclusions.

7.2 Main findings

In all studies, we explored the effects of maternal anxiety and mindfulness on offspring's neurocognitive functioning. In chapter 3, our first study was presented that investigated this aim. In this study, we examined the effects of maternal anxiety and mindfulness during pregnancy on infant's neural responses to sounds. To this end, we recorded EEG signals while we presented the infants with an auditory oddball paradigm and extracted ERPs. We found that higher maternal anxiety was associated with higher N250 amplitudes to the frequently presented standard sounds, whereas higher maternal mindfulness was associated with lower N250 amplitudes to these sounds. Maternal mindfulness was also positively associated with the P150 amplitude to standard sounds. These results suggest that the infants prenatally exposed to higher levels of maternal anxiety devoted more attentional resources to frequently occurring irrelevant sounds and that exposure to maternal mindfulness exerted, at least partly, opposite effects on the infants' cortical auditory processes.

In chapter 4, we focused on the effects of maternal mindfulness during pregnancy on infantile socio-emotional development and temperament and the possible mediating role of maternal anxiety in this relation. We found that higher maternal mindfulness during pregnancy was associated with fewer infant self-regulation problems and less infant negative affectivity. Mediation analysis showed that maternal anxiety mediated the association between maternal mindfulness and infant self-regulation problems. These results suggest that high maternal dispositional mindfulness during pregnancy may have positive effects on infant development, potentially via reducing anxiety symptoms in pregnant women.

The study presented in chapter 5 investigated the effect of maternal anxiety and mindfulness on affective picture processing in four-year-olds using the event-related potential method. We found a positive association between maternal anxiety during pregnancy and the amplitudes of the LPP for neutral pictures in the middle and late time window at central and anterior locations, but no association between the LPP amplitudes to unpleasant pictures and maternal anxiety during pregnancy. We did not find any associations between maternal mindfulness and the LPP amplitudes. The results indicate that children prenatally exposed to higher maternal anxiety devote more attentional resources to neutral pictures then children exposed to lower maternal anxiety, but not to unpleasant pictures.

Finally, chapter 6 presented a study in which we re-analysed data collected in the study presented in chapter 3 to examine the effects of maternal anxiety and mindfulness on the functional brain network configuration of infants. Although we found some interaction effects between maternal anxiety and sex on the brain network measures, these results did not survive statistical correction for postnatal maternal anxiety (at the age 9 months). Based on these results we have to conclude that we failed to find evidence for altered functional brain network configuration in infants prenatally exposed to maternal anxiety. However, methodological issues may have decreased the power of the analysis to find significant associations.

Taken together, the dissertation provides evidence that prenatal exposure to maternal anxiety is related to altered neurocognitive functioning in the offspring, including altered auditory attention, socio-emotional functioning, temperament, and affective processing. Previous studies associated prenatal exposure to maternal anxiety with altered brain structure (Buss *et al.*, 2010; Buss *et al.*, 2012; Qiu *et al.*, 2015b; Rifkin-Graboi *et al.*, 2015) and function in the offspring (Harvison *et al.*, 2009; Mennes *et al.*, 2009; Otte *et al.*, 2015). Here we propose that the results of chapter 3, 4 and 5, and those of previous studies, are in line with the notion that children prenatally exposed to higher levels of maternal anxiety show increased vigilance for potentially threatening stimuli. Vigilance is defined in this dissertation as an overall increase in “readiness” to detect stimuli in the environment or, in other words, a lower response threshold (Whalen, 1998).

7.3 Increased vigilance: a working hypothesis

Cues in the intrauterine environment of fetus exposed to maternal anxiety may guide the adaptation of the fetal phenotype, resulting in a variety of alterations in brain functioning. In the context of developmental plasticity, these alterations in brain functioning can be seen as adaptations to prepare the offspring for the predicted stressful, potentially threatening or fear-inducing environment (Gluckman and Hanson, 2007; Gluckman *et al.*, 2008; Frankenhuis and Del Giudice, 2012). Changes in the function and structure of the exposed fetal brain may later develop into characteristics that serve as an advantage in fear-inducing environments.

Vigilance for potentially threatening stimuli may be a good candidate for such an evolved characteristic, because it could facilitate faster fight or flight response of the individual increasing his/her chances of survival. Several researchers also proposed increased vigilance as a possible adaptive characteristic of children prenatally exposed to maternal anxiety (Kapoor *et al.*, 2006; Talge *et al.*, 2007; Glover, 2011). Neuroimaging studies have consistently identified the amygdala, (dorsolateral) prefrontal cortex, and hippocampus as key structures for vigilance and threat perception (Whalen, 1998; Lipka *et al.*, 2011; Dejean *et al.*, 2015; Dunsmoor and Paz, 2015; Fox *et al.*, 2015; Ironside *et al.*, in press). Interestingly, preclinical studies have demonstrated an association between prenatal stress and alterations in similar brain regions several decades ago (e.g., for the amygdala Cratty *et al.*, 1995). Recently, these associations were also found in humans prenatally exposure to maternal anxiety (Rifkin-Graboi *et al.*, 2015), maternal depression (Rifkin-Graboi *et al.*, 2013; Qiu *et al.*, 2015a) and early life stress (Malter Cohen *et al.*, 2014; Grant *et al.*, 2015; Thomason *et al.*, 2015).

Taken together, these studies support a hypothesis in which prenatal exposure to maternal anxiety causes alterations in the structure and function of brain regions related to threat perception (i.e., amygdala, hippocampus, and prefrontal cortex). These, in turn, lead to higher vigilance in the affected children. Increased vigilance can subsequently result in higher risk for child behavioral and emotional problems. Although parts of this model have a strong empirical basis, such as the relation between maternal anxiety during pregnancy and behavioral and emotional problems in children (e.g., Van den Bergh and Marcoen, 2004; Van den Bergh *et al.*, 2007; Loomans *et al.*, 2011), other parts are still lacking empirical support. In addition, the hypothesis as a whole should be tested in future research. A graphical presentation of this newly proposed hypothesis, the "increased vigilance hypothesis", is shown in Figure 7.1.

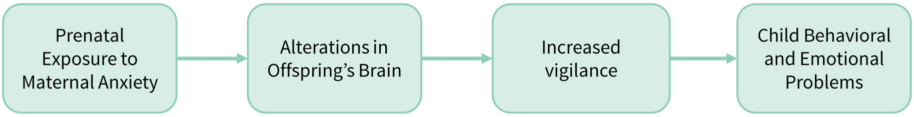

Figure 7.1. *Graphical representation of the increased vigilance hypothesis*

The results of the current dissertation contribute to the empirical investigation of the proposed hypothesis. First of all, the results of **chapters 3 and 5**, both contribute to the "increased vigilance hypothesis" as the increased attention allocation to both frequently occurring, standard sounds and neutral pictures in infants/children prenatally exposed to maternal anxiety could be viewed as increased vigilance in these infants and children. Both results could point to a lower threshold for (unconscious) threat appraisal when confronted with neutral stimuli. In a fear-inducing environment, the benefits of detecting real threat in a seemingly neutral situation may outweigh the costs of misinterpreting neutral situations as threatening. However, increased vigilance may ultimately lead to higher risk for developing behavioral and emotional disorders later in life, especially when the postnatal environment does not match the expected (Gluckman and Hanson, 2006; Frankenhuis and Del Giudice, 2012). In fact, previous research found that vulnerability to anxiety primarily stems from a lower threshold for appraising threat, which can be viewed as hypervigilance (Mogg *et al.*, 2000). Building on this, it is tempting to speculate that evaluating relatively innocuous stimuli as having higher subjective threat may place children prenatally exposed to maternal anxiety at a higher risk for internalizing problems later in life.

The results of **chapter 4** also seem to support this idea, as we found that maternal anxiety during pregnancy was related to more infant self-regulation problems and negative affectivity. Previous studies have found similar results (Van den Bergh, 1990; Van den Bergh, 1992; Austin *et al.*, 2005; Henrichs *et al.*, 2009; Baibazarova *et al.*, 2013). Negative affectivity is considered one of the personality traits most relevant to affective disorders and it is highly correlated with self-reported anxiety and depression (Tortella-Feliu *et al.*, 2010; Iqbal and Dar, 2015). Research has shown that infants with high negative affectivity display altered threat processing (Nakagawa and Sukigara, 2012). In addition, negative affectivity has been associated with altered amygdala function in relation to threat perception (Vizueta *et al.*, 2012). Vizueta *et al.* (2012) speculated that the heightened amygdala response to fearful stimuli in individuals with high negative affectivity may reflect processes related to vigilance, wariness, and anticipation of potential threats. Taken together, it makes sense to argue that increased vigilance leads to negative affectivity in infancy, which may predispose these infants to developing anxiety symptoms or an anxiety disorder later in life.

7.4 Maternal mindfulness as protective factor?

The results of the studies presented here showed inconsistent results on the influence of maternal mindfulness during pregnancy on child outcomes. In chapter 3, we showed that infants prenatally exposed to maternal mindfulness devote less attentional resources to frequently occurring, neutral sounds and in chapter 4 we demonstrated that maternal mindfulness during pregnancy is associated with less infant self-regulation problems and less negative affectivity. These results point to possible positive effects of maternal mindfulness during pregnancy in infants. However, in the study presented in chapter 5 we did find some effects of maternal mindfulness during pregnancy on the child affective processing, but the result became non-significant after statistically controlling for postnatal maternal mindfulness. Further, the study in chapter 6 failed to find an effect of maternal mindfulness during pregnancy on infant brain network connectivity. However, no significant effects of maternal anxiety on infant functional brain network configuration has been found either.

Several explanations of the inconsistent results are possible. First of all, it might be that maternal mindfulness during pregnancy does not exert influence beyond infancy, but only affects infant brain functioning before childhood. Another explanation for these inconsistent results may be that maternal mindfulness, in contrast to maternal anxiety, has no specific prenatal effects, but exerts its influence (also) in the postnatal period. The studies presented in chapters 3, 4, and 6 did not statistically control for postnatal maternal mindfulness as this measure was not available in infancy. Hence, with the present data we are unable to rule out that the positive results of prenatal exposure to maternal mindfulness are actually caused by postnatal mindfulness.

Based on the results of this dissertation it is not possible to draw strong conclusions on the potential beneficial effects of maternal mindfulness during pregnancy for the unborn child. Nevertheless, even if maternal mindfulness has no specific prenatal effects, it can still be useful during pregnancy to reduce maternal anxiety symptoms. The results of the study presented in chapter 4, demonstrating that maternal mindfulness influenced child outcomes via reduction of maternal anxiety during pregnancy, support the use of maternal mindfulness interventions to reduce maternal psychological distress during pregnancy. More research is necessary, however, to evaluate such interventions and the benefits for both mother and child.

7.5 Underlying mechanisms of DOBHaD

Although the empirical basis for the association between maternal psychological distress during pregnancy and child outcomes seems robust, the underlying mechanisms of this association are largely unknown. In this section, we describe three prominent and often-proposed mechanisms: the hypothalamic-pituitary-adrenal (HPA-) axis and its end product, cortisol, the placenta and the involvement of the 11β-HSD2 enzyme, and the role of epigenetics. Other proposed mechanisms, not discussed in this dissertation, include compromised maternal immune functioning, increased catecholamines (i.e., adrenalin and noradrenalin), altered intestinal microbiota, and changed health behaviors (For a recent review, see Beijers *et al.*, 2014; Jašarević *et al.*, 2015; Rakers *et al.*, in press).

Maternal HPA axis and cortisol

The most often proposed mechanisms in the relation between maternal stress during pregnancy and child outcomes is the involvement of the hypothalamic-pituitary-adrenal (HPA-) axis and its end product, cortisol (Beijers *et al.*, 2014). When a pregnant woman is exposed to stress, the HPA axis is activated, resulting in the release of multiple hormones, including cortisol. There is evidence for a strong correlation between cortisol in maternal and fetal compartments as maternal cortisol is able to cross the placenta (Sarkar *et al.*, 2007). Exposure to cortisol is essential for normal maturation of many parts of the central nervous system during fetal development. However, excessive levels of cortisol in the fetal circulation may alter neurotransmitter activity and synaptic plasticity resulting in changes in subsequent brain functioning (Harris and Seckl, 2011). Therefore, excessive levels of cortisol induced by maternal anxiety can be viewed as a potential mediator in the association between maternal anxiety and child outcomes.

Animal research has provided clear support for this hypothesis (Harris and Seckl, 2011; Beijers *et al.*, 2014). In human studies, however, only modest, if any, associations were found between the mother's mood and cortisol concentrations (Hompes *et al.*, 2012; Salacz *et al.*, 2012; Baibazarova *et al.*, 2013; Hellgren *et al.*, 2013; Voegtline *et al.*, 2013). In addition, inconsistent results were found for the association between maternal cortisol during pregnancy and child outcomes. In a recent review, Zijlmans *et al.* (2015) concluded that cortisol may not to be the main underlying mechanism in the relation between maternal anxiety during pregnancy and child outcomes. Most likely, cortisol plays a part in the interplay between several underlying mechanisms in a complex and indirect way and the study of the HPA-axis as mechanisms should therefore be supplemented with that of other mechanisms.

Placental function and the 11β-HSD2 enzyme

An important mechanisms to complement the HPA-axis explanation is placental functioning, since perturbations in the maternal environment must be transmitted across the placenta in order to affect the fetus (Jansson and Powell, 2007; Fowden *et al.*, 2008; O'Donnell *et al.*, 2009). A key placental enzyme in this context is 11β-HSD2, which prevents the majority of maternal cortisol from crossing the placenta by converting cortisol to the inactive cortisone (Wyrwoll *et al.*, 2011). Importantly, previous research has found higher correlations between maternal and amniotic fluid cortisol levels in anxious woman compared to non-anxious women, which suggest that maternal anxiety during pregnancy can increase the placental permeability to cortisol (Glover *et al.*, 2009). Indeed, O'Donnell *et al.* (2012) reported an association between maternal mood during pregnancy and down regulation of placental 11β-HSD2.

Maternal anxiety may also alter the function of the placenta in other ways, independent of HPA-axis function, such as via alterations in uterine blood flow. Several studies have shown that maternal anxiety in pregnancy is associated with reduced blood flow in umbilical or uterine arteries (Sjöström *et al.*, 1997; Teixeira *et al.*, 1999), compromising oxygen and nutrient delivery to the fetus. More recent studies have failed to replicate these results, however (Kent *et al.*, 2002; Monk *et al.*, 2012a). Many other factors of placental functioning may play a role, but, unfortunately, the placenta as a potential mechanism of the developmental programming effect of maternal anxiety is not yet sufficiently understood (O'Donnell *et al.*, 2009; Beijers *et al.*, 2014).

Epigenetics

Recently, insights from the field of epigenetics gave rise to another possible mechanism: epigenetic modifications, which are molecular mechanisms that alter gene activity without changing their DNA sequence (Babenko *et al.*, 2015). These modifications can cause altered gene expression in multiple tissues, including the brain, with consequences for the functioning and connectivity of neural circuits. Therefore, they may underlie alterations in neurodevelopment seen in offspring of highly anxious pregnant women (Van den Bergh, 2011; Monk *et al.*, 2012b; Babenko *et al.*, 2015). To date, several human studies have reported epigenetic changes in children prenatally exposed to maternal anxiety (Mulligan *et al.*, 2012; Schroeder *et al.*, 2012; Hompes *et al.*, 2013; Cao-Lei *et al.*, 2014; Cao-Lei *et al.*, 2015), supporting the idea that prenatal exposure to maternal anxiety alters the typical pattern of gene expression in the fetus.

Epigenetic modifications may also modulate the fetal phenotype in interaction with the above mentioned mechanisms; for example, via altering placental functioning (Monk *et al.*, 2012b). A recent study showed associations between maternal stress and epigenetic modifications of the placental 11β-HSD2 gene in rats (Jensen Peña *et al.*, 2012), suggesting that epigenetic pathways may underlie the above mentioned downregulation of this enzyme found in highly anxious women (O'Donnell *et al.*, 2012). In addition, maternal stress hormones (i.e., cortisol and catecholamines) have an important role in epigenetic modifications as they can signal the type, severity and duration of the environmental cues (Fowden and Forhead, 2009). Building on this, maternal cortisol (and other stress hormones) may be able to guide adaptations of the fetal phenotype to the expected environment via epigenetic modification of the placental phenotype. Exploration of epigenetic mechanisms, especially in interaction with previously proposed mechanisms, may provide more insight into the underlying mechanisms linking prenatal exposure to maternal stress and child outcomes.

7.6 Possible alternative explanations of DOBHaD effects

Beside the above mentioned mechanisms that may modulate programming of the fetal phenotype, other mechanisms may also (partly) explain our results. In this section we describe genetic heritability and postnatal influences as possible alternative explanations for the results of this dissertation.

Genetic transmission

The design of the studies included in the dissertation does not rule out that shared genetic factors may partly explain the association between maternal anxiety during pregnancy and altered offspring brain functioning. Genetic factors may influence both maternal predisposition to experience anxiety during pregnancy and the offspring's brain functioning (Rice *et al.*, 2010). However, human studies evaluating the consequences of stressful life events on child outcomes, such as natural disasters, suggest that the effects of prenatal anxiety cannot be explained by genetic predispositions alone (Laplante *et al.*, 2008; Cao-Lei *et al.*, 2015). Daskalakis *et al.* (2013) introduced the "three-hit concept" of vulnerability and resilience, in which they present the idea suggesting that genetic predispositions (hit-1) may interact with early-life environmental inputs, such as prenatal exposure to maternal anxiety (hit-2) to modulate programming of phenotypes with differential susceptibility to later-life challenges (hit-3). This concept suggests that genetic transmission and early life stress may interact and that both contribute to an altered phenotype of the offspring. Hence, when interpreting the results of this dissertation, it is important to take into account that the alterations in brain functioning related to prenatal exposure to maternal anxiety may be partially explained by a genetic predisposition from the parents.

Postnatal influences

Although we statistically controlled for the possible influence of postnatal maternal anxiety, other postnatal factors may also account for the association between maternal anxiety during pregnancy and child outcome. More specifically, the child may be exposed to adverse factors in the postnatal environmental associated with prenatal maternal anxiety, such as increased maternal postnatal depression (Heron *et al.*, 2004; Austin *et al.*, 2007), increased parenting stress (Misri *et al.*, 2010), and negative attitudes of the mother towards parenthood (Hart and McMahon, 2006). In addition, research examining postnatal modification (i.e., by mother-child interactions) of the effects of maternal anxiety during pregnancy has shown that maternal prenatal and postnatal factors may act in concert to shape the outcome in the offspring (Bergman *et al.*, 2010; Grant *et al.*, 2010). Therefore, future studies should not only control for potential confounding factors, but also investigate the role that these factors may play in the pathway from prenatal exposure to maternal anxiety to behavioral and emotional problems later in life (Schlotz and Phillips, 2009) (see section 7.7. below).

7.7 Directions for future research

The results presented in this dissertation contribute to the current knowledge concerning the developmental origins of behavior, health and disease, but also stimulates the development of future research questions. In this section, we discuss directions for future research to complement and extend the research presented in this dissertation.

Exploring increased vigilance

Future research is needed to explore the increased vigilance hypothesis presented in this dissertation (see Figure 7.1.). First of all, as stated above, only parts of the model are supported by empirical evidence, not the hypothesis as a whole. Although our results contribute to an empirical basis for the increased vigilance hypothesis, future mediation analyses should test the model and its proposed causal chain in longitudinal cohort studies. Secondly, future research should explore the working hypothesis of increased vigilance by testing different types of (threat) simulation. Previous research on threat processing has demonstrated that when threat exceeds a certain threshold, it captures attention in everyone, regardless of the anxiety level of the participant (Mogg and Bradley, 1998; Wilson and MacLeod, 2003). It might therefore be that the unpleasant pictures used in the study presented in chapter 5 exceeded the threshold for threat and therefore captured high attention in all infants/children. To test this hypothesis, future studies should include ambiguous or mild versus high threat stimuli in their design. We expect that children prenatally exposed to high maternal anxiety will devote more attentional resources towards the ambiguous or mildly threatening stimuli than children prenatally exposed to low levels of maternal anxiety, while there will be no differences for the unambiguous, highly threatening stimuli.

Thirdly, other paradigms could also be used to investigate whether children prenatally exposed to maternal anxiety show increased vigilance. For example, eye-tracking studies could examine whether prenatal exposure to maternal anxiety is associated with altered eye-movements when viewing pictures/scenes. Previous research on vigilance using eye-tracking has demonstrated that hypervigilance is characterized by intense scanning of the environment, as seen in an increase of the number of eye-movements, and that increased scanning operates both in the presence and in the absence of threatening stimuli (Richards *et al.*, 2014).

Focus on potential protective factors

Most research in the context of the DOBHaD hypothesis has focused almost exclusively on the effect of negative factors, such as maternal anxiety, stress, and depression. Only very few studies investigated the consequences of exposure to positive maternal factors (Pluess *et al.*, 2012; Stapleton *et al.*, 2012). The results of this dissertation contribute to this relatively unexplored part of DOBHaD, but need to be extended. Most importantly, future research should identify protective factors that prevent the fetus from the effects of exposure to high levels of maternal anxiety by, for instance, decreasing the risk for experiencing anxiety during pregnancy.

We recommend to first exploring the influence of these factors in longitudinal cohort studies using mediation analyses (cf. chapter 4). Next, the identified factors should be incorporated in interventions for anxious pregnant women and evaluated in randomized controlled studies. Several studies so far have investigated the effect of mindfulness interventions during pregnancy on the anxiety level of the mother (Vieten and Astin, 2008; Duncan and Bardacke, 2010; Sriboonpimsuay *et al.*, 2011; Guardino *et al.*, 2013), but none of these studies have included child measures as outcome. Besides maternal mindfulness, other protective factors that could be considered are partner support and maternal coping strategies (Graignic-Philippe *et al.*, 2014).

Focus on potential benefits of developmental programming by early adversity

As mentioned before in this dissertation, the fetal phenotype is adapted to the environment in utero to increase survival of the unborn child during pregnancy and after (Bateson *et al.*, 2004). Maternal anxiety is therefore hypothesized to modulate developmental programming in a way that promotes those characteristics that increase the survival of the unborn child in fear-inducing environments. In this dissertation, we found evidence that children prenatally exposed to maternal anxiety have evolved increased vigilance. In a fear-inducing environment, increased vigilance may provide benefits to the child. Although many researchers discussed possible benefits hypothetically (e.g., Glover, 2011; Frankenhuis and Del Giudice, 2012; Del Giudice, 2014), no study to date has directly investigated whether prenatally stressed children show characteristics that are beneficial in some environments/situations. This is probably due to the predominant view that that early adversity, such as maternal anxiety, is ‘toxic’ and disrupts normal development of the offspring (Frankenhuis and Del Giudice, 2012; Del Giudice, 2014). We argue that this view should be reconsidered and attention should be paid to the possibility that children prenatally exposed to higher levels of maternal anxiety are adapted to specific situations, i.e., stressful and fear-inducing situations, and may therefore outperform children prenatally exposed to lower levels of maternal anxiety when examined in these specific situations.

For future research we therefore propose to explore the potential benefits of prenatal exposure to maternal anxiety in specially designed paradigms that simulate these specific situations. To increase the ecological validity, these investigations would benefit from the use of virtual reality techniques. Besides increased vigilance, these benefits may include enhanced exploration (due to higher distractibility and impulsivity), a willingness to break rules (due to higher conduct disorder traits), and an increased ability to fight intruders or predators (due to increased aggression and faster maturation).

Measuring maternal anxiety

The women included in this dissertation were selected from the normal Dutch population and therefore only a small amount of women experienced extreme levels of anxiety. Although the results of this dissertation clearly show that (subclinical) increases of maternal anxiety during pregnancy can have an impact on the child's brain and behavioral functioning, it might be interesting to investigate the effect of extreme levels of maternal anxiety. Only few studies have examined the impact of extreme anxiety/stress. For example, studies investigating maternal psychiatric disorders during pregnancy on child outcomes are sparse or used measures that are not valid for diagnosis (Alder *et al.*, 2007). Also, research on the impact of subjective maternal stress during extreme conditions, such as disasters, war or poverty, are also limited (Walder *et al.*, 2014; Cao-Lei *et al.*, 2015). Future research should consider investigating populations with more extreme levels of maternal anxiety in addition to the 'normal' populations with low to medium anxiety levels.

Controlling for postnatal influences

As already mentioned in section 7.6., most studies that investigated the consequences of maternal anxiety during pregnancy on child outcomes have statistically controlled for maternal postnatal anxiety and birth outcomes, including the studies in this dissertation. Still, a major complication of these studies is the fact that the relevant outcome measure (e.g., brain imaging) has been collected after birth and is therefore confounded by postnatal influences. One way to control for postnatal influences is with randomized controlled trials in which half of the pregnant women receive a stress-reduction intervention and the other have care as usual. Still, this does not eliminate the influence of postnatal impact but controls it by distributing it evenly over the two experimental groups.

To completely eliminate influences of the postnatal environment, measurement of the offspring's brain should be done before birth, *in utero*. Although techniques are available to do this, such as fetal magnetoencephalography (MEG), no study to date has used these techniques for the investigation of developmental programming (Schleussner *et al.*, 2001). Very recently, cutting-edge functional magnetic resonance imaging (fMRI) techniques have become also available for this purpose. The first fMRI studies of the fetal brain have proven the effectiveness of this technique for quantifying the fetal brain

(Schopf *et al.*, 2012; Thomason *et al.*, 2013). The potential of fetal MEG and fMRI for the investigation of early life influences on the development of the fetal brain is huge and it is likely to produce major discoveries.

Although eliminating postnatal influences may be necessary to prove the influence of maternal anxiety during pregnancy, investigating the moderating role of the postnatal environment for developmental programming may also be of importance, especially for the development of postnatal interventions designed to prevent the effects of prenatal stress. As mentioned in section 7.6., several studies have demonstrated that the postnatal environment can moderate the effects of prenatal exposure to maternal anxiety (Bergman *et al.*, 2010; Grant *et al.*, 2010). For future research on the effect of early life influences on child outcomes, we therefore recommend to include prenatal measurements of the fetal brain as well as postnatal measurements in order to be able to disentangle pre- and postnatal influences.

7.8 Implications for public health policy

The results of this dissertation complement a large body of research clearly showing that the intrauterine environment is important for later life functioning (Bock *et al.*, 2015). Despite the fact that the underlying mechanisms are still unclear, our results confirm that the nine months before birth should not be disregarded. Midwives, obstetricians, and general practitioners should therefore inform (future) pregnant women that the development of a child does not start at birth but at conception (and even before; Rodgers *et al.*, 2013; Zaidan *et al.*, 2013). Because many pregnant women do not seek help from mental health services midwives have a crucial role in the identification, support, and referral of women experiencing mental health problems. A recent investigation of student midwives in the UK revealed that they often under-estimated the risk of women with existing mental health problems, felt ill-prepared, and lacked confidence in caring for them (Jarrett, 2015). Although there has been no similar research in the Netherlands, these results emphasize the need for better informing and training midwives.

A short screening tool should be incorporated in routine prenatal care to target women in need for intervention. Also, effective interventions should become available for them. Our results showed beneficial effects of mindfulness for both mother and child and thus suggest mindfulness as an effective component of stress-reduction interventions for pregnant women experiencing anxiety and worries. Such interventions may provide pregnant women diagnosed with anxiety disorders with an alternative to pharmacological interventions. Alternatives are highly desired, since psychopharmacological treatment during pregnancy has been associated with detrimental effects for the fetus, such as higher risk for premature birth and low birth weight (Huang *et al.*, 2014) and reduced fetal head growth (El Marroun *et al.*, 2012). Moreover, stressing the beneficial effect of being mindful during pregnancy provides a more positive and possibly more effective message to pregnant women than stressing the potential dangers of being anxious.

7.9 General conclusion

We provided neurophysiological evidence that prenatal exposure to maternal anxiety is associated with altered neurocognitive functioning in the offspring. We also showed some indications for beneficial effects of maternal mindfulness during pregnancy, but more empirical investigation is required to prove this. A new hypothesis was proposed, the “increased vigilance hypothesis”. It suggests that prenatal exposure to maternal anxiety causes alterations in structure and function in the offspring’s brain which, in turn, leads to increased vigilance for detecting potential threat in the environment. Although useful in fear-inducing environments, increased vigilance may predispose children prenatally exposed to maternal anxiety to higher risk for developing behavioral and/or emotional problems later in life. Future research should provide more empirical tests of the increased vigilance hypothesis, however, since parts of the proposed causal chain lack (consistent) empirical evidence.

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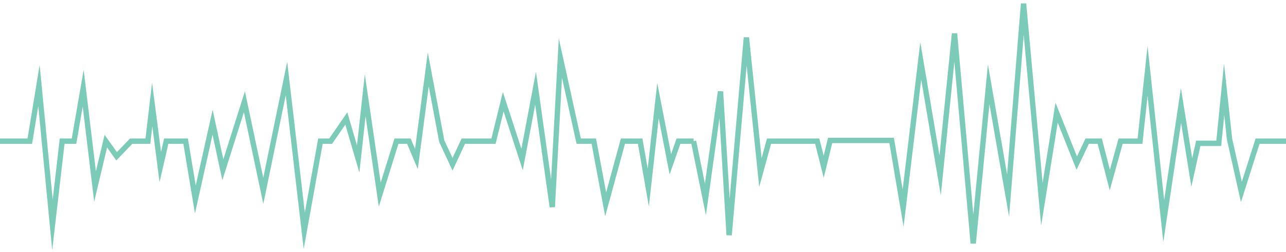
Summary



In **chapter 1** we introduced the concept of developmental origins of health and disease (DOHaD) – a field of research that focusses on the short- and long-term consequences of early life influences on health and risk for disease. We argued that the fetal brain may be particular sensitive to developmental programming since the brain is subject to dramatic developmental processes during the prenatal life period and introduced the developmental origins of *behavior*, health and disease (DOBHaD) hypothesis. The central aim of this dissertation was to examine the effect of exposure to maternal anxiety during pregnancy on neurocognitive functioning in infants and young children. Because research in the DOBHaD field almost exclusively focused on the effects of negative maternal states and traits we also included a potential positive/protective factor in our study: maternal mindfulness.

As described in **chapter 2**, the data for this dissertation was gathered as part of a longitudinal cohort study conducted at the Tilburg University, The Netherlands, following mothers and their children from pregnancy onwards. Prenatal data was collected three times during pregnancy, once in each trimester, while postnatal data was collected when the child was 2-4 months, 9 months and 4 years of age.

In **chapter 3**, we examined the effects of maternal anxiety and mindfulness during pregnancy on infant auditory attention. We found that infants exposed to higher levels of maternal anxiety during pregnancy devoted more attention to frequently occurring, standard sounds. The opposite was found for infants exposed to maternal mindfulness. These results indicate that heightened maternal anxiety during pregnancy may be associated with altered attentional processing in the offspring in that these infants devote increased attentional resources to uninformative sounds.

In **chapter 4**, we focused on the effects of maternal mindfulness during pregnancy on the socio-emotional development and temperament of the infant and the possible mediating role of maternal anxiety in this relation. The results suggested that high maternal trait mindfulness during pregnancy may have positive effects on infant socio-emotional functioning and temperament, potentially via reducing anxiety symptoms in pregnant women.

The effect of maternal anxiety and mindfulness on affective picture processing in four-year-olds was investigated in **chapter 5**. We found that children prenatally exposed to higher maternal anxiety devote more attentional resources to neutral but not to unpleasant pictures. These findings seem in line with the findings of chapter 3, as they both point to altered attentional processing in children prenatally exposed to maternal anxiety. More specifically, both studies show that prenatally exposed children devoted more attentional resources to uninformative, neutral stimuli (i.e., frequently presented standard sounds and neutral pictures of scenes).

For the purpose of **chapter 6** we re-analyzed data used in the study presented in chapter 3 to examine the effects of maternal anxiety and mindfulness on the functional brain network connectivity of infants. Although we found some interaction effects between maternal anxiety and sex on the brain network measures, these results did not survive statistical correction for postnatal maternal anxiety (at the age 9 months). Based on these results we have to conclude that we failed to find evidence for altered functional brain network configuration in infants prenatally exposed to maternal anxiety. It is unclear whether this was due to a genuine lack of impact of maternal anxiety during pregnancy on the infant's brain network configuration or due to methodological issues.

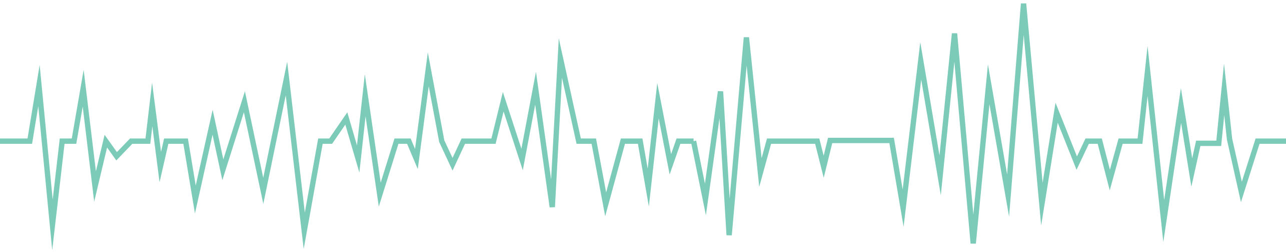
Finally, a new hypothesis, the "increased vigilance hypothesis", was proposed in **chapter 7** to integrate our results with previous findings. The hypothesis suggests that maternal anxiety during pregnancy alters the structure and function of the offspring's brain to adapt the unborn child to a potentially fear-inducing postnatal environment, resulting in increased vigilance for threat – a trait that is useful for survival in such environments. However, increased vigilance may predispose children prenatally exposed to maternal anxiety to higher risk for developing behavioral and/or emotional problems later in life.

We discussed that maternal anxiety during pregnancy may alter the structure and function of the developing brain via a complex interplay between biological mechanisms, including the hypothalamus-pituitary-adrenalin (HPA)-axis, placental functioning, and molecular mechanisms that alter gene activity without changing the DNA sequence (epigenetics). We also pointed out that shared genetic background of the mother and the child and postnatal influences may also (partly) explain our findings.

For future research, we recommend to test the proposed hypervigilance hypothesis by experimenting with different types of (threat) simulation, other research methods (i.e., eye-tracking), and other task paradigms. Secondly, future research should identify protective factors, besides maternal mindfulness, that are able to decrease or eliminate the effects of exposure to high levels of maternal anxiety on the fetus. Thirdly, the results of this dissertation show that moderate (subclinical) increases in maternal anxiety during pregnancy affect offspring's neurocognitive functioning. It may also be interesting to examine the effect of more extreme levels of maternal anxiety, such as anxiety disorders or extreme stress (e.g., war, poverty). Finally, we argued that future research should find better ways to control for postnatal influences. A good way to eliminate postnatal influences in future studies is to measure the brain *in utero*, for example with fetal fMRI measurements. The potential moderating influence of postnatal influences on developmental programming should also be investigate in future research, as it may open windows for early postnatal interventions.

Finally, our results have implications for public health policy. First, we recommend that midwives, obstetricians, and general practitioners should inform pregnant women that the development of a child does not start at birth but at conception or even before conception. Second, a short screening tool should be incorporated in routine prenatal care to target women in need for intervention. Finally, since our results showed beneficial effects of mindfulness for both mother and child, we suggest mindfulness as a component of interventions for pregnant women experiencing anxiety and worries. Such interventions may provide pregnant women prone to or diagnosed with anxiety disorders with an alternative to pharmacological interventions.

Samenvatting



In **hoofdstuk 1** hebben we het concept 'developmental origins of health and disease' (DOHaD) geïntroduceerd – een onderzoeksveld dat zich richt op de korte- en lange termijn gevolgen van invloeden in de vroege levensjaren op gezondheid en het risico op ziektes. We bespraken dat het foetale brein mogelijk bijzonder sensitief is voor programmeren tijdens de ontwikkeling omdat het foetale brein enorme ontwikkelingsprocessen doormaakt tijdens de prenatale periode. Vervolgens introduceerden we de hypothese 'developmental origins of *behavior*, health and disease (DOBHaD)'. Het centrale doel van deze thesis was het onderzoeken van de effecten van blootstelling aan angst van de moeder tijdens de zwangerschap op het neurocognitieve functioneren van baby's en jonge kinderen. Omdat onderzoek in het DOBHaD veld bijna volledig gefocust is op negatieve factoren van de moeder, hebben wij ook een potentiele positieve/beschermende factor toegevoegd aan onze studie: mindfulness van de moeder.

Zoals beschreven in **hoofdstuk 2** is de data van deze dissertatie verzameld als onderdeel van een longitudinale cohort studie uitgevoerd aan Tilburg University die moeders vanaf het eerste trimester van de zwangerschap volgt. Prenatale data is verzameld op drie tijdstippen tijdens de zwangerschap, één keer in elk trimester, en postnatale data is verzameld toen het kind 2-4 maanden, 9 maanden en 4 jaar oud was.

In **hoofdstuk 3** hebben we het effect van angst en mindfulness van de moeder tijdens de zwangerschap op auditieve aandacht van baby's onderzocht. We vonden dat baby's die blootgesteld waren aan hogere angst van de moeder tijdens de zwangerschap meer aandacht besteedden aan frequent gepresenteerde, standaard geluiden. Het omgekeerde was gevonden voor baby's die tijdens de zwangerschap blootgesteld waren aan hogere mindfulness van de moeder. De resultaten geven aan dat hogere angst van de moeder tijdens de zwangerschap mogelijk geassocieerd is met veranderingen in aandachtsprocessen in de nakomelingen, namelijk dat ze meer aandacht lijken te besteden aan niet-informatieve geluiden.

In **hoofdstuk 4** hebben we ons gericht op de effecten van mindfulness van de moeder tijdens de zwangerschap op de sociaal-emotionele ontwikkeling en het temperament van de baby's en de mogelijke mediërende rol van angst van de moeder in deze relatie. De resultaten suggereerden dat een hoge mate van mindfulness als karaktertrek van de moeder tijdens de zwangerschap een positief effect heeft op de sociaal-emotionele ontwikkeling en het temperament van de baby, mogelijk via het verlagen van angst symptomen bij zwangere vrouwen.

Het effect van angst en mindfulness op de verwerking van emotionele afbeeldingen in vierjarigen werd in **hoofdstuk 5** onderzocht. We hebben gevonden dat kinderen die tijdens de zwangerschap blootgesteld waren aan hogere angst van de moeder meer aandacht besteden aan neutrale afbeeldingen, maar niet aan onplezierige afbeeldingen. Deze bevindingen lijken op één lijn te staan met de bevindingen van hoofdstuk 3, aangezien ze beiden wijzen op veranderingen in aandachtsprocessen in kinderen blootgesteld aan angst van de moeder tijdens de zwangerschap. Meer specifiek wijzen beide studies erop dat prenataal blootgestelde kinderen meer aandacht besteden aan niet-informatieve, neutrale stimuli (frequent gepresenteerde, standaard geluiden en afbeeldingen van neutrale scenes).

Voor het doel van **hoofdstuk 6** hebben we de data van de studie gepresenteerd in hoofdstuk 3 opnieuw geanalyseerd om het effect van angst en mindfulness van de moeder tijdens de zwangerschap op de functionele hersennetwerk connectiviteit van baby's te onderzoeken. Ondanks dat we enkele interactie effecten vonden tussen angst van de moeder en sekse van de baby op de hersennetwerk maten, bleven deze resultaten niet overeind na statistische correctie voor postnatale angst van de moeder (op de leeftijd van 9 maanden). Gebaseerd op deze resultaten moeten we concluderen dat we geen bewijs hebben gevonden voor veranderingen in het functionele hersennetwerk van baby's die prenataal blootgesteld zijn aan angst van de moeder. Het is onduidelijk of dit veroorzaakt is door een werkelijk ontbreken van een effect van angst van de moeder tijdens de zwangerschap op het hersennetwerk van baby's of door methodologische tekortkomingen.

Ten slotte werd een nieuwe hypothese, de 'verhoogde vigilantie hypothese', geopperd in **hoofdstuk 7** om onze resultaten te integreren met voorgaande bevindingen. De hypothese veronderstelt dat angst van de moeder tijdens de zwangerschap de structuur en functie van de hersenen van de baby veranderd zodat het ongeboren kind zich kan aanpassen aan de potentieel angst-opwekkende omgeving na de geboorte, met als gevolg een verhoogde vigilantie voor gevaar – een karaktereigenschap die nuttig is voor het overleven een dergelijke omgeving.

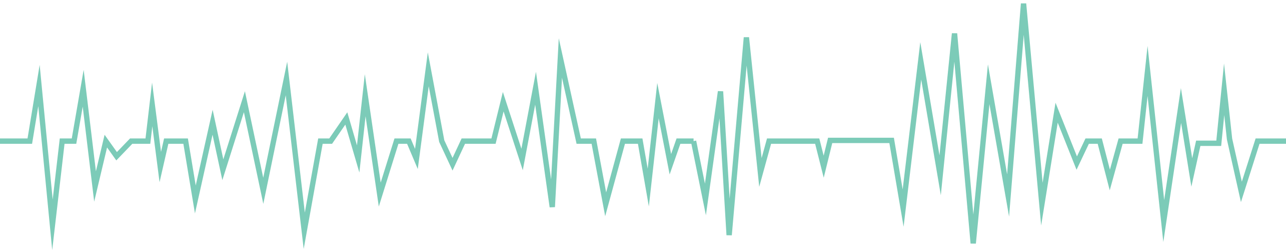
We hebben besproken dat angst van de moeder tijdens de zwangerschap de structuur en functie van het ontwikkelende brein mogelijk kan veranderen via een complexe samenwerking van biologische mechanismen, inclusief de hypothalamus-hypofyse-bijnier (HPA)-as, het functioneren van de placenta en moleculaire mechanismen die de activiteit van genen veranderen zonder de structuur van het DNA aan te passen (epigenetica). Ook hebben we benadrukt dat een gedeelde genetische achtergrond van moeder en kind en postnatale invloeden mogelijk ook (gedeeltelijk) onze bevindingen kunnen verklaren.

Voor vervolg onderzoek bevelen wij aan om de verhoogde vigilantie hypothese te toetsen door te experimenteren met verschillende soorten (bedreigende) simulatie, andere onderzoeksmethodes (zoals eye-tracking) en andere taak paradigma's. Ten tweede zou toekomstig onderzoek beschermende factoren moeten identificeren, naast mindfulness van de moeder, die in staat zijn om de effecten van prenatale blootstelling aan hoge angst van de moeder op de foetus te verminderen of te elimineren. Ten derde, de resultaten van deze dissertatie hebben laten zien dat gematigde (subklinische) verhoging van angst bij zwangere vrouwen het neurocognitief functioneren van de nakomelingen beïnvloed. Het zou ook interessant zijn om het effect van meer extreme angst bij zwangere vrouwen te onderzoeken, zoals angststoornissen of extreme stress (bijvoorbeeld tijdens oorlog of ernstige armoede). Ten slotte bespraken we dat toekomstig onderzoek een betere manier moet vinden om te controleren voor postnatale invloeden. Een goede manier om postnatale invloeden te elimineren in toekomstig onderzoek is door het meten van het brein *in utero*, bijvoorbeeld met behulp van foetaal fMRI onderzoek. De potentiële mediërende invloed van de postnatale omgeving voor de ontwikkeling zou ook onderzocht moeten worden in vervolg onderzoek, aangezien het mogelijk deuren opent voor vroege postnatale interventies.

Tot slot hebben onze resultaten implicaties voor het volksgezondheidsbeleid. Ten eerste raden wij verloskundigen en (huis)artsen aan om zwangere vrouwen te informeren over het feit dat de ontwikkeling van hun kind niet begint bij de geboorte maar bij conceptie of zelfs vóór conceptie. Ten tweede denken wij dat een kort screeningsinstrument geïntegreerd zou moeten worden in de routine van prenatale zorg om de vrouwen te identificeren die baat hebben bij een interventie. Omdat onze resultaten gunstige effecten lieten zien van mindfulness voor zowel moeder als kind, raden wij aan dat mindfulness wordt opgenomen als component in interventies voor zwangere vrouwen die angst en zorgen ervaren. Een dergelijke interventie kan zwangere vrouwen die gevoelig zijn voor angst klachten of gediagnostiseerd zijn met een angststoornis een alternatief bieden voor farmaceutische interventies.

Appendix:

Differences between human auditory event-related potentials (AERP) measured at 2 and 4 months after birth



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Abstract

Infant auditory event-related potentials (AERP) show a series of marked changes during the first year of life. These AERP changes indicate important advances in early development. The current study examined AERP differences between 2- and 4-month-old infants. An auditory oddball paradigm was delivered to infants with a frequent repetitive tone and three rare auditory events. The three rare events included a shorter than the regular inter-stimulus interval (ISI-deviant), white noise segments, and environmental sounds. The results suggest that the N250 infantile AERP component emerges during this period in response to white noise but not to environmental sounds, possibly indicating a developmental step towards separating acoustic deviance from contextual novelty. The scalp distribution of the AERP response to both the white noise and the environmental sounds shifted towards frontal areas and AERP peak latencies were overall lower in infants at 4 compared to at 2 months of age. These observations indicate improvements in the speed of sound processing and maturation of the frontal attentional network in infants during this period.

Introduction

Infant auditory event-related potentials (AERPs) are often used to index the early development of information processing (for reviews, see Csibra et al., 2008; Kushnerenko et al., 2013; Leppänen et al., 2004). AERPs are based on non-invasive recording of brain activity and they are elicited even in the absence of conscious attention making them a useful tool for infant research. Because they do not require behavioral responses, AERPs elicited by identical stimulation can be compared across age groups irrespective of the rapidly changing behavioral capabilities of young infants. The functionality of processing rapidly presented sound sequences, sound discrimination, and categorization are important prerequisites of auditory perception including speech and language acquisition (e.g., Benasich et al., 2002; Leppänen et al., 2002).

The morphology of AERP responses undergoes large changes with learning and maturation of the infantile nervous system during the first year of life (Fellman and Huotilainen, 2006; Kushnerenko et al., 2002a; Kushnerenko et al., 2002b; Leppänen et al., 2004; Morr et al., 2002). The infant brain undergoes profound structural and functional maturation, such as synaptogenesis (Huttenlocher, 1984) and increases in myelination in the temporal lobe (Deoni et al., 2011). These, as well as the effects of early learning are reflected in morphological and functional changes in the infantile AERP responses. Mapping typical and atypical developmental trajectories of information processing requires the characterization of AERP changes occurring within relatively short periods of time. In our review of developmental AERP changes during the first year of life (Kushnerenko et al., 2013), we noticed a dip in the number of electrophysiological studies targeting auditory processes during the first few months of infancy. Whereas much effort has been invested into characterizing AERPs at birth and at ca. 6 month, little is known about the typical AERP development between these two time points. He et al. (2009) suggested that the ability to detect pitch change similarly to adults probably emerges between 2 and 4 months of age. Moreover, previously Gomes et al. (2000) have shown that between 2 and 4 months of age, infants orient more to novel visual stimuli. However, it is yet unknown whether or not this also applies to the auditory modality. Thus, this short time period seems to be critical in the development of sound processing. The current study has been aimed at characterizing the development of AERPs during this period.

The auditory oddball paradigm allows assessing AERPs for both frequent repetitive and rare deviant sounds. Although by tradition, many previous studies focused on the differential response to rare and frequent sounds [the mismatch response (MMR); Alho et al., 1990; Dehaene-Lambertz and Gliga, 2004; Morr et al., 2002], it can be misleading in a developmental comparison, because this approach assumes that the developmental AERP changes for the frequent and infrequent sound *per se* (often of quite different acoustic makeup) have been identical, and thus the development of the difference response can be separately assessed. It is possible that a part of the previously observed dramatic developmental MMR changes during the first year of life (see Kushnerenko et al., 2002b; Leppänen et al., 2004; Morr et al., 2002) could have originated from separate developmental alterations of the AERPs elicited by the frequent and infrequent sounds employed. Because we aimed to assess AERP differences between two age groups for acoustically widely different sounds (white noise segments and environmental sounds), the frequent standard and some of the rare deviant sounds were qualitatively different. Therefore, we took the approach of separately assessing the responses to these sounds, testing the elicitation of MMR only when the standard and the deviant were identical sounds.

Large spectral changes (such as white noise and novel sounds appearing within a sequence of a repeating complex tone) have been found to reduce inter-individual variability and to improve the replicability of AERP responses in infants (Kushnerenko et al., 2002b; Kushnerenko et al., 2007), and might therefore provide a reasonably reliable assessment of typical AERP development. However, to date, only a few studies focused on the maturation of the processing of rare white noise and novel sounds. In newborn infants, Kusnherenko and collagues (2007) obtained similar responses for these two types of sounds in the context of a frequent tone stimulus. Háden and colleagues (2013) showed in neonates that whereas the morphology of the AERPs elicited by environmental sounds depended on the context (environmental sounds presented alone vs. amongst repeating complex tones), only the amplitude of the AERPs elicited by white noise segments was affected by the same manipulation. Otte and colleagues (2013) then obtained different AERP responses to white noise and novel sounds presented in the context of a repeating complex tone at 2 months of age. Using the same stimulus paradigm in the current study as Otte and colleagues (2013), we will look for further developmental steps in separating acoustic deviance from contextual novelty.

The present study explores the maturation of AERPs elicited in the context of the auditory oddball paradigm, comparing infants of 2 and 4 months of age. We expected that the separation of contextual novelty and acoustic deviance widens from 2 to 4 month of age and thus the difference between the AERPs elicited by white noise and environmental sounds to become larger during this period. Using Otte and colleagues' (2013) stimulus paradigm also allowed an investigation of the processing violations of a temporal regularity by recording responses to infrequent shortenings of the otherwise constant inter-stimulus interval (ISI). Auditory temporal features carry important information relevant to speech perception, such as stress and prosody. Recent evidence suggests that impairments in temporal processing skills are associated with developmental dyslexia (e.g., Flaugnacco et al., 2014). Thus, mapping the typical development of AERPs elicited by violating a temporal regularity may be useful as an early indicator of development in language acquisition.

Methods

The study was approved by the 'Central Committee on Research involving Subjects' and was conducted in full compliance with the Helsinki declaration. All mothers and fathers signed an informed consent form after the goals and procedures of the study had been explained to them. The experiment was conducted within the framework of a prospective study assessing the long-term effects of prenatal exposure to maternal anxiety, i.e. the Prenatal Early Life Stress (PELS)-study. Here we focus on comparing AERPs between the full 2- and 4-month-old groups of awake infants. Data obtained at 2 months of age have been previously reported by Otte et al. (2013), who compared the ERP responses between sleeping and awake infants.

Participants

Participants from a typical (i.e., non-clinical) population had been recruited from a general hospital and four midwives' practices: 178 women had been recruited before their 15th week of pregnancy and 12 women between the 15th and the 22nd week of their pregnancy. The women were followed up during their pregnancies and were invited for postnatal observations either 2 or 4 months after the birth of their baby. From the 91 2-month-olds and the 43 4-month-olds who participated in the ERP measurements, we included a total of 36 2-month-olds (19 girls) and 26 4-month-olds (12 girls) in the current study. These 2-month-olds had a mean age of 9.59 weeks (SD = .87), a mean birth weight of 3470 g (SD = 508) and a mean gestational age of 39.78 weeks (SD = 1.36). The 4-month-olds had a mean age of 17.96 weeks (SD = 3.48), a mean birth weight of 3443 g (SD = 354) and a mean gestational age of 39.73 (SD = 1.19).

The data obtained from infants who fell asleep during the experiment (40 2-month-olds and 9 4-month-olds) were excluded from the analysis because previous results suggested that the infants' state of alertness affects the AERP responses (e.g. Friederici, Friedrich, & Weber, 2002; Otte, et al., 2013) and we did not have sufficient number of sleeping 4-month olds to assess the state of alertness effects. In addition, from the 2-month-old group, nine infants were excluded because of too few (< 40) acceptable EEG epochs (due to excessive movements/artefacts), two because of excessive crying, and another four due to technical problems. From the 4-month-old group, four infants were excluded because of too few acceptable EEG epochs, three due to excessive crying, and one because the infant had been born prematurely. There were no significant differences among the excluded and included infants in gestational age or birth weight. All infants were healthy and had passed an otoacoustic emission-based screening test for hearing impairments, performed by a nurse from the infant health care clinic between the 4th and the 7th day after birth.

Stimuli and procedure

The infants were presented with an auditory oddball sequence composed of four types of sound events: A complex tone of 500 Hz base frequency presented with .7 probability following an ISI (offset-to-onset interval) of 300 ms (the "standard" tone); the same tone following an ISI of 100 ms (.1 probability; the "ISI-deviant"); a white noise segment (.1 probability; 300 ms ISI); and various environmental sounds (.1 probability; 300 ms ISI; "novel sounds"). Standard and ISI-deviant tones were constructed from the 3 lowest partials, with the intensity of the second and third partials set 6 and 12 dB lower, respectively, than that of the base harmonic. The novel sounds were 150 unique environmental sounds (e.g., door slamming, dog barking, etc.) and they were presented only once during the experiment to maintain their novelty throughout. The short ISI was chosen because larger MMR amplitudes were reported with faster rather than slower presentation rates for 2-month and 4-month-old infants (He, Hotson, & Trainor, 2009). The common stimulus duration was 200 ms including 10 ms rise and 10 ms fall times, resulting in an onset-to-onset interval of 500 ms preceding the standard tones, white noise and novel sounds, and 300 ms preceding the ISI-deviant tones; the common intensity was 75 dB (SPL). Sequences consisted of 1500 stimuli presented in a pseudorandom order with the restriction that novel/white noise stimuli were always preceded by two or more standard tones or a combination of a standard tone and an ISI-deviant. Further, consecutive ISI-deviants were always separated from each other by at least two standards or by a standard tone combined with either a white noise or a novel sound. The sequences were divided into 5 blocks of 300 stimuli, each, which were presented to the infants in a counterbalanced order. The duration of each stimulus block was approximately 2.5 minutes resulting in a total of 12.5 minutes for the whole recording.

The experiment took place in a dimly lit and sound-attenuated room at the Developmental Psychology Laboratory of the university. The complete procedure including electrode placement and removal, EEG recording, and necessary breaks lasted for approximately 60 minutes. During the EEG recording, infants sat or lay on their parent's lap between two loudspeakers placed at a distance of 80 cm from each side of the infant's head. The whole experimental procedure was recorded with two cameras: One placed behind and the other in front of the infant and the parent. The camera recordings were used to determine whether the baby was crying or moving.

ERP measurement and data processing

EEG was recorded with Biosemi Active Two amplifiers (www.biosemi.com) with a sampling rate of 512 Hz and filtered by a 5th order sinc filter with the -3 dB point at 1/5th of the sample rate (~102 Hz). Sixty-four Ag/AgCl electrodes were placed on the infant's scalp according to the International 10-20 system. Two reference electrodes were placed on the left and right mastoids; these were later mathematically combined to produce an average mastoids reference derivation (Luck, 2005).

Data were analysed using Brain Vision Analyzer 2.0.2 using the ActiView software (Brain Products GmbH). The signals were filtered (phase shift-free Butterworth filters) off-line with a bandpass of 1.0 – 30 Hz (slope 24 dB). These filter settings were chosen to make the results comparable with the study by Otte et al. (2013) and to add compatible evidence to the maturational database created by previous studies (e.g., Brannon et al., 2004; He et al., 2007; Kushnerenko et al., 2007).

Subsequently the data were segmented into epochs of 600 ms duration including a 100 ms pre-stimulus period. Epochs with a voltage change exceeding 150 μV within a sliding window of 200 ms duration as well as those including changes that exceeded the rate of 100 μV/ms at any electrode were rejected from further analysis. On average, the number of remaining trials included for analysis for the four stimulus types were as follows: standard: 600 (2-month-olds) vs. 601 (4-month-olds); ISI-deviant: 86 (2-month-olds) vs. 87 (4-month-olds); white noise: 85 (2-month-olds) vs. 86 (4-month-olds); novel sounds: 86 (2-month-olds) vs. 88 (4-month-olds). Infants with less than 40 trials for any of the four stimulus categories were rejected from further analysis (see the *Participants* section above). Next, ERPs for each infant were averaged separately for the four different stimulus types and baseline-corrected to the average voltage in the 100 ms pre-stimulus period.

The time windows for peak detection were selected on the basis of visual inspection of the group-average ERPs from the electrodes F3, Fz, F4, C3, Cz, C4, P3, Pz, and P4, separately for the standard and the three oddball stimuli. The following time windows were used for peak latency measurements in individual infants: for the standard (see Figure 1), a time window of 180-250 ms was set for the positive peak corresponding to P2; for the ISI-deviant (see Figure 2) a time window of 175-275 ms was set for the negative and another of 350-500 ms for the positive peak corresponding to the N250 and P350, respectively; for the white noise sound (see Figure 3), a window was set at 100-200 ms for the first positive peak corresponding to the early part of the bifurcated infantile P2 (P150), another one at 175-275 ms for the negative-going peak corresponding to the N250, and a third one at 300-450 ms for the second positive peak (P350); for the novel sound (see Figure 4), a time window of 250-450 ms was set for the positive peak corresponding to the infant P3a waveform. Peaks were termed in accordance with the nomenclature set up by Kushnerenko et al. (2002a,b; 2007). Note that the time windows of the white-noise P350 and novelty P3a extend over the onset of a possible following ISI-deviant tone. However, because the average amplitude of the ISI-deviant response in the overlap period is low (below 1 μV) and the overlap occurs only for ca. 10% of the white-noise and novel sounds, the bias caused by the overlap is below 3% of the measured amplitudes and it is approximately equal between the two age groups. Peaks were detected automatically as the highest/lowest point within the respective time windows. Average amplitudes were measured as the mean voltage in 60 ms long intervals centred on the peaks of the group-averaged response, separately for the two age groups.

In addition, for illustration purposes, topographical maps were created for the components showing significant ERP scalp distribution differences between 2- and 4-month-olds for the white noise and novel sounds. From the 64 electrodes, P9, P10, O1, Oz, O2, and Iz had to be excluded, because almost all infants showed a large number of artefacts on these electrodes (this was probably caused by the round shape of the EEG cap not matching well with the infants' typical shape at the back of the head). When necessary, the signals recorded from the included electrodes were interpolated by spherical spline interpolation (order = 4, degree = 10, and lambda = 1E-05; Perrin et al., 1989).

Statistical analysis

For comparing the two age groups on peak latency and amplitude measures at the nine electrode sites, mixed model repeated-measures ANOVAs with 'Anterior vs. Posterior' (frontal, central, parietal) x 'Laterality' (left, medial, right) as within-subjects factors, and 'Age-Group' (2 months and 4 months) as a between-subject factor were carried out for each stimulus type (standard, ISI-deviant, noise, and novel sound) and component. All analyses were controlled for gestational age at birth (GA) and birth weight (BW) of the infants by including these variables into the analysis as covariates. These covariates were included because previous studies, especially those of prematurely born infants, showed effects of gestational age at birth and birth weight on auditory ERPs (e.g., Hövel et al., 2014) and brain development (Ment and Vohr, 2008). Only effects including the Age-Group factor are interpreted. For the significant Age-Group × Anterior vs. Posterior interactions post-hoc tests were conducted by separate ANOVAs of Age-Group for the frontal (F3, Fz, F4), central (C3, Cz, C4), and parietal arrays of electrodes (P3, Pz, P4). For the significant Age-Group by Laterality interactions, ANOVAs of Age-Group were separately conducted for the left (F3, C3, P3), middle (Fz, Cz, Pz), and right array of electrodes (F4, C4, P4). GA and BW were always corrected for in post-hoc tests, too.

For assessing whether the brain of 2-month and 4-month-old infants distinguished the ISI-deviant from the standard stimulus, we subtracted the response to the standard from that of the ISI-deviant and then compared the difference against zero by repeated-measure ANOVAs with 'electrodes' (all nine electrodes) as a within-subject factor, separately for each age group and peak.

All statistical analyses were performed using SPSS 19.0 for Windows.

Greenhouse-Geisser correction was applied where appropriate (ε correction factors reported). All significant (α = .05) results involving the age-group factor are reported, together with the partial η^2 effect size values.

Results

Table 1 shows the group-mean peak latencies and mean amplitudes measured from 60-ms long windows centered at the mean peak latency (μV) averaged over all nine electrodes for all stimuli, separately for the 2- and 4-month-olds.

Table 1. *Group-average peak latencies (ms) and mean amplitudes measured from 60-ms long windows centered on the mean peak latency (μV) averaged over the nine analyzed electrodes, separately for the 4 stimulus types, the ERP peaks (rows) and for the 2- and 4-month-old infants (columns).*

Stimulus type and ERP peak	Peak latency (SD)		Mean amplitude (SD)	
	2-month-olds (N=36)	4-month-olds (N=26)	2-month-olds (N=36)	4-month-olds (N=26)
Standard tone				
P2	214 (3)	206 (4)	.2 (.3)	.5 (.3)
ISI-deviant				
N250	216 (7)	210 (8)	-2.1 (.4)	-2.2 (.5)
P350	435 (6)	411 (7)	.7 (.5)	1.6 (.6)
White noise				
P150	167 (4)	148 (5)	2.5 (.5)	2.2 (.6)
N250	221 (4)	224 (5)	1.2(.6)	-.4 (.7)
P350	383 (5)	365 (6)	4.3 (.6)	3.5 (.7)
Novel				
P3a	308 (7)	325 (8)	5.2 (.6)	5.1 (.7)

Standard tone

The standard tone elicited a fronto-centrally distributed P2 in both age groups (Figure 1). Although a significant Age-Group x Anterior vs. Posterior interaction was found for the peak latency [$F(2;120)=4.61$, $p<.05$, $\eta^2=.071$, $\varepsilon=.764$], this interaction was no longer significant after controlling for gestational age at birth and birth weight. The ANOVA of the mean amplitudes yielded a significant Age-Group × Anterior vs. Posterior interaction [$F(2;108)=5.00$, $p<.05$,

η^2=.085, ε=.838]. Post hoc tests showed a trend for a difference between 2- and 4-month-olds at the central electrodes, with a larger amplitude for 4-month-olds [*F*(1;54)=3.72, *p*=.06, η^2=.064], but no significant difference at the frontal and parietal electrodes. Controlling for gestational age and birth weight did not change this result.

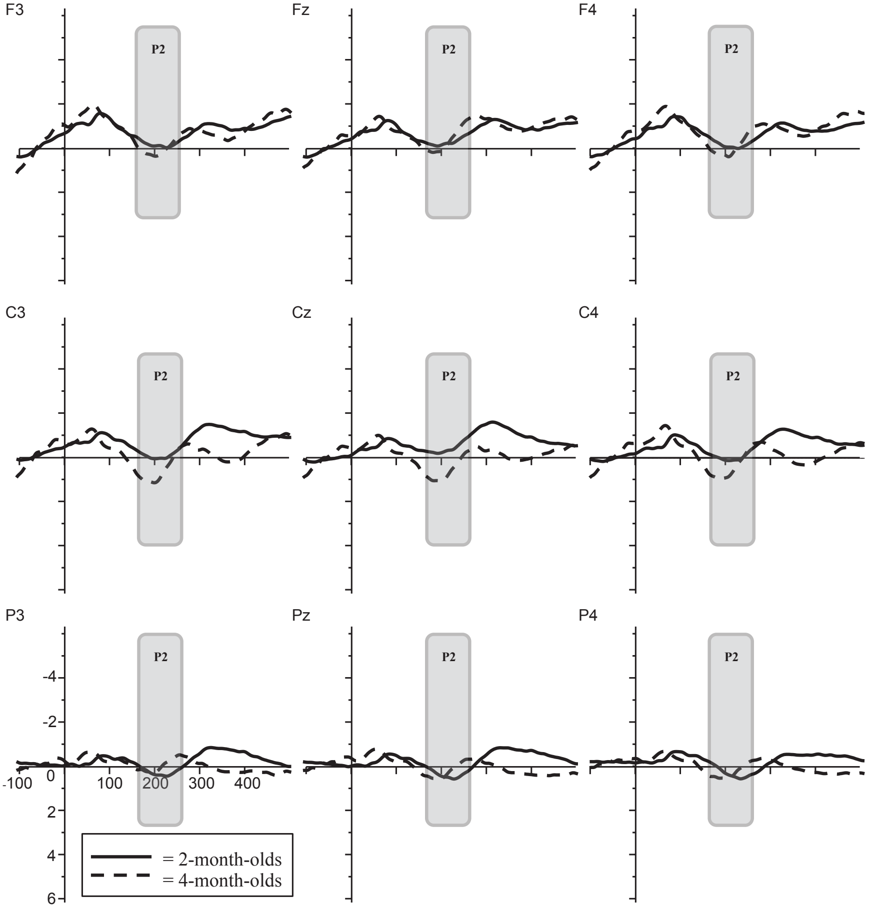

Figure 1. *Group-average ERP responses elicited by the standard tones in the 2- (solid line) and 4-month-old infants (dashed line) over frontal, central and parietal electrodes. The grey box at Cz indicates the time window within which the "P2" amplitudes were measured.*

ISI-deviant

Figure 2 shows that in both age groups, ISI-deviants elicited a fronto-centrally distributed negative-going wave (N250) followed by a similarly distributed positive-going wave (P350).

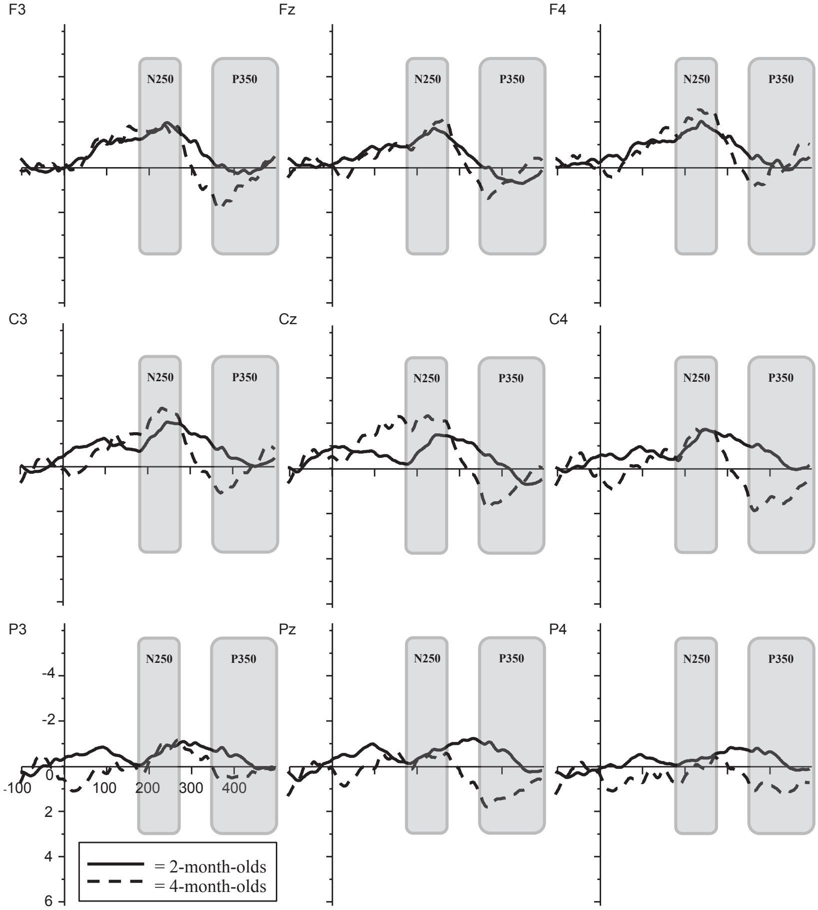

Figure 2. *Group-average ERP responses elicited by the ISI-deviant in the 2- (solid line) and 4-month-old infants (dashed line) over frontal, central and parietal electrodes. The grey boxes at Cz indicate the time windows within which the "N250/MMR" and "P350" amplitudes were measured.*

Age had a significant effect on the peak latency of the P350, with a shorter peak latency for the 4- than the 2-month-olds [$F(1;60)=8.05$, $p<.01$, $\eta^2=.118$].

Controlling for gestational age and birth weight did not alter this effect. Because the ISI-deviant sound is identical to the standard sound, for this rare sound we also tested whether the violation of the temporal regularity elicited a significant MMR response. The response to the ISI-deviant significantly differed from the standard-stimulus response for both age groups and latency ranges: N250 [*F*(1;35)=29.13, *p*<.001 and *F*(1;25)=13.53, *p*<01, in the 2- and the 4-month-old group, respectively] and P350 [*F*(1;35)=-3.263, *p*<.01 and *F*(1;25)=7.74, *p*<.01, respectively]. Thus, violating a temporal regularity elicited significant MMR responses in both age groups.

White noise

White noise sounds elicited a waveform with the following peaks: the P2 dissociated into P150 and P350 separated by the emerging N250 (Figure 3A). Figure 3A shows that in the 4-month-olds, the presence of an N250 component is more apparent than in the 2-month-olds. Thus the N250 appears to become prominent between 2 and 4 month of age. This interpretation is supported by the finding of a significant interaction between ‘Age Group’ and ‘Laterality’ for the N250 peak amplitude [*F*(2;108)=3.25, *p*<.05, η^2=.057, ε=.970]. Post-hoc test indicated that the amplitude differs between 2- and 4-month-olds mainly over the right hemisphere [*F*(1;54)=4.29, *p*<.05, η^2=.074], with a positive mean value for the 2-month-olds and a negative one for the 4-month-olds [1.4 vs. -.7 μV].

Figure 3A shows that the P150 and the P350 responses to white noise peaked significantly earlier in the 4- than in the 2-month-olds: for P150 [148 vs. 168 ms, respectively; *F*(1;60)=7.39, *p*<.01, η^2=.120] and for P350 [362 vs. 383 ms, respectively; *F*(1;54)=6.00, *p*<.05, η^2=.100]. For the P350 amplitude, a significant Age group by Anterior vs. Posterior interaction was obtained [*F*(2;108)=5.31, *p*<.05, η^2=.089, ε=.675]. Post-hoc tests revealed larger amplitudes in the 2- compared with the 4-month-olds at the parietal electrodes [*F*(1;54)=7.57, *p*<.01, η^2=.123], but not at the frontal or central electrodes. Figure 3B shows that in 2-month-olds, P350 peaks over parietal areas; in contrast, in 4-month-olds, this response appears to have shifted more towards frontal areas with very low amplitudes over parietal areas.

To examine whether the P350 amplitude difference between 2- and 4-month-olds was due to the emergence of the negative N250 peak, we performed correlation analysis (Pearson correlation) between the N250 and P350 amplitudes averaged over all electrodes and pooling the two age groups. The analysis revealed a significant positive correlation between the amplitudes measured from the N250 and P350 latency ranges [$r(1;62)=.65$, $p<.001$]. This result shows that when the N250 is not prominent (i.e., the voltage in the N250 range is positive), the P350 amplitude is large. When the N250 emerges (smaller positive or negative values) reducing or even eliminating the positivity in its range, the P350 amplitude is reduced.

Novel sounds

Novel sounds elicited a slow positive-going waveform (the P3a; Figure 4A). A significant Age-Group × Anterior vs. Posterior interaction was found for the P3a amplitude [$F(2;108)=12.84$, $p<.001$, $\eta^2=.192$, $\varepsilon=.766$]. Post-hoc tests revealed a significant parietal difference between the two age groups with lower P3a amplitudes at 4 than at 2 month of age [$F(1;60)=7.58$, $p<.01$, $\eta^2=.123$]. This pattern of results suggests that the P3a response to novel sounds has shifted towards frontal areas in 4- compared to 2-month-olds (see Figure 4B).

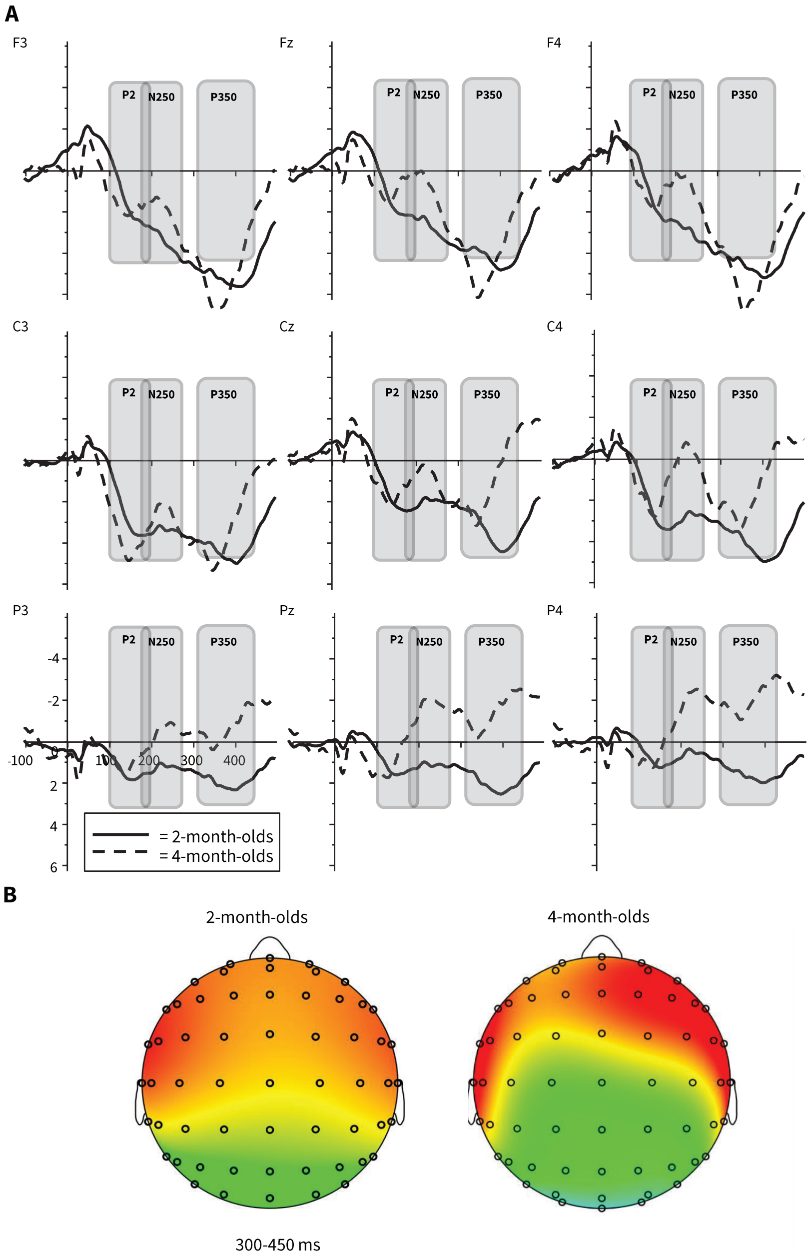

Figure 3 (***A***) *Group-average ERP responses elicited by the Noise sounds in the 2- (solid line) and 4-month-old infants (dashed line) over frontal, central and parietal electrodes. The grey boxes at Cz indicate the time windows within which the "P2", "N250", and "P350" amplitudes were measured. And* (***B***) *topographical maps for the P350 amplitude elicited by white noise segments in 2- (left) and 4-month-old infants (right), measured over 300-450 ms time period. The common color scale is below the left panel.*

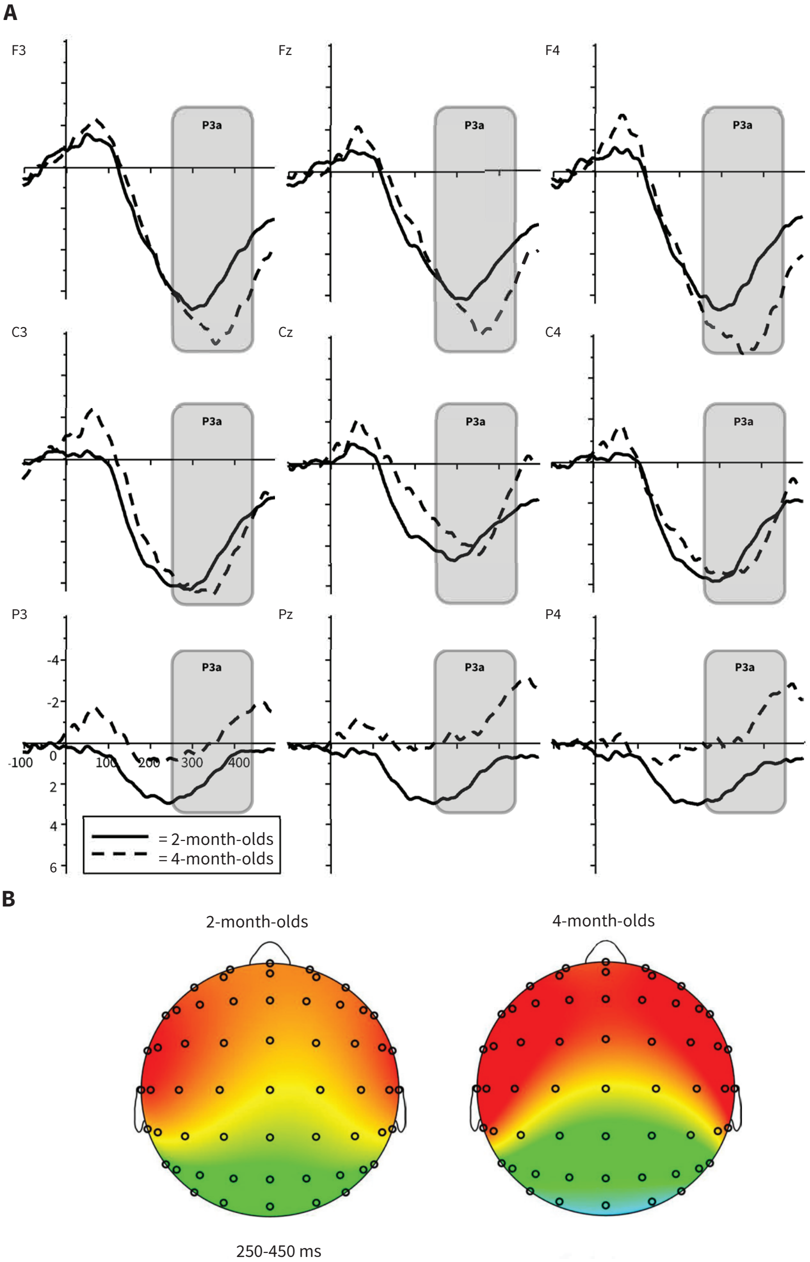

Figure 4 ***(A)*** *Group-average ERP responses in elicited by the Novel sounds in the 2- (solid line) and 4-month-old infants (dashed line) over frontal, central and parietal electrodes. The grey box at Cz indicates the time window within which the"P3a" amplitudes were measured. And (****B****) topographical maps for the P3a amplitude elicited by novel sounds in 2- (left) and 4-month-old infants (right), measured over 250-450 ms time period The common color scale is below the left panel.*

Discussion

The current study showed AERP differences between typically developing 2- and 4- months-olds for a repetitive complex tone and three rare sound events. We found that by 4 month of age morphological differences appeared between the responses elicited by white noise and novel sounds compared to the responses obtained at 2 months. This result supports the hypothesis that the processing of contextual novelty and wide acoustic deviance becomes increasingly separated within this period.

The findings suggest that the N250 displays marked development between 2 and 4 months after birth. As shown in Figure 4, novel sounds elicited a P3a-like prolonged positive response (cf., Kushnerenko et al., 2002a,b; 2007). In contrast, white noise sounds elicited the P150-N250-P350 response complex (Kushnerenko et al., 2013; Figure 3), which became more prominent by 4 months compared to the response pattern at 2 months: Significantly more negative N250 amplitude was found over the right hemisphere in the 4- as compared to the 2-month-olds with no corresponding difference in the preceding P150 peak. The emergence of the N250 between these two ages in response to spectrally rich, widely deviant sounds (white noise segments) but not in response to unique novel sounds is consistent with the hypothesis of increased separation between processing acoustic deviance and contextual novelty (Kushnerenko et al., 2013). The fact that one should take into account, however, is that the infant brain might distinguish these two sets of sounds by differences in the spectral contents (environmental sounds have typically narrower bandwidth than white noise segments) and/or by difference in temporal structure (environmental sounds typically showed spectral changes over time, whereas the white noise segments did not). Differential processing of environmental sounds and white-noise segments (but not the emergence of the N250) has also been observed in neonates (Háden et al., 2013).

ISI deviants elicited significant MMR responses in both age groups, but no age-related difference was obtained between the responses to the deviant events in the MMR latency range. The peak latency of the P350 was lower in 4 months compared to 2 months old infants. Although the AERP responses look visually different, with more pronounced peaks in 4- compared to 2-month-olds, these differences did not reach statistical significance. This could be due to high between subject variability of AERP responses for this stimulus type. This suggests that the development of processing shortening of the inter-stimulus interval progresses less uniformly during this period than the processing of spectrally rich sounds.

The significant latency decrease found for several AERP responses from 2 to 4 month of age is in line with the previous reports (e.g., Cheour et al., 1998; Jing and Benasich, 2006) and it suggests faster processing in 4- than in 2-month-olds. Faster processing is assumed to be due to processes such as increasing myelination in the developing nervous system (see Dehaene-Lambertz et al., 2010).

We also found topographical differences between some of the ERPs elicited in the two age groups. Firstly, we found a difference in the scalp topography between 2- and 4-month-olds for the standard tones with a larger ERP response over central electrode sites for 4- compared to 2-month-olds. For the novel and the white noise sound, a difference in scalp topography was found for the P350/P3a with larger positive voltage on parietal electrode sites for 2- compared to 4-month-olds. The topographical maps of these components (Figures 5 and 6) seem to indicate a developmental shift from 2 to 4 months of age towards frontal areas with a corresponding decrease of the positive voltage over parietal areas. The latter result is compatible with those of Kushnerenko et al. (2002a) demonstrating a developmental decrease of the P350 from 3 to 6 months of age (for a review, see Kushnerenko et al., 2013). These authors suggested that the decrease of the P350 was due to its overlap with the growing N250 component. Our post-hoc correlation analysis confirmed this suggestion: Higher (more negative) N250 amplitudes coincided with lower (less positive) P350 amplitudes. The interpretation of the assumed adult equivalents of these components (e.g., Escera et al., 2000) suggests that the mature version of these responses index the involvement of the frontal attention network.

Controlling for gestational age at birth and birth weight resulted in the elimination of a few of the initially statistically significant effects, suggesting that some differences in the variance of the AERPs of these two age groups are partly explained by the level of the infants' maturity at birth. While controlling for these covariates is often done in ERP-studies on prematurely born infants (Fellman et al., 2004; Hövel et al., 2014), many ERP-studies on infants born to term do not control for them. Our results, obtained in full-term infants, indicate that even in typically developing infants one should add these covariates to the statistical analyses when comparing between two groups of infants (such as between two age groups).

Conclusion

We found significant differences in AERPs within the short time period between 2 and 4 month after birth. This finding suggests that during this period, substantial maturation and learning takes place in the infant auditory information processing system. Data are consistent with the notion of early developmental improvements of infantile abilities in specifying their responses to novel auditory events, representing the temporal structure of sound sequences, and increasing the speed of sound processing. The emergence of the N250 AERP component was the most prominent AERP difference between the two tested age groups. This AERP development helps in understanding the developmental trajectory of auditory information processing in early infancy.

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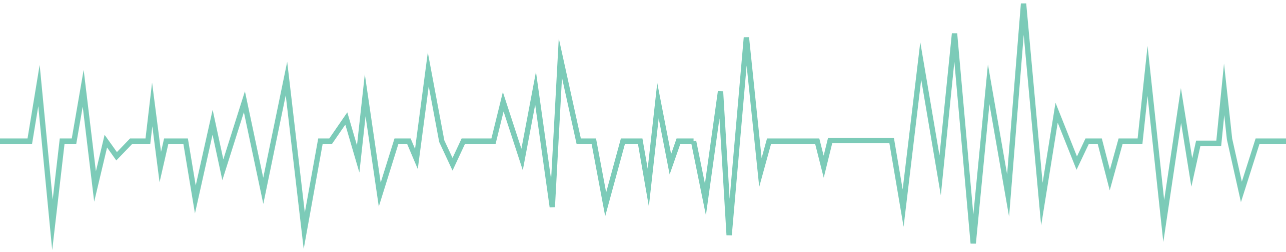

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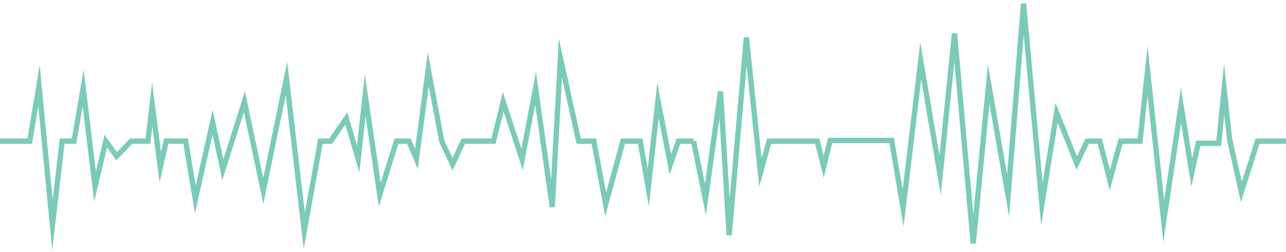
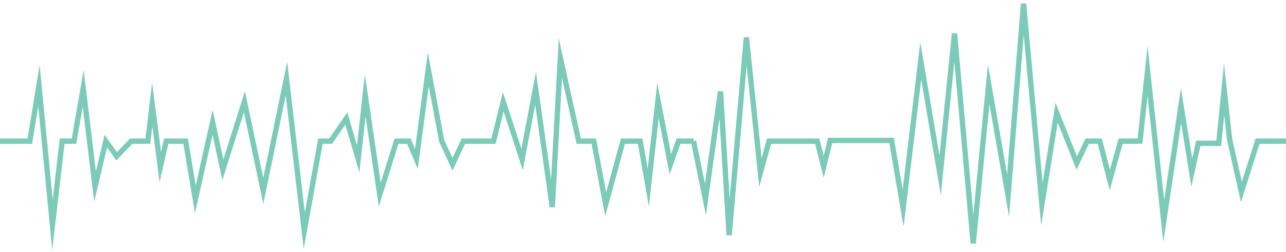
Abbreviations



AERP	Auditory event-related potential
DOHaD	Developmental Origins of Health and Disease
DOBHaD	Developmental Origins of Behavior, Health and Disease
EEG	Electroencephalography
ERP	Event-related potential
ISI	inter-stimulus-interval
MMR	mismatch response

List of publications



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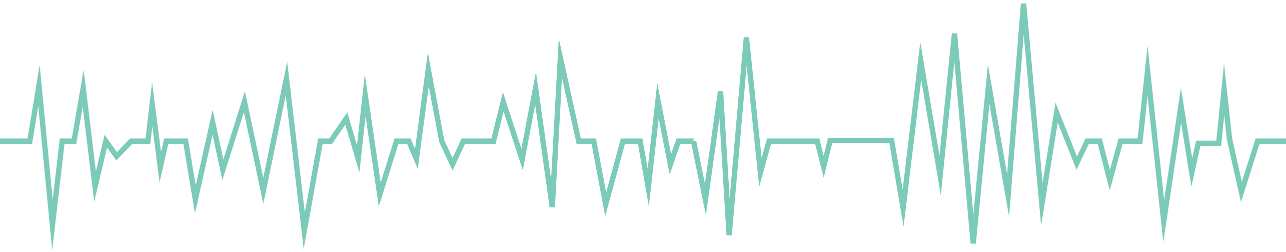
van den Heuvel, M.I., & Van den Bergh, B.R.H. (in preparation). Association between maternal psychological distress and cortisol during pregnancy: a multilevel analysis.

List of presentations



Oral presentations

van den Heuvel, M.I., Huse, K., Platzer, M., Schwab, M., & Van den Bergh, B.R.H. Epigenetic Modifications Associated With Maternal Anxiety During Pregnancy: DNA-methylation in Buccal Cells of 4-year-olds. *Invited talk at Jena University Hospital, Jena, Germany, September 2015.*

van den Heuvel, M.I. Prenatal Stress and (Neuro)behavior of Infants and Children. Results From the BrainAGE Project in Tilburg. *Invited talk at the Symposium 'Prenatal Stress and Brain Disorders Later in Life' in Berlin, Germany, September 2015.*

van den Heuvel, M.I., Donkers, F.C.L., Hillebrand, A., & Van den Bergh, B.R.H. Maternal Anxiety During Pregnancy is Associated With Less Integrated Brain Networks in 9-month-old Girls. *Presentation in paper session at the Society for Research on Child Development (SRCD) Biennial Meeting, Philadelphia, Pennsylvania, USA, March 2015.*

van den Heuvel, M.I., Winkler, I., Otte, R.A., Braeken, M.A.K.A., Donkers, F.C.L., Van den Bergh, B.R.H. Maternal mindfulness and anxiety during pregnancy affect infants' neural responses to sounds. *Invited talk at Donders Discussions Conference, Donders Institute Radboud University Nijmegen, The Netherlands, Oct 2014.*

van den Heuvel, M.I., Winkler, I., Otte, R.A., Braeken, M.A.K.A., Donkers, F.C.L., Van den Bergh, B.R.H. Maternal mindfulness and anxiety during pregnancy affect infants' neural responses to sounds. *Paper presented at Bi-annual meeting Dutch Society of Developmental Psychology (VNOP) conference, Wageningen, The Netherlands, May 2014.*

van den Heuvel, M.I. Maternal stress during Pregnancy and Brain Development of the Child: a Longitudinal Study. *Presentation about PhD-project at Baby Brain & Cognition meeting, Utrecht, The Netherlands, April 2014.*

van den Heuvel, M.I. The Baby Brain. *Invited presentation at Junior Science Café, Science Café, Tilburg, The Netherlands, May 2013.*

van den Heuvel, M.I., Otte, R.A., Braeken, M.A.KA., Winkler, I., Van den Bergh, B.R.H. Is anxiety during pregnancy related to early sensory cognitive processes in two-month-old infants? *Paper presented at Bi-annual meeting Dutch Society of Developmental Psychology (VNOP) conference, Wageningen, The Netherlands, May 2012.*

Poster presentations

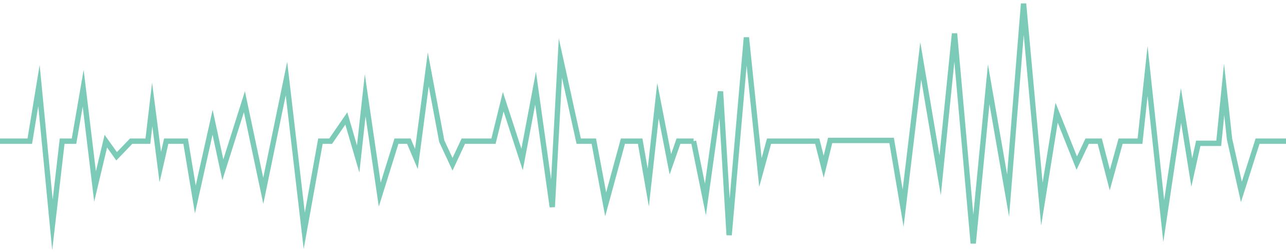
van den Heuvel, M.I., Johannes, M.A., Henrichs, J., Van den Bergh, B.R.H. Maternal Mindfulness During Pregnancy As Related to Infant Socio-emotional Development and Temperament: The Mediating Role of Maternal Anxiety. *Poster presented at the Society for Research on Child Development (SRCD) Biennial Meeting, Philadelphia, Pennsylvania, USA, March 2015.*

van den Heuvel, M.I., Winkler, I., Otte, R.A., Braeken, M.A.K.A., Donkers, F.C.L., Van den Bergh, B.R.H.M. Maternal mindfulness and anxiety during pregnancy affect infants' neural responses to sounds. *Poster presented at Developmental Origins of Health and Behavior (DoHAD) Conference, Nov 2013, Singapore, Singapore.*

van den Heuvel, M. I. , Winkler, I., Otte, R. A., Braeken, M. A. K. A., van der Wal, J. J. M., Donkers, F. C. L., van den Bergh, B. R. H. M. Maternal mindfulness and anxiety during pregnancy affect infants' neural responses to sounds. *Poster presented at Society for Psychophysiological Research (SPR) conference, Oct 2013, Firenze, Italy.*

van den Heuvel, M.I., Winkler, I., Otte, R.A., Braeken, M.A.K.A., Donkers, F.C.L., Van den Bergh, B.R.H.M. Maternal depressive symptoms during pregnancy alter auditory information processing in the two-month-olds offspring. *Poster presented at Central European Conference on Cognitive Development, January 9-12, 2013, Budapest, Hungary*

Biography



Marion van den Heuvel, daughter of Frans van den Heuvel en Thérèse van den Heuvel-de Beer, was born on 20th of January 1988 in Tilburg (The Netherlands). She is happily married to Robin Zwiers. After she graduated from high school at the St. Odulphus Lyceum in Tilburg (The Netherlands), she obtained her Bachelor in Health Sciences in 2009 at Maastricht University, with a major in Mental Health Sciences. In 2012 she graduated cum laude from the Research Master in Social and Behavioral Sciences at Tilburg University. During her Research Master she did a research internship at the University of East London (UK). In September 2012 she joined the BrainAGE project as PhD student at the department of Developmental Psychology at Tilburg University. During her PhD she obtained funding to visit the Leibniz Institute for Age Research in Jena (Germany), a partner of the BrainAGE project, for a short research internship. After finishing her PhD at Tilburg University, Marion will continue her research in the field of developmental programming as a postdoctoral researcher at Wayne State University in Detroit (USA). Marion was awarded the RoBUST postdoctoral fellowship of Wayne State University to work with Dr. Moriah Thomason in the Perinatology Research Branch of the Eunice Kennedy Shriver National Institute of Child Health and Human Development of the National Institutes of Health (NIH) at Wayne State University.