UNIVERSITY OF TURKU

Master's Degree Programme in Human Neuroscience

ASSOCIATION OF INFANT FRONTAL EEG ASYMMETRY WITH PRENATAL MATERNAL PREGNANCY-RELATED ANXIETY

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ABSTRACT

High levels of anxiety during pregnancy are associated with behaviour problems and increased risk of health difficulties in the offspring. Understanding how prenatal anxiety affects brain structure and function may help clarify its potential implications in neurodevelopment. Frontal electroencephalographic (EEG) alpha asymmetry has been used as an index for differences in approach/withdrawal tendencies and negative affect after birth. The data for this master thesis was collected from a sub-sample (n=105) of the FinnBrain Birth Cohort Study. The aim was to investigate the associations between frontal EEG asymmetry in newborns and prenatal maternal anxiety levels, measured with the Pregnancy-Related Anxiety Questionnaire (PRAQ-R2), during pregnancy. No significant associations were found between asymmetry scores and PRAQ-R2. Since this questionnaire measures only pregnancy-related anxiety, future studies should investigate potential effects of maternal prenatal anxiety in newborns considering more general aspects of maternal anxiety.

Keywords: frontal EEG asymmetry, newborn EEG, brain development, anxiety, pregnancy, pregnancy-related anxiety.

Contents

1. Introduction

1.1.   Approach/withdrawal tendencies and frontal brain activity

One of the core dimensions of human behaviour can be divided into two opposed motives: approach and avoidance. Emotions always contain approach and/or withdrawal components that determine how we react when exposed to a certain stimulus. Research has demonstrated that a specific phenomenon termed frontal EEG asymmetry is a reliable biomarker for differences in these motivation-related tendencies (Davidson et al., 1979; Davidson, 1983, Allen & Reznik, 2015). Frontal EEG asymmetry is defined as the difference between left and right alpha activity over the frontal regions of the brain, and it has been associated with patterns of emotion processing, motivation, temperament and psychopathology (Allen & Reznik, 2015; Harmon-Jones et al., 2010). More specifically, frontal cortex activity has been linked with both predispositions to respond to emotional stimuli and the consequent emotional changes (Harmon-Jones, Harmon-Jones, Serra & Gable, 2011). Already decades ago, the study of patients with damage to the right or left frontal cortex demonstrated the involvement of prefrontal cortical regions in approach and withdrawal motivation (Goldstein, 1939; Sutton & Davidson, 1997; Tomarken & Keener, 1998). One interpretation for frontal asymmetry is that individuals presenting a tendency to greater right frontal resting EEG activity might be more sensitive to fear-inducing stimuli. This characteristic way of responding is what Davidson (1990) called affective style. He was the first to propose that an individual's affective style is moderated by frontal brain activity and reflected in a frontal EEG asymmetry index. Alpha has been the most widely investigated frequency, but there are other frequency bands associated with emotional-dependent changes. Delta frequency has been linked to the positive/negative aspect of emotions but in an opposite manner to that of alpha (Ahern & Schwartz, 1985). Some other examples are the association between theta frequency band and

hedonic stimulation (Maulsby, 1971), beta and apprehension (Berkhout, Walter & Adey, 1969) or total power and better performance in emotional memory tasks (Harman & Ray, 1977).

Frontal EEG asymmetry research can be divided into two major research approaches. The first approach studies frontal asymmetry during rest, with no experimental alterations of the emotional state (Davidson, 1994; Sutton and Davidson, 1997). Therefore, the asymmetry works as a trait variable that can be related to psychological constructs or psychopathology (Blackhart, Minnix and Kline, 2006; Nusslock et al., 2011; Papousek, Reiser, Weber, Freudenthaler, Schulter, 2012). The second approach tries to manipulate emotional state and examine changes in frontal EEG asymmetry index (Harmon-Jones and Sigelman, 2001; Killeen and Teti, 2012). Although some researchers have tried this, most of frontal EEG asymmetry studies have examined differences in left versus right hemisphere activity during resting state. Results from some of these studies suggested withdrawal-related psychopathology interactions, but contradictory results were found in experiments with depression and anxiety symptom ratings (Quinn, Rennie, Harris, Kemp, 2014; Meyer et al., 2015).

Following the second approach, some studies tried to investigate frontal EEG asymmetry by inducing certain emotional alterations and consequently provoking changes in frontal EEG patterns. Videos evoking fear or disgust caused greater right frontal activity in infants (Davidson et al., 1990; Jones & Fox, 1992), whereas videos evoking happiness (happy faces) caused greater left frontal activity (Davidson & Fox, 1982). Children's self-regulation is thought to be influenced by reactivity to positive or negative affects and their modulation. This modulation can be observed in basic approach and withdrawal behaviours in very young infants (Rothbart, 1989). This incipient modulation skills might be partly influenced by frontal EEG activity.

In resting state, extended regions of the brain display electrical oscillations within the alpha frequency band (8–13 Hz in adults), so this is the reason why alpha power has been used

as an index for frontal EEG asymmetry (Davidson et al.,1990). Alpha power is typically operationalized in lower frequencies in young children (6–9 Hz) and those lower frequencies in the developing brain are assumed to be equivalent to adult alpha (Stroganova, Orekhova & Posikera, 1999; Marshall, Bar-Haim, & Fox, 2002). Since resting state is characterised by higher EEG power values in the alpha frequency, less alpha power would therefore indicate greater brain activity in the underlying area. According to the relative difference between left and right EEG alpha power, less alpha power in right frontal areas is associated with withdrawal emotions, whereas more alpha power correlates with approach tendencies (Davidson & Fox, 1982; Fox, 1991; Jones & Fox, 1992; Harmon-Jones, 2003; Harmon-Jones, Gable, Peterson, 2010; Saby & Marshall, 2012).

Are these EEG patterns of activation something we acquire through experience or a cortical configuration we are born with? According to Fox's model (1991), frontal EEG asymmetry in the alpha frequency band is present from birth. When it comes to studying this asymmetry index – already correlated with avoidance or fearful tendencies in infants – high levels of maternal prenatal anxiety may be an important factor to take into consideration. There is evidence that high levels of psychosocial stress and prenatal maternal cortisol are associated with fearful and reactive behaviours in the offspring (Davis et al., 2007; Davis & Sandman, 2010; Smith & Bell, 2010).

1.2. Maternal prenatal stress

Prenatal stress is known to modify the developing brain already during the embryonic stage. Even though stress is a generic term, prenatal stress is frequently defined as maternal depression and/or anxiety that are related to child behavioural, emotional and cognitive development. Prenatal exposure to maternal stress influences the developing hypothalamic pituitary adrenal (HPA) axis and it has significant effects in the baby's cortical wiring and health later in life (Kapoor, Petropoulos, & Matthews, 2008). Prenatal maternal depression

causes physiological changes in the intrauterine environment that may lead to long-term impact on the baby. These changes have been associated with an increased risk for behavioural, cognitive and socio-emotional problems (Buss et al., 2012; Waters, Hay, Simmonds, van Goozen, 2014).

Frontal EEG asymmetry research in newborns has focused mainly on maternal depression. Results have suggested that infants ftom depressed women had greater relative right frontal activity than those born to non-depressed women (Dawson et al., 1997; Field et al., 2004; Lusby, Goodman, Bell & Newport, 2014). In contrast, infants of non-depressed mothers tend to exhibit more symmetrical activation across hemispheres (Gustafsson, Grieve, Wemer, Desai & Monk, 2018). Field et al. (1995) examined 3- to 6-month-old infants aiming to find an association between maternal depressive symptoms and greater relative right frontal activity during a neutral condition and they found support for this hypothesis in 10 out of 17 babies. These findings have been replicated with infants of mothers diagnosed with depression. Infants whose mothers were clinically depressed showed greater relative right frontal EEG asymmetry compared to infants of non-depressed mothers (Dawson et al., 2001; Diego et al., 2006). In a more recent study, Lusby et al. (2014) recorded 83 infants' EEG during baseline, feeding, and play situations at 3 and 6 months of age and found that maternal depressive symptoms during pregnancy predicted variation in their EEG asymmetry scores.

Gustafsson et al. (2018) were the first ones to gather infant EEG data in the hospital right after birth aiming to examine prospectively maternal prenatal depression associations with EEG patterns in newborns. They found a correlation between depressive symptoms in 18 pregnant women (quantified by the Hamilton Rating Scale for Depression) and their babies' sleep EEG. Their results suggest that prenatal depressive symptomatology is associated with greater relative right-frontal alpha asymmetry during quiet sleep. Since this asymmetry was observed only a few hours after delivery, they demonstrated that maternal depression may have

a significant impact on the developing brain in utero, even before any other postpartum factors start influencing the offspring.

Despite depression's critical role in neurodevelopment, not many studies have tried to examine this prenatal cortical wiring processes focusing on anxiety, and we currently have little knowledge regarding the interaction between prenatal anxiety, not necessarily accompanied with depression, and the baby's frontal EEG asymmetry after birth or in early life.

1.3. Prenatal anxiety

Anxiety disorders are the most common mental disorders. There are over 60 million anxiety disorders diagnoses per year only in the European Union (prevalence estimated at 14%) (Wittchen et al. 2011). They are characterised by continuous nervousness, excess worry and fears, excessive activation of the autonomic nervous system and sometimes even panic attacks. These symptoms usually lead to avoidance behaviour towards things that provoke them. Anxiety is also a part of normal life and it is not always considered a disorder, so it can be hard to differentiate between normal and pathological anxiety. Pathological anxiety is required to be continuous and it should considerably complicate everyday life. Anxiety disorders are a prevalent issue in pregnant women (Lee et al., 2007; Teixeira, Figueiredo, Conde, Pacheco, Costa, 2009). There is evidence of higher obsessive-compulsive disorder prevalence in pregnant women (2.07%) compared to the general population (1.08%) (Russell, Fawcett & Mazmanian, 2013). Pregnancy entails many life changes and transitions, and the tendency to be anxious can render pregnancy very stressful. Although pregnancy is associated with higher rates of, for instance, generalized anxiety disorder (Matthey and Ross-Hamid, 2011), a significant amount of variation in anxiety during pregnancy cannot be explained by specific anxiety disorders (Orr et al., 2007).

Pregnant women differ in how strong they worry about labour pains and may present what we know as fear of childbirth, or they can be more concerned about the health of the baby or the inevitable physical changes their body will suffer (Huizink et al. 2004). In some cases, this continuous worrying may lead to high levels of anxiety and adverse health effects on both the mother and baby (Nicholson et al. 2006). Interestingly, the effects of maternal anxiety during pregnancy are not limited to physical health problems in the baby. High anxiety levels during pregnancy have been linked to neonates' more inconsistent behavioural state (restless babies, crying more) as compared to those of non-anxious mothers (Van den Bergh 1990). In another study, pregnancy-related anxiety predicted child negative affectivity after birth and had a distinctive influence on the developing fetus (Blair et al., 2011). Additionally, other researchers have found that pregnancy-related anxiety might be even a better predictor of potential health problems in the baby than general anxiety (Reck et al. 2013).

Since we know that anxiety during pregnancy can significantly affect the offspring, we need more research to understand its scope and consequences. This study considers maternal prenatal pregnancy-related anxiety and its association with offspring outcome determined with EEG by observing EEG asymmetry in frontal, central and parietal pairs of electrodes. Central and parietal electrodes were included in the analyses to obtain better resolution of the extent of anxiety-related asymmetry.

2. Aims and hypothesis

Maternal prenatal depression has a disruptive impact on neurodevelopmental trajectories and it significantly affects frontal EEG asymmetry in newborns as demonstrated by previous studies. Evidence suggests that less alpha power in right frontal areas is associated with withdrawal tendencies and more sensitivity to negative affect. However, additional

variables, such as prenatal anxiety, may similarly influence resting baseline asymmetric activity. Is there an association between pregnancy-related anxiety and greater right-frontal alpha asymmetry? We hypothesized that children whose mothers had elevated pregnancy-related anxiety may show greater relative difference between left and right EEG alpha power.

This study aims to shed more light on the importance of the prenatal environment in shaping children's neurobehavioral trajectories. Even subclinical levels of maternal depression influence brain development in the womb (Gustafsson et al., 2018) and prenatal anxiety could play a similar role in determining cortical wiring processes in children. The study of frontal EEG asymmetry in infants promises to give more knowledge regarding fundamental properties of infant emotion and motivation, and it may serve as a potential biomarker for the development of affective disorders later in life.

3. Materials and Methods

3. 1. Participants

The infants participating in this study were recruited from FinnBrain Birth Cohort, located in South-Western Finland. They focus on prospective effects of early life environment and genetics on child neurodevelopment and health. Families were recruited between December 2011 and April 2015 in their first trimester ultrasound visit at gestational week (gwk) 12. The total number of children in the study is n=3837. The parents gave their written informed consent for the infants to participate in the studies, approved by the Ethics Committee of the Hospital District of Southwest Finland and the Ethics Committee of the Hospital District of Helsinki and Uusimaa. The study protocol followed the Declaration of Helsinki.

FinnBrain acquired anxiety scores from mothers and EEG data from newborns (n=151, 76 boys and 75 girls). The infants were all born full-term between 37 and 42 weeks of gestation and their birth weights were between 2635 and 4770 g. The inclusion criteria were that the infants were healthy (Apgar score of 7–10 at 5 min), had stable physical condition and normal hearing. Women needed to have sufficient knowledge of Finnish or Swedish in order to fill in the study questionnaires.

For data preprocessing, 31 EEG files were not available, and 15 more subjects were excluded due to some corrupted files. The final study population was n=105.

3.2. Methods

3.1.1. EEG recording

As previously stated, EEG asymmetry can be considered both a trait individual difference or a consequence of a manipulated state, and emotional responses may alter frontal EEG asymmetry scores. Hence, EEG asymmetry research requires to avoid any kind of state manipulation before or during EEG recording. (Blackhart, Kline, Donohue, LaRowe, Joiner, 2002).

Sleep EEG was recorded 0–34 days postpartum. It was continuously recorded for 2-10 minutes during infant sleep without any acoustic stimulation other than hospital sounds. The 16 electrodes were positioned according to the 10–20 system. Electrode impedances were tested in the beginning of the experiment and kept below 20kΩ. EEG was recorded with a sampling frequency of 500 Hz. Electrical signals were continuously recorded with BrainAmp amplifiers (BrainProducts GmbH, Gilching, Germany) with a bandwidth of 0.01–100 Hz.
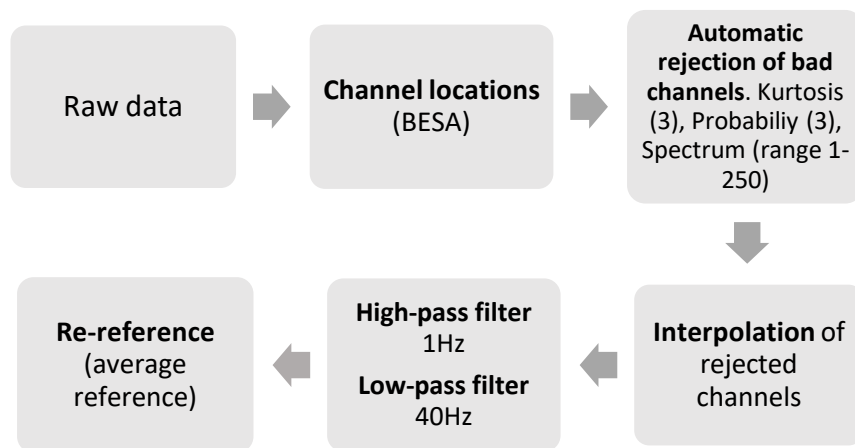
3.1.2. Preprocessing

Figure 1. Data preprocessing stages.

EEGLAB is a Matlab toolbox for processing EEG, MEG and other electrophysiological data and developed by Swartz Center for Computational Neuroscience at the University of San Diego (Delorme & Makeig, 2004). EEGLAB was used in signal preprocessing so that data could be better manipulated with other Matlab tools. Channel locations were determined using BESA default spherical coordinates. The so-called "bad" channels have distributions of values that differ too much from a normal distribution than other channels, so they can have a great impact when average reference is employed. These problematic channels were automatically rejected in terms of kurtosis, probability and spectrum range (see Figure 1 for threshold criteria). After channel interpolation, high pass (1Hz) and low pass (40Hz) filters were implemented. The main cause of artifacts in the very low frequencies is usually heartbeat signal (2-4 Hz), but it is known that low frequencies are more predominant in young infants' EEG data. For this reason, the high pass filter limit was set in 1Hz. Finally, the data were re-referenced using average reference.
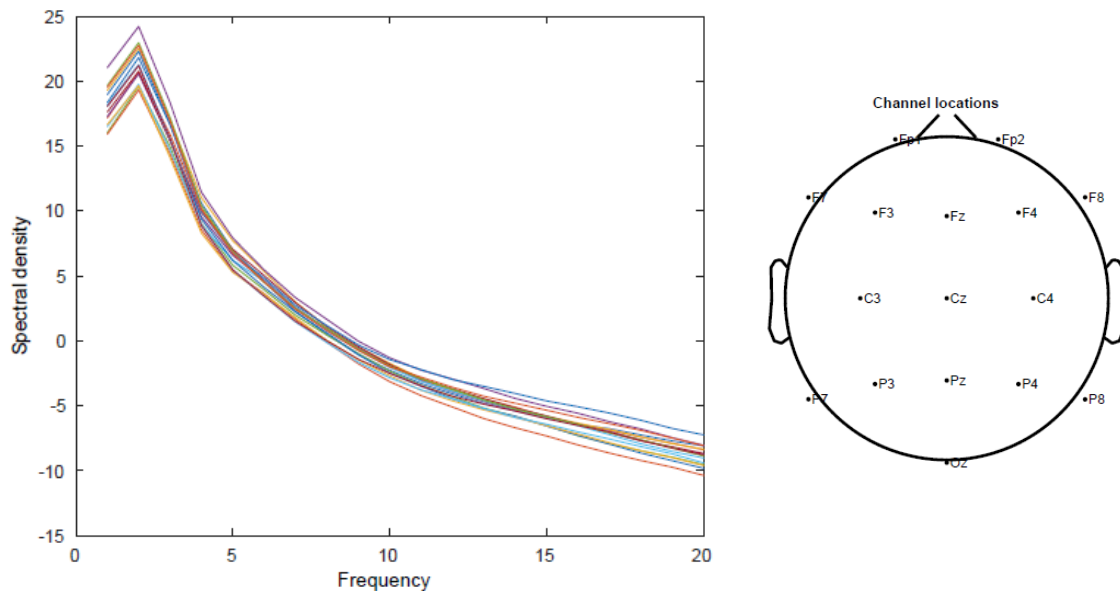
Figure 2. Mean spectra in the 16 channels.

3.1.3. Asymmetry calculation

Since untransformed power values tend to be positively skewed (Allen, Coan, & Nazarian, 2004), alpha power at any given site is first natural log transformed. Then, the difference score (ln[right]-ln[left] alpha power) represents the relative activity at homologous right and left sides. According to EEG literature, brain activity is indicated by lower EEG power values in the alpha frequency band. Thus, an asymmetry score of '0' represents total symmetry between hemispheres, a negative score (more alpha power in the left side) reflects greater right frontal activation, and a positive score (more alpha power in the right side) reflects greater left frontal activation. This log-difference score also helps to correct individual differences that might influence signal amplitude (Eshel, Witman, Rosenfeld, Abboud, 1995).

This present study focused on prefrontal and frontal electrode pairs (Fp2-Fp1, F4-F3) that correspond to regions widely studied throughout the asymmetry literature as well as two extra pairs of channels, central and parietal electrodes (C4-C3, P4-P3), that may neighbour

frontal activity in newborns' EEG data and provide better resolution of the extent of anxiety-related asymmetry. Young infants usually have a dominant frequency between 6 and 9 Hz (Marshall, Bar-Haim, & Fox, 2002), and this frequency band is thought to approximate the alpha band in adults and has been used in all previous studies of infant frontal asymmetry. However, mean spectra from our data (see Figure 2) did not reflect this frequency dominance. Instead, it showed a peak around 2-3 Hz that might be a byproduct of the high pass filter set in 1Hz. Given the possible developmental increase of alpha rhythm frequency, this characteristic peak might not be observable in newborn EEG.

3.1.4.   Pregnancy-Related Anxiety Questionnaire (PRAQ-R2)

The 10-item self-report Pregnancy-Related Anxiety Questionnaire-Revised (PRAQ-R) (Huizink et al., 2014) is a shortened version of the 34-item PRAQ (van den Bergh, 1990). Scores range from 1 (definitely not true) to 5 (definitely true). It has been widely used to assess pregnancy-related anxiety in women who are about to give birth for the first time (nulliparous women). It has good psychometric values and predictive validity for birth and childhood outcomes. However, this revised version of PRAQ has a problem: it was not designed to be administered in women who have already experienced pregnancy and labour in the past (parous women), as particularly one item of the questionnaire is not relevant for women who gave birth before (see Table 1). Therefore, PRAQ-R2 was adapted to suit nulliparous and parous women with a new item to replace the problematic one if needed (*"I am anxious about the delivery"* instead of *"I am anxious about the delivery because I have never experienced one before"*). PRAQ-R2 items can be ordered into three subscales:

1. Fear of giving birth (4 items): *"I am worried about the pain of contractions and the pain during delivery."*

2. Worries about bearing a physically or mentally handicapped child (3 items): "*I sometimes think that our child will be in poor health or will be prone to illnesses.*"

3. Concern about own appearance (3 items): "*I am worried about my enormous weight gain.*"

Table 1. English version of PRAQ-R/R2 used by FinnBrain, containing both the old item only for nulliparous women (8) and the new replacing item applicable for all pregnant women regardless previous experiences with pregnancy (1).

1. I am anxious about the delivery. *	1 2 3 4 5
2. I am worried about the pain of contractions and the pain during delivery.	1 2 3 4 5
3. I am worried about the fact that I shall not regain my figure after delivery.	1 2 3 4 5
4. I sometimes think that our child will be in poor health or will be prone to illnesses.	1 2 3 4 5
5. I am concerned about my unattractive appearance.	1 2 3 4 5
6. I am worried about not being able to control myself during labour and fear that I will scream.	1 2 3 4 5
7. I am worried about my enormous weight gain.	1 2 3 4 5
8. I am anxious about the delivery because I have never experienced one before. **	1 2 3 4 5
9. I am afraid the baby will be mentally handicapped or will suffer from brain damage.	1 2 3 4 5
10. I am afraid our baby will be stillborn, or will die during or immediately after delivery.	1 2 3 4 5
	1 2 3 4 5

The time points of assessment considered in this study were gestational weeks 24 and 34. However, the scores at these two time points were strongly correlated (rho=.767) so an average score was calculated.

3.2. Statistical analysis

Asymmetry literature combines research examining frontal asymmetry as a predictor or predicted variable. In this study, frontal asymmetry was examined as the outcome, with the assumption that the probability of this score depends on values in other predictor variable: anxiety. Four mixed-effects regression analyses, one for each pair of electrodes, were conducted in Matlab to examine the relationship between the fluctuation of maternal pregnancy-related anxiety and EEG asymmetry scores. Also IBM SPSS statistical software (version 25.0) was used to calculate descriptive data.

Regression models were built with asymmetry scores as predicted variable and two fixed-effects predictor variables, PRAQ-R2 average score (24 gwk and 34 gwk) and frequency band (delta, 1-2 Hz; theta, 2-6 Hz; alpha 6-11 Hz; beta, 11-19 Hz). Both predictor variables' main effects were considered as well as the interaction between them. A random effect for the subject ID (random intercept) was added to characterise the idiosyncratic variation that is due to individual differences. The threshold for statistical significance was $p<0.05$.

Table 2. Information about the participants included in these analyses.

Variable		Total n = 105	Boys n = 55	Girls n = 50
Mothers				
BMI (body mass index)	mean (SD)	24.2 (4.5)	23.9 (4.8)	24.5 (4.1)
Mothers age, years	mean (SD)	30.2 (4.1)	30.3 (4.9)	30.1 (3.4)
PRAQ score 24gwk	mean (SD)	23.7 (7.4)	23.9 (6.4)	23.5 (8.6)
PRAQ score 34gwk	mean (SD)	22.9 (6.3)	22.6 (5.2)	23.3 (7.5)
PRAQ average score	mean (SD)	23.6 (6.9)	23.7 (5.9)	23.6 (7.9)
Children				
Gestational weeks	mean (SD)	39.9 (1.4)	39.8 (1.5)	40.1 (1.1)
Birth weight, g	mean (SD)	3579 (496)	3626 (534)	3529 (450)

4. Results

4.1. Asymmetry scores distribution

Power from 1 to 20 Hz was then extracted from the spectrum from each pair of electrodes in order to depict the asymmetry values in all the frequency bands included in the analyses. The spectopo function of EEGLAB gives the power value in dB and it plots the power spectral density of the selected window size at all channels, each traced line representing a participant. Asymmetry scores were calculated by subtracting the natural log transformed scores (ln[Right] - ln[Left]) for each homologous left and right electrodes (Fp2-Fp1, F4-F3, C4-C3 and P4-P3). Negative values on this index reflect relatively greater right activity (Figure 3).
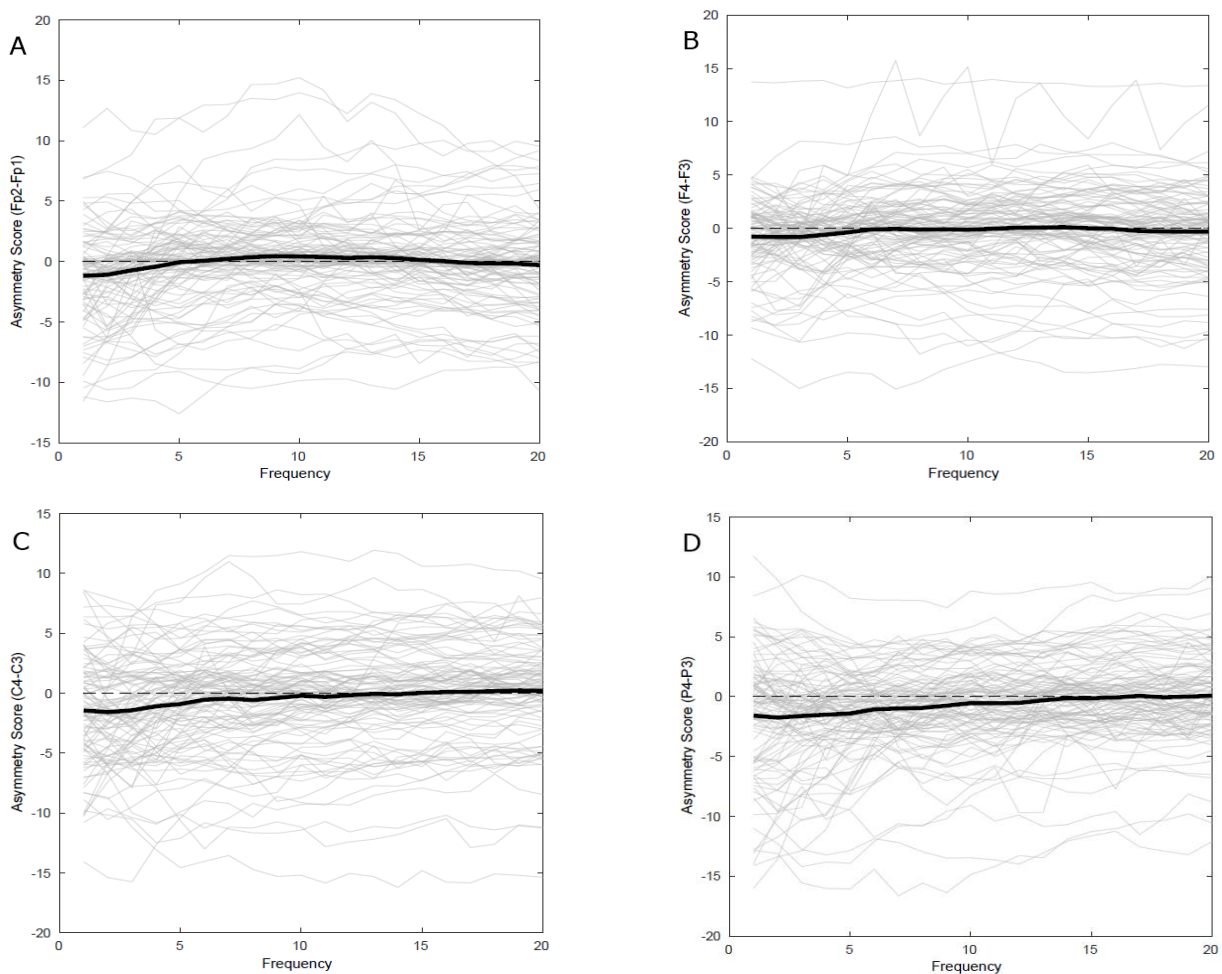

Figure 3: A–D: Asymmetry scores distributions. Scores distribution in prefrontal electrodes (A), scores distribution in frontal electrodes (B), scores distribution in central electrodes (C), scores distribution in parietal electrodes (D).

4.2. Regression analysis

No significant associations were found between asymmetry index and PRAQ scores. There was no difference in the interaction with alpha compared with other frequency bands. The effect closest to statistical significance was the coefficient obtained from the interaction of beta frequency band * PRAQ on parietal asymmetry score (p=0.1, Table 6). Alpha frequency band * PRAQ interaction was insignificant in both prefrontal (p=0.52, Table 3) and frontal (p=0.14, Table 4) electrodes, so both frontal pairs failed to show noteworthy effects.

However, alpha frequency band predictor effect was significant in frontal electrodes (p=0.046, Table 4), which is concordant with previous frontal EEG asymmetry research, as alpha frequency band is known to be the best predictor for frontal asymmetry variations. The effect was partially visible in beta frequency band too, where the results were relatively close to be significant (p=0.055).

Table 3. Fixed effects coefficients (95% CIs). Prefrontal electrodes (Fp2-Fp1).

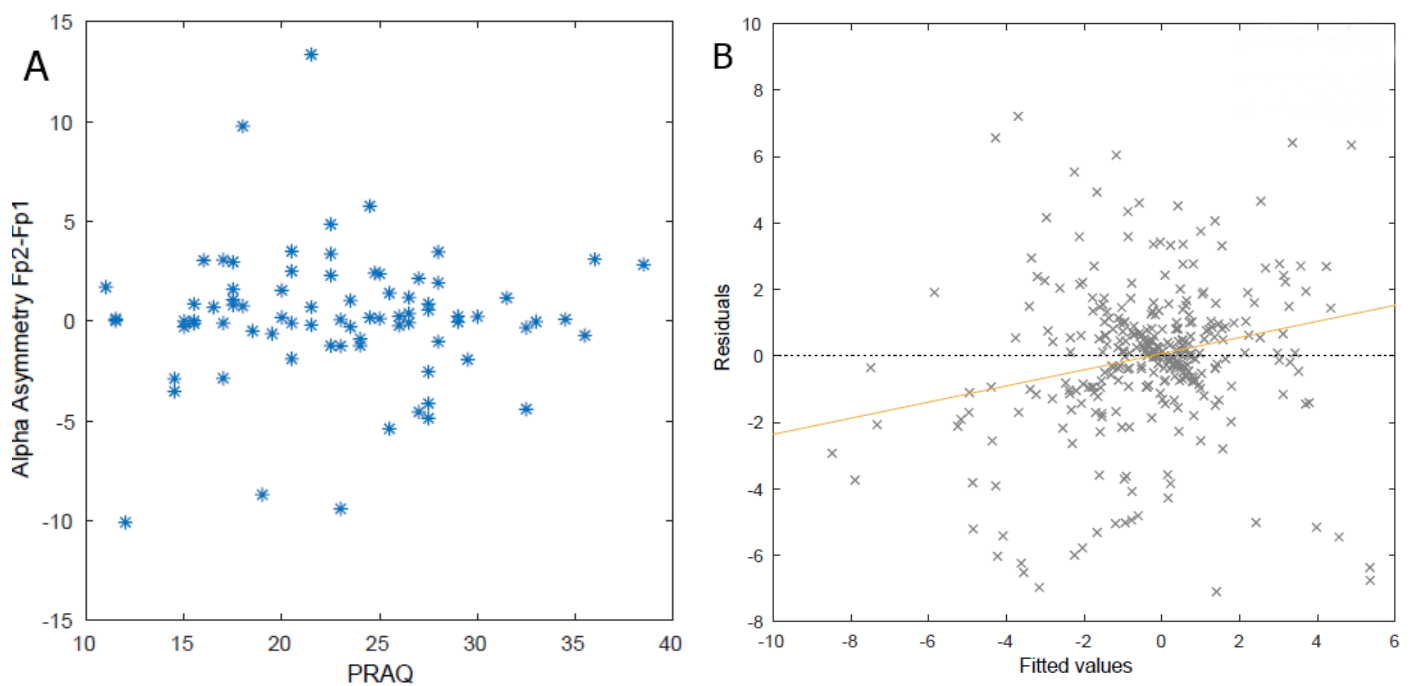| Variable	Estimate	SE	t value	p value
Intercept	-0.8711	1.418	-0.6143	0.5395
Theta	0.6344	1.6197	0.3917	0.6956
Alpha	0.1464	1.6197	0.0904	0.9280
Beta	-0.7886	1.6197	-0.4869	0.6267
PRAQ	-0.0035	0.0591	-0.0591	0.9529
Theta x PRAQ	-0.0019	0.0682	-0.0289	0.9770
Alpha x PRAQ	0.0439	0.0682	0.6429	0.5208
Beta x PRAQ	0.1470	0.0682	1.1348	0.2573

Figure 4. A–B: Regression results in prefrontal electrodes (Fp2-Fp1). Scatter plot representing correlation between alpha asymmetry scores in prefrontal electrodes and PRAQ (A). Regression residuals vs. fitted values (B).

Table 4. Fixed effects coefficients (95% CIs). Frontal electrodes (F4-F3).

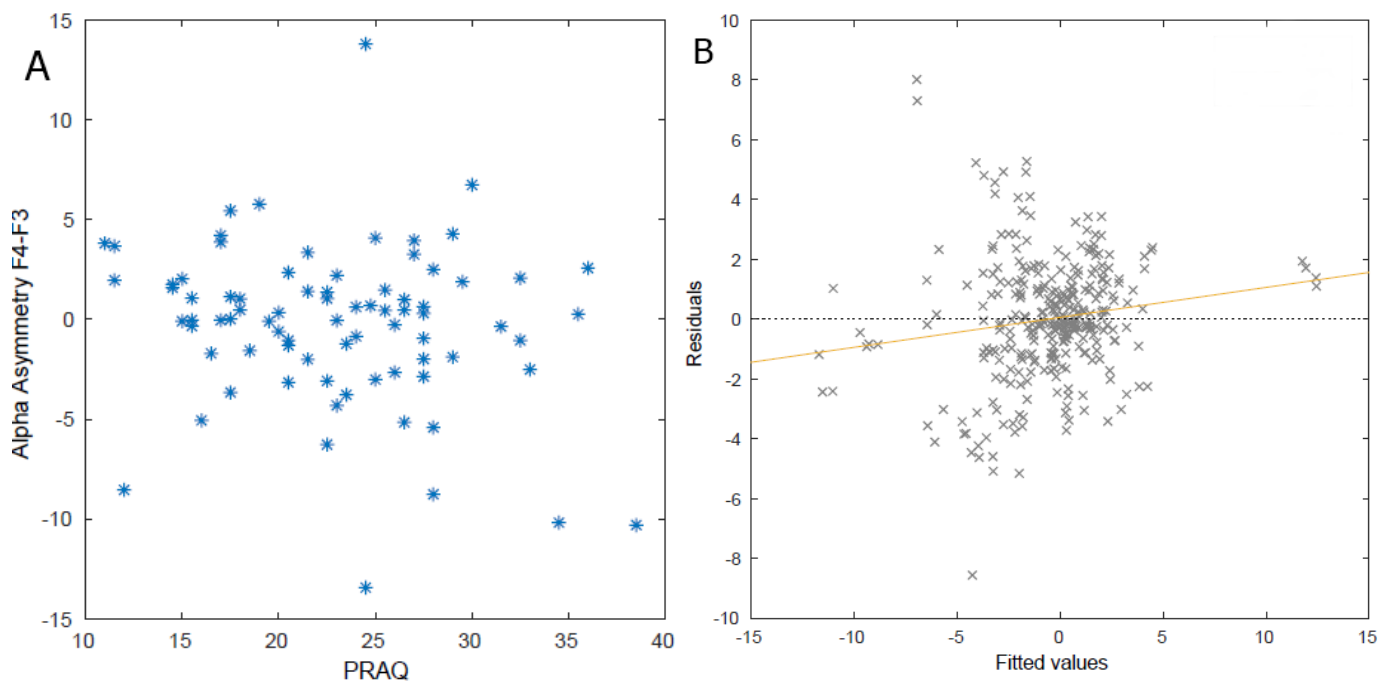| Variable	Estimate	SE	t value	p value
Intercept	1.048	1.3615	0.7697	0.4420
Theta	1.985	1.383	1.4353	0.1522
Alpha	0.8121	1.383	2.002	0.0461*
Beta	0.3558	1.383	1.9223	0.0555
PRAQ	-0.0630	0.0556	-1.6005	0.1105
Theta x PRAQ	-0.0295	0.0583	-1.2713	0.2046
Alpha x PRAQ	-0.0093	0.0583	-1.4767	0.1408
Beta x PRAQ	0.0133	0.0583	-1.3807	0.1684

Figure 5. A–B: Regression results in frontal electrodes (F4-F3). Scatter plot representing correlation between alpha asymmetry scores in frontal electrodes and PRAQ (A). Regression residuals vs. fitted values (B).

Table 5. Fixed effects coefficients (95% CIs). Central electrodes (C4-C3).

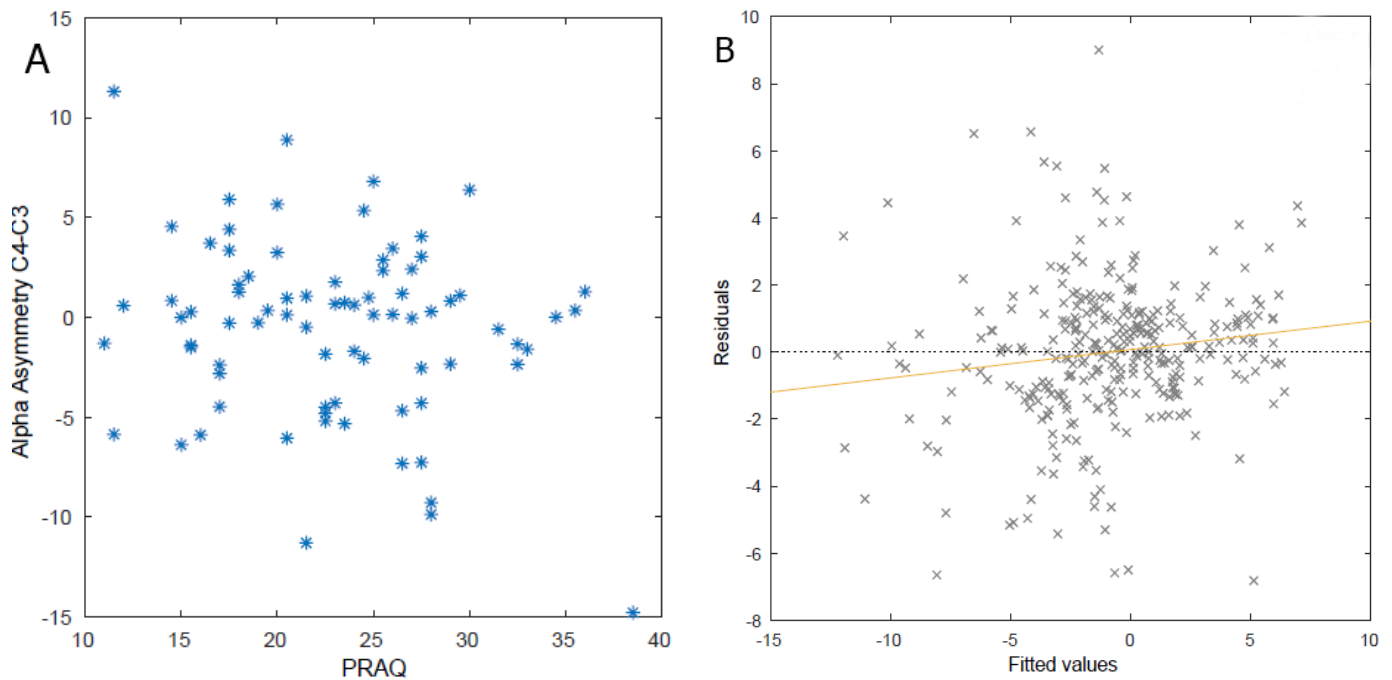| Variable	Estimate	SE	t value	p value
Intercept	0.2145	1.4764	0.1453	0.8846
Theta	1.2619	1.4754	0.8553	0.3930
Alpha	2.5813	1.4754	1.7495	0.0812
Beta	2.4604	1.4754	1.6676	0.0964
PRAQ	-0.0749	0.0601	-1.2466	0.2135
Theta x PRAQ	-0.0393	0.0622	-0.6315	0.5282
Alpha x PRAQ	-0.0656	0.0622	-1.0559	0.2918
Beta x PRAQ	-0.0407	0.0622	-0.6553	0.5128

Figure 6. A–B: Regression results in central electrodes (C4-C3). Scatter plot representing correlation between alpha asymmetry scores in central electrodes and PRAQ (A). Regression residuals vs. fitted values (B).

Table 6. Fixed effects coefficients (95% CIs). Parietal electrodes (P4-P3).

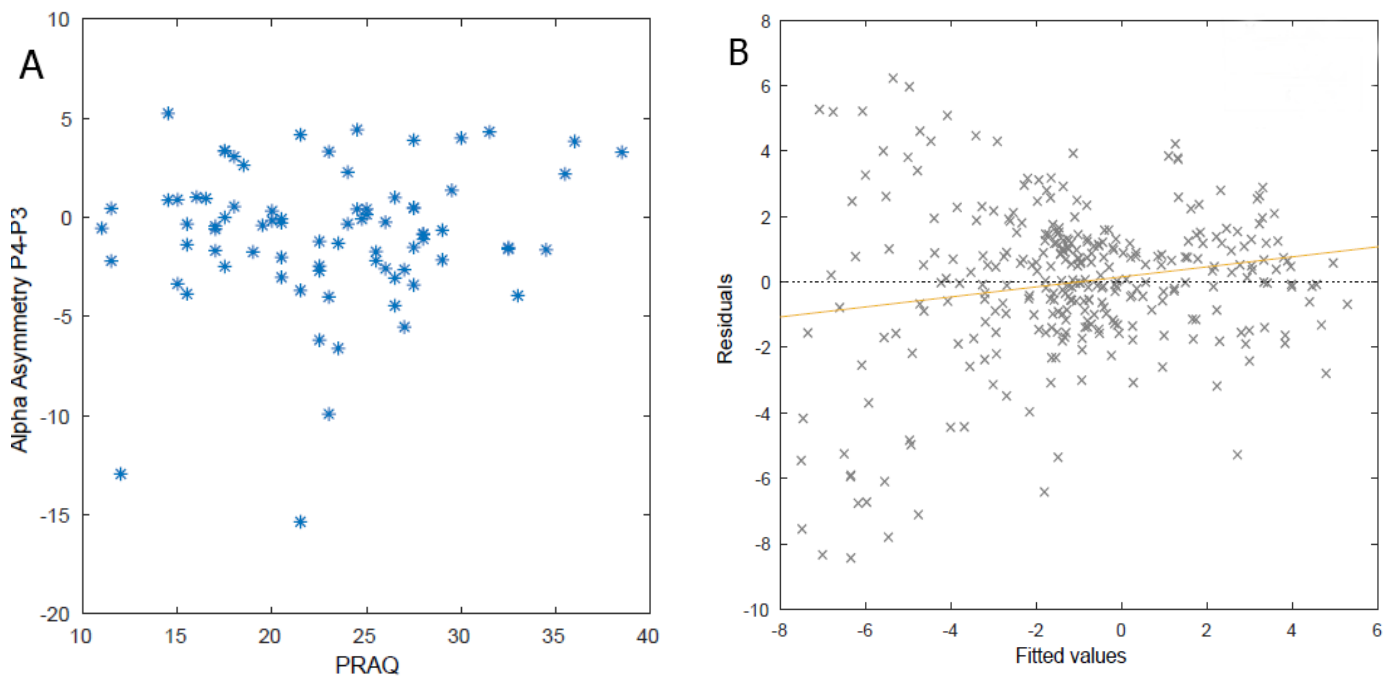| Variable	Estimate	SE	t value	p value
Intercept	-2.8066	1.4975	-1.8742	0.0618
Theta	-0.9889	1.6159	-0.6120	0.5410
Alpha	-1.1906	1.6159	-0.7368	0.4618
Beta	-1.376	1.6159	-0.8516	0.3951
PRAQ	0.0656	0.0619	1.0597	0.2901
Theta x PRAQ	0.0468	0.0681	0.6876	0.4922
Alpha x PRAQ	0.0786	0.0681	1.1544	0.2492
Beta x PRAQ	0.1108	0.0681	1.6267	0.1048

Figure 7. A–B: Regression results in parietal electrodes (P4-P3). Scatter plot representing correlation between alpha asymmetry scores in parietal electrodes and PRAQ (A). Regression residuals vs. fitted values (B).

5. Discussion

The association of maternal depression symptomatology on prenatal neurodevelopment, and specifically frontal EEG asymmetry, has been widely studied in the past (Field et al., 2004; Allen & Reznik, 2015; Gustafsson et al., 2018). Results suggest that prenatal depressive symptomatology is associated with greater relative right frontal alpha asymmetry. Nevertheless, the definition of prenatal stress englobes not only depression but also anxiety. The present study tried to investigate the relationship between maternal pregnancy-related anxiety and frontal EEG asymmetry. In accordance with previous depression research, it was hypothesised that children from mothers with higher pregnancy-related anxiety would show greater right frontal alpha asymmetry. Contrary to our hypothesis, no associations between alpha frequency band and anxiety scores were observed. Results in the four pairs of electrodes included in the analyses failed to reach significance for this interaction. The predicted outcome, based on the assumption that a negative result in the asymmetry index indicates higher levels of activity in right frontal areas, should have shown a negative slope between PRAQ scores and alpha power in frontal electrodes (the higher PRAQ, the more negative alpha asymmetry score). On the contrary, the results reflect even the opposite tendency (e.g. prefrontal electrodes).

However, there are some strengths of the study that should be mentioned. First, the null result obtained does not seem to reflect insufficient statistical power, as the analysed sample in this study was bigger than those in other newborn EEG asymmetry studies carried out so far (Gustaffson et al. (2018) included data from a subsample of only 18 women). Some studies did, in fact, have bigger samples (e.g. Dawson et al. 1997, n=117), but the recruited infants were much older (13-15 months old). Moreover, only the alpha frequency band effect on asymmetry scores in frontal electrodes was found to be significant (p=0.046), which supports the idea of alpha being the most relevant frequency band in frontal asymmetry index. Secondly,

anxiety was measured in two different time points (24 and 34 gwk) and then an average score was calculated. Considering more than one time point may be beneficial since repeated measurements offer a more representative view of the mother's general state during pregnancy. Besides, infant sleep is considered to be a more homogenous state in comparison with other resting state measurements, as attention and orientation cannot be controlled in awake babies. Lastly, the majority of infant EEG asymmetry research has focused on studying asymmetry in alpha frequency over the frontal electrodes, but our study examined delta, theta and beta different frequency bands as well (in four different electrode pairs). There is evidence that other spectral changes are involved in emotional responses. In frontal areas, delta and total band power have been suggested to be lateralized for positive and negative emotions, and theta band has been linked to the general arousal characteristics of the emotions (Ahern & Schwartz, 1985; Lin, Duann, Chen & Jung, 2010). Therefore, our findings enrich previous research by examining broader aspects of infant EEG.

5.1. Limitations of the study

Several limitations need to be pointed out. The weak association between maternal pregnancy-related anxiety and infant EEG could be a consequence of peculiarities in new-born brain functioning, such as possible differences in dominant frequencies in newborns compared with adults or even older infants. There is variation in the use of specific frequency bands across studies, so power in young infant brains might reflect distinct components across development There is still no consensus determining what frequency bands could best discriminate infants of anxious or non-anxious mothers in early life. In addition, the number of babies in quiet or active sleep was not known. In other studies, this has been treated as a relevant factor and experts say it should be taken into consideration in the analysis. Gustafsson et al. (2018) found that the association between depressive symptoms and greater right frontal

asymmetry was significant only during quiet sleep. There could be methodological reasons behind this finding, such as less movement during quiet sleep, which leads to more accurate data. It should also be noted that PRAQ-R2 is measuring a really specific type of anxiety (only pregnancy-related), so future studies should try to replicate these findings using more general instruments and consider more aspects of anxiety.

Moreover, due to time constraints, the models had a narrow perspective and did not include other variables that may have an impact in frontal EEG asymmetry, such as maternal pharmacological ongoing treatments, maternal depression, infant age, gender or even mode of delivery. Studies examining the heritability of frontal asymmetry have determined that this pattern of EEG asymmetry may only relate to anxiety and depression risk in females, but not in males (Smit, Posthuma, Boomsma and de Geus, 2007), suggesting sex differences might play a significant role. Thus, gender might be an important covariate when studying the interaction between anxiety and neurodevelopment. Anxiety influence on brain development is a complex phenomenon and so are the variables measuring it in early infancy. This is probably a multifactorial process, so it is necessary to clarify more predictor variables in order to decrease noise.

6. Conclusion

This thesis aimed to investigate the interaction between pregnancy-related anxiety and frontal EEG alpha asymmetry. Our null result might reflect the need of a more thorough approach, which should consider some other possible confounding variables. Besides, alpha frequency rhythm changes continuously across development, so its interaction with anxiety may be inconsistent in newborns and older infants. In any case, it was a step towards understanding the role of prenatal anxiety in neurodevelopment. Frontal EEG asymmetry has

been associated with patterns of emotion processing and the ability to react to stressful environments or approach reinforcing stimuli. These are essential factors in the process of developing self-regulation skills and may in part determine future risks for certain affective disorders later in life. These results are a first glimpse of the complex wiring process that takes place inside the human brain while we are still in the womb.

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