Prenatal exposure to maternal anxiety affects neurocognition in the first year of life

Otte, R.A.

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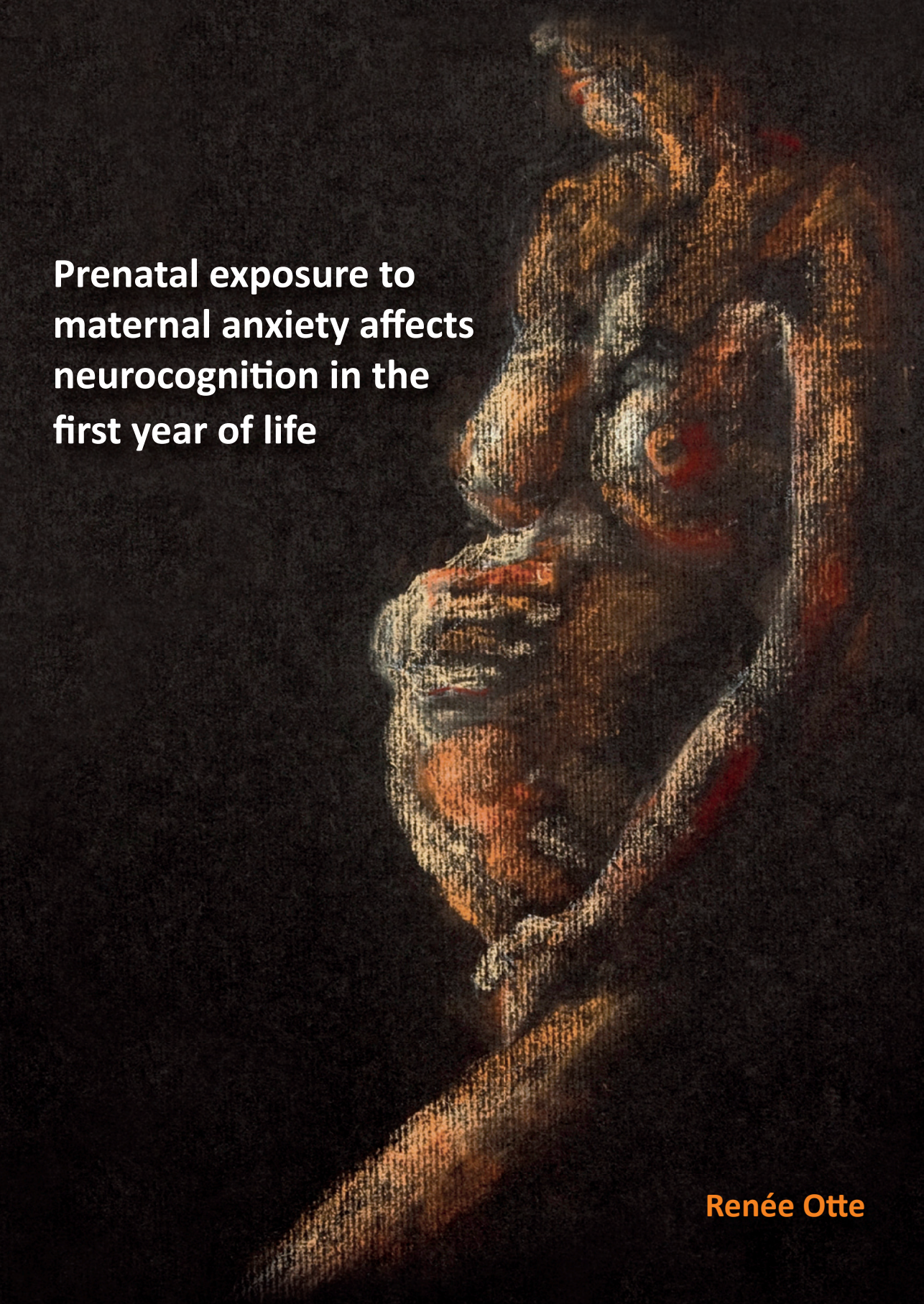

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Prenatal exposure to maternal anxiety affects neurocognition in the first year of life

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Promotores:	Prof. dr. B.R.H.M. Van den Bergh Prof. dr. I. Winkler
Overige leden:	Prof. dr. S. Vanhatalo Prof. dr. J.H.M. Vroomen Dr. E.V. Kushnerenko Dr. M. Mennes

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List of Abbreviations

A

ADHD	Attention Deficit Hyperactivity Disorder
AERP	Auditory event-related potential
Anx-EP	Anxiety experienced during early pregnancy
ANS	Autonomic Nervous System

B

BMI	Body Mass Index
BNBAS	Brazelton Neonatal Behavioral Assessment Scale
BSID	Bayley Scales of Infant Development

C

CRH	Corticotropin-releasing hormone
CRL	Crown-rump length

D

DOHaD	Developmental origins of health and disease

E

EEG	Electro-encephalogram

ERP	Event-related potential

F

FF	Fearful face-fearful voice
FH	Fearful face-happy voice
FH+	Family with a history of language learning impairments
FH-	Family without a history of language learning impairments
fMRI	functional Magnetic Resonance Imaging

H

5-HT	5-hydroxytryptamine (serotonin)
5-HTTP	5-hydroxytryptamine transporter
HF	Happy face-fearful voice
HH	Happy face-happy voice
HPA	Hypothalamic-Pituitary-Adrenal (-axis)

I

ISI	Inter-stimulus interval

M

MDI	Mental developmental index (of the BSID)
MMN	Mismatch negativity
MMR	Mismatch response

N

Nc	Negative component

NIRS	Near Infrared Spectroscopy

P

Pc	Positive compoment
PDD-NOS	Pervasive developmental disorder-Not otherwise specified
PELS	Prenatal Early Life Stress
PRAQ	Pregnancy-related Anxiety Questionnaire

R

RT	Reaction time

S

STAI	Spielberger State-Trait Anxiety Inventory
SCL	Symptom Checklist-90
SSRI	Selective serotonin reuptake inhibitor

Part A

General introduction and methods

1

General introduction

Development is a plastic process, which refers to the systematic continuities and changes that occur as a function of the on-going dialogue between the individual and his environment (Entringer et al., 2010; Gottlieb, 1997; Shaffer, 2002). During no other period does this process take place at a greater pace than during life in the womb. In roughly 9 weeks time, all body parts and major organ systems are formed; in another 15 to 18 weeks, survival outside the womb becomes possible. After just 40 weeks of gestation, the fertilised egg has changed from a one-cell zygote into a human being with ca. 200 billion cells (Shaffer, 2002).

With the speed of development come sensitive periods of cell proliferation, migration, and differentiation, during which the foetus is highly susceptible to environmental cues or experiences. A sensitive period has been defined as a limited time window in development during which the presence or absence of a particular experience or cue exerts a strong effect over structure or function, even beyond that point in development (Bornstein, 1989; Knudsen, 2004). In typical development, environmental cues during sensitive periods help program the normal developmental trajectory of the foetus (DiPietro, 2012; Van den Bergh, 2007, 2011a). Adverse cues, however, such as maternal undernutrition, alcohol use, and experiencing of stress and anxiety, potentially result in altered programming - or: reprogramming - of structure and function in cells, tissues, and organ systems. This, in turn, may affect psychophysiological functioning, and increase an individual's vulnerability to regulation problems at behavioural, emotional, and cognitive levels from the foetal period to at least early adulthood. (Barker, 1998; Mennes, 2008; Nathanielsz, 1999; Räikkönen et al., 2011; Van den Bergh, 2011a,b). The processes involved have been termed perinatal (re)programming and constitute the core of research in the field of developmental origins of health and disease (DoHAD). The focus

of this dissertation is the neurocognitive outcome of infants who have been exposed to maternal anxiety during their development in the womb, in the tradition of research in the DoHAD field.

1.1 Brain development and reprogramming effects

> Our brains are the brains that were shaped by experience, our live are the lives that began as babies, our consciousness is the consciousness that reaches back to childhood
>
> Alison Gopnik, 2009

Like all organs and organ systems, the brain is subject to sensitive periods of development. It is one of the first to develop during pregnancy: as early as 3 weeks after conception a primitive form of the human brain is present in the foetus. Approximately 3 months post-conception, the brain has taken on its distinctive human shape and about 4 months later gyri and sulci start to form (Kolb and Whishaw, 2001). At birth, in structural terms the neonate's brain resembles that of an adult; all gross anatomical features are present (Gazzaniga et al., 2002). Thus, although brain development continues after birth and even into adolescence (Gopnik, 2009; Kolb and Whishaw, 2001), researchers have suggested that the brain is particularly susceptible to reprogramming effects during the foetal period (Räikkönen et al., 2011; Van den Bergh, 2011a).

A number of studies have shown associations between intra-uterine exposure to atypical environmental cues and reprogramming of neurocognitive development. For instance, Bellinger et al. (1987) studied early cognitive development in infants and toddlers between 6 and 24 months of age in relation to prenatal exposure to lead. They found that infants who had been prenatally exposed to high levels of lead ($\geq$ 10 $\mu g/dL$; based on lead levels in umbilical cord blood) had lower scores on the Mental Development Index (MDI) of the Bayley Scales of Infant Development (BSID; Bayley, 1969), and the effect did not depend on the postnatal capillary blood lead levels. Also, prenatal iron deficiency, as inferred from serum ferritin concentrations at birth, has been associated with impaired auditory recognition memory in newborns (Beard, 2008; Georgieff, 2008; Georgieff et al., 2002; Siddappa et al., 2004). Another example is provided by Streissguth et al. (1983), who examined the effects of maternal alcohol intake during pregnancy on newborn infants. They demonstrated reduced habituation to auditory and visual stimuli 27 hours after birth, as measured by the Brazelton Neonatal Behavioral Assessment Scale (BNBAS; Als et al., 1977).

1.2 Neurocognitive development and prenatal exposure to maternal anxiety

In addition to prenatal iron deficiency, exposure to lead, and maternal alcohol intake, the developing foetus may be influenced by the psychological status of the mother. Over the last decades, an increasing amount of evidence has been gathered linking prenatal exposure to high levels of maternal anxiety and altered neurocognitive outcome in the offspring. As part of a prospective longitudinal study that began almost 30 years ago, pregnant women have filled out standardised psychological anxiety questionnaires (Van den Bergh et al., 1989). Cognitive development of the children was investigated at the ages of 15, 17, and 20 years. Results suggested that adolescents from mothers who had experienced high levels of anxiety during the 12th to 22nd week of gestation had difficulties with endogenous, but not exogenous[1], cognitive control processes (Mennes et al., 2006, 2009; Van den Bergh et al., 2006, 2005), which were suggested to be related to subtle changes in four prefrontal clusters, including the inferior frontal junction (Mennes, 2008).

Another prospective longitudinal study examined 5-year-old's neurocognitive functioning using a simple reaction time (RT) task, and a choice RT task with a compatible[2] and an incompatible[3] part (Loomans et al., 2012). The authors found that the higher the level of maternal anxiety the children had been prenatally exposed to, the larger the intra-individual variability in RT they displayed. In addition, children in the upper 10% of prenatal maternal anxiety had a longer mean RT and showed more intra-individual variability in RT in the incompatible trials of the choice RT task.

In the longitudinal study by Brouwers et al. (2001), prospectively collected anxiety scores from pregnant women were related to infant development as measured by the BSID. The results showed that at 2 years of age, toddlers who had been exposed to high levels of maternal anxiety during late pregnancy had lower MDI scores compared with those exposed to lower levels of maternal anxiety. In the review by Räikkönen et al. (2011) other examples of longitudinal studies on prospectively measured maternal anxiety during pregnancy and infant neurocognitive outcome can be found.

[1] Endogenous control refers to control that has to be exerted and maintained from within a person, i.e. from internal cognitive processes. In contrast, exogenous control refers to control that is triggered by an external event.

[2] Correct responses to the compatible trials consisted of clicking the right mouse button with the right forefinger for stimuli presented on right side of a fixation cross, and by clicking the left mouse button with the left forefinger for stimuli presented on the left side of a fixation cross.

[3] Correct responses to the incompatible trials consisted of clicking the right mouse button with the right forefinger for stimuli presented on the left side of a fixation cross, and by clicking the left mouse button with the left forefinger for stimuli presented on the right side of a fixation cross.

1.3 Measuring maternal anxiety during pregnancy

A number of instruments can be employed for assessing maternal anxiety during pregnancy. In a recent review, Nast et al. (2013) reported usage of 11 different measures in the period 1999-2009. Not all of them measured the same type of anxiety. For example, whereas the Beck Anxiety Inventory (Beck and Steer, 1990) is a measure of somatic and panic-related anxiety symptoms (e.g. feeling hot, nervous; Beck et al., 1988), the Pregnancy-Related Anxiety Questionnaire (PRAQ) specifically focuses on anxiety related to being pregnant (e.g. fear for integrity of the foetus, fear of pain during delivery; Van den Bergh, 1989). In this dissertation, we were interested in the influence of the actual state of anxiety ('state anxiety') during pregnancy on infant neurocognitive outcome in a non-clinical sample. The state anxiety subscale of the Spielberger State-Trait Anxiety Inventory (STAI; Spielberger et al., 1970) provides a valid measure of the intensity of transitory anxiety in response to present, real life stress. In addition, previous studies found state anxiety, measured between 15 and 22 weeks of pregnancy, to be a good predictor of offspring neurocognitive outcome (Mennes et al., 2006, 2009; Van den Bergh et al., 2005, 2007). Therefore, in the studies presented in the coming chapters, the state anxiety subscale of the STAI was used for assessing maternal anxiety during pregnancy.

In addition to the STAI, the anxiety subscale of the Symptom Checklist-90 (SCL; Derogatis and Cleary, 1977; Derogatis et al., 1973) was administered in our test population. This subscale was added to investigate whether or not another measure of anxiety - i.e. one that is more focused on bodily symptoms of anxiety, such as trembling and heart pounding - would yield similar results as the STAI.

1.3.1 Timing of prenatal exposure to maternal anxiety

Another factor that is likely related to specific neurocognitive outcome, is the timing of exposure to maternal anxiety. As was mentioned earlier, the brain is especially susceptible to (atypical) environmental cues during sensitive periods in development. However, susceptibility may differ per pregnancy trimester and per brain structure. Because different structures follow different developmental trajectories, one can expect exposure to maternal anxiety during different trimesters to result in different offspring outcomes. Exposure during early-mid rather than late gestation has been associated with specific changes in neurocognitive functioning in several previous studies (Davis and Sandman, 2010; Mennes et al., 2009; Rodriguez and Bohlin, 2005; Van den Bergh et al., 2006). Therefore, in this dissertation we focused on maternal state anxiety measured during the earlier stages of pregnancy, i.e. between 9 and 15 weeks gestational age (GA).

1.3.2 Factors possibly confounding the effects of maternal anxiety

If a mother is experiencing anxiety during (early) pregnancy, chances are that she will also feel them after giving birth. This is supported by previous research, reporting that anxiety shows considerable stability from pregnancy through the postpartum period (Grant et al., 2008; Heron et al., 2004; Lee et al., 2007). In other words, postpartum exposure to maternal anxiety could also influence neurocognitive functioning. Therefore, to disentangle effects of anxiety during and after pregnancy, the analyses described in this dissertation statistically controlled for anxiety experienced by the mother after pregnancy.

In addition to associations with prenatal exposure to anxiety, relations have been found between maternal alcohol intake and smoking during pregnancy, and offspring neurocognitive outcome (e.g. see Kodituwakku, 2007; Rodriguez and Bohlin, 2005). Thus, these factors could potentially confound associations between neurocognitive findings and maternal anxiety. Therefore, we also controlled for them in our analyses.

1.4 Event-related potentials and prenatal exposure to maternal anxiety

Most studies on prenatal exposure to maternal anxiety have made use of behavioural measures to study associations with offspring neurocognitive outcome. However, although these instruments can be used to make inferences about the underlying brain functionality, they do not measure actual brain functioning (Mennes et al., 2009). Therefore, in the study by Mennes et al. (2009) mentioned earlier, neurocognitive functioning in 17-year-olds prenatally exposed to anxiety was investigated by means of event-related brain potentials (ERPs). An ERP is a neural response embedded within the electroencephalogram (EEG; i.e. a recording of electrical activity from the scalp) that is associated with specific sensory, cognitive, and/or motor events, and which can be extracted from the continuous EEG.

The most commonly used method of extracting ERPs relies on averaging the segments of the EEG signal aligned with each other with respect to the eliciting event (e.g. an external stimulus). The underlying assumption is that the signal sums together the unchanging response to the event and an independent ergodic "noise" resulting from the brain activity unrelated to the event. Under these conditions, the response to the event is not affected by the averaging procedure, whereas the noise is reduced in proportion to the square root of the number of segments averaged. From this follows that extracting the responses by the averaging method requires several repetitions of the same event. In practice, in adults 40-200 repetitions are needed, except for the earlier smaller-amplitude responses, which typically require many more repetitions (Luck, 2005). Several other methods exist for extracting ERP responses from the EEG signal; each relying on a different set of assumptions. Its robustness and compatibility with other investigations makes

averaging a reasonable choice.

The averaged ERP waveform consists of a sequence of peaks and troughs - positive and negative voltage deflections - which are referred to as peaks or components (Luck, 2005). The components have been given names that refer to their polarity and their position within the waveform (e.g. P1 for the first positive peak, or N450 for the negative peak 450 ms after stimulus-onset).

An advantage of ERPs over behavioural measures is that ERPs provide a measure of processing that connects the sensory input and the response that can separate, for instance, perceptual- and response-related processes. Also, as ERPs have a very high temporal resolution, timing of neurocognitive processes can be studied with millisecond accuracy (Luck, 2005). In addition, ERPs can be recorded in the absence of a behavioural response (Nelson and Bloom, 1997), even for unattended stimuli (Sussman, 2007), which makes them quite suitable for studying neonates and infants. Finally, an additional benefit is gained by allowing direct comparison across age groups. In contrast, the rapidly developing motor capabilities of infants during the first year of life make it difficult to find common behavioural methods for measuring, e.g., 2- and 9-month-olds.

Since studying the development of neurocognitive processes helps to gain insight into the neural bases of human perception, emotion and cognition, recent years have seen an increased interest in the use of ERPs to examine these processes in infants (de Haan, 2007). Despite this, the study by Mennes et al. (2009) stands alone thus far in having used ERPs to investigate these processes in the context of prenatal exposure to prospectively measured maternal anxiety. Thus, in the studies described in this dissertation, we attempted to fill this gap in the literature by studying neurocognitive functioning in infants prenatally exposed to maternal anxiety by means of ERPs.

1.4.1 Factors possibly confounding neurocognitive measures in infants

Previous research found that several factors may influence the morphology of (infant) ERPs. For instance, ERPs have been found to change as a function of the sex of the subject (Lavoie et al., 1998). Moreover, a number of studies on reprogramming of development after prenatal exposure to atypical environmental cues found sex differences in offspring outcome (for a review see e.g. Glover and Hill, 2012). Thus, sex of the infants could influence our results. Therefore, in all analyses presented in this dissertation, we investigated whether effects were different for boys and girls.

Other factors that could exert an influence on the ERP concern birth weight and GA. For example, a study by Fellman et al. (2004) demonstrated that infants born small for GA (birth weight as a proxy for intra-uterine distress) show atypical ERPs to rare sounds presented in a sequence of familiar sounds. The authors also described differences in ERPs between infants born at term and preterm infants, showing that variation in GA at birth can result in differential ERPs.

Another factor that is known to affect the ERP is age at testing. As an example, Kushnerenko et al. (2002) studied infant auditory ERPs from birth to the age of 12 months, and reported profound developmental changes over the first year of life. In addition, although ERPs can be recorded in sleeping infants, the infant's state of alertness may result in differential ERPs, for instance in polarity of the components (Friederici et al., 2002). To take the potential effect of these factors described above into account, we statistically controlled for them in our analyses.

1.5 Aims and outline of this dissertation

1.5.1 Aims

This dissertation focuses on mothers and infants who have been recruited within the context of an on-going longitudinal study on the short- and long-term consequences of Prenatal Early Life Stress (PELS study). The general aim of the studies presented in this dissertation is to provide insight into the association between prenatal exposure to maternal anxiety and neurocognitive outcome of the infant, as measured by ERPs, during the first year of life. Part B contains two general chapters describing the paradigms that were used to investigate neurocognitive functioning in infants. Employing an auditory oddball paradigm, we examined the ability of 2-month-old infants to process rapid sequences of sounds, and with an audiovisual paradigm we studied multimodal processing of emotional information in 9-month-olds. Part C then addresses the core aim of this dissertation. The ability to process sound sequences in 2-month-olds will be related to exposure to prenatal maternal anxiety, and a possible link with language development will be proposed. We will also look into the question whether effects found for the 2-month-olds are still present by the time the infants reach 9 months. Finally, we describe effects of prenatal exposure to maternal anxiety on the ability to process emotional information from facial and voice stimuli.

The following research questions will be discussed:

- Are 2-month-olds able to identify violations of temporal regularities in a repetitive auditory sequence, and does state of alertness affect these processes? (Chapter 3)
- Do 9-month-olds process emotional (fearful and happy) vocalisations differently when they have been primed with a visual stimulus conveying the same, versus a different emotion? (Chapter4)
- To what extent does prenatal exposure to maternal anxiety during early pregnancy influence information processing in 2-month-old infants? (Chapter 5)
- To what extent does prenatal exposure to maternal anxiety during early pregnancy influence information processing in 9-month-old infants? (Chapter 6)
- How do early life experiences influence the ability to process emotional information from facial and voice information? (Chapter 7)

1.5.2 Outline

This dissertation is divided into 8 chapters. Following the present introductory chapter (Chapter 1), Chapter 2 explains the study protocol of the PELS study, including information on participant recruitment, and methods of data collection and analysis. In chapters 3 to 7, the research questions as presented above will be examined. Finally, a general discussion and a summary of the findings are presented in Chapter 8.

2 Participants and methods

2.1 Participants

Participants for the PELS study were pregnant women, their partners, and, after birth, their infants. The participants were recruited between April 2009 and September 2010 through a hospital and four midwife's practices in and in close vicinity to Tilburg, The Netherlands. For initiating collaboration with these institutions, the researchers first contacted them by telephone and gave a short explanation on the study and its design. If an institution was interested, a follow-up appointment was made during which the aims and goals of the study were set forth in more detail. This information was also handed over in the form of an information letter. The request put to the institutions was two-fold: the researchers first asked for permission to address the institution's patients or clients [1] about the study, and, second, if permission was given, they asked for assistance with the recruitment. Assistance comprised of 1) promoting the study in a few sentences during a client's first visit to the institution, and 2) collecting contact information from interested clients and once a week sending the contact information to the researchers in pre-paid envelopes. One of the five midwife's practices that were contacted ("De Vlinder") did not wish to cooperate in the study. The other four practices ("Eva", "De Zon" "Lente", "Isis") as well as the Sint Elizabeth hospital gave their consent.

On receiving the contact information from potential participants, the researchers called them to make an appointment at their convenience and in a location of their choosing (at home, at Tilburg University, at work, etc...). All steps of the study

[1] From this point onward I will use the word "client" to refer to both patients and clients.

were then explained in full, in accordance with the Declaration of Helsinki. A letter containing this information was also given. If both parents(-to-be) agreed to participate in the study, and to let their infant participate after its birth, they both signed an informed consent form.

A total of 190 women and their partners consented to participate. One hundred and seventy-eight of them were recruited between their 9th and 15th week of pregnancy (M GA=11.96; SD=1.73) and 12 between their 16th and 23nd week (M GA=17.33; SD=2.10). The infants (including two pairs of twins) were born between September 23rd 2009 and March 24th 2011. Demographical data for the mothers (at recruitment) and their infants can be found in Table 2.1.

2.2 Procedure

The study consisted of five sessions conducted at different times, referred to as T1 to T5, of which the first 3 took place during pregnancy and the last two after the birth of the infants. Through midwives and hospital nurses information on the delivery was also collected. T1, T2, and T3 were administered at a GA of respectively 9 - 15, 16 - 23, and 30 - 37 weeks. Participants were invited for T4 when the infants were either between 8 and 11 weeks of age or 4 to 5 months old. At T5, finally, the infants were aged 9 to 11 months.

Sessions T1 to T3 mostly took place at the participants' homes, unless the participant preferred meeting somewhere else, e.g. at work, at her parents' home, or at the researchers' office at Tilburg University. Both postpartum measurements took place at the Tilburg University Babylab. During the five sessions, different types of data were collected from the parents and, after birth, from their baby to relate prenatal exposure to maternal anxiety to infant neurocognitive outcome. In Table 2.2 an overview is presented of the data collected during each session.

2.3 Dependent variables

We measured ERPs for indexing the infants' neurocognitive functioning. In the studies described in Chapter 3, 5 and 6 of this dissertation ERPs were elicited in response to the sounds delivered in an auditory oddball task with 1 frequent (standard) tone and three types of infrequent (deviant) sounds. In the studies in Chapter 4 and 7 ERPs were recorded in response to auditory and visual stimuli presented within a multimodal emotion paradigm. In all studies, ERPs were obtained with slight variation of the common methods described below. Details per study can be found in Chapter 3 through 7.

Materials for the collection of infant brain data were provided by BioSemi (www.biosemi.com, Amsterdam, The Netherlands). Head caps with 64 electrode locations positioned according to the revised version of the International 10-20 system were used, with low impedance, highly conductive electrode gel pasted on the scalp beneath the electrodes. Electrodes placed on the left and right mastoids,

		N	%	*M (SD)*
Mothers		190		
Age				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 at home	1	.5	
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	
Currently has a job	yes	176	92.6	
	no	14	7.4	
Family income	$<$ 2100	9	4.7	
(monthly, in €)	2200 - 3600	38	20.0	
	$>$ 3600	131	68.9	
	Don't want to disclose	10	5.3	
	Missing	2	1.1	
Assisted pregnancy	No	153	80.6	
	Hormonal therapy	7	3.7	
	Intra-uterine insemination	4	2.1	
	In vitro fertilisation	15	7.9	
	Intracytoplastmatic sperm injection	8	4.2	
	Other	3	1.6	
Primigravida		74	38.9	
Has ever miscarried^o		47	24.7	
Being treated for:	Depression	1	.5	
	Personality problems	1	.5	
Has been treated in the	Depression	18	9.5	
past for:	Anxiety problems	7	3.7	
	Personality problems	2	1.1	
	PDD-NOS	1	.5	
	Postpartum stress disorder	1	.5	
	Eating disorder	2	1.1	
	ADHD	1	.5	
Infants		192		
Sex	Boy	92	48.2	
	Girl	96	49.7	
	Missing	4	2.1	
Birth weight (grams)				3444 (519)
GA at birth (weeks)				39.69 (1.6)

* Dutch/Bolivian (N=1), Dutch/Morroccan (N=2), Dutch/Romanian (N=1), Dutch/Russian (N=1), Dutch/Turkish (N=1)

^{o} Before 12 weeks GA

Table 2.1: Demographical data for the mothers (at recruitment) and their infants

		T1	T2	T3	Birth	T4	T5
Maternal data	Questionnaires	x	x	x		x	x
	Heart rate variability (math task)	x		x			
	Heart rate variability (24-hours)	x	x	x			
	Heart rate variability (baseline)						x
	Cortisol from saliva	x	x	x		x	x
	Cortisol from hair	x		x		x	x
	Data on delivery				x		
Paternal data	Questionnaires	x	x	x		x	x
Infant data	Data on birth				x		
	Questionnaires (for both parents)					x	x
	Bayley Scales of Infant Development						x
	Laboratory Temperament Assessment Battery (Fear subscale)						x
	Auditory ERP task					x	x
	Audio-visual ERP task						x
	Heart rate variability (ERP task)					x	x
	Cortisol from saliva					x	x
	Cortisol from hair						x

Table 2.2: Overview of data collected at T1 to T5

respectively, were used for off-line referencing. In addition, two electrodes were placed on the infants' chest for measuring the electrocardiogram (ECG).

During electrode placement the infant was lying in his/her parent's arms or sitting in their lap. Two experimenters prepared the infant for testing, while a third experimenter provided distraction as needed. After finishing preparations, the EEG signal was inspected for noise. As BioSemi uses active electrodes with a very low input impedance[2], any remaining noise on the channels could be reduced by adding extra gel to the electrode cavity or to the on-line CMS-DRL reference (see below).

The continuous EEG was recorded with BioSemi ActiveTwo amplifiers with a sampling rate of 512 Hz[3]. The standard BioSemi reference (CMS-DRL) was used[4] and off-line preprocessing was done with BrainVision Analyzer software (BVA), versions 2.0.1 and 2.0.2 (http://www.brainproducts.com, Brain Products, Munich, Germany). In BVA, EEG signals were filtered and re-referenced to the mathematically combined mastoids (Luck, 2005). The data were then segmented into epochs of 600 or 1000 ms length, including a 100 or 200 ms long pre-stimulus interval (see the exact value at the description of each study). The latter was later used as

[2] see http://www.biosemi.com/active_electrode.htm for details

[3] On-line filtering options are set automatically in the BioSemi software. See http://www.biosemi.com/faq/adjust filter.htm and http://www.biosemi.com/faq/adjust samplerate.htm for details

[4] see www.biosemi.com/faq/cms&drl.htm for details

the baseline for amplitude measurements. The epochs were averaged separately for each condition and stimulus type, excluding epochs with (movement) artifacts. Data from infants with too few acceptable responses for any one of the conditions were skipped for further analysis.

Group-averaged (difference) waveforms were calculated in BVA and areas of interest were chosen based on visual inspection of these waveforms on electrode sites where the effects were largest or predicted to be largest. Mean response amplitudes in microvolts for each infant and electrode site under investigation were entered in the statistical analysis (see "Data analysis").

2.4 Predictor

For the purpose of this dissertation, maternal anxiety experienced during pregnancy was operationalised as the sum score of the items on the anxiety subscales of the Dutch versions of the Spielberger State-Trait Anxiety Inventory (STAI; Spielberger et al., 1970; van der Ploeg, H.M. et al., 1980) and, separately, the Symptom Checklist-90 (SCL; Arrindell and Ettema, 2003; Derogatis and Cleary, 1977; Derogatis et al., 1973). The anxiety subscale of the STAI was chosen because previous studies found it to be a good predictor of offspring neurocognitive outcome (Mennes et al., 2006, 2009; Van den Bergh et al., 2005, 2007). The anxiety subscale of the SCL was used to investigate whether another measure of anxiety would yield results similar to STAI.

The STAI is a self-report questionnaire with the subscales Trait and State Anxiety. Both scales consist of 20 items which can be rated on a Lickert scale from 1 (not at all/almost never) to 4 (very much/almost always). Whereas trait anxiety refers to a disposition or proneness to react with anxiety, the state anxiety subscale provides a measure of the intensity of transitory anxiety in response to present, real life stress (Spielberger, 1975). Therefore, maternal state (and not trait) anxiety was used as a measure of maternal anxiety during pregnancy. Examples of items on the State scale are "I feel secure", "I am worried" and "I feel indecisive". In the studies described in this dissertation, Cronbach's α for the state anxiety subscale ranged between .905 and .930, indicating good internal consistency.

The SCL is also a self-report instrument. The questionnaire quantifies current psychopathological symptoms in terms of nine domains, of which anxiety is one. The anxiety subscale measures more generalised anxiety experienced within the last 4 weeks, with complaints related to increased vegetative arousal (bodily symptoms), general symptoms such as nervousness, more specific symptoms such as panic attacks, and cognitive symptoms such as fearful thoughts (Arrindell and Ettema, 2003; Derogatis and Cleary, 1977). The 10 items are rated on a 5-point Lickert scale, ranging from 0 (not at all) to 4 (extremely). Cronbach's α for this subscale was .861 for the study in Chapter 7. Examples of items are: "Heart pounding or racing", "Trembling", and "Spells of terror or panic".

2.5 Covariates

To take potential confounding effects into account, we controlled for the following covariates in the studies described in this dissertation:

- Maternal factors: postpartum anxiety levels, alcohol intake (continuous), and maternal smoking (dichotomous) during pregnancy (gathered through self-report);
- Infant factors: sex and GA at birth (obtained from hospital and midwives' medical files); birth weight controlled for GA (computed by regressing GA on birth weight); the infant's age at testing.

The continuous covariates were first correlated to the ERP data and only added to the ANCOVAs if significant correlations were found. As just a few mothers were smokers, we controlled for maternal smoking by checking whether or not exclusion of the corresponding infant data erased (or yielded) significant findings

2.6 Data analysis

Preprocessed data for all five studies were analysed with SPSS (SPSS 17.0, 18.0 and IBM SPSS 19.0). For analyses without maternal state anxiety during pregnancy as a predictor, repeated measures ANOVAs were run on the ERP amplitudes. When analysing associations between maternal state anxiety and infant ERP data, repeated measures ANCOVAs were used, with the maternal state anxiety score entered as a continuous predictor variable. When the sphericity assumption was violated, Greenhouse-Geisser correction was used, and the corresponding ϵ correction factor is given in the description. In addition, the partial η^2 is always given as an indication of the explained variance.

Part B

Measuring infant neurocognitive functioning

3

Detecting violations of temporal regularities in waking and sleeping two-month-old infants

R.A. Otte, I. Winkler, M.A.K.A. Braeken, J.J. Stekelenburg,
O. van der Stelt, & B.R.H. Van den Bergh.

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Abstract

Correctly processing rapid sequences of sounds is essential for developmental milestones, such as language acquisition. We investigated the sensitivity of two-month-old infants to violations of a temporal regularity, by recording event-related brain potentials (ERP) in an auditory oddball paradigm from 36 waking and 40 sleeping infants. Standard tones were presented at a regular 300 ms inter-stimulus interval (ISI). One deviant, otherwise identical to the standard, was preceded by a 100 ms ISI. Two other deviants, presented with the standard ISI, differed from the standard in their spectral makeup. We found significant differences between ERP responses elicited by the standard and each of the deviant sounds. The results suggest that the ability to extract both temporal and spectral regularities from a sound sequence is already functional within the first few months of life. The scalp distribution of all three deviant-stimulus responses was influenced by the infants' state of alertness.

Keywords: Infants; event-related potential (ERP); mismatch negativity (MMN); auditory oddball paradigm; ISI-deviant stimulus; state of Alertness

3.1 Introduction

The perception and representation of timing in the human brain has been fascinating researchers for a long time. Temporal processes have been separated into different time scales from circadian rhythms to processes in the millisecond range (Mauk and Buonomano, 2004) and numerous studies were carried out to gain insight into mechanisms underlying temporal processing in the brain (e.g. Ivry and Spencer, 2004; Koch et al., 2009; Lewis and Miall, 2009; Mauk and Buonomano, 2004). Most studies into the subject have been conducted on adults and pre-school and school-age children. Less is known, however, about the temporal processing abilities of infants. Extending our knowledge about these processes is fundamental for understanding developmental milestones, such as language acquisition, in which temporal processes play an important role. For example, in some languages phoneme duration may distinguish between minimal pairs of words (Peterson and Lehiste, 1960). Also, several studies showed that the ability to accurately process the temporal characteristics of rapidly presented sequences of sounds is critical for analysing and segmenting spoken language (Benasich et al., 2006; Benasich and Leevers, 2002; Fitch et al., 2001; Tallal et al., 1985). Therefore, the aim of the current study was to test whether infants are sensitive to violations of temporal regularities, i.e. to unpredictable changes in the timing of auditory stimulus delivery.

Using cardiac responses and behavioural measures, it has been shown that infants are sensitive to some temporal stimulus parameters and have a degree of control over timing their actions. For example, Pouthas and colleagues (1996) found that newborns and two-month-olds could learn to time pauses between non-nutritive sucks. Jusczyk et al. (1983) and Eilers et al. (1984) obtained evidence showing that two-month-old infants accurately discriminated sounds that differed by a few hundred milliseconds in duration. In five-month-old infants, Chang and Trehub (1977) demonstrated discrimination between multi-tone patterns of identical tonal components but different temporal arrangements of the tones.

Studies using electrophysiological methods have also provided insights into stimulus processing in infants. A number of these studies measured infant analogues (Alho et al., 1990) of the mismatch negativity (MMN; Näätänen et al., 1978) event-related brain potential (ERP). The MMN is a cortical response to deviations from the regular features of a sound sequence (for a recent review, see Näätänen et al., 2011) and it has been suggested to reflect processes evoked by failed auditory predictions (Winkler, 2007). The MMN is most often studied in the auditory oddball paradigm, in which a repeating sound is occasionally exchanged for a different sound. However, violations of complex regularities can also evoke the MMN (Näätänen et al., 2001).

The MMN component has been extensively studied in adults. Relevant for the current study are the MMN results regarding the detection of violations of temporal regularities. The MMN has been elicited by occasional decreases and increases in stimulus duration (Näätänen et al., 1989; Winkler et al., 1996), shortenings of the stimulus onset asynchrony (SOA; onset-to-onset interval) and the inter-stimulus

interval (ISI; offset-to-onset interval; Nordby et al., 1988; Takegata et al., 2001), stimulus omissions (Yabe et al., 1997), and infrequent changes in the temporal structure of complex sounds (Grimm and Schröger, 2005; Winkler et al., 1998) and sound patterns (Müller and Schröger, 2007; Takegata et al., 2005; Winkler and Schröger, 1995). These studies provided evidence that the various temporal aspects of auditory stimulation are encoded in the memory underlying the MMN response and that the MMN is elicited by violations of temporal expectations (for a review, see Czigler et al., 2003).

MMN experiments are quite suitable for infant studies, because, in contrast to most other ERP components, MMN-like discriminative ERP responses ('mismatch responses'; MMRs) can be obtained very early in infancy (Cheour, 2007), they require no behavioural response (Nelson and Bloom, 1997), and are elicited by unattended stimuli also (Sussman, 2007). Furthermore, the component can be recorded in waking as well as in sleeping infants (Kushnerenko et al., 2001b), although mixed results have been found as to whether or not the infant's state of alertness influences the MMR (see for example Cheour et al., 1998; Friederici et al., 2002; Hirasawa et al., 2002).

Despite the advantages, only a relatively small number of MMN experiments tested violations of temporal regularities in infants. Kushnerenko et al. (2001a) showed that infants aged 2-6 days are sensitive to increases in tone duration, evident in changes in their N2 responses. Also, Kushnerenko et al. (2001b) found that neonates are able to discriminate duration changes in speech sounds, demonstrated by a negative inflection in their ERP wave. Winkler et al. (2009b) obtained an MMR to violations at the downbeat of a rhythmic sound pattern in newborn infants. Finally, results from an experiment by Brannon et al. (2004) suggested that ten-month-old infants can accurately detect changes of a temporal interval within a sequence of tones.

These studies showed that infants and even neonates have some sense of timing and they react to temporal deviations with a discriminative response comparable to the adult MMN. The goal of the current study was to shed light on whether infants can identify violations of temporal regularities in a repetitive auditory sound sequence, by testing whether they detect occasional shortenings of the otherwise uniform ISI. In addition, since some studies suggested that the MMR may vary as a function of the infant's state of alertness (Friederici et al., 2002) and approximately half of the infants were asleep during the EEG recording, the effects of the state of alertness on the MMR were examined by comparing the ERP responses between waking and sleeping infants. The stimulus paradigm, developed for our ongoing longitudinal study, was adapted from the one designed by Kushnerenko et al. (2007), adding ISI deviants to the rare environmental (contextually novel) and white noise sounds (high spectral deviance) embedded in a regular sequence of a repetitive complex tone. The effects of the infants' state of alertness on the responses to the rare environmental and white noise sounds will also be presented.

3.2 Methods

3.2.1 Subjects

Subjects were 76 infants whose mothers have been taking part in a longitudinal study on prenatal early life stress (PELS project). The study was approved of by the Medical Ethical Committee of St. Elisabeth Hospital in Tilburg, The Netherlands. Informed consent was obtained from all mothers and fathers in accordance with the Declaration of Helsinki.

For the PELS project, a total of 190 pregnant women had been recruited, of whom 178 before their 15th and 12 between their 15th and 23rd week of pregnancy. Recruitment took place at a general hospital and four midwives' practices in and around Tilburg, The Netherlands. Pregnancies were dated using crown-rump length (CRL) around the 12th week of pregnancy measured by a professional, or the last menstrual period when CRL data were unavailable. The women were followed up during their pregnancies and were invited for postnatal observations either two or four months after the birth of their babies. Ninety-one women visited the lab for testing when their babies (54 girls) were aged two months and of these recordings, data from 76 infants (46 girls) were suitable for analysis. Data from 15 infants were excluded from the analyses due to crying (2), excessive movements/artifacts (9), and technical problems (4). The mean age of the remaining infants at testing was 9.6 weeks ($M = 70.1$ days, $SD = 6.2$ days). Mean gestational age and mean birth weight were 39.9 weeks ($SD = 10.5$ days) and 3454 g ($SD = 474$ g), respectively. All infants were healthy and had passed a screening test for hearing impairments, performed by a nurse from the infant health care clinic, between the 4th and 7th day after birth. During testing in our lab 36 of the infants were awake (20 girls) and 40 were asleep (26 girls).

3.2.2 Stimuli

The stimulus sequences consisted of 4 types of tones - one standard and three deviants - each with 10 ms rise and fall times and of 200 ms duration. Stimuli had an intensity level of 75 dB and were delivered at a uniform 300 ms ISI (offset-to-onset), except for the ISI-deviant events (see below). The standard sound was presented at a probability of 0.7 and the three types of deviants with a probability of 0.1, each. The standard was a complex tone constructed from the 3 lowest partials. The fundamental frequency was 500 Hz and the intensity of the second and third partials was 6 and 12 dB lower, respectively, than that of the first one. One deviant was identical to the standard sound, but preceded by 100 ms instead of 300 ms ISI ('ISI-deviant'). The other two deviant types (spectral deviants) were white noise segments ('white noise sound') and environmental sounds ('novel sounds', 150 different ones), such as a barking dog and a door bell. Each novel sound was delivered only once during the experiment to maintain novelty throughout.

Sounds were presented in a semi-random order with the restriction that both white noise and novel sounds were always preceded by at least two standard

sounds or a combination of a standard sound and an ISI-deviant. Also, consecutive ISI-deviants were always separated from each other by at least two standards or by a standard combined with either a white noise or novel sound. In total, 1150 standard sounds were presented and 150 deviants of each type. The stimuli were divided into five blocks of 300 stimuli, each and presented with short breaks in between. The order within the five stimulus blocks was separately randomized, and their presentation order was counterbalanced across subjects.

3.2.3 Procedure

The infants were tested at the developmental psychology lab at Tilburg University, The Netherlands, in a dimly lit and sound-attenuated room. During the experiment, parents were seated in a chair facing a pair of speakers while holding the infant in their arms. The speakers were placed 60 cm apart, both ca. 80 cm from the baby's head. The parent-child dyad was observed through a pair of cameras and notes were taken on whether the baby was quiet, crying, awake or asleep and whether or not he/she was sucking a pacifier, the parent's finger, a toy, etc. As some authors (e.g. Friederici et al., 2002) found that in infants the MMRs to deviant sounds changed as a function of the state of alertness, we divided our sample into a waking and a sleeping subgroup and state of alertness was later used as a between-subjects factor in the analyses. The monitored behaviour in combination with the online electroencephalography (EEG) signal was used to determine in which state of alertness the baby was during each stimulus block: awake or asleep with active (REM) and quiet (non-REM) sleep collapsed into a single category. Only data recorded during those stimulus blocks in which the infant was either awake or asleep throughout the whole period were analysed.

Before the start of the experiment, the infants were familiarised with the standard sound, the ISI-deviant and the white noise segments. The novel sounds were not included in this pre-test making sure that they were indeed new to the infants during the actual experiment. The standard sound thus became a 'frequent familiar' stimulus, the white noise sound and the ISI-deviant 'infrequent familiar' and the novel sounds 'infrequent unfamiliar' stimuli (Richards, 2003).

3.2.4 Data acquisition and analysis

The EEG was recorded with Biosemi ActiveTwo amplifiers (www.biosemi.com) with 512 Hz sampling rate using a head cap with 64 electrode locations placed according to the revised version of the International 10-20 system. Two reference electrodes were placed on the left and right mastoids, respectively. Off-line, the EEG signals were filtered with a 1 to 30 Hz band-pass filter (slope 24 dB) and a 50 Hz notch filter and were segmented into 600 ms epochs, including a 100 ms pre-stimulus interval as the baseline. The epochs were averaged separately for each deviant stimulus type, excluding epochs with sample-to-sample voltage steps larger than 100 μV or in which the amplitude range exceeded 120 μV in a sliding window of 200 ms anywhere within the whole epoch. Data from infants with less

than 40 acceptable responses for any one of the three deviants were removed from further analysis (9 infants). Difference waveforms were calculated by subtracting the response to the standard sound separately from the response elicited by the ISI-deviant, the white noise sound, and the novel sound.

As the MMR was expected to be localised mostly over frontal (F3,Fz,F4) and central (C3,Cz,C4) electrode sites, for each deviant sound type, the grand-averaged difference waveforms were averaged together from these areas for locating the MMR responses separately for the three deviants. Although the overall morphology of the deviance-related ERP responses was compatible with that found by Kushnerenko et al. (2007), there were large latency differences between the three types of deviants. The most prominent response was a broad positive-going difference waveform which was measured between 410 and 470 ms for the ISI-deviants, 385 and 445 ms for the white noise sounds and 275 and 335 ms for the novel sounds. An earlier negative-going difference waveform could also be observed in the ISI-deviant response with similar, although less pronounced deflections discernible for the other two deviants. However, the absolute voltage values of these responses were mostly positive[1]. Therefore, for assessing the amplitudes of these negative-going waveforms, we subtracted the mean voltage in the 20 ms window at the start of the waveform (centred on the preceding positive going peak) from the mean voltage in the 20 ms window centred on its peak. Short windows were used because the reference positive-going peaks were typically quite narrow and thus symmetric measurements required both windows to be short. For the ISI-deviant, an onset window of 65-85 ms and a peak window of 215-235 ms were used. For the white noise sounds, these windows were 156-176 ms and 187-207 ms, respectively, and for the novel sound, 185-205 ms and 207-227 ms windows were used.

Elicitation of the difference responses (the MMRs) was tested by comparing the mean frontal (F3,Fz,F4) amplitude of each deviant's difference wave in each measurement interval to zero using two-tailed Student's t tests, separately for the two states of alertness (2 states of alertness x 3 deviants x 2 measurement intervals = 2 x 6 t tests). Holm-Bonferroni (Holm, 1979) correction was used to control for increases in Type I errors due to multiple comparisons. Then, for testing the effects of the state of alertness on the amplitude and scalp distribution of the deviant-minus-standard difference responses over sites F3, Fz, F4, C3, Cz, C4, P3, Pz and P4, ANOVAs were run with 'Deviant Type' (ISI-deviant, white noise sound, novel sound) x 'Anterior vs. Posterior' (frontal, central, parietal) x 'Laterality' (left, medial, right) as within-subjects factors and 'State of Alertness' (awake vs. asleep) as a between-subjects factor, separately for the two measurement intervals. Greenhouse-Geisser correction was used where applicable and the ϵ correction

[1]Note that the ERP effects of the preceding standard stimulus are shifted by 200 ms for the ISI deviants. This misalignment is probably responsible for the positive waveform at t = 0 as well as positive shift reducing the absolute (negative) amplitude values. However, because the standard-stimulus response is mainly flat by 500 ms post-stimulus, this does not substantially bias the peak-to-peak measurements of components, as they were measured from latency ranges past 500 ms from the onset of the preceding standard stimulus.

factor given, together with the partial η^2 effect size when describing the results.

3.3 Results

Figures 3.1 and 3.2 present the grand-averaged difference waveforms (frontal, central, and parietal sites) for the three types of deviants in waking and sleeping in-

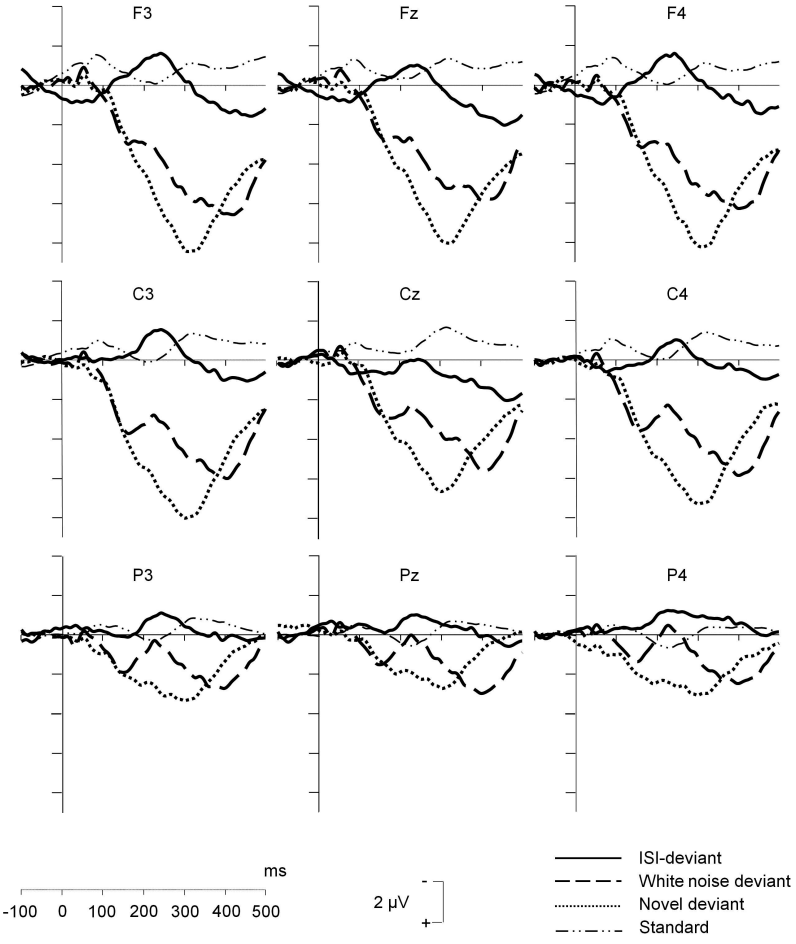

Figure 3.1: Group-average (36 waking infants) difference waveforms elicited by the ISI-deviant (solid line), white noise (broken line) and novel sounds (dotted line). Stimulus onset is at 0 ms. Amplitude calibration is at the bottom of the figure.

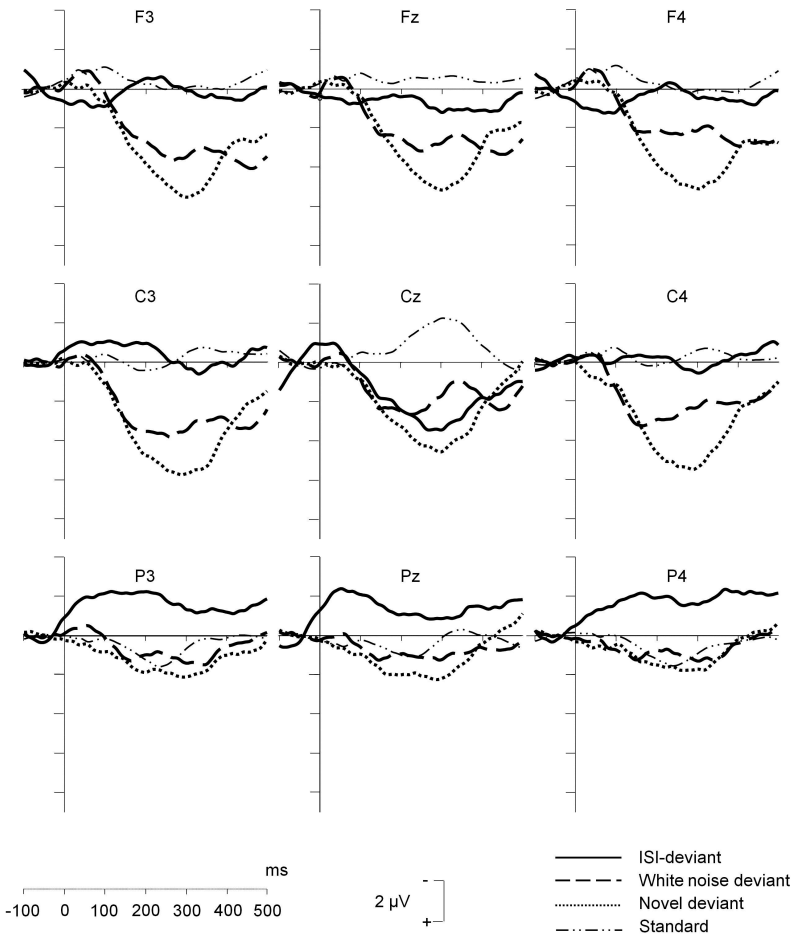

Figure 3.2: Group-average (39 sleeping infants) difference waveforms elicited by the ISI-deviant (solid line), white noise (broken line) and novel sounds (dotted line). Stimulus onset is at 0 ms. Amplitude calibration is at the bottom of the figure.

fants, respectively. Grand-averaged waveforms in response to the standard sound can also be found in the figures.

As was expected on the basis of previous research, the white noise and novel sounds elicited large positive responses. In contrast, the most prominent difference response elicited by the ISI-deviant was a negative-going peak followed by a somewhat smaller late positive-going wave. For all three deviants, the highest-amplitude responses appeared on fronto-central electrode sites. In the sleeping

infants the amplitudes seemed to be attenuated compared to those in the waking infants.

These observations are supported by the results of the two-tailed t tests comparing the deviant to the standard responses (see Table 3.1). Both the white noise and novel sound elicited a significant positive-going MMR, both in the waking and the sleeping infants. Neither of these deviants, however, yielded a significant negative-going response. The ISI-deviant, on the other hand, elicited significant MMRs of both types (positive- and negative-going waveforms), but only in the waking and not in the sleeping infants.

Negative-going wave	State of alertness	Awake		Asleep	
		MMR	t(35)	MMR	t(38)
	ISI-deviant	-1.63	-2.624*	-.12	-.188
	White noise sound	-.21	-.691	.09	.376
	Novel sound	.49	2.549	.43	2.850
Positive-going wave	State of alertness	Awake		Asleep	
		MMR	t(35)	MMR	t(38)
	ISI-deviant	1.32	2.491*	.46	.895
	White noise sound	5.83	7.194***	2.79	3.302**
	Novel sound	7.56	10.728***	5.08	7.815***

Note: $^{*}p < .05$, $^{**}p < .005$, $^{***}p < .001$

Table 3.1: Results of one-tailed t tests for significant MMRs

Table 3.2 summarises the results of the ANOVA for the positive- and negative-going MMRs. Response amplitudes and scalp distributions were significantly different for the ISI-deviant and the novel and white-noise sounds for both MMR responses (main effects of Deviant Type and interactions between Deviant Type and the Anterior vs. Posterior and/or the Laterality factors). There was a significant main effect of the State of Alertness in the positive-going MMR and a trend for the same effect in the negative-going MMR. Also, a number of interaction effects between the State of Alertness and the within-subjects factors describing the scalp distribution of the responses have been found, as illustrated by Figures 3.3 and 3.4. In general, the State of Alertness-related changes in the scalp distributions of the MMR responses were different for the ISI-deviant on the one hand and the white noise and novel sounds on the other. More specifically, for the negative-going MMR, State of Alertness had a larger effect on the response to the ISI-deviant, whereas for the positive-going MMR, State of Alertness had a larger effect on the response to the white noise and novel sounds.

3.4 Discussion

The current study tested whether two-month-old infants detect small (200 ms) deviations in the regular inter-stimulus interval in an auditory oddball paradigm with

Negative-going wave	Source	df	F	p	ϵ	η^2
	State of alertness	1,74	3.84	$.054^{+}$		.049
	IWN	2,148	3.90	$.044^{*}$	.61	.050
	IWNxState	2,148	1.86	n.s.	.61	
	FCP	2,148	4.50	$.021^{*}$	.76	.057
	FCPxState	2,148	1.39	n.s.	.76	
	LMR	2,148	17.17	$.000^{***}$	.98	.188
	LMRxState	2,148	4.12	$.018^{*}$	.98	.053
	IWNxFCP	4,296	13.19	$.000^{***}$	.46	.151
	IWNxFCPxState	4,296	1.08	n.s.	.46	
	IWNxLMR	4,296	14.78	$.000^{***}$	.67	.167
	IWNxLMRxState	4,296	4.37	$.007^{**}$	.67	.056
	FCPxLMR	4,296	3.54	$.008^{*}$	.95	.046
	FCPxLMRxState	4,296	.94	n.s.	.95	
	IWNxFCPxLMR	8,592	2.34	$.043^{*}$	.61	.031
	IWNxFCPxLMRxState	8,592	1.24	n.s.	.61	
Positive-going wave	Source	df	F	p	ϵ	η^2
	State of alertness	1,74	9.89	$.002^{**}$		.118
	IWN	2,148	49.74	$.000^{***}$	.93	.402
	IWNxState	2,148	1.30	n.s.	.93	
	FCP	2,148	92.55	$.000^{***}$	.76	.556
	FCPxState	2,148	.74	n.s.	.76	
	LMR	2,148	3.22	$.049^{*}$	.88	.042
	LMRxState	2,148	.39	n.s.	.88	
	IWNxFCP	4,296	9.01	$.000^{***}$	.75	.109
	IWNxFCPxState	4,296	3.27	$.022^{*}$	.75	.042
	IWNxLMR	4,296	5.95	$.000^{***}$	.91	.074
	IWNxLMRxState	4,296	.33	n.s.	.91	
	FCPxLMR	4,296	.57	n.s.	.88	
	FCPxLMRxState	4,296	.14	n.s.	.88	
	IWNxFCPxLMR	8,592	5.59	$.000^{***}$	.87	.070
	IWNxFCPxLMRxState	8,592	1.56	n.s.	.87	

Note: $^{+}p < .1$, $^{*}p < .05$, $^{**}p < .005$, $^{***}p < .001$, n.s. not significant
IWN: Deviant type (ISI-deviant, white noise sound, novel sound), FCP: Anterior vs. Posterior (frontal, central, parietal), LMR: Laterality (left, medial, right

Table 3.2: Results of omnibus ANOVAs on both negative- and positive-going waves.

one standard and three deviant sounds. We found significant differences between ERP responses elicited by the standard sound and the ISI-deviant stimulus. This suggests that infants detect even presentation rates and represent them as a regular aspect of the stimulation. Thus adult-like mechanisms for detecting violations

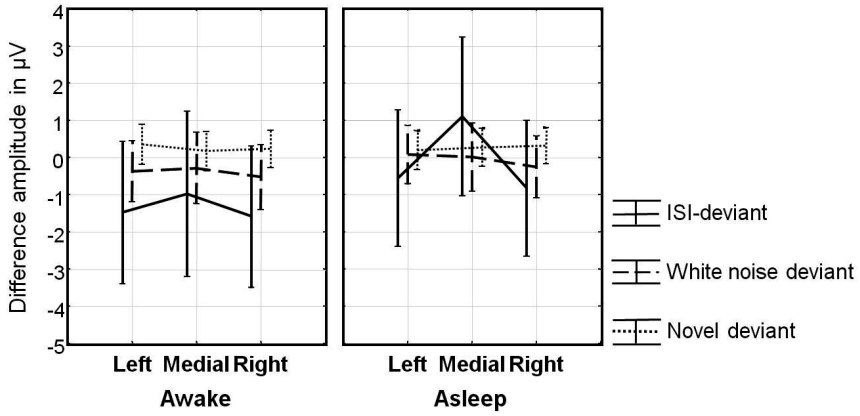

Figure 3.3: Interaction between Deviant Type, Laterality and the State of Alertness for the negative-going MMR.

in auditory temporal patterns may already be functional in two-month-old infants. This ability is a necessary prerequisite of extracting and representing temporal structure from sound sequences (such as musical rhythm; Winkler et al., 2009b and, in general, for constructing auditory objects (Winkler et al., 2009a) that span multiple discrete sounds (such as a series of footsteps).

Our results are generally in accordance with the findings of Brannon et al. (2004), who found in ten-month-old infants an early negative deflection at 120-240 ms post-stimulus over frontal brain areas and a late anterior positivity in the ERP difference wave in response to stimuli with deviant onset-to-onset intervals. We also found a negativity in response to our ISI-deviant, most clearly defined in the waking infants, at 215-235 ms from stimulus onset and a late positivity at fronto-central electrode sites. One may speculate that the slightly earlier responses on frontal electrodes found in older infants represent faster temporal analysis due to maturation of the brain (e.g. increased myelination).

As can be seen in figures 1 and 2, the morphology of the responses elicited by the ISI-deviant is quite different from those elicited by the white noise and novel sounds. Whereas the infants responded with a large positive MMR to these latter two deviants, the response to the ISI-deviant is of lower amplitude and - perhaps more importantly - starts with a negative-going discriminative response. This difference was most prominent for the waking infants. In adults, similar findings have been obtained for ISI-deviants. Ford and Hillyard (1981) found that occasionally shortening the ISI between two stimuli in an otherwise isochronous sequence resulted in a large negative waveform peaking 135-220 ms from stimulus-onset. The authors offered two explanations: this response could be unique to stimuli presented earlier than predicted or it could be a manifestation of a more general ERP response to deviance. Nordby et al. (1988), using pitch-deviant and time-deviant

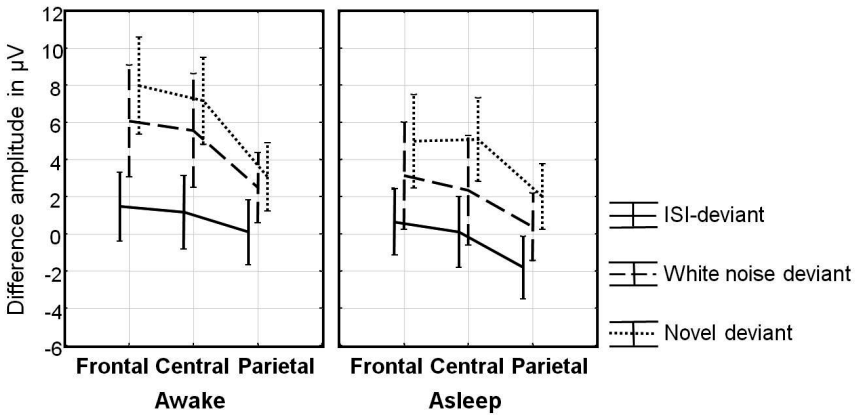

Figure 3.4: Interaction between Deviant Type, Anterior vs. Posterior and the State of Alertness for the positive-going MMR.

tones, concluded that both deviants elicited the MMN and that the differences between the MMNs elicited by these two types of deviants may stem from differences in their discriminability or salience. However, since then, several studies showed that the MMN is not a unitary process; different deviations activate partly separate neural circuits (e.g., Giard et al., 1990), specifically, spectral and temporal deviations result in MMNs with different generator structure (Alain et al., 1999; Takegata et al., 2001). Further, deviance-related responses may appear quite early and may be partly generated subcortically (Grimm and Escera, 2012; Slabu et al., 2010). The same may be true for infants: at least partly different neural circuits may detect stimuli deviating in temporal versus spectral characteristics from a standard sound. However, the infant MMR cannot be regarded as a full equivalent of the adult MMN (see, e.g., Kushnerenko et al., 2007). Therefore, drawing conclusions on the basis of the component structure of the adult MMN may be misleading.

Another difference between the current temporal and the two spectral deviants is that the latter widely deviate from the standard. In adults, these wide deviants are usually termed as "novel" sounds, acknowledging that they usually categorically differ from the standard (context) sounds (Escera et al., 2000; Friedman et al., 2001; Polich, 2007). When presented amongst tonal standards, sounds with wide distributed spectral contents activate large fresh networks in the auditory system and thus elicit high-amplitude responses, only a part of which can be regarded as being related to deviance detection. This is true both for the adult and infant responses (see, e.g., Kushnerenko et al., 2007). The responses observed in the current study are fully compatible with those previously reported for newborn infants Kushnerenko et al. (2007). In adults, the P3a component, which is a characteristic part of the novelty response, is thought to be involved in redirecting attention

to the incoming stimulus (Escera et al., 2000; Friedman et al., 2001). Although there are speculations as to whether the large positive response observed in infants could be a precursor of the adult P3a, this assumption is difficult to substantiate. Similarly to the deviance-related response, the novelty response in neonates is not a full analogue of the response observed in adults.

The responses obtained in this study were influenced by the infants' state of alertness, although the effect was less dramatic than that found in a previous study (Friederici et al., 2002). In general, for all three deviants we saw smaller responses in sleeping than in waking infants. The state of alertness primarily affected the scalp distribution of the main MMR response for each type of deviance: the negative-going MMR for the ISI and the positive-going MMR for the spectral deviants (white noise and novel sound). It should be noted, however, that our categorisation of the various behavioural states, as allowed by the recording methods and the sample size, may not be sufficiently elaborate for distinguishing between the effects of the various states of alertness (such as quiet sleep, active sleep, drowsy, quiet awake, active awake) on the ERP responses. Pooling together, separately, different sleep and waking states may have distorted some of the effects of the state of alertness. Research focused on the different states of alertness is needed to study these effects in more detail.

As mentioned earlier, the current results support the notion that the perceptual abilities of infants may be somewhat less developed in terms of details, but they are not qualitatively different from that of adults. Therefore, together with similar findings (e.g. Alho et al., 1990; Cheour et al., 1997; Cheour-Luhtanen et al., 1996; Winkler et al., 2009b) they provide the perceptual basis for many important areas of learning occurring during the first year of life, such as developing the basic means of communication, object representation and motor control. In addition, as our results are based on a rather large group of infants compared to most similar studies conducted previously - thus increasing the reliability of the data - they can potentially be used as normative information for studying typically developing children.

Assessing infants' ability to detect violation of temporal regularities may also be useful for future clinical applications. For example, some studies showed that the amplitude of the MMN-like ERPs elicited by frequency deviance was reduced in newborns and infants with cleft palate. This reduction appeared to remain constant over time (Čeponienė et al., 2000; Cheour et al., 1999). Further, Holopainen et al. (1997) found that the MMN to frequency deviance was attenuated in young children with developmental aphasia. Hence, the MMN/MMR can potentially be used to identify developmental problems such as learning difficulties and speech impairments very early, even before they can be detected behaviourally (e.g. Weber et al., 2005). More research is needed, however, normative as well as clinical, before the MMR component can be used in clinical settings.

The present study investigated whether infants aged two months can discriminate between stimuli which have been presented with different inter-stimulus intervals. Our findings show that they are indeed able to detect deviations as small as 200 ms. These results suggest that the ability to detect violations of temporal reg-

ularities develops very early and is already functional within the first few months of life. The distribution of the response to both temporal and spectral deviations is influenced by the state of alertness of the infants.

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4

Multimodal processing of emotional information in 9-month-old infants I: Emotional faces and voices

R.A. Otte, F.C.L. Donkers, M.A.K.A. Braeken,
& B.R.H. Van den Bergh.

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Highlights

- Processing of audiovisual information is important for communication with others
- We studied processing of fearful and happy face/voice pairs in 9-month-old infants
- Fearful facial expressions modulated the processing of fearful and happy voices
- The infants in the current study displayed a bias for fearful stimuli

Abstract

Making sense of emotions manifesting in human voice is an important social skill which is influenced by emotions in other modalities, such as that expressed by the corresponding face. Although processing emotional information from voices and faces simultaneously has been studied in adults, little is known about the neural mechanisms underlying the development of this ability in infancy. Here we investigated multimodal processing of fearful and happy face/voice pairs using event-related potential (ERP) measures in a group of 84 9-month-olds. Infants were presented with emotional vocalisations (fearful/happy) preceded by the same or a different facial expression (fearful/happy). The ERP data revealed that the processing of emotional information appearing in human voice was modulated by the emotional expression appearing on the corresponding face: infants responded with larger auditory ERPs after fearful compared to happy facial primes. This finding suggests that infants dedicate more processing capacities to potentially threatening than to non-threatening stimuli.

Keywords: Infant; multimodal processing; audiovisual processing; event-related potential (ERP); emotion perception; affective priming

4.1 Introduction

Humans are social beings, often living together in close quarters and as such communication with others is a significant part of our day-to-day life. In this social context human voices are one of the most important stimuli in our auditory environment. They not only convey semantic information, but also information about one's identity and emotional state (Belin et al., 2004; Grossmann, 2010; Latinus and Belin, 2011). That voices are special is emphasised by the existence in the adult human brain of voice-selective regions along the upper bank of the superior temporal sulcus dedicated to the processing of human vocal sounds - both speech and non-speech vocalisations (Belin et al., 2000; Kreifelts et al., 2009). The amygdala, inferior prefrontal cortex, and insula have been found to be involved in the processing of affective information in voices (Belin et al., 2004; Blasi et al., 2011). How emotional information from vocalisations is processed partly depends on information from other modalities, such as visual input from facial expressions. For example, de Gelder and Vroomen (2000) and de Gelder et al. (2002) found that in adults both recognition and judgement of emotion in voices is modulated by consciously as well as unconsciously recognised emotion in faces, providing evidence that the brain uses information from both modalities to interpret emotions. The goal of the current study was to examine whether this is also true for 9-month-old infants: do they process emotional vocalisations differently when they have been primed with a visual stimulus conveying the same, versus a different emotion?

From a developmental perspective, studying how emotional information from faces may modulate the processing of emotional content from voices is important, for example for understanding how interpersonal skills develop, such as interaction with others by reading their emotions (Grossmann, 2010; Walker-Andrews, 1997). The ability to process emotional information from different modalities simultaneously appears to develop quite early in human life. Behavioural experiments, for instance, have found that by 3 to 5 months of age recognition of affect emerges in bimodal stimulation, first in familiar and then in unfamiliar contexts and persons (Kahana-Kalman and Walker-Andrews, 2001; Walker-Andrews, 1997), as evidenced by discrimination between happiness, anger and sadness (Flom and Bahrick, 2007; Leppänen and Nelson, 2008). For successful differentiation at this age, though, it is necessary that there is temporal synchrony between face and voice, i.e. speech should be played in synchrony with lip movement (Flom and Bahrick, 2007). From 7 months of age infants are able to detect happiness, anger and sadness acros audiovisual modalities without needing temporal synchrony between faces and voices (Flom and Bahrick, 2007; Soken and Pick, 1992; Walker-Andrews, 1997).

To date, electrophysiological measures such as event-related potentials (ERPs) have been seldom used to study multimodal processing of emotional information in infancy. However, as ERPs can be recorded in the absence of a behavioural response (Nelson and Bloom, 1997), even for unattended stimuli (Sussman, 2007), they are quite suitable for studying emotion processing in infants. Indeed, in previous infant research on emotional faces (e.g. de Haan et al., 2003; Leppänen

et al., 2007, emotional voices (Grossmann et al., 2005) and emotional face/voice pairs (Grossmann et al., 2006), the use of ERPs helped to gain insights into the development of the underlying mechanisms. For example, in the study by Grossmann et al. (2006), the authors presented their 7-month-old subjects with a happy or angry static facial expression (a prime). After a 400 ms-delay, a word was spoken in an emotionally congruent or incongruent tone. Faces remained visible until the end of the presentation of the word. The authors found that the emotionally incongruent condition elicited a larger Negative Component (Nc) around 500 ms post-stimulus. In contrast, the emotionally congruent condition elicited a larger Positive Component (Pc) approximately 800 ms after stimulus onset. (Grossmann et al., 2006) concluded that the attenuation of the Nc and enhancement of the later Pc reflected recognition of the familiar/expected face/voice pairs, and that the infants had thus recognised and processed emotions from both modalities.

To our knowledge, the study by (Grossmann et al., 2006) is the only ERP study with infants in which processing of emotional auditory stimuli is studied in the context of emotional facial expressions, instead of the other way around. However, as the auditory system develops earlier than the visual system (Anderson and Thomason, 2013; Anderson et al., 2001), from a developmental perspective, emotional vocalisations may be just as relevant as facial expressions in the first months of life. This is supported by findings that 5-month-olds do respond to emotional vocalisations in the absence of facial emotional expressions, but not vice versa (Fernald, 1993). Also, Caron et al. (1988) found that 5- to 7-month-olds rely more on auditory than visual input when discriminating emotional expressions. In addition, results from a study by Mumme et al. (1996) suggested that information from the mother's voice alone, but not from her face only, can be sufficient in guiding 12-month-olds' behaviour in ambiguous situations. Therefore, in the current study, we examined the processing of emotional vocalisations (fearful and happy) in a large group (N = 84) of 9-month-old infants after priming them by a visual stimulus conveying the same, versus a different emotion.

We hypothesised that 1) the emotional quality of the visual prime (happy vs. fearful) will modulate the response to the following voice; and 2) that emotional (in)congruency between the visual prime and the following voice will modulate the ERP response to the latter.

4.2 Methods

4.2.1 Subjects

Subjects were 84 infants (one pair of twins) and their mothers from a normal (i.e. non-clinical) population who have been taking part in a longitudinal study on prenatal early life stress (PELS project). The study was approved by the Medical Ethical Committee of the St. Elizabeth Hospital in Tilburg, The Netherlands. Informed consent was obtained from all mothers and fathers in accordance with the Declaration of Helsinki. Detailed information on the cohort and its recruitment

has been described previously in Otte et al. (2013a).

In short, the cohort consists of 190 women - and their partner and child - who have been recruited during pregnancy, either before 15 weeks gestational age (GA; N = 178) or between week 16 and 22 (N = 12) of gestation, from a general hospital and four midwives' practices in Tilburg, The Netherlands. Women were followed up three times during their pregnancies (measurement waves T1, T2 and T3, respectively) and were invited to the lab for postpartum observations both 2 to 4 months (T4) and 9 to 11 months (T5) after giving birth. Here, we report the results from infants measured at T5; data collected at T4 have been discussed elsewhere (Otte et al., 2013a; van den Heuvel et al., 2013).

At T5 147 of the original 190 women came in for testing with their infant (one pair of twins). Forty-three women did not participate in this measurement wave, because of drop out before T5 (N = 32), because they could not be reached in time (6), they were (N = 1) or their infant was (N = 2) too ill, they had miscarried around T2 (N = 1), or their infant had passed away (N = 1). Three of the 147 mothers had delivered prematurely, and 1 mother had delivered a baby small for gestational age (GA; i.e. birth weight $<$2500 g at term delivery). Data for infants of these mothers (N = 4) were excluded from analysis beforehand. Data for an additional 60 of the remaining 144 infants were later excluded because of too little remaining data after removing invalid trials (i.e. with movement artifacts and where the infant had not looked at the stimulus; N = 33), fussiness (N = 13), and technical problems (e.g. severe problems with mastoids; N = 14). This attrition rate (41.2%) is similar to other infant ERP studies (DeBoer et al., 2007). All infants were healthy and had passed a screening test for hearing impairments (evoked otoacoustic emission), performed by a nurse from the infant health care clinic, between the 4th and 7th day after birth. The mean age at testing of the 84 infants (45 girls) included in the sample was 303 days (SD=14 days). Mean GA and mean birth weight were 39.9 weeks (SD=8.7 days) and 3477 g (SD=464 g), respectively.

4.2.2 Stimuli

4.2.2.1 Visual stimuli

Visual stimuli were 18 colour photos of 9 Caucasian women in frontal view, each expressing both happiness and fear. Only female and Caucasian identities had been chosen so as to avoid any sex or ethnicity differences from influencing the infant ERPs (e.g. see Ramsey et al., 2005; Vogel et al., 2012. The emotional faces had been cut out from their original background and pasted onto a black background. Four identities were taken from the validated NimStim Face Stimulus Set (http://www.macbrain.org/ resources.htm[1]). Their fearful and happy expression

[1]Development of the MacBrain Face Stimulus Set was overseen by Nim Tottenham and supported by the John D. and Catherine T. MacArthur Foundation Research Network on Early Experience and Brain Development. Please contact Nim Tottenham attott0006@tc.umn.edu for more information concerning the stimulus set.

had been recognised at least 75% of the time in the validation study. In Figures 4.1A and 4.1B examples of a happy and fearful face from the NimStim set can be found. An additional 5 identities were included from a database with emotional facial stimuli from the Cognitive and Affective Neurosciences Laboratory at Tilburg University, The Netherlands. This was done because we wanted to include as many identities as possible to minimise the possibility that potential effects would be due to the identity itself, and the NimStim set did not offer more than 4 female Caucasian identities whose fearful and happy expression survived the validation process. The stimuli from the Tilburg database had been validated in a pilot study in which they were rated for emotion (fear, happiness, anger, neutral, surprise, sadness and disgust), intensity (scale from 1 to 5) and positive/negative affect (scale from 1 to 5) by at least 8 participants. For the 5 identities used for this study both the emotion fear and happiness were correctly recognised by the raters 80% of the time or more. In Figures 4.1C and 4.1D examples of a happy and fearful face from the Tilburg set can be found.

4.2.2.2 Auditory stimuli

Auditory stimuli were voice recordings of six women expressing fear and happiness with non-verbal vocalisations. We chose to use non-verbal vocalisations as to minimise automatic semantic or verbal processing (Van den Stock et al., 2008). Because not all vocalisations had been recognised well enough in the validation study (see below), for two of the identities we only used the fearful vocalisation, for two of them we only used the happy vocalisation and for two of them we used vocalisations of both emotions, resulting in 4 fearful and 4 happy auditory stimuli. These stimuli were provided by the Cognitive and Affective Neurosciences Laboratory at Tilburg University also, and were recorded, processed and validated as described by Van den Stock et al. (2008). Semi-professional actors were asked to make a frightened or happy sound, based on a specific script describing situations such as an attack by a robber. Audio recordings were made at a 44.1 KHz sampling rate and an intensity level of 75 dB. They were originally of 800 ms duration, and were shortened to 500 ms for the experiment described here. The sounds were validated in a pilot session by 15 participants, who were instructed to categorise as accurately and as fast as possible the emotion expressed by voices (fear or happiness). All stimuli used here had been correctly recognised 80% of the time or more.

4.2.3 Procedure

Each of the 18 visual stimuli was paired with both the fearful and happy auditory stimuli. This created a relatively large set of face/voice compounds, minimising the chance that potential effects would be caused by specific compounds. The 144 compounds comprised four experimental conditions: happy face-happy voice (HH), fearful face-fearful voice (FF), happy face-fearful voice (HF), and fearful face-happy voice (FH). They were presented twice during the experiment (288

Figure 4.1: Examples of a typical happy (A) and fearful (B) visual expression from the NimStim dataset, and of a typical happy (C) and fearful (B) visual expression from the Tilburg dataset.

trials), divided in four blocks of 72 stimuli, each. The presentation order within each of the blocks was randomised and the order between the blocks was counterbalanced. The blocks were presented with small breaks in between as needed.

During stimulus presentation the infant was seated on its parent's lap in a dimly lit and sound-attenuated room. The parent-infant dyad were seated behind a desk with a computer screen (CRT VGA, 21 inch, 1280 x 1024, 100 Hz) at a distance of approximately 70 cm from the eyes. The visual stimuli measured 18.5 x 22.5 cm and the horizontal and vertical visual angles were 7.53^o and 9.13^o, respectively. Auditory stimuli were presented through speakers positioned on either side of the

screen, and at a distance of approximately 90 cm from the infant's head. To prevent the parent from influencing the infant's ERP responses by unconsciously reacting to the stimuli, he or she was wearing head phones through which classical music was playing. Two cameras filmed the experimental session and these data were later used to code whether the infant had looked at a specific trial or not (see also "Data acquisition and analysis" section 4.2.4).

Each stimulus block started with the sound of a laughing baby and the presentation of a red dot growing bigger and smaller in the centre of the computer screen to attract the infant's attention. When the infant was looking at the screen, the experimenter started the first trial. Each trial lasted 1400 ms and started with the presentation of a visual stimulus that lasted 900 ms, after which an auditory stimulus was presented. A larger interval between presentation of faces and voices compared to that in the study by Grossmann et al. (2006) was chosen, as to decrease chances that late responses to the visual stimulus would interfere with responses to the auditory stimulus. The visual stimulus remained in place until the auditory stimulus was played out (after 500 ms). Each trial was followed by an inter-trial interval with variable duration (between 600 and 1000 ms) to reduce temporal predictability. During this interval, the screen was black.

When the infant looked away from the screen, the experimenter tried to recapture his/her attention by presenting an attractive moving figure in the centre of the screen. As soon as the infant was looking at the screen again, the experiment continued. The experiment was concluded either after all 288 stimuli had been presented or when the infant became too fussy to continue.

4.2.4 Data acquisition and analysis

EEG was recorded with BioSemi ActiveTwo amplifiers (BioSemi, Amsterdam, The Netherlands) with a sampling rate of 512 Hz[2]. Infants wore head caps with 64 electrode locations positioned according to the revised version of the International 10-20 system. The standard BioSemi reference(CMS-DRL) was used (see www.biosemi.com/faq/cms&drl.htm for details) and two additional electrodes were placed both on the left and right mastoid. Off-line, these were mathematically combined to produce an average mastoids reference derivation (Luck, 2005).

Before the data were processed further, independent raters inspected the data and scored per infant per trial whether or not he or she had indeed looked at the visual stimulus. All trials were scored by 2 different raters (there were 4 raters in total) and these scorings were afterwards compared. Agreement between raters was between 81% and 99% and was 95% on average. Whenever scorings differed, the trials concerned were re-inspected and scored again. If there was still doubt about whether the infant had actually seen the stimulus, the trial was excluded. Trials during which an infant was crying were also excluded. Only EEG signals

[2]On-line filtering options in the BioSemi software are set automatically and depend on the chosen sampling rate. See http://www.biosemi.com/faq/adjust filter.htm and http://www.biosemi.com/faq/adjust samplerate.htm

from trials during which the infant was looking at the stimulus were used for data analysis.

The EEG data were analysed using BrainVision Analyzer software (Brain Products, Munich, Germany). The continuous EEG signals were filtered off-line, with a 0.1 to 20 Hz band-pass filter (slope 24 dB), and an additional 50 Hz notch filter to make sure all 50 Hz noise would be removed. The signals were then segmented into 1000 ms-long epochs, time-locked to the onset of the auditory stimulus. The 200 ms before auditory stimulus onset were used as the baseline. The epochs were averaged separately for each of the four conditions (FF, HH, HF, FH). Epochs with sample-to-sample voltage steps exceeding 80 μV were excluded, as were epochs with amplitudes exceeding 150 μV in any 200 ms-long window within the whole epoch, and those with amplitudes below 0.5 μV in any 100 ms-long window within the whole epoch. Data from infants with less than 14 acceptable responses for any one of the four condition were removed from further analysis (33 infants, see also "Subjects" section 4.2.1). The average number of available trials per infant was 36.4 for FF (SD = 12.6), 37.5 for HH (SD = 12.0), 36.6 for HF (SD = 11.8), and 37.1 for FH (SD = 12.8).

Time windows for analysis were selected based on visual inspection of the grand average waveform for all responses combined at electrode sites F3, Fz, F4, C3, Cz, C4 (see Figure 1), where responses were largest. The following windows, each centred around a peak in the grand average waveform, were chosen: 120-200 ms, 200-260 ms, 290-430 ms, 380-540 ms, and 620-680 ms post-stimulus (from this point on referred to as P150, N250, P350, N450, and P650, respectively). These first four areas correspond to the infant P150-N250-P350-N450 ERP complex in response to auditory stimuli described by Kushnerenko et al. (2002), who found that this pattern is already identifiable in auditory ERPs from birth.

Amplitudes measured from the above mentioned windows were analysed by means of a 2 x 3 x 2 x 2 repeated measures ANOVAs design (run in IBM SPSS 19.0), separately for each window. Within-subjects factors were "Frontal-central" (frontal, central), "Laterality" (left, medial, right), "Visual Prime" (fearful, happy), and "Auditory Emotion" (fearful, happy). As we were especially interested in effects of priming by the visual stimulus on the auditory stimulus, interactions and main effects including the factor Visual Prime were further analysed by means of post-hoc tests. Greenhouse-Geisser correction was used where necessary and the ϵ correction factor is given, together with the partial η^2 effect size.

4.3 Results

The grand-averaged waveform for all responses combined can be found in Figure 4.2. Figure 4.3 shows the grand-averaged responses per condition. For investigating our hypotheses only effects involving Visual Prime will be interpreted here, because this factor represents effects of the visually presented emotion on the processing of the emotional sounds (see Table 4.1 for a full list of statistical information).

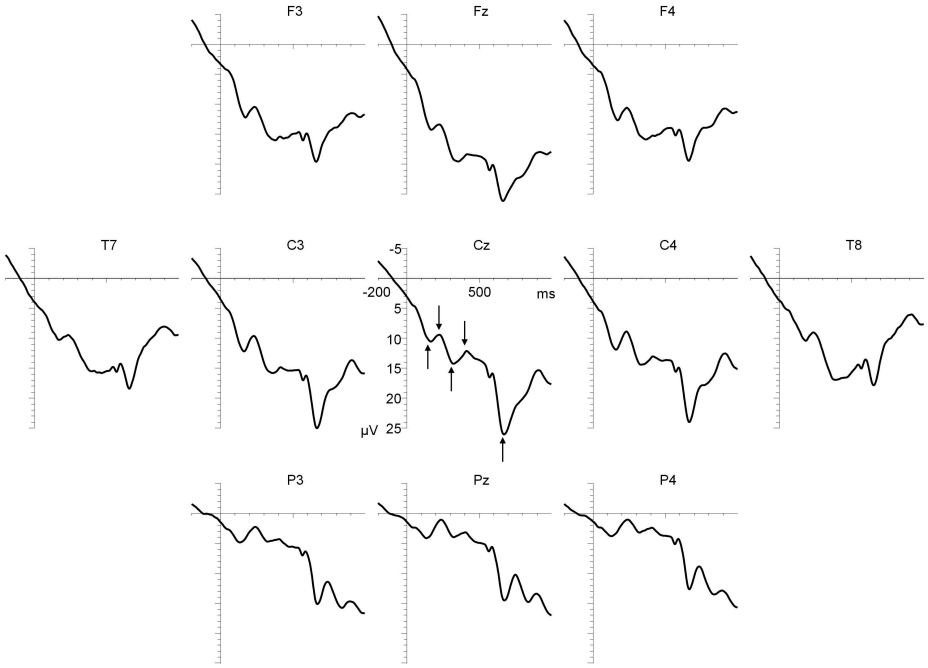

Figure 4.2: Grand-averaged waveform for all 4 conditions (FF, HH, FH, HF) combined. Arrows indicate the P150, N250, P350, N450 and P650 area, respectively. Stimulus onset is at 0 ms. Amplitude calibration is at Cz.

4.3.1 Results for P150 (120-200 ms post-stimulus)

The 4-way ANOVA yielded an interaction between the factors Visual Prime and Frontal-Central. Post-hoc tests showed that if the visual stimulus had been fearful, the auditory responses had larger positive amplitudes on central electrode sites than when the visual stimulus had been happy ($t(83) = 2.032; p < .001$). On frontal electrodes sites response amplitudes for the fearful and happy visual stimuli did not significantly differ from each other ($t(83) = .935; p > .05$).

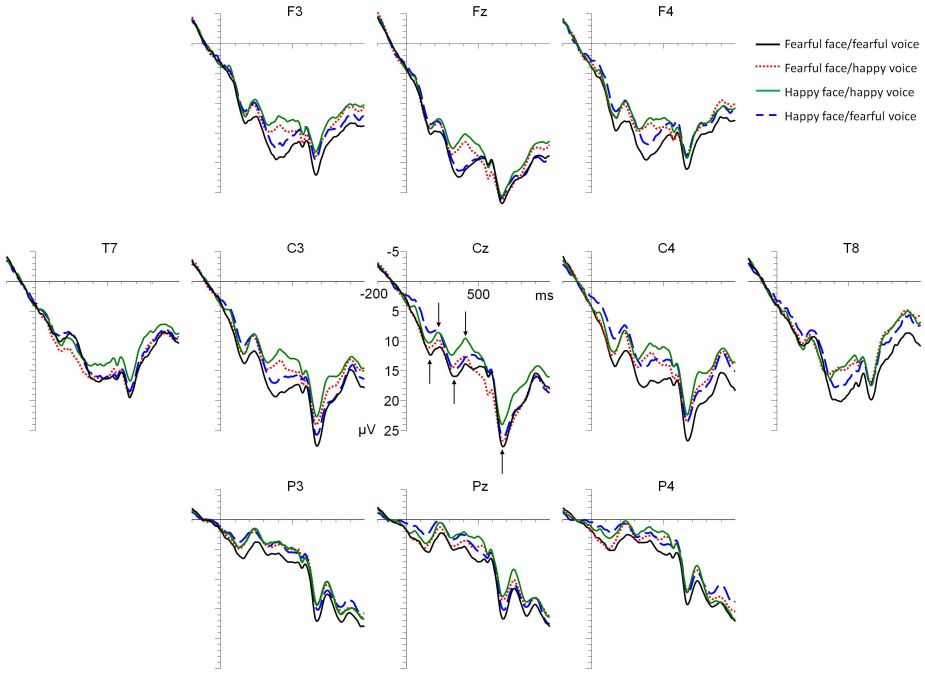

Figure 4.3: Grand averages in response to condition FF (solid line), HH (short-striped line), FH (dotted line), and HF (long-striped line), respectively. Arrows indicate the P150, N250, P350, N450 and P650 area, respectively. Stimulus onset is at 0 ms. Amplitude calibration is at Cz.

4.3.2 Results for N250 (200-260 ms post-stimulus)

The analysis yielded a main effect for the factor Visual Prime. Post-hoc tests showed that when the visual stimulus had been fearful, responses to the auditory stimulus had been of smaller negative-going amplitudes than when the visual stimulus had been happy($t(83) = 1.855; p < .05$).

4.3.3 Results for P350 (290-430 ms post-stimulus)

There was a main effect for Visual Prime, and post-hoc tests showed that when the visual stimulus had been fearful, responses to the auditory stimulus had been of higher positive amplitudes than when the visual stimulus had been happy ($t(83) = 1.783; p < .05$).

Latency range (ms)	Factor(s)	*df*	*F*	*p*	ϵ	η^2
120-200	Frontal-Central	1,83	5.204	.025		.059
	Visual Prime	1,83	8.762	.004		.095
	Frontal-Central x Laterality	2,166	23.078	.000	.931	.218
	Frontal-Central x Visual Prime	1,83	6.295	.014		.071
200-260	Frontal-Central	1,83	10.325	.002		.111
	Laterality	2,166	4.511	.015	.910	.052
	Visual Prime	1,83	8.494	.005		.093
	Frontal-Central x Laterality	2,166	10.966	.000	.928	.117
290-430	Frontal-Central	1,83	7.780	.007		.086
	Visual Prime	1,83	6.500	.013		.073
	Auditory Stimulus	1,83	12.625	.001		.132
	Frontal-Central x Laterality	2,166	18.759	.000		.184
380-520	Frontal-Central	1,83	5.937	.017		.067
	Visual Prime	1,83	6.570	.012		.073
	Auditory Stimulus	1,83	10.747	.002		.115
	Frontal-Central x Laterality	2,166	13.741	.000		.142
	Frontal-Central x Laterality x Auditory Stimulus	2,16	3.717	.026		.043
620-680	Laterality	2,166	4.957	.008	.810	.056
	Frontal-Central x Laterality	2,166	9.004	.000		.098

Table 4.1: Significant main effects and interactions

4.3.4 Results for N450 (380-520 ms post-stimulus)

For the N450 results similar to those for the N250 were found. The analysis yielded a main effect for Visual Prime, which was explained by smaller negative-going responses to the auditory stimuli when the preceding facial expression had been fearful ($t(83) = 1.974; p < .05$).

4.3.5 Results for P650 (620-680 ms post-stimulus)

No significant main or interaction effects for Visual Prime were found.

4.4 Discussion

The current study investigated in 9-month-old infants the processing of emotional (fearful and happy) auditory vocalisations after priming them with a facial expression conveying the same, versus a different emotion. We found that the infants

responded with larger P150 and P350 amplitudes, and smaller N250 and N450 amplitudes to the auditory stimuli when they had been primed with a fearful as compared to with a happy facial expression. These findings confirm our first hypothesis that the presentation of an emotional facial expression can modulate the processing of a vocal expression in 9-month-olds. Contrary to our expectation, we did not find evidence for our second hypothesis. Emotional (in)congruency between the visual prime and the following voice did not differentially modulate the ERP response to the latter: when the facial expression had been fearful, responses to both the fearful (congruent) and the happy (incongruent) auditory vocalisations were modulated in the same way compared to when the facial expression had been happy.

Our findings are in accordance with previous studies on audiovisual processing of emotional information in both adults and infants suggesting that how the brain processes emotional information from voices can be modulated by an emotional expression in faces (de Gelder et al., 2002; de Gelder and Vroomen, 2000; Grossmann et al., 2006). As the results were most pronounced for the fearful stimuli, they are also compatible with studies on a negative bias in infants, which showed that "infants attend more to, are more influenced by, and use to a greater degree negative rather than positive facets of their environment" (Vaish et al., 2008, p383).

In the current study, data from a large group of infants (N = 84) were included in the analysis. In addition, the infants attended the visual stimuli quite well (73.2% on average), and we were able to retain a relatively large average number of stimuli per condition (37.3). Therefore, the finding that fearful facial expressions modulate the processing of emotional vocalisations appears to be robust.

In contrast to previous findings, infants in the current study did not respond differentially to emotional auditory vocalisations after priming with a visual stimulus conveying the same, versus a different emotion. There are several possible reasons for this negative finding. 1) The fearful facial stimuli may have elicited higher arousal levels than the happy facial stimuli, such that response amplitudes to the following auditory stimuli were stronger regardless of the type of emotion. This corresponds to previously mentioned findings that infants display a negativity bias to fearful stimuli (Vaish et al., 2008). In the context of the current experiment, however, it does not explain why no (in)congruency effects were found for auditory stimuli following a happy expression. 2) The infants may not have recognised the fearful or the happy expression in the voices and therefore did not match voices to faces expressing the same emotion. Indeed, although there are findings that infants can discriminate between fearful and happy facial expressions from the age of 7 months (Kotsoni et al., 2001; Nelson and De Haan, 1996), studies reporting recognition of fearful vocalisations are scarce and they were conducted in older infants (Mumme et al., 1996). However, since the largest responses were found for the fearful congruent condition (e.g. fearful face-fearful voice), this explanation does not seem plausible. Also, it does not account for the lack of effects for happy/happy face/voice compounds. 3) The interval between the presentation of the face and the voice might have been too long. As mentioned in the "Methods

section 4.2, we chose a 900 ms interval to avoid any overlap from the late ERP responses to the faces with the early ERP responses to the voice. Because of the duration of the interval, perhaps the infants did not interpret the faces and the following voice as a pair. Thus, although processing of the auditory stimulus was influenced by the preceding face, possibly no processing of faces and voice as a compound took place. 4) As the same voices and faces appeared in different combinations in the current experiment, the 9-month-olds may not have been able to associate voices with a particular face. So, on hearing a certain voice, they may have experienced this voice as not belonging to the face that had been (and was being) presented, obviating the need for the face and voice to emotionally match each other. Future studies in which the interval between the facial and vocal expression is shorter, and in which a facial identity is always accompanied by the same vocal identity could help answer the question why infants did not respond differentially to emotionally similar versus dissimilar face/voice pairs.

To conclude, the current study investigated multimodal processing of fearful and happy face/voice pairs in 9-month-old infants by means of ERPs. Analysis revealed that the processing of fearful and happy vocalisations was modulated by fearful facial expressions: infants responded with larger auditory ERPs after having been primed by a fearful visual stimulus. The findings provide evidence for a negative bias in infants, confirming previous results that infants, just like adults, dedicate more processing capacities to potentially threatening than unthreatening stimuli in their surroundings.

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Part C

Prenatal exposure to maternal anxiety affects infant neurocognition in the first year of life

5

Prenatal exposure to maternal anxiety and information processing in two-month-old infants: An auditory ERP study

R.A. Otte, I. Winkler, M.A.K.A. Braeken,
M.I. van den Heuvel, & B.R.H. Van den Bergh.

Brain Research, under review

Abstract

Atypical events occurring during critical periods of prenatal life may induce reprogramming of prenatal and neonatal development. In the current study we examined in two-month-old infants the influence of exposure to maternal anxiety during early pregnancy (Anx-EP) on information processing related to language development. Seventy pregnant women filled out a State-Trait Anxiety Inventory between their 9th and 15th pregnancy week. Two months after birth, auditory event-related potentials (AERPs) were recorded in their infants using an oddball paradigm with a standard tone and three types of deviants: inter-stimulus interval (ISI) deviants, rare white noise and novel sounds. Analysis showed that exposure to higher levels of Anx-EP was associated with larger response amplitudes elicited by the standard and the identical ISI-deviant tones. No similar effects were found for the white noise and novel sounds. Infants from highly anxious mothers thus appear to process sounds low on new information more extensively than infants born to mothers with lower levels of anxiety during their pregnancy. The poorer language acquisition observed in other studies in children prenatally exposed to high levels of maternal anxiety may be related to the more extensive processing of sounds with low information contents.

Keywords: Pregnancy; state anxiety; infants; prenatal exposure; information processing; auditory event-related potentials

5.1 Introduction

During critical periods of life in the womb and shortly after birth, environmental influences can shape (or program) the developing organism by influencing gene regulatory mechanisms. Atypical events may induce reprogramming of prenatal and neonatal development, and these processes have been termed perinatal (re)programming (Barker, 1998; Godfrey and Barker, 2001; Nathanielsz, 1999; Van den Bergh, 2011a,b). For example, several reviews concluded that exposure to high levels of maternal stress and anxiety during pregnancy increased the likelihood of regulation problems at behavioural, emotional, and cognitive levels. These effects on the offspring were observed from the foetal period to early adulthood (Mennes, 2008; Mennes et al., 2009; Räikkönen et al., 2011; Talge et al., 2007; Van den Bergh et al., 2005), even after controlling for potential confounders, such as alcohol consumption and smoking during pregnancy, socio-economic status, and postpartum levels of maternal stress and anxiety. The goal of the current study was to assess the influence of exposure to maternal anxiety during early pregnancy (Anx-EP) on information processing in young infants.

One of the cognitive findings from previous research was that exposure to prenatal maternal stress and anxiety is related to poorer language skills (Henrichs et al., 2011; King and Laplante, 2005; Laplante et al., 2004, 2008). Critical to learning language is to correctly track, process, and categorise rapidly presented acoustic stimuli (Aslin, 1989; Benasich et al., 2006; Benasich and Leevers, 2002; Fitch et al., 2001; Tallal et al., 1985). Several studies found that difficulties in processing rapid successions of auditory cues are associated with delays or disruptions of language acquisition and development in infancy and (early) childhood (Benasich et al., 2006; Guttorm et al., 2005, 2010; Molfese and Molfese, 1985, 1997). Although it is hypothesised that a complex interaction between genetic and environmental risk factors acting very early in life underlies the development of these difficulties (Benasich and Leevers, 2002; Bishop, 2006), no studies have yet tested how exposure to Anx-EP affects the processing of rapidly presented sounds in infants.

To our knowledge, only two studies have been conducted for testing associations between pre- or perinatal maternal anxiety and sensory-cognitive functioning in the offspring. In the first, Harvison and colleagues (2009) examined the effects of the mother's postpartum anxiety on the auditory event-related potentials (AERPs) elicited by her or a stranger's voice in newborn infants. Newborns from mothers who had experienced high anxiety displayed higher-amplitude negative frontal slow waves in response to a female stranger's voice compared to their mother's voice. In contrast, newborns from mothers with low anxiety showed the opposite pattern. The second was a prospective study conducted by Mennes et al. (2009) who studied ERPs in adolescents whose mother's state anxiety had been measured between their 12th and 22nd week of pregnancy. The ERP data showed an enlarged early frontal P2a component in adolescents of mothers whose state anxiety score had been higher than 43, but not in adolescents of mothers with scores below 43. This Anx-EP effect was only found during an endogenous cog-

nitive control task with a high cognitive load (i.e., a gambling task) and not during an exogenous cognitive control task (i.e., a go/no go task). Results of both studies suggest that pre- and perinatal exposure to maternal anxiety can be associated with neurophysiologically detectable differences in the offspring, at least from birth onwards and up until late adolescence.

To examine the role of Anx-EP on auditory processes critical for language acquisition in infants, we measured maternal anxiety prospectively between the 9th and 15th week of pregnancy and measured AERPs elicited by rapidly presented sequences of sounds in the infants at the age of 2 months in a longitudinal design. We focused specifically on maternal anxiety in the early stages of pregnancy, based on prior research suggesting that exposure during early rather than late gestation is related to changes in cognitive functions (Malaspina et al., 2008; Mennes et al., 2006, 2009; Van den Bergh, 2011b; Van den Bergh et al., 2006). In addition, because some studies suggested that AERPs in infants are modulated by the state of alertness (Friederici et al., 2002; Otte et al., 2013a), this variable was used as a between-subjects factor in the analyses. Because the current study represents one of the first efforts to investigate auditory information processing in the context of prenatal exposure to anxiety, our hypothesis was of an exploratory nature. If Anx-EP is associated with developmental reprogramming in the infant brain affecting the processing of rapid sequences of sound, then we should find that some part of the inter-subject variability of AERPs measured in 2-month-old infants can be explained by differences in Anx-EP.

5.2 Results

Figures 5.1 to 5.4 show the group-averaged AERP responses for each stimulus type, separately for infants of women with high, medium, and low Anx-EP scores. Note that the figures are for illustration purposes only as Anx-EP was entered as a continuous predictor variable into the statistical analyses. In Table 5.1, the mean amplitudes and standard deviations for each selected latency range and stimulus type are given for the whole group (N=70). The significant results of the ANCOVAs are summarised in Table 5.2.

For the standard tone (Figure 5.1), the ANCOVAs revealed a significant main effect of Anx-EP, both for the 170-270 ms and the 210-360 ms latency range (measurement ranges are marked on the figures), where higher levels of Anx-EP were associated with larger negative response amplitudes. Both effects remained significant ($p<.05$) after controlling for the covariates described in Methods. Scatter plots of the associations can be found in Figure 5.5A and 5.5B, respectively.

The effects of Anx-EP on the early negativity in the ISI-deviant were similar to those found for the standards, although less widely distributed and somewhat less pronounced (Figure 5.2). A significant three-way Anterior-Posterior x Laterality x Anx-EP interaction was found, which was not explained by the covariates tested. A scatter plot of the association can be found in Figure 5.5C. For unravelling this interaction, we first ran six two-way ANCOVAs, separately, between Anx-EP and

	Standard sound		White noise sound		
Site	170-270	210-360	50-150	140-240	300-400
F3	-.29 (2.20)	-.35 (2.16)	-.66 (3.12)	2.32 (3.76)	4.02 (4.45)
Fz	-.55 (2.18)	-.75 (2.15)	-.14 (3.23)	2.14 (4.10)	3.03 (4.35)
F4	-.31 (2.19)	-.35 (2.07)	-.44 (3.25)	2.13 (3.95)	3.32 (4.35)
C3	.02 (2.45)	-.46 (2.26)	-.67 (3.21)	3.64 (4.10)	3.43 (4.54)
Cz	-1.12 (2.43)	-1.67 (2.25)	.48 (3.39)	2.01 (3.76)	1.28 (4.89)
C4	-.25 (2.14)	-.68 (1.95)	.86 (2.87)	3.09 (3.52)	2.77 (5.00)
P3	.81 (1.95)	.38 (1.67)	.47 (2.68)	1.76 (3.61)	1.62 (3.23)
Pz	.62 (1.92)	.09 (1.71)	.55 (2.75)	1.49 (3.36)	1.18 (3.39)
P4	.86 (1.66)	.56 (1.54)	.54 (2.58)	1.45 (3.60)	1.76 (3.58)
	ISI-deviant		Novel sound		
Site	80-140	200-250	30-70	150-400	
F3	-.81 (3.21)	-1.11 (3.14)	-1.10 (1.42)	4.33 (3.32)	
Fz	-.62 (3.58)	-.85 (3.57)	-.84 (1.43)	3.71 (3.56)	
F4	-.64 (3.39)	-.91 (3.23)	-1.05 (1.40)	4.31 (3.59)	
C3	-1.23 (3.32)	-.81 (3.27)	-.53 (1.49)	4.86 (3.66)	
Cz	-.19 (3.72)	.46 (3.83)	-.34 (1.76)	2.41 (3.74)	
C4	-.85 (3.50)	-.59 (3.13)	-.59 (1.44)	4.25 (3.76)	
P3	-1.26 (2.84)	-.60 (2.77)	0.06 (1.13)	2.00 (2.83)	
Pz	-1.49 (2.72)	-.40 (3.01)	.20 (1.32)	1.76 (2.74)	
P4	-1.28 (2.81)	-.57 (2.96)	-.06 (1.06)	1.97 (2.65)	

Table 5.1: Mean amplitudes (SD) for each latency range (ms) per stimulus type and electrode site

the frontal, central, parietal, left, medial and right electrode arrays. Bonferroni correction was used to control for increases in type I errors due to multiple comparisons. These post hoc tests revealed a significant interaction between Anx-EP and the electrodes on the right side of the head (F4, C4, P4; $F(2, 134) = 5.932; p < .05; \eta^2 = .081$)). Testing the effects of Anx-EP separately on the F4, C4, and P4 amplitudes showed that for F4, higher levels of Anx-EP were associated with larger positive amplitudes ($F(1, 67) = 3.446; p < .1; \eta^2 = .049$), whereas no similar effect was found for C4 and P4. No significant effects of Anx-EP have been found for the 200-250 ms latency range.

No significant amplitude effects related to Anx-EP were found for the white noise (Figure 5.3) and the novel sound (Figure 5.4).

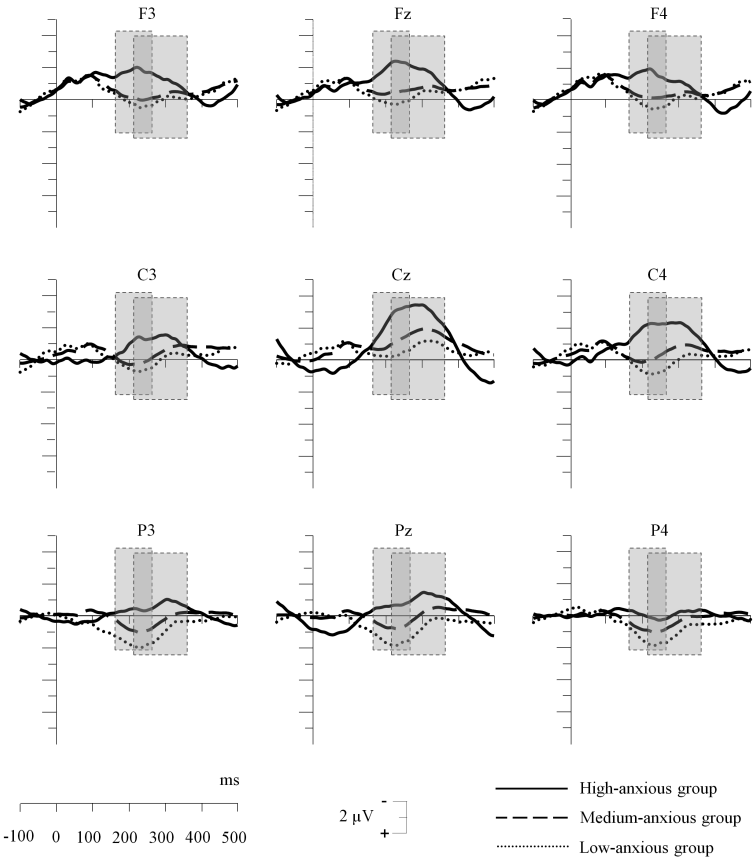

Figure 5.1: Group-averaged AERP responses for the standard tone for infants exposed to low (dotted line), medium (broken line) and high (solid line) levels of Anx-EP. Stimulus onset is at 0 ms. Amplitude calibration is at the bottom of the figure. Grey boxes mark the measurement ranges analysed.

5.3 Discussion

In this study, we examined the relationship between Anx-EP and the infant's evoked responses to sounds delivered in an auditory oddball paradigm. We found significant effects of Anx-EP on some of the AERP responses: Higher levels of Anx-EP were associated with larger amplitudes in response to the frequent standard tones and the ISI-deviants. In contrast, no significant Anx-EP-related effects were obtained for the novel and rare white noise sounds.

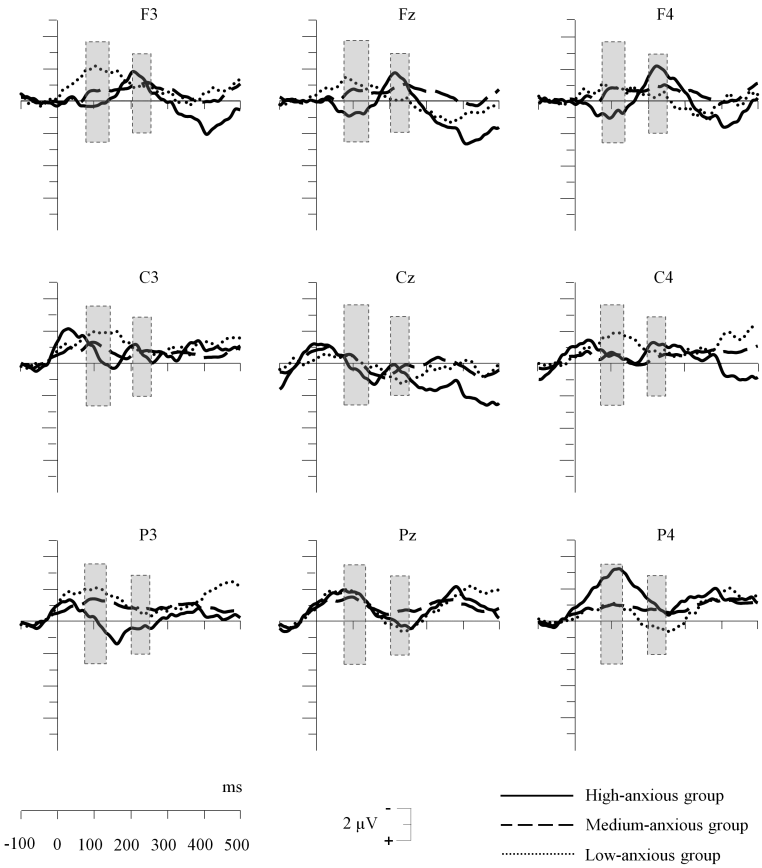

Figure 5.2: Group-averaged AERP responses for the ISI-deviant for infants exposed to low (dotted line), medium (broken line) and high (solid line) levels of Anx-EP. Stimulus onset is at 0 ms. Amplitude calibration is at the bottom of the figure. Grey boxes mark the measurement ranges analysed.

Thus, our data showed that some part of the inter-subject variability of AERPs measured in 2-month-old infants can be explained by differences in Anx-EP. This finding is compatible with the perinatal programming hypothesis: maternal anxiety during pregnancy may affect the development of neural networks, leading to the observed differences in sound processing. Overall, our data are in line with those of two previous studies on pre- and perinatal anxiety using ERP measures. First, Harvison et al. (2009) also reported neurophysiologically-based differences

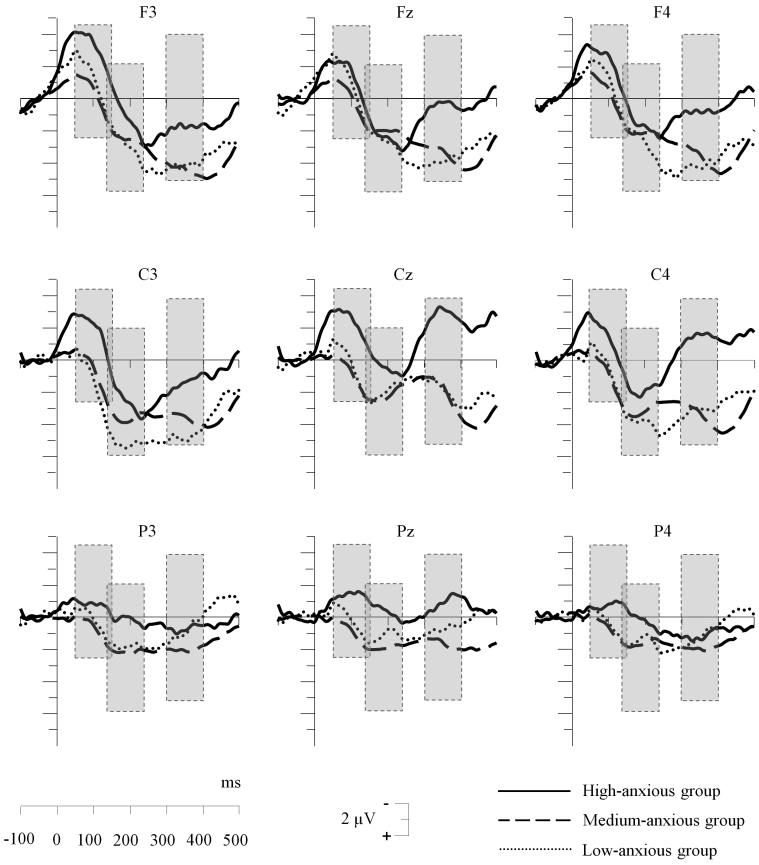

Figure 5.3: Group-averaged AERP responses for the white noise sound for infants exposed to low (dotted line), medium (broken line) and high (solid line) levels of Anx-EP. Stimulus onset is at 0 ms. Amplitude calibration is at the bottom of the figure. Grey boxes mark the measurement ranges analysed.

in brain activity in response to auditory stimuli between infants from high versus low anxious mothers. Our results provide stronger evidence, however, because in contrast to Harvison's study, we measured maternal anxiety prospectively, i.e. during pregnancy, and controlled for potential covariates. In addition, due to measuring a much larger group of infants, our findings appear to be more robust. Second, although the paradigm described here cannot be compared directly to that used byMennes et al. (2009), results may be interpreted in a similar way. Exposure to higher levels of Anx-EP was associated with larger amplitudes in response to

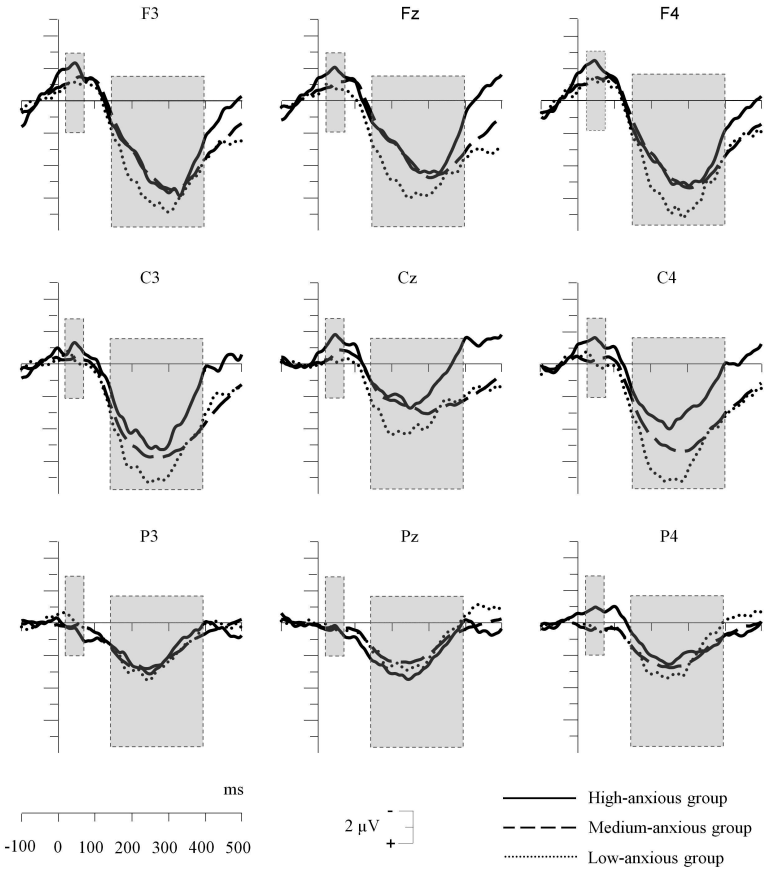

Figure 5.4: Group-averaged AERP responses for the novel sound for infants exposed to low (dotted line), medium (broken line) and high (solid line) levels of Anx-EP. Stimulus onset is at 0 ms. Amplitude calibration is at the bottom of the figure. Grey boxes mark the measurement ranges analysed.

the stimuli 1) that had been presented to the infants most frequently (the 500 Hz tone, which was used as the standard and the ISI-deviant) and therefore contained little new information (current study) and 2) to task-irrelevant stimuli erroneously perceived as containing task-relevant information (Mennes et al., 2009).

The infants' AERP responses to the standard and the identical ISI-deviant tones were larger and showed morphological differences with higher Anx-EP experienced by their mother. No such AERP differences were seen in response to the

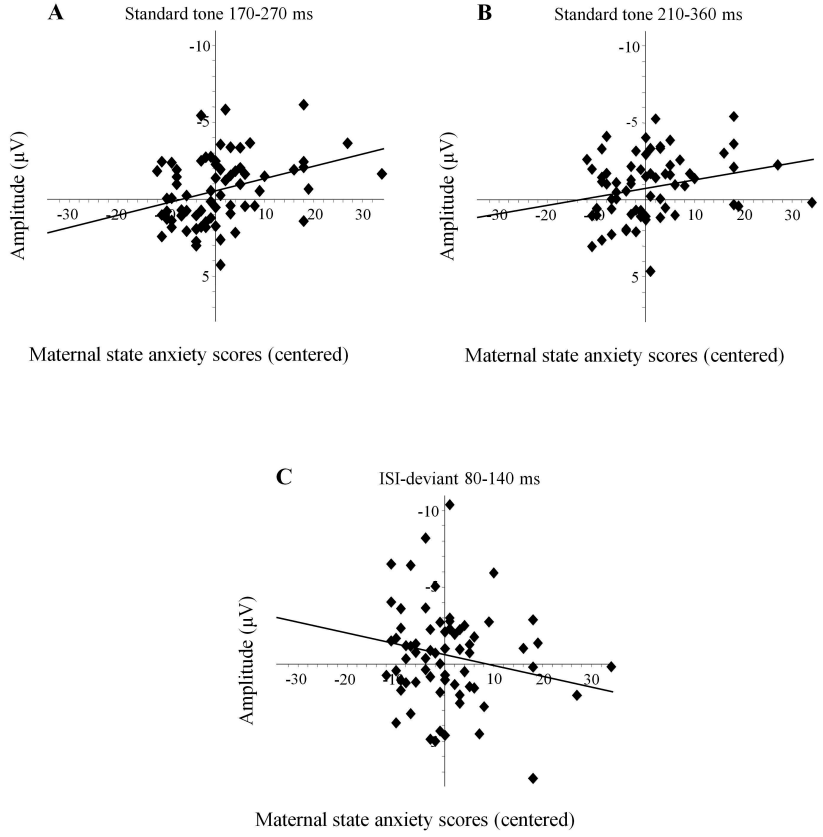

Figure 5.5: The association between Anx-EP and individual AERP response amplitudes for the 170-270 ms (A) and the 210-360 (B) ms latency range for the standard sound and the 80-140 ms latency range for the ISI-deviant (C) at electrode site Fz.

more complex white noise and novel sounds. This stimulus-specific difference in the response patterns suggests that infants exposed to higher levels of Anx-EP may have processed the frequent sound differently from infants of less anxious mothers, whereas they processed the rare acoustically deviant sounds similarly to them. One explanation is that higher levels of Anx-EP in the mother are associated with increases in processing capacities committed to less informative sound events in the infants. An interesting question is whether these differences are indicative of later difficulties in language learning, which are suspected to be related to problems in processing rapid sound sequences (Benasich and Leevers, 2002;

Benasich et al., 2002; Molfese and Molfese, 1985, 1997; Tallal et al., 1985). If our interpretation regarding the over-processing of uninformative sounds is correct, this would indeed cause problems in learning auditory cues within rapid sound sequences. Committing too much capacity to less informative sensory events decreases the amount left for processing the more informative ones. This problem is exacerbated by high temporal event densities, such as when a sound sequence is presented at a fast rate.

Results described in a study by Benasich et al. (2006) provide corroborative evidence for the above suggestion. The authors presented 6-month-old infants from families with (FH+) and without a history of language learning impairments (FH-) with standard and deviant tone pairs. They showed that the FH+ infants process the standard sounds differently from the FH- infants. Their data fit our interpretation of the AERP differences associated with exposure to higher levels of Anx-EP, including a delayed N250 in the FH+ compared to the FH- infants, which was found to be inversely related to language outcome at 24 months. Thus, judging from the similarity with our AERP results, one may speculate that in our group, too, exposure to higher levels of Anx-EP will be associated with less optimal language abilities at 24 months. A follow-up study is of course needed to test whether this speculation holds true.

Another remaining question is what mechanisms underlie the differences in information processing between infants prenatally exposed to different levels of Anx-EP. Several animal studies (Coe et al., 2003; Schneider et al., 2008, for reviews see Nathanielsz, 1999 and Weinstock, 2008) and one study in humans (Gitau et al., 2001) have found evidence for the role of maternal stress hormones that may enter the foetus by crossing the placental barrier and affect concentrations of neurotransmitters, such as dopamine, norepinephrine, and serotonin. In this way, typical neuronal migration and differentiation may be perturbed, leading to alterations in brain circuitry or synaptic functioning (Coe et al., 2003; Schneider et al., 2008; Weinstock, 2008). Furthermore, there is increasing evidence that environmental influences may give rise to epigenetic changes, i.e. to changes in the pattern of genes that are expressed or silenced (Malaspina et al., 2008). Higher levels of maternal anxiety might trigger commonly present but unexpressed genes (Rice et al., 2007), or, alternatively, prenatal environmental influences could induce gene silencing, e.g., by DNA methylation (Mill and Petronis, 2008). Future studies may try to identify the epigenetic gene regulatory mechanisms that are influenced by Anx-EP, leading to altered gene expression in prenatal development.

It is important to note that 30% and 40% of the variance in the occurrence of anxiety disorders can be explained by genetic variation (Leonardo and Hen, 2006). Although factors beyond genes play an important role in the development of these disorders, we note that in our study the infants of highly anxious mothers could have been genetically endowed with vulnerability for developing anxiety disorders. The observed associations, therefore, may reflect this vulnerability or be an expression of an anxious disposition as well. In this light, it would be interesting to also test the mothers using the same paradigm and examine whether they show similar responses as their infants. In the same vein, there appears to be a heritable

component in disorders related to difficulties in rapid auditory processing, such as language learning impairment and dyslexia (Bishop, 2002, 2006; Schumacher et al., 2007). Thus, more research is needed to disentangle the complex relationship between genetic factors, perinatal (re)programming, and anxiety on the one hand and language-related disorders and rapid auditory processing, on the other.

In conclusion, the present study investigated the relationship between prenatal exposure to maternal anxiety during pregnancy and rapid auditory processing abilities at the age of 2 months. Our results suggest that exposure to higher levels of Anx-EP is associated with more extensive processing of sounds with low information contents. This association may underlie the poorer language acquisition observed in children born to mothers who experienced high levels of Anx-EP. Future research could focus on slower maturation of the neural sources contributing to the observed ERP components and/or on weaker habituation processes to specify the underlying mechanisms.

5.4 Experimental procedures

5.4.1 Participants, stimuli, and procedure

Seventy infants and their mothers participated in the experiment. They are part of a group participating in a longitudinal study on Prenatal Early Life Stress (the PELS project). Details about the cohort and procedures regarding data collection and test administration have been provided elsewhere (Otte et al., 2013a).

In short, 190 pregnant women filled out questionnaires in each trimester (T1 to T3) on their emotions and state of mind, and two months after giving birth they and their infants (N=91) were invited to the developmental psychology lab (Babylab) at Tilburg University, The Netherlands, for postnatal observations (T4). Forty-three women did not participate in these observations because they dropped out before T4 (N=27), could not be reached in time (N=11), or their infant was seriously ill (N=4) or had passed away (N=1). As mentioned in the introduction, we focused on the effects of anxiety measured between the 9th and 15th week of pregnancy (T1) in this study, because previous research only found effects of maternal anxiety occurring during the early period of pregnancy on cognitive functions (Mennes et al., 2006; Van den Bergh, 2011b; Van den Bergh et al., 2006). T1 questionnaire data were missing for 6 of the 91 participants invited at T4, because the questionnaire had not been returned (N=1) or because of recruitment after T1 (N=5). The remaining 85 women had filled out the questionnaire at an average gestational age (GA) of 13.79 weeks (SD=1.32 weeks).

At T4, the infants were administered an auditory oddball paradigm with stimulus sequences consisting of one frequent standard tone (p = 0.7) and three infrequent deviant sounds (p = 0.1, each). All stimuli had 10 ms rise and fall times and were of 200 ms duration. Their intensity level was 75 dB and they were delivered with a uniform 300 ms inter-stimulus interval (ISI, offset-to-onset), except for the 'ISI-deviant' events (see below). The standard was a complex tone of 500

Hz composed of its 3 lowest partials with the intensity of the second and third partials being 6 and 12 dB lower, respectively, than that of the first one. The 'ISI-deviant' was identical to the standard sound, but preceded by 100 ms instead of the regular 300 ms of silence. The other two deviant types were white noise segments ('white noise') and environmental sounds ('novel sounds', 150 unique ones, such as a doorbell and a barking dog). A total of 1150 standard and 150 deviant sounds of each type (1500 sounds, altogether) were divided into five blocks of 300 stimuli, each and delivered to the infants with short breaks between them. Each sequence was pseudo-randomized, with the restriction that both white noise and novel sounds were always preceded by at least two standard sounds or a combination of a standard sound and an ISI-deviant. Also, consecutive ISI-deviants were always separated from each other by at least two standards or by a standard combined with either a white noise or novel sound. During the experiment, parents were seated in a chair in the dimly lit sound-attenuated room of the Babylab, facing a pair of speakers while holding the infant in their arms. The loudspeakers were placed 60 cm apart, both ca. 80 cm from the infant's head.

Recordings from 70 infants (40 girls) were suitable for analysis. Data from 15 infants were excluded due to crying ($N = 2$), excessive movements/artifacts ($N = 9$), or technical problems ($N = 4$) during testing. Thirty-five infants were awake during testing (19 girls) and 35 were asleep (21 girls). Demographical statistics of the mothers and their infants are given in Table 5.3.

5.4.2 Measuring Anx-EP

Anx-EP was assessed with the State Anxiety subscale of the Dutch version of the Spielberger State-Trait Anxiety Inventory (STAI; Spielberger et al., 1970; van der Ploeg, H.M. et al., 1980. Through self-report, the subscale provides a measure of the intensity of transitory anxiety in response to real life stress (Spielberger, 1975). Cronbach's α for the subscale was .93 in the current study.

To control for postpartum anxiety (Anx-PP), Spielberger's State Anxiety subscale was also administered at T4, at about the same time the mother and her infant were invited to the Babylab for postnatal observations. Data from 6 mothers were missing at T4 and a Latent Class model for multilevel data sets (Vermunt, 2008; Vermunt et al., 2008) was used to multiply impute (Rubin, 1987) the missing item scores. We checked whether significant effects remained intact after adding the imputed item scores to the analyses.

5.4.3 Data acquisition and AERP measurements

The EEG was recorded with Biosemi ActiveTwo amplifiers with 512 Hz sampling rate using a head cap with 64 electrode locations placed according to the revised version of the International 10-20 system. Two reference electrodes were placed on the left and right mastoids, respectively. Off-line, the EEG signals were filtered with a 1 to 30 Hz band-pass filter (slope 24 dB) and a 50 Hz notch filter. The pre-processed signals were segmented into 600 ms-long epochs, including a 100

ms pre-stimulus interval which was later used as the baseline. The epochs were averaged separately for the standard and the three deviant stimulus types, excluding epochs with sample-to-sample voltage steps exceeding $80\mu V$ or the overall amplitude range exceeding $150\mu V$ in any 200 ms-long window within the whole epoch. Data from infants with less than 40 acceptable responses for any one of the four stimulus types were removed from further analysis (9 infants, see also "Participants, stimuli and procedure"). On average 663 stimuli per infant were available for the standard tone, 94 for the white noise, 94 for the ISI-deviant, and 95 for the novel sound.

For selecting the AERP time windows for the analyses, we first formed groups of high (Anx-EP$\geq$42; $N = 9$), medium (23$<$Anx-EP$<$42; $N = 50$), and low (Anx-EP$\leq$23; $N = 11$) Anx-EP scores. After visual inspection of the group-averaged waveforms (Figures 5.1-5.4) on electrode sites F3, Fz, F4, C3, Cz, C4, P3, Pz and P4, time windows with the largest difference between the high-vs-low-anxiety groups were selected for the quantitative analyses, because these latency ranges were expected to show a high amount of variation related to Anx-EP. Because Anx-EP was entered as a continuous predictor in the statistical analyses (see below; i.e., the groups themselves were not used for hypothesis testing), the medium Anx-EP group was not involved in this preliminary analysis, which was conducted only to bootstrap the ERP measurements. The 'post-hoc' manner of selecting time windows was chosen because: 1) as stated earlier, very little work is available on the association between prenatal exposure to maternal anxiety and infant auditory information processing, which makes it difficult to a priori determine windows of interest; 2) our hypothesis was of an exploratory nature, and the current way of selecting time windows allowed us to examine differences wherever they emerged. The following time windows were selected: for the standard tone (Figure 5.1), a window from 170 to 270 ms and another one from 210 to 360 ms; for the white noise (Figure 5.3), a window from 50 to 150 ms, a second one from 140 to 240 ms, and a third one from 300 to 400 ms; for the ISI-deviant (Figure 5.2), a window from 80 to 140 ms and another one from 200 to 250 ms; for the novel sound (Figure 5.4), a window from 30 to 70 ms and one from 150 to 400 ms.

5.4.4 Statistical analyses and covariates

Repeated measures mixed-mode ANCOVAs were run on the mean AERP amplitudes, separately for each stimulus type and time window, with the structure 'Anterior-Posterior' (frontal, central, parietal) x 'Laterality' (left, medial, right) as within-subjects factors, and 'State of Alertness' (awake vs. asleep) as a between-subjects factor. Anx-EP was entered as a continuous predictor variable to the model. It was not added as a between-subjects factor with the levels high, medium and low anxiety, because this would result in groups of different sizes (e.g. $N = 9$, $N = 50$, and $N = 11$, respectively) and because previous research has shown that categorisation of a continuous variable can lead to problems such as loss of effect size, power and/or information about individual differences (Altman and Royston, 2006; MacCallum et al., 2002; Royston et al., 2006). The following covariates

were added to the ANCOVAs to control for their potential influence: Anx-PP and maternal smoking and alcohol intake during pregnancy (gathered through self-report); sex and GA at birth (obtained from hospital and midwives medical files); birth weight controlled for GA (computed by regressing GA on birth weight); and the infant's age at T4. The continuous covariates were first correlated to the AERP data and only added to the ANCOVAs if significant correlations were found. As just 2 mothers had smoked, we controlled for maternal smoking by checking whether or not exclusion of the corresponding infant data erased (or yielded) significant findings. Mean or percentage values of the covariates are included in Table 5.3.

All significant results including Anx-EP are reported. Main effects and interactions including only other factors are not relevant to the current question and were omitted from the main text for easier reading. (They are, however, included in Table 5.2 for completeness.) Greenhouse-Geisser correction was used where applicable and the ϵ correction factor is given in Table 5.2, together with the partial η^2 effect size.

Acknowledgements

The authors are grateful to the parents and infants for their continued participation in our study and to the students who helped with the data collection. In addition, the authors thank Dr. Elena Kushnerenko, for her help in designing the oddball paradigm, and Daniel van der Palm, for his help with the multiple imputation of missing postpartum data.

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Standard sound: 170-270 ms latency range

	Unadjusted model					Adjusted model[1,3]				
Source	df	*F*	*p*	ϵ	η^2	df	*F*	*p*	ϵ	η^2
Anx-EP	1,67	7.675	.007		.103	1,61	6.089	.016		.091
FCPxStatAl	2,134	7.687	.002	.78	.103	2,122	8.209	.001	.78	.119
LMRxStatAl	2,134	4.880	.017		.059	2,122	4.744	.023		.060
FCPxLMRxStatAl	4,268	2.978	.026	.87	.043	4,244	3.734	.009	.86	.058

Standard sound: 210-360 ms latency range

	Unadjusted model					Adjusted model[3,5]				
Source	df	*F*	*p*	ϵ	η^2	df	*F*	*p*	ϵ	?2
Anx-EP	1,67	5.147	.027		.071	1,65	6.092	.016		.086
FCPxStatAl	2,134	5.023	.012	.82	.070	2,13	4.943	.013	.83	.071
LMRxStatAl	2,134	10.778	.000		.139	2,13	9.654	.000		.129
FCPxLMRxStatAl	4,268	4.193	.005	.85	.059	4,26	4.503	.003	.87	.065

White noise sound: 50-150 ms latency range

–

White noise sound: 140-140 ms latency range

	Unadjusted model				
Source	df	*F*	*p*	ϵ	η^2
FCPxLMRxStatAl	4,268	2.607	.036		.037

White noise sound: 140-140 ms latency range

	Unadjusted model				
Source	df	*F*	*p*	ϵ	η^2
FCP	2,134	4.029	.026	.85	.057
FCPxStatAl	2,134	7.827	.001	.85	.105
FCPxLMRxStatAl	4,268	3.738	.006	.89	.046

ISI-deviant sound: 80-140 ms latency range

	Unadjusted model					Adjusted model[3,5]				
Source	df	*F*	*P*	ϵ	η^2	df	*F*	*P*	ϵ	η^2
FCPxLMR	4,268	4.538	.003	.86	.063	4,26	1.077	.364	.86	.016
FCPxLMRxAnx-EP	4,268	4.268	.004	.86	.060	4,26	4.216	.004	.85	.061

ISI-deviant sound: 200-250 ms latency range

–

Novel sound: 30-70 ms latency range

–

Novel sound: 150-400 ms latency range

–

Abbreviations: Anx-EP = maternal state anxiety during early pregnancy; FCP = Frontal,Central,Parietal; LMR = Left,Medial,Right; StatAl = State of Alertness; ISI = Inter-Stimulus Interval

Unadjusted model: 'Anterior-Posterior' x 'Laterality' as within-subjects factors, Anx-EP as a continuous predictor variable, 'State of Alertness' as a between-subjects factor

Adjusted model: Unadjusted model with control for covariates. Covariates include one or more of the following variables: maternal state anxiety during the postpartum period[1]; maternal alcohol intake during pregnancy (continuous)[2]; infant's gestational age in days at birth[3]; infant's sex[4]; infant's birth weight controlled for gestational age at birth[5]; and infant's age in days at T4[6]. Inclusion or exclusion of mothers who had smoked during pregnancy did not change any of the results reported here. We did not correct for covariates when significant effects did not pertain to associations with maternal state anxiety.

Table 5.2: Significant main effects and interactions for the Unadjusted and Adjusted ANOVAs

		N	*%*	*M (SD)*
Mothers		70		
Age at T1 (years)				32.07 (4.15)
Age at T4 (years)				32.79 (4.06)
State anxiety at T1				32.66 (9.31)
State anxiety at T4				31.42 (6.66)
Marital status	Married	37	52.9	
	Cohabiting	32	45.7	
	Single	1	1.4	
Educational level	Primary or secondary	2	2.9	
	General vocational training	20	28.6	
	Higher vocational training	27	38.6	
	University degree or higher	21	30.0	
Family income	$<$ 2100	2	1.9	
(monthly, in €)	2200 - 3600	16	22.9	
	$>$ 3600	51	72.9	
	Dont want to disclose	1	1.4	
Primigravida		26	37.1	
Has ever miscarried		19	27.1	
Smoking during pregnancy		2	2.9	
Drinking alcohol during pregnancy*		6	8.6	
Infants		70		
Sex	Boy	30	42.9	
	Girl	40	57.1	
Birth weight (grams)				3470 (469)
GA at birth (weeks)				39.9 (1.3)
Age at T5 (days)				70.2 (6.1)

GA = Gestational Age

*From 1 glass of wine or liquor during the whole pregnancy to maximally 3 glasses a month

Table 5.3: Sample characteristics of participating women and their infants

6

Prenatal early life stress and auditory information processing in 9-month-old infants

R.A. Otte, I. Winkler, M.A.K.A. Braeken,
& B.R.H. Van den Bergh.

In preparation

Abstract

Exposure to atypical events during critical periods of life in the womb, such as maternal anxiety, may induce reprogramming of prenatal and neonatal development. Studying biologically-based markers of reprogramming early in life could help elucidate the mechanisms underlying reprogramming. Therefore, in the current study we examined the relationship between maternal anxiety during early pregnancy (Anx-EP) and auditory information processing in 9-month-old infants by means of an auditory oddball paradigm with a standard tone and three types of deviants: inter-stimulus interval (ISI) deviants, rare white noise segments and novel sounds. We found significant positive associations between the level of Anx-EP and infant's response amplitudes to stimuli with low information contents (standard and ISI-deviant). For rare, more informative stimuli no effects of exposure to Anx-EP were found.

Keywords: Prenatal exposure; state anxiety; pregnancy; infants; auditory event-related potentials; auditory information processing; oddball paradigm

6.1 Introduction

Development refers to the process of systematic continuities and changes occurring as a function of the on-going dialogue between the individual and its environment (Gottlieb, 1997; Shaffer, 2002). During no other period does this process take place at such a great pace as during life in the womb. With the speed of development come sensitive periods during which the foetus is highly susceptible to (adverse) environmental conditions, such as maternal under-nutrition, diabetes, stress and anxiety or alcohol use, potentially leading to altered programming or reprogramming of structure and function in cells, tissues, and organ systems. This, in turn, may alter social-emotional, psychophysiological (including neurocognitive) functioning, increasing an individual's vulnerability to regulation problems at behavioural, emotional, and cognitive levels, which can be observed from the foetal period to early adulthood (see e.g. Barker, 1998; Entringer et al., 2010; Mennes et al., 2006; Nathanielsz, 1999; Räikkönen et al., 2011; Sandman et al., 2011; Van den Bergh, 2011a; Van den Bergh et al., 1989, 2005). Although a large body of research has been dedicated to studying perinatal programming, only a few studies identified biologically-based markers of reprogramming early in life. As these markers could help to elucidate the processes of reprogramming, in the current study we examined the relationship between maternal anxiety during early pregnancy (Anx-EP) and auditory information processing in 9-month-old infants by means of event-related potentials.

Some previous studies have examined auditory information processing in the context of exposure to maternal stress and anxiety during pregnancy. For example, in a study on depressed pregnant women, the foetuses displayed delayed habituation to vibro-acoustic stimulation (Allister et al., 2001). The authors remarked that being less able to respond to and process stimulation may affect postpartum information processing. These findings are compatible with results from studies by Streissguth et al. (1983) and Potter et al. (2000), who examined the effects of maternal alcohol intake and cocaine use during pregnancy, respectively, on newborn infants. Streissguth and colleagues (1983) demonstrated reduced habituation to auditory and visual stimuli 27 hours after birth. The cocaine-exposed neonates in the study by Potter and colleagues (2000) showed impaired habituation to an auditory habituation-dishabituation paradigm, suggesting slower speed for auditory information processing.

Few studies have made use of brain measures such as auditory event-related potentials (AERPs) for assessing the effects of maternal anxiety on auditory information processing in infancy. AERPs have very high temporal resolution making it possible to study the timing of neurocognitive processes with millisecond accuracy(Luck, 2005). They can be recorded in the absence of a behavioural response (Nelson and Bloom, 1997), even for unattended stimuli. Thus AERPs are suitable for studying neonates and infants (Luck, 2005; Nelson and Bloom, 1997). Harvison et al. (2009) examined the effects of perinatal anxiety in the mother on the AERPs elicited by her voice or that of a stranger in newborn infants. Whereas newborns from mothers with low anxiety displayed a slow negative frontal wave-

form of higher amplitude to their mother's compared to a female stranger's voice, newborns from mothers with higher anxiety showed the opposite pattern.

As part of our on-going research project on prenatal early life stress, in an earlier study we presented 2-month-old infants with an auditory oddball sequence (Otte et al., 2013b). Three different rare deviants were delivered together with the frequent standard tones: the standard tone preceded by a shorter inter-stimulus interval (ISI-deviant), white noise segments, and novel sounds. Results suggested that compared to infants from low anxious mothers, infants of high anxious mothers processed sounds with low information contents (i.e. the standard tones) more extensively (Otte et al., 2013b). Together with the findings by Harvison et al. (2009), the results provide evidence that pre- and perinatal exposure to maternal anxiety may be associated with neurophysiologically detectable differences in the infants.

The goal of the current study was to examine the effects of exposure to Anx-EP on auditory information processing in infants aged 9 months, by means of an oddball paradigm with standard tones, ISI-deviant sounds, white noise deviants and novel deviants. Because the study represents one of the first efforts to investigate auditory information processing in the context of prenatal exposure to anxiety, our hypotheses were of an exploratory nature. Basing on our previous work with 2-month-olds, we hypothesised that exposure to high levels of prenatal maternal anxiety would be associated with more extensive processing of sounds with low, but not with high information contents in the auditory oddball paradigm. That is, we expected higher amplitudes in response to the standard and ISI-deviant sounds, but not the novel and white noise, with prenatal exposure to higher levels of maternal anxiety.

6.2 Methods

6.2.1 Subjects

Measurements were conducted with 84 infants (one pair of twins; 43 girls) and their mothers who have been taking part in our longitudinal cohort study on prenatal early life stress. The total group of participants of which this subgroup is a part has been described previously in Otte et al. (2013a). They represent a sample from a normal (i.e. non-clinical) population.

One hundred and ninety pregnant women enrolled in the project between April 2009 and September 2010. Most of them were in their 9th to 15th week of pregnancy ($N = 178$), and a small group was recruited when they were 16 to 22 weeks pregnant ($N = 12$). In each pregnancy trimester (T1 to T3) the women filled out standardised anxiety and stress questionnaires. In the current study, we focused specifically on maternal anxiety experienced in the early stages of pregnancy (T1), based on prior research suggesting that exposure during early rather than late gestation can be associated with specific changes in cognitive functions as measured with both behavioural and physiological (e.g. EEG) measures (Malaspina

et al., 2008; Mennes et al., 2006, 2009; Van den Bergh, 2011a; Van den Bergh et al., 2006). None of the women described here were under treatment or using medicines for psychological or psychiatric problems at T1. Both at 2/4 months (T4) and at 9 months (T5) after birth, the mothers and their infants were invited for postnatal observations. Here, we report the results from infants measured at 9 months of age; data collected at T4 have been discussed elsewhere (Otte et al., 2013b; van den Heuvel et al., 2013). Forty-five of the 84 infants included in the current study had been tested with the same experimental paradigm (see "Stimuli and procedure") at 2 months of age (Otte et al., 2013b). An additional 16 infants were tested with the same paradigm at the age of 4 months (van den Heuvel et al., 2013). Figure 6.1 describes the inclusion and exclusion of participants for the current study. All infants had been screened for hearing impairments (evoked otoacoustic emission) and found healthy by a nurse from the infant health care clinic in the first week postpartum. Demographical statistics of the infants and their mothers are given in Table 6.1.

6.2.2 Measuring Anx-EP

Anx-EP was measured with the Dutch version of the Spielberger State-Trait Anxiety Inventory (STAI; Spielberger et al., 1970; van der Ploeg, H.M. et al., 1980) between the 9th and 15th week of pregnancy (T1; see also "Subjects" section). The STAI is a self-report questionnaire with the subscales Trait and State Anxiety. Both scales consist of 20 items which can be rated on a scale from 1 (not at all/almost never) to 4 (very much/almost always). Trait anxiety refers to a disposition or proneness to react with anxiety. In contrast, the state anxiety subscale provides a measure of the intensity of transitory anxiety in response to real life stress Spielberger (1975). Therefore, the state anxiety sum scores ("State Anxiety") were used as a measure of Anx-EP in this study. Cronbach's α for the State subscale was .916.

Postpartum exposure to maternal anxiety could also influence auditory information processing in the infants (Grant et al., 2008; Heron et al., 2004; Lee et al., 2007). Therefore, Spielberger's state anxiety subscale was administered at T5, at about the same time when the mother and her infant were invited for postnatal observations. We could then statistically control for this factor by adding it as a covariate to the analysis (see "Data recording and analysis").

State Anxiety data were unavailable for three mothers at T1, either because they had been recruited after T1 ($N = 2$) or because they had not returned the questionnaire ($N = 1$). In addition, 20 mothers did not fill out the questionnaire at T5. The missing State Anxiety sum scores were imputed by means of the Expectation-Maximization method in IBM SPSS Statistics 19.0. Variables used for the imputation were State Anxiety sum scores measured at other time points; e.g. when the State Anxiety sum score was missing for T1, the sum scores from T2, T3, T4 and T5 - if available - were used to estimate the missing score. If State Anxiety sum scores were missing for more than 2 time points, the participant was excluded from further analysis ($N = 3$, see Figure 6.1).

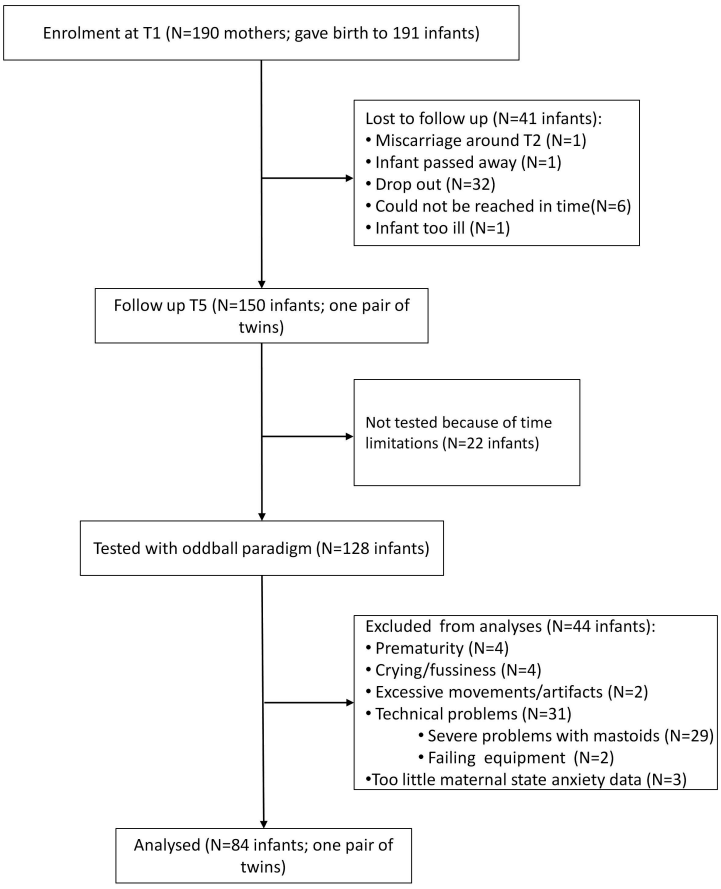

Figure 6.1: Flowchart describing the inclusion and exclusion of participants for the current study.

6.2.3 Stimuli and procedure

We administered the same auditory oddball paradigm at T5 as we did at T4 and which has been described in Otte et al. (2013a). The infants were presented with stimulus sequences consisting of standard tones (p = 0.7) and three types of deviant sound events (p = 0.1, each). All stimuli were of 200 ms duration and were delivered at a 300 ms ISI (offset-to-onset interval), except for the ISI-deviant events (see below). The standard was a complex tone constructed from the 3 lowest partials. The fundamental frequency was 500 Hz and the intensity of the second and third partials was 6 and 12 dB lower, respectively, than that of the first one. The "ISI-deviant" was identical to the standard sound, but it was preceded by 100 ms instead

		N	%	*M (SD)*
Mothers		83		
Age at T1 (years)				32.39 (3.80)
Age at T5 (years)				33.73 (3.80)
State anxiety at T1				32.82 (8.89)
State anxiety at T5				31.92 (7.66)
Marital status	Married	44	53.0	
	Cohabiting	39	47.0	
Educational level	Primary or secondary	5	6.0	
	General vocational training	23	27.7	
	Higher vocational training	36	43.4	
	University degree or higher	19	22.9	
Family income	$<$ 2100	3	3.6	
(monthly, in €)	2200 - 3600	18	21.7	
	$>$ 3600	56	67.5	
	Dont want to disclose	6	7.2	
Primigravida		30	36.1	
Smoking during pregnancy		3	3.6	
Drinking during pregnancy*		6	7.2	
Has been treated in	Depression	8	9.6	
the past for	Anxiety problems	3	3.6	
	Personality problems	2	2.4	
	PDD-NOS	1	1.2	
	Postpartum stress disorder	1	1.2	
Infants		84		
Sex	Boy	41	48.8	
	Girl	43	51.2	
Birth weight (grams)				3497 (475)
GA at birth (weeks)				39.96 (1.2)
Age at T5 (days)				303.6 (13.7)

GA = Gestational Age; PDD-NOS = pervasive developmental disorder-not otherwise specified

*From 1 glass of wine or liquor during the whole pregnancy to maximally 3 glasses a month

Table 6.1: Sample characteristics of participating women and their infants

of the regular 300 ms of silence. The other two deviant types were white noise segments ("white noise") and environmental sounds ("novel sounds", 150 unique sounds, such as a doorbell and a barking dog). A total of 1050 standard and 150 deviant sounds of each type (1500 sounds, altogether) were divided into five blocks of 300 stimuli, each and delivered to the infants with short breaks between them. Each sequence was pseudo-randomised, enforcing at least two sounds of the other types to intervene between two consecutive deviants of the same type. During the

experiment, parents were seated in a chair in a dimly lit sound-attenuated room, facing a pair of speakers with the infant sitting on their lap or lying in their arms. The loudspeakers were placed 60 cm apart, both ca. 80 cm from the infants head. The infants were given toys without sounds to play with or were entertained by an experimenter who, for instance, played (silent) peek-a-boo or who blew bubbles.

6.2.4 Data recording and analysis

EEG was recorded with Biosemi ActiveTwo amplifiers with a sampling rate of 512 Hz[1]. The standard BioSemi reference (CMS-DRL) was used (see www.biosemi.com/faq/cms&drl.htm for details). Infants wore head caps with 64 electrode locations positioned according to the revised version of the International 10-20 system. In addition, electrodes were placed both on the left and right mastoid and these were mathematically combined (off-line) to produce an average mastoids reference derivation (Luck, 2005).

The EEG signals were filtered off-line, with a 1 to 30 Hz band-pass filter (slope 24 dB), and a 50 Hz notch filter to make sure all 50 Hz noise would be filtered out. The pre-processed signals were segmented into 600 ms-long epochs, including a 100 ms pre-stimulus interval, which was later used as the baseline. The epochs were averaged separately for the standard and the three deviant stimulus types, excluding epochs with sample-to-sample voltage steps exceeding 80 μV or the overall amplitude range exceeding 150 μV in any 200 ms-long window within the whole epoch. Data from infants with less than 40 acceptable responses for any one of the four stimulus types were removed from further analysis (2 infants, see Figure 6.1). The average number of available stimuli per infant was 753 for the standard tone, 108 for the novel sound, 108 for the white noise segments and 108 for the ISI-deviant.

For selecting time windows for amplitude measurements, we formed groups of high (State Anxiety ≥ 42; $N = 13$) versus low/medium (State Anxiety < 42; $N = 71$) maternal Anx-EP. As responses to the auditory stimuli were expected to be most clearly defined on top of the head, the group-averaged waveforms (Figures 6.2 to 6.5) for electrode sites F3, Fz, F4, C3, Cz, C4, P3, Pz and P4 were visually inspected. Time windows showing the largest difference between the high-vs-low/medium-anxiety groups were selected for the quantitative analyses, because these latency ranges were expected to show a high amount of variation related to the mother's Anx-EP. This 'post-hoc' manner of selecting time windows was chosen because: 1) as stated earlier, very little work is available on the association between prenatal exposure to maternal anxiety and infant auditory information processing, which makes it difficult to a priori determine windows of interest; 2) our hypotheses were of an exploratory nature, and the current way of selecting time windows allowed us to examine differences wherever they emerged. The following time windows were selected: for the standard tone (Figure 6.2), a window from

[1]On-line filtering options in the BioSemi software are set automatically based upon the sampling rate. See http://www.biosemi.com/faq/adjust filter.htm and http://www.biosemi.com/faq/adjust samplerate.htm

230 to 300 ms; for the novel sound (Figure 6.3), a window from 250 to 400 ms; for the white noise (Figure 6.4), a window from 280 to 320 ms; for the ISI-deviant (Figure 6.5), a window from 140 to 190 ms and one from 320 to 400 ms.

AERP amplitudes measured from these windows were analysed by means of repeated measures ANCOVAs, separately for each stimulus type and time window. Within-subjects factors were 'Anterior-Posterior' (frontal, central, parietal) and 'Laterality' (left, medial, right). Important to emphasise is that the maternal prenatal state anxiety score at T1 ('State Anxiety') was entered as a continuous predictor variable. It was not included as a between-subjects factor (e.g. high versus low/medium anxiety), because dichotomising the variable would result in groups of different sizes (e.g. $N = 13$ versus $N = 71$) and because previous research has shown that dichotomisation of a continuous variable can lead to problems such as loss of effect size, power and/or information about individual differences (Altman and Royston, 2006; MacCallum et al., 2002; Royston et al., 2006).

We controlled for the following 8 covariates: infant's state of alertness at T5 (awake or asleep, both quiet and active sleep; $N = 6$; determined by combining monitored behaviour and the online EEG signal); maternal smoking and alcohol intake during pregnancy (gathered through self-report at T2; the latter as a continuous variable); maternal postpartum anxiety; infant sex and GA in days at birth (obtained from hospital and midwives medical files); infant birth weight controlled for GA (computed by regressing GA on birth weight); and the infant's age in days at T5. Categorical covariates were one at a time added to the model to control for their potential influence. Continuous covariates were first correlated with the AERP data and only added to the ANCOVAs if significant correlations were found. As only 3 mothers were smokers, we controlled for maternal smoking by checking whether or not exclusion of the corresponding infant data changed the statistical significance of any effect or interaction. Mean/percentage values of the covariates are included in Table 6.1.

In Table 6.2, all significant effects are reported, both for the unadjusted ANCOVA model described above and for the adjusted model with control for potential covariates. Numbers in superscript in the 'adjusted model' column indicate which covariates were controlled for and, given our procedure, also which continuous covariates had correlated with the AERP measures. Since only main effects and interactions including State Anxiety are relevant to the current research question, significant results including other factors (e.g. interactions between state of alertness and electrode site) are not discussed in the body of the article for easier reading, but they can be found in Table 6.2. Greenhouse-Geisser correction was used where applicable (uncorrected degrees of freedom and corrected p-values are given) and the ϵ correction factor can be found in Table 6.2, together with the partial η^2 effect size.

6.3 Results

Group-averaged AERP responses for each stimulus type are shown in figures 6.2 to 6.5, separately for infants of mothers with high versus medium/low State Anxiety[2]. All significant results, with F- and p-values, are given in Table 6.2.

Standard tone: 230-300 ms latency range

	Unadjusted model					Adjusted model[3,7]				
Factor(s)	df	F	p	ϵ	η^2	df	F	p	ϵ	η^2
FCPxLMRxAnx-EP	4,328	2.719	.030		.032	4,316	2.511	.042		.031

Novel sound: 250-400 ms latency range

	Unadjusted model									
Factor(s)	df	F	p	ϵ	η^2	df	F	p	ϵ	η^2
FCP	2,164	4,608	0.24	.66	0.53					

White noise sound: 280-320 ms latency range

	Unadjusted model				
Factor(s)	df	F	p	ϵ	η^2

—

ISI-deviant sound: 140-190 ms latency range

	Unadjusted model					Adjusted model[1,2,3,7]				
Factor(s)	df	F	p	ϵ	η^2	df	F	p	ϵ	η^2
Anx-EP	1,82	6.025	.016		.068	1,78	5.779	.019		.069

ISI-deviant sound: 320-400 ms latency range

	Unadjusted model					Adjusted model[1,2,3,7]				
Factor(s)	df	F	p	ϵ	η^2	df	F	p	ϵ	η^2
FCPxLMR	4,328	3.413	.009		.040					
FCPxLMRxAnx-EP	4,328	3.269	.012		.038	4,312	2.648		.034	.033

Abbreviations: Anx-EP = maternal state anxiety during early pregnancy; FCP = Frontal,Central,Parietal; LMR = Left,Medial,Right; StatAl = State of Alertness; ISI = Inter-Stimulus Interval
Unadjusted model: 'Anterior-Posterior (FCP)' x 'Laterality (LMR)' as within-subjects factor; Anx-EP as a continuous predictor variable
Adjusted model: Unadjusted model with control for covariates. Covariates include one or more of the following variables: maternal state anxiety during the postpartum period[1]; maternal alcohol intake during pregnancy (continuous variable)[2]; infant's sex[3]; infant's gestational age (in days) at birth[4]; infant's birth weight controlled for gestational age at birth[5]; infant's age (in days) at T5[6]; infant's state of alertness during testing at T5[7]. Inclusion or exclusion of mothers who had smoked during pregnancy did not change any of the result reported here. We did not correct for covariates when significant effects did not pertain to associations with maternal state anxiety.

Table 6.2: Significant main effects and interactions for the Unadjusted and Adjusted ANCOVAs

For the standard sound (Figure 6.2), a significant three-way Anterior-Posterior x Laterality x State Anxiety interaction was found, which remained intact ($p < .05$) after controlling for the covariates discussed in the "Data recording and analysis"

[2]Note that the figures are for illustration purposes only. State Anxiety was entered as a continuous predictor variable into the statistical analyses.

section. For unravelling the three-way interaction, we first ran six two-way interactions, separately, between State Anxiety and the frontal, central, parietal, left, medial and right electrode arrays. Bonferroni correction was used to control for increases in type I errors due to multiple comparisons. The post hoc tests revealed

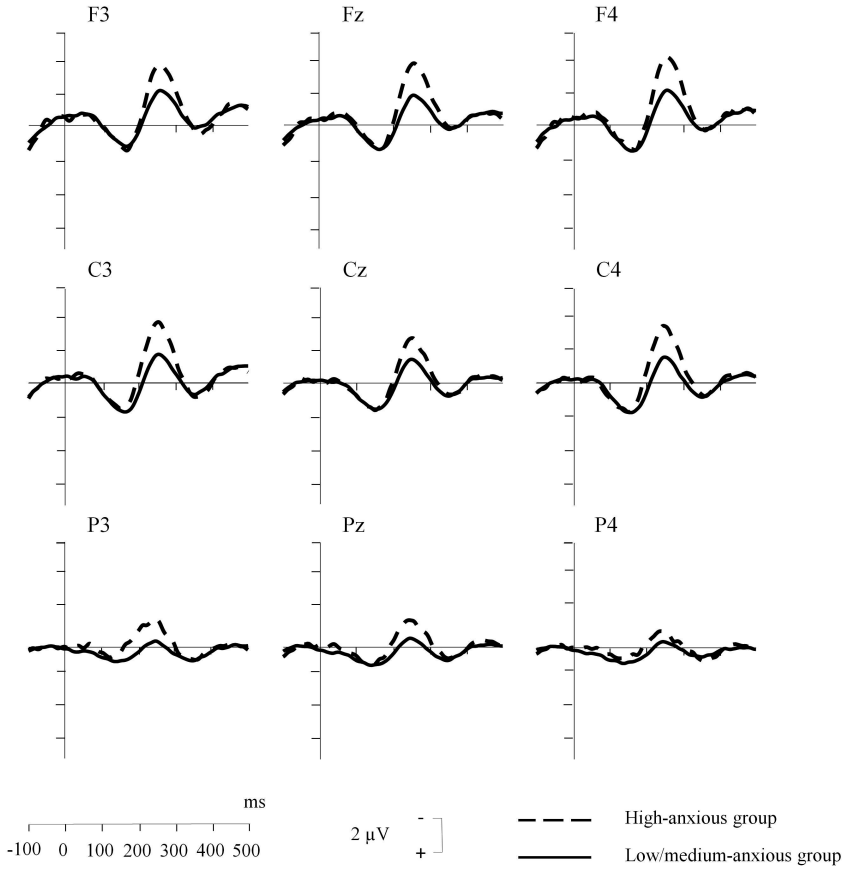

Figure 6.2: Group-averaged waveforms elicited by the standard tone for the infants exposed to high (broken line) versus low/medium(solid line) levels of Anx-EP. Stimulus onset is at 0 ms. Amplitude calibration is at the bottom of the figure.

a significant interaction between State Anxiety and the electrodes on the right side of the head (F4, C4, P4; $F(2, 164) = 6.941; p < .05; \eta^2 = .078; \epsilon = .083$). Testing the effects of State Anxiety separately on the F4, C4, and P4 amplitudes showed that for F4 and C4, higher levels of State Anxiety were associ-

ated with larger amplitudes [F4 ($F(1,82) = 4.996; p < .05; \eta^2 = .057$) and C4 ($F(1,82) = 4.227; p < .05; \eta^2 = .049$)], whereas no similar effect was found for P4.

No significant associations were found between State Anxiety and the AERP amplitude measures for either the novel sounds or the white noise segments (Figures 6.3 and 6.4, respectively).

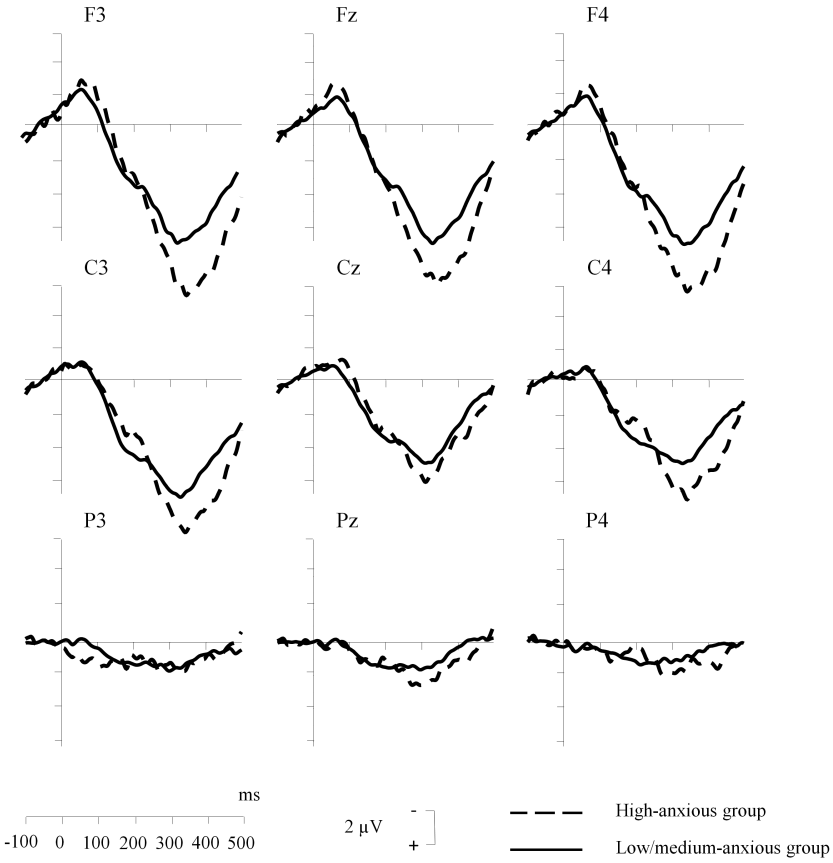

Figure 6.3: Group-averaged waveforms elicited by the novel sound for the infants exposed to high (broken line) versus low/medium(solid line) levels of Anx-EP. Stimulus onset is at 0 ms. Amplitude calibration is at the bottom of the figure.

For the ISI-deviant (Figure 6.5), the analysis revealed a main effect of State Anxiety for the first positive waveform (140 - 190 ms), with higher scores on

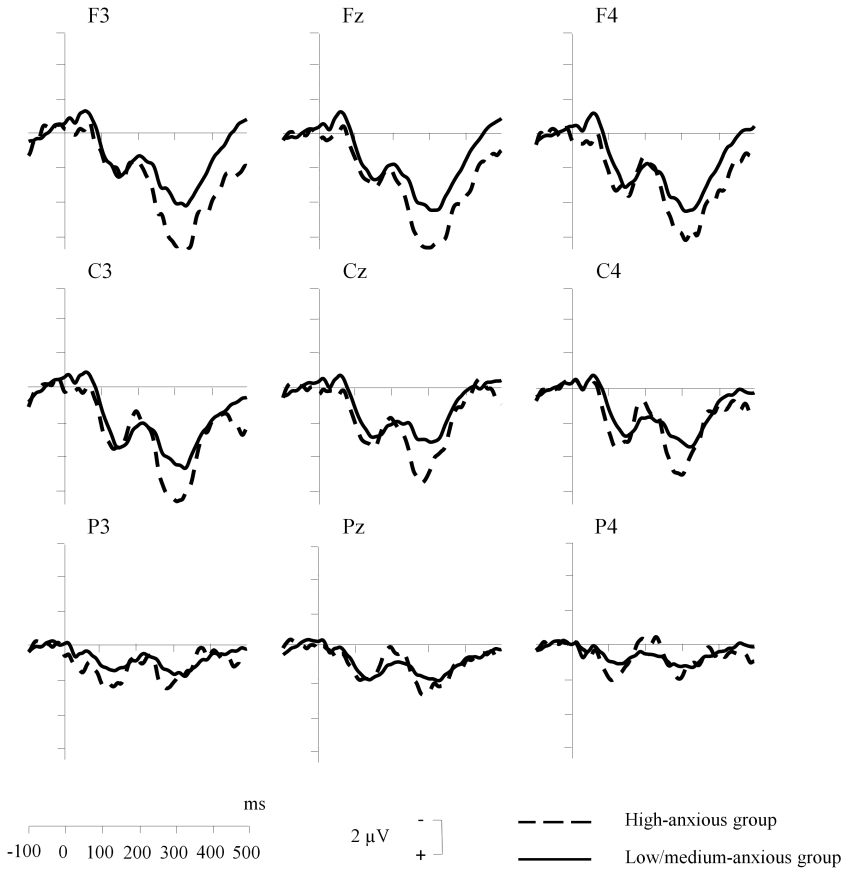

Figure 6.4: Group-averaged waveforms elicited by the white noise segments for the infants exposed to high (broken line) versus low/medium(solid line) levels of Anx-EP. Stimulus onset is at 0 ms. Amplitude calibration is at the bottom of the figure.

State Anxiety associated with larger amplitudes. In addition, we found a significant three-way Anterior-Posterior x Laterality x State Anxiety interaction for the second positivity (320 - 400 ms). Both effects remained significant after controlling for the effects of covariates described earlier ($p < .05$). For the three-way interaction, we ran six two-way interactions separately between State Anxiety and the frontal, central, parietal, left, medial and right electrode arrays. Again, Bonferroni correction was used to control for increases in type I errors due to multi-

ple comparisons. The post hoc tests revealed a significant interaction between the frontal electrode locations (F3, Fz, F4) and State Anxiety ($F(2, 164) = 8.950; p < .001; \eta^2 = .098$). Testing the effects of State Anxiety separately on the F3, Fz, and F4 amplitudes showed that for F4, higher levels of State Anxiety were associated with larger amplitudes [F4 ($F(1, 82) = 5.372; p < .05; \eta^2 = .061$)]. No similar significant effects were obtained for F3 and Fz.

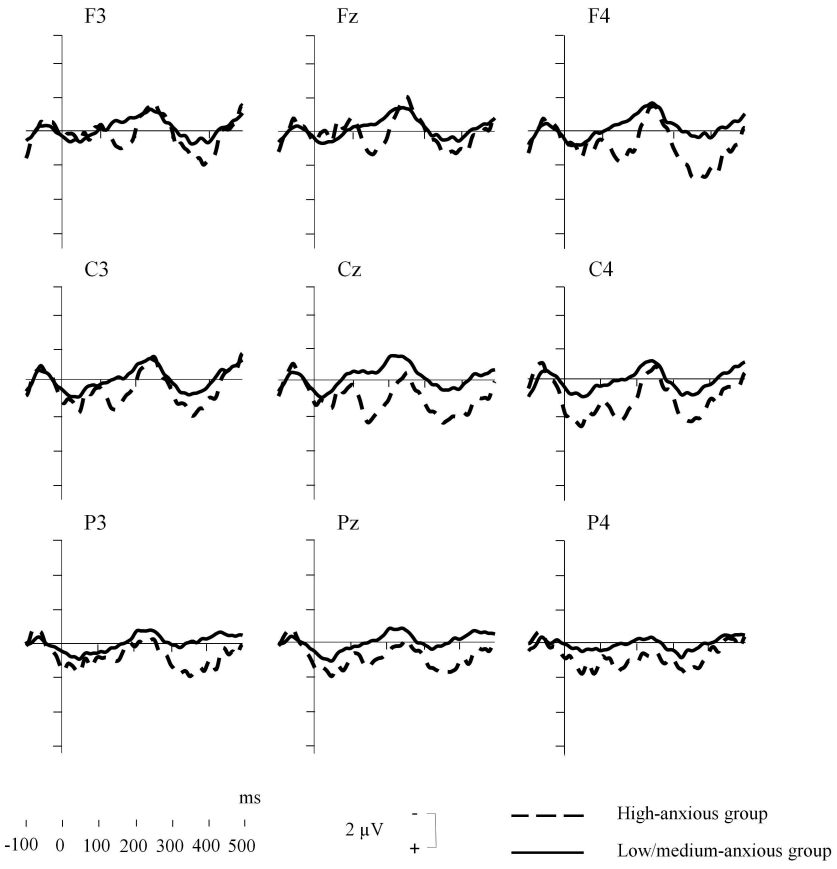

Figure 6.5: Group-averaged waveforms elicited by the ISI-deviant for the infants exposed to high (broken line) versus low/medium(solid line) levels of Anx-EP. Stimulus onset is at 0 ms. Amplitude calibration is at the bottom of the figure.

6.4 Discussion

The current study examined the relationship between Anx-EP and auditory information processing in 9-month-old infants in an auditory oddball paradigm. The analyses yielded evidence supporting our hypothesis that exposure to high levels of Anx-EP is associated with more extensive auditory information processing of low-content stimuli. We found that response amplitudes to the standard tone and ISI-deviant - i.e. sounds with low information contents - were higher the more state anxiety a mother had experienced. In contrast, responses to infrequent white noise and novel sounds were unrelated to Anx-EP.

Our results thus suggest that the between-subject variability of the responses obtained in an auditory oddball task in 9-month-olds can partially be explained by exposure to Anx-EP. These findings are compatible with the perinatal programming hypothesis, according to which in utero exposure to maternal anxiety may lead to reprogramming of structure and function in cells, tissues, and organ systems, potentially accounting for the observed differences in auditory information processing. Also, the results are generally in line with previous research relating exposure to perinatal anxiety to auditory information processing, such as the study by Harvison et al. (2009) who found neurophysiologically detectable differences in sound processing between infants exposed to low versus higher levels of perinatal maternal anxiety. In addition, the data correspond to the findings showing slower habituation to sounds in the offspring of mothers with alcohol (Streissguth et al., 1983) or cocaine intake (Potter et al., 2000) during pregnancy. One may speculate that the neural mechanisms responsible to adaptation to background (uninformative) sounds may be especially sensitive to various adverse effects during the early phase of pregnancy.

Although findings from the current study with 9-month-olds are comparable to those of our study with 2-month-olds (Otte et al., 2013b), they are not identical, especially for the responses to the standard sound. In the 2-month-olds, for the standard sound an early negativity was found around 100 ms post-stimulus, that had a much longer latency for infants exposed to higher levels of Anx-EP levels. In the 9-month-old group, in contrast, the longer latency for infants exposed to higher levels of Anx-EP had disappeared and instead they showed a larger N250-like waveform. The shortening of latencies is a general tendency of the early development of ERP responses (de Haan, 2007; Picton and Taylor, 2007). It appears as if in this respect, 2-month-old infants exposed to higher levels of Anx-EP were somewhat behind in development compared infants exposed to lower levels of Anx-EP, but they caught up with them by the age of 9 months.

Common to the two ages was the higher response amplitude elicited by sounds with low information content. One possible explanation for these findings is that infants of highly anxious mothers may have delayed or impaired habituation in comparison with infants from low-anxious mothers, which would correspond to findings from e.g. Allister et al. (2001); Potter et al. (2000); Streissguth et al. (1983) and Field et al. (2004). An alternative explanation is that the infants display a more mature response, as several studies have related increases in response am-

plitude to increases in age (Bisiacchi et al., 2009; Kushnerenko et al., 2002). This partly corresponds with findings from Jing and Benasich (2006), who described increases in amplitude (e.g. in the N250 component) to auditory stimuli from 3 months onward. However, they also found that this increase reached a maximum at around 9 months of age, after which amplitudes steadily decreased with increasing age of the infants. Thus, as the infants in the current study were 9.9 months on average, the larger response amplitudes for infants in the high-anxiety group could point to less mature responses as well. There is as yet too little literature available to decide for sure what the findings mean in terms of maturation.

More important, perhaps, is the question what mechanism(s) may mediate or underly the relationship between exposure to Anx-EP and pre- and postnatal infant development. Research in animals has shown that high levels of Anx-EP may promote the release of hormones such as glucocorticoids, which can cross the placental barrier and may alter the synthesis and/or release of certain neurotransmitters (Maccari et al., 2003; Nathanielsz, 1999; Weinstock, 2008). Results from previous studies point to a potential role of dopamine. For example, a series of studies of rhesus monkeys that were stressed during pregnancy showed that their offspring displayed less habituation to repetitive tactile stimuli and increased D2 receptor binding in the striatum (Roberts et al., 2004; Schneider et al., 2008). Roy et al. (2007) suggested that increases in dopamine receptors and transporters may be caused by alterations in information flow due to defective myelin. This is supported by the results of Church et al. (2012), who exposed pregnant rats to corticosteroid treatment, which in humans is given to pregnant women at risk for preterm birth to support infant brain and lung maturation. The authors found evidence for poor auditory sensitivity and prolonged neural transmission along central and peripheral auditory pathways in the prenatally exposed offspring, potentially caused by impaired myelination and neural growth. Thus, exposure to high levels of anxiety combined with other environmental influences may result in epigenetic changes in the expression of genes that influence myelination (e.g. neuregulin 1), which in turn changes levels of available dopamine. This may be one mechanism underlying the differences in auditory information processing described here (Gluckman et al., 2007; Mill and Petronis, 2008; Rice et al., 2007; Roy et al., 2007).

In the current study data from a rather large group of infants were included in the analysis. In addition, we could control for a number of potential confounders, such as postpartum maternal anxiety, increasing reliability and validity. Also, as participants in the study came from a mainly healthy population, results can be generalised to the common population. A drawback of the study is that no genetic material has been analysed. Therefore, the effects of genes and heritability of anxiety could not be controlled for.

In summary, then, the current study tested the relationship between prenatal exposure to Anx-EP and auditory information processing in 9-month-old infants. Similarly to the results obtained for 2-month-old infants, we found that the higher the level of Anx-EP a mother had experienced, the stronger her infant's responses to stimuli with low information contents. For rare more informative stimuli no

effects of exposure to Anx-EP were found.

Acknowledgements

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7

Multimodal processing of emotional information in 9-month-old infants II: Prenatal exposure to maternal anxiety

R.A. Otte, F.C.L. Donkers, M.A.K.A. Braeken,
& B.R.H. Van den Bergh.

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Highlights

- Processing of multimodal emotional information is important for daily interactions;
- Atypical influences very early in life may affect the development of this ability;
- We studied this ability in the context of prenatal exposure to maternal anxiety ;
- Prenatal exposure to anxiety was related to higher arousability in 9-month-olds;
- The infants also showed more extensive processing of more familiar emotional voices.

Abstract

The ability to read emotional expressions from human face and voice is an important skill in our day-to-day interactions with others. How this ability develops may be influenced by atypical experiences early in life. Here, we investigated multimodal processing of fearful and happy face/voice pairs in 9-month-olds prenatally exposed to maternal anxiety, using event-related potentials (ERPs). Infants were presented with emotional vocalisations (happy/fearful) preceded by emotional facial expressions (happy/fearful). The results revealed larger P150 amplitudes in response to fearful vocalisations when infants had been exposed to higher levels of anxiety, regardless of the type of visual prime, which may indicate higher arousability to fearful vocalisations. In addition, higher levels of anxiety were associated with smaller auditory P350 and larger N450 amplitudes in response to happy, more familiar, vocalisations, indicating increased attention. This suggests that prenatal exposure to maternal anxiety may be related to more extensive processing of emotional vocalisations infants are more familiar with.

Keywords: Perinatal programming; anxiety; infant; event-related potential (ERP); multimodal processing; emotion perception

7.1 Introduction

The ability to read each other's emotional expression is an important skill in our day-to-day interactions with others. In most social situations, emotional information can be inferred from more than one modality simultaneously, such as from both the face and the voice of the other person. Previous research has found that emotional information from one modality, such as a face, may influence how emotional information from another modality, such as a voice, is processed and perceived. For example, de Gelder and Vroomen (2000) and de Gelder et al. (2002) found that in adults, both recognition and judgement of emotions from voice can be modulated by consciously as well as unconsciously recognised emotion in the corresponding face. Developmental studies have shown that in infants, too, emotional faces may affect processing of emotional voices and the other way around (e.g. Flom and Bahrick, 2007; Grossmann et al., 2006; Walker-Andrews and Lennon, 1991; for a review, see Grossmann, 2010). What has not been studied, however, is the potential effect of early life influences on the ability to detect and process emotional audiovisual information. If the development of the auditory or the visual system is altered or even disrupted, for instance by atypical maternal psychological mood during pregnancy, this may change how information is processed for both modalities. The goal of the current study, therefore, was to examine whether early life experiences, in the form of prenatal exposure to maternal anxiety, influence the ability to process emotional information from face and voice.

Over the last decades, evidence has become available that exposure to atypical or adverse environmental factors during gestation may reprogram the genetically based neural architecture of the brain, thereby changing the developmental pathway of neurocognitive processes (part of the 'perinatal programming hypothesis'; see e.g. Weinstock, 2008 and Räikkönen et al., 2011 for reviews). For instance, iron deficiency during life in the womb, as inferred from serum ferritin concentrations at birth, has been associated with impaired auditory recognition memory in newborns, possibly due to disruptions of the myelination process (Beard, 2008; Georgieff, 2008; Georgieff et al., 2002; Siddappa et al., 2004). In the same vein, the psychological status of the mother during pregnancy may influence offspring neurocognitive development. In evidence of this, associations have been found between prenatal exposure to maternal stress and anxiety, and alterations in infant, child, and adolescent neurocognition (Charil et al., 2010; Entringer et al., 2010; Mennes et al., 2006; Otte et al., 2011; Sandman et al., 2011; Van den Bergh, 2011a; Van den Bergh et al., 2005). In light of these findings, we pose the question whether multimodal processing of emotional stimuli can also be related to in utero exposure to maternal anxiety.

To answer this question, we studied multimodal processing of emotional information in 9-month-old infants by means of event-related potentials (ERPs). Until now, behavioural measures have most often been used to study both multimodal processing of emotional information in infants, and infant neurocognitive outcome following prenatal exposure to maternal anxiety. ERPs, however, have a number of advantages over behavioural measures. First, ERPs provide a continuous mea-

sure of information processing from stimulus exposure to until after elaboration of the response, which allows for assessment of which stage(s) of processing are affected by a specific experimental manipulation (Luck, 2005). Second, ERPs have a very high temporal resolution, and therefore the timing of neurocognitive processes can be studied with millisecond precision (Luck, 2005). Third, ERPs can be recorded in the absence of a behavioural response (Nelson and Bloom, 1997), even for unattended stimuli (Sussman, 2007). This makes them quite suitable for studying actual brain functioning in neonates and infants, instead of making inferences about neurocognitive functioning based on behavioural measures.

The paradigm for the current study was based on an ERP study by Grossmann et al. (2006), who investigated in 7-month-old infants the processing of angry and happy vocalisations following the presentation of an emotionally congruent or incongruent facial expression (prime). The authors found that the emotionally incongruent condition elicited a larger Negative component (Nc) around 500 ms post-stimulus. In contrast, the emotionally congruent condition elicited a larger Positive component (Pc) approximately 800 ms after stimulus onset. Grossmann et al. (2006) concluded that the attenuation of the Nc and enhancement of the later Pc reflected recognition of the familiar/expected face/voice pairs, and that the infants had thus recognised and processed emotions from both modalities.

To our knowledge the study by Grossmann et al. (2006) is the only ERP study with infants in which the processing of emotional auditory stimuli following an emotional visual prime is investigated, instead of the other way around. However, since the auditory system develops earlier than the visual system (Anderson and Thomason, 2013; Anderson et al., 2001), from a developmental perspective, emotional vocalisations may be just as relevant as facial expressions in the first months of life. Thus, to supplement the existing literature with data on the processing of emotional vocalisations in infancy, in the current study (and in our related paper entitled "Multimodal processing of emotional information in 9-month-old infants I: Emotional faces and voices") we focused on effects of emotional facial expressions on the processing of emotional auditory stimuli (in the context of prenatal exposure to maternal anxiety).

Following Grossmann et al. (2006) we used both a positive and a negative emotion (happiness and fear), and investigated the processing of auditory vocalisations following an emotional (happy/fearful) visual prime. In contrast to Grossmann et al. (2006), fear instead of anger was used as the negative emotion. We had several reasons for this. First, we hypothesised that as anxiety shows considerable stability from pregnancy through the postpartum period Grant et al. (2008); Heron et al. (2004); Lee et al. (2007), infants exposed to high levels of maternal anxiety in the womb would also be exposed to higher levels of postpartum maternal anxiety, possibly resulting in differences in how they process fearful stimuli compared to infants exposed to lower levels of maternal anxiety. Second, research has found that 1) exposure to maternal anxiety is associated with more anxiety in the offspring (Lupien et al., 2009; O'Connor et al., 2002b; Van den Bergh and Marcoen, 2004); 2) individuals with high state anxiety respond stronger to fearful stimuli (Bishop, 2002); and 3) high trait anxiety has been related to altered processing of

emotional information from face and voice (Koizumi et al., 2011). Studying responses to fearful stimuli may yield insights into how these findings relate to each other. Finally, infants display increased attention to fearful stimuli, at least from 7 months old onwards (Kotsoni et al., 2001; Montague and Walker-Andrews, 2001; Peltola et al., 2009).

As this study represented the first effort to relate prenatal exposure to maternal anxiety to processing of multimodal emotional information, our hypothesis were of an exploratory nature. We hypothesised that higher levels of maternal anxiety during pregnancy 1) would be associated with larger responses to fearful auditory stimuli; and 2) would most strongly affect responses to auditory stimuli which had been preceded by visual stimuli conveying the same (versus a different) emotion, reflecting a relationship between prenatal exposure to maternal anxiety and multimodal processing of emotional information.

7.2 Methods

7.2.1 Subjects

Subjects were 82 infants (one pair of twins) and their mothers from a normal (i.e. non-clinical) population who have been taking part in a longitudinal study on prenatal early life stress (PELS project). The study was approved by the Medical Ethical Committee of St. Elizabeth Hospital in Tilburg, The Netherlands. Informed consent was obtained from all mothers and fathers in accordance with the Declaration of Helsinki. Detailed information on the cohort and its recruitment has been described previously in (Otte et al., 2013a).

In short, the cohort consists of 190 women - and their partner and child - who have been recruited during pregnancy, either before 15 weeks gestational age (GA; $N = 178$) or between week 16 and 22 ($N = 12$) of gestation from a general hospital and four midwives' practices in Tilburg, The Netherlands. Women were followed up three times during their pregnancies (measurement waves T1, T2 and T3, respectively) and were invited to the lab for postpartum observations both 2 to 4 months (T4) and 9 to 11 months (T5) after giving birth. Here, we report the results from infants measured at T5; data collected at T4 have been discussed elsewhere (Otte et al., 2013a; van den Heuvel et al., 2013).

At T5 147 of the original 190 women came in for testing with their infant (one pair of twins). Forty-three women did not participate in this measurement wave, because of drop out before T5 ($N = 32$), because they could not be reached in time (6), they were ($N = 1$) or their infant was ($N = 2$) too ill, they had miscarried (N=1) around T2 or their infant had passed away ($N = 1$). Three of the 147 mothers had delivered prematurely,1 mother had delivered a baby small for GA (e.g. birth weight $<$2500 gram at term delivery), and 2 mothers had been under treatment for psychological problems at T1 (depression and personality problems, respectively), potentially influencing the questionnaire data collected at that time. Data for infants of these mothers ($N = 6$) were excluded from analysis

beforehand. Data for an additional 60 of the remaining 142 infants were later excluded because of too little remaining data after removing invalid trials (e.g. with movement artefacts, and during which the infant had not looked at the stimulus; $N = 33$), fussiness ($N = 13$), and technical problems (e.g. severe problems with mastoids; $N = 14$). This attrition rate (42.2%) is similar to other infant ERP studies (DeBoer et al., 2007). All infants were healthy and had passed a screening test for hearing impairments (evoked otoacoustic emission), performed by a nurse from the infant health care clinic, between the 4th and 7th day after birth. Demographical statistics of the infants, and their mothers, are given in Table 7.1.

		N	%	M (SD)
Mothers		81		
Age at T1 (years)				32.39 (3.80)
State anxiety at T1				32.82 (8.89)
State anxiety at T5				31.92 (7.66)
Marital status	Married	45	55.6	
	Cohabiting	37	43.2	
	Single	1	1.2	
Educational level	Primary or secondary	7	8.0	
	General vocational training	18	20.7	
	Higher vocational training	42	48.3	
	University degree or higher	20	23.0	
Family income	$<$ 2100	3	3.4	
(monthly, in €)	2200 - 3600	19	21.8	
	$>$ 3600	62	71.3	
	Dont want to disclose	3	3.4	
Primigravida		35	40.2	
Smoking during pregnancy		3	3.4	
Drinking during pregnancy*		7	8.0	
Has been treated in	Depression	8	9.2	
the past for	Anxiety problems	4	4.6	
	Personality problems	2	2.3	
	PDD-NOS	1	1.1	
Infants		82		
Sex	Boy	38	46.3	
	Girl	44	53.7	
Birth weight (grams)				3474 (569)
GA at birth (weeks)				39.9 (1.3)
Age at T5 (days)				303.1 (14.1)

GA=Gestational Age; PPD-NOS=Pervasive Developmental Disorder-Not Otherwise Specified

*From 1 glass of wine or liquor during the whole pregnancy to maximally 3 glasses a month.

Table 7.1: Sample characteristics of participating women and their infants

7.2.2 Predictor

Maternal anxiety during pregnancy was measured before 15 weeks GA, with Dutch versions of the anxiety subscales of the Spielberger State-Trait Anxiety Inventory (STAI; Spielberger et al., 1970; van der Ploeg, H.M. et al., 1980, and the Symptom Checklist-90 (SCL; Arrindell and Ettema, 2003; Derogatis and Cleary, 1977; Derogatis et al., 1973. We specifically focused on early pregnancy, because previous research associated exposure during early rather than late gestation with specific changes in offspring cognitive functions as measured with behavioural (i.e. neuropsychological) as well as with physiological (i.e., ERP and fMRI) measures (Malaspina et al., 2008; Mennes et al., 2006, 2009; Van den Bergh et al., 2006).

7.2.2.1 Anxiety subscale from the STAI

The STAI is a self-report questionnaire with the subscales "Trait" and "State" Anxiety. Both scales consist of 20 items which can be rated on a Lickert scale from 1 (not at all/almost never) to 4 (very much/almost always). Whereas trait anxiety refers to a disposition or proneness to react with anxiety, the state anxiety subscale provides a valid measure of the intensity of transitory anxiety in response to real life stress (Spielberger, 1975). Therefore, maternal state (and not trait) anxiety was used as a measure of prenatal maternal anxiety. The subscale's Cronbach's α was .902 in the current study. Examples of items on the State scale are: "I feel secure", "I am worried" and "I feel indecisive".

7.2.2.2 Anxiety subscale from the SCL-90

The SCL, too, is a self-report instrument. The questionnaire quantifies current psychopathological symptoms in terms of nine primary constructs, of which anxiety is one. The anxiety subscale measures current feelings of more generalised anxiety, with complaints related to increased vegetative arousal, general symptoms such as nervousness, more specific symptoms such as panic attacks, and cognitive symptoms such as fearful thoughts (Arrindell and Ettema, 2003; Derogatis and Cleary, 1977). The 10 items are rated on a 5-point Lickert scale, ranging from 0 (not at all) to 4 (extremely). Cronbach's α for this subscale was .861 in the current study. Examples of items are: "Heart pounding or racing", "Trembling", and "Spells of terror or panic".

7.2.2.3 Postpartum anxiety

To control for postpartum anxiety, the state anxiety subscales of both the STAI and the SCL were also administered at T5, at about the same time the mother and her infant were invited to the lab for the T5 observations. Anxiety data were unavailable for 4 mothers at T1 because of recruitment after this first data collection wave. In addition, 17 mothers did not return the questionnaire at T5. The missing anxiety sum scores were imputed, separately per questionnaire, by means of the Expectation-Maximization method as implemented in IBM SPSS Statistics

19.0.Variables used for the imputation were anxiety sum scores measured at other time points; e.g. when the anxiety sum score was missing for T1, the available anxiety sum scores from the same questionnaire (so STAI data for imputing STAI data, and SCL data for imputing SCL data) measured from T2, T3, T4 and/or T5 were used to estimate the missing score.

7.2.3 Stimuli

7.2.3.1 Visual stimuli

Visual stimuli were 18 colour photos of 9 Caucasian women in frontal view, each expressing both happiness and fear. Only female and Caucasian identities had been chosen so as to avoid any sex or ethnicity differences from influencing the infant ERPs (e.g. see Ramsey et al., 2005; Vogel et al., 2012). The emotional faces had been cut out from their original background and pasted onto a black background. Four identities were taken from the validated NimStim Face Stimulus Set (http://www.macbrain.org/ resources.htm[1]). Their fearful and happy expression had been recognised at least 75% of the time in the validation study. Examples of a typical happy and fearful expression from the NimStim dataset can be found in Figures 7.1A and 7.1B, respectively.

An additional 5 identities were included from a database with emotional facial stimuli from the Cognitive and Affective Neurosciences Laboratory at Tilburg University, The Netherlands. This was done because we wanted to include as many identities as possible to minimise the possibility that potential effects would be due to the identity itself, and the NimStim set did not offer more than 4 female Caucasian identities whose fearful and happy expression survived the validation process. The stimuli from the Tilburg database had been validated in a pilot study in which they were rated for emotion (fear, happiness, anger, neutral, surprise, sadness and disgust), intensity (scale from 1 to 5) and positive/negative affect (scale from 1 to 5) by at least 8 participants. For the 5 identities used for this study both the emotion fear and happiness were correctly recognised by the raters 80% of the time or more. In Figures 7.1C and 7.1D examples of a happy and fearful face from the Tilburg dataset can be found.

7.2.3.2 Auditory stimuli

Auditory stimuli were voice recordings of six women expressing fear and happiness with non-verbal vocalisations. We chose to use non-verbal vocalisations as to minimise automatic semantic or verbal processing (Van den Stock et al., 2008). Because not all vocalisations had been recognised well enough in the validation study (see below), for two of the identities we only used the fearful vocalisation,

[1]Development of the MacBrain Face Stimulus Set was overseen by Nim Tottenham and supported by the John D. and Catherine T. MacArthur Foundation Research Network on Early Experience and Brain Development. Please contact Nim Tottenham at tott0006@tc.umn.edu for more information concerning the stimulus set.

Figure 7.1: Examples of a typical happy (A) and fearful (B) visual expression from the NimStim dataset, and of a typical happy (C) and fearful (B) visual expression from the Tilburg dataset.

for two of them we only used the happy vocalisation and for two of them we used vocalisations of both emotions, resulting in 4 fearful and 4 happy auditory stimuli. These auditory stimuli were provided by the Cognitive and Affective Neurosciences Laboratory at Tilburg University, too, and were recorded, processed and validated as described by Van den Stock et al. (2008). In short, semi-professional actors were asked to make a frightened or happy sound, based on a specific script describing situations such as an attack by a robber. The 800 ms audio recordings

were made at a 44.1 KHz sampling rate and were shortened to 500 ms for the experiment described here. The sounds were validated in a pilot session by 15 participants, who were instructed to categorise as accurately and as fast as possible the emotion expressed by the voice (fear or happiness). All stimuli used here had been correctly recognised 80% of the time or more.

7.2.4 Procedure

Each of the 18 visual stimuli was paired with both the fearful and happy auditory stimuli, resulting in 144 face/voice compounds and four experimental conditions: happy face-happy voice (HH), fearful face-fearful voice (FF), happy face-fearful voice (HF), and fearful face-happy voice (FH). The compounds were presented twice during the experiment (288 trials), divided in four blocks of 72 stimuli, each. The presentation order within each of the blocks was randomised, and the order between the blocks was counter-balanced. The blocks were presented with small breaks in between as needed.

During stimulus presentation the infant was seated on its parent's lap in a dimly lit and sound-attenuated room of the Tilburg University Babylab. The parent-infant dyad were seated behind a desk with a computer screen (CRT VGA, 21 inch, 1280 x 1024, 100 Hz) at a distance of approximately 70 cm from the infant's eyes. The visual stimuli measured 18,5 x 22,5 cm and the horizontal and vertical visual angles were 7.53^o and 9.13^o, respectively. Auditory stimuli were presented through speakers positioned on either side of the screen, and at a distance of approximately 90 cm from the infant's head. To prevent the parent from influencing the infant's ERP responses by unconsciously reacting to the stimuli, he or she was wearing head phones through which classical music was playing. Two cameras filmed the experimental session and these data were later used to code whether the infant had looked at a specific trial or not (see also "Data acquisition and analysis" section 7.2.5).

Each stimulus block started with the sound of a laughing baby, and the presentation of a red dot growing bigger and smaller in the centre of the computer screen to attract the infant's attention. When the infant was looking at the screen, the experimenter started the first trial. Each trial lasted 1400 ms and started with the presentation of a visual stimulus that lasted 900 ms, after which an auditory stimulus was presented. The visual stimulus remained in place until the auditory stimulus was played out (after 500 ms). Each trial was followed by an inter-trial interval with variable duration (between 600 and1000 ms) to reduce temporal predictability. During this interval, the screen was black. When an infant looked away from the screen, the experimenter tried to recapture the infant's attention by presenting an attractive moving figure in the centre of the screen. As soon as the infant was looking at the screen again, the experiment continued. The experiment was concluded either after all 288 stimuli had been presented or if the infant became too fussy to continue.

7.2.5 Data acquisition and analysis

EEG was recorded with BioSemi ActiveTwo amplifiers (BioSemi, Amsterdam, The Netherlands) with a sampling rate of 512 Hz[2]. Infants wore head caps with 64 electrode locations positioned according to the revised version of the International 10-20 system. The standard BioSemi reference (CMS-DRL) was used (see www.biosemi.com/faq/cms&drl.htm for details) and two additional electrodes were placed both on the left and right mastoid. Off-line, these were mathematically combined to produce an average mastoids reference derivation (Luck, 2005).

Before the data were processed further, independent raters inspected the data and scored per infant per trial whether or not he or she had indeed looked at the visual stimulus. All trials were scored by 2 different raters (there were 4 raters in total) and these scorings were afterwards compared. Agreement between raters lay between 81% and 99% and was 95% on average. Whenever scorings differed, the trials concerned were re-inspected and scored again. If there was still doubt about whether the infant had actually seen the stimulus, the trial was excluded. Trials during which an infant was crying were also excluded. Only EEG signals from trials during which the infant was looking at the stimulus were used for data analysis.

The EEG data were analysed using BrainVision Analyzer software (Brain Products, Munich, Germany). The continuous EEG signals were filtered off-line, with a 0.1 to 20 Hz band-pass filter (slope 24 dB), and an additional 50 Hz notch filter to make sure all 50 Hz line noise would be removed. The signals were then segmented into 1000 ms-long epochs, time-locked to the onset of the auditory stimulus. The 200 ms before auditory stimulus onset were used as the baseline. The epochs were averaged separately for each of the four conditions (FF, HH,HF, FH). Epochs with sample-to-sample voltage steps exceeding 80 μV were excluded, as were epochs with amplitudes exceeding 150 μV in any 200 ms-long window within the whole epoch, and those with amplitudes below 0.5 μV in any 100 ms-long window within the whole epoch. Data from infants with less than 14 acceptable responses for any one of the four conditions were removed from further analysis (33 infants, see also "Subjects" section 7.2.1). The average number of available trials per infant was 36.5 for condition FF ($SD = 12.6$), 37.5 for HH ($SD = 11.9$), 36.6 for HF ($SD = 11.7$), and 37.1 for FH ($SD = 12.7$).

Time windows for analysis were selected based on visual inspection of the grand average waveform for all responses combined at electrode sites F3, Fz, F4, C3, Cz, C4 (see Figure 2), where responses were largest. The following windows, each centred around a peak in the grand average waveform, were chosen: 120-200 ms, 200-260 ms, 290-430 ms, 380-540 ms, and 620-680 ms post-stimulus (from this point on referred to as P150, N250, P350, N450, and P650, respectively). These first four areas correspond to the infant P150-N250-P350-N450 ERP pattern in response to auditory stimuli described by Kushnerenko et al. (2002), who found

[2]On-line filtering options in the BioSemi software are set automatically based upon the sampling rate. See http://www.biosemi.com/faq/adjust filter.htm and http://www.biosemi.com/faq/adjust samplerate.htm

that this pattern is already identifiable in auditory ERPs from birth.

To answer our research question, amplitudes measured from the above mentioned windows were analysed by means of a 6 x 2 x 2 repeated measures ANCOVAs design (run in IBM SPSS 19.0), separately for each window. Within-subjects factors were "Electrode" (F3, Fz, F4, C3, Cz, C4), "Visual Prime" (fearful, happy), and "Auditory Emotion" (fearful, happy). Continuous predictor variables ('covariates' in SPSS's ANCOVA) were state anxiety measured with the STAI (StatAnx), and more generalised anxiety measured with the SCL (GenAnx). Separate analysis were run for both predictor variables. We chose not to enter StateAnx and GenAnx as between-subjects factors (e.g. high versus low anxiety), because dichotomising the variables would result in groups of different sizes, and because previous research has shown that dichotomisation of a continuous variable can lead to problems such as loss of effect size, power and/or information about individual differences (Altman and Royston, 2006; MacCallum et al., 2002; Royston et al., 2006). Post-hoc tests were run to further investigate any significant main/interaction effect. We were especially interested in effects of priming by the visual stimulus (Visual Prime) on the auditory stimulus (Auditory Emotion) in the context of exposure to anxiety, because this factor represents effects of the visually presented emotion on the processing of the emotional sounds. However, as our research question and hypotheses were of an exploratory nature, we ran post-hoc tests for all effects including StateAnx and GenAnx.

Previous research has shown that several factors may affect and/or confound both infant ERPs and the association between prenatal exposure to atypical cues and infant cognitive outcome (e.g. see Fellman et al., 2004; Heron et al., 2004; Kodituwakku, 2007; Kushnerenko et al., 2002; Lavoie et al., 1998; Rodriguez and Bohlin, 2005; Streissguth et al., 1983). Therefore, we controlled for the following factors by adding them, one at a time, as covariate to the analyses: maternal alcohol intake during pregnancy (continuous) and postpartum anxiety; and infant sex, birth weight (controlled for gestational age), gestational age and age at testing. The continuous factors were first correlated to the ERP data and only added when significant correlations were found. The dichotomous covariates were added as between-subjects factor to the analyses. As only three mothers had smoked during pregnancy, we controlled for this factor by running the analyses with and without infants from smoking mothers, and then examining whether effects remained the same. Greenhouse-Geisser correction was used where necessary and the ϵ correction factor is given, together with the partial η^2 effect size in Table 7.2.

7.3 Results

The grand-averaged waveform for all responses combined, on which selection of windows for analysis was based (see "Methods" section 7.2), can be found in Figure 7.2. As there were no significant effects involving StatAnx, only findings for GenAnx will be reported here. For investigating our hypotheses, all main and interaction effects including the latter factor will be interpreted (see Table 7.2 for

120-200 ms latency range					
Factor(s)	*df*	*F*	*p*	ϵ	η^2
Electrode	5,4	2.750	.048	.56	.033
Visual Prime	1,8	3.896	.052		.046
Auditory Emotion x GenAnx	1,8	3.568	.063		.043
Visual Prime x Auditory Emotion	1,8	4.215	.043		.050

200-260 ms latency range					
Factor(s)	*df*	*F*	*p*	ϵ	η^2
Electrode	5,4	3.504	.019	.55	.042
Visual Prime	1,8	6.408	.013		.074

290-430 ms latency range					
Factor(s)	*df*	*F*	*p*	ϵ	η^2
Electrode	5,4	3.495	.019	.55	.042
Visual Prime	1,8	6.040	.016		.070
Auditory Emotion x GenAnx	1,8	4.249	.043		.050

380-520 ms latency range					
Factor(s)	*df*	*F*	*p*	ϵ	η^2
Electrode	5,4	2.245	.092	.55	.027
Visual Prime	1,8	4.132	.045		.049
Auditory Emotion x GenAnx	1,8	2.941	.090		.035

620-680 ms latency range					
Factor(s)	*df*	*F*	*p*	ϵ	η^2
Auditory Emotion x GenAnx	1,8	2.973	.089		.036
Visual Prime x Auditory Emotion x GenAnx	1,8	3.167	.079		.038

Table 7.2: Significant main and interaction effects for GenAnx

statistical information). However, significant results involving other factors can be found in Table 7.2.

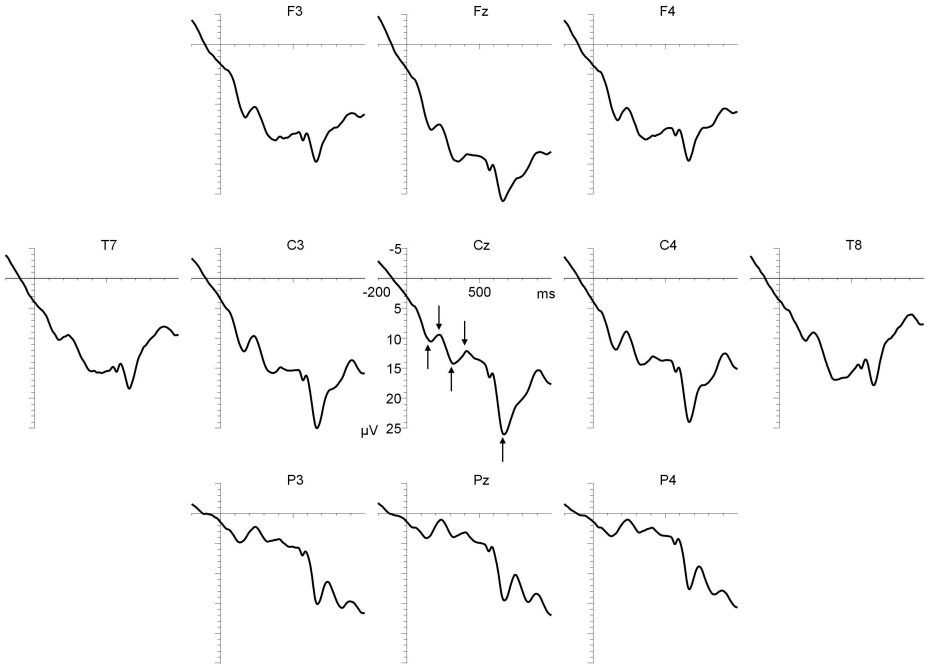

Figure 7.2: Grand-averaged waveform for all 4 conditions (FF, HH, FH, HF) combined. Arrows indicate the P150, N250, P350, N450 and P650 area, respectively. Stimulus onset is at 0 ms. Amplitude calibration is at Cz.

7.3.1 Results for GenAnx

7.3.1.1 Results for the P150 (120-200 ms post-stimulus)

There was a small interaction effect between Auditory Emotion and GenAnx. This interaction was caused by a positive association between GenAnx and fearful vocalisations (larger positivity) versus a negative association between GenAnx and happy vocalisation (smaller positivity). The interaction effect remained intact after controlling for the covariates described in the methods section (7.2). A plot of the interaction can be found in Figure 7.3A.

There were no associations between Visual Prime and GenAnx.

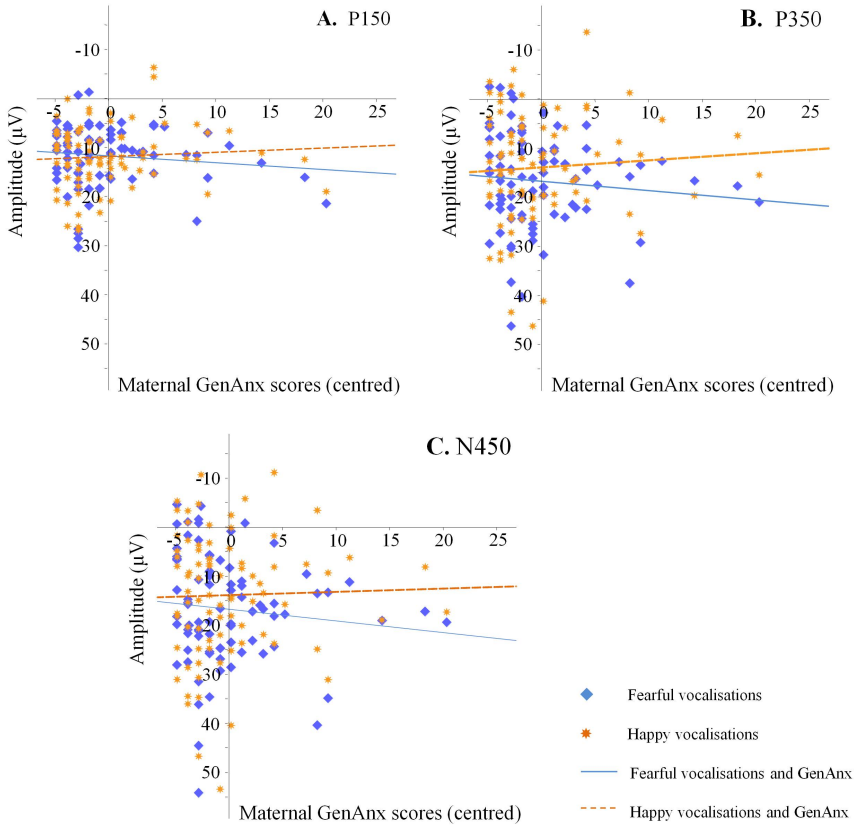

Figure 7.3: Significant interactions between GenAnx (measured with the SCL) and Auditory Emotion (fearful vocalisations: solid line; happy vocalisations: broken line) for the P150 (A), the P350 (B), and the N450 (C). Maternal SCL scores have been centred on the mean.

7.3.1.2 Results for the N250 (200-260 ms post-stimulus)

The analysis yielded no effects with the factor GenAnx.

7.3.1.3 Results for the P350 (290-430 ms post-stimulus)

There was an interaction effect between Auditory Emotion and GenAnx, which was caused by a positive association between GenAnx and fearful vocalisations (larger positivity) versus a negative association between GenAnx and happy vocalisation (smaller positivity). After controlling for post-partum anxiety, the inter-

action effect remained, but became smaller (p-value increased from .043 to .052, see Table 7.2). None of the other covariates affected the interaction, the plot of which can be found in Figure 7.3B.

Results revealed no significant associations between Visual Prime and GenAnx.

7.3.1.4 Results for the N450 (380-540 ms post-stimulus)

Again, there was a small interaction effect between Auditory Emotion and GenAnx, caused by the positive association between GenAnx and fearful vocalisations (smaller negativity), and the negative association between GenAnx and happy vocalisations (larger negativity). The interaction effect remained intact after controlling for the covariates mentioned. A plot of the interaction can be found in Figure 7.3C.

No associations were found between Visual Prime and GenAnx.

7.3.1.5 Results for the P650 (620-680 ms post-stimulus)

There was a small 2-way interaction between Auditory Emotion and GenAnx, and a small 3-way interaction between Auditory Emotion, Visual Prime, and GenAnx. Post hoc analysis on the higher order (i.e. 3-way) interaction showed a negative association between GenAnx and happy vocalisations following a fearful expression (smaller positivity). In contrast, for happy vocalisations following a happy expression, and for all fearful vocalisations (so both following a happy and fearful expression) a positive association with GenAnx was found (larger positivity). After controlling for postpartum anxiety, both the 2- and 3-way interaction disappeared.

The analysis did not yield associations between Visual Prime and GenAnx.

7.4 Discussion

The current study investigated in 9-month-old infants the processing of emotional (fearful and happy) face/voice compounds in the context of prenatal exposure to maternal anxiety. For the P150, the P350, and the N450 we found associations between the level of maternal anxiety, as measured with the SCL, and processing of emotional vocalisations. More specifically, for the P150 and P350, statistical analyses revealed that the higher the level of maternal anxiety had been, the larger the positivity was in response to the fearful vocalisations, and the smaller in response to happy vocalisations. For the N450, higher levels of maternal anxiety were related to a smaller negative-going response to the fearful vocalisations, and to a larger negative-going response to happy vocalisations. These associations remained intact after controlling for several covariates, such as maternal postpartum anxiety. The results confirmed our first hypothesis that higher levels of maternal anxiety during pregnancy are associated with larger responses to fearful auditory stimuli. The current study did not provide evidence for our second hypothesis that

the association between exposure to maternal anxiety and responses to the audiovisual stimuli would be stronger for auditory stimuli that followed visual stimuli conveying the same (versus different) emotional information.

Our results suggest that the between-subject variability in responses to the emotional vocalisations in an audiovisual paradigm with emotional face/voice pairs can be partially explained by prenatal exposure to maternal anxiety. These findings are compatible with the perinatal programming hypothesis, which postulates that exposure to an atypical or adverse environment during pregnancy may alter the developmental pathway of the foetus and child. They are also in line with previous studies that found associations between prenatal exposure to maternal anxiety and offspring neurocognitive outcome (e.g. Bergman et al., 2007; Davis and Sandman, 2010; Mennes et al., 2006; Otte et al., 2011; Van den Bergh et al., 2005, 2012, 2013.

The analysis revealed a positive association between prenatal exposure to higher levels of maternal anxiety and P150 amplitudes (120-200 ms after stimulus onset) elicited by the fearful auditory stimuli (regardless of the type of visual prime). Kushnerenko et al. (2002) proposed that the "infant P150" is a precursor of the adult P1, which shows poor sound-feature specificity but is sensitive to arousal (Čeponienė et al., 2003; Erwin and Buchwald, 1986). The adult P1 is therefore assumed to be involved in feature non-specific aspects of stimulus processing, like stimulus detection, facilitation/inhibition, and sensorimotor integration (Čeponienė et al., 2003). As such, prenatal exposure to high levels of maternal anxiety may be associated with a higher level of arousability (i.e. being more easily aroused), reflected in the larger P1 responses to fearful auditory stimuli. This interpretation is in line with the finding that infants who have been exposed to higher compared to lower levels of maternal anxiety display more anxiety themselves (Lupien et al., 2009; O'Connor et al., 2002b; Van den Bergh and Marcoen, 2004), and therefore may be more easily aroused by threatening stimuli (Kim et al., 2011; Rosen and Schulkin, 1998).

For the P150 and the P350 we found, as hypothesised, larger responses to the fearful compared to the happy auditory stimuli with increasing levels of maternal anxiety. In contrast, for the N450 we found smaller (negative-going) responses to the fearful stimuli. This may be because N450 responses to the fearful voice (especially when following a fearful face) originated from higher positive values, and were not strong enough to result in a larger negativity compared to the happy vocalisations. Alternatively, it is possible that since infants are generally more familiar with happy than fearful emotions (see e.g. Grossmann, 2010), a 'negative shift' in response to the more familiar stimuli, indicating increased attention (Purhonen et al., 2004), may have yielded the smaller positive P350 and larger negative-going N450 amplitudes in response to the happy stimuli - and conversely the larger positive P350 and smaller negative-going N450 in response to fearful stimuli. As higher levels of anxiety were associated with a more pronounced negative shift for the happy vocalisations (e.g. smaller P350 amplitudes and more negative N450 amplitudes), the results suggest that compared to infants prenatally exposed to lower levels of maternal anxiety, infants exposed to higher levels may

be more sensitive to this negative shift for familiar stimuli. This corresponds to research findings relating prenatal exposure to higher levels of maternal anxiety to more extensive processing of less informative (or more familiar) sounds, as compared to novel or more informative sounds (Otte et al., 2013b; Van den Bergh et al., 2012, 2013).

Interestingly, whereas we found effects for anxiety as measured with the anxiety subscale of the SCL, we did not find effects with the state anxiety subscale of the STAI. In two former studies, however, significant associations were found in both 2- and 9-month-old infants between anxiety measured with the STAI and the processing of less informative sounds (Otte et al., 2013b; Van den Bergh et al., 2012, 2013). These differential findings are actually not very surprising, since the state anxiety subscale of the STAI and the anxiety subscale of the SCL appear to measure an overlapping, yet distinct anxiety construct (see "Methods" section 7.2). Taking the present and former results together suggests that feelings of more generalised anxiety during pregnancy may affect processing of familiar emotional vocalisations, while higher levels of state anxiety may affect processing of sounds with low(er) information contents. However, replication or follow-up studies are needed to test whether this hypothesis holds true.

In the current study, no evidence was found for an association between prenatal exposure to maternal anxiety and alterations in the processing of multimodal emotional information. One possible explanation is that the present paradigm was not sensitive enough to measure the association. In evidence of this, only for the P150, instead of for all areas under investigation, a (small) interaction between Visual Prime and Auditory Emotion was found (see Table 7.2), indicating integration of the emotional information from face and voice. In other words, maybe no multimodal processing had taken place, precluding conclusions on how maternal anxiety may affect the processes involved. Future studies could develop a more sensitive paradigm, so that firmer conclusions may be drawn about the possible association between prenatal exposure to anxiety and processing of emotional information from face and voice.

Some drawbacks of this study should be mentioned. First, in this paper we have referred to studies showing that infants who have been exposed to higher compared to lower levels of maternal anxiety display more anxiety themselves. Unfortunately, as we did not include infant anxiety scores in the current analyses (e.g. parent- or observer-reported scores), we cannot be certain that the infants exposed to higher levels of maternal anxiety indeed did experience more anxiety. Follow up research could take infant fearfulness and/or temperament into account when studying responses to emotional audiovisual stimuli.

Second, the effects we found were not very large. The p-values ranged between .043 and .097, and the partial η^2 values between .034 and .050. However, results remained intact after controlling for a number of potential confounders, including postpartum anxiety levels. In addition, we investigated a rather large group of infants ($N = 82$), who attended the visual stimuli quite well (73.2% on average), and we were able to retain a relatively large average number of stimuli per condition (37.3). This suggests that although the effects we found may be small, they

appear to be robust.

In summary, the current study investigated multimodal processing of fearful and happy face/voice pairs in infants in the context of prenatal exposure to maternal anxiety. Analysis revealed higher P150 amplitudes in response to fearful vocalisations when infants had been exposed to higher levels of anxiety, suggesting these infants may be aroused more easily than infants exposed to lower levels of anxiety. In addition, smaller P350 and larger N450 amplitudes in response to happy vocalisations were found, indicating increased attention for stimuli the infants are more familiar with. Infants prenatally exposed to higher levels of maternal anxiety appeared to be more sensitive to this overall increase in negativity in the P350 and N450, and thus to dedicate more processing capacity to more familiar, or less informative, emotional vocalisations.

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Part D

General discussion

8 General discussion

This dissertation described the first results of an on-going, longitudinal research project on the short- and long-term consequences of Prenatal Early Life Stress (PELS), in the tradition of research in the field of the developmental origins of health and disease (DoHAD, see Chapter 1). Within the PELS project, we focused on effects of prenatal exposure to maternal anxiety on the neurocognitive outcome of infants in their first year of life. We will first summarize the main findings of the five studies included in the thesis. Results will be related to the core aims of the dissertation and a common working hypothesis for explaining the main results will be proposed. We will then attempt to describe the brain structures possibly involved and the mechanisms that may underlie our findings, taking into account the notion of sensitive periods and individual differences. Next, the strengths and limitations of the dissertation will be listed. Finally, directions for future research and clinical implications will be discussed.

8.1 Overview of the main findings

The first two chapters described paradigms we used for studying infant neurocognitive functioning. In Chapter 3, an auditory oddball paradigm was discussed with one standard tone ('standard') and three types of deviants: the standard tone presented with a deviant inter-stimulus interval ('ISI-deviant'); white noise segments ('white noise'); and environmental sounds ('novel sound'). The main objective of this study was to investigate whether the brain of 2-month-old infants can detect violations of temporal regularities. This question was addressed by studying deviant-minus-standard responses delienating the mismtach response (MMR) to

deviant auditory events. An additional objective was to study MMRs in the context of the infants' state of alertness (i.e. awake or asleep). The main findings were that 1) the brain of 2-month-old infants indeed detects violations of a temporal regularity, as evidenced by a significant MMR appearing on frontal electrode sites; and 2) the infants' state of alertness influenced the scalp distributions of the MMR.

In *Chapter 4*, results of a multimodal paradigm with happy and fearful face/voice pairs were presented. The study's research question was whether 9-month-old infants process emotional vocalisations differently after they have been primed with an emotionally congruent or incongruent visual stimulus. The analyses revealed that responses to the vocalisations were modulated by the type of emotion in the preceding facial expression: fearful faces yielded larger auditory ERP responses than happy faces. As there was no interaction between the type of visual and auditory emotion, we found no evidence for differential processing of emotionally congruent versus incongruent fearful/ happy face/voice pairs. Results were discussed in terms of a 'negativity bias', i.e. larger response amplitudes after presentation of sounds signalling potential danger.

In the three following chapters the core questions of the dissertation were addressed by examining data from the oddball and audiovisual paradigm in the context of prenatal exposure to maternal anxiety. The goal of the study described in *Chapter 5* was to assess the influence of exposure to maternal anxiety during early pregnancy (as measured with the STAI; see Methods, Chapter 2), on information processing in 2-month-old infants. Our analyses yielded significant positive associations between maternal anxiety and response amplitudes to the standard and the ISI-deviant tones (i.e. larger response amplitudes with higher levels of anxiety). No effects on the ERP responses to white noise and novel sounds were found. The standard and the ISI-deviant were acoustically identical (they only differed in the interval preceding the tone), and together had been presented 80% of the time in the oddball sequences. These sounds carry less information (surprise) than the other two sounds appearing in the sequences. Therefore, we proposed that whereas all infants process white noise and novel sounds similarly, infants exposed to higher levels of maternal anxiety process sounds with low information contents more extensively. As the results showed similarities to those obtained from infants from families with a history of language learning impairments (Benasich et al., 2006), we further proposed that the association may represent a precursor for later problems with language development.

The study described in *Chapter 6* investigated responses to the oddball paradigm in 9-month-old infants in relation to prenatal exposure to maternal anxiety, again, measured with the STAI. Similar results were obtained to those in the 2-month-olds: significant positive associations were found between levels of maternal anxiety and response amplitudes to the standard and ISI-deviant (i.e. larger response amplitudes), but not to the white noise and novel sounds. This supported our proposal that maternal anxiety influences the processing of familiar, and therefore less informative sounds.

Finally, *Chapter 7* addressed the question how early life experiences may in-

fluence the ability to process emotional information from facial and voice input in 9-month-old infants. The analysis revealed that maternal anxiety as measured with the SCL (see Methods, Chapter 2), but not with the STAI, was positively related to the amplitude of the ERP responses to the fearful vocalisations, and negatively to those evoked by the happy vocalisations. For the time window approximately 120-200 ms post-stimulus (P150), effects were explained in terms of increased arousability in response to fearful stimuli in infants prenatally exposed to higher levels of maternal anxiety. Smaller P350 amplitudes combined with larger N450 amplitudes (larger overall negativity; 'negative shift') in response to happy vocalisations suggested increased attention for emotional auditory stimuli infants are generally more familiar with (i.e. happy stimuli). Infants prenatally exposed to higher levels of maternal anxiety appeared to be more sensitive to this negative shift, and to dedicate more processing capacity to familiar emotional vocalisations.

8.2 Less efficient habituation: a working hypothesis

> A fox who had never yet seen a lion, when he fell in with him for the first time in the forest was so frightened that he was near dying with fear. On his meeting with him for the second time, he was still much alarmed, but not to the same extent as at first. On seeing him the third time, he so increased in boldness that he went up to him and commenced a familiar conversation with him.
>
> Æsop's Fables; cited by Thompson (2009)

In the three chapters making up the core of this thesis a positive association was found between levels of maternal anxiety during pregnancy and processing of less informative or more familiar stimuli. In Chapter 5 and 6, this was evidenced by more extensive processing of (larger ERP amplitudes to) sounds that had been presented most often by infants of more anxious pregnant women. In Chapter 7, this was evidenced by a stronger negative shift in response to familiar emotional sounds (i.e. sounds that infants encounter quite frequently in daily interactions with their parents/caregivers). These results could also be interpreted the other way around: infants prenatally exposed to lower levels of maternal anxiety responded with smaller ERP amplitudes to repeated presentation of the same/similar stimuli. Thus, in comparison with prenatal exposure to lower levels of maternal anxiety, it may not so much be that exposure to higher levels of anxiety was related to more processing, but rather that it was related to a smaller decrease in processing of a familiar stimulus. Taken together, this suggests that prenatal exposure to anxiety may result in alterations in habituation. We thus propose that there is a negative association between prenatal exposure to maternal anxiety and the degree to which habituation to repeated stimulus presentation takes place.

Thompson and Spencer (1966) described habituation as a decrement in responsiveness to a stimulus after repeated presentations. In addition, they summed up 8 common characteristics of habituation:

1. If the stimulus is withheld, the response tends to recover over time;
2. If repeated series of habituation training and spontaneous recovery are given, habituation becomes successively more rapid;
3. Other things being equal, the more rapid the frequency of stimulation, the more rapid and/or more pronounced is habituation;
4. The weaker the stimulus, the more rapid and/or more pronounced is habituation. Strong stimuli may yield no significant habituation;
5. The effects of habituation training may proceed beyond the zero response level (i.e. additional habituation training given after the response has disappeared or reached a stable habituated level may still yield effects);
6. Habituation of response to a given stimulus may exhibit stimulus generalisation to other stimuli;
7. Presentation of another (usually strong) stimulus results in recovery of the habituated response (dishabituation);
8. Upon repeated application of the dishabituatory stimulus, the amount of dishabituation produced habituates.

The authors proposed that these characteristics combined may serve as an operational definition of habituation. However, it is as yet unclear whether or in what way the nine criteria for habituation were met here.

Results from previous studies provide corroborating evidence for the hypothesis that prenatal exposure to atypical cues may affect habituation processes. For example, Fride et al. (1986) found that in rats, prenatal exposure to unpredictable stress was related to a decrease in habituation to stressful stimuli. Also, a few studies with humans have presented data on the association between prenatal exposure to stressors and changed habituation in the offspring. As described in Chapter 6, foetuses of depressed pregnant women displayed delayed habituation and recovery to a vibroacoustic stimulus (Allister et al., 2001). In addition, Streissguth et al. (1983) demonstrated reduced or no habituation to auditory and visual stimuli 27 hours after birth in infants prenatally exposed to alcohol. Comparable results were obtained in cocaine-exposed neonates in a study by Potter et al. (2000),who showed impaired habituation and recovery to an auditory habituation-dishabituation paradigm.

Studies with clinical populations have also found evidence for alterations in habituation. Perry et al. (2007) compared a group of adults diagnosed with autistic disorder with a group of typically developing adults. They found that the adults with autism needed more trials before they habituated to a startle response.

Guiraud et al. (2011) tested 9-month-old infants at risk for autism (i.e. with an older sibling with autism) with an auditory oddball paradigm. Compared to low-risk infants, the high-risk infants showed less habituation to standard sounds and a reduced sensitivity to changes in frequency. A large body of research with schizophrenic patients provides evidence that this population, too, shows impaired habitation in response to repeated presentations of startling stimuli (e.g. Geyer et al., 1990; Ludewig et al., 2003; Takahashi et al., 2008), although some studies did not find impaired habituation (Quednow et al., 2008).

What these clinical populations have in common with the studies presented here is that researchers have hypothesised the genesis of schizophrenia and autism to be related to atypical influences (e.g. viral infections) during critical windows of foetal brain development (e.g. Beversdorf et al., 2005; Brown and Derkits, 2010; King, 2011; Kinney et al., 2008; Mednick et al., 1988; Susser et al., 1996). Thus, as mentioned earlier in this dissertation, the importance of sensitive periods during development in the womb is stressed.

Less obvious is what causes the presumed changes in habituation. Possibly, prenatal exposure to higher levels of maternal anxiety leads to higher offspring anxiety, and arousability or vigilance levels (see e.g. Kim et al., 2011; Van den Bergh and Marcoen, 2004), such that the brain responds to every stimulus that is being presented. A related possibility is that the brain is unable to modulate the repeated intrusion of irrelevant stimuli (see e.g. Massa and O'Desky, 2012; Weikum et al., 2012) and therefore does not habituate to repeated stimulus presentation. Alternatively, or perhaps at the same time, memory traces (see e.g. Winkler, 2007) of preceding stimuli might not be 'strong' enough. That is, perhaps the brain cannot remember well enough that certain stimuli have already been presented. In the same vein, maybe habituation processes are altered because of an impairment in the brain for comparing and matching identical stimuli and concluding that the one stimulus is exactly the same as the other (see e.g. Baldeweg, 2006). Before turning to structures and mechanisms potentially involved in the hypothesised alterations in habituation, in the next paragraph we will take a closer look at sensitive periods, in combination with individual differences.

8.3 Sensitive periods and individual differences

8.3.1 Sensitive periods

As our first assessment of the pregnant women took place between the 9th and 15th week of pregnancy (13.55 weeks GA on average), we could examine maternal psychological status during early pregnancy in relation to infant cognitive functioning. Although several previous studies reported that stress and anxiety during early-to-mid rather than late pregnancy were more strongly related to altered infant neurocognitive outcome (e.g. Davis and Sandman, 2010; Mennes et al., 2009; Rodriguez and Bohlin, 2005; Van den Bergh et al., 2006), others found effects associated with the later stages of pregnancy (e.g. Brouwers et al., 2001; O'Connor

et al., 2002a). These different findings show that there is still no consensus about what the sensitive periods are during which development of brain structures and networks are most likely to be affected by prenatal exposure to maternal anxiety. This is actually not surprising, since different structures and networks in the brain follow a different developmental trajectory. Thus, prenatal exposure to maternal anxiety may have differential outcomes depending on which structures/networks were developing at the time of exposure and in what stage of development they were.

Coordinated by large numbers of interacting genes (Van den Bergh, 2011a), specific areas of the central nervous system begin to form with the neurogenesis and migration of cells in the forebrain, midbrain, and hindbrain. More caudal structures, such as the pons and medulla (hindbrain), generally start developing earlier than more rostral structures, such as the neocortex and hippocampal formation (forebrain) (Rice and Barone Jr, 2000). Based on rat studies, Rice and Barone Jr (2000) estimated at what time during human foetal development neurogenesis occurs in the major brain structures, i.e. when the neurons are born that will eventually form those structures.

According to their estimates, during the first half of the first pregnancy trimester neurons are born in the spinal cord, medulla, pons and pallidum. Later, but still in the first trimester, neurogenesis takes place in tegmentum, thalamus and hypothalamus, and still somewhat later in the piriform and entorhinal cortex, and the subiculum. In the cerebellum, tectum, striatum, amygdala, neocortex, limbic cortex, and hippocampal areas CA1 to CA3 and dentate gyrus neurogenesis starts in the first trimester, but lasts into the next trimester(s); genesis of neurons in the cerebellum and the dentate gyrus extends even into the postpartum period. The authors stated that the developing brain structures are especially vulnerable to (toxic) external agents during the phase of active proliferation, adding that when proliferation is disrupted, migration and differentiation are often also altered. That is precisely why authors such as Meyer et al. (2007) have reasoned that atypical influences or cues during early rather than late gestation may be especially harmful: early in gestation atypical cues may not only interfere with cell proliferation and differentiation, but may also predispose the developing nervous system to alterations in subsequent cell migration, target selection, and synapse maturation, leading to a cascade of (subtle) alterations in brain and behaviour.

8.3.2 Individual differences

Important to note is the fact that although the different organ systems follow a developmental trajectory which is roughly the same for all humans (Rice and Barone Jr, 2000; Sesma and Georgieff, 2003), there are important individual differences. Developmental plasticity, that is, the degree to which an individual is malleable or susceptible to environmental reprogramming influences, may differ from person to person. Consequently, not all individuals in utero exposed to the same atypical environmental cues have the same outcome. This is possibly related to, or caused by, differences in genetic makeup, and/or the interplay between genes

and the (micro-)environment that may differ per individual (Belsky and Pluess, 2009a,b; Meaney, 2010).

As an example, gene-environment interaction studies ('GxE' studies) have found that the polymorphism in the promoter gene encoding for the serotonin transporter (i.e. long versus short 5-HTTP allele) moderates the effect of atypical influences during early development on offspring outcome (Barry et al., 2008; Caspi et al., 2003). With respect to anxiety, Pluess et al. (2011) demonstrated that whether or not infants displayed negative emotionality after prenatal exposure to maternal anxiety depended on which polymorphism of the 5-HTTP allele they were carrying. However, the finding by Pluess et al. (2011) has not (yet) been replicated, so additional studies are necessary to confirm the results.

From an evolutionary perspective, it makes sense that there are individual differences in susceptibility to atypical environmental cues, as variation within a group increases the chance that the group will survive (Glover, 2011). In this context, different concepts and theories on individual differences have been put forward. As an example, both Belsky's 'differential susceptibility' (Belsky, 1997a,b, 2005; Belsky and Pluess, 2009a) and Boyce and Ellis' 'biological sensitivity to context' (Boyce and Ellis, 2005) predict that some individuals are more susceptible than others to both adverse *and* beneficial environments. This means that, for instance, those individuals who are especially vulnerable to adversity may also be those who especially benefit from positive experiences (Belsky and Pluess, 2009b; Van den Bergh, 2011a).

Along the same lines, Nederhof and Schmidt (2012) made an effort to integrate two hypotheses on early life adversity and later life outcomes - the "cumulative stress hypothesis" (see e.g. Monroe and Simons, 1991) and the "mismatch hypothesis" (see e.g. Frankenhuis and Del Giudice, 2012; Schmidt, 2011) - by taking into account individual differences in sensitivity to programming. The cumulative stress hypothesis postulates that atypical or aversive experiences early in life predispose individuals to be more vulnerable to aversive challenges later in life. The mismatch hypothesis, on the other hand, states that atypical or aversive cues early in life may reprogramme an individual so that he is better able to deal with aversive environments later in life. If the later environment differs from the early environment, however, a mismatch occurs, which may predispose an individual to less optimal outcomes[1].

With these two hypotheses as outset, Nederhof and Schmidt (2012) combined the factors 'heritable variation', 'developmental experience' and 'the timing of experience' and proposed that this combination determines the size of programming effects in each individual. Thus, based on how susceptible someone is to reprogramming effects in response to atypical environmental cues, some individuals are

[1] A good example illustrating the importance of a match between the early and later environments comes from the Bicylus butterfly, which during development as a larva can adapt to the environment it is expected to hatch in as a butterfly. When a larva develops in 18^{o}C, the resulting butterfly will be able to survive a long, dry season; when it develops in 25^{o}C, the butterfly is equipped to live in a warm and wet environment. If a mismatch occurs between the expected and the actual environmental circumstances, chances for survival decrease dramatically (Brakefield et al., 1996; Nederhof and Schmidt, 2012).

at greater risk for adverse outcomes when early life stress and stress later in life accumulate (accumulative stress hypothesis), while others are at greater risk if a mismatch occurs between their early and later environments (mismatch hypothesis).

Key observations from this and the former section are that in the population described in this dissertation prenatal exposure to maternal anxiety has likely had differential effects on infant outcome, related to when during pregnancy maternal anxiety was experienced, in combination with the infant's (genetic) susceptibility to atypical environmental cues. In section 8.6.1 we will come back to the concept of individual differences when discussing directions for future research. In the next section, we turn to mechanisms potentially underlying the effects observed in the studies presented here, after exploring which brain structures in our population may have been affected by prenatal exposure to anxiety.

8.4 Structures involved and underlying mechanisms

8.4.1 Structures involved

The results described in the core chapters of this dissertation point to potential reprogramming of auditory information processing related to memory for 'regularity representations' (Winkler, 2007; Chapters 5 and 6) and to vocal emotional processing (Chapter 7), which we proposed to be related to alterations in habituation. As such, and taking into account the period of pregnancy we studied ($\pm$ 9-15 weeks GA), one might speculate that in our population, prenatal exposure to anxiety may have affected the development of structures such as the primary auditory cortex (processing of incoming sounds), the hippocampus (memory for regularity representations), and amygdala (memory and processing of emotional sounds), and/or connections and networks to and from these structures.

This speculation is corroborated by a large body of animal research. For instance, studies with rats and monkeys have shown that exposure to high levels of prenatal stress is associated with alterations in the hippocampus, such as significant decreases in overall hippocampal volume (Coe et al., 2003; Szuran et al., 1994), decreases in cell proliferation in the hippocampal dentate gyrus (Lemaire et al., 2000, 2006), and alterations in spine density in the hippocampal stratum radiatum, CA1 and CA3 regions (Ishiwata et al., 2005; Martínez-Téllez et al., 2009). Also, results from rat studies suggest that exposure to high levels of stress during foetal development may result in permanent alterations in neuronal activity in the amygdala (for a review, see e.g. Weinstock, 2008). Evidence for effects of exposure to prenatal stress and anxiety on the primary auditory cortex is less readily available, although Koenig et al. (2005) described that rats stressed during gestation showed disruptions in auditory sensory gating (i.e. filtering out redundant or unnecessary auditory stimuli in the brain; see also Cromwell et al., 2008). In addition, quite recently Hunter et al. (2012) found similar results in human infants. P50 auditory sensory gating was recorded in infants (mean age 76 days) from mothers with

an anxiety disorder during pregnancy (retrospectively diagnosed), and from mothers who used antidepressants during pregnancy. The authors found that infants from anxious mothers who had not used antidepressants displayed less habituation during the auditory gating task. In infants whose anxious mothers had used antidepressants this performance deficit was attenuated.

8.4.2 Underlying mechanisms

The above gives a possible answer to the question which brain structures related to habituation processes might be affected by prenatal exposure to maternal stress and anxiety. A remaining question is in what way these structures could have been affected in the studied group. Since there are no neural connections between the mother and her foetus, the effects must be mediated in another way, such as through alterations in placental blood flow and transient hypoxia (i.e. increased uterine artery resistance, leading to reduced blood flow to the foetus) and/or stress hormones (Monk et al., 2011; Mulder et al., 2002; Weinstock, 2008).

8.4.2.1 Cortisol and the HPA-axis

Within the DoHAD field, a stress hormone often suggested to be involved in the context of prenatal exposure to stress and anxiety and offspring outcome is the glucocorticoid cortisol. Experiencing both stress and anxiety have been associated with increased levels of cortisol (e.g. see Dickerson and Kemeny, 2004; Kirschbaum et al., 1993; Mancuso et al., 2004; Pruessner et al., 1999; Sarkar et al., 2008), of which the release in the human body is mediated by the hypothalamic-pituitary-adrenal (HPA-) axis. During pregnancy, cortisol is essential for foetal development, as it, for instance, promotes lung maturation and extra-uterine lung function. Excess levels, however, have been found to adversely affect the foetus (for reviews see e.g. Harris and Seckl, 2011; Weinstock, 2008), possibly in the manner described below.

Maternal anxiety during pregnancy activates the HPA-axis, increasing maternal levels of circulating corticotropin-releasing hormone (CRH) and cortisol. This, then, up-regulates placental CRH production and release into the foetal bloodstream, leading to higher cortisol concentrations in the foetal plasma. Importantly, whereas hypothalamic CRH production is suppressed by anxiety-induced cortisol through a negative feedback loop, placental CRH is increased by cortisol. This results in a positive feedback loop, as placental CRH can reach the pituitary gland and stimulate the production of adrenocorticotrophin hormone, and in turn, cortisol (Gangestad et al., 2012; Monk et al., 2011; Mulder et al., 2002; O'Donnell et al., 2012). In this way, prenatal exposure to anxiety results in progressively higher CRH levels in the foetal plasma (Charil et al., 2010). Upon reaching the foetal brain, placental CRH could influence neuronal differentiation and function(ing) at different stages of development by affecting areas rich in CRH receptors, such as para-hippocampal and limbic areas (e.g. hippocampus, amygdala; Charil et al., 2010; Weinstock, 2008), possibly impairing memory functions such as the memory

for regularity representations related to repeated presentation of auditory (possibly emotional) stimuli.

8.4.2.2 Cortisol and placental 11β-HSD2

A drawback of the mechanism described above is that several researchers reported only rather modest associations between a mother's mood during pregnancy and her cortisol level (Davis and Sandman, 2010; Obel et al., 2005; Sarkar et al., 2006 and see Glover et al., 2010 and O'Donnell et al., 2012 for reviews), so it is likely that other mechanisms are (also) at work. The placenta has been suggested to play a central role in the relation between prenatal exposure to anxiety and infant (neurocognitive) outcome, as it regulates the exchange of e.g. nutrients, hormones and waste product between mother and child. An important placental enzyme in this context is 11β-HSD2. A function of the enzyme is to convert cortisol into its inactive metabolite cortisone, protecting the foetus from excess exposure to the glucocorticoid. Inhibition of 11β-HSD2 has been demonstrated to contribute to low birth weight and intra-uterine growth restriction (e.g. see Causevic and Mohaupt, 2007; McTernan et al., 2001; Shams et al., 1998; Wächter et al., 2009), and also to dose-related impairments in cognitive and behavioural development (Räikkönen et al., 2009)[2].

Maternal stress and anxiety during pregnancy have been found to down-regulate placental 11β-HSD2 activity, resulting in increased exposure of the placenta and foetus to cortisol (Harris and Seckl, 2011; O'Donnell et al., 2012; Wyrwoll et al., 2011). Even in the absence of increases in maternal cortisol levels during pregnancy, or lack of evidence for a correlation between cortisol and maternal anxiety, this mechanism could explain how prenatal exposure to anxiety might lead to increased fetal exposure to cortisol (O'Donnell et al., 2012). Exposure to cortisol has been associated with alterations in synaptogenesis, neurotransmitter function, and glucocorticoid receptor expression in the developing brain (Monk et al., 2011), which might eventually lead to altered infant neurocognitive outcome.

8.4.2.3 Neurotransmitters

On a neurochemical level, several neurotransmitters have been implicated in the association between prenatal exposure to maternal anxiety on offspring outcome in general, and, in some cases, on altered habituation processing in particular. For example, as discussed in Chapter 6, Schneider et al. (2008) and Roberts et al. (2004) reported decreased habituation to repetitive tactile stimuli and increased D2 (dopamine) binding in the striatum in prenatally stressed rhesus monkeys. Roy et al. (2007) suggested that alterations in information flow due to defective myelin may have changed levels of available dopamine. Defective myelin could cause abberations in auditory sensitivity and result in prolonged neural transmission along

[2]The paper by Räikkönen et al. (2009) provided indirect evidence, by relating maternal liquorice consumption during pregnancy to child outcome. Liquorice contains glycyrrhiza, which inhibits human placental 11β-HSD2.

central and peripheral auditory pathways (Church et al., 2012), possibly resulting in the decreases in habituation observed in the studies presented here.

In addition, Field et al. (2003) found higher levels of norepinephrine and lower levels of dopamine as measured from urine samples in highly anxious compared to less anxious pregnant women, and subsequently in their neonates after birth. Norepinephrine is a vasoconstrictor, and Teixeira et al. (1999) reported an association between maternal anxiety and increased uterine artery resistance, leading to reduced blood flow to the foetus, although other studies did not find this association (for a review, see Monk et al., 2011).

Also serotonin is thought to be involved, which during development functions as a trophic factor (Monk et al., 2011), affecting critical developmental processes such as cell migration, neuronal division, and synaptogenesis (Monk et al., 2011). Atypical levels of serotonin (5-HT) or the serotonin transporter (5-HTTP) related to maternal anxiety-like symptoms during pregnancy, may alter these processes (Ansorge et al., 2004; Monk et al., 2011; Morrison et al., 2002). A study by Simpson et al. (2011) with rats found that perinatal changes in 5-HT levels, by administration of citalopram (a selective serotonin reuptake inhibitor; SSRI), altered tonotopic organisation and receptive field properties in primary auditory cortex. Similarly, changes in 5-HT levels could have affected tonotopic organisation in the infants described in this dissertation, and through aberrant auditory functioning could have led to alterations in habituation to auditory stimuli.

Moreover, modulation in 5-HT levels has been found to affect the balance in local excitatory-inhibitory circuitry (Levitt, 2003; Stanwood and Levitt, 2004; Weikum et al., 2012). Thus, maternal anxiety-induced changes in 5-HT levels (Ansorge et al., 2004; Monk et al., 2011; Morrison et al., 2002) may have influenced excitatory-inhibitory circuitry in the auditory cortex. This would likely result in altered neurocognitive outcome and may relate to our suggestion in section 8.2 that in infants prenatally exposed to high levels of maternal anxiety the brain has difficulties with modulating the repeated intrusion of irrelevant stimuli, and therefore does not habituate to repeated stimulus presentation.

8.4.2.4 Epigenetic changes

Another potential mechanism underlying the findings described in this dissertation may come from epigenetic changes in gene expression (i.e. potentially heritable changes without genetic modifications in the DNA itself) resulting from prenatal exposure to maternal anxiety - possibly in conjunction with altered levels of (placental) CRH, and 11β-HSD2 and neurotransmitter activity. Increases or decreases in DNA methylation associated with gene silencing or expression, respectively, may be sufficient to cause heritable, phenotypic changes (Van den Bergh, 2011a). In other words, prenatal exposure to maternal anxiety might alter the typical pattern of gene expression, in that commonly expressed genes remain un-expressed or are silenced, while genes that should be silenced become expressed.

That experiences very early in life can reprogram gene expression, is supported by the much-cited findings from Francis et al. (1999) and Weaver et al. (2004).

They showed that in rats increased maternal care (e.g. licking and grooming and arch-back nursing) was associated with epigenetic changes in DNA methylation in the rat pups.

McGowan et al. (2009) reported similar results on differences in the quality of parental care and epigenetic changes in humans. In an extraordinary postmortem study with suicide victims, they found that, compared to 'control' victims, victims with a history of childhood abuse showed epigenetic differences in a neuron-specific glucocorticoid receptor. In a more recent human study from colleagues investigating PELS hypotheses in a Belgium population, Hompes et al. (2013) reported associations between prospectively measured maternal emotional status during pregnancy and the methylation state of the human glucocorticoid receptor (NR3C1 gene) in the infant (DNA methylation was analysed in genomic DNA from cord blood). More specifically, the authors demonstrated that maternal anxiety during the first and second trimester has an influence on the methylation state of important sites of exon 1F NR3C1 promoter[3], resulting in decreased expression of NR3C1. Hompes et al. (2013) remarked that exon 1F is highly expressed in the human hippocampus, and that they therefore expect this decrease to lead to a specific reduction of NR3C1 in hippocampal sites. This reduction may be related to impaired memory (for regularity representations), and, in turn, may result in decreases in habituation to repeated auditory stimulation.

8.4.2.5 Heritability

A complementary mechanism is that the associations observed in this dissertation can be accounted for by genetically inherited pathways, in that genetically heritable factors may influence both a maternal predisposition to experiencing anxiety, and to offspring outcomes (Rice et al., 2010). Indeed, between 30 to 40% of the variance in the occurrence of anxiety disorders can be explained by genetic variation (Leonardo and Hen, 2006). Also, in a study with primary school-aged and adolescent twins heritability of trait anxiety was found to be approximately 45% (Legrand et al., 1999)[4]. As no genetic material has been analysed in the studies presented in this dissertation, no direct evidence can be given for this alternative - or complementary - explanation of the results. However, if alterations in habituation are a 'symptom' of a tendency to respond with state anxiety, one can infer that, just like their infants, mothers in the PELS study with higher levels of state anxiety will also show decreased habituation in response to repeated stimulus presentation. A follow-up study investigating both mother and child can be envisioned to test this hypothesis.

[3] Oberlander et al. (2008) showed that methylation of the NR3C1 gene 1F promoter in DNA from human cord blood is related to prenatal exposure to maternal emotional stress.

[4] Note that we did not use trait, but state anxiety in the studies described in this dissertation. However, the correlation between state and trait anxiety was relatively high in the PELS population (i.e. between .58 and .81 for the different measurement points, with an average of .74). In addition, it is perceivable that, just like anxiety disorders, a proneness to state anxiety is also heritable.

8.4.3 Summary of underlying mechanism

In summary, several co-occurring and partly interdependent mechanisms may mediate the relationship between prenatal exposure to maternal anxiety and decreased habituation to repeated auditory stimuli presentation. Examination of the literature suggests that, likely among other mechanisms, altered myelinisation, shifts in excitatory−inhibitory circuits, alterations in memory function, and altered tonotopic organisation may play a central role. These mechanisms, in concert with individual differences and genetic heritability, are hypothesised to underlie the differential processing of familiar (emotional) sounds observed in infants exposed to high levels of maternal anxiety during development in the womb.

8.5 Strengths and limitations

The biggest strength of the PELS study is its prospective, longitudinal design. That is, maternal stress and anxiety were measured from the first trimester of pregnancy onwards, as well as during the second and third trimester. Two measurements after pregnancy allowed for data collection of postpartum emotional status, and outcome of the infants at two different ages. This design enabled us to both link anxiety levels during pregnancy to infant neurocognitive outcome, and to do this while controlling for anxiety after pregnancy. In addition, we were able to control for other potential confounders, such as maternal smoking and alcohol use during pregnancy, gestational age (GA), and birth weight controlled for GA. That our effects remained intact after taking the potential effects of these confounders into account, suggests that our findings are quite robust.

Another strength of the studies described here is that instead of using behavioural measures and inferring associations, we were able to use ERPs as a direct measure of infant neurocognitive functioning. The groups of infants we were able to study with ERPs in the different chapters described here were relatively large compared to other infant ERP studies. This likely increased power and reliability of the results. Also, because the results are based on a large data set, our results can be potentially used as normative information for studying children from a normal (i.e. non-clinical) population.

It is important to note that whereas ERPs have a high temporal resolution, the spatial resolution is quite poor. That is, localising where in the infant brain an ERP component has been generated is very complex and represents a number of problems (see e.g. Luck, 2005 for an elaboration). Therefore, at the current time, we are unable to make firm claims about which structures and networks have generated the ERPs elicited by the stimuli presented in the studies reported in the dissertation. Using methods such as near infra red spectroscopy (NIRS) or functional magnetic resonance imaging (fMRI) in combination with ERPs could yield answers to this question.

An additional strength of our study is that instead of dividing our sample in groups of infants exposed to high versus low levels of anxiety, anxiety was in-

cluded as a continuous predictor in our analysis. In this way, several (statistical) problems could be avoided. First, since approximately 19.6% of the general Dutch population suffers from an anxiety disorder during their lifetime (Graaf et al., 2010), drawing a random selection of subjects from the population is likely to result in sample containing relatively few anxious subjects. This was also true for our study: at T1 16.5% of our sample - or 29 women - reported high levels of state anxiety (i.e. sum score of 42 or higher). Dividing our sample into a group of infants prenatally exposed to high versus low levels of maternal anxiety would have resulted in unequal group sizes, potentially confounding the results. Second, dichotomisation of the predictor variable would have resulted in loss of information about individual differences in the original distribution; loss of effect size and power; the potential to overlook nonlinear relationships; and loss of measurement reliability, as demonstrated by MacCallum et al. (2002). Avoiding the problems mentioned in this paragraph has yielded more reliable and robust results, with larger effect sizes and power.

A limitation of the studies presented in this dissertation is that we did not administer paradigms specifically designed to test habituation. That is, we did not study whether the infants' ERP responses habituated to repeated stimulus presentation and recovered after the stimulus had been withheld for some time. Thus, our proposal that there is a negative association between prenatal exposure to maternal anxiety and the amount to which habituation to repeated stimulus presentation takes place constitutes a working hypothesis that should be further tested in future research. In section 8.6.4.1 an example is given of how habituation processes could be investigated.

An additional limitation is that of weak external validity; i.e. the mothers in our study had a higher educational level than the general Dutch population: in 2012 28% of the Dutch women were highly educated (i.e. higher vocational training or university degree) compared to 64% in our sample (CBS, 2012). Results, therefore, are not representative for the whole Dutch population. Nevertheless, the results we found do appear to be robust for the sample studied, so they are likely at least representative for young families with an above-average education.

Another drawback is that no genetic material has been analysed. In relation to this, the mothers included in the different studies have not been tested with the ERP paradigms described in Chapter 3 and 4. Also, the anxiety level of the infants was not taken into account. Combined, this makes it difficult to make inferences about the extent to which genes and heritability could have influenced the results presented here. In the next paragraph an example is given of how some of these factors could be taken into account in future research.

8.6 Future directions

8.6.1 Long-term sequelae

Because of time limitations, for the current dissertation we were unable to follow up the PELS infants beyond 9-11 months of age. Therefore, it is as yet uncertain whether the results described here remain stable over time. Also, due to their tender age the infants had not yet reached important developmental milestones, such as talking. This prevented us from testing whether in our population the differences in auditory information processing are indeed related to, for instance, language performance, as was predicted in Chapter 5. Therefore, an important future direction is following up the infants into later ages. A number of questions could then be addressed (more thoroughly), such as whether the initial differences in the developmental trajectory of the infants of high and low anxious pregnant mothers remain stable over time or whether they change, and if yes, how; how the initial differences would be associated with later development; whether and how the differences evoke different interaction patterns with significant others and within different learning contexts; how they might be related to changes in brain networks on the one hand, and later cognitive, emotional and behavioral development on the other hand; and how individual differences relate to each of these questions. A few examples of follow-up research are explored below.

The PELS study has been partly based on the study by Mennes (2008; which had been started by Van den Bergh, 1989), who related prenatal exposure to maternal anxiety to cognitive control in the adolescent offspring, both by means of neuropsychological (e.g. go/no go task) and neurophysiological (ERP, fMRI) measures. Mennes (2008, p.183) concluded that prenatal exposure to maternal anxiety is related to endogenous cognitive control (see Chapter 1) and the physiological brain processes associated with this cognitive function. He suggested that these findings could be related to the prefrontal cortex and its associated circuitry (see also Mennes, 2008; Mennes et al., 2006, 2009). Thus far, 'our' PELS infants have not been tested with paradigms specifically designed to measure prefrontal cortex functioning. To be able to compare (ERP) results across both Mennes' and our cohort, follow-up research could focus on prefrontal cortex functioning in the PELS cohort, and link this to our hypothesis that prenatal exposure to maternal anxiety is negatively associated with habituation. According to Massa and O'Desky (2012), the frontal cortex is necessary for habituation of attention to objects in the peripheral environment that are not targeted for action. This suggests that in a follow-up study of the PELS cohort a relation between prenatal exposure to maternal anxiety, altered habituation and involvement of the prefrontal cortex may be expected.

Another conclusion that Mennes (2008, p. 180) drew, was that the effects he described showed important similarities with core symptoms of attention deficit-hyperactivity disorder (ADHD), such as impulsivity. A follow-up study could investigate whether in the PELS cohort, too, an association can be found between prenatal exposure to maternal anxiety and ADHD-like symptoms, and how this relates to our finding that prenatal exposure to anxiety affects habituation processes.

While some authors found associations between ADHD or ADHD-like symptoms and decreased habituation, both in human studies and in rat models of ADHD (e.g. Jansiewicz et al., 2004; Zhuang et al., 2001) other authors did not (e.g. Iaboni et al., 1997; Lubar et al., 1995). A follow-up study may shed more light on whether or not an association exists between ADHD(-like symptoms) associated with prenatal exposure to maternal anxiety on the one hand, and altered habituation on the other, and how that, in turn, relates to individual differences in developmental trajectory.

8.6.2 Individual differences and GxE studies

In section 8.3.2 we mentioned that susceptibility to reprogramming may differ from person to person (Belsky, 1997a,b, 2005; Belsky and Pluess, 2009a; Boyce and Ellis, 2005) and also depend on (differences between) the prenatal and postpartum environment (Frankenhuis and Del Giudice, 2012; Monroe and Simons, 1991; Nederhof and Schmidt, 2012; Schmidt, 2011). Therefore, investigating individual differences in relation to an individual's (changing) environment is a venue worth exploring in future research. As an example, through PELS data collected during pregnancy, an assessment could be made of the prenatal context the infant developed in, in terms of maternal mood (complaints), pregnancy-related risk factors (e.g. high maternal body mass index (BMI) and/or blood pressure), and socioeconomic status. Such assessments could also be made for postpartum measurements T4 and T5, and again in a follow-up study at a later time, during which DNA samples could be analysed from both the child and its parents. Longitudinal data analysis might then characterise whether and how the infant's/child's environment has changed over time from the prenatal period onwards. This information could be related to the infant/child neurocognitive outcome, to his/her genetic makeup (e.g. by replicating studies such as those by Pluess et al., 2011) and that of the parents (by 'comparing' DNA from parent and child), and to epigenetic changes (e.g. by replicating studies such as those by Hompes et al., 2013).

Since for humans still relatively little is known about what constitutes high susceptibility to reprogramming, it is likely that a study like the one proposed above would be of an exploratory nature and pose more than a few challenges. Perhaps, when more is known about the epigenetic and genetic mechanisms related to susceptibility to reprogramming, it will become easier to design studies such as the one described here. Until that time, experimental work with animals in which (genetically) different strains/lines can be selected and compared, may help to gain insights into associations between individual differences and individuals' environments.

8.6.3 Measuring maternal anxiety: STAI versus SCL

In this dissertation, maternal anxiety was measured during and after pregnancy with the state anxiety subscale of the STAI and the anxiety subscale of the SCL. Both subscales measure (relatively) current state of anxiety, although the STAI subscale enquires about what is experienced at the moment the questionnaire is

filled out, and the SCL subscale what has been experienced within the last 4 weeks. Another difference is that whereas the STAI subscale measures transitory anxiety in response to real life stress, the SCL subscale measures more generalised anxiety, including bodily, cognitive, and specific symptoms (see Chapter 2).

That the subscales measure a partly overlapping, yet distinct anxiety construct is reflected in the correlation between the scales' sum scores. In the total PELS population correlations were approximately between .45 and .60 (average .55) for the different measurement points[5], indicating low to moderate correlations. Moreover, differences in the association with infant ERP data were found for both subscales. Chapter 5 and 6 showed that maternal anxiety as measured with the STAI affects, both in 2- and 9-month-old infants, the processing of sounds of low information contents embedded in a rapid sound sequences. Data presented in Chapter 7 suggest that this measure of anxiety does not affect the processing of familiar emotional sounds within an audiovisual context. In contrast, maternal anxiety as measured with the SCL was associated with increased attention for the familiar emotional sounds, as evidenced by a negative shift in the auditory ERPs. Taken together, these results suggest that the STAI and SCL are related to different outcomes of infant neurocognition. However, this suggestion warrants careful further investigation and as such might form a topic for follow up research.

At the same time, the above suggestion raises the question whether bodily felt anxiety symptoms (such as trembling and heart pounding) act via a different etiological pathway than more 'cognitive' items (such as worrying and fearful thoughts). Since associations have been found between both anxiety and mindfulness during pregnancy on the one hand and heart rate variability on the other (Braeken et al., 2012, 2011), autonomic nervous system (ANS) functioning is likely to play a role, too, in the relation between prenatal exposure to anxiety and infant outcome. Thus, another interesting line of future research would be to explore this relation further and include measures of the ANS in DoHAD-oriented research.

8.6.4 Mismatch response

Chapter 3 of this dissertation described an auditory oddball paradigm with standard and deviant sounds designed to elicit the Mismatch Response (MMR), the infant analogue of the adult Mismatch Negativity (MMN[6]). The MMR can be assessed from the deviant-minus-standard difference waveforms - for more accurate control procedures, see Kujala et al. (2007). In Chapter 5 and 6 the same oddball paradigm was used as that in Chapter 3, but instead of examining the MMR, by choice, the original (unsubtracted) responses to the auditory stimuli were investigated.

Originally, the intention had been to investigate the MMR in the context of prenatal exposure to maternal anxiety. Several previous studies with children had

[5]Note that the N differed per measurement point and questionnaire, for instance, at T4 114 mothers filled out the STAI and 112 the SCL.

[6]Both MMR and MMN are not to be confused with the mismatch hypothesis described in sections 8.3.2 and 8.6.2.

found the MMN to be sensitive to atypical development, as evidenced, for example, by the finding that MMNs of smaller amplitude are elicited in distractible children (Cheour, 2007). Therefore, we had hypothesised that if prenatal exposure to maternal anxiety were associated with altered processing of auditory stimuli, this would manifest in changes in the MMR. However, when analysing the data, ERPs in response to the standard sound turned out to be significantly associated with the level of anxiety the mother had experienced during pregnancy. In other words, higher levels of anxiety were associated with larger response amplitudes to the standard. We deemed it inappropriate to create and analyse difference waves when the responses to the standard differed with different levels of anxiety, and we therefore opted to work with the original responses. Future studies could direct an effort into investigating if and how MMR/MMN would be affected when the response to the standard sound in an oddball paradigm depends on the predictor variable. This could be achieved by employing an assessment of the MMR/MMN that does not rely on subtracting between responses elicited by different sounds presented with different overall probabilities (see below).

8.6.4.1 MMN/MMR and habituation

Taking the above into account, MMN/MMR paradigms could be employed to further explore the nature of the findings described in this dissertation. For instance, a paradigm specifically designed to measure habituation could consist of the consecutive presentation of structured sequences of tones (stimulus trains) of different frequencies, with each stimulus train containing the same frequency tones only (for example: AAAAAABBBBBBCCCCCC...; where 'A', 'B', and 'C' represent tones of different frequencies). In this way, the first tone of a train is always a deviant, which eventually becomes a standard within that train through repeated presentation. A decrease in MMN/MMR amplitude from the first difference (response to first sound minus second sound) to the last difference (response to first sound minus last sound) within a train would indicate habituation has taken place. If our hypothesis about the negative association between prenatal exposure to maternal anxiety and habituation holds, we would expect that higher levels of maternal anxiety are related to less habituation over repeated stimulus presentation in the offspring.

8.6.4.2 MMN/MMR and ADHD(-like) symptoms

The detection of a mismatch between a standard and a deviant appears to trigger an involuntary shift of attention (passive attention), and therefore can be regarded as an index for pre-attentive mechanisms (Cheour, 2007). As such, the MMN/MMR could also be used to investigate ADHD(-like) symptoms, like impulsivity and distractibility, which have been related to prenatal exposure to maternal anxiety (see section 8.6.1). For example, in children with ADHD, smaller amplitude MMNs have been reported (reviewed in Cheour, 2007; see also e.g. Kemner et al., 1998; Rothenberger et al., 2000; Sawada et al., 2008), and some evidence was found

for augmentation of the MMN by administration of methylphenidate (a medicine given to enhance mental focus in ADHD patients). In future studies with the PELS cohort, ADHD-like symptoms related to prenatal anxiety exposure could be tested by for instance relating performance on some relevant behavioural tasks to the MMN collected through a simultaneously presented oddball paradigm. We may expect the performance of children with more ADHD-like symptoms to be more affected by intrusion of distracting deviants in the oddball paradigm.

8.7 Clinical implications

The associations we found between prenatal exposure to maternal anxiety and infant neurocognitive outcome appear to be quite robust, and therefore warrant attention from professionals working with pregnant women (e.g. midwives, gynaecologists, nursery nurses, etc; here referred to as 'clinicians'). Importantly, care should be taken that not only negative consequences of prenatal exposure to maternal anxiety are highlighted. Indeed, there often are two sides to the same coin[7] - as is nicely illustrated by Figure 8.1, presenting the positive characteristics of what Schut (2013) refers to as "creative scatterbrains"[8]. Thus, we argue that positive consequences, such as those depicted in 8.1, and the importance of supporting, sensitive parenting that may modify the negative effects (see e.g. Kaplan et al., 2008) should also be addressed, in an effort to avoid creating feelings of guilt in pregnant women who experience anxiety and/or stress despite being told that this might be bad for the development of their baby.

Taking positive outcomes and factors potentially modifying the negative effects into account, clinicians can create awareness among their clients of the association between maternal mood and child outcome, for example through (preconception) counselling and psycho-education for women in the childbearing age and their partners. That councelling sessions may have beneficial effects, is evidenced by results from a study by Williams et al. (2012), suggesting that preconception counselling is associated with positive maternal behaviors that increase the likelihood of a healthy woman, pregnancy, and infant. In addition to psycho-education, counselling sessions could include advice on what to do if a women experiences mood complaints or starts experiencing them during pregnancy.

To be able to intervene as early as possible, we advise clinicians to screen for anxiety by means of questionnaires, such as the SCL and STAI, during intake as well as during the course of pregnancy and in the postpartum period. As some authors reported effects of pregnancy-related anxiety on infant and child outcome (e.g. Buss et al., 2011; Huizink et al., 2003), questionnaires specifically measuring

[7] A quote famous among the Dutch is Johan Cruijff's "Ieder nadeel heb z'n voordeel" [Every disadvantage has its advantage]".

[8] Schut (2013) dubbed the term "creative scatterbrain" to describe someone who is both creative and chaotic. The creative part of the term can refer to artistic creativity, but also to e.g. thinking out of the box. With the scatterbrain part of the term she means to refers to being absent-minded, having jumbled thoughts, or working on several things at the same time. In Schut's view, individuals who have AD(H)D, dyslexia or autism or who are highly gifted can be called creative scatterbrains.

Positive Characteristics

● Dyslexia	● ADHD	● Highly giftedness	● Autism
- Creative	- Creative	- Creative	- Creative
- Spatial awareness	- Spontaneous	- Intelligent	- Eye for detail
- Technical	- Emphatic	- Good in linguistics	- Honest
- Photographical memory	- Strong intuition	- Sense of humour	- Realistic
- 'Filmic' memory	- Having fun and a sense of humour	- Motivated	- Objective
- Quick reasoning	- Good in finding new solutions	- Set in his/her own ways	- Perfectionist
- Overseeing complex situations quickly	- Strong in crisis situations	- Artistic	- Thinking analytically
- Good in organising	- Never angry for long	- An ear for music	- Being concrete
- Good in drawing	- Energetic	- Knowledgeable	- Living according to rules
- Rich fantasy	- Open	- Good in organising	- Deductive reasoning
- Thinking in images	- Passionate and enthusiastic	- Good in concentrating	- Calculating
	- Honest	- Inventive in finding explanations	- Focused on facts

Figure 8.1: Positive characteristics of "creative scatterbrains"

this construct during pregnancy could also be administered (e.g. the PRAQ; Van den Bergh, 1989). If screening outcomes reveal elevated levels of anxiety, several steps could be taken. Depending on the severity and whether or not there is a clear cause for the complaints, clinicians could for instance advice the client to cut back hours at work and take more rest. In addition or alternatively, clients could benefit from taking relaxation, mindfulness or yoga lessons, since these have been found to beneficially influence (anxious) pregnant women (see e.g. Bastani et al., 2005; Satyapriya et al., 2009; Vieten and Astin, 2008). In case of severe complaints, clients could be referred for psychological or psychiatric support.

Nederhof and Schmidt (2012) offered an additional suggestion for psychologists/psychiatrists, as some of their clients may be more vulnerable to cumulative stressors, and others may be at greater risk for mismatch (see also section 8.3.2). Trying to intervene from a psychopathological perception, Nederhof and Schmidt (2012) explain, might cause a maladaptation from the evolutionary-biological perspective, potentially creating a mismatch between the client and her environment. Thus, the authors advise that when treating (pregnant) clients, individual differences in susceptibility to reprogramming, and past and current environments should be taken into account. This view is supported by a paper from Jensen et al. (1997), who emphasised taking into account an individual's fit(s) with his/her environment(s). They advised psychologists/psychiatrists to help find his/her client (an) environment(s) that "may reduce the adaptive strain on [an individual's] nervous system whose set-point may be at the other pole from the environment in which he or she finds himself or herself" (Jensen et al., 1997, p. 1675).

A final implication for clinical practice, from a somewhat different perspective, can be derived from Chapter 3 and section 8.6.4 of the current chapter. The MMR described in Chapter 3 assessed infants' ability to detect violations of temporal regularities. Potentially this measurement can be used to identify developmental problems or their precursors, such as problems with auditory attention, learning difficulties, and speech and language impairments very early (e.g. see Benasich et al., 2006; Kushnerenko et al., 2013; Molfese and Molfese, 1985, 1997), even before they can be detected behaviourally (Weber et al., 2005). Furthermore, as mentioned in Cheour (2007), the MMR could in the future be used as a diagnostic or prognostic tool for impairments in clinical peadiatric population, such as infants at risk for/with autism, ADHD, and cochlear implants. In the context of (suspected) prenatal exposure to maternal anxiety, the MMR could be used to examine whether prenatal reprogramming has taken place (i.e. whether development appears to take/have taken an atypical course) and whether intervention is desired. More research is needed, however, normative as well as clinical, before the MMR component can be used in clinical settings.

8.8 Concluding remarks

In this dissertation effects of prenatal exposure to maternal anxiety were studied in relation to neurocognitive outcome of infants in their first year of life. We found a positive association between levels of maternal anxiety and processing of familiar, non-informative sounds, pointing to possible changes in processes underlying habituation to repeated stimulus presentation (e.g. defective myelinisation, shifts in excitatory-inhibitory circuits, altered tonotopic organisation, impaired memory function). Follow up research is needed to investigate the exact mechanism(s) underlying the present findings; whether the effects remain stable over time and whether they do so in the same way for different individuals and over different environmental contexts; and whether they are related to emotional, behavioural and

cognitive outcome at later ages. We recommend the use of ERP studies, potentially in combination with NIRS and fMRI measurements, and in-depth study of developmental trajectories to examine these questions.

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Summary, publications and acknowledgements

Summary

Development refers to the continuities and changes that occur as a function of the on-going dialogue between the individual and his environment. During no other period does this process take place at a greater pace than during life in the womb. With the speed of development come sensitive periods during which the foetus is highly susceptible to environmental cues or experiences. In typical development, environmental cues help program the normal developmental trajectory of the foetus. Atypical cues, however, such as maternal under-nutrition, or experiencing of stress and anxiety, potentially result in altered programming - or: reprogramming - of structure and function in cells, tissues, and organ systems. This, in turn, may affect psychophysiological functioning, and increase an individual's vulnerability to regulation problems at behavioural, emotional, and cognitive levels from the foetal period to at least early adulthood. The focus of this dissertation is the neurocognitive outcome of infants who have been exposed to maternal anxiety during their development in the womb.

In the studies presented in this dissertation we used event-related potentials (ERPs) as a direct measure of infant neurocognitive functioning. An ERP is a neural response embedded within the electroencephalogram (EEG; a recording of electrical activity from the scalp) that is associated with specific sensory, cognitive, and/or motor events, and which can be extracted from the continuous EEG by means of averaging. Since studying the development of neurocognitive processes helps to gain insight into the neural bases of human perception, emotion and cognition, recent years have seen an increased interest in the use of ERPs to examine these processes in infants. Despite this, few other studies have been conducted in which ERPs were used to investigate these processes in the context of prenatal exposure to prospectively measured maternal anxiety. Thus, we attempted to fill this gap in the literature by studying neurocognitive functioning in infants prenatally exposed to maternal anxiety by means of ERPs.

The first two chapters of this dissertation described paradigms we used for studying infant neurocognitive functioning. In Chapter 3, an auditory oddball paradigm was discussed with one standard tone ('standard') and three types of deviants: the standard tone presented with a deviant inter-stimulus interval ('ISI-deviant'); white noise segments ('white noise'); and environmental sounds ('novel sound'). The main objective of this study was to investigate whether the brain of 2-month-old infants can detect violations of temporal regularities. Results suggested that 1) the brain of 2-month-old infants indeed detects violations of a temporal regularity; and 2) the infants' state of alertness influenced the scalp distributions

of the response to the standard tone and the deviants.

In Chapter 4, results of a multimodal paradigm with happy and fearful face/voice pairs were presented. The study's research question was whether 9-month-old infants process emotional vocalisations differently after they have been primed with a facial expression conveying the same versus a different emotion. The analyses revealed that responses to the vocalisations were modulated by the type of emotion in the preceding facial expression: fearful faces yielded larger auditory ERP responses than happy faces. Results were discussed in terms of a 'negativity bias', i.e. larger response amplitudes after presentation of sounds signalling potential danger.

The goal of the study described in Chapter 5 was to assess the influence of exposure to maternal anxiety during early pregnancy on information processing in 2-month-old infants, by means of the oddball paradigm described in Chapter 3. Our analyses yielded significant positive associations between maternal anxiety and response amplitudes to the standard and the ISI-deviant tones (i.e. larger response amplitudes with higher levels of anxiety). No effects on the ERP responses to white noise and novel sounds were found. The standard and the ISI-deviant were acoustically identical (they only differed in the interval preceding the tone), and together had been presented 80% of the time in the oddball sequences. These sounds carry less information (surprise) than the other two sounds appearing in the sequences. Therefore, we proposed that whereas all infants process white noise and novel sounds similarly, infants exposed to higher levels of maternal anxiety process sounds with low information contents more extensively.

The study described in Chapter 6 investigated responses to the oddball paradigm in 9-month-old infants in relation to prenatal exposure to maternal anxiety. Similar results were obtained to those in the 2-month-olds: significant positive associations were found between levels of maternal anxiety and response amplitudes to the standard and ISI-deviant (i.e. larger response amplitudes), but not to the white noise and novel sounds. This supported our proposal that maternal anxiety influences the processing of familiar, and therefore less informative, sounds.

Finally, Chapter 7 addressed the question how early life experiences may influence the ability to process emotional information from facial and voice input in 9-month-old infants, by means of the paradigm described in Chapter 4. The analysis revealed that maternal anxiety was positively related to the amplitude of the ERP responses to the fearful vocalisations, and negatively to those evoked by the happy vocalisations. Effects were explained in terms of increased arousability in response to fearful stimuli in infants prenatally exposed to higher levels of maternal anxiety. Also, smaller P350 amplitudes combined with larger N450 amplitudes (larger overall negativity; 'negative shift') in response to happy vocalisations suggested increased attention for emotional auditory stimuli infants are generally more familiar with (i.e. happy sounds that infants encounter quite frequently in daily interactions with their parents/caregivers). Infants prenatally exposed to higher levels of maternal anxiety appeared to be more sensitive to this negative shift, and to dedicate more processing capacity to familiar emotional vocalisations.

In sum, in the three chapters making up the core of this thesis (Chapter 5, 6

and 7) a positive association was found between levels of maternal anxiety during pregnancy and processing of less informative/more familiar stimuli. In Chapter 5 and 6, this was evidenced by more extensive processing of (larger ERP amplitudes to) sounds that had been presented most often by infants of more anxious pregnant women. In Chapter 7, this was evidenced by a stronger negative shift in response to familiar emotional sounds (i.e. happy stimuli). Taken together, this suggests that prenatal exposure to anxiety may result in alterations in habituation. We therefore proposed that there is a negative association between prenatal exposure to maternal anxiety and the degree to which habituation to repeated stimulus presentation takes place.

Several co-occurring and partly interdependent mechanisms may mediate the relationship between prenatal exposure to maternal anxiety and decreased habituation. Examination of the literature suggests that, likely among other mechanisms, altered myelinisation, shifts in excitatory-inhibitory circuits, alterations in memory function, and altered tonotopic organisation may play a central role. These mechanisms, in concert with individual differences and genetic heritability, are hypothesised to underlie the differential processing of familiar (emotional) sounds observed in infants exposed to high levels of maternal anxiety during development in the womb. Follow up research is needed to investigate the exact mechanism(s) underlying the present findings; whether the effects remain stable over time and whether they do so in the same way for different individuals and over different environmental contexts; and whether they are related to emotional, behavioural and cognitive outcome at later ages. We recommend the use of ERP studies, potentially in combination with NIRS and fMRI measurements, and in-depth study of developmental trajectories to examine these questions.

Samenvatting

Ontwikkeling verwijst naar de opeenvolging van veranderingen die plaatsvinden in functie van de voortdurende wisselwerking tussen een individu en zijn omgeving. Tijdens geen andere periode in het menselijk leven gaat dit proces zo snel als gedurende de ontwikkeling in de baarmoeder. Doordat de ontwikkeling op zo'n hoog tempo verloopt, ontstaan gevoelige perioden waarin een foetus extra vatbaar is voor prikkels uit de omgeving. Kenmerkend voor een normale ontwikkeling is dat deze omgevingsprikkels het ontwikkelingsverloop van de foetus helpen vormgeven of 'programmeren'. Atypische prikkels, zoals ondervoeding bij de moeder of gevoelens van stress en angst, kunnen het ontwikkelingsverloop beïnvloeden - of: herprogrammeren -, wat kan leiden tot veranderingen in de structuur en de functie van cellen, weefsels en organen. Deze veranderingen kunnen op hun beurt psychofysiologisch functioneren wijzigen en kwetsbaarheid vergroten voor regulatieproblemen op het niveau van gedrag, emotie en cognitie. De focus van dit proefschrift is de neurocognitieve ontwikkeling van baby's die tijdens hun ontwikkeling in de baarmoeder zijn blootgesteld aan angst van hun moeder.

In de studies die in dit proefschrift worden beschreven, zijn Event-Related Potentials (ERPs) gebruikt als directe maat voor het neurocognitieve functioneren van baby's. Een ERP is een hersenresponse, die optreedt in reactie op specifieke sensorische, cognitieve en/of motorische prikkels (bijvoorbeeld een bepaald geluid). Door gebruik van middeling kunnen ERPs worden geëxtraheerd uit het elektroencephalogram (EEG), een opname van de hersenactiviteit die gemaakt wordt aan de buitenzijde van de schedel. Onderzoek met behulp van ERPs naar de ontwikkeling van neurocognitieve processen kan inzicht bieden in de neurale basis van menselijke perceptie, emotie en cognitie. Dat is een reden dat ERPs de laatste jaren steeds populairder zijn geworden bij onderzoek naar de ontwikkeling van deze processen bij baby's. Desondanks zijn er nog maar enkele studies uitgevoerd waarin ERPs werden gebruikt om deze processen te bestuderen in de context van blootstelling aan prospectief gemeten angst van de moeder tijdens de zwangerschap. Onze studies vormen een poging het gat in de literatuur te vullen, door aan de hand van ERPs het neurocognitieve functioneren te bestuderen van baby's die in de baarmoeder zijn blootgesteld aan angst van de moeder.

De eerste twee hoofdstukken in dit proefschrift bevatten een beschrijving van de paradigma's (experimenten) die we hebben gebruikt om het neurocognitieve functioneren van baby's te onderzoeken. In Hoofdstuk 3 is een oddball- ('de vreemde eend in de bijt') paradigma besproken, waarin 4 soorten geluid werden aangeboden: één standaardtoon ('standaard') en drie typen oddballs/deviante ge-

luiden. De eerste oddball was hetzelfde als de standaardtoon, maar werd aangeboden met een interstimulusinterval ('ISI-deviant') dat korter is dan normaal; het tweede type oddball waren segmenten witte ruis ('witte ruis') en het derde waren omgevingsgeluiden ('novel'). Het doel van deze studie was te onderzoeken of de hersenen van baby's van 2 maanden schendingen kunnen detecteren in de temporele regelmaat van een reeks stimuli. Dit betekent dat we wilden weten of de hersenen van de baby's reageren wanneer een geluid plotseling eerder wordt aangeboden dan verwacht (dus als de temporele regelmaat doorbroken wordt). De resultaten suggereren dat 1) de hersenen van baby's van 2 maanden inderdaad reageren op een schending van de temporele regelmaat; en 2) dat wáar de hersenreactie het duidelijkste zichtbaar is, afhankelijk is van of de baby wakker is of slaapt, in combinatie met het type geluid dat werd aangeboden.

In Hoofdstuk 4 zijn resultaten gepresenteerd van een paradigma met angstige en blije stemmen en gezichten. De onderzoekvraag van de studie in dit hoofdstuk was of baby's van 9 maanden emotionele stemmen anders verwerken, wanneer net daarvoor een gezicht is aangeboden met dezelfde of juist een andere emotie. De analyses lieten zien dat de hersenreactie op emotioneel stemgeluid afhankelijk is van de emotie op het eerder getoonde gezicht: angstige gezichten lokten een grotere auditieve ERP uit dan blije gezichten. De resultaten lijken te wijzen op een 'negativiteitsbias', oftewel, op grotere responseamplitudes na aanbieding van geluiden die waarschuwen voor potentieel gevaar.

Het doel van het onderzoek beschreven in Hoofdstuk 5 was vaststellen wat de invloed is van blootstelling aan angst van de moeder tijdens de zwangerschap op informatieverwerking door baby's van 2 maanden oud. Hiervoor hebben we het oddballparadigma uit Hoofdstuk 3 gebruikt. De analyses wezen op een positieve associatie tussen angst bij de moeder en de grootte van de reactie op de standaardtoon en de ISI-deviant: hoe hoger de angst van de moeder, hoe groter de hersenresponse. Voor de novel en witte ruis vonden we geen verschillen. De standaard en ISI-deviant klonken exact hetzelfde (alleen het interval voorafgaand aan de tonen verschilde) en werden samen 80% van de tijd aangeboden in het oddballparadigma. Doordat ze zo vaak zijn aangeboden, bevatten deze geluiden minder nieuwe informatie dan de novel en de witte ruis. Samengenomen betekent dit mogelijk dat 1) alle baby's de witte ruis en novel op dezelfde manier verwerken; en 2) dat hoe hoger de angst van de moeder geweest is, hoe uitgebreider baby's geluiden verwerken die weinig nieuwe informatie bevatten.

In de studie die is beschreven in Hoofdstuk 6, onderzochten we hersenreacties van baby's van 9 maanden met het oddballparadigma uit Hoofdstuk 3 en bekeken we of deze reacties samenhangen met blootstelling aan angst van de moeder tijdens de zwangerschap. We vonden resultaten die vergelijkbaar zijn met die van de baby's van 2 maanden: er waren significante positieve associaties tussen het angstniveau van de moeder tijdens de zwangerschap en de amplitude van de hersenresponse op de standaard en ISI-deviant (oftewel: grotere amplitudes), maar niet op de witte ruis en de novel. Deze bevinding ondersteunt onze suggestie uit Hoofdstuk 5, dat angst bij de moeder tijdens de zwangerschap van invloed is op de verwerking van bekende geluiden die minder nieuwe informatie bevatten.

Tenslotte werd in Hoofdstuk 7 de vraag onderzocht of vroege ervaringen van invloed zijn op de verwerking van informatie uit emoties van het gezicht en de stem. Hiervoor onderzochten we baby's van 9 maanden, aan de hand van het paradigma dat werd beschreven in Hoofdstuk 4. Uit de analyses kwam naar voren dat angst van de moeder positief gerelateerd is aan de responseamplitude van angstige geluiden en negatief aan die van blije geluiden. De resultaten vormen een aanwijzing voor verhoogde arousal ('onrust/opwinding') in reactie op angstige stimuli bij kinderen van wie de moeders erg angstig zijn geweest tijdens de zwangerschap. Daarnaast wijzen de kleinere P350 (een bepaalde piek in de ERP) in combinatie met de grotere N450 (samen met de kleinere P350 wijzend op een grotere negativiteit; 'negative shift') in reactie op blije geluiden, op een toename in de aandacht voor emotionele geluiden waarmee baby's over het algemeen meer bekend zijn (zoals blije geluiden waarmee baby's vaak in aanraking komen in de dagelijkse interactie met hun ouders/verzorgers). Baby's die in de baarmoeder zijn blootgesteld aan een hoger angstniveau, waren gevoeliger voor deze 'negative shift' en lijken bekende emotionele geluiden uitgebreider te verwerken.

Samengevat vonden we in de 3 belangrijkste hoofdstukken van dit proefschrift (hoofdstukken 5 tot en met 7) een positieve samenhang tussen de hoeveelheid angst die de moeder tijdens de zwangerschap heeft ervaren, en de verwerking van bekende of minder informatieve stimuli door haar baby op 2 en 9 maanden. Dit bleek in hoofdstuk 5 en 6 doordat baby's van hoogangstige moeders geluiden die minder nieuwe informatie bevatten, uitgebreider verwerkten. In hoofdstuk 7 bleek dit doordat deze baby's een sterkere 'negative shift' vertoonden in reactie op bekende (blije) emotionele geluiden. Samengenomen suggereert dit dat prenatale blootstelling aan angst van de moeder zou kunnen leiden tot veranderingen in habituatie ('gewenning') bij haar baby. We stellen daarom dat er een negatieve associatie bestaat tussen blootstelling aan angst van de moeder tijdens de zwangerschap enerzijds en de mate waarin bij haar kind habituatie plaatsvindt in reactie op herhaalde aanbieding van stimuli anderzijds.

Verschillende mechanismen mediëren mogelijk de relatie tussen prenatale blootstelling aan angst en een afname van habituatie. De bestaande literatuur suggereert dat - hoogstwaarschijnlijk in combinatie met andere mechanismen - een rol is weggelegd voor veranderingen in myelinisatie, verschuivingen in hersencircuits die gerelateerd zijn aan opwekken en onderdrukken van reacties (excitatie/inhibitie), veranderingen in geheugenfunctie(s) en veranderingen in de tonotopische organisatie (organisatie van hersengebieden op basis van geluidsfrequentie). Onze hypothese is dat deze mechanismen, samen met individuele verschillen en genetische kwetsbaarheden, ten grondslag liggen aan de andere manier van het verwerken van geluiden die we hebben geobserveerd bij kinderen die tijdens hun ontwikkeling in de baarmoeder zijn blootgesteld aan hoge doses angst van hun moeder. Vervolgonderzoek is nodig om na te gaan welke mechanismen nu precíes het fundament vormen van de huidige resultaten. Daarnaast kan in vervolgonderzoek worden bestudeerd of de hier beschreven effecten stabiel blijven over de tijd, of dat op dezelfde manier gebeurt voor verschillende personen en in verschillende contexten, en of de resultaten gerelateerd zijn aan emotie, gedrag en cognitie op latere

leeftijd. We adviseren het gebruik van ERP-studies, eventueel in combinatie met NIRS of fMRI, en grondig onderzoek naar het verloop van de ontwikkeling om deze vragen verder te onderzoeken.

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