Connecting the dots:

Relating the infant brain network to infant behavior

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CONNECTING THE DOTS

RELATING THE INFANT BRAIN NETWORK TO INFANT BEHAVIOR

De punten met elkaar verbinden

De relatie tussen netwerken in het babybrein en hun gedrag

(met een samenvatting in het Nederlands)

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CHAPTER 1

General Introduction

Infants exhibit exceptional development in complexity of behavior during their first year of life. This growth in behavior is mirrored in the brain, the first year of life is marked by an explosion in neural growth and optimization of existing brain structure (Ball et al., 2014; Keunen, Counsell, & Benders, 2017b; Lodygensky, Vasung, Sizonenko, & Hüppi, 2010; van den Heuvel et al., 2014), through the strengthening of important connections and the pruning on non-used connections (Innocenti & Price, 2005). Special attention to explain behavioral development is given towards the development of the human connectome: the collection of functionally distinct areas in the brain and their interconnectivity. Complex behaviors likely require the optimized integration of information processed throughout the brain. Studying the connectome during the first year of life could provide us valuable insight into why certain behaviors develop when.

One such complex type of behavior that shows considerable growth in the first year of life is social competency mostly. At birth, infants mostly interact from a distance. Looking at familiar and new faces, analyzing sounds and smells. This changes rapidly, aided by motor skill development. At two months, infants start to respond to their environment by utilizing smiling and laughter (Wolff, 1987). At three months, infants recognize faces and clear neural substrates of face processing can be found (de Haan, Johnson, & Halit, 2003; Halit, de Haan, & Johnson, 2003; Sangrigoli & De Schonen, 2004). At five months, infants start to babble towards their parents and show an increased affinity for the mother's voice (de Boysson-Bardies, Sagart, & Durand, 1984; Oller & Eilers, 1988). At 9 months, infants show the first signs of joint attention, actively trying to let others attend to what they are attending to and vice versa (Eggebrecht et al., 2017; Kopp & Lindenberger, 2011; Mundy, Card, & Fox, 2000). Lastly, during the last weeks of their first year of life, infants even start to participate in game-like constructs.

Understanding how these social behaviors arise is of critical importance, not only to better understand which conditions must be met for these behaviors to develop, but also, to better understand how these behaviors are represented in human cognition, both in adults and in children. This dissertation aims to study the development of the functional connectome during infancy to detect early markers for social competency.

The following sections deal with a short outline of the study of the development of the human connectome and the use of graph theory to describe this development. Subsequently, the development of the social brain will be shortly discussed. Thereafter, I will discuss the use of EEG in infants to study the development of both functional brain networks and social competency. Lastly, an outline of this dissertation is presented.

1.1 Analyzing and quantifying brain networks

Whole-brain networks are complex. Comparing networks across subjects or following the development of networks over time within a subject can therefore be difficult. To quantify the core characteristics of these networks graph theory can be used (for review see Bullmore & Sporns, 2009). In the graph-theoretical framework, networks are composed of nodes and edges. Nodes are centers of information and edges are the connections between the nodes. The determination of what is defined as a node in neuroscientific research depends on neuroimaging method. Opposed to (f)MRI connectivity research, where nodes are usually defined as anatomically distinct areas based on anatomical or functional atlases, within EEG connectivity research each electrode with its specific scalp-based location is used as nodes. To calculate the edges between the nodes, a measure of signal correlation is used. In EEG, phase difference consistency is often seen as a measure of connectivity as synchronized oscillatory activity allows for an optimized flow of information between two regions (Fell & Axmacher, 2011).

It can be useful to divide graph characteristics into two camps: ones quantifying network integration and ones quantifying network segregation processes (Rubinov & Sporns, 2010; Sporns, 2013; Zhao, Xu, & He, 2019). In brain networks, segregation usually represents the ability of the network to have anatomically near brain areas closely working together to perform specialized functions.

To quantify the segregation ability of a network the clustering coefficient and the modularity are often calculated (see figure 1.1). The

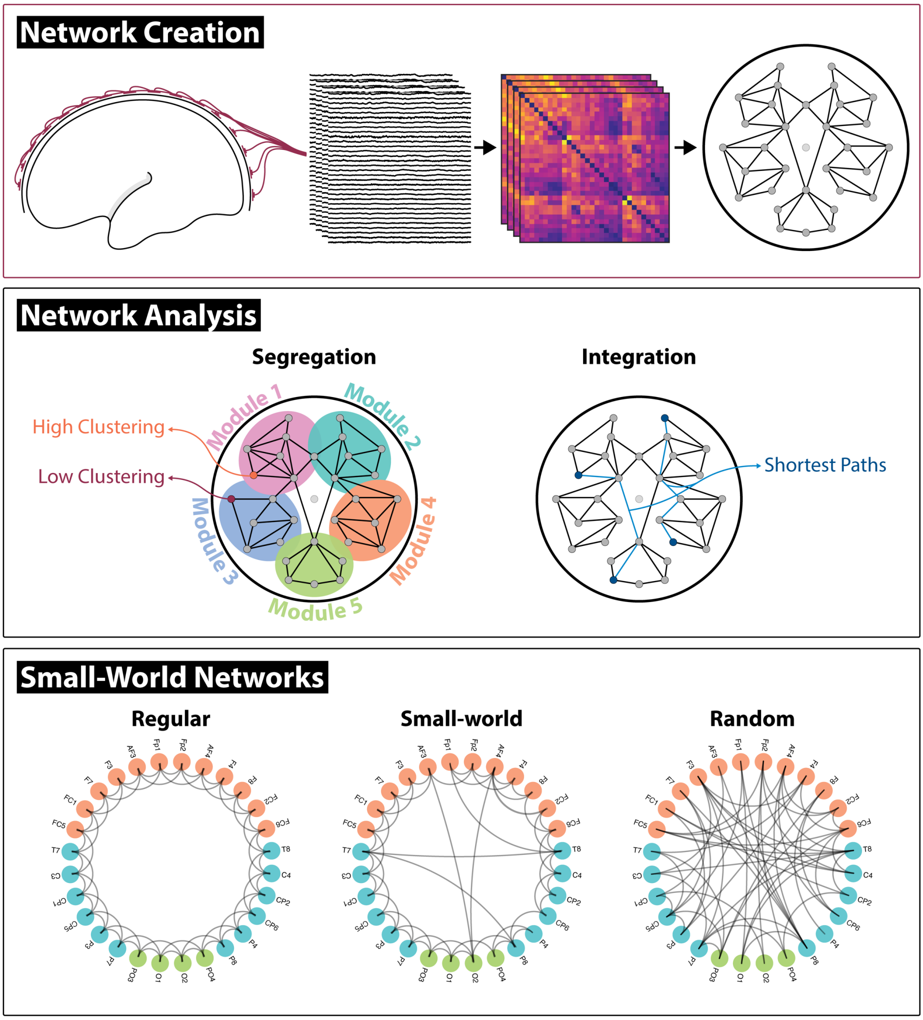

Figure 1.1 Analyzing functional EEG networks. To analyze functional networks in infants, the following steps are taken. Firstly, Network creation (panel 1) by measuring the EEG-signal, then calculation connectivity between electrode sites and storing this information in an adjacency matrix. This can be visualized as a graph. Here shown fictionalized, top down, with the electrodes being nodes and the connectivity between the electrodes being edges. In panel 2, two of the most important global characteristics of a network are visualized: segregation and integration. Networks with high integration and high segregation are small-world networks (panel 3). Here visualized as circle graphs, with neighboring nodes in the graph also neighboring in the circle graph. A small-world graph is a shown as a midpoint between a completely regular graph, with all neighboring electrodes being connected and a fully random graph.

clustering coefficient determines the tendency of interconnectedness of neighboring nodes in a network. That is to say: if a node is connected to two other nodes, how large is the chance that these other nodes are also connected? Modularity quantifies whether the network follows a modular structure. In these networks, communities of nodes show strong interconnectedness, but weak connectivity to other nodes in different communities (Newman, 2004).

In contrast to segregation, integration of a network pertains to the ability of different parts of the network to communicate easily. The most commonly used characteristics to describe this trait are characteristic path length and global efficiency. Characteristic path length is the average of all the shortest path lengths in the network, where a path is the sum of the number of edges or weights between two nodes. Global efficiency is the inverse of the characteristic path length. A network with high global efficiency and low characteristic path length has a high global transfer efficiency and integrates information easily.

These seemingly opposing network traits are vital for the optimized processing of information. On the one hand, nodes need to form cliques to optimize communication for specialization. However, the information processed by these cliques needs to be easily conveyed and integrated into the information of other communities of nodes within the network. An optimized topology of a network should therefore possess a high clustering coefficient and a relatively low characteristic path length. This topology is referred to as small-worldness and is commonly found in biological, social, and traffic networks (Watts & Strogatz, 1998). The small-worldness of a network is calculated as the ratio of the normalized clustered coefficient and the normalized characteristic path length. With a random network being used as a null network for these normalizations. Networks with a small-worldness much larger than 1 are referred to as small-world networks.

1.2 The development of the infant functional connectome

Right after birth, the infant functional connectome already shows considerable similarities with the mature connectome. Neonatal brain networks show highly efficient small-world topologies and modularity structure (De Asis-Cruz, Bouyssi-Kobar, Evangelou, Vezina, & Limperopoulos, 2015; Fransson, Åden, Blennow, & Lagercrantz, 2011; Gao et al., 2011). Gao and colleagues found among a sample of 147 sleeping infants that global efficiency and local efficiency increased over the course of the first year of life. They noted that the development of long-range connections especially increased global connectivity (Gao et al., 2011). These findings were replicated in a MEG-study by Berchicci and colleagues (Berchicci, Tamburro, & Comani, 2015), who found that the infant sensory-motor network showed marker increases in global and local efficiency throughout infancy. Both these studies underline the increase of network segregation and integration during the first year of life.

The main hypothesis for the development of connectivity during the first year of life is the "local to distributed" developmental pattern of the human brain (Fair et al., 2009; Johnson, 2000). At birth, infants' brain connectivity is mostly focused on short-range connections. The connectome is therefore highly fractured. As mentioned before, during the first year of life both global and local efficiency increase, but global efficiency makes the most improvement. Long-range connections linking distributed brain regions become strengthened. It is hypothesized that after birth environmental stimuli encourage the cooperation of many different brain areas. Therefore, increasing the need for network integration (Vértes & Bullmore, 2015).

1.3 The relationship between the functional connectome and behavior

The relationship between the infant connectome and cognition is not fully understood. Most of the current studies focus on predicting cognition later in life or predicting atypical development based on neonatal connectomes. Keunen and colleagues found that neonate structural connectivity segregation and integration could predict Performance IQ at age 5 (Keunen et al., 2017a). Wee and colleagues found that clustering coefficients in certain neonatal brain areas could be associated with internalizing and externalizing behavior in early childhood (Wee et al., 2017). From a functional point of view, most studies have looked into the connectivity from and to specific brain areas. Behavioral inhibition at two years old has been associated with default mode network connectivity in neonates (Sylvester et al., 2018). An EEG-coherence study by Kuhn-Popp and colleagues found that left-hemispheric coherence could predict individual language skills at age 4 (Kühn-Popp, Kristen, Paulus, Meinhardt, & Sodian, 2015). Lastly, functional connectivity has also been related to atypical development. Orekhova and colleagues showed that infants at risk for ASD who later get ASD show hyperconnectivity in the alpha band compared to infants at risk for ASD who do not get ASD later in life (Orekhova et al., 2014).

These results show the potential of infant brain network analysis to conceive early biomarkers for cognition and atypical behavior. However, while predicting cognition is undeniably valuable, following the development of the connectome concurrently with the development of cognition is vital to better understand how one affects the other. Complex behaviors require brain areas to effectively communicate and integrate all available information into one unified construct. Conversely, experience with complex behaviors further optimizes and enhances brain network structure through selective pruning and the enhancement of important connections. Closely following this unending feedback loop might yield valuable insight into how complex behaviors develop and what can go wrong. To study this, some requirements have to be met. In the next section, I will go over the outline of this dissertation to attempt to tackle this difficult question.

1.4 Outline of this dissertation

To relate individual developmental differences in cognition to developmental differences in the infant connectome, a large sample size of infants is needed. This could pose some issues. The infants need to be studied multiple times throughout their first year of life. As infants cannot be reliably scanned in an MRI-scanner unless they are neonates, the measurements need to be done using EEG. Measuring functional connectivity with EEG is not uncommon, but it is important to check the reliability of our measures. From previous research, we know that graph characteristics can be reliably detected based on fMRI measurements (Andellini, Cannatà, Gazzellini, Bernardi, & Napolitano, 2015; Wang et al., 2011) and EEG measurements in adults (Hardmeier et al., 2014). It is currently unclear, however, whether the same holds for infants. Infant EEG data is notoriously noisy, due to the researcher's inability to instruct the infant. Additionally, as infants will not tolerate watching a black screen or close their eyes for extended periods of time, a movie will be shown to occupy the infant. In what way this affects the network measurements is currently unknown. The detection of reliable and stable measures is vital for the measures to be used as possible biomarkers for cognition. Therefore, in **Chapter 2** we explore this question, by testing the same group of infants twice – one week apart – and comparing the found network measures.

Similar issues arise due to the size of the sample used in this dissertation. As mentioned before, infant EEG data can be noisy compared to adult data. This effect could be exacerbated due to the use of a large population of infants, as this necessitates the use of a multitude of research assistants, testing locations, and testing environments. Understanding what the influence is of these external conditions on EEG data quality is therefore essential. Additionally, the magnitude of the sample allows us to test the effect of some external factors on data quality that were until now untested. In **Chapter 3**, we, therefore, test the influence of external factors (e.g. Season, Time of testing, Research Assistant, Age) on EEG data quality. We end that chapter with recommendations that we take heed to in the remainder of the dissertation

Social behavior can be related to the infant's connectome in multiple ways. In **Chapter 4**, we study how the networks in the infant brain process social information. Here we compare EEG whole-brain network characteristics when an infant is watching a social video with when an infant is watching a non-social video. Most importantly, we analyze whether the difference between the processing of social versus non-social cues develops over time, by comparing infants of 5-months-old with the same infants at 10-months-old.

In **Chapter 5**, we study the relationship between social behavior and the infant connectome in a different way. By following the development of infant brain network integration and segregation over the first year of life and relating this development to the development of social competency and infant temperament. Cross-lagged panel models allow for the detection of whether the brain network influences behavior later in the first year or vice versa.

Finally, in **Chapter 6**, the recommendations, and issues with the use of EEG in infant connectivity research in **Chapter 2 &** 3, the outcomes of how social cues are processed by the infant brain (**Chapter 4**), and how social competency is related to the development of the human connectome (**Chapter 5**), will be discussed. Conclusions will be drawn based on the findings in this dissertation and future directions will be extensively discussed.

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CHAPTER 2

Test-retest Reliability of EEG Network Characteristics in Infants

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BV, RH, CK designed the study. Data was collected by research assistants at the KKC. BV, RH analyzed the data. BV, RH, CK interpreted the data. BV drafted the paper. BV, RH, CK revised the paper

2.1 Abstract

Functional EEG networks in infants have been proposed as useful biomarkers for developmental brain disorders. However, the reliability of these networks and their characteristics has not been established. We evaluated the reliability of these networks and their characteristics in ten-month-old infants. Data was obtained during two EEG sessions one week apart and was subsequently analyzed at delta (0.5-3 Hz), theta (3-6 Hz), alpha1 (6-9 Hz), alpha2 (9-12 Hz), beta (12-25 Hz), and low gamma (25-45 Hz) frequency bands. Connectivity matrices were created by calculating the phase lag index between all channel pairs at given frequency bands. To determine the reliability of these connectivity matrices, intra-class correlations were calculated of global connectivity, local connectivity, and several graph characteristics. Comparing both sessions, global connectivity, as well as global graph characteristics (characteristic path length and average clustering coefficient) are highly reliable across multiple frequency bands; the alpha1 and theta band having the highest reliability in general. In contrast, local connectivity characteristics were less reliable across all frequency bands. We conclude that global connectivity measures, but not local connectivity measures are highly reliable network measures in infants.

2.2 Introduction

The brain is a complex network consisting of highly interconnected regions. During early childhood, these networks develop at a rapid pace. Electroencephalography (EEG) can be used to study this early development of functional networks (Boersma et al., 2013; Orekhova et al., 2014). The high temporal resolution of EEG allows for the study of high-frequency oscillatory brain activity, while the infant is relatively unrestricted in its movements. Synchronized oscillatory activity allows for optimized flow of information between two regions (Fell & Axmacher, 2011) and therefore studying oscillatory brain activity, either at rest or during a task, gives insight in underlying functional connectivity and brain networks. Oscillatory brain activity ranges from ultraslow oscillations (0.05 Hz) to fast transient oscillations (up to 500 Hz) (Buzsáki, 2004). Infant EEG has limited functionality in the detection of high-frequency oscillations, as contamination with muscle-induced high-frequency artifacts is difficult to remove. Therefore, most developmental EEG researchers focus on slower oscillatory activity, including delta (0.1-3 Hz), theta (3-6 Hz), alpha (6-12 Hz), beta (12-25 Hz), and low gamma (25-45 Hz) bands. Functionally distinct networks can be found at these different frequency bands, which is most notably represented in the spatial scale of oscillatory synchrony, which ranges from several centimeters in slow oscillations (Schoffelen, 2005) to micrometers in ultrafast oscillations (Izhikevich, 2001).

Functional brain networks and characteristics have been used in the past to study differences between typical and atypical brain development. In autism spectrum disorder (ASD) for example, global connectivity (the averaged connection strengths of the whole brain network) tends to be deteriorated at lower frequencies, which is compensated by increased global connectivity at higher frequencies (Boersma et al., 2013; Orekhova et al., 2014; O'Reilly, Lewis, & Elsabbagh, 2017; Peters et al., 2013; Righi, Tierney, Tager-Flusberg, & Nelson, 2014). Similarly, children with attention-deficit hyperactivity disorder show an increase in frontal low alpha connectivity and a decrease in frontal high alpha connectivity (Murias, Swanson, & Srinivasan, 2007).

Comparing these networks on a global connectivity level has shown usefulness. However, to better understand the differences between these complex networks on a detailed level, a graph theoretical framework can be used (Albert & Barabási, 2002; Bullmore & Sporns, 2009), which simplifies the network into nodes (centers of information or – in the case of EEG connectivity – EEG sensors) and edges (connections between the nodes). With this mathematical approach, several metrics can be calculated describing certain aspects of a network. The most commonly used network metrics are the characteristic path length (Lw), the average clustering coefficient (Cw) and the small-worldness index (SWI). The characteristic path length is the average shortest path length between all nodes in the network. A shorter characteristic path length generally indicates a higher global efficiency in networks. The average clustering coefficient describes the number of clusters in a network. Higher clustering generally indicates higher local efficiency in networks. Small-world networks are networks in which both short path lengths and high clustering are present. As such, small-worldness is calculated as the ratio between the normalized clustering coefficient and the normalized path length. All of these characteristics have been connected to several neurodevelopmental disorders, like ASD (Peters et al., 2013; Rudie et al., 2013; Tsiaras et al., 2011) and ADHD (Ahmadlou, Adeli, & Adeli, 2012).

While these connectivity and graph measures show potential as biomarkers to detect atypical development, biomarkers are only useful if they have a low inter-subject variability and a high test-retest reliability (Hardmeier et al., 2014). Several studies have shown that this is the case for adult EEG/MEG networks (Deuker et al., 2009; Hardmeier et al., 2014; Kuntzelman & Miskovic, 2017). Whether this also holds true for infants, however, is currently unknown. For the early detection of neurodevelopmental disorders, it is especially vital that network measures are reliable during infancy. Therefore, in this study, we set out to determine the test-retest reliability and inter-subject variability for functional EEG network measures, created by task-dependent continuous EEG in infants.

2.3 Methods & Materials

2.3.1 Subjects & Procedure

77 ten-month-old infants, recruited from communal registers in the Netherlands, participated in the study. The final sample consisted of 60 infants (29 males, at first visit: mean age = 301 days, range = 272-342, at second visit: mean age = 308 days, range = 279-349). During the EEG recording infants were seated in a high chair and watched two different one-minute videos on a computer screen, three separate times. The first video depicted social stimuli with singing women as the subject, the second video depicted non-social stimuli of toys that were moving without human interference, earlier used in a study by Jones and colleagues (Jones, Venema, Lowy, Earl, & Webb, 2015). The parents/guardians received information about the study beforehand and signed an informed consent form before the start of the first session. The medical ethical committee of the University Medical Center Utrecht approved the study (application number: 14-221). Children received a toy after participation.

2.3.2 EEG Acquisition

EEG was recorded using a cap with 32 electrodes (ActiveTwo system, BioSemi) positioned according to the international 10/20 system, at a sampling rate of 2048 Hz. A Common Mode Sense (CMS) and Driven Right Leg (DRL) electrode were used to provide an active ground. In addition, two mastoid electrodes (EXG1 & EXG2) were placed behind the ears and one ocular electrode under the eye (EXG3).

2.3.3 EEG Analysis

EEG data was analyzed exclusively using Matlab, by means of the FieldTrip toolbox (Oostenveld, Fries, Maris, & Schoffelen, 2011). The original 2048 Hz data was down sampled to 512 Hz, using chip interpolation and band-pass filtered at 0.1-70 Hz with a two-way Butterworth filter. Artifacts were removed from the continuous EEG. Artifacts were defined as an absence of signal, clipping, muscle artifacts and excessive noise. Channels were removed if more than 50 percent of the signal in a channel contained artifacts. Bad channels were removed from both sessions of a subject. The cleaned data was used for further analysis.

2.3.4 Connectivity calculation

The cleaned data for each subject was bandpass filtered into 6 bands: delta (0.1-3 Hz), theta (3-6 Hz), alpha1 (6 – 9 Hz), alpha2 (9 – 12 Hz), beta (12 – 25 Hz), and gamma (25 – 45 Hz). Since individual theta and alpha peaks are influenced by development, alpha1 and theta bands were chosen to encompass all theta and alpha peaks +/- 1 Hz. The resulting data was cut into 5s. epochs. 20 random epochs were picked per subject per session. For each epoch, connectivity between pairs of electrodes (32*31/2 = 496) was calculated with the phase lag index (PLI) and the debiased weighted PLI, both relying on the same principle of phase locking or phase synchrony (Tass et al., 1998). The phase lag index (PLI), proposed by Stam et al., (Stam, Nolte, & Daffertshofer, 2007), describes the asymmetry of the distribution of phase differences between pairs of signals:

$$\text{PLI} = \left|\langle sign\left[\sin\left(\Delta\varphi(tk)\right)\right]\rangle\right| \ ,$$

where $\Delta\varphi$ is the instantaneous phase difference between signals at time point t for k = 1 ... N per epoch (N = 5*512 = 2560), determined using the Hilbert transformation. || stands for absolute values, <> for the mean values and the sign for a signum function (phase difference is either -1, 0, or 1). The resulting PLI can range from 0 to 1. Volume conductance, the effect that multiple electrodes register activity from the same source, plays a minimal role in the PLI. Activity from a single source will appear in both electrodes as having a phase difference of exactly zero. Since the PLI indexes the stability of phase leaping or lagging, a phase difference of zero will lead to a PLI of zero.

The debiased weighted (dwPLI) PLI is an adjustment of the PLI developed by Vinck and colleagues (Vinck, Oostenveld, van Wingerden, Battaglia, & Pennartz, 2011). The PLI is weighted by the amount of lag between the two signals, thereby limiting the influence of near zero phase differences. This minimizes the amount of false positive connectivity between near zero phase difference signals, which could be caused by noise in the data. Since infant data is notorious for its noisiness, the dwPLI is included as well. Our used version of the weighted PLI also debiases the connectivity based on the number of epochs, since infant data likely involves few trials. This debiasing can cause the dwPLI to be negative and, therefore, ranges from -1 to 1.

2.3.5 Graph Analysis

Several graph measures were calculated using the acquired individual connectivity matrices. The complete weighted matrices were used, eliminating the need for arbitrary thresholds. The following graph measures were calculated using the brain connectivity toolbox (Sporns & Rubinov, 2010) (table 2.1): average clustering coefficient (Cw), characteristic (average shortest) path length (Lw); and small-worldness index (SWI, calculated as the ratio between normalized Cw and normalized Lw). Both the averaged clustering coefficient and the characteristic path length are normalized to limit the influence of global connectivity on these characteristics.

Table 2.1 Graph measures references and formulae

Name		Formula	Reference
Average clustering coefficient	Cw	$\mathrm{Cw} = \frac{1}{n}\sum_{i\in N}\frac{2t_i}{k_i(k_i-1)}$	(Onnela, Kaski, & Kertész, 2004)
Characteristic path length	Lw	$\mathrm{Lw} = \frac{1}{n}\sum_{i\in N}\frac{\sum_{j\in N, i\neq j} d_{ij}}{n-1}$	(Dijkstra, 1970)
Small-worldness Index	SWI	$\mathrm{S} = \frac{\mathrm{C}/\mathrm{C}_{\mathrm{rand}}}{\mathrm{L}/\mathrm{L}_{\mathrm{rand}}}$	(Watts & Strogatz, 1998)

2.3.6 Statistical Analysis

The test-retest reliability was determined differently across three different steps of the analysis (figure 2.1). At the most basic level (step 1, figure 2.1a), the complete connectivity matrices were compared over sessions by calculating the Pearson's correlation coefficient (R). The reliability of the connectivity measures of steps 2 and 3 (figure 2.1b and c) were calculated by comparing sessions through an intra-class correlation (ICC) (Shrout &

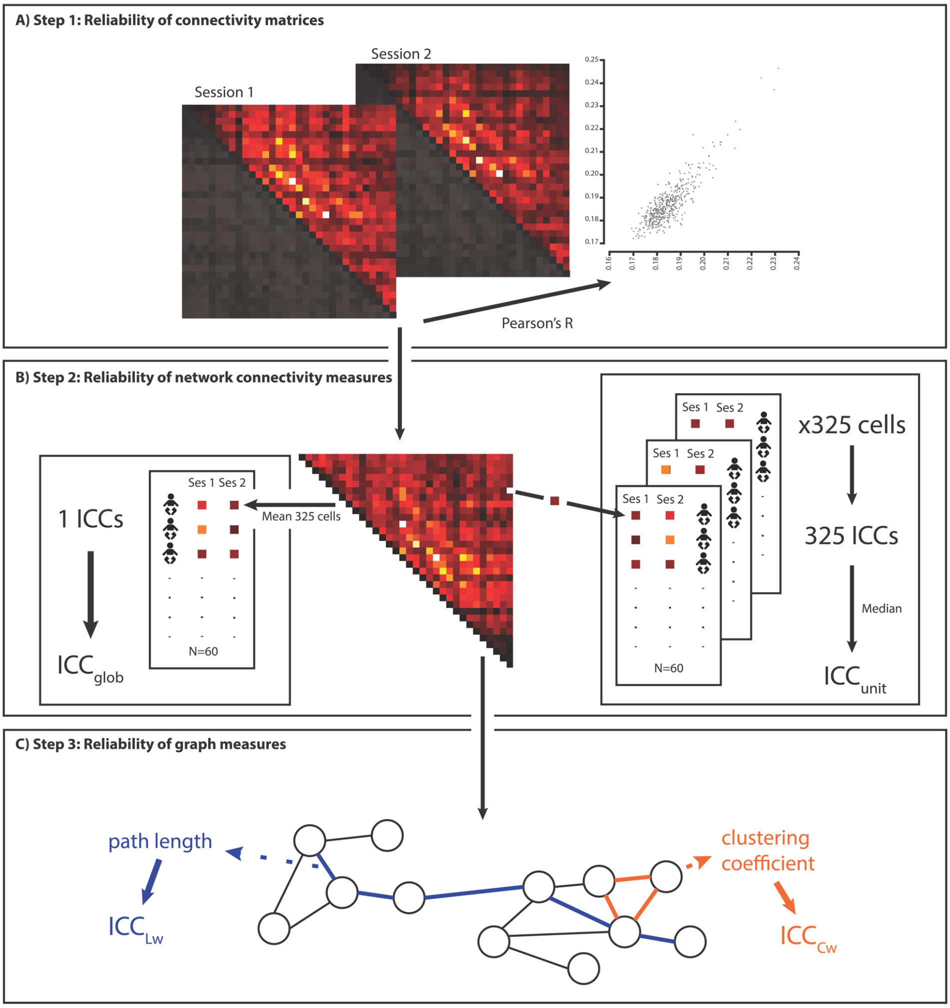

Figure 2.1 Overview of the different steps in network analysis and their respective reliabilities. This figure shows the complete steps of network analysis and graphically depicts the reliabilities calculated for each step. A) reliability at the most fundamental level, in which connectivity matrices are correlated over sessions for each subject, for each frequency band. B) reliability of global (left) and local, 'unit-wise' (right), connectivity. C) graph theoretical representation of the network and several graph characteristics, which are compared over sessions.

Fleiss, 1979; Weir, 2005), which uses a one-way ANOVA to determine the mean squared error (MS_e) and the between object (subject) variance (MS_r). Shrout & Fleiss (1979) describe six distinct statistical models which carry the name, of which we are using an ICC(3,1) two-way mixed effect model, similar to other studies on the reliability of graph measures (Hardmeier et al., 2014; Hatz et al., 2016). ICC values were calculated using:

$$\mathrm{ICC} = \frac{\mathrm{MS_r} - \mathrm{MS_e}}{\mathrm{MS_r} + (\mathrm{k} - 1)\mathrm{MS_e}}\ ,$$

where k is the number of measurements per subject.

We assessed the reliability of both global and local (dw)PLI connectivity matrices (step 2, figure 2.1b). The global PLI/dwPLI (ICC-$_{\mathrm{glob}}$) was calculated by averaging over all 325 electrode pairs of each subjects' matrix, creating one value per subject per frequency band per session. A single ICC value per frequency band was calculated by comparing session 1 vs session 2. The local PLI/dwPLI unit-wise reliability was determined by calculating an ICC value per electrode pair over all subjects' session 1 vs session 2, creating 325 ICC values. Since these values did not follow a normal distribution, the median was taken as the single reliability value ($\mathrm{ICC_{unit}}$). To summarize, the reliability of the global PLI/dwPLI is the reliability of all connections combined, while the unit-wise reliability is the median reliability of all the reliabilities of individual connections. To test the influence of noisy connections with low connectivity, an average connectivity matrix was calculated based on all connectivity matrices from both sessions. The top 25th percentile of connections were selected based on connectivity strength and the unit-wise reliability calculation was performed using only these connections for each subject (Guo et al., 2012).

To test the reliability of the graph measures (Cw, Lw, and SWI), values were calculated for each subject, per session, per frequency band (step 3, figure 2.1c). An ICC was used to calculate the reliability of these graph measures over sessions. In accordance to previous research on graph metrics, we report ICC values below 0.4 as low reliability, $0.4 < \mathrm{ICC} < 0.6$ as mediocre reliability, $0.6 < \mathrm{ICC} < 0.75$ as good reliability and an ICC of 0.75+ as excellent reliability (Hardmeier et al., 2014; Jin, Seol, Kim, & Chung, 2011). To understand the effect of outliers, a bootstrapping procedure with replacement and 10000 permutations was used to estimate the 95% confidence intervals for both COV and ICC values, similarly used by Hardmeier and colleagues (Hardmeier et al., 2014). For a clear overview of the reliability tests, please refer to figure 2.1. Lastly, for both the connectivity and graph measures the inter-subject variability was determined using the coefficient of variation (ratio between mean and standard deviation).

2.3 Results

2.3.1 Reliability of connectivity matrices

The results of the correlation of the connectivity matrices across sessions are presented in figure 2.2. Correlation coefficients range widely and the median of the coefficients is generally low. There is little difference between the reliability of dwPLI and PLI connectivity matrices, showing ranges of respectively 0.1 - 0.37 and 0.03 - 0.33.

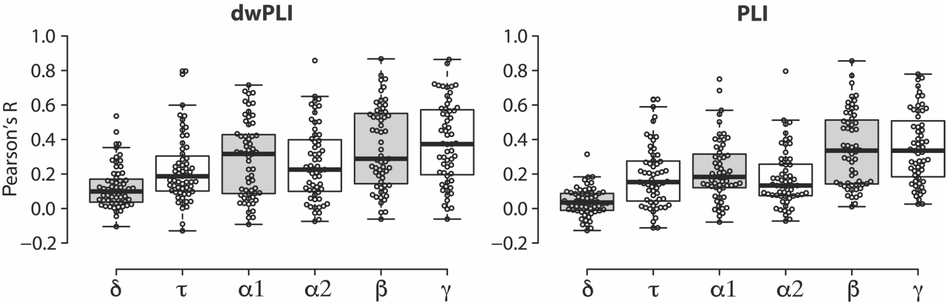

Figure 2.2 Connectivity matrix correlation coefficients for all frequency bands. Boxplot of all individual connectivity matrix correlations for session 1 vs session 2, shown for delta (d), theta (t), alpha1 (a1), alpha2 (a2), beta (b), and gamma (g). The left graph shows the correlation coefficients for the connectivity matrices calculated with the dwPLI, the right graph shows the PLI calculated connectivity matrices. Correlations range widely, but the median of the correlations within each frequency band is low. Plotted with BoxplotR (Spitzer, Wildenhain, Rappsilber, & Tyers, 2014)

2.3.2 Reliability of network connectivity measures

Inter-subject variability (table 2.2) of global PLI was low for all frequency bands ($0.02 < \mathrm{COV}_{\mathrm{glob}} < 0.12$). Reliability of global PLI (table 2.3) was excellent for theta, alpha1, and alpha2 frequency bands ($0.84 < \text{ICC}_{\text{glob}} < 0.91$), mediocre to good for delta, gamma and beta frequencies ($0.60 < \text{ICC}_{\text{glob}} < 0.72$). Global PLI values at session 1 differed significantly between frequencies (F: 772, $p<0.00001$). All frequency bands differed significantly from each other ($p<0.00001$), except for alpha1 and alpha2 global connectivity values ($p = 0.11$).

Compared to global PLI, inter-subject variability of global dwPLI (table 2.2) was higher ($0.91 < \text{COV}_{\text{glob}} < 1.15$) and reliabilities were lower, with theta, alpha1, alpha2 frequencies showing good to excellent reliability ($0.75 < \text{ICC}_{\text{glob}} < 0.91$) and delta, beta, and gamma frequencies having a poor reliability ($-0.29 < \text{ICC}_{\text{glob}} < 0.74$) (table 2.3). Also, note the wider 95% confidence intervals for the dwPLI calculated global connectivity. Therefore, dwPLI is excluded from this point onwards in the results to prevent misinformation.

The reliability of local, unitwise, PLI connectivity was lower than global PLI, with the median ICC showing mediocre to good reliability in the theta and alpha1 frequency band ($0.50 < \text{ICC}_{\text{unit}} < 0.62$) and delta, alpha2, beta and gamma frequency bands showing poor reliability ($0.07 < \text{ICC}_{\text{unit}} < 0.27$). The reliability of unit-wise connectivity improved considerably when using the 25th top percentile of the on average strongest

Table 2.2 Inter-subject variability of global connectivity with 95% confidence intervals

		Delta	Theta	Alpha1	Alpha2	Beta	Gamma
PLI		**0.02**	**0.12**	**0.09**	**0.05**	**0.05**	**0.05**
	95% CI	0.02-0.03	0.09-0.15	0.08-0.11	0.02-0.06	0.04-0.06	0.03-0.06
dwPLI		**0.92**	**1.11**	**0.73**	**1.15**	**0.76**	**0.91**
	95% CI	0.47–1.18	0.89-1.33	0.60-0.89	0.64-1.42	0.60-0.89	0.64-1.11

Table 2.3 Test-retest reliability of global connectivity with 95% confidence intervals

		Delta	Theta	Alpha1	Alpha2	Beta	Gamma
PLI		**0.60**	**0.91**	**0.84**	**0.86**	**0.72**	**0.61**
	95% CI	0.38 - 0.73	0.82 - 0.95	0.71 - 0.92	0.61 - 0.93	0.52 - 0.83	0.32 - 0.74
dwPLI		**-0.29**	**0.82**	**0.75**	**0.91**	**0.74**	**0.49**
	95% CI	-1.51-0.11	0.69 - 0.90	0.54 - 0.87	0.39 - 0.97	0.47 - 0.88	0.28 - 0.73

connections over both sessions (figure 2.3A), with both theta and alpha1 having good reliability ($0.62 < ICC_{unit} < 0.73$), alpha2, beta and gamma having mediocre reliability ($0.41 < ICC_{unit} < 0.48$). Delta local connectivity reliability is still poor ($ICC_{unit} = 0.18$). The distribution of unit-wise reliability showed a considerable spread in ICC values. All frequency bands were skewed towards the higher ICC values, with alpha1 and theta frequency bands being most pronounced (figure 2.3B).

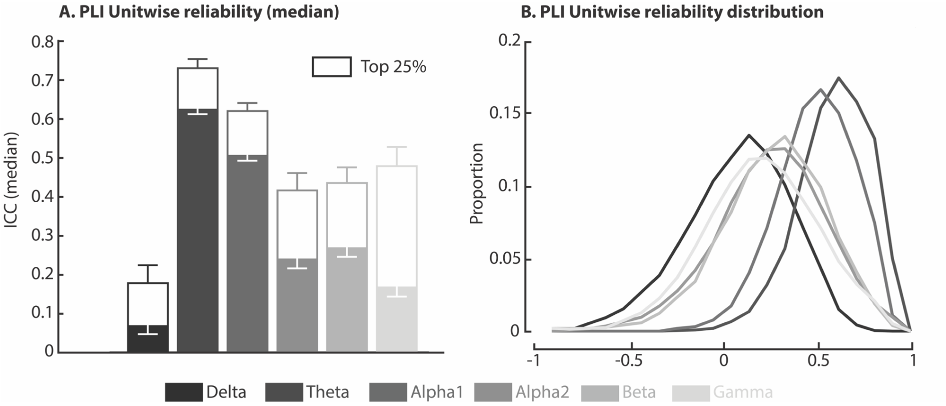

Figure 2.3. Reliability of unit-wise PLI. A) Bar plot of ICC values for unit-wise reliability per frequency band with theta and alpha1 showing the highest reliability. All frequency bands show marked improvement when only the on average strongest 25% of connections are used. Errorbars represent 2SE. B) Distribution of ICC values for all frequency bands. All frequency bands show a distribution skewed towards the positive ICC values. This is most pronounced in the theta and alpha1 frequency band.

2.3.3 Reliability of graph measures

Table 2.4 shows the reliability of graph measures calculated from the PLI matrices. PLI average clustering coefficient (Cw) was excellently reliable across alpha1, alpha2 and theta frequency bands ($0.84 < ICC_{Cw} < 0.91$) and was mediocre to good in reliability in delta, beta and theta frequency bands ($0.59 < ICC_{Cw} < 0.73$). Lw_{nrm} showed excellent reliability across theta, alpha1, apha2, and gamma frequency bands ($0.84 < ICC_{Lw} < 0.89$) and mediocre to good reliability in delta, theta, and beta frequency

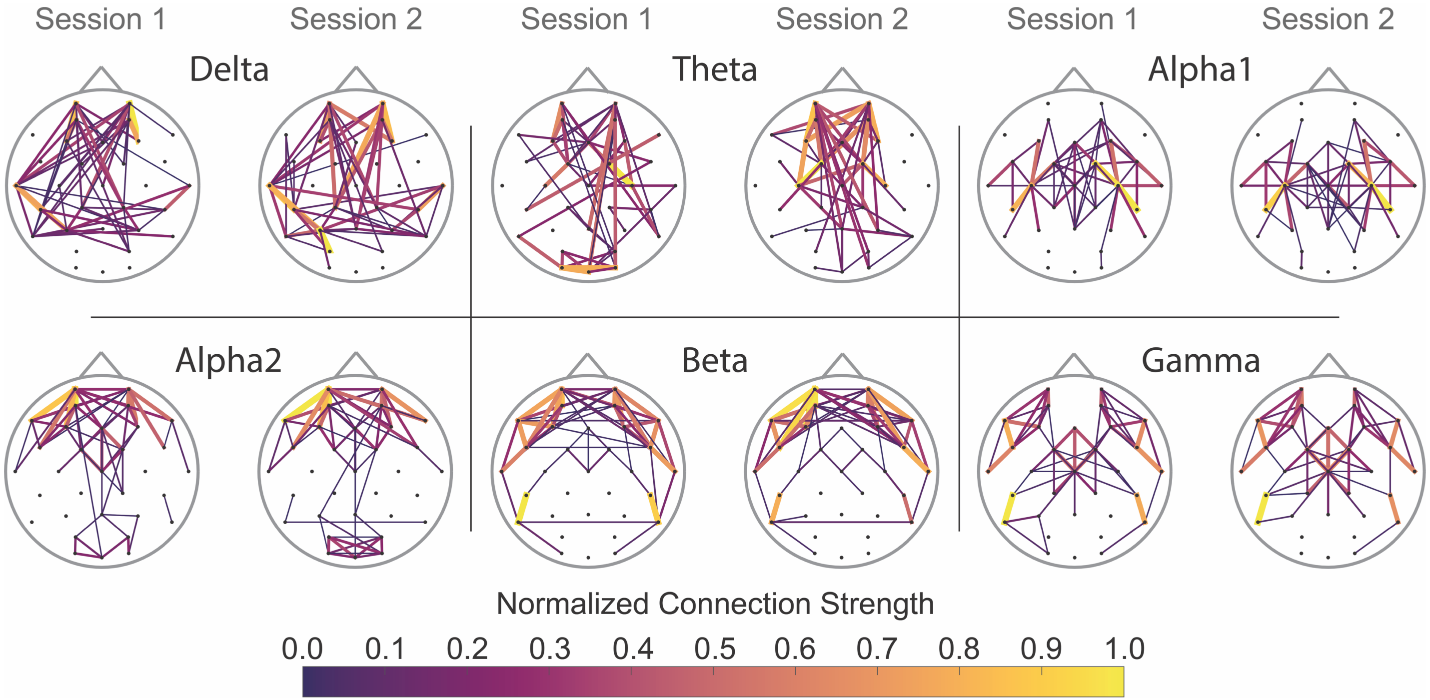

Figure 2.4 Average connectome per frequency band per session. The averaged connectomes with the 12 percent strongest connections show great similarity between sessions. Yellow, thicker lines depict higher normalized connection strength, blue, thinner lines depict lower normalized connection strength. Note the higher dependency on long range connections in slower oscillations.

bands ($0.53 < \mathrm{ICC}_{\mathrm{Cw}} < 0.72$). The small-worldness index (SWI) was least reliable, with mediocre reliability in theta and alpha1 frequency bands ($0.56 < \mathrm{ICC}_{\mathrm{SWI}} < 0.67$) and poor reliability in the delta, alpha2, beta, and gamma frequency bands ($0.13 < \mathrm{ICC}_{\mathrm{SWI}} < 0.25$. During session 1, not all networks showed small-worldness (range: $0.\ 9869 < \mathrm{SWI} < 1.02$). Average connectomes were created for both sessions for all frequency bands, which shows a strong similarity in strongest connections and connection strength between session 1 and 2 (Figure 2.4).

Table 2.4 ICC Reliability of PLI graph measures

		Delta	Theta	Alpha1	Alpha2	Beta	Gamma
$\mathrm{Cw}_{\mathrm{nrm}}$		**0.59**	**0.91**	**0.84**	**0.87**	**0.73**	**0.62**
	95% CI	0.32-0.73	0.81-0.95	0.81-0.91	0.63-0.93	0.53-0.84	0.37-0.75
$\mathrm{Lw}_{\mathrm{nrm}}$		**0.53**	**0.89**	**0.84**	**0.84**	**0.72**	**0.59**
	95% CI	0.19-0.71	0.79-0.94	0.72-0.92	0.63-0.92	0.53-0.84	0.33-0.75
SWI		**0.25**	**0.56**	**0.67**	**0.21**	**0.14**	**0.13**
	95% CI	-0.02-0.54	0.36-0.73	0.40-0.83	-0.47-0.71	-0.07-0.34	-0.10-0.49

2.4 Discussion

In this paper, we showed for the first time that infant functional brain network characteristics can be reliable, by determining the test-retest reliability and the inter-subject variability of infant functional EEG connectivity across a 1-week period. Overall, reliabilities of global connectivity characteristics were high, while more local characteristics showed lower, though still acceptable reliabilities. Characteristics calculated with the connectivity matrices of theta and alpha1 frequency bands were most reliable. This pattern of reliability is similar to earlier studied reliability of adult network characteristics.

Broadly, the reliability of EEG networks can be assessed on three levels, which coincide with three steps of network analysis: The reliability of 1) the complete connectivity matrices, 2) global and local functional connectivity measures gathered from these matrices and, 3) graph characteristics gathered from the graphs created from these matrices. Firstly, we reported that connectivity matrices correlate poorly over sessions. Reliabilities of complete connectivity matrices have, to our knowledge, never been reported for EEG networks in adults nor infants. It is thus difficult to compare our reliabilities to other studies. Most other studies focus on the reliability of steps two and three of connectivity analysis: global and local connectivity measures; and graph characteristics.

Secondly, we found excellent test-retest reliability of global connectivity, the average of all connections in a connectivity matrix. Local, unit-wise, reliability showed a wide range of test-retest reliabilities across most frequency bands, ranging from connections with very low reliability to connections with excellent reliability. This is in concurrence with several adult MEG/EEG reliability studies. Hardmeier and colleagues reported excellent global connectivity reliability in their eyes-closed resting state EEG study, in theta, alpha1, and alpha2 frequency bands, while local inter-regional connectivity ranged from poor to excellent across all frequency bands (Hardmeier et al., 2014). Deuker and colleagues found good test-retest reliability for MEG global connectivity during eyes-open resting state and excellent reliability during an n-back task in theta and alpha frequency bands (Deuker et al., 2009). Lastly, Jin and colleagues found moderate to high test-retest reliability in eyes-open and closed MEG resting state global connectivity, in theta and alpha frequency bands (Jin et al., 2011).

Thirdly, the reliability of global first order graph metrics tested in this study ranged from moderate to excellent, with both average clustering coefficient (Cw) and characteristic path length (Lw) being excellently reliable across theta, alpha1 and alpha2 frequency bands. This is also found in other EEG network reliability studies. Previously mentioned Hardmeier and colleagues also tested the reliability of graph metrics and found excellent reliabilities for both Cw and Lw in theta, alpha1 and alpha2 bands (Hardmeier et al., 2014). More recently, Kuntzelman & Miskovic tested adults during an eyes-closed resting state EEG paradigm, comparing global and local graph measures on coherency and dwPLI. They reported good reliability of global dwPLI metrics in theta, alpha1 and alpha2 frequency bands (Kuntzelman & Miskovic, 2017).

Across the study, we report lower reliabilities for delta, beta and gamma frequency bands than for theta, alpha1, and alpha2 frequency bands. This is in concurrence with several previously mentioned studies in which lower beta and gamma reliabilities (Hardmeier et al., 2014; Jin et al., 2011; Kuntzelman & Miskovic, 2017); and lower delta reliabilities (Deuker et al., 2009; Kuntzelman & Miskovic, 2017) were found. Most commonly, the lower reliability of higher frequency bands is explained by the dichotomy between higher and lower frequency bands, where higher frequency bands are more involved in establishing cognitive representation, while lower frequencies are more anatomically constrained (Bassett & Bullmore, 2006). This constraint could aid higher reliabilities over sessions. Also, both theta and alpha have been suggested to be important for processing attention (Aftanas & Golocheikine, 2001; Klimesch, Doppelmayr, Russegger, Pachinger, & Schwaiger, 1998) and top down control (Engel, Fries, & Singer, 2001). Since our task could specifically target these systems, the resulting higher signal to noise ratio in these frequency bands could result in more reliable networks. Lastly, the higher prevalence of muscle artifacts in the higher frequency bands could limit reliability, especially in children. The small-worldness index (SWI) is also less reliable in our study, which is in concurrence with previous studies (Hardmeier et al., 2014; Kuntzelman & Miskovic, 2017). Since small-worldness is calculated using both clustering coefficient and path length, and both these characteristics vary independently across sessions, a combination of these variances in the SWI (SWI) could contribute to a lower reliability for the SWI.

The overall spatial resolution has a large influence on test-retest reliability with global connectivity characteristics being highly reliable, while local connectivity characteristics are somewhat less reliable. This study also shows that different steps of the analysis yield different reliabilities. Most interestingly, lowly reliable connectivity matrices generate highly reliable connectivity and graph characteristics, which can be explained in several ways. Firstly, it is possible that some lowly connected, noisy connections are present in the full connectivity matrices, which are averaged out in global connectivity characteristics. Secondly, brain networks fluctuate in activity over time (Chang & Glover, 2010). It is possible that, comparing multiple sessions, the state of the network is different, but the underlying characteristics and anatomy are equal. Thirdly, a difference in fixing the EEG cap over sessions could lead to a rotation in connectivity matrices over sessions (Hatz et al., 2016) and lastly, an unknown covariate, that remains stable over sessions, could influence network characteristics, but not connectivity matrices. It is, currently unknown which of these explanations (or a combination of these explanations) is correct and future research is needed to further understand the relationship between unreliable connectivity matrices and reliable connectivity characteristics.

It is important to note that reliability does not imply validity and that this study, therefore, does not allow conclusions on the validity of these measures. It is currently unknown how tightly these measures reflect true cortical and subcortical brain connectivity. This becomes more difficult with EEG, which is restricted to measuring activity at the sensor level. While resting state oscillations have been found to be connected to resting-state connectivity gathered from functional MRI data (Laufs, 2008; Mantini, Perrucci, Del Gratta, Romani, & Corbetta, 2007), in our study, due to the difficulty of doing resting-state research with infants, we opted for a continuous video stimulus. While this makes it more difficult to understand how these network characteristics are reflected in the structural connectome, it comes with the added benefit of minimizing the variance over sessions, thereby possibly improving reliability. This is also reflected in the study of Deuker and colleagues, where task-dependent connectivity measures were shown to be more reliable than resting state connectivity measures (Deuker et al., 2009). In addition, previous research has shown the influence of global connectivity on both characteristic path length and average clustering coefficient. Therefore, the high reliability of both these metrics in this study could be explained through the high reliability of global connectivity. Even normalizing these graph metrics does not completely eradicate this problem and future research is therefore necessary to understand the exact implications of this (van den Heuvel et al., 2017).

While the validity of these measures can be disputed, previous research has shown the potential of network characteristics as biomarkers of neurodevelopmental disorders. Orekhova and colleagues found that while comparing infants at risk for ASD, global connectivity was related to whether or not an infant actually developed ASD (Orekhova et al., 2014). Boersma and colleagues found similar results when comparing toddlers with ASD to toddlers without ASD (Boersma et al., 2013). Others have noted differences in graph characteristics in adults suffering from ASD (Belmonte et al., 2004) and ADHD (Ahmadlou et al., 2012). This, together with the here reported excellent reliability of graph and connectivity measures in theta, alpha1 and alpha2 frequency bands in infants, underlines the potential of using these measures to detect neurodevelopmental disorders at an earlier age, conceivably increasing our fundamental knowledge on how these disorders develop and could possibly be treated.

2.5 Conclusions

This study showed for the first time that global and to a lesser extent local PLI connectivity measures in infants are reliable over a one-week period. We recorded EEG from infants twice, one week apart, while they were watching social and non-social videos. We found that when comparing the resulting PLI networks, global network measures are stable over time. Reliable global network measures could play a vital role in finding biomarkers for several disorders. The unrestrictive nature and the relative ease of an EEG recording make it especially useful to detect these network characteristics at a very young age, giving us important insight in the development of these disorders, possibly making early detection and intervention possible.

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CHAPTER 3

Limiting Data Loss in Infant EEG: Putting hunches to the test

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BV, CJ designed the study. Data was collected by BV & research assistants at the KKC. BV analyzed the data. BV, CJ interpreted the data. BV, CJ drafted the paper. BV, CJ revised the paper

3.1 Abstract

EEG is a widely used tool to study the infant brain and its relationship with behavior. As infants usually have small attention spans, move at free will, and do not respond to task instructions, attrition rates are usually high. Increasing our understanding of what influences data loss is therefore vital. The current paper examines external factors to data loss in a large-scale on-going longitudinal study (the YOUth project; 1279 five-month-olds, 1024 ten-months-olds, and 109 three-year-olds). Data loss is measured for both continuous EEG and ERP tasks as the percentage data loss after artifact removal. Our results point to a wide array of external factors that contribute to data loss, some related to the child (e.g., gender; age; head shape) and some related to experimental settings (e.g., choice of research assistant; time of day; season; and course of the experiment). Data loss was also more pronounced in the ERP experiment than in the EEG experiment. Finally, evidence was found for within-subject stability in data loss characteristics over multiple sessions. We end with recommendations to limit data loss in future studies.

3.2 Introduction

Infancy is a developmental period that marks clear changes in behavior and the brain. Electroencephalography (EEG) is an oft-used method to study these changes during infancy for two reasons. The high temporal resolution of EEG allows researchers to study closely linked brain-behavior correspondences. That is, to study whether and when infants can perceive contrasts between certain stimuli, researchers rely on changes in the EEG signal as a proxy for changes in behavior. Also, EEG can be used in an easy-going and non-threatening environment while infants are not required to make overt responses or to follow task instructions. Consequently, infant EEG research has a long history dating back to the 1930s when the development of sleep and awake EEG rhythms was studied in infants (Smith, 1938).

With the advance of neuroimaging techniques, the last decades saw an additional increase in studies relying on infant EEG (Azhari et al., 2020; Reid, 2012). However, measuring EEG in infants is not a straightforward task, and comes with its own challenges, often resulting in high attrition rates (Noreika et al., 2020; Stets et al., 2012). Fortunately, there is literature focusing on explaining and improving the methodology (cf. e.g., Bell & Cuevas, 2012; De Haan, 2007; Stets, Stahl, & Reid, 2012; Thierry, 2005). Our article aims to attribute to this literature by examining how factors beyond paradigm-specific parameters contribute to data attrition in infant EEG studies. Before we elaborate on the methodology of infant EEG, we will give a short overview of how EEG data can be used to better understand the developing brain.

Infant EEG researchers can derive several measures from the EEG signal. One of the most commonly used measures is the Event-related potential (ERP). In ERP paradigms, subjects are presented with certain types of stimuli numerous times while EEG is recorded. The ERP represents the averaged brain activity patterns to one type of stimuli within a short time window, beginning at the onset of a stimulus ('time-locked'). Researchers then compare ERPs of different types of stimuli to understand whether infant brains can differentiate between different stimuli. With this paradigm, one can, for instance, observe whether and when infants can distinguish faces from other objects. This has been widely studied in adults, who show distinct differences in the peak around 170ms after stimulus onset (N170) (Kanwisher et al., 1997). A similar peak is found in infants but slightly delayed after stimulus onset. Six-month-olds show differences in facial and object processing 290ms after stimulus onset (Haan et al., 2002; Halit et al., 2004), which indicates that while infants do show distinct differences in the processing of faces versus other objects, it is not fully matured. This finding encapsulates the promise of using ERP as a behavioral proxy but is certainly not the only field for which ERPs have been used. ERPs have also been extensively used to study, among others, the development of language (Junge et al., 2012; Kuhl, 2010; Peña et al., 2010), emotions (de Haan et al., 2004; Leppänen et al., 2007), and joint attention (Kopp and Lindenberger, 2011; Striano et al., 2006).

The study of brain waves is a different EEG measure which specifically exploits the high temporal resolution of EEG (oscillations). Studying the oscillatory patterns of brain activity follows the hypothesis that synchronized oscillatory activity allows for an optimized flow of information between two regions (Fell and Axmacher, 2011). Therefore, it is likely that areas in the brain exhibiting similar oscillatory activity patterns are communicating or allowing for communication. These oscillatory activity patterns oscillate in functionally distinct frequency bands. For EEG, the frequency bands are (in order from low to high-frequency oscillations) the delta, theta, alpha, beta, and gamma frequency bands. In infants, EEG power analysis has been used to better understand, among others, the development of working memory (Bell and Wolfe, 2007), joint attention skills (Mundy et al., 2000), and motor development (Cuevas et al., 2014). More recently, EEG oscillatory information along with network analysis has been used to study the development of infant brain connectivity. Global network strength (the average connectivity in the whole brain), for example, has been related to autistic spectrum disorder symptoms: results show that infants who develop autism later in life exhibit higher global connectivity in theta and alpha frequency bands (Orekhova et al., 2014), while toddlers show lower global connectivity in the beta frequency band (Boersma et al., 2013). These global network measures are reliable in infants (van der Velde et al., 2019).

The aforementioned studies shortly sketch the breadth of possibilities in using EEG to study the infant brain and its development. One of the downsides in using EEG in infants, however, is the high rate of attrition. As it may be time-consuming and costly to recruit infants and their parents, high attrition rates might contribute to the fact that many infant (EEG) studies are underpowered (Bell and Cuevas, 2012; Frank et al., 2017; Noreika et al., 2020), increasing the likelihood of drawing false conclusions or resulting in non-replicable findings (Button et al., 2013). There are several reasons why attrition is high in studies testing infants. Awake infants cannot be instructed to remain attentive over the full course of an experiment, and the earlier the experiment is terminated before completion (because an infant will start crying, refuses to sit still, or falls asleep), the more likely an infant becomes excluded from further analysis. Of course, high attrition rates are found in any type of research involving awake infants (Frank et al., 2017; The ManyBabies Consortium, 2019). However, EEG seems to have especially high rates of attrition even when compared to other infant study designs (Stets et al., 2012), with an average attrition rate of 49.2 percent based on 149 ERP articles published between 1990 and 2010.

This begs the question: what causes such high attrition factors in infant EEG studies? Ideally, future studies can use such information to minimize attrition rates during recording. Previous studies have aimed to shed some light on this question and determined several important factors that influence attrition rates. A recent meta-analysis comparing different paradigms on their attrition rates revealed that task-specific factors are a major influence, with auditory and audio-visual ERP studies resulting in markedly lower attrition rates than studies yielding visual ERP studies (Stets et al., 2012). Also, child characteristics partly explain the likelihood of attrition. For instance, infant temperament plays a role, with infants who are exhibiting more negative temperament showing higher rates of attrition (Marshall et al., 2009).

Additionally, Slaughter and Suddendorf found infant age to predict infant fussiness, with younger infants showing higher rates of fussiness, which they attributed to younger infants being more likely to fall asleep during the experiment (Slaughter and Suddendorf, 2007). While infant fussiness is a major reason to exclude infants in any kind of infant research, there is reason to believe that EEG recordings might place an additional burden on children's willingness to complete the task: Some infants do not tolerate the designated headgear (net or cap) while other infants lose the required attention due to the repetitive nature of EEG paradigms (Slaughter and Suddendorf, 2007).

This additional burden on infants in the case of EEG causes trials to be contaminated by artifacts caused by movement, absence of data, or tiredness. To obtain a sufficient number of trials one solution might be to prolong experiments, thus anticipating data loss. However, ERP components can alter due to habituation processes (Stets and Reid, 2011). Moreover, it is important to note that there is no 'golden standard' in what the field considers the minimum number of clean trials required for a reliable. It varies from paradigm to paradigm and is also dependent on the variable of interest. For instance, there is evidence that an ERP-component related to visual attention ('Nc-component') is already visible in 7 trials (Stets & Reid, 2011), while a modulation of the ERP-component related to auditory processing ('mismatch-negativity') might require over 100 trials (Cheour et al., 1998). Therefore, prolonging experiments is not always ideal, and, as such, it is important to limit data loss from the onset in any experiment.

3.2.1. The current study: motivations and goals

This study aims to improve the process of infant EEG data collection by extending the literature on data attrition described in the previous paragraphs. Most of the previous work into origins of data loss compared attrition rates over various smaller studies, through (meta)-reviewing. In this study, we will focus on one large longitudinal study to examine factors that vary between individuals and which possibly contribute to data loss. This allows us to study relationships between factors that differ for each infant and their respective data loss. Some of these analyses aim to replicate previous findings while others serve to confirm researchers' hunches to ideal circumstances of testing. To examine such effects, each factor needs to have enough variation to warrant further inspection. As such, large-scale studies are required since these studies invariably show high variability in testing conditions and environments, as keeping these steady over extended periods of time is impossible.

The large-scale study used here is the YOUth study (Onland-Moret et al., 2020). YOUth fits our requirements for various reasons. It consists of two separate EEG-experiments – an ERP-study on face discrimination and a continuous video EEG-experiment on social versus non-social discrimination. Therefore, the YOUth study allows us to compare the effects of factors on attrition rates in two different tasks, one visual and one audio-visual, thus assessing whether any observed factor is viable across tasks (i.e., generalizable to other tasks) or whether it is task-specific. Additionally, the YOUth study is a longitudinal study, with infants visiting multiple times between the age of 5 months and 6 years. The study is on-going and aims to include 3000 children. At the time of writing, we have included 1279 five-month-olds, 1024 ten-months-olds, and 109 three-year-olds. This allows us to not only study the effects of a wide range of external factors on data loss but also enables us to assess whether longitudinal effects are working on attrition (e.g., some children are more prone to data loss than others).

This large-scale study will be used to determine whether data loss can be predicted based on several external factors. We use data loss as the dependent variable here, as the failure to meet the requirement to have a certain number of clean trials is one of the foremost reasons for attrition. What is important to note, however, is that high data quality does not equal low data loss. As mentioned earlier, in some ERP paradigms, a low amount of trials at the start of the experiment can provide similar or better results than when too many trials are used (Stets and Reid, 2011). Nevertheless, most experiments are constructed in such a way to yield a reasonable number of trials within a reasonable amount of testing. Of course, it remains questionable what is reasonable: there is no golden standard in the minimum number of trials. Nevertheless, at least for continuous EEG paradigms, the main assumption is that more clean data leads to higher reliability (Fraschini et al., 2016).

In the current paper, we examine various factors possibly related to data loss. Our factors of interest can be categorized into three groups. First, we focus on factors related to the infant, namely the gender, age, head shape, and the general well-being of the infant. The second group of factors related to the experimental conditions out of control of the subject: time of testing, the season of testing, whether the subject participated in other tasks before the EEG measurements, and research assistant (RA) present. Finally, as this study has a longitudinal design, we examined the stability of some data loss measurements within-subjects, namely the likelihood of data loss and attrition across visits.

In short, this paper is meant to illustrate the impact of a range of factors on data loss in infant EEG paradigms. Some of these factors of interest have been put forward as ‘hunches’ based on our own (subjective) experience but have never been put to a test: for example, the effect of season and time slot of testing. Other factors have already been proven in the literature, and we aim here to replicate them. Please note that our study is by no means meant as a complete overview of all factors possibly influencing infant EEG data loss. Moreover, although we will be analyzing two separate paradigms, one visual and one audio-visual, we do not know how well these results generalize to other studies, paradigms, locations, and setups. What we aim to achieve is to broaden our understanding of what factors could influence data loss. Therefore, this paper could prove useful for both novel researchers venturing into the world of infant EEG and experienced EEG researchers. Both novel infant and experienced EEG researchers can use these findings to set up new studies, taking heed to here described influential data loss factors and trying to keep these factors optimal over the course of their study. Additionally, experienced researchers can use this paper to better understand the data loss issues likely influencing their own datasets to detect possible biases during analysis.

3.3 Methods

3.3.1 Participants

The YOUth study is a longitudinal cohort study consisting of two large cohorts differing in age range. The YOUth Baby & Child cohort follows infants from 20-24 weeks gestational age until the age of seven years while the YOUth Child & Adolescent cohort follows children from the age of 8 until the age of 16 years. Both behavioral and cognitive development is tracked through numerous tasks and methods (e.g. eye-tracking, EEG, MRI, questionnaires). The YOUth study was approved by the Medical Research Ethics Committee of the University Medical Center Utrecht and all participants' parents provided written informed consent. A brief overview of the YOUth study including the measurements conducted at each timepoint is available from https://www.uu.nl/en/research/youth-cohort-study (cf. Onland-Moret et al., this issue).

The current study only uses data from the YOUth Baby & Child cohort, since this is the only cohort in which EEG was measured, in young children from 5 months onwards. In total, 1278 5-month-old infants, 1046 10-month-old infants, and 104 3-year-old toddlers were included. The lower amount of 3-year-olds is due to the fact that measurements for the latter have started only recently, and data for all waves is still on-going. Data of 3-year-olds are only included in the data loss comparison between waves. All other analyses are done with only the 5 and 10-month-old infants. As attrition and data loss are fundamental elements of our study design, no infants were excluded from our analysis. Table 3.1 and Table 3.2 show the demographic and attrition information for our study, for the EEG and ERP paradigm, respectively. Attrition due to fussiness was counted when the infant was excluded from the analysis for having too little (or no) data due to the infant being too tired or inattentive, started crying or moving too much, or refused to wear the cap. Note that for these tables we categorized infants in the attrition group using a conservative threshold (data loss over 75 percent), but that in the remainder of the paper data loss is used as a continuous variable. Attrition due to experiment(er) error was counted when the RA logged this or when the resulting data file was corrupted. Attrition rates are 27% or even lower, which is somewhat below the expected range (Stets et al., 2012).

Table 3.1 Demographic and attrition information – Continuous EEG

	Gender	Total	Attrition		Exp. Error		Fussiness		Age (in days)	
		N	N	%	N	%	N	%	mean	sd
5m		1278	342	26.8	41	3.2	301	23.4	166.7	23.4
	Boy	628	160	25.5	18	2.9	142	22.6	166.7	23.0
	Girl	650	180	27.7	23	3.5	157	24.2	166.7	23.8
10m		1046	240	22.9	46	4.4	194	18.5	315.7	24.4
	Boy	514	111	21.6	20	3.9	91	17.7	316.3	24.4
	Girl	523	127	24.3	26	5	101	19.3	315.2	24.4
3y		104	15	14.4	6	5.8	9	8.7	957.8	161.2
	Boy	51	9	17.6	3	5.9	6	11.8	954.5	168.9
	Girl	50	6	12.0	3	6	3	6	961.1	154.6

Table 3.2 Demographic and attrition information – ERP

Wave	Gender	Total	Attrition		Exp. Error		Fussiness		Age (in days)	
		N	N	%	N	%	N	%	mean	sd
5m		1278	328	25.7	34	2.7	294	23	166.7	23.4
	Boy	628	154	24.5	14	2.2	140	22.3	166.7	23.0
	Girl	650	174	26.8	20	3.1	154	23.7	166.7	23.8
10m		1035	261	25.2	41	4	220	21.3	315.7	24.4
	Boy	514	128	24.9	19	3.7	109	21.2	316.3	24.4
	Girl	521	133	25.5	22	4.2	111	21.3	315.1	24.4
3y		101	10	9.9	3	3	7	6.9	957.8	161.2
	Boy	51	6	11.8	2	3.9	4	7.8	954.5	168.9
	Girl	50	4	8	1	2	3	6	961.1	154.6

3.3.2 Apparatus and stimuli

EEG was recorded using a cap with 32 electrodes (ActiveTwo system, BioSemi) positioned according to the international 10/20 system, at a sampling rate of 2048 Hz. A Common Mode Sense (CMS) and Driven Right Leg (DRL) electrode were used to provide an active ground. During the EEG recording, all infants and toddlers were seated 65 cm from a computer screen.

The presentations of the two experiments were in a fixed order. The first experiment was the ERP experiment in which children saw pictures of faces with neutral expressions and houses. Pictures were presented for 1000ms, and the ISI was 700 – 1000 ms. There were 96 trials: 48 in the neutral face condition (4 x 12 models) and 48 in the house condition (4 x 12 houses). Order of stimuli was pseudo-randomized: per block of 24 trials (4 blocks in total), all pictures appeared once in a randomized order. Between blocks and whenever the infant was not looking at the screen, the experimenter played additional sounds or video clips as attention getters. The task lasted approximately 3-4 minutes.

The other experiment was a continuous EEG experiment, which consisted of two, one 1-minute long, videos repeated three times. One video depicted singing women, while the other depicted moving toys without human interference. In between videos, short breaks were taken (5 in total) after which the new video was started. Similar videos were used earlier in a study by Jones and colleagues (Jones, Venema, Lowy, Earl, & Webb, 2015). During both experiments, research assistants were allowed to pause the task if the child got too fussy. This task lasted 6-7 minutes. Tasks could be stopped at any time if either parent or child prevented continuation.

3.3.3 Data loss calculation

The calculation of data loss was similar for both tasks. EEG data were analyzed exclusively using MATLAB, using the FieldTrip toolbox (Oostenveld et al., 2011). The original 2048 Hz data were downsampled to 512 Hz, using chip interpolation and band-pass filtered at 0.1–70 Hz with a two-way Butterworth filter. A notch-filter at 50 Hz was used to remove the background mains hum. The common average was used as a reference. For the ERP task, epochs were created based on stimulus presentation which led to 96 one-second epochs cut from 200ms before stimulus presentation until 800ms after stimulus presentation. For the continuous EEG task, the data was divided into 360 equal one-second epochs.

Data loss calculation was kept as analogous as possible to the regular cleaning of EEG data. Trials were rejected based on the following criteria: amplitude (>+-250uV); jumps; kurtosis (>7); and absence of data. Jumps were detected using the FieldTrip toolbox[1]. Thresholds were chosen based on commonly used thresholds in earlier EEG studies. However, both maximum amplitude and kurtosis are subjective thresholds (which researchers can disagree on). To prevent eventual influence of subjectivity in choosing thresholds on data loss calculation, we also calculated data loss with a wide range of thresholds, ranging from stringent (amplitude > +-100uV & kurtosis > 3) to lenient (amplitude > +- 300 & kurtosis > 8). Outside these ranges, almost all data were respectively removed or included, which makes determining differences between factors impossible. We did not observe any noticeable differences depending on our choice of rejection criteria: results were similar regardless of whether we used more stringent or more lenient thresholds. This is not surprising as correlations between data loss values found for each subject for different leniency in data loss calculation methods were high ($0.83 < r < 0.91$). We, therefore, decided to maintain our relatively lenient thresholds for artifact rejection.

All trials with artifacts based on the criteria mentioned above in any channel were counted as bad trials ($N_{badtrials}$). Data loss (DL) was calculated as the percentage of bad trials of all expected trials separate for the ERP and the continuous experiment with the following formula:

$$DL = \frac{N_{badtrials}}{N_{expectedtrials}} * 100$$

, with $N_{expectedtrials} = 96$ in the case of the ERP task and $N_{expectedtrials} = 360$ in the case of the continuous task. Channels with more than 40 percent data loss were considered 'bad channels' and removed. Bad channels were interpolated using weighted averaged neighboring clean channels.

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[1] Using *ft_ artifact_jump* with standard options

Whenever there were more than two bad channels, we removed the entire subject was removed entirely, and set the data loss of that particular subject to 100 percent. Therefore, we expected two peaks in data loss distributions. One ‘low-data-loss’ peak for subjects who have successfully completed the experiment with limited to no data loss (around 20 percent data loss) and one ‘high-data-loss peak for subjects who showed little to no clean data (around 100 percent data loss). A graphical summary of the calculation of data loss is shown in Figure 3.1.

3.3.4 Creating groups based on factors

Demographic information of all infants tested can be found in Table 3.1. Since the number of infants tested per factor changes (number of infants tested by selected RAs are less than infants tested during a type of season), all number of infants per analysis are separately mentioned in the appropriate figures. For every analysis, infants were grouped according to factor. The following child-related factors were used to group infants:

1. Gender (for more info on demographics see Table 3.1 and Table 3.2).
2. Age. We grouped age by the wave as used in the YOUth project (5-month-old vs. 10-month-old vs. 3-year-old). Note that since we only recently started testing 3-year-old toddlers, the number of subjects is considerably lower (for more info on demographics see Table 3.1 and Table 3.2).
3. Head shape. This was logged by the RA present during the testing day. There are four possible options: normocephaly (regular skull shape), brachycephaly (shorter than usual skull shape), plagiocephaly (skull with a flat spot), and scaphocephaly (elongated skull). Note that these are simplified denominations and RAs were only asked to group infants based on the category which best represented the infant’s head shape
4. General well-being of the infant. The RA asked the parent after testing whether the child was experiencing a typical day, or whether there was something amiss. There were three possible

answers: "My child is having a typical day", "my child is ill", and "my child is tired / did not sleep well"

The following setting related factors were used to group infants:

1. Time of EEG experiment. We grouped these into four time slots: early morning testing (08:00-10:00), late morning testing (10:00 – 12:00), early afternoon testing (12:00-14:00), and late afternoon testing (14:00+). The latter was exceedingly rare and was therefore discarded from the study.
2. Order of testing during the test day. Besides the EEG-testing, the infant also took part in an eye-tracking session and a parent-child interaction session. Therefore, the EEG session could be the first, second, or third session (cf. Onland-Moret et al., this issue).
3. Season of testing: spring, summer, autumn, winter.
4. Research assistant (RA). Our approach is similar to the one employed by Hessels & Hooge (2019), who assessed the influence of RA in the YOUth cohort on data loss in the eye-tracking sessions for those RAs who tested at least 33 infants per wave. In our case, there were four RAs that tested clearly more infants than the others: these four (coded RA1, RA2, RA3, and RA4) tested over 40 5- and 10-month-old infants. In addition, to observe whether RAs improve with increasing experience, we tested the effect of time of RA on data loss over time for all RAs.
5. Task length. To study the influence of task duration on data loss, we logged the average amount of data loss as the task progressed. This allowed us to follow the progression of data loss and whether or not taking breaks in between trials played a positive role in limiting data loss throughout the experiment. Breaks in the continuous EEG experiment were breaks in between videos, during which a new video was started up. Breaks in the ERP experiment were videos used as attention grabbers shown every 24 trials.

For the following factors, stability over session was determined:

1. Attrition due to fussiness. As Tables 3.1 and 3.2 show for each wave, several children were to be excluded for further analysis, even though they participated in other sessions (i.e., eye tracking or parent-child interactions). In all cases, too little (or no) data was clean enough for analysis. Attrition due to fussiness of the infant was counted when the task was stopped either by the RA or parent, due to excessive movement, refusal to wear the cap, inattentiveness, sleepiness, or crying. When too much noise was detected in the data for analysis (either through too few trials surviving cleaning or more than 2 channels being noisy) and the infant was logged as restless or crying, this child was also to be counted as attrition due to fussiness.
2. Cap refusal. A subset of too fussy infants, but only those who specifically did not start the EEG-experiment but did participate in other experiments during the day.
3. Data loss. For those infants who participated in the tasks, we categorized them based on the proportion of data loss: a low group (the lowest 50 percent of data loss) and a high group (the highest 50 percent of data loss).

To prevent unreliable visual and statistical comparisons, only the categories of the categories within a factor which included more than 40 subjects were used for visualization and statistical analysis.

3.3.5 Data visualization and statistics

Data loss for infant and setting-related factors was visualized using a kernel-smoothed density plot, using MATLAB, with automatically determined bandwidth. The kernel-smoothing is used to increase the ease of visual comparisons between groups. Data is plotted using the gramm MATLAB toolbox (Morel, 2018). As mentioned earlier, data loss distributions have two distinct peaks: one around 20 percent data loss and another one around 100 percent data loss. Therefore, data is non-normally distributed, which is why we used non-parametric tests to compare groups. For this, we used the Kruskal-Wallis H test (Kruskal and Wallis, 1952),

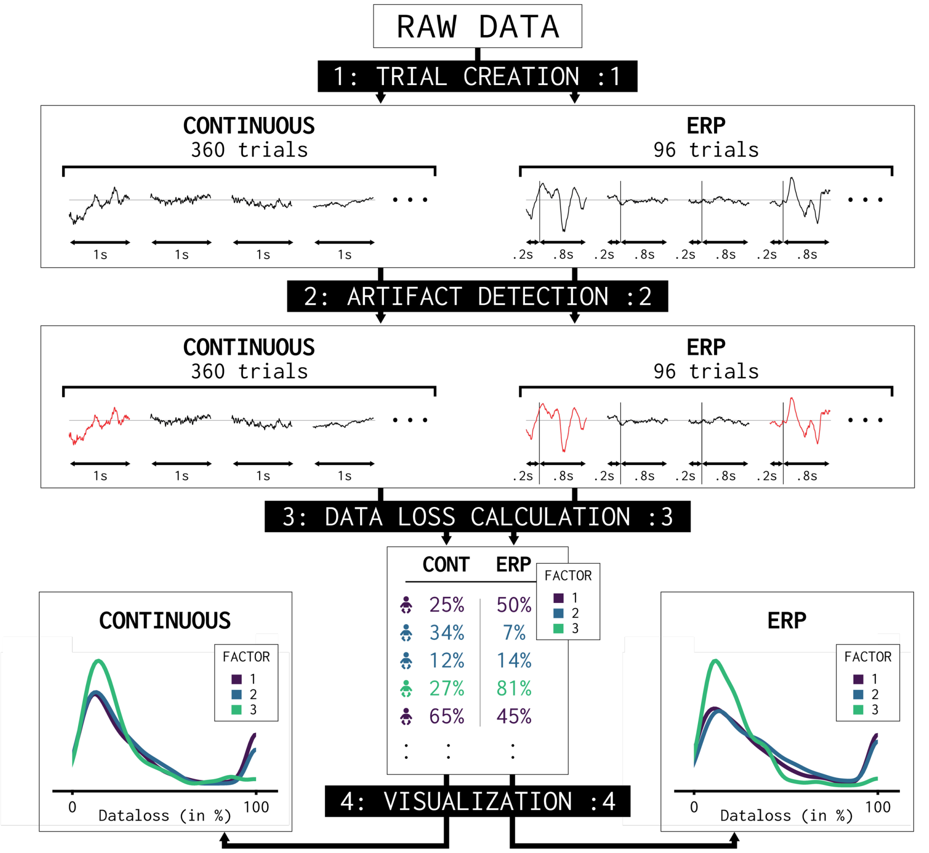

Figure 3.1. Graphical overview of analysis design. Data is analyzed in four steps. 1) Raw data is cut into 1s trials for both the continuous and ERP task, resulting in 360 and 96 trials respectively. 2) Jump, noise and flatline artifacts are detected and trials containing artifacts are selected. 3) Data loss is calculated by calculating the percentage of trials containing artifacts over the total expected trials (360 in the case of the continuous experiment and 96 in the case of the ERP experiment). 4) Subjects are grouped based on factor and the data loss distributions are visualized using a probability density function. The probability density functions plotted in the visualization step are made using the gramm toolbox (Morel, 2018). The *stat_ density.m* function is based on the standard *ksdensity.m* function in matlab.

which is an extension of the Mann-Whitney U test (Mann and Whitney, 1947) and can be used for comparing two or more independent samples of equal or different sample sizes. To test the effect of experience on RA data loss, a linear regression was performed with experience as a running number of the number of infants tested by the RA. To test the stability of longitudinal factors over the course of the entire study, cross-tabulation was used to visualize results and chi-square tests of independence were used to determine correlations between the categorical variables.

3.4 Results

We carried out analyses and created figures for both the EEG and the ERP experiments. Most figures show distributions of data loss. The data loss of each infant is one value in each distribution. Higher amounts of data loss imply noisier data. Therefore, distributions with its center of gravity further towards the left signify generally cleaner data.

Since both tasks yielded similar results for most comparisons, we decided to present only the results for the continuous EEG task in the main article, as these results were determined using more trials. Only when there was a difference between the tasks we present both results in the main article. The results for the ERP task are in the supplementary materials.

3.4.1 Influence of child-related factors on data loss

Distributions of data loss for four different factors regarding the child tested are shown in figure 3.2. Figure 3.2A presents the different data loss distributions for different genders. Data loss was nearly identical across genders. For both genders, there are bimodal distributions of data loss, with clear peaks around 15 and 100 percent data loss. The peaks around 100 percent appear similar, while the early peak is more pronounced for boys. In other words, boys show a slight increase in lower amounts of data loss compared to girls. A Kruskal-Wallis H test using gender as a fixed factor resulted in a significant effect of gender at the $p<.01$ level ($\chi^2(1) = 7.282$, $p = 0.007$), indicating that boys have slightly lower data loss compared to girls.

Figure 3.2B visualizes the data loss distributions for the different waves as used in the YOUth study. Infants in both the 5- and 10-month-old

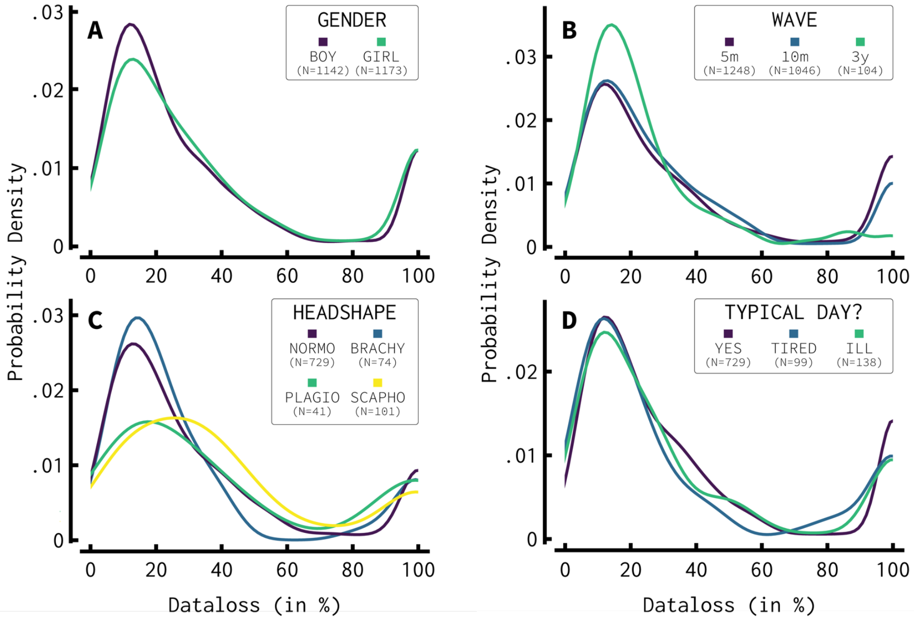

Figure 3.2. Influence of infant related factors on infant EEG data loss. This Figure shows probability density functions of data loss for different factors relating to infants. Note that all distributions show two distinct peaks. One at 100 percent, indicating all infants discarded from the data set, either due to very noisy data or due to too many bad channels; and one at +/- 15 percent indicating the group of infants who very successfully participated. A) The data loss distributions for boys and girls: boys have a larger likelihood to have lower data loss. B) The data loss distributions between waves show a large effect for 3-year-old toddlers, who show markedly improved data loss. Data loss between 5-month-old and 10-month old waves is similar. C) Data loss distributions for different head shapes (normocephaly, brachycephaly, plagiocephaly, and scaphocephaly). Large differences can be seen in data loss distributions, with both plagiocephalic and scaphocephalic infants showing markedly higher and highly varying data amounts of data loss. D) Data loss distributions for ill or tired children showed no clear effect on data loss distributions compared to infants who participated during a typical day.

waves show very similar data loss distributions. The 3-year-old wave, however, revealed marked improvement. Three-year-old toddlers were less likely to be 100 percent discarded and more likely to show lower amounts of data loss. The fixed factor wave was tested using the Kruskal-Wallis H test, which found a significant effect at the $p<.05$ level ($\chi^2(2) = 8.925$, $p = .012$).

The data loss distribution for different head shapes of participating infants is depicted in figure 3.2C. Data loss distributions show distinct differences. The peak at 100 percent data loss is similar across head shapes. However, both normocephalic and brachycephalic infants show a higher likelihood of low data loss, with a higher peak of around 15 percent. Both plagiocephaly and scaphocephaly show peaks further to the right and highly variable amounts of data loss. This effect was tested to be significant at the $p<.01$ level ($\chi^2(3) = 11.832$, $p = .008$).

Lastly, figure 3.2D shows the effect of any subtle problems the infant might have had during testing according to the parent present. The parent was asked whether the child had been ill, was tired, or was experiencing a typical day. Both tiredness and illness showed no marked effect on data loss distributions. A Kruskal-Wallis H test yielded no significant results. ERP data showed similar results and are depicted in Supplementary Figure 3.1.

3.4.2 Testing related factors on data loss

Figure 3.3 shows distributions related to the timing of the experiment. As can be seen in Figure 3.3A, data loss distributions were different across EEG timeslots. This is more visible in the peak resembling the lower data loss group: early EEG testing (between 8 a.m. and 10 a.m.) leads to a higher likelihood for the infant to be in the lower data loss group. The difference in timeslots is not apparent in the second peak, which resembles those infants with a 100 percent data loss. This suggests that the time of day only affects data loss for those infants who complete the tasks, but not for those infants for whom the task was terminated prior to completion. A Kruskal-Wallis H test with timeslot as fixed factor yielded a significant result at the $p<.01$ level ($\chi^2(2) = 12.023$, $p = .002$).

The influence of experiment order is presented in Fig 3b. Recall that during the test day, each infant participates in three sessions in random order: the EEG-experiments, an eye-tracking session, and a parent-child interaction (PCI)-session. Each other session usually takes 10-20 minutes. To limit the influence of early morning testing, which is strongly correlated

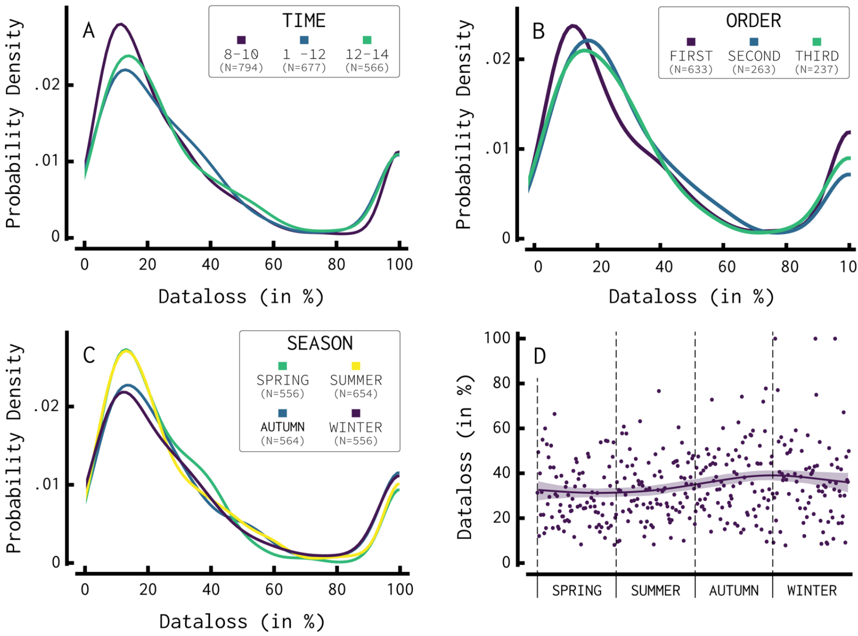

Figure 3.3 Influence of timing related factors on infant EEG data loss. This figure shows probability density functions of data loss for different factors relating to the timing of testing. A) shows that starting time influenced data loss, with early starting infants (between 8 a.m. and 10 a.m.) showing cleaner EEG data. The low data loss peak is slightly displaced to the left and a higher peak indicates a higher portion of infants has low data loss early in the morning. Time slots did not cause differences in infants with 100 percent data loss. B) shows that order of testing has limited influence on data loss: whether EEG was the first, second, or third task of the day, data loss was relatively similar. C) shows that season of testing also considerably influenced data loss. Infants showed lower data loss in sunny months in the Netherlands with a higher portion of infants showing low data loss and a lower portion of infants showing 100 percent data loss. D) shows the data loss of the study, averaged over year. Each dot represents the average data loss per day. A clear increase in data loss can be seen around late autumn/early winter. A smoothed line was drawn, which can be used as a visual aid (using Eilers and Marx' method with automatic lambda (Eilers & Marx, 2002).

to EEG being the first experiment tested, only infants who had their EEG-experiment after 10 a.m. were taken into account. Figure 3.3b shows that there is little difference in data loss distributions whether the infant participates in the EEG-experiment first, second, or last during a test day. The Kruskal-Wallis H test yielded no significant results. The ERP data showed similar results and are depicted in Supplementary Figure 3.3.

Figure 3.3C visualizes the effect of season of testing on data loss. Warmer seasons in the Netherlands (spring and summer) show markedly lower amounts of data loss compared to colder seasons (autumn and winter). This effect was visible in both peaks: in a higher amount of lower data loss infants and lower amounts of 100-percent data loss infants. The Kruskal-Wallis H test confirmed this effect to be significant at the $p<.01$ level ($\chi^2(1) = 7.011$, $p = .008$). Figure 3.3D adds to this by showing the average data loss per day for every day in the year. A clear bump can be seen in the autumn and winter months. Supplementary Figure 3.2 shows this effect throughout the entire study (so not averaged per year), with clear bumps in data loss at the start and end (winter and autumn) of 2016, 2017, 2018, and 2019, compared to spring and summer each year.

Figure 3.4 shows data distributions across different research assistants (RAs). The only RAs included here are RAs who tested at least 40 infants of both 5-month-wave and 10-month-wave infants. The four panels show the distributions of data loss for infants in these waves (top and bottom), for both the continuous and the ERP task (left and right).

The continuous task (left panels) reveals large differences in data loss distributions between RAs, with RA 3 and RA4 outperforming RA1 and RA2 across age groups. Peaks of infants with low data loss can be seen further to the left. Clearly, RA4 has a distinctly lower 100 percent data loss peak compared to the other RAs. Kruskal-Wallis H tests were conducted, separately for each of the waves. The effect of RAs on the 5-month-old data loss was found to be significant at the $p<.01$ level ($\chi^2(3) = 11.549$, $p = .009$). A significant effect was also found for the effect of RAs on the 10-month-old data loss at the $p<.001$ level ($\chi^2(3) = 20.207$, $p < .001$).

The influence of RAs on data loss during the ERP task is shown in the right panels of Figure 3.4. In contrast to the continuous EEG sessions, we note that there were only limited differences between RAs in the 5-month-old infants. However, clear differences can be seen at the 100 percent peak, with RA4 showing lower proportions of infants with 100 percent data loss. Also, a slight displacement can be seen in the low data loss infant peak with RAs 1 and 2 being slightly more shifted to the right. Differences in

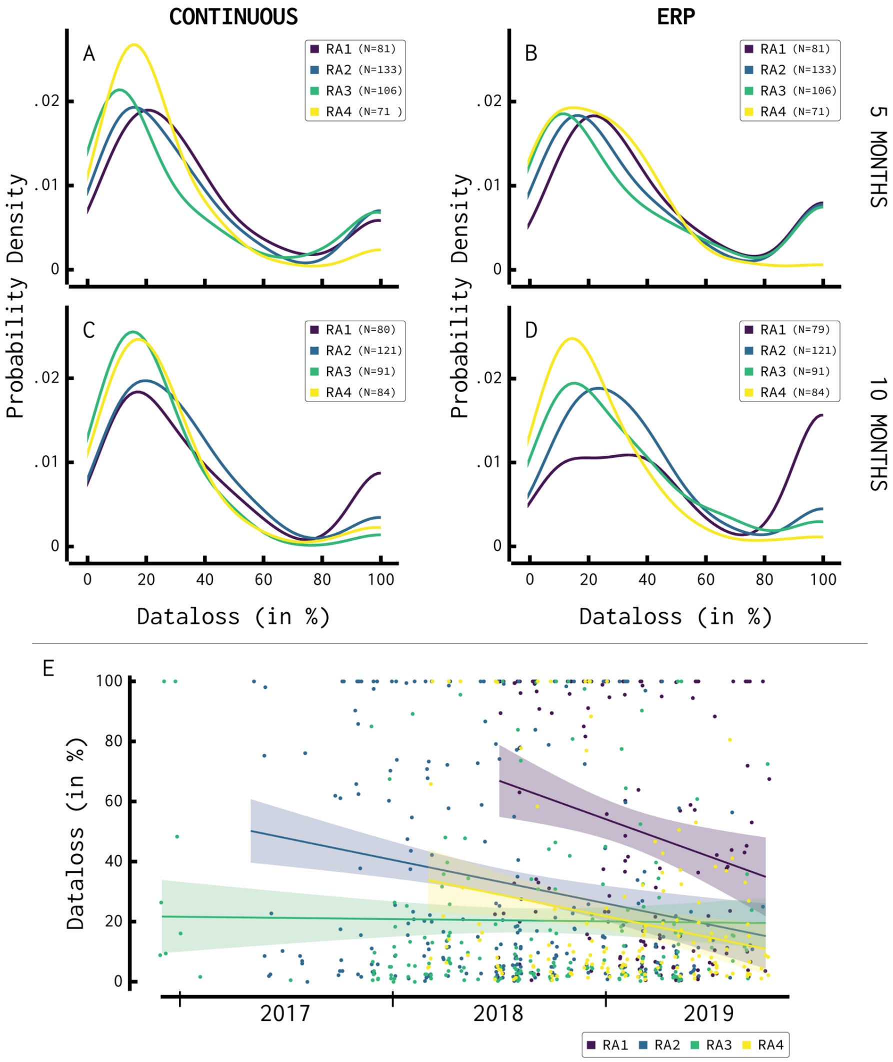

Figure 3.4 The influence of four research assistants (RAs) on data loss. Shown RAs have tested at least 40 infants in the 5 and 10-month waves in both continuous and ERP tasks. Clear influence of RA can be seen across all tasks and age groups. RA3 and 4 continuously outperform RA1 and 2, with low data loss peaks shifted to the left in both the continuous and ERP task. The proportion low data loss is also higher for RA1 and 2 in the continuous task and the ERP task with 10-month-old infants. The ERP task shows lower influence of RA in the 5-month-old wave compared to the 10-month-old wave. Ranking of assistants remains consistent over age groups and tasks (RA4 > RA3 > RA2 > RA1). E) shows the data loss per previously studied RA over time. A clear downward trend can be detected for three of the four studied RAs. Indicating an effect of experience on data quality per RA.

the 10-month-old wave are more distinct with once again RA3 and RA4 outperforming RA1 and RA2. Kruskal-Wallis H tests yielded significant results at the p<.01 level in the 5-month-old wave ERP data ($\chi^2(3) = 13.268$, $p = .004$) and at the p<.001 level in the 10-month old wave ERP data ($\chi^2(3) = 40.984$, $p < .001$).

What can be clearly seen from these distributions is that the ranking between RAs remains similar across tasks and waves (RA3 and 4 versus RA1 and 2), with RA4 outperforming all other RAs across tasks and waves, and RA1 performing worst across all tasks and waves. It is therefore likely that RA is the driving factor in data loss caused here, as RA performance appears consistent across waves and tasks.

Figure 3.4E shows data loss for 5 and 10-month-old infants for each of the four RAs as a function of time across the years, capturing their experience. Clear effects of experience can be seen in three of the four RAs: data loss decreases over time. To test this the effect of experience a linear regression was calculated using RA experience of all RAs (not just the four RAs mentioned above) to predict data loss. The following significant regression equation was found (F(1,2199) = 10.849), p<0.01, $R^2 = 0.005$).

Figure 3.5 shows the effect of the length of the task on data loss. The top panel shows data loss of trials throughout the entire continuous EEG experiment while the lower panel repeats this for the ERP experiment. Recall that during the continuous EEG experiment, six one-minute-long videos are presented. In between videos, each infant can take a short break as the new video is started. Figure 3.5A reveals a clear effect of the breaks: they coincide with a decrease in data loss. A second finding is that there is only a minor upward trend indicating a higher likelihood of data loss over the course of the entire experiment. Comparison between the five- and ten-month-old infants further shows that both age groups perform rather similar over the course of the entire experiment.

Figure 3.5B shows data loss as a function of the course of the task, for the ERP experiment. Every 24 trials mandatory breaks are taken by showing a short video clip ('attention getter'). We can see the effect of these breaks, as a decrease in data loss right after the break. However, this

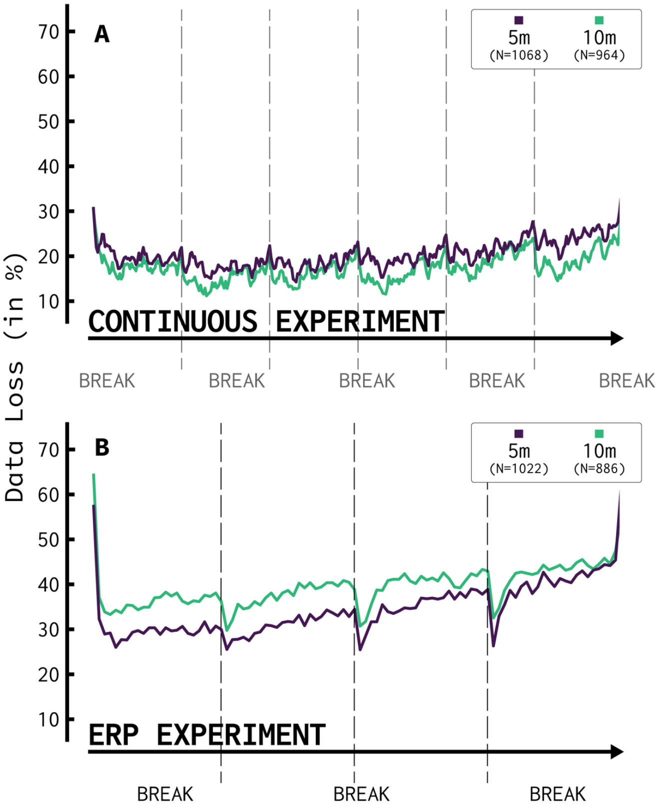

Figure 3.5 Data loss over the course of the entire experiment. A) The continuous experiment consists of 2 unique videos, repeated 3 times. After each video a short break is experienced during the starting up of the new video. Clear upward trends can be spotted over the course of each separate video. This upward trend is reset after each break. A slight upward trend over the course of the entire experiment can also be detected. Both 5 and 10-month-old infants showed similar data loss over time. B) The ERP experiment consists of 96 trials, with mandatory breaks after every 24 trials, during which a video is shown. Here also a clear effect of break can be seen, with stark decreases in data loss after each break, especially later in the experiment. Contrary to the continuous experiment, however, the improvement does not last for much longer than 2 trials, which indicates that data loss did not reset. 5-month-old infants seem to outperform 10-month-old infants, but data loss increased more sharply over the course of the experiment in the 5-month-old infants.

effect appears more short-lived. Thus, the ERP experiment differs from the continuous experiment in that the upward trend in data loss proceeds shortly (almost immediately) after the break. Moreover, compared to the EEG experiment, the ERP experiment witnesses a more pronounced increase in data loss over the course of the experiment. It appears that both groups of infants react differently to the stimuli they are viewing. Differences between 5 and 10-month-old infants become smaller over the course of the ERP-experiment, but 5-month-old infants consistently show lower amounts of data loss than the 10-month-old infants do. Note that for both paradigms the first and last trials show a larger proportion of data loss, which possibly indicates an effect of starting and quitting the experiment.

Table 3.3 Cross table of included vs excluded based on fussiness

		Session 2			
		Included		Excluded	
		N	%	N	%
Session 1	Included	515	86.7	128	82.1
	Excluded	86	14.3	28	17.9

3.4.3 Longitudinal effects and effects of entire study on data loss

We determined stability between 5 and 10-month-waves of several data loss characteristics by creating cross tables of these characteristics and performing a chi-square test for independence to check whether there is a relationship between the categorical variables. Table 3.3 is a cross table comparing the stability of infants who were either included or excluded due to fussiness over sessions. No significant relationship was found ($X^2(1, N = 757) = 1.28$, $p =$ n.s.). Table 3.4 is a cross table of cap refusal of the 5-month-olds' and 10-month-olds' sessions. A significant relationship was found ($X^2(1, N = 980) = 4.4$, $p < .05$), indicating that cap refusal in the first session influences the likelihood of cap refusal in session two and vice versa. Please note, however, that cap refusal is rare, and that only 3 / 980 infants refused the EEG cap in both sessions.

Table 3.5 is a cross table for the amount of data loss. Data loss is categorized into high data loss (being in the group with the 50% highest data loss) and low data loss (being in the group with the 50% lowest data loss). A chi-square test for independence yielded a significant result ($X^2(1, N = 672) = 6.3$, $p < .05$), indicating some stability over sessions for these categories. However, a correlation showed no relationship between individual values of data loss over both sessions ($r = 0.070$).

Table 3.4 Cross table of cap refusal

		Session 2			
		Yes		No	
		N	%	N	%
Session 1	Yes	3	6.8	19	2.0
	No	41	93.2	917	98.0

Table 3.5 Cross table of amount data loss

		Session 2			
		Low		High	
		N	%	N	%
Session 1	Low	197	56.8	153	47.1
	High	150	43.2	172	52.9

3.4 Discussion

Attrition rates in infant EEG are usually high. While previous research has given a clear overview of which factors affect attrition rates similarly across studies, little is known about the factors contributing to data attrition within a study. In this paper, we showed that there is a wide array of factors influencing data attrition in one large-scale study. These factors can vary between subjects, possibly changing outcome measures and results, which may lead to biased conclusions.

The factors influencing data attrition described in this study can be broadly divided into three groups: child-related factors, testing-related factors, and longitudinal (study-specific) factors. Three child-related factors were found to influence data loss: gender, age, and head shape. Four testing-related factors were found to influence data loss: time of testing, the season of testing, research assistant present during the experiment, and task length all had considerable influence on data. Lastly, data attrition rates of the first session of testing were found to be related to the second session of testing, underlining possible longitudinal biases in terms of data loss. The influence of all these factors was found irrespective of which EEG task analyzed, even though data loss was found to be lower in the continuous EEG experiment presenting audio-visual video clips compared to the ERP paradigm presenting still images. Below we first discuss the main findings concerning our three factors of interest, then we will discuss the limitations and future directions of this study and lastly, we will formulate recommendations based on the results described here to minimize data loss in future infant EEG studies.

3.4.1 The impact of child-related factors on EEG data loss

By comparing data loss between the age groups in our study, we found a slight influence of age on data loss. The oldest group (three-year-old children) showed markedly lower data loss, but between 5- and 10-month-old infants, the difference in data loss was negligible. This is in contradiction with the finding of Slaughter and Suddendorf (2007), who described younger infants having slightly higher attrition rates compared to older infants, which they explained by a higher rate of sleepiness of younger infants during testing. What is likely is that in our study the higher likelihood of tiredness for younger infants during testing is offset by the higher likelihood of resistance to preparation procedures or restlessness in older infants (Hoehl and Wahl, 2012), resulting in similar data loss across waves. Support for this reasoning comes when we compare our results to data quality for the same set of children in a different session: eye tracking. Hessels and Hooge showed that for the eye-tracking sessions in the YOUth Infant & Child cohort there was a limited difference in data loss between 5 and 10-month-old infants, but that quality significantly improved for the three-year-olds (Hessels and Hooge, 2019).

Surprisingly, data loss was lower for boys than for girls. We did not anticipate this finding. We tentatively speculate post-hoc here that research assistants might find it easier to approach and handle boys, which speeds up capping, whereas they are more careful and considerate in capping girls. This fits with findings that mothers, too, behave differently with their daughters than with their sons (Clearfield and Nelson, 2006). A different possibility might have to do with differences in head circumference. Boys have generally larger heads during infancy (Niklasson and Albertsson-Wikland, 2008), which we also, on average, found in our study (boys: 44.8 cm +/-1.9, girls: 43.5 +/- 2.0). Larger heads could increase the fit of the cap and therefore decrease data loss.

Another factor we examined was the impact of infant head-shape. Irregularity of head shape causes a higher possibility of data loss in infants, which is probably caused by poorer cap fit. It is important to note that it is currently unknown how well this result generalizes towards other EEG equipment types. In our experience, irregular head shape of an infant affects data loss in electrodes towards the edges of the cap most negatively. This is likely caused by an increase or decrease in pressure on the electrode sites. Net-saline equipment could also be affected by this, but further research is necessary. For cap-gel equipment, this is evidence that the development of infant specialized EEG-equipment is warranted. One possible solution could be the creation of caps specifically designed for the most common head shape irregularities (flat-spotted back head, elongated head shape). While this increases costs in terms of material needed, this could be counterbalanced by a lower probability of data loss. Future testing is needed to understand whether creating caps for a wider range of head shapes increases data loss.

Finally, we asked whether parents' judgments on the suitability of the child for this particular testing day affected data loss. We did not observe noticeable effects here: 'regular days' showed similar patterns as 'days when children were judged to be tired'; or when parents reported that their child had just recovered from illness, like a cold. The most likely explanation for this effect is that infants who are too ill or too tired will not come in for testing and, as such, only slightly ill or tired infants participate. It is therefore essential that the lab is flexible and allows for rescheduling of appointments whenever the parents feel it is necessary. Small changes in the well-being of an infant will apparently not influence the likelihood of data loss.

3.4.2 The impact of measurement-related factors on EEG data loss

We also examined a wide range of factors regarding the testing arrangements. Above all findings, the choice of research assistant (RA) influenced data loss in our infant sample. The RA-dependency of infant

EEG data underlines the importance of close monitoring of data loss. Even in the YOUth project, which has a rigorous training program for new RAs, with live-monitoring sessions, it is impossible to level the playing field between RAs in terms of data loss. In addition to the differences between RAs in data loss, a positive effect of experience was also found. Experience (as in the number of infants tested) was negatively related to data loss, both for the four RAs (Figure 3.6B) and for all RAs together.

A possible explanation for this effect of RA on data loss is that some RAs are more proficient in capping. Shortening capping times presumably enables children to have more energy and attention for what is coming next (i.e., the experiment), which enhances the likelihood of infants successfully completing the experiment. Note, however, there is no research yet examining the factor of capping time on data loss. Secondly, some RAs might prove better at calming both infants and parents in potentially stressful situations, resulting in less data loss. Also, some assistants might be more likely to intervene in the experiment when the EEG signal is deteriorating or when the child signals a need for a break (to e.g. eat a breadstick). Lastly, it is also possible that some RAs simply need more time to become proficient enough to limit data loss. Figure 3.6B hints at this with start data loss wildly differing between RAs, but all seemingly trending towards the same data loss limit. It is important to mention that none of the RAs tested here underperformed based on our expectations. We expected a dropout rate of 20-30%, which all these RAs complied with. The main difference was caused by two overperforming RAs (data loss dropout rate of ≈ 10%). Specifically studying the outperforming RAs could provide us with valuable information to limit future data loss.

What should be seen as a limiting factor is that studying the effect of RA on data loss is difficult as many other hidden factors could be driving differences in data loss. For example, some RAs could only have tested in the summer months, only during the early hours of the day, or only tested on certain weekdays. In our case, we only picked RAs who have tested for an extended period of time during the entirety of the study and are available throughout the day. So, the mentioned factors would likely not affect the outcome here, but there might be currently unknown factors that drive these differences. Therefore, it will be valuable to know how well this result translates to other studies.

A second factor that impacted data loss was the time of onset of the EEG task. Earlier hunches were confirmed that early morning testing lowered data loss. This is likely to be caused by early morning testing fitting better in most infants' sleep/eat-schedules, with most young infants waking and eating early. An early morning participant will, therefore, more likely to be well-rested and well-fed. Also, an early morning participant will have had fewer chances to be overwhelmed by experiences that are out of the ordinary.

Besides the time of day, we also found an influence of the season in which testing took place. Infants generally performing better during the warmer months in The Netherlands (spring and summer). One possible explanation for this is that during the warmer months in The Netherlands, infants have a possible lower likelihood of colds or flu. Even when parents indicate that their child can participate even though he or she just recovered from an illness such as the flu, it is our experience that these children can be irritable and do not tolerate capping. A different explanation of why data loss is lower in spring and summer could be related to the higher humidity during the summer months. Humidity affects skin impedance during a measurement (Clar et al., 1975). Therefore, higher humidity can increase the ease of signal transmission between the electrode and the scalp, lowering the possibility of data loss. Opposingly, an increase in high humidity can also increase sweating, which in turn can cause sweating artifacts (White and Van Cott, 2015).

Lastly, the factor of time elapsed during a task impacted data loss. The more the task progressed, the higher the likelihood there was data loss. This effect was heightened in the ERP design, which showed a clear increase in data loss over the course of the experiment. These results are in line with earlier studies, which found results from ERP studies to change over the course of an experiment (Nikkel and Karrer, 2009; Stets et al., 2012). Both of our experiments revealed a positive effect of breaks: with the continuous experiment resetting in data loss likelihood after every break while the ERP experiment only witnessed a short improvement in chance on data loss, after which it reverted to the original data loss progression. This could be caused by infants getting habituated to the ERP paradigm. The ERP paradigm has little changes over the course of the experiment, while the continuous EEG paradigm changes constantly over the course of every video. This underlines the importance of the development of new EEG paradigms specifically designed for infants. It is vital to not only consider the choice of stimuli but also how to implement new ways to take breaks. In the continuous experiment, the restarting of a new video seemed to have a desirable effect, while the showing of a short movie clip did not cause a similar long-lived effect in the ERP experiment.

3.4.3 The impact of longitudinal factors on EEG data loss

Some evidence was found for stability of data loss over waves. Data attrition due to fussiness was not found to be stable over sessions. However, infants who were categorized as high data loss in one session were more likely to be categorized similarly in the other session and vice versa. This ties in with the earlier found relationship between infant temperament and data attrition (Marshall et al., 2009), where infants with a negative temperament showed higher attrition rates than infants with a positive temperament. This could prove problematic in longitudinally designed studies, as infants with certain character traits would have biases in data availability. This might influence outcomes and in turn, can lead us to draw wrong conclusions when comparing infants. Moreover, it questions whether all results can be generalizable to the whole population.

Similar results are found in cap refusal: when an infant refused to participate in the experiment in one session, he or she was more likely to refuse to participate in the other session as well. This result might be used for future researchers to decide to call on an infant who has refused to participate in a study before since there is a higher likelihood of cap refusal. What is important to note, however, is that cap refusal in our study was exceedingly rare. So rare that only 3 infants (of the in total 980 tested) refused the cap twice.

3.4.4 Limitations of the current study

Some caveats have to be taken into account when judging the abovementioned conclusions. First and foremost, we studied each factor in isolation. As a result, the exact relationship between the interplay of factors described here and data loss remains difficult to characterize. Moreover, there may be other, more fundamental, factors affecting the factors described here. Perhaps the differences between RAs are all to be explained if we realize that some RAs frequently start at 10 am or whether some RAs only work on some workdays. We have checked these specifically and found them to be untrue, but there could be countless other factors influencing data loss, stretching beyond our current imagination. Therefore, while our results suggest that some factors can explain data loss, these factors may share a common origin currently unknown.

Secondly, this study focuses only on the external factors related to infant and setting but ignores any factors related to technical issues arising from using EEG in infants. For instance, the two most popular techniques currently used are cap-gel and net-saline systems. Since our study only uses cap-gel equipment, we cannot systematically compare these techniques on data loss (DeBoer et al., 2007; Johnson et al., 2001). Readers, therefore, have to be careful generalizing these results to other EEG techniques. This is especially true for head shape, which could have different effects on net-saline equipment. Also, researchers vary widely in how they pre-process the data (e.g., in choice of reference electrode; filtering, and trial rejection criteria). We did not manipulate the choice of analyses here since we felt this would go beyond the scope of the paper. There are other papers available that examine the consequences of different pre-processing steps or analyses (Luck, 2010). Future research is warranted to examine whether these technical issues influence infant EEG data loss differently.

Lastly, it might be tempting to equate data loss to data quality, but it is not. Future research is necessary to better understand what constitutes acceptable data for different tasks. On the one hand, previous studies have found that studying more infants with fewer trials can sometimes yield better and more reliable results in infant ERP studies (Stahl et al., 2010; Stets and Reid, 2011). On the other hand, in face-processing ERP tasks, ERPs appear more reliable when more trials are included (Munsters et al., 2019). For EEG connectivity, current evidence seems to point to more data equates to more reliable results (Fraschini et al., 2016; van der Velde et al., 2019), but it is unknown whether this always holds true. Recall that there is no such thing as a golden standard for minimum amount of required data in an EEG study. Therefore, future studies are warranted to increase our understanding in which cases, which factors have to be taken into account to increase result replicability and quality.

3.4.5 General recommendations

Our results presented here, in addition with the earlier studies on data loss and attrition in infant EEG (Hoehl and Wahl, 2012; Slaughter and Suddendorf, 2007; Stets et al., 2012), lead us to provide the following recommendations for developmental EEG researchers. These recommendations can hopefully be used in future studies to ease the gathering and analysis of infant EEG data.

Firstly, it is important to understand that there is a wide range of factors that potentially influence data loss in infant EEG studies. Some of these factors are well-established factors, like the length of the experiment, age, or child temperament, but there are also external influences that researchers are less likely able to control, like the season of testing or infant gender. It is especially important to understand that these uncontrollable external factors could be represented in your different groups in a biased way. For example, in a longitudinal study, one could study a group of young infants in the summer and then re-test them 6 months later in the winter. This could increase data loss specifically in the re-test group, possibly biasing outcomes in comparing the two groups. Also, this could lead to increased data attrition, limiting the power of your longitudinal analysis.

It is likely that a far wider range of factors than described in this study influence data loss and data attrition. Keeping this in mind when analyzing studies is vital, especially with data-driven approaches, like

Hidden Markov Models and machine learning. Such factors potentially could mask our understanding of why differences between groups exist. Differences can likely arise from differences in data loss due to these external factors. Either from biases in the representation of certain infant characteristics or through lower reliability of measures gathered with increased data loss. Keeping a running tally of data loss and attrition can aid one's understanding of when and why certain important events happen.

Secondly, when studying the infant brain through EEG, we advise limiting the use of different research assistants. Not only did we observe influences from the research assistant present during testing on the amount of data loss, but we also saw that experience greatly reduced variation across RAs coupled with an overall decrease in data loss. In large-scale studies (like the YOUth project) it is not always possible to rely on a small set of RAs. In these cases, closely monitor your assistants. In the YOUth project, we have set up a running tally of data loss for each assistant. This allows a better understanding of which conditions data loss happens and also to intervene if necessary. More importantly, it allows us to learn from those assistants who are exceeding our expectations.

Thirdly, it is important to understand that controlling for data loss is not always feasible. If data loss is biased in the amount between your groups, controlling for it could hide the true outcome. Moreover, increased data loss might lead to increased attrition. Having to test extra infants in certain groups to ensure equal population sizes could bias certain groups by increasing the likelihood of infants with better temperament being in certain groups. Therefore, ensuring environmental factors are least likely to cause data loss should be one of the most important points in designing infant EEG studies. We summarize these points as follows:

1. Focus on testing early in the day, paying heed to infant eat and sleep schedules.
2. Preferably test in the summer or spring months.

3. Keep the experiment short and interesting. Create infant-specific stimuli, with breaks. Use auditory (or audio-visual) stimuli if possible.
4. Limit the number of research assistants used and designate them to EEG recordings only if possible

Finally, it is unknown how well the results described here generalize to other infant EEG studies. We believe we can only advance our understanding of what factors contribute to data loss when more studies are more explicit in reporting their numbers of attrition and data loss. This echoes the recommendations put forward in the meta-analysis on data attrition by Stets and colleagues (2012), who reported that many studies prior to 2012 did not include such information. Failure to include these types of information limits our understanding of the origins and reasons for data loss. Moreover, it could mask biases within data sets. We, therefore, recommend that studies should report attrition rates split, at least, by age groups and gender. Ideally, to further improve general insights into the data loss of infant EEG studies, studies should include data loss distributions to visualize differences or highlight potential biases between groups.

These recommendations are not only meant for researchers, but also for editors and reviewers of developmental journals. The field requires new guidelines to which infant EEG researchers need to adhere to when publishing their data. Especially, as data sets become larger and cross-lab collaborations increasingly common, we need better insights into the quality and biases in which individual data sets are collected.

3.5 Conclusions

Data loss in infant EEG is costly, as it leads to underpowered infant EEG studies. One (undesirable) solution to add power would be to test more infants, but this requires time, money, and easy access to infants. Another solution would be to create a testing environment that limits data loss. It is therefore of the utmost importance to design infant EEG studies specifically with limiting data loss in mind. Many decisions that researchers make to limit data loss are based on their hunches. This study put these hunches and other factors to the test, by comparing data loss distribution across several factors related to the setting or the infant itself. These factors have to be kept in mind when designing and analyzing new infant EEG studies. We hope that this article not only informs the reader but also progresses the debate on the topic of EEG data loss in infants.

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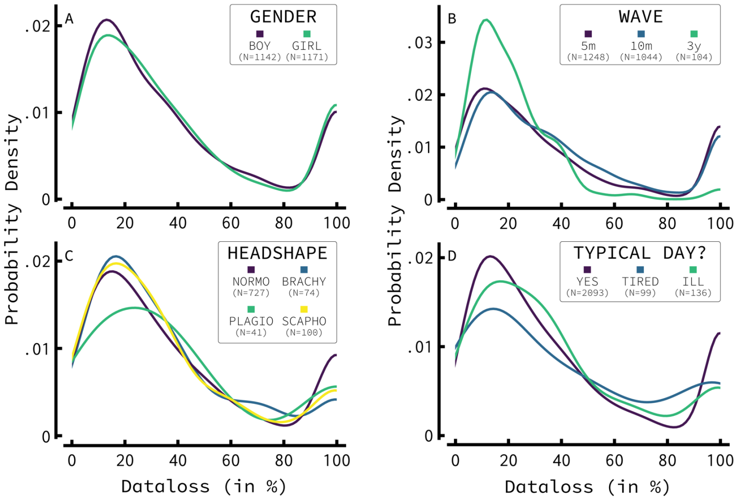
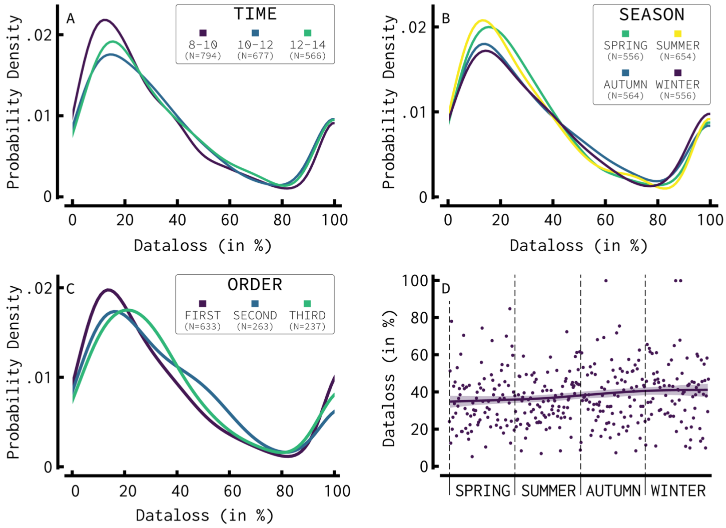
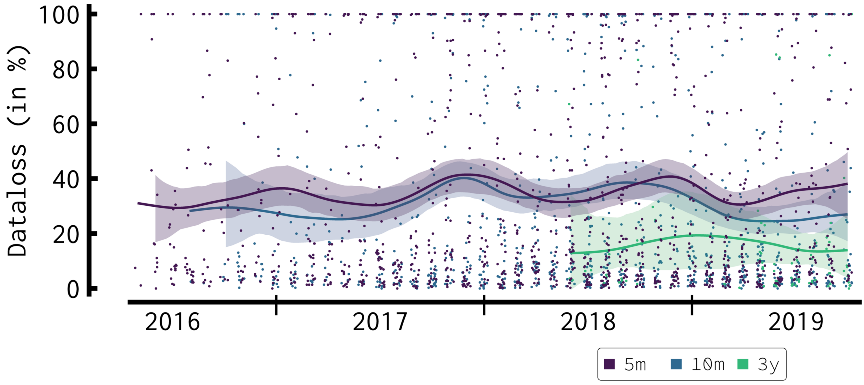
Appendix I: Supplementary figures



Supplementary figure 3.1. Effects of infant-related external factors on ERP data quality. Plots with data loss distributions of all external factors also tested with continuous EEG data. Gender, wave, head shape, and typical day all show similar plots as with continuous EEG data, underlining the influence of these factors on data quality.



Supplementary figure 3.2. Effects of timing-related external factors on ERP data quality. Plots with data loss distributions of all external factors also tested with continuous EEG data. Time of onset of testing, order, and season all show great overlap with the continuous EEG plots. Once again, a distinct bump in data loss can be seen in figure D) around autumn and winter.



Supplementary figure 3.3. Data loss over the course of the entire study. Data loss over the course of the entire study is shown here. Every dot is a measurement of a single subject in a particular month. Data is slightly jittered to increase visibility of most dots. A smoothed line of the averages per month is shown here. A cyclic rhythm can be seen with lower data loss in the spring and summer months (middle of each year) and higher data loss at the start and towards the end of each year (winter and autumn). This rhythm can be found across age groups.

CHAPTER 4

The emergence of a theta social brain network during infancy

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Author contributions:
BV, CK designed the study. Data was collected by BV & research assistants at the KKC. BV analyzed the data. BV, TW, CK interpreted the data. BV drafted the paper. BV, TW, CK revised the paper

4.1 Abstract

Infants' socio-cognitive ability develops dramatically during the first year of life. From the perspective of ontogeny, the early development of social behavior allows for parent-child attachment, which in turn enhances survival. Thus, it is theorized that the development of social behavior, driven by social brain networks, forms the core of developmental acquisitions during this period. Further, understanding the maturation within the neural networks during social development is crucial to obtain a better grasp of the development of social developmental disorders. Therefore, we performed a longitudinal study in 854 infants measured at around 5 and 10 months to map the development of functional networks in the brain when infants were processing social and non-social videos. Using EEG, we focused on the frequency bands most commonly connected to social behavior: theta and alpha. We found that alpha networks remained relatively stable over the first year of life and showed no selectivity for social versus non-social stimuli, theta networks, showed strong global reconfigurations. The development of the theta networks progressed from a parietal occipital network in early infancy to a frontoparietal network towards the end of the first year of life. This reconfiguration coincided with selectivity for social versus non-social stimuli, with infants approaching the end of their first year of life showing increased synchronicity of theta communication when watching social videos versus non-social videos. Our findings provide strong evidence for the involvement of a frontoparietal theta network in the development of the social brain.

4.2 Introduction

Human social behavior develops dramatically during the first year of life. After birth, a newborn shows little social capabilities, but during the development between 4 and 12 months of age, infants start to exhibit signs of joint attention, vocal communication, and participate in complex social constructs, like games. These dramatic developmental changes in behavior are accompanied by equally dramatic changes in the brain, which triples in size during the first year of life (Huttenlocher and Dabholkar, 1997; Huttenlocher and de Courten, 1987; Innocenti and Price, 2005; Keunen et al., 2017). Young infants cannot tell us whether they possess certain social capabilities. Therefore, to study the onset of these capabilities, it is useful to detect whether unique neural correlates exist when processing certain social stimuli. For example, the finding that the brain of an infant already responds differently to faces versus houses at three months of age provides evidence that young infants are already primed to detect and process these important social cues (de Haan et al., 2001). Detecting and processing faces, however, is just one facet of social development. As an infant develops, social behavior becomes more complex, requiring input from a wide range of brain areas.

Therefore, when linking the development of the processing of complex cues with neuronal changes, researchers often focus on the development of infant brain networks. After birth, the network features of the infant's brain show many of the macroscopic properties of an adult brain, with the exception that the infant brain has an overabundance of connections (Huttenlocher and de Courten, 1987) and is unoptimized. Therefore, during childhood and later development, brain networks are optimized through the process of selective pruning of unimportant connections and the strengthening of important (and often long-range) connections (Huang et al., 2015; Yap et al., 2011). This optimization process from a localized to an integrated brain network is already well underway during infancy (Fair et al., 2009; Huang et al., 2015; Tóth et al., 2017), possibly allowing for more complex behaviors to arise over the course of the first year of life.

In this paper, we focus on the development of social behavior during infancy, by analyzing underlying brain networks and communication patterns when infants view complex social stimuli. Early frameworks tackling the relationship between the development of behavior and the brain emphasized the maturation of dedicated brain areas as a prerequisite for specific behavior (Atkinson, 1984; Johnson, 1990), some emphasizing the role of experience herein (Shadmehr and Holcomb, 1997; Spencer et al., 2009). However, more recently, the interactive specialization (IS) framework highlighted the interrelationship between anatomically distinct brain areas (Johnson, 2011). In the IS framework, brain areas respond to a wide array of non-specific cues in the undeveloped brain. During development, interactions between brain areas cause these responses to be more specific towards certain stimuli. Therefore, new behaviors in infants will not arise through the onset of activity in one single region but through changes in the activity of multiple brain regions. Following this framework, the emergence of complex social behaviors coincides with the emergence of a so-called social brain network, involved in processing social cues.

In adults, several areas thought to be involved in social processing have been grouped as the ‘social brain’ (Adolphs, 2008; 2003; 2001; 1999). This ‘social brain’ consists of several areas in the prefrontal and the parietal cortex. Most importantly, the fusiform face area, superior temporal sulcus, and the orbitofrontal cortex. Trying to connect the social brain to development, Johnson and colleagues predicted that during infancy the differences in brain activity patterns towards social and non-social stimuli become more distinct in areas associated with the social brain and that the social brain matures as a whole during infancy, rather than maturing region by region (Johnson et al., 2005). Work underlining this increase in social specialization in the infant's brain is extensive. For example, Farroni and colleagues used fNIRS and found evidence for social cortical specialization as early as a few days after birth (Farroni et al., 2013). Several ERP studies show similar results: infants start showing different ERP responses towards upside down versus right side up faces between 6 and 12-months (Haan et al., 2002; Halit et al., 2003); and start showing distinctly different brain activity patterns in response to fearful faces compared to neutral faces at 6-months but not at 3-months-of age (Hoehl and Striano, 2010). These studies provide compelling evidence that the social brain is undergoing specialization during the first year of life. However, evidence for the interactive part of the IS framework is less commonly described. Most notably, little is known whether entire networks become more specialized towards social stimuli over time. In this study, we, therefore, aim to test this hypothesis by testing infant brain networks in response to animate and inanimate movies in a large-scale longitudinal study.

Networks in the infant's brain can be studied using the synchronicity of oscillatory EEG rhythms to determine the likelihood of communication between distinct brain areas, as synchrony in oscillatory activity patterns allows for optimized communication between two distinct regions (Fell and Axmacher, 2011). These oscillatory rhythms can be divided into separate frequency bands. We focus on theta and alpha rhythm networks, as these networks can be reliably measured in infants (van der Velde et al., 2019). The theta rhythm (3-6 Hz) is commonly associated with social processing. Theta power is greater in infants looking at neutral faces compared with smiling faces (Bazhenova et al., 2007) and is increased during infant-directed speech (Orekhova et al., 2006). Most notably, an earlier study comparing the oscillatory response to inanimate versus animate movies found a general increase of theta power that became more pronounced between 6 and 12 months of age (Jones et al., 2015).

Alpha (6-9 Hz) oscillations have also been connected to social processing but in a less specific manner. Alpha power is thought to be an indication of brain ‘idling’. Therefore, alpha suppression is an indication of increased activity in that brain area. For example, observing goal-directed actions showed this suppression of alpha power in 8 to 16-month-old infants (Montirosso et al., 2019; Southgate et al., 2009). Also, during live eye contact, alpha band-activity desynchronizes in infants of 6-months-old (Hoehl et al., 2014). Evidence for the involvement of alpha frequency communication in social behavior was also found in infants at risk for ASD, with infants later developing ASD showing distinctly higher alpha connectivity during infancy (Orekhova et al., 2014).

The abovementioned studies show the undoubted connection between the development of social processing and oscillatory activity patterns, but to our knowledge, no longitudinal studies have actively measured the development of functional social brain networks during infancy in a large population. To assess the underlying neurobiology of the emerging social brain, we utilize EEG to study alpha and theta waves in a large group of infants twice during the first year of life. Functional networks were created using EEG data (theta and alpha bands) gathered from infants watching naturalistic social and non-social videos, similar to the ones used in Jones and colleagues (2015). We set out to test the hypothesis by Johnson and colleagues (2011) that the social brain emerges during infancy and that the social brain network increasingly specializes during infancy. Therefore, we expect a significant reconfiguration of the functional connectome during infancy (between 4-10 months of age). We expect this reconfiguration to coincide with an increased specificity of global network strength comparing networks of social and non-social cues.

4.3 Methods and materials

4,3.1 Participants

The YOUth study is a longitudinal cohort study consisting of two large cohorts differing in age range. The YOUth Baby & Child cohort follows infants from 20-24 weeks gestational age until the age of seven years. The YOUth Child & Adolescent cohort follows children from the age of 8 until the age of 16 years. Both behavioral and cognitive development is tracked through numerous tasks and methods (e.g. eye-tracking, EEG, MRI, questionnaires). The YOUth study was approved by the Medical Research Ethics Committee of the University Medical Center Utrecht and all participants' parents provided written informed consent. A brief overview of the YOUth study including the measurements conducted at each timepoint is available from https://www.uu.nl/en/research/youth-cohort-study (Onland-Moret et al., 2020)

The current study only uses data from the YOUth Baby & Child cohort, since this is the only cohort in which EEG was measured in young children from 5 months onwards. In total, 849 5-month-old infants and

642 10-month-old infants were included. Table 4.1 shows the demographic and attrition information of all subjects in our study. Attrition due to fussiness was counted when the infant was excluded from the analysis for having too little (or no) data due to the infant being too tired, inattentive, started crying, moved too much, or refused to wear the cap. Attrition due to experiment(er) error was counted when the RA logged this or when the resulting data file was corrupted. Attrition rates are well into the expected range (Stets et al., 2012). Only subjects with EEG valid -data sets for both sessions were used in the longitudinal analyses. Table 4.2 shows the demographic information of these subjects.

Table 4.1 Cross-sectional demographic and attrition information

	Gender	Total	Attrition		Exp. Error		Fussiness		Age (in days)	
		N	N	%	N	%	N	%	mean	sd
5m		849	125	14.7	27	3.2	98	11.5	167.2	22.9
	Boy	421	63	15	17	4	46	10.9	168.3	23.0
	Girl	428	62	14.5	10	2.3	52	12.1	166.2	22.7
10m		642	76	11.8	26	4	50	7.8	315.7	24.7
	Boy	318	32	10.1	14	4.4	18	5.7	316.8	25.0
	Girl	324	44	13.6	12	3.7	32	9.9	314.6	24.5

Table 4.2 Longitudinal demographic information

Session	Gender	N	Age (mean)	Age (sd)
5m		428	315.1	25.2
	Boy	227	315.3	25.5
	Girl	201	314.9	24.9
10m		428	167.3	22.3
	Boy	227	168.0	21.7
	Girl	201	166.5	23.0

4.3.2 Task Design

Infants were seated at 65 cm from a computer screen. Either on the lap of the parent or, when possible, in a highchair. Infants watched videos of either social or non-social content. Each video was 60 seconds in length and repeated 3 times. In total, the infants viewed 6 videos with the tasks interleaved. The initial video was randomized between subjects. The social video depicted women singing nursery rhymes and the non-social video showed toys that were moving without human interference (meaning that no hands were present on the screen). All moving toys were toys that could move by themselves (for example a spinning top). These movies were highly standardized and adapted from earlier work (Jones et al., 2015; Orekhova et al., 2014; Tomalski et al., 2013) by translating them to Dutch. The videos have earlier been used to detect reliable networks and characteristics (Haartsen et al., 2020; van der Velde et al., 2019). Beyond the content of the videos, the videos were kept as similar as possible. Both videos had comparable luminance and included similar amounts of motion and sound. The videos were meant to provide infants with typical experiences. In between videos, the infant was allowed a short break during the startup of the next video (see Figure 4.1A).

4.3.3 EEG Acquisition & Cleaning

EEG was recorded using a cap with 32 electrodes (ActiveTwo system, BioSemi) positioned according to the international 10/20 system, at a sampling rate of 2048 Hz. A Common Mode Sense (CMS) and Driven Right Leg (DRL) electrode were used to provide an active ground.

EEG data was analyzed using MATLAB, using the FieldTrip toolbox (Oostenveld et al., 2011). The original 2048 Hz data was down sampled to 512 Hz, using chip interpolation and band-pass filtered at 0.1-70 Hz with a two-way Butterworth filter. Artifact removal was similar to work done earlier in our lab (van der Velde et al., 2019). The continuous EEG data was cut into 1s. trials. Trials were selected for removal if they: contained too high amplitudes (> 250 uV); contained jumps (detected with *ft_jump_removal*.m); were excessively non-normal (kurtosis > 7); contained flatlining electrodes (inverse of variance > 0.1); or contained excessive noise (variance > 1500). The trials before and after bad trials were also selected for removal. Channels were removed if more than 40 percent of the trials in a channel contained artifacts. If no more than two bad channels were found in a single subject, the two channels were interpolated utilizing weighted averaging of the neighboring channels. If more than two bad channels were found in a subject's EEG dataset or if the two bad channels in question were neighbors, that subject's session was removed from the analysis. After cleaning, the continuous parts of the clean EEG data longer than 3 seconds were cut into 3-second trials. The sampling points to be included were stored for later use. The PLI can be prone to overestimation bias if the total amount of trials is low. Therefore, subjects were required to have at least 30 trials in the non-social condition and 30 trials in the social condition (see Figure 4.1B). After cleaning, 125 5-month-old infants and 76 10-month-old infants were removed (see Table 4.1). The remaining 724 5-month-old infants and 566 10-month-old infants were used for further analysis. Data quality characteristics of the EEG of the entire cohort are reported elsewhere (van der Velde and Junge, 2020).

4.3.5 Network calculation

Network calculation was similar to earlier studies of our group (van der Velde et al., 2019). To limit the influence of filter edge-effects, the raw EEG data was bandpass filtered into theta (3 up to, but not including 6 Hz.) and low alpha (6 up to, but not including 9 Hz.) frequency bands. Since individual theta and alpha peaks are influenced by development, alpha1 and theta bands were chosen to encompass all theta and alpha peaks +/- 1 Hz. After filtering, the three-second trials were created based on the earlier stored sampling data for clean trials. For each epoch, connectivity between pairs of electrodes (32*31/2 = 496) was calculated with the phase lag index (PLI), relying on the same principle of phase locking or phase synchrony (Tass et al., 1998).

The PLI was chosen over the debiased weighted PLI (Vinck et al., 2011), as previous work has shown the PLI to be slightly more reliable in an infant population (van der Velde et al., 2019). The PLI, proposed by Stam and colleagues, (Stam et al., 2007), describes the asymmetry of the distribution of phase differences between pairs of signals:

$$\mathrm{PLI} = \left|\langle sign\left[\sin\left(\Delta\varphi(tk)\right)\right]\rangle\right| \ ,$$

where $\Delta\varphi$ is the instantaneous phase difference between signals at time point t for k = 1 ... N per epoch (N = 5*512 = 2560), determined using the Hilbert transformation. | | stands for absolute values, <> for the mean values, and the sign for a signum function (phase difference is either -1, 0, or 1). The resulting PLI can range from 0 to 1. One of the key strengths of the PLI is that it is relatively resistant to volume conductance. Activity from a single strong source will likely appear in both electrodes as having a phase difference of close to zero. Maybe jumping from phase lagging to phase leaping every once in a while, under the influence of noise. This centering around zero phase lag will cause the PLI to be calculated as zero. The PLI is certainly not completely immune to volume conduction. It is, however, one of the most important tools we have to limit the influence of it on calculated connectivity.

The 496 connectivity values per epoch were used to create an adjacency matrix for every epoch for a subject. These adjacency matrices were used to calculate three separate subject connectivity matrices for both frequency bands: 1) An average of the entire experiment, 2) an average over the entire social experiment, and 3) an average over the entire non-social experiment (see Figure 4.1C). In addition to these adjacency matrices, global network strength was calculated for each subject connectivity matrix by averaging all connections in the network.

4.3.6 Network visualization

Grand averages were calculated for each of the six categories, by averaging each subject's connectivity matrix for each category over all subjects. To visualize these grand averaged connectivity matrices, connectomes were created using in-house MATLAB scripts. Two separate visualization processes were undertaken. In the first process, the grand averaged connectivity matrices were thresholded, to only include the strongest 25 percent of all connections. The remaining connections were visualized as connectomes based on electrode location. In the second process, neighboring nodes were grouped and connections between these nodes averaged to create average connectivity between areas. The following areas were created through averaging: Frontal (F), consisting of electrodes Fp1, Fp2, AF3, AF4, Fz; Left Frontal (LF), consisting of electrodes F3, F7, FC5, FC1; Right Frontal (RF), consisting of electrodes F4, F8, FC6, FC2; Central (C), consisting of electrodes C3, C4, CP1, CP2, Cz; Left Parietal (LP), consisting of electrodes T7, CP5, P7, P3; Right Parietal (RP), consisting of electrodes T8, CP6, P8, P4; and Occipital (O), consisting of electrodes PO3, PO4, O1, Oz, O2, Pz. A graphical overview of the grouping can be seen in Figure 4.1D. In the paper, these networks are labeled 'smoothed connectomes' to distinguish them from the full connectomes, since the process of taking together the connectivity of neighboring nodes essentially smoothes the connectome.

4.3.7 Spectral analysis

Previous work has underlined the possibility of overestimating and overinterpreting functional connectivity results due to the strong relationship between functional connectivity measures and EEG power (Demuru et al., 2020). This is even true for the phase lag index, which is calculated without the involvement of signal amplitude. However, increases in amplitude likely increase signal-to-noise ratios and with it the likelihood to calculate increases in connectivity. Therefore, we have included a spectral analysis and will use both theta and alpha power as dependent variables to compare to our main outcomes studying functional connectivity.

Spectral preprocessing was performed analogously to the spectral analysis in (Jones et al., 2015). Using MATLAB the cleaned epochs were subjected to an FFT, creating power spectra for all electrodes. Power values were averaged across all epochs and averaged across theta (3-6 Hz.) and alpha (6-9 Hz.) frequency bands. Only participants with enough clean trials and enough non-noisy channels were included in the analysis. The logged theta and alpha power was used for statistical analysis. The resulting power measures were strongly correlated with global connectivity. Theta power correlated strongly with theta global connectivity ($r = 0.61$) and alpha power correlated strongly with alpha global connectivity ($r = 0.75$).

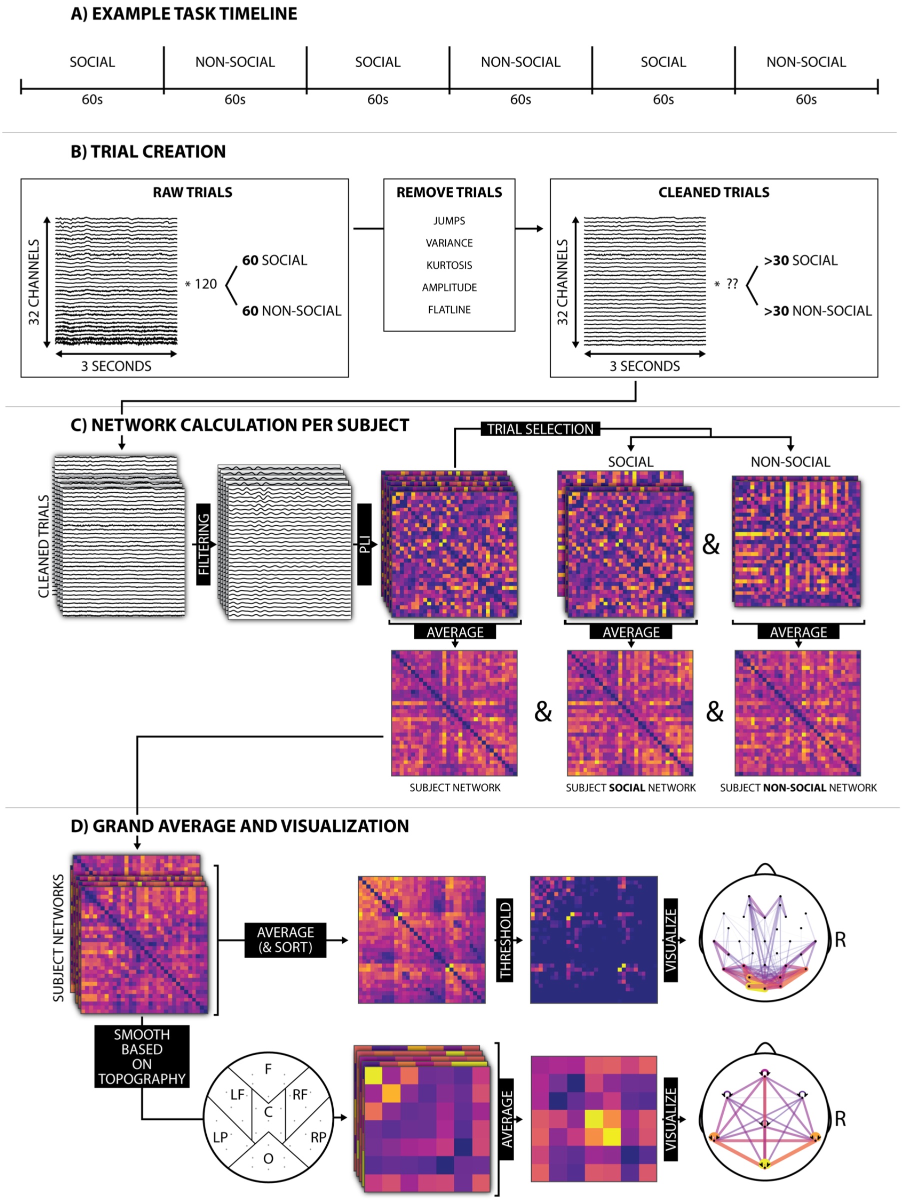
4.3.8 Statistical analysis

A major difficulty in assessing longitudinal effects is the almost unavoidability of missing data (Ibrahim and Molenberghs, 2009). Analyzing this type of data with a repeated measurements ANOVA (rmANOVA) forces the researcher to throw any subject away with missing data in one session, due to the rmANOVA requirement of MCAR (Missing Completely at Random). A linear mixed model (LMM), in contrast, assumes missing at random (MAR). This means, in practice, that even if a single session is missing, we can still use the participant's data points that were observed in other sessions for the analysis. Additionally, rmANOVAs assume sphericity and require independent variables to be categorical. Therefore, rmANOVAs are an effective procedure, easy to implement with statistical software, but only if the above assumptions are met, the variances between repeated measures are relatively equal, and we have complete data of all subjects. Since missing data is not MCAR in our study and additionally we include participant age (which is a continuous variable) as an independent variable, using a rmANOVA is not possible. Therefore, we opt to use LMMs to test our hypotheses.

LMMs can be seen as an extension of the linear regression models, but also include random effects and correlated errors (Bates, 2005; Jiang, 2007; McCulloch and Neuhaus, 2005). Similar to ANOVAs, LMMs include fixed effects (observed variables, like in our case connectivity strength towards social or toys videos; or time). The most common LMMs include at least a subject random effect. This random subject effect, also called random intercept, captures the differences in starting point between subjects. A subject that has a higher value in session 1 is assumed to also be higher in session 2. If necessary, additional random effects can be added to capture additional variance or correlation. This all allows for greater flexibility in the correlations between sessions. One additional important positive aspect of LMMs is that time does not have to be categorical: it can be added as a continuous measure. Therefore, in our case, where the age of infants is continuously noted in days, using an LMM provides an additional benefit (Krueger and Tian, 2004).

For our first research question, we evaluated how theta and low alpha networks developed over the first year of life. The research question can be split into two sub questions: 1) How does the average connectivity strength of EEG networks develop during the first year? 2) Does the network reconfigure over the first year, and if so, how? To answer these questions, we used the above described LMMs. The first sub question evaluates how average connectivity strength develops over infancy. We created two LMMs: one for theta average connectivity and one for low alpha average connectivity. With both LMMs, age was used as a fixed effect in addition to random intercept for participants. Since the PLI can induce a bias based on the number of trials, for each frequency band we compared two models: one with the number of trials as an extra fixed effect and one without. We used the Akaike information criterion (AIC) to select the most appropriate statistical model (lower is better). In both frequency bands, the model with the added fixed effect for the number of trials did not yield a significantly better model (theta: 3116.3 versus 3116.6; and alpha: 2990.0 versus 3007.2). The final model formula was: averageConnectivity ~age + (1|subject).

With the second sub question, we want to look at network reconfiguration over time. This is slightly different from our first sub question. If the first sub question determines that the network as on average increases in connectivity during the first year, we are not necessarily interested in that all the connections increase during this period. That would be redundant. What we are most interested in, is whether some connections become on average more or less important in the individual networks. Therefore, we center and scale the networks per subject for each session (also known as z-scoring). These z-scored connections are then used to perform LMMs. To do LMMs on all the connections in a network with 32 nodes would lead to multiple comparison issues. Therefore, we use the smoothed connectome of connectivity between 7 large areas in the brain (see above). In an undirected 7-node network, there are 28 connections. For each of the 28 connections, LMMs were fitted with age as a fixed effect, zscored connection as a dependent variable, and a random intercept for participant. This was done both for



theta and for low alpha networks. Once again, model fit was compared between the inclusion of the number of trials as a fixed effect or not. No models fitted better with the number of trials as a fixed effect. AIC of chosen models for theta connections varied between 2900.3 and 4520.9. For alpha connections, AIC varied between 2671.4 and 4596.8. Due to

Figure 4.1. Overview of analysis steps taken during this study. This figure shows all analysis steps taken during this experiment. From the gathering of data to the visualization of the eventual results. A) depicts an example video order during the experiment for a particular infant. The infant either started with the social or toy video and watched each video three times interleaved. B) Trials were created by cutting the 60s dataset in 20 3s trials for each video (so 20*6=120 trials in total) and were consequently discarded if problems were found with variance, kurtosis, jumps, absolute amplitude, or absence of data. C) Networks were created for each infant by filtering clean trials in theta and alpha frequency band. For each frequency band communication synchrony was calculated between electrodes using the PLI. Resulting adjacency matrices were created for the average synchrony over the entire task, the social videos, and the non-social videos. This results in 2 (frequency bands) * 3 (task options) = 6 adjacency matrices per subject. D) Grand averages were calculated using the average of all subject adjacency matrices for each of the 6 aforementioned categories. Connectomes were used to visualize data, either through thresholding or through averaging connectivity strength over neighboring electrodes.

space constraints, the AIC outcomes are in the supplementary material. The final formula use was connectionConnectivity ~age + (1|subject). All p-value thresholds were Bonferonni corrected.

For our second research question, we want to understand whether during development these theta and low alpha networks show an increase in sensitivity for social stimuli versus non-social stimuli. That is to say, whether network differences in processing social versus non-social cues become more distinct. Once again this question was first answered globally: are there differences in average connectivity strength in processing social cues versus non-social cues and how does this develop over the first year of life. And then locally: are there differences in individual connections in processing social cues versus non-social cues and how does this develop over the first year of life. We try to answer the first sub question by constructing an LMM with average connectivity (for social or non-social trials) as a dependent variable and age and social contrast (whether the infant was watching a social video or a toys video) as fixed effects. Once again, a random intercept for the participant was used. The inclusion for the number of trials did not yield a better fitting model (theta: AIC = -14469.88 versus AIC = -14449.14; and alpha: AIC = -13955.92 versus AIC = -13929.38). The final formula used was averageConnectivity ~age * socialContrast + (1|subject).

For the second sub question, we constructed a similar LMM as in the first sub question, but now for every connection in the smoothed connectome separately. The 28 LMMs were created both for the theta and the low alpha networks. No improvement was found when adding the number of trials to the model fit. For theta AIC varied between -13215.97 and -10619.19. For low alpha, AIC varied between -14263.85 and -11261.27. Due to space constraints, the AIC outcomes are in the supplementary material. The final formula used was connectionConnectivity ~age * socialContrast + (1|subject). All p-value thresholds were Bonferonni corrected.

Analyses were performed using the lmer4 package in R (Bates et al., 2014; 2007). Relative effect sizes were computed following Brysbaert and Stevens (2018). Effect size of $d < 0.2$ were deemed small, $0.2 < d < 0.5$ were deemed small-medium, $0.5 < d < 0.8$ were deemed medium-large, and $d > 0.8$ were deemed large.

For the models including an interaction, pairwise contrasts were calculated for every month of age of a subject. Since data is non-normal, these pairwise contrasts were calculated using the Wilcoxon signed-rank test. P-value thresholds were Bonferroni corrected. Since Cohen's d calculated effect size is calculated using means, which is non-optimal in non-normal data, Pearson's R was used to calculate the effect size. We used the following notions for effect size: $r < 0.1$ is small effect size, $0.1 < r < 0.3$ is small-medium effect size, $0.3 < r$ 0.5 is medium-large effect size, and $r > 0.5$ is large effect size.

4.4 Results

4.4.1 The development of global network strength over time

For global network strength, the estimated subject variance was 0.11 with an estimated residual variance of 0.67. For low alpha global network strength, the estimated subject variance was 0.22 with an estimated residual variance of 0.47. For the theta global network strength LMM, a significant main effect was found for infant age in days ($\hat{\beta} = 0.48$, $p < 0.0001$, $d = 1.35$). For the low alpha global network strength LMM, a significant main effect of infant age in days was also found ($\hat{\beta} = 0.53$, p <0.0001, d $= 1.89$). See table 4.3 for the summary of the results.

Table 4.3 Linear Mixed Model with age (in days) as a fixed effect and global network strength as the dependent variable

Frequency Band	Param.	Estimate	Test (df)	p*	Effect size
Theta	Age	0.48	t = 19.47 (833.21)	<0.0001	d = 1.35
Low Alpha	Age	0.53	t = 25.31 (715.96)	<0.0001	d = 1.89

** Bonferroni corrected*

4.4.2 The reconfiguration of the theta network

Figures 4.2 and 4.3 show the development of network topology in the infant's brain between 4-months-old and 11-months-old. The low alpha network configuration remains relatively stable over time, with high frontocentral connectivity. The theta network, however, reconfigures from an occipitoparietal network towards a frontoparietal network. To overcome the problems of choosing a threshold to depict networks, Figure 4.3 shows simplified "smoothed" network topologies. Connections are averaged according to the location on the scalp. Here a clear reconfiguration in the theta network can be seen as well, with clear interconnectivity appearing at 9 months old between the frontal areas and the left and right parietal areas.

To focus on the reconfiguration of the network, we evaluated the developmental pattern of individual connections in the network. To simplify the analysis, we used the smoothed networks, consisting of 28 connections in total. Above, we showed that age has a strong effect on global connectivity. To control for general connectivity strength increase due to age, individual networks were scaled and centered. A high value of connectivity now indicates that the connection is relatively more important compared to the other connections in the individual's network.

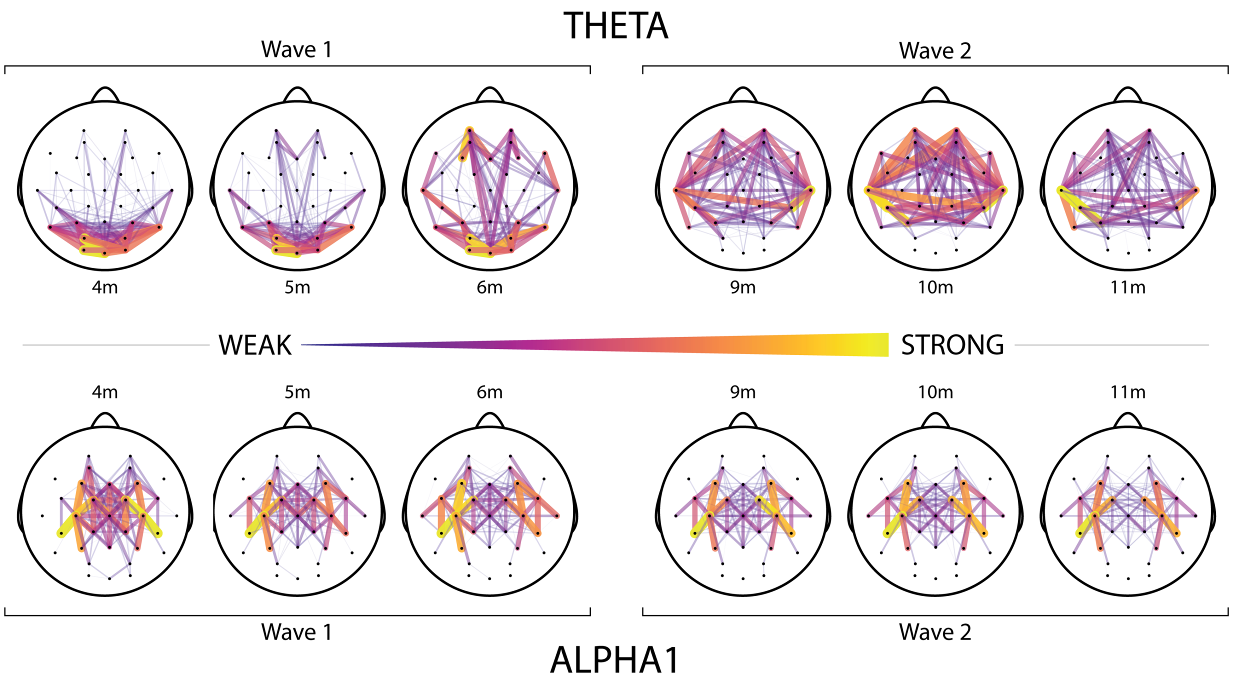


Figure 4.2 Normalized thresholded network topologies during infant development. Depicted here average network topologies between 4 and 11 months old viewed top down. The networks are normalized per age group to enhance the focus on network configuration instead of network strength. Stronger connections are thicker and more yellow. The low alpha networks show little reconfiguration. The theta networks do show a distinct reconfiguration from an occipitoparietal network towards a frontoparietal network. This switch seems to come online gradually, with 5 and 6-month-old infants already showing distinctly more frontoparietal connectivity compared to the 4-month-old infants. Note that infants were tested in 2 waves (4 to 6-month-old and 9 to 11-month-old), so every infant is represented twice in this figure.

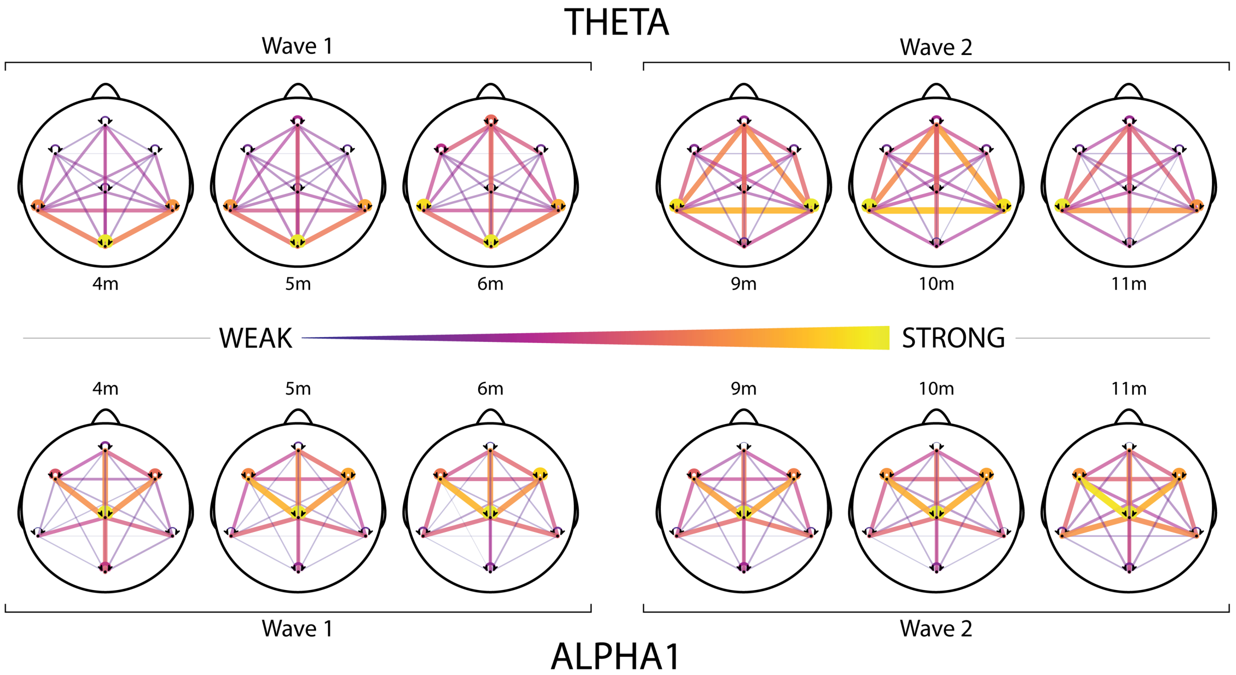


Figure 4.3 Normalized smoothed network topologies. Depicted here smoothed average network topologies between 4 and 11 months old viewed top down. Connections are averaged according to location on the scalp (see figure 1d). Double ended arrows represent interconnectivity within a certain area. Low alpha shows a clear pattern across the first year of development, with high connectivity within the central areas and all areas with central areas in the brain. For the theta networks, however, a clear reconfiguration takes place with the occipitoparietal network slowly morphing into a frontoparietal network, which can be clearly spotted from 9-months-old onwards with a clear triangle of connectivity between the frontal and left and right parietal areas in the brain. Also note the increase in interconnectivity between parietal areas.

The LMMs used single connection strength as the dependent variable, with age (in days) as a fixed variable and random intercepts for subject.

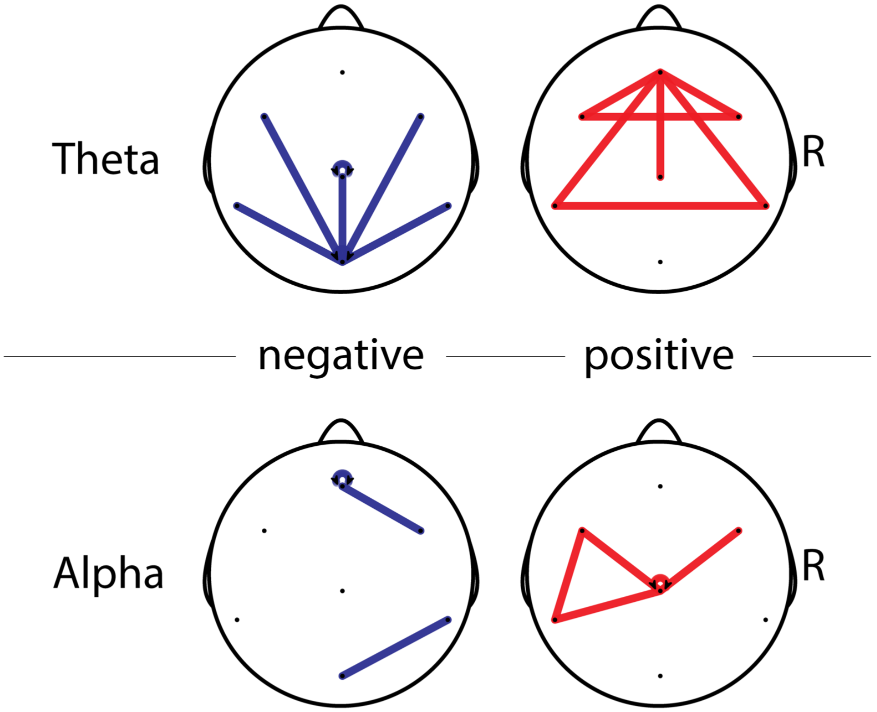


Figure 4.4. Development of important connections during infancy. Depicted here are the connections of the smoothed connectomes which become significantly more or less important in the connectome during the first year of life. A linear mixed model was run over each connection with normalized connection strength as dependent variable and age as fixed variable. The red connections indicate a significant increase in importance at the $p<0.0001$ level (Bonferroni corrected). The blue connections indicate significant decrease in importance at the $p<0.0001$ level (Bonferroni corrected). For theta, a clear switch can be seen, where connections to the occipital cortex become less important during the first year of life and the frontoparietal connection become more important. In the alpha network the opposite is true, where the already important frontocentral connectivity becomes increasingly prominent, while other connections become less involved.

The results are shown in Figure 4.4. The red connections indicate an increase in relative connectivity at the $p<0.0001$ level (Bonferroni corrected), the blue connections indicate a decrease in relative connectivity at the $p<0.0001$ level (Bonferroni corrected). In line with the earlier visualizations of the networks, over the first year of life, a clear reconfiguration can be seen in the theta network, from an occipitoparietal network towards a frontoparietal network. In the alpha network, such a change was not found. If anything, the frontocentral network becomes more prominent over time. For the full linear mixed models of all 28 connections and both frequency bands, please refer to the supplementary materials.

4.4.3 The infant EEG network and social processing

To understand whether this reorganization of the theta network coincided with an increase in sensitivity for the processing of social stimuli, we again utilized LMM (see Table 4.4 & Table 4.5). This time, global connectivity when watching social or toy videos was used as the dependent variable. Since we were interested in whether the development of the theta network increased sensitivity for social processing, we used both ‘age’ and ‘social versus toy’ contrasts as fixed effects. Once again, a random intercept for subject was included.

For the theta networks (Table 4.4), the estimated subject variance was 0.33 with an estimated residual variance of 0.47. Not surprisingly, a significant main effect was found for age ($\hat{\beta}$ = 0.37, $p < 0.0001$, $d = 0.81$). Additionally, a significant main effect was found for the social versus toys contrast ($\hat{\beta}$ = 0.24, $p < 0.0001$, $d = 0.42$). Lastly, and most importantly, a significant interaction effect was found between age and social contrast ($\hat{\beta}$ = 0.19, $p < 0.0001$, $d = 0.35$) indicating that as the infant's brain develops, the difference in theta network synchronicity increases. Further analyzing this effect, we performed 6 post-hoc Wilcoxon signed-rank tests, separating the infants per age in months. We found that from 6 months onwards, a significant difference in global connectivity strength in the social versus non-social condition could be found. From 9-months-old onwards, a medium-large effect was detected ($0.42 < r < 0.52$). For more detailed statistics, please see Table 4.4.

We performed a similar LMM analysis for the low alpha networks. The estimated subject variance was 0.22 with an estimated residual variance of 0.47. A significant main effect was found for age ($\hat{\beta} = 0.52$, p <0.0001, d $= 1.40$). No effect for social contrast or interaction effect between age and social contrast was found. Since we did not find an interaction effect, no post-hoc analyses were performed. For more detailed statistics, please see Table 4.5. Figure 4.5 shows the predicted global strength over the first year of life, based on these LMMs. A clear interaction effect can be seen in the theta networks, but not in the low alpha networks.

Table 4.4. Linear mixed model with age and social vs toys videos as the fixed effects and theta global connectivity as the dependent variable (pairwise contrasts are indented).

Parameter	Estimate	Test (df)	p*	Effect size
Age	0.37	t = 17.27 (1924.88)	<.0001	d = 0.81
Social	0.24	t = 8.58 (1503.96)	<.0001	d = 0.42
Age X Social	0.19	t = 6.80 (1500.11)	<.0001	d = 0.35
04m (Social vs Toys)		Z = 0.95	n.s.	r = 0.10
05m (Social vs Toys)		Z = 2.05	n.s.	r = 0.11
06m (Social vs Toys)		Z = 2.83	<0.05	r = 0.24
09m (Social vs Toys)		Z = 7.07	<.0001	r = 0.49
10m (Social vs Toys)		Z = 7.79	<.0001	r = 0.52
11m (Social vs Toys)		Z = 5.34	<.0001	r = 0.42

** Bonferroni corrected*

Table 4.5. Linear mixed model with age and social vs toys videos as the fixed effects and low alpha global connectivity as the dependent variable (pairwise contrasts are indented).

Parameter	Estimate	Test (df)	p*	Effect size
Age	0.52	t = 30.26 (1852.00)	<.0001	d = 1.40
Social	-0.06	t = -0.74 (1545.80)	n.s.	d = -0.04
Age X Social	-0.01	t = -0.38 (1542.76)	n.s.	d =0.01

** Bonferroni corrected*

We once more zoomed in on the interaction effect between age and the contrast type (social versus toy) to find out which connections, between which areas in the brain showed a similar interaction effect over the first year of life. With the theta networks, 28 LMMs with the *age (in days)* $\times$

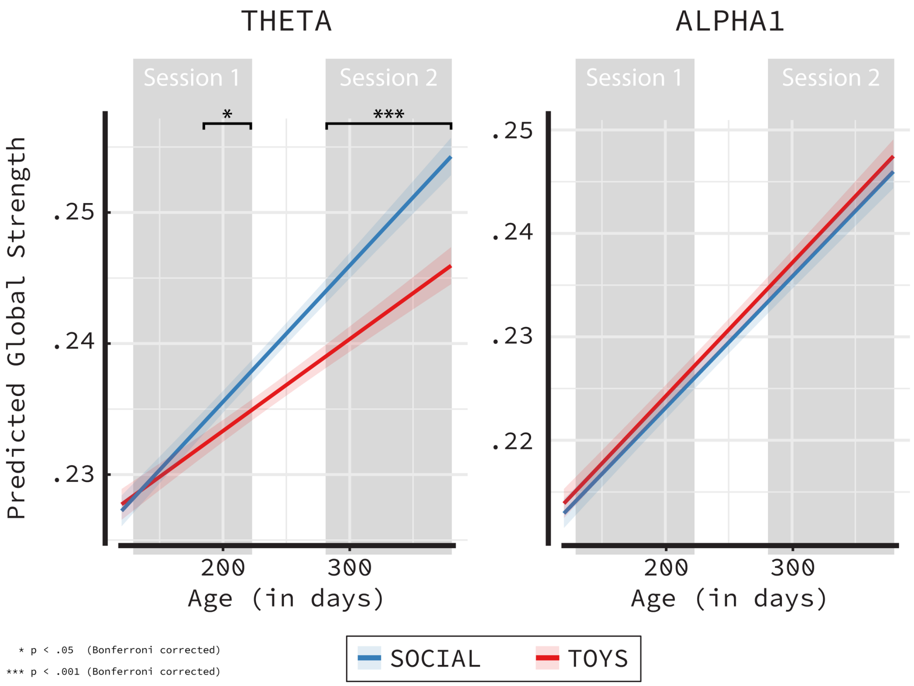


Figure 4.5. Interaction effect age and watching social videos. During the first year of life, theta global connectivity differences between watching social and toys videos become more distinct. This effect is significant from 6 months onwards but becomes more pronounced over the first year of life. No such effect can be seen in alpha1 global connectivity. Session 1 and 2 are depicted as light gray to visualize the age range the prediction of the LMM is based on.

social contrast interaction effect with single connectivity strength as the dependent variable were calculated for each connection in the smoothed connectivity matrix. The resulting findings are presented in Table 4.6. The significance levels are Bonferroni corrected for multiple comparisons. Significant interaction effects were found in connections throughout the brain, except for the interconnectivity in the frontal regions. We plotted the connections with at least a small-medium effect size (d>0.2) in Figure 4.6. Figure 4.6 nicely illustrates the frontoparietal triangle showing and the occipitoparietal connections both having a significant interaction effect. For the alpha networks, no significant interaction effects were found for single connections. Therefore, the resulting LMMs are presented in the supplementary materials.

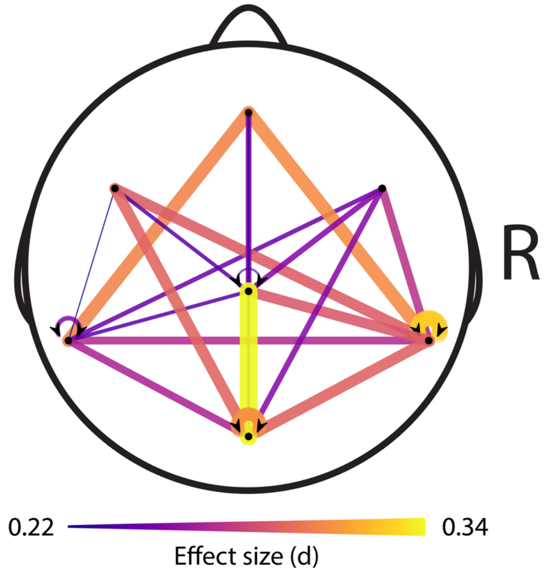


Figure 4.6. The theta connections with significant age x social interaction effects. Depicted here are the connections with a significant interaction effect between age and social contrast. These connections developed an increase in synchronicity for social stimuli over the course of the first year of life. Connections are scaled in color and thickness according to their effect size. Only connections with an effect size higher than the cut off for small effect size (0.2) are shown. Opposed to the development of the theta connectome, social specialization happens all throughout the brain, with the exception of frontal interconnectivity. Note the clear frontoparietal triangle and the strong connectivity between the occipital and the parietal areas.

4.4.4 Infant EEG power and social processing

To provide a comparison, similar models were calculated with theta and alpha power as dependent variables. For the theta power, the estimated subject variance was 0.49 with an estimated residual variance of 0.29. A significant main effect was found for age ($\hat{\beta} = 0.46$, $p < 0.0001$, $d = 1.47$). Additionally, a significant main effect was found for the social versus toys contrast ($\hat{\beta} = 0.17$, $p < 0.0001$, $d = 0.39$). Lastly, a significant interaction effect was found between age and social contrast ($\hat{\beta} = 0.14$, $p < 0.0001$, $d = 0.29$). Note that both the scaled estimation and the effect size of theta

Table 4.6 Linear mixed model interaction effect between the fixed effects of age and social vs toys with theta individual connections as the dependent variable

Connectivity Pair		Estimate	Test (df)	p*	Effect size
Frontal	Frontal	-0.01	t = -0.19 (1761.49)	n.s	d = -0.01
Frontal	Left Frontal	0.08	t = 2.76 (1730.68)	n.s	d = 0.13
Frontal	Right Frontal	0.10	t = 3.53 (1739.68)	< .05	d = 0.17
Frontal	Left Parietal	0.18	t = 6.47 (1720.44)	< .0001	d = 0.31
Frontal	Right Parietal	0.18	t = 6.49 (1701.93)	< .0001	d = 0.31
Frontal	Central	0.15	t = 5.36 (1721.69)	< .0001	d = 0.26
Frontal	Occipital	0.15	t = 5.02 (1781.50)	< .0001	d = 0.24
Left Frontal	Left Frontal	0.10	t = 3.26 (1722.31)	< .05	d = 0.16
Left Frontal	Right Frontal	0.10	t = 3.74 (1725.42)	< .01	d = 0.18
Left Frontal	Left Parietal	0.13	t = 4.66 (1725.74)	< .0001	d = 0.22
Left Frontal	Right Parietal	0.17	t = 6.14 (1716.51)	< .0001	d = 0.30
Left Frontal	Central	0.14	t = 5.07 (1727.79)	< .0001	d = 0.24
Left Frontal	Occipital	0.19	t = 6.29 (1767.83)	< .0001	d = 0.30
Right Frontal	Right Frontal	0.10	t = 3.48 (1726.44)	< .05	d = 0.17
Right Frontal	Left Parietal	0.15	t = 5.18 (1724.39)	< .0001	d = 0.25
Right Frontal	Right Parietal	0.15	t = 5.74 (1707.95)	< .0001	d = 0.28
Right Frontal	Central	0.15	t = 5.35 (1723.18)	< .0001	d = 0.26
Right Frontal	Occipital	0.16	t = 5.41 (1740.73)	< .0001	d = 0.26
Left Parietal	Left Parietal	0.15	t = 5.23 (1721.07)	< .0001	d = 0.25
Left Parietal	Right Parietal	0.15	t = 5.65 (1696.48)	< .0001	d = 0.27
Left Parietal	Central	0.14	t = 5.06 (1722.69)	< .0001	d = 0.24
Left Parietal	Occipital	0.17	t = 5.71 (1764.39)	< .0001	d = 0.27
Right Parietal	Right Parietal	0.20	t = 6.94 (1707.56)	< .0001	d = 0.34
Right Parietal	Central	0.17	t = 6.10 (1714.08)	< .0001	d = 0.29
Right Parietal	Occipital	0.19	t = 6.30 (1776.24)	< .0001	d = 0.30
Central	Central	0.15	t = 4.89 (1751.40)	< .0001	d = 0.23
Central	Occipital	0.22	t = 7.30 (1750.73)	< .0001	d = 0.35
Occipital	Occipital	0.21	t = 6.65 (1763.05)	< .0001	d = 0.32

** Bonferroni corrected*

power are lower for the interaction effect compared to interaction effect with theta global connectivity as dependent variable.

We performed a similar LMM analysis for the low alpha power. The estimated subject variance was 0.44 with an estimated residual variance of 0.22. A significant main effect was found for age ($\hat{\beta} = 0.59$, p $<$0.0001, d $=$ 1.87). No effect for social contrast or interaction effect between age and social contrast was found. Additionally, tables and figures detailing this interaction effect were created. These can be found in the supplementary materials. Lastly, a figure was create showing the scalp topography of alpha and theta power in response to the social and toys videos. This figure can also be found in the supplementary materials.

4.5 Discussion

Our goal was to describe the relationship between the development of social capabilities and the development of the infant's brain. Since behavior is embedded within functioning networks in the human brain, and since there is considerable social cognitive development within the first year of life, we focused on the development of infant brain networks over this period. We described the development of alpha and theta networks in the infant's brain with its relationship to the processing of social stimuli. As a measure of the total synchronicity of the brain, we used global connectivity. We found that theta networks, but not alpha networks, show increased sensitivity in differentiating social versus non-social videos, with an increase in global connectivity found when looking at social compared to non-social videos. Notably, this increase was only found in infants when they reached approximately 9-months of age. This period of time coincided with a striking reconfiguration of the theta network. This reconfiguration, in addition to the increased sensitivity to social videos, provides important evidence for the emergence of the social brain during infancy.

4.5.1 Network development

Both alpha and theta networks show a general increase in network strength. This indicates greater synchronicity between brain regions, which likely reflects increase neural communication between distant brain areas and is related to the global maturation of neural networks. Supporting this, Xie and colleagues (Xie et al., 2018) studied sustained attention in infants and found that global connectivity both in the alpha and in theta networks increased over the course of development during the first year of life. Maturation of global networks during infancy has also been shown in fMRI studies (Gao et al., 2015; 2014). This is possibly caused by an increase in myelination, which progresses at a rapid pace during the first year of life (Keunen et al., 2017). Together with the strengthening of important connections, this could lead to increased global connectivity. It is important to note that increases in network strength do not necessarily indicate the optimization of networks. Increases in theta have been connected to immaturity (Orekhova et al., 2006), however, during the first year of life increases in theta communication appear to be related to typical development.

Together with a global increase in synchronicity, theta networks also underwent considerable reconfiguration during the first year of life. Theta synchronicity evolved from a predominant occipitoparietal network to a more balanced pattern that included the addition of a frontoparietal network. Prior studies have found that frontoparietal brain networks are related to attention (Gao et al., 2015), working memory (Short et al., 2013), and, noteworthy for this study, the social brain (C. D. Frith, 2007; U. Frith and C. Frith, 2010). Figure 4.3 shows this especially clear, with a clear increase in the synchrony in the triangle between the prefrontal cortex and the bilateral parietal cortex.

The change in network configuration occurred quite rapidly. This supports the hypothesis of Johnson and colleagues (2005), who hypothesized that through the lens of the IS framework, networks should mature in unison rather than area by area. Note, however, that we did not study the infants consistently for all ages and that the sudden shift in configuration coincides with a 3-month hiatus between waves of data collection (between 6 and 9-months-old). Thus, we do not know how rapidly the theta network develops within this 3-month window of time. Future research is warranted to better understand the precise temporal characteristics and importantly, environmental factors that contribute to the functional network organization in the infant's brain.

Per our hypothesis, the theta network showed a considerable reconfiguration of important connections in the whole brain network. The alpha network, however, did not show this reconfiguration. On average alpha showed the strongest synchronicity in the frontocentral areas of the brain. As the infant developed, these frontocentral connections in the network strengthened and became an even more important focal point of the network. The alpha network did show a similar increase in global connectivity as the theta network. This underlines the uniqueness of the reconfiguration of the theta network during infancy.

4.5.2 Social selectivity

In addition to the considerable reorganization of the theta network in the infant's brain, we also showed that this reorganization coincided with an increase in global connectivity when watching social stimuli compared to non-social stimuli. This increase only was identified in the 10-month-old infants, and not in the 5-month-old infants, so after the reorganization took place. Increases in global connectivity are an important indication that areas in the brain increase in communication with each other when looking at social images. As global connectivity is also found to be a reliable brain network metric (van der Velde et al., 2019), this is important evidence that the theta network is connected to the development of the social brain.

This is in agreement with earlier work from Jones and colleagues (2015), who studied EEG-power in response to similar videos as used in the current paper and found that theta power increased when looking at social videos versus non-social videos. However, contrary to their work, in our paper increases in synchronicity were found throughout the entire brain network (excepting frontal interconnectivity) and not specifically in parietal and frontal regions. A possible explanation for this is that the reorganized theta network has a strong backbone of frontal and parietal regions, these communications are projected throughout the brain, leading to significant differences in synchronicity in the whole-brain network. Extra evidence for this topography of theta power while watching social and non-social videos can be found in the current paper. As our spectral analysis yielded very similar results compared to Jones and colleagues' (2015) work (supplementary materials). The alpha network did not show any selectivity for social versus non-social stimuli, with synchronicity between signals remaining equal for both the 5-month-old and the 10-month-old infants.

4.5.3 Limitations and future directions

The above-described work comes with some limitations. First, it is difficult to determine whether the network 'comes online' suddenly (as hypothesized by the IS framework, Johnson, 2011). We demonstrate that the reconfiguration of the core theta network takes place between 6 and 9 months of age, but how rapidly within this period of time is not known. Additionally, infants in the current study were tested twice, limiting the ability to test non-linear trajectories of brain network development. Future studies, with greater temporal sampling, will shed light on these issues.

Also, in our study, we did not find any relationship between alpha synchronicity and social development. What is important to note, however, is that studies that did find a relationship between alpha depression and social processing were using live conditions of social interaction (Jones et al., 2015). Future research is necessary to study in what way the development of alpha connectivity influences social brain processing during infancy, including the addition of live social interactions.

Lastly, it is important to note that it is difficult to pull apart EEG power and EEG connectivity. As mentioned before, there is a clear relationship between functional connectivity measures and EEG power. This is also the case in our paper. Therefore, we have included spectral results for inspection. Both effect sizes and estimates are lower in the models based purely on power measurements compared to global connectivity measures, but it is currently unclear whether this means that connectivity measures tell us more than just the power measures. Related to this is the possibility of entrainment caused by speech during the social videos (Leong et al., 2017). This would cause EEG power to increase, but not due to connectivity. However, this should not be the case in our study, as we find similarly structured networks in both the social and the toys condition. Indicating that the measurement of communication in the social condition does not just arise due to entrainment. Additionally, entrainment should cause brain waves to have the exact same phase, which should lower their measured PLI connectivity.

4.6 Conclusion

We showed that theta networks in the brains of infants undergo a large reconfiguration during the first year of life; from an on average parieto-occipital network towards a frontoparietal network. This reconfiguration coincides with an increase in sensitivity for social versus non-social stimuli, which is represented by an increase in synchronicity of brain activity when looking at social videos compared to non-social videos. The alpha network neither shows this reconfiguration or this increase in sensitivity for social stimuli. This provides important evidence for the development of the social brain network during the first year of life and its probable relationship to theta communication. Also, this study provides evidence for the interactive specialization framework (Johnson, 2011), as the reconfiguration appears to develop in unison, rather than piecewise between different regions. Future studies can hopefully shed light on whether this theta network reconfiguration can be used to predict behavioral outcomes and whether enhancing these network connections in children with delayed social development can improve their social cognitive abilities.

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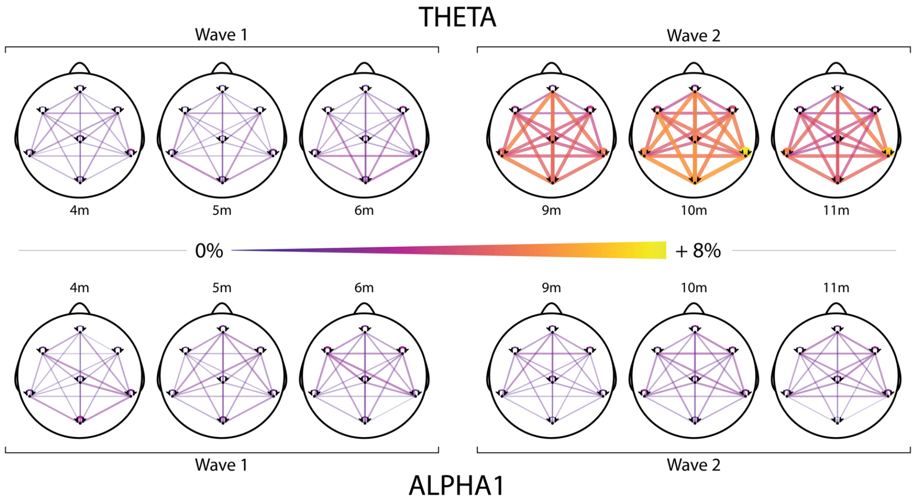
Appendix I: Supplementary Materials

Supplementary table 4.1. Linear mixed model interaction effect between the fixed effects of age and social vs toys with low alpha individual connections as the dependent variable

Connectivity Pair		Estimate	Test (df)	P*	Effect size
Frontal	Frontal	-0.03	t = -0.99 (1707.61)	n.s	d = -0.05
	Left Frontal	-0.02	t = -0.81 (1718.15)	n.s	d = -0.04
	Right Frontal	-0.04	t = -1.72 (1717.95)	n.s	d = -0.08
	Left Parietal	-0.02	t = -0.74 (1714.52)	n.s	d = -0.04
	Right Parietal	0.00	t = -0.18 (1715.89)	n.s	d = -0.01
	Central	-0.02	t = -0.85 (1722.11)	n.s	d = -0.04
	Occipital	-0.01	t = -0.28 (1718.89)	n.s	d = -0.01
Left Frontal	Left Frontal	0.00	t = -0.08 (1724.86)	n.s	d = 0.00
	Right Frontal	-0.06	t = -2.43 (1726.34)	n.s	d = -0.12
	Left Parietal	-0.02	t = -0.89 (1725.70)	n.s	d = -0.04
	Right Parietal	-0.04	t = -1.43 (1716.22)	n.s	d = -0.07
	Central	-0.02	t = -0.83 (1728.30)	n.s	d = -0.04
	Occipital	-0.02	t = -0.97 (1729.56)	n.s	d = -0.05
Right Frontal	Right Frontal	-0.02	t = -0.66 (1732.10)	n.s	d = -0.03
	Left Parietal	-0.02	t = -0.75 (1718.20)	n.s	d = -0.04
	Right Parietal	0.00	t = -0.15 (1720.53)	n.s	d = -0.01
	Central	-0.02	t = -0.73 (1730.09)	n.s	d = -0.03
	Occipital	0.00	t = 0.08 (1727.34)	n.s	d = 0.00
Left Parietal	Left Parietal	0.00	t = -0.15 (1706.78)	n.s	d = -0.01
	Right Parietal	-0.03	t = -1.15 (1711.05)	n.s	d = -0.06
	Central	-0.03	t = -1.10 (1727.66)	n.s	d = -0.05
	Occipital	0.02	t = 0.92 (1716.71)	n.s	d = 0.04
Right Parietal	Right Parietal	-0.02	t = -0.54 (1714.35)	n.s	d = -0.03
	Central	-0.01	t = -0.34 (1727.03)	n.s	d = -0.02
	Occipital	0.05	t = 1.96 (1721.53)	n.s	d = 0.09
Central	Central	-0.03	t = -1.16 (1724.31)	n.s	d = -0.06
	Occipital	0.01	t = 0.58 (1729.93)	n.s	d = 0.03
Occipital	Occipital	0.13	t = 1.37 (1723.85)	n.s	d = 0.07

** Bonferroni corrected*



Supplementary figure 4.1. Smoothed connectome differences between social and non-social videos. The difference in connectivity strength (in %) for the smoothed connectomes is depicted. Very little to no difference in connection strength between social and non-social stimuli can be detected in the low alpha frequency band and in the theta frequency band at 5-months-old. In the 10-month-old wave, however, large differences can be found across the entire connectome up to 8% difference in connectivity strength in the parietal areas.

Supplementary table 4.2. Linear mixed model with age and social vs toys videos as the fixed effects and theta power as the dependent variable

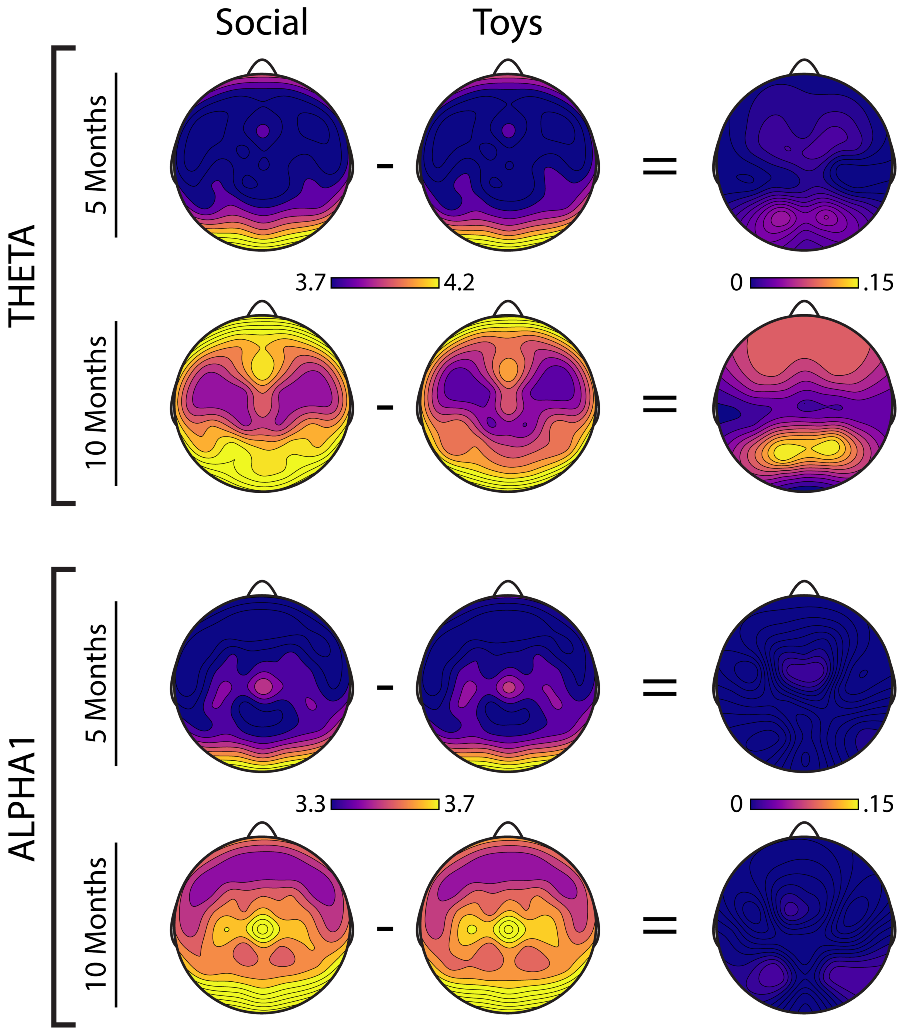
Parameter	Estimate	Test (df)	p*	Effect size
Age	0.46	t = 31.51 (1836.63)	<.0001	d = 0.81
Social	0.17	t = 7.72 (1607.49)	<.0001	d = 0.39
Age × Social	0.14	t = 5.82 (1608.03)	<.0001	d = 0.29

** Bonferroni corrected*

Supplementary table 4.3. Linear mixed model with age and social vs toys videos as the fixed effects and alpha power as the dependent variable

Parameter	Estimate	Test (df)	p*	Effect size
Age	0.59	t = 31.51 (1836.63)	<.0001	d = 1.87
Social	-0.04	t = 7.72 (1607.49)	n.s.	d = -0.11
Age × Social	-0.02	t = 5.82 (1608.03)	n.s.	d = 0.01

** Bonferroni corrected*



Supplementary figure 4.2. Scalp topography of log power during social and non-social videos and the difference between the two conditions. This figure is a recreation of a figure of Jones and colleagues (2015). We replicate their findings in that theta power while watching social videos is higher in frontal and parietal areas compared to non-social videos. This is only true at 10-months-old. We find no differences in alpha power between social and non-social videos.

CHAPTER 5

Relating the development of infant behavior to infant brain networks

In Preperation:
van der Velde, B., White, T.J., Geeraerts, S.B., & Kemner, C. (in prep). Relating the development of infant behavior to infant brain networks

Author contributions:
BV, CK designed the study. Data was collected by BV & research assistants at the KKC. BV analyzed the data. BV, TW, SB, CK interpreted the data. BV drafted the paper. BV, TW, SB, CK revised the paper

5.1 Abstract

INTRODUCTION: Infant behavior becomes increasingly complex during the first year of life. Complex behavior likely requires a multitude of brain areas to communicate efficiently. Here, we used a prospective population-based cohort to determine the longitudinal relationship between the infant brain network and infant behavior.

METHODS: We studied nearly 1000 infants in two sessions during their first year of life. Networks were created from task EEG data, filtered into theta and alpha frequency bands. Global network strength and small-worldness were compared over sessions. Social competence and infant temperament development were measured using the ASQ:SE and the IBQ-SF questionnaires respectively. Cross-lagged panel models and linear mixed-effects models were used to describe the associations between behavioral and brain network measures.

RESULTS: Brain networks showed expected patterns of maturation during the first year of life with increases in global connectivity and small-worldness. Brain networks and behavior showed a complex relationship. Early brain connectivity predicted infant orienting/regulation later in life. However, early infant orienting/regulation was also related to higher network optimality later in life. Other behavioral measures yielded no significant relationships.

CONCLUSIONS: We found evidence for the complex interaction between brain networks and infant regulation/orienting. This two-way relationship could cause a positive feedback loop. Where increasingly optimized brain networks allow for increasingly complex behaviors which in turn optimize brain networks.

5.2 Introduction

The infant's brain develops strikingly during the first year of life, with the brain tripling in size (Gilmore, Knickmeyer, & Gao, 2018). This structural neural development coincides with the largest behavioral development in the human lifespan. The study of this relationship, between the development of brain and behavior during the first year of life, has been one of the focal points of developmental psychological research. Especially prominent are studies relating the development of certain skills to the development of certain brain areas (de Haan, Johnson, & Halit, 2003; Halit, de Haan, & Johnson, 2003; Mundy, Card, & Fox, 2000). However, during the first year of life infants start exhibiting increasingly complex behaviors. These types of behaviors need input from a wide variety of functionally specialized brain areas to be efficiently integrated into one unified construct to respond successfully (Adolphs, 2001; Miller, 2000; Pessoa, 2008). Unsurprisingly, in the past years, researchers have become increasingly interested in the infant brain network to explain behavior (Mundy & Jarrold, 2010; van der Velde, White, & Kemner, 2021).

Two key developing behaviors during infancy – social behavior and self-regulation - likely have a complicated relationship with developing brain networks. For successful social interaction, the infant needs to review and integrate many different cues, including - but not limited to - social cues (Alden & Wallace, 1995), emotional cues (Lopes, Salovey, Côté, Beers, & Petty, 2005), and sensory cues. Good communication between functionally distinct brain areas is therefore required for successful social interactions. Conversely, however, exhibiting these complex behaviors also optimizes the brain. Through experience and learning oft-used connections get strengthened and unused connections get pruned (Keunen, Counsell, & Benders, 2017). Social development is especially vital for this since most infant learning happens through social interactions (Grusec, 1994). Therefore, improved social skills will optimize brain functioning - not only between areas important for social interactions - but for the brain as a whole. So, this relationship between social competency and the brain network seems to be reciprocal: mature brain networks allow for social behavior, which in turn optimizes this whole-brain network. A similar relationship between brain and behavior can be theorized for a behavior vital for successful social interaction: self-regulation (Adolphs, 2003; Nigg, 2017).

The relationship between brain networks and social and self-regulatory development has seen an increase in interest over the past decades. Yet, most prior research ignores this reciprocal relationship during development. The aim of this current study is therefore to investigate this two-way relationship between the development of social behavior and self-regulation during the first year of life with the development of the infant brain network, using a longitudinal cross-lagged panel model.

5.2.1 Development of the brain network

Structurally, most of the macrostructures of the infant brain network are already present at birth (Ball et al., 2014; van den Heuvel et al., 2014). The neonate brain network is, however, still unoptimized. The first years of life are therefore mostly spend on optimization, through the selective pruning of rarely used connections and the strengthening of oft-used connections (Keunen et al., 2017). Functionally, a similar strive for optimization has been shown. Activity patterns in distinct brain areas become more focal and stronger during development, indicating an increase in specialization (Durston et al., 2006). This coincides with increased integration of brain information, through increased reliance on long-range connections in the developing connectome (Fair et al., 2009).

To calculate this optimization of the brain network, a graph-theoretical framework is often used. In graph theory, networks consist of nodes (the centers of information) and edges (the connections between the nodes). A network structure consisting of many clusters (neigboring nodes are strongly interconnected) and a relative ease of going from one part of the network to another (small average path length) is generally seen as an optimal network, since it combines two key features of any network: specialization and integration. This optimality is captured in the small-worldness index (Watts & Strogatz, 1998).

While neonate brain networks do show some semblance of small-worldness (Fransson et al., 2007), many studies have found evidence for further optimization during childhood. In an EEG-network study, Boersma and colleagues showed that the clustering increases between the ages of 5 and 7 years (Boersma et al., 2011). Similar studies have found general decreases in the path length between the ages of 2 and 6 years (Bathelt, O'Reilly, Clayden, Cross, & de Haan, 2013; Power, Fair, Schlaggar, & Petersen, 2010). In infants, similar effects have been found, with the clustering coefficient increasing and average path length decreasing between 6 and 12 months old (Xie, Mallin, & Richards, 2018). In short, while the infant brain already shows some small-worldness, the brain network further optimizes during development.

5.2.2 The current paper

In this study, we set out to research the link between changes in the infant brain network and behavior. To increase the generalizability of this study, a large population-based sample of infants was tested multiple times to quantify individual changes in the brain network. For this, we used the population-based YOUth project, whose sample measures the typical Utrecht child (Onland-Moret et al., 2020). To study large groups of infants a relatively non-intrusive neuroscientific method is needed. EEG can be used to study functional networks, both in adults and infants. EEG signals acquired from different electrodes on the scalp can be filtered into separate frequency bands and in these frequency bands networks can be created based on the likelihood of areas under the scalp communicating. In a previous cross-sectional study with 10-month-old infants and testing the network characteristics of path length, clustering coefficient, and small-worldness showed that in alpha and theta frequency bands, we found reliable metrics over sessions (van der Velde, Haartsen, & Kemner, 2019). Additionally, both theta and alpha networks have been related to social (Orekhova et al., 2014; van der Velde et al., 2021) and self-regulation development (Bell, 2012; Broomell, Savla, & Bell, 2019; Swingler, Willoughby, & Calkins, 2011).

To study this two-way relationship, this study will use cross-lagged panel models to analyze the relationship between behavior and the connectome (Hamaker, Kuiper, & Grasman, 2015). A cross-lagged panel model is a type of structural equation model that specifically determines the pathway in a relationship between two variables, i.e. whether one variable drives change in the other or vice versa. This model could therefore explain whether more complex behaviors in session one will explain more optimized brain network functioning in session two or vice versa.

5.3 Methods and materials

5.3.1 Participants

The YOUth study is a longitudinal cohort study consisting of two large cohorts differing in age range. The YOUth Baby & Child cohort follows infants from 20-24 weeks gestational age until the age of six. Both behavioral and cognitive development is tracked through numerous tasks and methods (e.g., eye-tracking, EEG, MRI, questionnaires). The YOUth study was approved by the Medical Research Ethics Committee of the University Medical Center Utrecht and all participants' parents provided written informed consent. A brief overview of the YOUth study including the measurements conducted at each timepoint is available from https://www.uu.nl/en/research/youth-cohort-study_(Onland-Moret et al., 2020).

In total, 1008 5-month-old and 758 10-month-old infants were included. Table 5.1 shows the demographic and attrition information for our study, for the EEG and ERP paradigms, respectively. Attrition due to fussiness was counted when the infant was excluded from the analysis for having too little (or no) data due to the infant being too tired or inattentive, started crying or moving too much, or refused to wear the cap. Attrition due to experiment(er) error was counted when logged or when the resulting data file was corrupted. Attrition rates are 27% or lower, which is somewhat below the expected range (Stets, Stahl, & Reid, 2012). For more information on the EEG-data quality in the YOUth project please refer to an earlier paper by van der Velde and Junge (van der Velde & Junge, 2020).

5.3.2 EEG Acquisition

EEG was recorded using a cap with 32 electrodes (ActiveTwo system, BioSemi) positioned according to the international 10/20 system, at a sampling rate of 2048 Hz. A Common Mode Sense (CMS) and Driven Right Leg (DRL) electrode were used to provide an active ground.

Table 5.1 Demographic and attrition information

Wave	Gender	Tested	Attrition		Analyzed	Age (in days)	
		N	N	%	N	mean	sd
5m		1008	270	26.8	738	167.0	23.3
	Boy	493	128	26	365	167.7	23.0
	Girl	515	142	27.6	373	166.4	23.7
10m		758	197	26	561	316.4	25.2
	Boy	374	96	25.7	278	317.5	25.2
	Girl	384	101	26.3	283	315.3	25.2

5.3.3 EEG Analysis

EEG data was analyzed exclusively using MATLAB, using the FieldTrip toolbox (Oostenveld, Fries, Maris, & Schoffelen, 2011). The original 2048 Hz data was downsampled to 512 Hz, using chip interpolation and band-pass filtered at .1-70 Hz with a two-way Butterworth filter. Artifacts were removed from the continuous EEG. Artifacts were defined as the absence of signal, clipping, muscle artifacts, and excessive noise. Channels were removed if more than 40 percent of the signal in a channel contained artifacts. Bad channels were removed for both sessions of a subject. If no more than two bad channels were found in a single subject, the two channels were interpolated utilizing weighted averaging of neighboring channels. If more than two bad channels were found, the subject was removed from further analysis. The cleaned data was used for further analysis.

5.3.4 Connectivity calculation

The cleaned data for each subject was bandpass filtered into 6 bands: delta (.1-3 Hz), theta (3-6 Hz), alpha1 (6 – 9 Hz), alpha2 (9 – 12 Hz), beta (12

– 25 Hz), and gamma (25 – 45 Hz). Since individual theta and alpha peaks are influenced by development, alpha1 and theta bands were chosen to encompass all theta and alpha peaks +/- 1 Hz. The resulting data was cut into 3s. epochs. For each epoch, connectivity between pairs of electrodes (32*31/2 = 496) was calculated with the phase lag index (PLI) and the debiased weighted PLI, both relying on the same principle of phase locking or phase synchrony (Tass et al., 1998).

The phase lag index (PLI), proposed by Stam and colleagues, (Stam, Nolte, & Daffertshofer, 2007), describes the asymmetry of the distribution of phase differences between pairs of signals:

$$\text{PLI} = \left|\langle sign\left[\sin\left(\Delta\varphi(tk)\right)\right]\rangle\right| \quad ,$$

where $\Delta\varphi$ is the instantaneous phase difference between signals at time point t for k = 1 ... N per epoch (N = 5*512 = 2560), determined using the Hilbert transformation. || stands for absolute values, <> for the mean values, and the sign for a signum function (phase difference is either -1, 0, or 1). The resulting PLI can range from 0 to 1. Volume conductance, the effect that multiple electrodes register activity from the same source, plays a minimal role in the PLI. Activity from a single source will appear in both electrodes as having a phase difference of exactly zero. Since the PLI indexes the stability of phase leaping or lagging, a phase difference of zero will lead to a PLI of zero.

5.3.5 Graph Analysis

Several graph measures were calculated using the acquired individual connectivity matrices. The following graph measures were calculated using the brain connectivity toolbox (Rubinov & Sporns, 2010) (table 5.1): global connectivity, average clustering coefficient (Cw), characteristic (average shortest) path length (Lw); and small-worldness. To calculate network small-worldness, the small-world propensity (SWP) is used. This recently developed measure for small-worldness is in general more applicable to weighted brain networks, due to the incorporation of weighted estimates for clustering coefficient and path length. Additionally, it is density-independent, whereas with previous definitions of small-worldness a decrease of small-worldness could be seen as the density increased. Since weighted non-thresholded networks were used in this paper, the use of SWP is preferred. Networks with a SWP of .4 or higher are considered small-world networks. For more technical information, please refer to the paper of Muldoon and colleagues (Muldoon, 2016).

5.3.6 Behavioral measures

Infant temperament was assessed using the Dutch translation of the Infant Behavior Questionnaire Short Form (IBQ-SF) (Putnam, Helbig, Gartstein, Rothbart, & Leerkes, 2014). In this questionnaire, 94 items are rated on a Likert-type scale of 1 to 7. A not applicable option is present for when parents have not observed the behavior in the child. The items can be subdivided into 15 categories of child temperament: Activity Level, Distress to Limitations, Approach, Fear, Duration of Orienting, Smiling and Laughter, Vocal Reactivity, Sadness, Perceptual Sensitivity, High Intensity Pleasure, Low Intensity Pleasure, Cuddliness, Soothability, and Falling Reactivity. These categories are commonly grouped into three factors. Surgency/Extraversion (Activity, Approach, Vocal Reactivity, High Intensity Pleasure, Smiling and Laughter, and Perceptual Sensitivity) generally measures impulsiveness and positive affect; Negative Affect (Sadness, Distress to Limitations, Fear, and Falling Reactivity) refers to the predisposition of the child to experience negative feelings; and Orienting/Regulation (Low Intensity Pleasure, Cuddliness, Duration of Orienting, and Soothability) with refers to the ability of the infant to regulate attention and behavior.

To assess social competence, we used the ages and stages – social emotional questionnaire (ASQ:SE). Information from the 5-month-old infants was obtained using the 6 months ASQ:SE (19 items) and 10-month-old infants' information was obtained using the 12 months ASQ:SE (19 items). Parents were required to indicate per item whether their child does the item “Most of the Time”, “Sometimes”, or “Never/Rarely”. Responses are coded as 0, 5, or 10 points respectively, with higher total points being indicative of problems in social-emotional development, while low scores suggest less problem behavior.

5.3.7 Statistical analysis

To test our hypothesis regarding the development of the network and possible sex differences, linear mixed effect models are used. A major difficulty in assessing longitudinal effects is the problem of missing data (Ibrahim & Molenberghs, 2009). Analyzing longitudinal data with missing values using an ANOVA (rmANOVA) forces the researcher to exclude subjects with missing data in one session, due to the rmANOVA requirement of MCAR (Missing Completely at Random). A linear mixed model (LMM), in contrast, assumes missing at random (MAR). This means, in practice, that even if a single session is missing, we can still use the participant's data points that were observed in other sessions for the analysis. Additionally, rmANOVAs assume sphericity and require independent variables to be categorical. Therefore, rmANOVAs are an effective procedure, easy to implement with statistical software, but only if the above assumptions are met, the variances between repeated measures are relatively equal, and complete data of all subjects are available. Since missing data is not MCAR in our study and additionally we include participant age (which is a continuous variable) as an independent variable, using a rmANOVA is not possible. Lastly, an ANOVA assumes normality. Likert scales and yes/no questionnaires rarely yield normal data. Therefore, we opt to use LMMs to test these hypotheses.

To test the development of network characteristics during the first year of life, a linear mixed model was built for each of these network characteristics in each frequency band with age and gender as dependent variables and participant as a random intercept. Both age and the dependent variables were centered and scaled. The formula for all models was: network characteristic ~ age * gender + (1|Subject). The Akaike information criterion (AIC) for the models was as follows; alpha strength: 3457.0, alpha SWP: 383.4, theta strength: 2835.5, and theta SWP: 337.2. Similarly, to test the development of the behavioral characteristics, a linear mixed model was built for each of these behavioral characteristics with age and gender as dependent variables and participant as a random intercept. Both age and the dependent variables were centered and scaled. The formula for all models was: behavioral characteristic ~ age * gender + (1|Subject). The Akaike information criterion (AIC) for the models was as follows; surgency: 2721.5, regulation/orienting: 2881.125, negative affect: 2904.145, and ages and stages social emotional: 2928.9.

Analyses were performed using the lmer4 package in R (Bates, Mächler, Bolker, & Walker, 2014). Relative effect sizes were computed according to Brysbaert and Stevens (Brysbaert & Stevens, 2018) and can be seen as analogous to Cohen's d. Effect sizes of $d < .2$ were deemed small, $.2 < d < .5$ were deemed small-medium, $.5 < d < .8$ were deemed medium-large, and $d > .8$ were deemed large.

To test our hypotheses relating behavior to brain development, we made use of cross-lagged panel models (CLPMs) using Mplus 8.4 (Muthén & Muthén, 2009) which tests which extend network characteristics and behavioral characteristics in session 1 predicted these characteristics in session 2. Since the data was non-normally distributed, maximum likelihood with robust standard errors (MLR) was used to estimate all CFAs. The SEM, which also included categorical variables, was estimated with a robust weighted least square estimator using a diagonal weight matrix (WLSMV) with theta parameterization.

Several issues needed to be taken into account when creating the cross-lagged panel models. First, age varied between subjects both in the first session and in the second session. Parents were allowed to come in with their infants at 4, 5, or 6 months old for the first session and at 9, 10, and 11-months old during the second session. Therefore, age at session 1 was added as a predictor for the variables at session 1, and age at session 2 was added as a predictor for the variables at session 2. Additionally, due to sex differences in the development of the infant brain network and infant behavior, our CLPMs might not be similar across sex. Based on earlier analyses, we assumed no differences between sexes and, therefore, constrained the model across sex. This yielded a good model fit for the theta network characteristics (AIC = 24645.6, $\chi2/df = 1.2$, CFI = .9770, RMSEA = .02 [0-.032], SRMR = .07) and the alpha network characteristics (AIC = 24177.8, $\chi 2/df = 1.1$, CFI = .994, RMSEA = .01 [0-.027], SRMR = .06). Since two separate models were run, p-values of both models were Bonferroni corrected. Note that this correction is too strict since the models are not independent. To test for the spuriousness of the relationships, the relationships were tested using 10000 bootstrap samples. For this, the recommended bias-corrected bootstrap was used (Shrout & Bolger, 2002), which does not assume normally distributed parameter estimates.

5.4 Results

The results are divided into three subsections: first describing the development of the infant brain network followed by the development of infant temperament and social competency. Lastly, the relationship between the infant brain network development and infant behavior is described.

Table 5.2. Theta - LMM theta network characteristics

Dependent Variable	Parameter	Estimate	Test (df)	p*	Effect size
	Age	.33	t = 12.37 (724)	<.0001	d = .81
Strength	Sex_{girl}	-.13	t = -2.88 (834)	.004	d = -.20
	Age * Sex_{girl}	-.03	t = -.82 (714)	n.s.	d = -.06
	Age	.22	t = 6.43 (730)	<.0001	d = .47
SWP	Sex_{girl}	-.01	t = -.20 (805)	n.s.	d = -.01
	Age * Sex_{girl}	-.05	t = 1.09 (719)	n.s.	d = -.08

** Bonferroni corrected*

Table 5.3 Alpha - LMM for alpha network characteristics

Dependent Variable	Parameter	Estimate	Test (df)	p*	Effect size
	Age	.51	t = 12.37 (672)	<.0001	d = 1.15
Global Strength	Sex_{girl}	.04	t = .66 (817)	n.s.	d = .05
	Age * Sex_{girl}	-.08	t = -1.59 (662)	n.s.	d = -.12
	Age	.34	t = 8.25 (751)	<.0001	d = .60
SWP	Sex_{girl}	.00	t = -.05 (821)	n.s.	d = -.01
	Age * Sex_{girl}	-.09	t = -1.54 (741)	n.s.	d = -.08

** Bonferroni corrected*

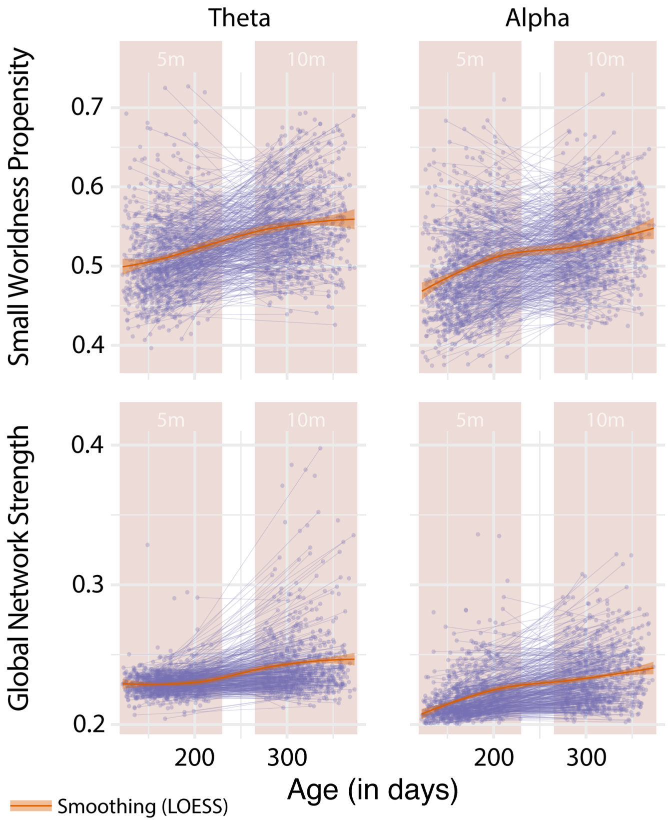


Fig 5.1 Spaghetti plots of development of network characteristics. Both small-worldness propensity (left) and network strength (right) can be seen increasing throughout the first year of life for both the theta and alpha brain networks. A smoothed LOESS curve is added as visual aid.

5.4.1 Development of the infant brain network

Three core characteristics were calculated for every participant for both their theta and alpha networks: Strength (average connectivity) and small worldness propensity (SWP). Linear mixed models were built with age and gender as dependent variables. Table 5.2 shows the results for the theta networks. For both characteristics, a main effect was found for age. Both strength (ß=.33, p<.0001, d=.81) and SWP (ß=.22, p<.0001, d=.47) showed increases over the course of the first year of life. A main effect for gender was only found for strength with girls showing lower strength compared to boys (ß=-.13, p=.004, d=-.20). No significant interaction effects between age and sex were found.

Table 5.3 shows the results for the alpha1 networks. Once again, for both characteristics, a main effect was found for age. Both strength (ß=.51, p<.0001, d=1.15) and SWP (ß=.34, p<.0001, d=.60) showed increases over the course of the first year of life. No significant main effects for gender or interaction effects between age and gender were found. Figure 5.1 shows a spaghetti plot of the development of network characteristics during the first year of life.

Table 5.4. Behavior - LMMs

Dependent Variable	Parameter	Estimate	Test (df)	p*	Effect size
Surgency	Age	.43	t = 12.43 (449)	<.0001	d = 1.17
	Sex$_{girl}$	.01	t = .18 (677)	n.s.	d =.01
	Age * Sex$_{girl}$	-.08	t = -1.59 (462)	n.s.	d = -.15
Regulation	Age	-.15	t = -3.93 (505)	<.0001	d = -.37
	Sex$_{girl}$	.09	t = 1.33 (694)	n.s.	d = .10
	Age * Sex$_{girl}$	-.04	t = -.73 (519)	n.s.	d = -.07
Neg. Affect	Age	.12	t = 3.18 (505)	.001	d = .28
	Sex$_{girl}$	.03	t = .37 (694)	n.s.	d = .03
	Age * Sex$_{girl}$	.06	t = 1.04 (519)	n.s.	d = .09
ASQ	Age	.23	t = 5.81 (506)	<.0001	d = .52
	Sex$_{girl}$	-.13	t = -1.94 (694)	n.s.	d = -.15
	Age * Sex$_{girl}$	-.03	t = -1.17 (519)	n.s.	d = -.10

**Bonferonni corrected*

5.4.2 Development of infant behavior

Similar to the creation of linear mixed models for network characteristics, we also built linear mixed models for the behavioral development tested using the infant behavior questionnaire (IBQ) and the ages and stages: social-emotional questionnaire. We used the IBQ subscales (regulation, negative affect, and surgency) and the ASQ scores as dependent variables. For all questionnaires, a main effect was found for age. Surgency (ß=.43, p<.0001, d=1.17), negative affect (ß=.12, p=.001, d=.28), and ASQ (ß=.23, p<.0001, d=.52) all significantly increased during the first year of life. Regulation (ß=-.15, p<.0001, d=-.37) significantly decreases during the first year of life. No main effects for gender or interaction effects between age and gender were found. See Table 5.4 for an overview of the LMMs for behavior and figure 5.2 for the spaghetti plots of the development of behavior during the first year of life.

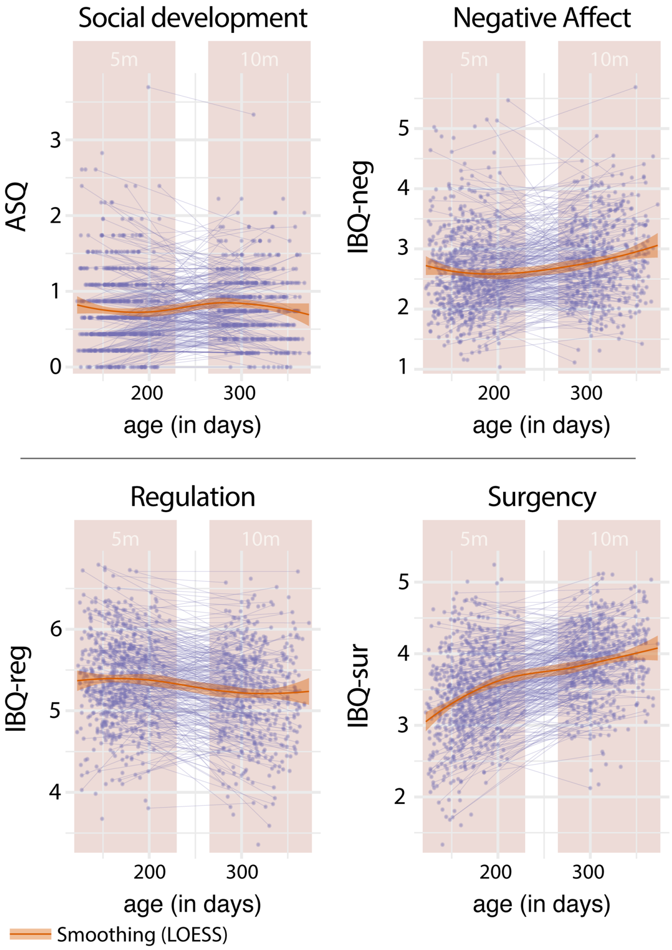


Fig 5.2. Spaghetti plots of development of behavioral characteristics. Both negative affect and surgency increase in the first year of life. Regulation decreases during the first year of life. Lastly, social development clearly shows the effect of age standardization. Different questionnaires were used for the 5 and 10-month-old infants. Therefore, the younger infants in both sessions scored higher (lower social competency).

5.4.3 The relationship between the developing infant brain network and infant behavior

The development of the network characteristics small-worldness propensity and network strength for both alpha and theta networks were related to behavioral development using cross-lagged panel models. The results are summarized in tables 5.5 & 5.6 and visualized in figure 5.3. Table 5.5 shows the lagged regressions for the theta network characteristics with the behavioral characteristics. All latent variables at session 1 predicted themselves significantly at session 2 (.26 < ß < .48, with p<.0001 for all). Of extra note are the lagged regressions between the brain measures SWP and strength and the behavioral measure regulation. Theta network strength negatively predicted regulation in session 2 (ß=-.121, p<.05). Opposingly, regulation in session 1 had a negative relationship with small-worldness propensity in session 2 (ß =-.157, p<.05). Within-session regressions were also calculated. Nearly all latent variables for behavioral scores were significantly related to each other relation except for surgency and negative affect 1 (-.428 < ß < .435). In session 2, all the standard errors for the latent variables for behavioral scores showed significant relationships (-.18 < ß < .22). For exact values, see supplementary materials and results.

Table 5.5. Theta – Lagged regressions - ß matrix

		Session 2					
		Strength	SWP	ASQ	Surgency	Neg.	Reg
Session 1	Strength	**.452*** **	-.003	.071	-.008	.062	**-.121***
	SWP	.033	**.258*** **	.003	-.023	-.017	.041
	ASQ	.014	-.039	**.326*** **	-.006	-.021	-.053
	Surgency	.070	.023	-.124	**.480*** **	.088	.027
	Neg. Affect	.020	-.028	.005	-.074	**.412*** **	-.094
	Regulation	-.002	**-.157****	-.117	.051	-.061	**.443*** **

Similar results were found for the alpha networks. All latent variables at session 1 predicted themselves significantly at session 2 (.269 < ß < .496, with p<.0001 for all). Of extra note is the lagged regression between the brain measures SWP and the behavioral measure regulation. Regulation in session 1 had a negative relationship with alpha 1 small-worldness propensity in session 2 (ß =-.142, p<.05). Nearly all latent variables for behavioral scores showed within-session relationships - except for surgency and negative affect - showed significant relationships in session 1 (-.428 < ß < .435). In session 2, all residuals of the latent variables for behavioral scores showed significant relationships (-.18 < ß < .22). These results are visualized in figure 5.3.

Table 5.6. Alpha – Lagged regressions - ß matrix

		Session 2					
		Strength	SWP	ASQ	Surgency	Neg	Reg
Session 1	Strength	**.373*****	.017	-.022	.013	.006	.044
	SWP	.058	**.271*****	.014	-.037	-.037	.074
	ASQ	-.043	.004	**.331*****	-.004	-.025	-.052
	Surgency	-.012	.016	-.139	**.482*****	.085	.031
	Neg. Affect	-.027	-.086	.003	-.076	**.416*****	-.103
	Regulation	-.106	**-.161***	-.115	.057	-.057	**.439*****

5.5 Discussion

Determining how infant behavior is related to infant brain functioning is of key importance to better understand typical and atypical development of infant cognition. Complex behaviors, like social competence or regulation, certainly require input from multiple brain areas. Thus, approaches that assess distributed networks, especially at a period of life of rapid brain development, are optimal. How these brain areas communicate, and work together is extremely informative. Thus, here we related the development of infant functional brain network characteristics to the development of two key facets of infant behavior: infant temperament and social competency.

There are two important things to consider when analyzing the relationship between brain networks and behavior. First, the relationship between the development of brain networks and the development of behavior is two-way: complex behaviors occur once areas in the brain start efficiently communicating, however, communication in the brain is optimized under the influence of experience. Thus, to study this bi-directional relationship we used cross-lagged panel models. Second, to study the effect of relatively small individual differences in brain functioning and infant behavioral development, large samples are required. Therefore, in this study, we followed more than 750 infants longitudinally during their first year of life.

This paper focused on three topics: First, we looked into the development of *functional brain networks* during the first year of life, by comparing EEG networks at 5-months-of-age with EEG networks at 10-months-of-age using graph theory to characterize and compare networks. We focused on theta and alpha networks, which have been shown to be critical during infancy (Hoehl, Michel, Reid, Parise, & Striano, 2014; Orekhova et al., 2014; Orekhova, Stroganova, Posikera, & Elam, 2006; van der Velde et al., 2021), with the hypothesis that functional brain networks both increased in connectivity during the first year of life and becomes increasingly optimized. Second, we looked into the development of infant behavior (temperament and social competency) during the same time period. We hypothesized that infant temperament, as measured with the Infant Behavior Questionnaire Short Form (IBQ-SF), would follow a similar developmental trajectory as in earlier studies, the subscales surgency and negative affect increasing and orienting/regulation decreasing. Lastly, we looked into the relationship between the development of the brain networks and behavior using cross-lagged panel models. Here we expected to find a complex relationship with both behavior affecting brain development later in infancy and brain development affecting behavioral development later in infancy. Here we first discuss the main findings concerning these three critical questions. Subsequently, we will discuss the limitations and future directions of this study.

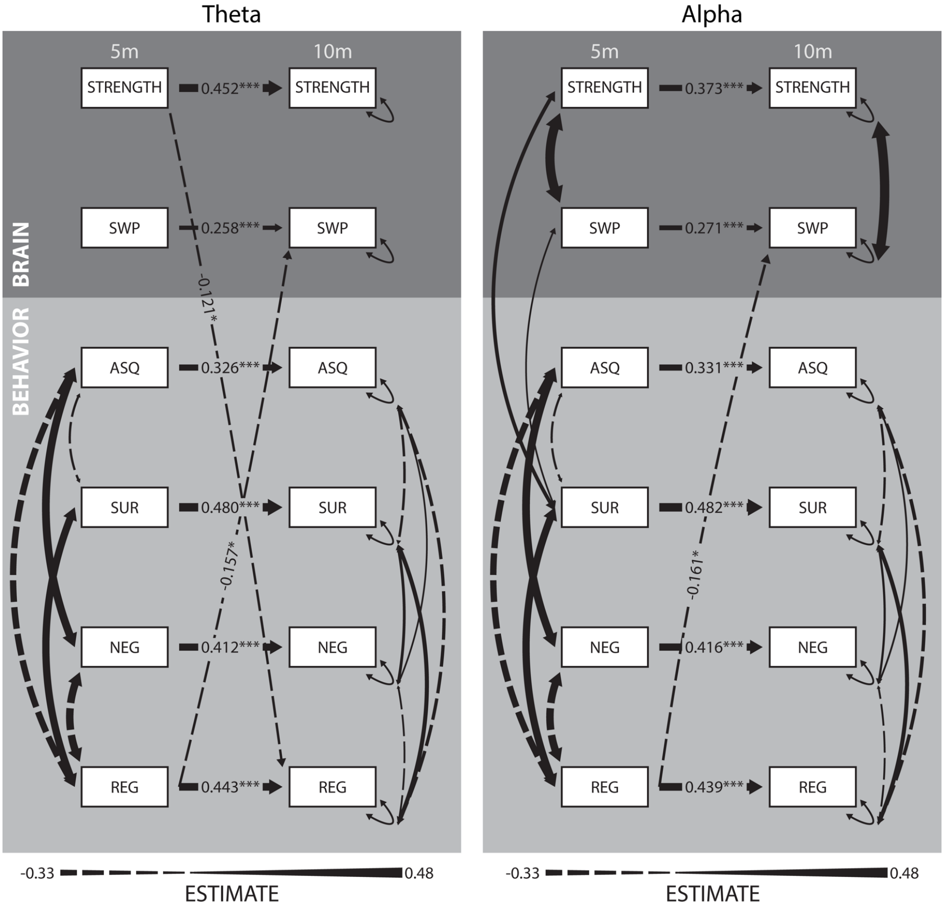


Figure 5.3. Cross-lagged panel models with only significant paths depicted. Both theta (left) and alpha (right) models network strength and small worldness (SWP) is related to social development (ASQ), surgency (SUR), negative affect (NEG), and regulation (REG) at an older age and vice versa. In both models, regulation at 5 months predicts brain network optimization (SWP) at 10 months. Additionally, theta network strength at 5 months predicts regulation at 10 months

5.5.1 The development of the infant brain network

Both alpha and theta brain networks saw considerable changes during the first year of life. Firstly, alpha and theta global connectivity significantly increased over time. Other studies, including our own, into the development of the infant brain network, have found similar results (Tóth et al., 2017; van der Velde et al., 2021). Similarly, studies into the development of alpha and theta power (which is related to, but not the same as, brain networks) show marked increases during the first year of life (Gabard-Durnam et al., 2019; Jones, Venema, Lowy, Earl, & Webb, 2015; Sankupellay et al., 2011). This provides insight into the neurobiology of infant development, as increases in functional connectivity indicate that brain areas becomes more in sync as the infant develops, and thereby communication between brain areas is improved (Keunen et al., 2017). This is likely caused by increases in structural connectivity, probably mostly through myelination (Dubois, Hertz-Pannier, Dehaene-Lambertz, Cointepas, & Le Bihan, 2006).

Both theta and alpha networks also showed distinct increases in small-worldness propensity during the first year of life. This is an indication that the infant brain network not only strengthens its communication between brain areas but also that the topology of brain areas involved in these brain networks becomes more optimized. Our results mirror earlier findings in infant fMRI studies (Batalle et al., 2012; De Asis-Cruz, Bouyssi-Kobar, Evangelou, Vezina, & Limperopoulos, 2015; Fransson et al., 2007; Gao et al., 2011; Huang et al., 2015),

Additionally and perhaps most encouragingly, we also found relatively high stability of these measures over the two sessions. Infants who showed high global network strength and/or high small-worldness at 5-months-old were also more likely to show high global network strength and/or high small-worldness at 10-months-old, thus remaining relatively stable in their developmental trajectory in relation to other infants. Previous work has already shown that global network measures can be reliably measured in 10-month-old infants (Haartsen, van der Velde, Jones, Johnson, & Kemner, 2020; van der Velde et al., 2019). However, to our knowledge, this is the first time this stability over an extensive period during infancy has been reported. This is evidence that these measures are distinct across subjects and relatively stable across lengthy periods of time. This underlines the usefulness of these measures to study brain development, explain individual differences in behavior, and identify changes related to environmental factors or emerging psychopathology.

5.5.2 The development of infant social and temperament behavior

In our study, we measured infant temperament using the IBQ:SF (Gartstein & Rothbart, 2003). Our findings mirror findings of earlier infant temperament studies (Gartstein & Rothbart, 2003; Putnam et al., 2014), with the subscales surgency and negative affect increasing during the first year of life and the subscale regulation/orienting decreasing during the first year of life. Additionally, we found that infant social competency decreased during the first year of life. However, this might be caused by the questionnaire used to determine social competency. This ASQ:SE questionnaire is normalized according to infant age. In this study, we used the 6-month-old and the 12-month-old versions of the questionnaire. Since the 6-month-old questionnaire was used for infants between 4 and 6-months-old and the 12-month-old questionnaire was used for infants between 9 and 11-months-old, it makes sense that on average infants showed lower social development at 10-months-old compared to 5-months old.

5.5.3 The relationship between the developing brain network and infant behavior

In short, clear developmental patterns of both behavior and brain networks emerged in our dataset, but how do these relate to each other? We studied this question using cross-lagged panel models, in which the paths between session 1 and session 2 (the so-called cross-lagged paths) are of special interest. Does the brain mostly influence behavior later in infancy, or does persisting in certain behaviors influence the brain later in infancy or both? The aforementioned behaviors measurements are modeled with two core brain characteristics: 1) Global network strength, as a general measure of brain network maturation due to myelination and increases in the synchronicity of communication, and 2) small-worldness, as a measure of optimality of brain networks.

We found that brain networks influence behavior later in infancy, but also that behavior also influences the later development of brain networks. We found that theta network strength at 5-months-of-age predicts orienting/regulation at 10-months. Conversely, we found that orienting/regulation at 5-months-of-age predicts theta network small-worldness at 10 months. Therefore, it seems that the total amount of theta network communication predicts the complexity of later behavior, but that the early complexity of behavior predicts later optimality of brain network structure. Global strength is a measure of total communication in a network. Therefore, an increase in global strength could indicate certain communication pathways becoming available, allowing for more complex behaviors to be exhibited. These new experiences, allowed for by the new pathways, in turn, will provide 'practice' for the network, and optimize its functioning. The alpha network saw similar relationships. However, only a lagged path from behavior to brain optimization was found with orienting/regulation predicting brain optimization later in life. Why the path *alpha brain network global strength to orienting/regulation* is present in the theta network later in infancy, but not in the alpha network is not fully understood. It is, however, possible that small-worldness tells us more about the general topology of the brain and that theta and alpha small-worldness are therefore inextricably linked. Global theta or alpha strength, however, tells us more about what type of communication the brain uses to convey messages. This might therefore have a more specific relationship with what the brain is doing during the measurement.

Interestingly, no lagged relationships between brain networks and social competency were found. This is surprising since previous work from our group found that the theta network reorganized during the first year of life and that with this reorganization came an increased (EEG) sensitivity to social stimuli (van der Velde et al., 2021). Therefore, we expected to find a relationship between the theta function brain network and social behavior. There are several possible explanations for this. First, EEG is an indirect measure of brain structure, with a low spatial resolution. This forces the researcher to focus on the global workings of the brain. More detailed brain optimization characteristics like the rich-club coefficient (Ball et al., 2014; van den Heuvel & Sporns, 2011) cannot be detected. Perhaps more detailed brain network descriptions are better matched to describe the relationship between the brain and these specific behaviors.

Additionally, measuring behavior through the use of parent-filled questionnaires is not ideal. Parent biases might creep in, and questionnaires do not always provide a valid representation of child behavior. This has been shown on countless occasions. For example, Parade & Leerkes (2008) showed that the gender effects were large when using the infant behavior questionnaire, with fathers interpreting fear differently compared to mothers. Additionally, they found that emotional state of mind, like depression, strongly influenced eventual scoring (Parade & Leerkes, 2008). Future studies, with objective or multi-informant measures for social competence and infant temperament, might be required to better understand the exact relationship between infant brain networks and infant behavior.

To our knowledge, this is the first study of bi-directional relationships between brain networks and behavior in a large, longitudinal sample of infants. This study can therefore be seen as a starting point for future work. For example, we have described several relationships between brain networks and typical behavior. Can these relationships also be found to detect and explain atypical behavior? Additionally, extra sessions would offer the opportunity to better detect non-linear longitudinal trends during development (i.e., U-shaped, exponential, logarithmic). with greater than two-time points, random intercept cross-lagged panel (RI-CLPM) models can be applied instead of the here used regular cross-lagged panel model. This RI-CLPM better represents the actual within-person relationships over time (Hamaker et al., 2015; Mulder & Hamaker, 2021). It would be interesting to study a limited population of infants regularly (for example weekly) to better assess developmental spurts and to allow for better non-linear model fits. This is, of course, intensive for infants and parents, but it could yield invaluable results in much greater details regarding the relationship between brain and behavior.

5.6 Conclusions

In this study, we followed for the first time a large population of infants to describe the development of network characteristics during the first year of life. We used an EEG-based analysis method of functional networks to determine the development of several graph characteristics. Both theta and alpha functional networks showed an increased optimization, through an increase in small-worldness and an increase in general network strength. Network characteristics increased in variability across sessions but remained relatively stable over time, indicating individual differences in the network topology followed a relatively stable development during infancy. The individual differences showed some predictive value in predicting behavioral development, with theta global network strength predicting infant regulation/orienting later in infancy and regulation/orienting predicting later network optimization. The current study shows the potential to explain behavior by studying the development of infant brain networks with EEG. In the future, we could use this information to detect biomarkers for typical and non-typical behavior, which could aid us in predicting and treating behavioral outcomes.

5.7 References

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CHAPTER 6

Discussion

In this dissertation, we aimed to explore the relationship between the development of networks in the infant's brain and infant behavior. Ultimately, asking the question whether differences in characteristics of infant brain networks could explain differences in social competency and behavioral control. This dissertation specifically focused on social competence and self-regulation during infancy, since both types of behavior develop considerably during the first year of life. Importantly, both behaviors likely have a two-way relationship with networks in the human brain. For example, complex social behavior cannot exist without a wide variety of brain areas communicating effectively. On the other hand, however, the ability to exhibit more complex social behaviors opens new avenues to experience new stimuli through social learning (Bandura & Walters, 1977; Grusec, 1994). A similar thing can be said for self-regulation. Additionally, atypical development of these behaviors has been commonly related to brain network dysfunction in older children and adults (f.e. Autism (Barttfeld et al., 2011; Belmonte et al., 2004; HansonCatherine, José, RamseyJoseph, GlymourClark, 2013; Orekhova et al., 2014) or ADHD (Ahmadlou, Adeli, & Adeli, 2012; Beare et al., 2017; Furlong et al., 2020; Murias, Swanson, & Srinivasan, 2007)). All these elements underline the importance of shedding light on the relationship between the development of these behaviors and the development of the infant brain network.

To study these relationships, however, we realized some methodological issues needed solving first. Most importantly, infant EEG data notably differs from adult EEG data. For example, infants cannot be instructed to watch the screen or to sit still. This, therefore, requires different tasks to be used and requires dealing with noisy data. Consequently, reliability of network measures needed to be checked for infant EEG. An additional methodological issue is related to the study of individual differences, relating individual differences in brain development to behavioral development requires a considerable sample size. This sample size makes it difficult to keep the environment steady across measurements. Therefore, understanding infant EEG data quality and what external factors influence it are of vital importance.

This chapter is divided into two sections. In the first section (Infant EEG data quality and its reliability), we will discuss our papers on the reliability of infant network characteristics and the quality of infant EEG data, integrating our findings into other existing studies. In the second section (Relating infant behavior to their brain networks), we will discuss the relationship between infant behavior and their brain networks, by first delving into how networks in the brain are used to process behavior inducing stimuli and later directly relating the development of behavior to the development of these networks.

6.1 Infant EEG data quality and its reliability

Infant EEG data cannot be equated to adult EEG data. Infants get fussy easily and do no heed instructions. Therefore, the resulting data is often noisy, task setups differ greatly and are often shorter in length to accommodate the need of the infant. The influence of these issues is exacerbated when testing large samples of infants, since keeping external factors constant is nearly impossible. In our studies, we wanted to related individual differences in the development of behavior to brain network characteristics, which requires large samples of infants. Therefore, we first set out to answer two core questions: 1) Are brain network characteristics calculated with infant EEG data reliable? And 2) Which external factors influence infant EEG data quality?

In **chapter 2**, we tried to answer the first question. We found that global metrics, metrics that are averaged over the entire brain, are generally highly reliable in both the theta and the low alpha frequency bands. Measures tested were global strength (average of all connections in the network), characteristic path length (average of all the shortest paths in the network), averaged clustering coefficient, and small-worldness. This finding is similar to several findings in the reliability of adult EEG brain networks (Deuker et al., 2009; Hardmeier et al., 2014). Local metrics were less reliable. This could be seen in individuals' adjacency matrices showing poor similarity over sessions, but also in the reliability of individual connections. Stronger connections did show higher reliability compared to on average weaker connections, lending credence to the oft-used thresholding of adjacency matrices to analyze brain networks. It does show, however, that we should be careful in trying to relate individual connections in the infant's brain to infant behavior.

In **chapter 3**, we showed that there is a wide array of factors influencing data attrition in one large-scale study. These factors can vary between subjects, possibly changing outcome measures and results, which may lead to biased conclusions. The factors influencing data attrition described in this study can be broadly divided into three groups: child-related factors, testing-related factors, and longitudinal (study-specific) factors. Three child-related factors were found to influence data loss: gender, age, and head shape. Four testing-related factors were found to influence data loss: time of testing, the season of testing, research assistant present during the experiment, and task length all had considerable influence on data. Lastly, data attrition rates of the first session of testing were found to be related to the second session of testing, underlining possible longitudinal biases in terms of data loss.

These findings serve as a sneak preview to hopefully entice the reader to do their own analyses of these and other external factors possibly influencing infant EEG data quality. These findings must be replicated in other labs all around the world. Only then can we come up with standardized testing methods for all infant EEG studies. Additionally, it is important to understand in what way these results generalize to other age groups. Are these findings just applicable to young infants or is there a large amount of overlap with older age groups?

6.1.1 Future directions and open questions

This above-described findings do not mean that we should be satisfied with our current knowledge in how to study the infant connectome using EEG. There are still a lot of open questions. One important example: what is the minimum amount of data required to calculate reliable EEG networks. In an fMRI study, White and colleagues (2014) showed that the calculation of several core networks in the child brain stabilized after 5½ minutes and therefore suggested an acquisition time of at least 5½ minutes for children. Similar studies are also necessary for infant EEG. Previous studies into infant ERP have found that testing longer does not necessarily increase the quality of the outcome (Stets & Reid, 2011). Is the same true for infant brain networks? Or is it similar to adult fMRI brain networks, where more data is better? And if so, what is the minimum amount of required data to get to these stable brain networks?

Additionally, it is important to understand the influence of infant EEG data quality on the reliability of these metrics. Contrasting the recommendation for adult EEG brain network data to use less, but cleaner data (Fraschini et al., 2016), in our studies, we purposefully did not deep clean the EEG data. Most importantly because deep cleaning EEG increases the change of inducing biases in your infant population. Harshly cleaning your EEG data will result in higher amount of attribution due to lack of remaining data in subjects. Since it is very likely that certain character traits in infants increase the likelihood of getting clean data, you will induce a bias in your eventual population towards these infants. Additionally, requiring completely cleaned limits the applicability of these methods to detect biomarkers for atypical development. Therefore, it was important to us that reliable network metrics could be found even with slightly noisy data. This is not to say that we should not be interested in what the effect is of noisy data on reliability. We found reliable global metrics, but could intense cleaning increase the reliability of local metrics? Future studies are required to determine the exact relationship between EEG data cleanliness and reliability.

In addition to poor local metrics, small-worldness also shows low reliability. Are there ways of increasing reliability for these metrics? Or could we use different metrics for network optimality that show higher reliability? For an alternative of small-worldness, we could focus our attention on the analysis of the minimum spanning tree. The minimum spanning tree is a subgraph constructed from a weighted network, in which all nodes are connected in such a way that the connection cost is minimized without forming cycles (Boersma et al., 2013; Tewarie, van Dellen, Hillebrand, & Stam, 2015). This is a method to overcome the subjective thresholding of networks. Tree hierarchy (Boersma et al., 2013), a metric calculated using this method, shows high similarities with small-worldness. Could these metrics prove more reliable? Future studies are necessary to better understand the limits and the possibilities of using graph metrics in infants.

Lastly, so far, we have only discussed the relationship between EEG data quality and reliability. However, in **chapter 3** of this dissertation we discuss several external factors that influence data loss. What this chapter does not tackle, however, is how these external factors influence outcome measures (either through data loss or not). Is it possible that time of day can influence global network strength? Could season of testing influence clustering coefficient? It is of the utmost importance that we improve our understanding on the effects of certain external factors on outcome measures, since unwittingly biases might creep into your dataset through these external factors when working with very young children. For example, when working with infants, the infant (in combination with the parent) decides when we can test. This could lead to older infants – who are more likely to be awake according to a strict schedule – to mainly come in at certain hours of the day (most likely early in the morning). If this is the case, it might be very difficult to disentangle whether the development causes differences in network measures or environmental factors are the culprit. Future studies testing these (and other) environmental factors is therefore vital to better estimate the validity of our results.

6.2 Relating infant behavior to infant brain networks

So, now we knew that a wide array of brain network metrics can be calculated reliably in a large sample of infants using EEG. Additionally, we knew some of the external factors – possibly biasing our outcomes – to look out for. With this information in mind, we tried to answer the core questions of this dissertation in chapter 4 and 5. In these chapters, we related the development of key infant behaviors to the development of brain network characteristics. As mentioned before, we were especially interested in the development social competence due to the possible two-way relationship: 1) increases in communication efficiency allow for more complex social behaviors, and 2) increases in more complex social behaviors allow for better functioning social learning, which in turn increases experience and optimizes brain network communication.

In **chapter 4** we investigated the functional connectome of infants during social and non-social processing. During EEG measurement, the infants were watching social and non-social videos. Using this EEG data, we calculated individual whole-brain networks at 5-months-old and 10-months-old. We noted a striking reorganization in the theta whole-brain network, changing – on average – from a parieto-occipital network towards a fronto-parietal network at 10-months-old. No such change was found in the alpha whole-brain network. Both networks did show a considerable increase of average network strength over that period of time. Strikingly, however, only the development of the theta network coincided with an increase in selectivity for social versus non-social stimuli. While watching social videos, the theta network in the infant brain showed a marked increase in synchronicity. This was only the case from 9-months onwards (once the network on average was reorganized) and not the case with alpha networks at any point in time.

This study provides evidence for the involvement of theta communication in the processing of social cues. This fits in nicely with earlier studies finding theta power to be related to social processing (Jones, Venema, Lowy, Earl, & Webb, 2015; Orekhova, Stroganova, Posikera, & Elam, 2006). Additionally, this study shows a seeming requirement for the reorganization of the theta network before it becomes sensitive towards social versus non-social stimuli. This fits nicely with the interactive specialization theory (Johnson, 2000; 2011; Johnson, Grossmann, & Cohen Kadosh, 2009), which hypothesizes that the switch from an infant being able to not do something to them being able to do that thing is not being caused by individual areas becoming mature, but by networks of brain areas coming online. Note that these results do not tell us anything about an individual's switch from a parieto-occipital network towards a frontoparietal network. Only that, on average in our population, this switch takes place somewhere between 6 and 9-months old. It might be very difficult to detect these changes in individuals and at least multiple sessions are needed to confirm reconfigurations in sole participants.

So, in short theta communication in the infant brain seems to be related to the processing of social cues. However, this leaves out an important piece of the puzzle: how does this development relate to the development of actual infant behavior? In **chapter 5**, we aimed to answer this question by following the infant's connectome during the second half of the first year of life and relating its characteristics to the stark development in social behavior and self-regulation.

We first just looked at the brain and found that infant brain networks showed increases of alpha and theta communication as the child grew older. However, not only was there more communication, but it also seemed that pattern of communication was better optimized, with increases of small-worldness found over the course of the first year of life. Lastly, as the child grew older, the brain seemed to increase its reliability on long-range connectivity. All these findings fit nicely within the existing body of literature done in smaller populations of infants, both in EEG (Gao, Alcauter, Smith, Gilmore, & Lin, 2015; Gao et al., 2009; Huang et al., 2015) and (f)MRI (Fransson, Åden, Blennow, & Lagercrantz, 2011; Smyser, Snyder, & Neil, 2011), where increases in global connectivity and communication optimization are commonly found over the course of the first year of life and the brain reorganizes from a local towards a global organization (Smyser et al., 2011).

This development of brain network characteristics was then related to the development of infant behavior using a special type of structural equation modelling: cross-lagged panel models. Cross-lagged panel models are useful tools to better understand two-way interactions. Of especial interest to us where the cross paths between brain and behavior measures. That is to say: does the behavior outcome at session 1 predict brain outcome at session 2 and vice versa. Our results showed, both in theta and alpha networks slight relationships between brain network characteristics and self-regulation. Theta network strength at session 1 predicted self-regulation at session 2. Conversely, self-regulation at session 1 predicted theta small worldness at session 2. Similar results were found in the alpha networks, with self-regulation at session 1 predicting alpha small worldness at session 2. These results might be viewed as evidence for a complex relationship between brain networks and behavior with both behavior influencing the optimization of the network and the existence of the network influencing complexity of behavior. It is important to note, however, that these relationships are very slight, which could limit the applicability of these findings on the level of the individual.

6.2.1 Future directions and open questions

One of the key facets in understanding the development of the infant brain network is to zoom in on the role of infant experience. There are multiple ways to tackle this issue, but one interesting approach could be to shine a light on the development of motor skills in infancy. The milestones of motor development have always been an important guide for parents to track their infant's development. However, the impact the reaching of these milestones has on other key skills, like social development, has not always been appreciated. Increased motor skill allows the infant to act on the world and interact with it in increasingly complex ways (Hofsten, 2004).

Studying motor development with a connectivity perspective makes sense. Motor development milestones create a sudden burst in new experiences in other key skills. These new skills also become increasingly motor-dependent. Especially the skills that pertain to the locomotion of the child influence social behavior strongly (Campos et al., 2000). This evidence extends to atypical social development, in which motor development is commonly affected as well (Leonard & Hill, 2014). Motor skill milestones could therefore be used as an important indicator for experiencing more complex social situations. Therefore, future studies could relate motor skill development and social competence longitudinally over the first year of life, with a mediating role of the infant brain network.

Extra care should be given towards understanding the order of development of these core features of infant cognition. Motor development is linked to optimization of infant brain networks (Marrus et al., 2018), social competence can be linked to the infant brain network (this dissertation), and social competence and motor development in infancy are inextricably linked (Leonard & Hill, 2014). However, it is currently unknown what (if any) is the main driver of these parallel processes. Does motor development drive further optimization of the infant brain network and with-it social competence? Does the optimization of the infant brain network drive motor skill milestones and social competence? Does motor development allow for more complex social experiences, and both increase optimization in the infant's brain? Or is the development of these skills and their underlying neural mechanisms more dynamic, consistently switching roles between driver and passenger.

The relationship between the infant brain, motor skills, and social behavior could also be studied more directly. Most studies, researching the infant's brain, do their best in ensuring the immobility of the infant. Motion creates artifacts in all our known imaging methods to be used in infants. This shuts off an important area of research into the social development of the child. Older infants rarely exhibit social behavior in absence of movement and not allowing for this movement during brain measurements limits our understanding of social behavior. Designing setups that include approaching and playing behavior while measuring EEG is therefore vital. Not because of increased ecological validity, because it is difficult to determine what real-world behavior is and whether or not there is such a thing as getting closer to this behavior in a lab setting (Holleman, Hooge, Kemner, & Hessels, 2020), but because the behaviors exhibited by infants become in such a setting become more complex and therefore require the input of more distinct brain areas. This would lead to a better understanding of how these brain areas distinct in function communicate during these complex tasks.

Unfortunately, this requires considerable methodological advances. Infants need to be able to move freely while being measured and artifacts caused by this motion should be minimal. However, there are possibilities on the horizon making this possible. Both from a hardware and a software perspective. The EEG hardware advancement most promising for these types of studies is the increase in the use of wireless EEG machines (f.e. the F1 EEG headset, the B-alert, or the Enobio). Not only do these headsets improve the possibility of moving around, but the removal of wires also removes an important inducer of motion artifacts. Early comparison studies between regular EEG headsets and wireless headsets show promising results (Debener, Minow, Emkes, Gandras, & De Vos, 2012; Hinrichs et al., 2020; Kam et al., 2019; Ratti, Waninger, Berka, Ruffini, & Verma, 2017). One key problem these wireless headsets do not fix is the muscle artifacts which will become more plentiful during motion. Here, advances in software, driven by the research into brain computer interfaces, could play an important role, to actively filter out muscular artifacts while measuring EEG (Elsayed, Zaghloul, & Bayoumi, 2017; Song & Sepulveda, 2018). If these issues are fixed, even to a limited extent, we could devise more complex social tasks, in which the child could move freely. This will allow us to better describe in what way the brain communicates during these complex social behaviors.

The influence of experience on the social brain network could also be studied from a different perspective. One that likely captures the imagination right now. The COVID-19 pandemic has affected the lives of many people on earth. One group of people, however, has the curious distinction of never having lived outside of the pandemic: young infants. While health-wise many infants will come out of the pandemic unscathed, changes in their social experiences will be numerous. Visits by friends and family have become less frequent and for extended periods of time, infant daycare was impossible for many infants in The Netherlands. This has undoubtedly influenced the heterogeneity of social experiences an infant faced in 2020. Studying the social brain network of these infants, paying extra attention to the optimized structure of these networks, and increases or decreases in synchronicity between social brain areas during the processing of social cues could shed light on the importance of variety in social experiences during the first year of life for the development of a healthy social brain.

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Appendices

APPENDIX I

Nederlandse Samenvatting

I.1. Aanleiding

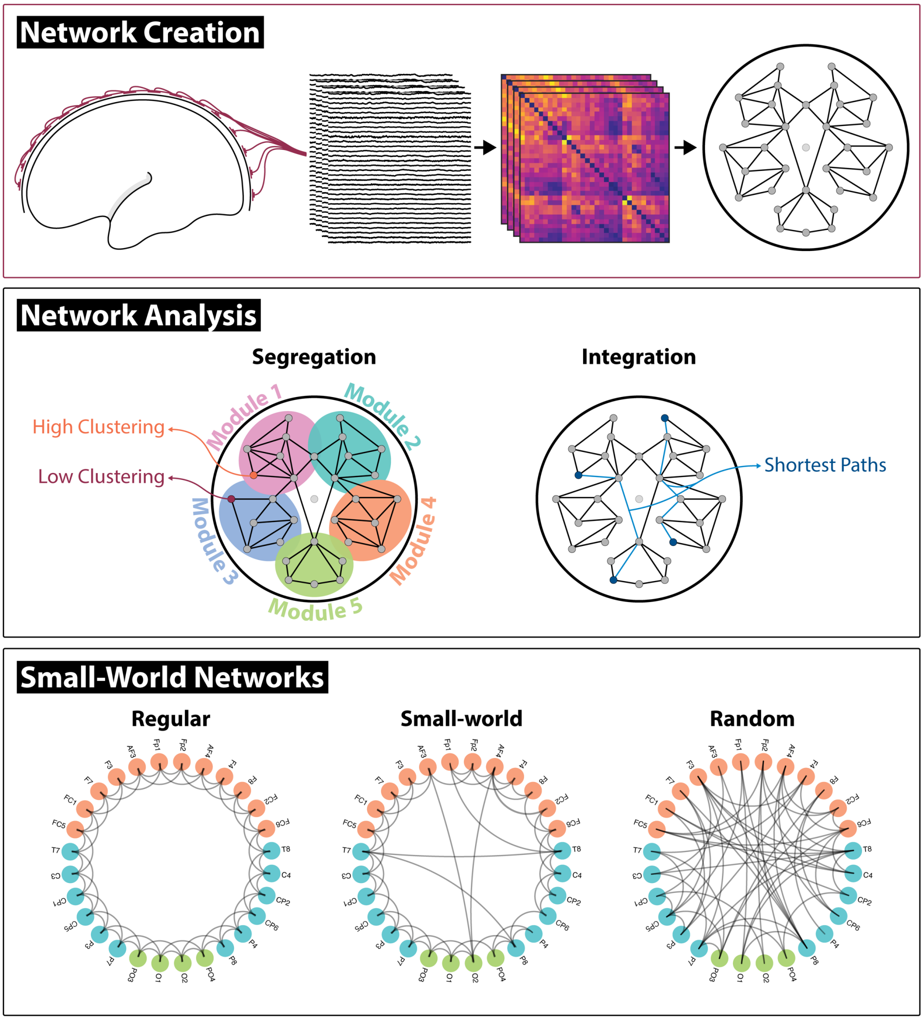
Het babybrein ontwikkelt zich razendsnel gedurende het eerste levensjaar. Dit is van groot belang aangezien een baby gebombardeerd wordt met steeds complexer wordende informatie. Een groot gedeelte van het leerproces van kinderen verloopt sociaal en wordt aangeleerd door of via personen in de omgeving van het kind. Het is dus begrijpelijk dat het verwerken van deze sociale informatie een belangrijke functie is van het jonge brein. Dit is een zeer complex proces, waarbij een persoon zich vele dingen kan afvragen. Wat is de emotie van de persoon naar wie ik kijk? Wat is de relatie van de persoon met zijn/haar omgeving? Praat de persoon tegen mij? Wijst de persoon iets aan? Veel verschillende gebieden in het brein zijn met deze onderdelen van sociaal gedrag bezig. Voor een optimale en snelle beslissing is goede communicatie tussen deze gebieden is vitaal. Gebieden in het brein die met elkaar verbonden zijn en communiceren noemen we ook wel brein netwerken. Als we willen weten hoe complex gedrag – zoals sociaal gedrag – zich ontwikkelt, is het begrijpen van de ontwikkeling van deze netwerken in het brein van groot belang.

Het bestuderen van netwerken in een babybrein is om verscheidene redenen niet gemakkelijk. Ten eerste is het meten van een babybrein ingewikkeld. Baby's zijn niet te instrueren, zitten niet stil, en kunnen maar beperkt hun aandacht ergens op vestigen. Als gevolg worden babybreinen vaak slapend of post-mortem bestudeerd. Hoewel dit tot interessante inzichten kan leiden op het gebied van de structuur van het brein, staat dit ver af van het brein onderzoeken terwijl het kind bepaald gedraag laat zien. Een tweede probleem van het bestuderen van netwerken in het brein is dat het vergelijken van netwerken zeer complex is. Er wordt niet gekeken naar een enkel gebied, maar vaak naar tientallen gebieden tegelijkertijd en naar de verbinding tussen deze gebieden. Het is zo erg lastig om vast te kunnen stellen wanneer netwerken fundamenteel verschillen van elkaar.

Het eerste probleem wordt vaak opgelost door EEG te gebruiken. Bij EEG worden elektrodes bevestigt op het hoofd van de proefpersoon die hersenactiviteit kunnen meten. EEG is relatief resistent tegen beweging, makkelijk te gebruiken met korte taken, en is relatief non-invasief. De perfect methode voor baby's dus. EEG heeft nog een ander bijkomend voordeel van het meten van breinactiviteit: EEG heeft een zeer goede temporele resolutie. Waar fMRI op het snelst een meting per second kan doen, kan EEG meer dan 2000 keer per seconde meten. Dit zorgt ervoor dat we beter onderzoek kunnen doen naar breinprocessen die een zeer korte tijdspanne in beslag nemen. Door deze goede temporele resolutie zijn we goed in staat de oscillaties van het brein te meten. Populaties van neuronen in het brein vuren vaak in oscillerende patronen. De frequentie van deze oscillaties kunnen verschillen. In het brein praten we vaak over de volgende frequentiebanden, geordend van traag naar snel: delta, theta, alfa, beta, en gamma oscillaties. De hypothese is dat het brein gebruikt maakt van deze verschillende frequentiebanden om optimaler te communiceren. Verschillende frequentiebanden worden dan ook toegeschreven aan andere cognitieve processen. Zo wordt alfa vaak gezien als inhiberend en theta en beta juist als exciterend. Dit wordt extra interessant bij het bestuderen van netwerken in het brein. Er wordt ervan uitgegaan dat op hetzelfde moment verschillende netwerken in het brein met elkaar communiceren in andere frequentiebanden om interferentie te voorkomen.

Voor het oplossen van het tweede probleem – het onderzoeken en vergelijken van netwerken is complex - wordt vaak gebruik gemaakt van *graph theory* (West, 2001). In *graph theory* worden netwerken gerepresenteerd als *nodes* (in het geval van het brein zou dit onze brein gebieden zijn) en *edges* (de verbindingen tussen de nodes, zie ook figuur 1). Met deze wiskundige benadering van een netwerk kunnen we verschillende karakteristieken van een netwerk berekenen.

Een belangrijke karakteristiek van een netwerk is hoe makkelijk het is om van een kant van het netwerk naar de andere kant van een netwerk te komen. Als deze karakteristiek, ook wel *karakteristiek pad lengte* of *globale efficiëntie* genoemd (Dijkstra, 1970), laag is dan is het makkelijk om van de ene kant naar de andere kant van het netwerk te komen en spreekt men van hoge integratie. Dit is zeer belangrijk voor het brein om goed te



Figuur 1 Netwerken en grafentheorie.

functioneren. Gebieden die ver van elkaar liggen moeten goed kunnen communiceren, informatie uitwisselen, en samenwerken om zo tot snelle beslissingen te kunnen komen.

Een tweede belangrijke karakteristiek van een netwerk is hoeveel clusters er zitten in het netwerk. Een cluster is een groep *nodes* die allemaal zeer sterk met elkaar verbonden zijn. Als deze karakteristiek, ook wel *cluster coëfficiënt* of *lokale efficiëntie* genoemd, hoog is zijn er relatief veel clusters aanwezig in het brein en wordt er gesproken van een hoge segregatie. Ook hiervan is het goed te verklaren waarom dit belangrijk zou zijn voor een menselijk brein. Gebieden die dicht bij elkaar liggen kunnen door sterke interconnectiviteit een specialistische taak uitvoeren. Als netwerken zowel een relatief hoge *cluster coëfficiënt* als een lage *karakteristiek pad lengte* hebben wordt er gesproken van een *small-world* netwerk (Watts & Strogatz, 1998) en dit wordt vaak de optimale organisatie van een netwerk genoemd.

Dus we hebben een veelgebruikte tool om het brein te meten – EEG – en een veelgebruikte tool om de netwerken te kwantificeren en te vergelijken – *graph theory.* Opvallend genoeg, echter, wordt er op dit moment nog weinig onderzoek gedaan naar de relatie tussen ontwikkelende breinnetwerken en de ontwikkeling van gedrag in het eerste levensjaar. Waarom is dit het geval? Dit komt voornamelijk, omdat er een aantal fundamentele problemen opgelost dienen te worden.

Ten eerste is het onduidelijk hoe betrouwbaar deze maten zijn. Als netwerken in het babybrein meerdere keren gemeten worden, zijn de uitkomstwaardes voor de fundamentele karakteristieken van het netwerk dan elke keer hetzelfde? En zijn deze waardes (relatief) uniek voor een baby? Alleen als aan deze twee eisen wordt voldaan, kun je op een zinnige manier, met de gemeten netwerken, gedrag voorspellen.

Ten tweede is het niet volledig duidelijk hoe EEG-data het beste afgenomen kan worden bij baby's. Er zijn veel aannames, veelal op basis van ervaring, maar deze aannames zijn niet uitvoerig getest. Het is van groot belang dat hier onderzoek naar gedaan wordt, want baby-onderzoek is kostbaar. Er is relatief veel uitval en data heeft veel ruis door baby's die niet stil zitten en niet hun aandacht vast kunnen houden. Duidelijk krijgen welke externe factoren van belang zijn om zo goed mogelijk EEG af te nemen is dus van groot belang.

Als laatste zijn verschillen in

breinnetwerken veelal klein – globaal gezien is praktisch elk brein hetzelfde. Dus om iets zinnigs te zeggen over de relatie tussen de ontwikkeling van het brein en gedrag dienen grote groepen baby's getest te worden. Dit neemt de nodige problemen met zich mee, zowel op het vlak van meten als analyseren. In ons geval zijn we specifiek geïnteresseerd in de ontwikkeling van gedrag en het brein. Dat maakt dit probleem nog groter. Niet alleen is een grote populatie baby's nodig, maar deze baby's moeten ook nog eens meerdere keren getest worden. Dit laatste probleem is opgelost door het opzetten en uitvoeren van het YOUth onderzoek (door Prof.dr Chantal Kemner, vele andere onderzoekers, en alle onderzoeksassistenten). Het YOUth onderzoek is een groot onderzoek wat loopt in de regio Utrecht waarin 3000 kinderen worden gevolgd vanaf nog voor dat ze geboren zijn totdat ze zes jaar oud zijn. In dit proefschrift wordt niet gebruik gemaakt van de volledige sample, maar in de meeste hoofdstukken wordt weldegelijk gewerkt met ongewoon grote populaties.

Dit proefschrift gaat in eerste instantie over het oplossen van probleem 1 en 2 beschreven in de alinea's hierboven. Daarna wordt er over gegaan op een toepassing van de netwerkanalyse in baby's om babygedrag te kunnen verklaren.

I.2. Methodologische problemen en oplossingen

In **hoofdstuk 2** beantwoorden we de eerste vraag hierboven beschreven: kunnen we netwerken bij baby's betrouwbaar meten? Mijns inziens zou dit altijd het begin van onderzoek moeten zijn. Kan ik datgeen wat ik meet wel vertrouwen?

Om dit te onderzoeken, werden 77 baby's van 10 maanden oud twee keer getest met precies een week ertussen. Tijdens beide testsessies werd er EEG afgenomen terwijl het kind naar een scherm aan het kijken was. Op het scherm werden twee verschillende video's afgebeeld. De eerste video was van zingende vrouwen, de tweede van bewegend speelgoed. De afgenomen EEG werd na elke sessie geschoond en gecontroleerd. Te ruisige proefpersonen (proefpersonen die te veel hadden bewogen of waarvan de meting om andere redenen niet was gelukt) werden verwijderd. Van de overblijvende 60 baby's werden de breinnetwerken geanalyseerd.

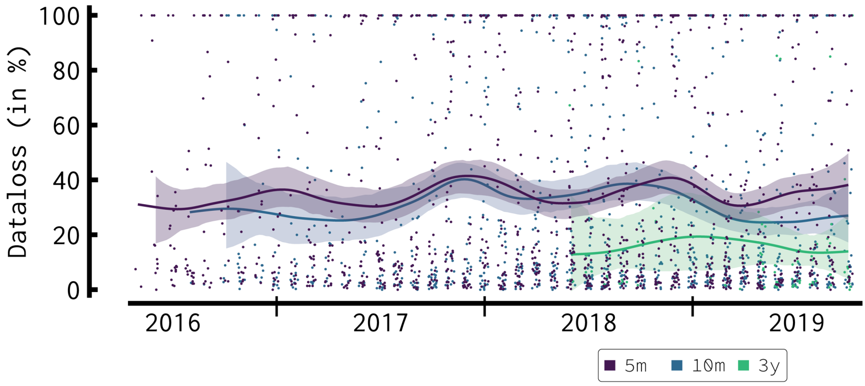
Breinnetwerken werden berekend voor de verschillende frequentiebanden (delta, theta, laag alfa, hoog alfa, beta, en gamma). Dit werd gedaan aan de hand van de *Phase Lag Index* (PLI) (Stam, Nolte, & Daffertshofer, 2007). Bij de PLI wordt gekeken hoe stabiel het faseverschil is tussen twee signalen. Des te hoger de stabiliteit, des te hoger de verwachting dat deze twee signalen met elkaar aan het communiceren zijn. Deze faseverschil stabiliteit werd voor het volledige experiment berekend tussen alle elektrodes die gemeten werden. In totaal gebruikten we 32 elektrodes, dus er werden per proefpersoon 32*31/2 = 496 connecties berekend. Met het resulterende netwerk berekenden we de volgende graaf karakteristieken: netwerksterkte (gemiddelde sterkte van het hele netwerk), karakteristiek pad lengte, cluster coëfficiënt, en small-worldness.

Onze bevindingen lieten zien dat in de theta en alfa frequentiebanden we betrouwbare netwerken konden meten. De netwerksterkte was betrouwbaar (ICC>0.8), de clustering coëfficiënt was betrouwbaar (ICC>0.8), karakteristiek pad lengte was betrouwbaar (ICC>0.8), en de small-worldness was redelijk betrouwbaar (ICC>0.6). Ook vonden we voldoende variantie tussen proefpersonen. Deze twee elementen: betrouwbare, maar variërende, netwerken zorgen ervoor dat we kunnen concluderen dat netwerken in baby's goed te meten zijn met EEG en dat de beschreven uitkomstmaten bruikbaar zijn om eventueel gedrag te verklaren.

Kortom, EEG-netwerken in de theta en alfa frequentiebanden zijn betrouwbaar, maar de andere frequentiebanden zijn een stuk minder betrouwbaar. Hoe komt dit? Het zou wel eens zo kunnen zijn dat de data te ruisig is. Dit is vooral een probleem in de hogere frequentiebanden. Daarom zijn we in **hoofdstuk 3** op onderzoek uitgegaan om EEG-datakwaliteit te maximaliseren en data verlies te minimaliseren. In dit hoofdstuk proberen we dus een antwoord te geven op het tweede probleem hierboven beschreven: Welke externe factoren zijn van invloed op de kwaliteit van EEG-data?

Voor dit onderzoek maakten we gebruik van de YOUth dataset. In totaal includeerden we 1278 5 maanden oude baby's, 1046 10 maanden oude baby's, en 104 3 jaar oude peuters. Vergelijkbaar met **hoofdstuk 2** werd de EEG-data gemeten terwijl de baby's en peuters naar verschillende video's aan het kijken waren. De verkregen data werd automatisch opgeschoond. Dat wil zeggen, slechte stukken EEG werden gedetecteerd en verwijderd. Per proefpersoon werd er berekend hoeveel procent van de data verwijderd werd.

Hierna konden we kijken of verschillende externe factoren invloed hadden op hoeveel data er werd weggegooid. We vonden verschillende interessante resultaten. Zo had de onderzoeksassistent die de EEG afnam een zeer grote invloed op het uiteindelijke dataverlies. Andere externe factoren die een invloed hadden op dataverlies waren leeftijd (ouder minder data verlies), geslacht (vrouwen meer dataverlies dan mannen), tijd van testen (hoe eerder, hoe beter), en hoofdvorm (atypische hoofdvormen leiden tot dataverlies). Het meest verrassend was het effect van seizoen: in de wintermaanden werd meer dataverlies vastgesteld dan in de zomermaanden (zie ook figuur 2).



Figuur 2. Data verlies gedurende de hele studie. Hier afgebeeld is de hoeveelheid data verlies gedurende de hele studie (vanaf 2016). Elk stipje in een losse proefpersoon. Een gemiddelde lijn is getekend om de resultaten beter zichtbaar te maken. Een duidelijk jaarlijks ritme is te zien met pieken in de wintermaanden (meer dataverlies). Dit effect is te zien voor alle leeftijden.

Ook keken we naar het effect van lengte van de taak op data verlies. Hier zag je (niet geheel verrassend) dat naarmate de taak langer duurde, er meer data verlies was. Wat zeker wel interessante was, was het gelimiteerde effect van een pauze. Na de pauze schoot het dataverlies omlaag, maar binnen 1 à 2 seconden was het dataverlies weer terug op het originele punt. Als laatste werden baby's vergeleken over meerdere sessies.

Hier vonden we kleine verbanden, waarbij baby's die eerder een slechte meetsessie hadden gehad een iets grotere kans hadden op nog een slechte sessie de tweede keer.

I.3. De relatie tussen gedrag en het babybreinnetwerk

Nu we wisten dat bepaalde netwerkmaten betrouwbaar waren, maar ook zicht hadden wat voor externe factoren invloed konden hebben op onze data, konden we aan de slag met de toepassing van de netwerkanalyse bij baby's. Zoals eerder vermeld wilden we graag weten hoe de ontwikkeling van sociaal gedrag gerelateerd was aan de ontwikkeling van het babybrein. Er zijn verschillende facetten aan sociaal gedrag. Een belangrijke tweescheiding kan gemaakt worden tussen het interpreteren en verwerken van sociale stimuli en het zelf daadwerkelijk uitvoeren van sociaal gedrag. In **hoofdstuk 4 & 5** werden deze twee verschillende onderdelen onderzocht. In beide gevallen wordt de ontwikkeling van sociaal gedrag gerelateerd aan de ontwikkeling van het babybreinnetwerk.

In **hoofdstuk 4** wordt de ontwikkeling van theta en alfa netwerken beschreven en wordt er onderzocht of deze netwerken anders reageren op sociale stimuli dan op niet-sociale stimuli. De resultaten van deze studie zijn zeer interessant. Theta netwerken laten namelijk een duidelijk ontwikkelingspatroon zien. Waar we bij jonge baby's vooral communicatie zien tussen de pariëtale en visuele gebieden, herconfigureert zich dit tot een netwerk waar vooral frontale en pariëtale gebieden met elkaar communiceren. Deze verandering is niet te zien in het alfa netwerk. Er lijkt dus een totale reconfiguratie van het theta breinnetwerk plaats te vinden tussen 5 en 10 maanden oud.

Tegelijkertijd met deze reconfiguratie begint het netwerk ook gevoeliger te worden voor sociale stimuli. De synchronisatie binnen dit netwerk verhoogd namelijk bij het zien van de sociale video's vergeleken met de niet-sociale video's. Dit gebeurt alleen bij de theta netwerken van 10-maanden-oude baby's. Deze verhoogde gevoeligheid is niet te zien binnen het alfa netwerk. Deze studie lijkt dus bewijs te hebben gevonden voor het ontstaan van een frontopariëtaal sociaal brein netwerk in de tweede helft van het eerste levensjaar.

Dus we weten nu dat babybreinnetwerken betrouwbaar zijn en dat ze gebruikt kunnen worden om het verwerken van bepaalde stimuli te verklaren. Het voornaamste stuk van de puzzel wat nu nog mist is hoe deze netwerken en de ontwikkeling daarvan nu precies gerelateerd zijn aan de ontwikkeling van gedrag. Hier gingen we verder op in, in **hoofdstuk 5**.

In deze studie volgde we zowel de gedrags- als de breinontwikkeling van 800 baby's gedurende hun eerste levensjaar. Qua gedrag waren we vooral geïnteresseerd in sociale ontwikkeling en regulatie (belangrijk voor emotie). We zijn vooral zo geïnteresseerd in sociaal gedrag, omdat een toename in sociaalheid baby's in staat stelt meer van de wereld te beleven. Leerprocessen zijn – zeker in de eerste levensjaren – veelal sociaal: ouders laten hun kind wat zien of kinderen willen samen met ouders/broertjes/zusjes iets doen.

De ontwikkeling van dit sociale gedrag zorgt er dus direct voor dat het kind meer ervaring krijgt met haar/zijn omgeving. Deze toename in ervaring heeft dan weer invloed op het brein van het kind. Hoe meer een kind bepaalde dingen ervaart, hoe optimaler gebieden in het brein gaan samenwerken. Echter het tegenovergestelde is ook waar. Zonder een (relatief) optimaal presterend breinnetwerk is complex gedrag niet mogelijk. Het is daarom waarschijnlijk dat er een wisselwerking ontstaat gedurende het eerste levensjaar waar complex gedrag het brein optimaliseert, maar het optimaliseren van het brein ervoor zorgt dat er meer complex gedrag uitgevoerd kan worden.

De hypothese vinden we terug in onze data. De sterkte van zowel theta als alfa netwerken bij 5 maanden oude kinderen voorspelt hun regulatie op 10 maanden oude leeftijd. Andersom voorspelt regulatie op 5 maanden oude leeftijd, small-worldness bij 10 maanden. Dus de ontwikkeling van het brein maakt complexer gedrag mogelijk en het complexere gedrag optimaliseert het brein. Belangrijk is wel om aan te geven dat deze relatie klein, doch significant, is en dat we geen relaties hebben gevonden tussen sociale ontwikkeling en het brein (dus alleen tussen regulatie een het brein).

I.3. Conclusies

In dit proefschrift onderzochten we de relatie tussen de ontwikkeling van sociaal gedrag en de ontwikkeling van het babybreinnetwerk. Voordat we deze vraag konden beantwoorden werd echter al snel duidelijk dat er eerst een en ander nodig was op methodologisch vlak. Daarom hebben we eerst de methode onderzocht en getracht te optimaliseren.

Door **hoofdstuk 2 & 3** zouden onderzoekers beter in staat moeten zijn keuzes te maken met betrekking tot het uitvoeren en analyseren van baby EEG-data om netwerken te meten. Hoofdstuk **4 & 5** geven een eerste aanzet van alles wat mogelijk is om te bestuderen vanuit een breinnetwerk perspectief in baby's. Hierin werd duidelijk dat theta communicatie gedurende het eerste levensjaar steeds belangrijker wordt voor het verwerken van sociale informatie. Ook werd er een wederkerige relatie gevonden tussen het babybreinnetwerk en gedrag, waarin complex gedrag gerelateerd is met een optimaler brein later in het eerste levensjaar. Het tegenovergestelde was echter ook waar, een meer ontwikkeld brein voorspelde complexer gedrag later in het eerste levensjaar.

I.4 Referenties

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APPENDIX II

Dankwoord

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De overige leden van de promotiecommissie, prof. dr. A.L. Van Baar, prof. dr. D.J.L.G. Schutter, prof. dr. M.J.N.L. Benders, prof. dr. H.E. Hulshoff

Pol, prof. dr. C.J. Stam, en prof. dr. J.L. Kenemans, wil ik graag bedanken voor het beoordelen van mijn proefschrift en de bereidheid zitting te nemen in mijn promotiecommissie.

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APPENDIX III

List of Publications

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Not in this dissertation

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Hofstee, M., van der Velde, B., Huijding, J., Endendijk, J., Kemner, C., Deković, M. (Under review) The Direct and Indirect Effects of Parenting Behaviors and Functional Brain Network Efficiency on Self-Regulation from Infancy to Early Childhood: A Longitudinal Mediation Model

APPENDIX IV

Curriculum Vitae

Bauke van der Velde was born on June 26th, 1989, in Leiden, The Netherlands. After graduating high school in 2007, he studied Bèta-Gamma with a major in Psychobiology at the University of Amsterdam and obtained his Bachelor of Science with honours in 2011, where he was awarded the IIS Science Award for best interdisciplinary bachelor thesis. He continued his education with the Master Brain and Cognitive Sciences at the University of Amsterdam. For his final master thesis, he moved to London for a year to study cue integration in children under dr. Tessa Dekker at the University College London. During his bachelor and master, Bauke worked as a research and MRI scan assistant at the Spinoza Center in Amsterdam and at the Birkbeck-UCL Centre for Neuroimaging in London. After graduating, Bauke started his PhD in 2015 at Utrecht University, in the Department of Experimental Psychology, studying the development of the infant brain network during the first year of life. Next to his research, he is involved as a teacher in creating and coordinating several psychology and UCU courses. Currently, Bauke is employed as a postdoctoral researcher at the Department of Experimental Psychology at Utrecht University where he studies the effects of COVID-19 measures on infant development