FUNCTIONAL CONNECTIVITY SUPPORTING EMOTION PERCEPTION, AND RESTING STATE NETWORKS IN TYPICALLY DEVELOPING NEONATES AND FOLLOWING PRENATAL EXPOSURE TO MATERNAL MOOD DISTURBANCES AND SSRIs

by

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Abstract

The first year of life is a period of dramatic structural changes in the brain. Along with structural changes, infants achieve significant behavioral milestones. The bridge between brain structure and behavior is strongly based on functional connections that enable intrinsic functioning and also develop emotion perception skills, both critical for early development. However, little is known about how these connections are functionally organized in infancy. Prenatal exposures to maternal mood disturbances and the use of selective serotonin reuptake inhibitor (SSRI) antidepressants play a crucial role in shaping infants’ development, although it remains unclear how these exposures are linked to infant developmental outcomes. In this thesis, I use task-based electroencephalography (EEG) and resting-state functional magnetic resonance imaging (rs-fMRI) combined with graph theory analysis to study the functional networks of emotion perception and the intrinsic functional connectivity of resting state networks (RSNs) in typically developing infants and in infants prenatally exposed to mood disturbances and SSRIs. I found that 8-to-10-month-old infants have network characteristics that are similar to adults when observing basic emotions (Chapter 3). Moreover, an increase in prenatal maternal mood symptoms was associated with reduced modularity only for negative emotions, while prenatal SSRI drug-exposure was associated with higher network modularity in observing both positive and negative emotions. In contrast, higher postnatal mood symptoms were associated with alterations in frontal hubs (Chapter 5). Prenatal mood disturbances were associated with alterations in intrinsic RSNs. Specifically, compared to the control group, infants exposed to prenatal maternal depression showed higher hub values of the left anterior-cingulate, insula, and caudate as well as higher hub values in the
amygdala (Chapter 7). Prenatal SSRI exposure associated both with higher hub values in Heschel’s gyrus (Chapter 7) and with hyperconnectivity of the putative auditory network (Chapter 6) possibly support shifts in language perception previously reported in infants exposed to prenatal SSRI. Collectively, these data indicate that the core functional organization for observing basic emotions is in place at 8-to-10 months of age. Further, maternal mood disturbances and SSRI exposure may differently shape early intrinsic and emotion perception functional organization, possibly leading to different developmental trajectories.
Lay Summary

How different brain regions functionally interact in infancy is poorly understood. The goal of this thesis was to study how an infant’s brain is functionally organized to support spontaneous brain function (during sleep) and emotion perception in typically developing infants. Another goal was to study how these functions are affected by prenatal maternal depression (PMD), treated with selective serotonin reuptake inhibitors (SSRI) antidepressants or untreated. Recorded brain activity of eight-month-old infants while observing emotions revealed that infants process basic emotions in a manner that is similar to adults. PMD-exposed infants showed an increased processing of negative emotions, and SSRI-exposed infants showed decreased emotional processing. Sleeping newborns exposed to PMD showed increased abilities to integrate information from regions that regulate emotions, and SSRI-exposed infants showed increased connectivity in auditory and language regions. Collectively, these results might have implications for infants’ socio-emotional and language development following PMD with or without SSRI.
Preface

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List of Abbreviations

5HT – 5-hydroxytryptamine receptors
AC-PC – anterior commissure-posterior commissure
ACC – anterior cingulate cortex
AD – absolute displacement
AFNI – analysis of functional neuroimages
AN – auditory/language network
ANOVA – analysis of variants
ASD – autism spectrum disorder
BAEP – brainstem auditory evoked potential
BDI – Beck depression inventory
BESA – brain electrical source analysis
BET – brain extraction tool
BOLD – blood oxygenation level dependent
BSI – Brief Symptom Inventory
CES-D – Center for Epidemiologic Studies Depression Scale
DL-PFC – dorsolateral prefrontal cortex
DMN – default-mode network
DTI – diffusion tensor imaging
EEG – electroencephalography
ENA33 – Edinburgh neonatal atlas
EPDS – Edinburgh postnatal depression scale
EPI – echo planar image
EPN – early posterior negativity
ERP – event related potentials
FA – fractional anisotropy
FEAT – fMRI expert analysis tool
FFA – fusiform face area
FHR – fetal heart rate
FL – frontal left
FLIRT – FMRIB’s linear image registration tool
FMIs – Freiburg Mindfulness Inventory
fMRI – functional magnetic resonance imaging
FPNL/R – left/right frontoparietal networks
FSL – fMRIB software library
FWHM – full width half mean
GA – gestational age
GLM – general linear model
GTA – graph theory analysis
HAM-D – Hamilton rating scales for depression
HPA – hypothalamic-pituitary-adrenal
IBQ – infant behavior questionnaire
ICA – independent component analysis
MCA – middle cerebral artery
MCFLIRT – motion correction FMRIB’s linear image registration tool
MD – mean diffusivity
MELODIC – multivariate exploratory linear decomposition into independent components
MINI – mini-neuropsychiatric interview
NAPI – neurobehavioral assessment of the preterm infant
Nc – negative central
OpM – occipito polar cortex medial
PCFDR – false discovery rate controlled partial correlation
PES – pregnancy experiences scale
PFC – prefrontal cortex
PL – parietal left
PLSR – projection to latent structures regression
RD – relative displacement
rs–fMRI – resting state functional magnetic resonance imaging
RSN – resting state networks
SA – salience network
SATI – State-Trait Anxiety Inventory
SCID-I – Structured Clinical Interview for DSM- IV Axis I Disorders interview
SD – standard deviation
SLC-90 – Symptom Checklist
SM – sensorimotor network
SNRI – selective-norepinephrine reuptake inhibitors
SSRI – selective serotonin reuptake inhibitors
STS – superior temporal sulcus
TAL – temporal anterior left
TPL – temporal posterior left
V1 – medial occipital network
V2 – occipital pole network
V3 – lateral visual/parietal network
WM – white matter
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Dedication

To my parents.

To Ran, Romy, and Ben.

To my grandma, Rivka
Chapter 1: Introduction

1.1 Rationale and goals

The first year of life is one of the most fascinating periods in which the brain undergoes tremendous growth and development. The growth of anatomical gray matter and anatomical connections create the foundations for establishing the intrinsic functional connections that support action, behavior, and cognition. These functional connections also support emotional development, which is critical in early infancy for developing the abilities to perceive others’ emotions and to make sense of the environment. However, it remains unclear how these connections are organized to support general development in early infancy and to support the development of emotion perception. Moreover, within the “prenatal programming framework,” the prenatal environment—particularly in the context of maternal mood disturbances and exposure to SSRI antidepressants—plays a crucial role in shaping the offspring’s development. However, the neural correlates of prenatal programming of the functional connectivity organization that support intrinsic and emotional development are not well understood.

In this thesis, I take advantage of advances in neuroimaging techniques, which enable a glimpse into the functional connectivity of infants in their first year of life. My first goal is to learn about the functional organization of networks that support emotional development, using an emotion perception task, and to learn about the intrinsic functional connectivity of networks during rest. In addition, I seek to investigate how the functional connectivity organization of these networks develops within a prenatal programming framework related to prenatal depression and SSRIs. In order to answer these questions, I use a data-driven approach (Independent Component Analysis (ICA)) and graph theory analysis. In the first project
(Chapter 3: 3), I focus on characterizing the functional networks of emotional perception during emotion observation, comparing between typically developing 8-to-10-month-old infants and adults using a task-based electroencephalography (EEG) of the observation of emotional faces. Thereafter, I discuss the influence of maternal depression and SSRI on an infant’s development and how neuroimaging techniques have been used to study the impact of these influences (Chapter 4). In the next project (Chapter 5), I focus on studying associations between maternal depression and prenatal SSRI and emotion perception in 8-to-10-month-old infants and employ an EEG-based measure of responses to emotional face observation. In the final chapters, I focus on studying differences in intrinsic functional connectivity between typically developing infants and infants exposed to depression and SSRI using ICA as an unbiased data-driven approach (Chapter 6) and graph theory analysis (Chapter 7). To this end, I use resting state functional magnetic resonance imaging (rs-fMRI) of newborn infants during natural sleep.

1.2 The development of functional connectivity

The first year of life is one of the most dynamic periods of brain development in the life span (Gao et al., 2015b). Early anatomical development begins in utero and includes neurogenesis, synaptogenesis, dendritic arborization, and axon formation. After birth, the brain volume continues to increase, mostly as a result of the growth in grey matter neural connections, long range axons, and myelination to form the anatomical structure of the brain (Gao et al., 2016) and to support the establishment of neuronal functional connectivity. Functional connectivity describes the temporal correlations between patterns of brain activation of anatomically separated regions (van den Heuvel & Pol, 2010), as opposed to physical
connections of white matter microstructure pathways that project among different brain regions (Roberts et al., 2013). It has been shown that in many cases, functional connections are structurally connected and linked, but they are not identical (van den Heuvel and Hulshoff Pol, 2010); the precise correlation among these functional connections remains unclear (Bullmore and Sporns, 2012). While it is known that structural brain networks need to support immense amounts of functional arrangements, the mechanisms that support this plasticity are unknown (Bullmore and Sporns, 2012). Functional connectivity characterizes functional interactions between delineated regions and can provide information regarding the level at which different regions or components of the brain are coupled in their activation to accomplish a certain task (Rogers et al., 2007). Performing a task activates brain regions that are functionally relevant to the task and are temporally correlated in their activation patterns, thereby creating functional networks (van den Heuvel and Hulshoff Pol, 2010). For example, an individual who taps his left and right fingers for several seconds will activate brain regions related to this “motor task,” such as the left and right motor cortices and the supplementary motor area (Biswal et al., 1995). Gazzaley, Rissman, & D’Esposito (2004) show that the working memory task of active maintenance of faces results in the activation of widely distributed brain regions such as the fusiform face area (FFA), dorsolateral/ventrolateral parts of the prefrontal cortex (PFC), premotor cortex, hippocampus, intraparietal sulcus, caudate nucleus, thalamus, and occipito-temporal regions. Functional connectivity is believed to represent one of the most feasible approaches to elucidating the link between brain structure and behavior (Gao et al., 2016).
1.2.1 Functional connectivity of emotion perception

The ability to perceive, recognize, and understand social stimuli is an essential prerequisite for developing social, cognitive, and emotional skills. Developing these abilities is at the core of almost every developmental achievement in infancy (Grossmann, 2010). In order to communicate with each other, humans observe others’ facial emotions, extracting emotional cues to guide their response in various social contexts (Frith, 2009). The mechanisms underlying the development of these abilities and how they develop in the first year of life have been extensively studied for decades (Batty and Taylor, 2003; Paolo Fusar-Poli et al., 2009; Haxby et al., 2000; Snyder et al., 2002). Most of the knowledge on the neural correlates of emotion processing in early infancy is based on EEG–ERP studies, which provide valuable information on specific components related to emotion perception (Grossmann et al., 2007; Leppänen and Nelson, 2009; Missana et al., 2014) (also see the Introduction section of Chapter 3: 3). One of the most important models for face processing is the two-process theory (Johnson, Senju, & Tomalski, 2015; Morton & Johnson, 1991). According to this theory, two processes are involved in the processing of faces: CONSPEC and CONLERN. CONSPEC relates to subcortical processes that contribute toward orientation to faces of the same species, accounting for the attentional bias exhibited in newborn infants for faces and face-like stimulation (Reynolds and Roth, 2018). CONLERN refers to processes of a cortical nature involved in face perception, such as face recognition and categorization, which are acquired over time and with experience. An important aspect of this model is that CONSPEC processes are believed to be innate, thereby biasing a newborn infant toward a face or face-like stimuli to ensure sufficient exposure; on the other hand, CONLERN processes build on these biases to fine tune neural networks for
processing faces (Johnson et al., 2015; Reynolds & Roth, 2018). CONSPEC involves subcortical regions like the Amygdala, and CONLERN involves cortical regions such as the fusiform face area, superior temporal sulcus (STS), medial prefrontal cortex, and orbitofrontal cortex. Leppänen & Nelson (2009) indicate an emotional face processing network by which the amygdala and orbitofrontal cortex receive visual information mainly through two pathways: The first path is information that is transferred from the fusiform gyrus and posterior superior temporal sulcus, and the second and more rapid path is information that is directly transferred from the early visual cortex. Moreover, they suggest that as the infant acquires more experience in observing emotional faces, this network becomes functional, thereby enabling an infant to distinguish between basic expressions and become attentionally biased toward salient expressions like fear (Leppänen and Nelson, 2009). At this stage, the network undergoes refinement processes that involve the stabilization and preservation of connections used more frequently on the one hand, and pruning the less relevant and infrequently used connections on the other hand (Cassia, Bulf, Quadrelli, & Proietti, 2014). Thus, behavioral studies imply that emotional processing is refined as the infant gains more experience in perceiving emotional facial expressions. However, thus far, evidence of neural correlates and the development functional connectivity organization in infants that support this remain limited.

Building on these models for face processing, experience and exposure to different social environments may play an important role in refining the “social network.” Indeed, studies of maltreated children provide strong evidence of how high frequency of exposure to negative faces in the close environment can shape emotional processing in infancy and childhood (Dannlowski et al., 2013; Tottenham et al., 2011). Results from functional magnetic resonance imaging
(fMRI) studies suggest that maltreatment during childhood is associated with increased activation of the amygdala for viewing negative expressions. Earlier studies have shown that while basic expressions such as happy, sad, and fearful were perceived similarly between control and children who were raised in an abusive environment, children in the abused group allocated more perceptual resources and showed increased sensitivity to angry expressions compared with control children (Pollak et al., 2000; Pollak and Kistler, 2002). This evidence of emotion-specific alterations in perception among maltreated children suggests that atypical experience might not have a broad effect on the basic organization of emotion perception but could result in a more subtle effect that is very specific to the nature of the exposure (Leppänen and Nelson, 2009). While more emphasis has been placed on how postnatal experiences shape the functional networks for emotion perception, less is known about how prenatal exposures shape the development of emotional networks. This is a critical question, as depression and use of antidepressant medications (i.e., SSRI) are common during pregnancy and have been shown to alter the socio-emotional development of the offspring (Brummelte et al., 2017; Gentile, 2015). The effects of maternal depression and maternal SSRIs on emotion perception are further discussed in section 4.4 and addressed in greater detail in Chapter 5.

1.2.2 Functional connectivity of resting-state networks

Even in the absence of any external task, such as during rest, spontaneous functional connectivity displays spatial patterns of activation similar to patterns displayed during task (Biswal et al., 1995), thereby yielding functionally relevant resting state networks (RSNs) (Damoiseaux et al., 2006). These RSNs are believed to maintain ongoing functional connectivity
during rest to improve efficiency and reaction time for the time when functional connectivity is needed for task performance (Damoiseaux et al., 2006). Studies among adults have shown that RSNs are consistently demonstrated in healthy adults and have potential functional relevance related to auditory, motor, visual, executive functioning, and memory (Damoiseaux et al., 2006; O’Reilly et al., 2012). Raichle et al. (2001) also demonstrate the existence of the default-mode network, which is attenuated during attention-demanding tasks, while its activity is enhanced during rest or while daydreaming, thereby reflecting a state of intrinsic brain activity. The importance of healthy functioning of RSNs has been further established by numerous studies that show that dysfunction of RSNs is associated with different brain disorders, such as that in Alzheimer disease (de Haan et al., 2009; Tahaei, Jalili, & Knyazeva, 2012), schizophrenia (Orliac et al., 2017), autism spectrum disorder (ASD) (Cerliani et al., 2015), and depression (Sheline, Price, Yan, & Mintun, 2010; Wang, Hermens, Hickie, & Lagopoulos, 2012). Emerging evidence from recent years has indicated the existence of fundamental RSNs already in early infancy (Gao et al., 2016) and even in the fetal stage to a certain extent (Doria et al., 2010). A series of recent fMRI studies have demonstrated that primary auditory and visual networks are functionally synchronized from birth, whereas higher-order networks become functional only later in life (Doria et al., 2010; Fransson et al., 2010, 2009; Gao et al., 2016, 2015b). It is suggested that the early development of basic sensory networks is crucial for establishing the foundations for the subsequent development of higher-order networks (Gao, Lin, Grewen, & Gilmore, 2016). Figure 1-1 depicts the development of RSNs in the first two years of life.
Development of the brain’s functional networks during the first year of life

Resting state networks evaluated at five time points are shown from the first to the fifth rows and the corresponding adult resting state networks are shown at the bottom row (green dots show the locations of seeds). Color bar indicates correlation strength. V1 = medial occipital network; V2 = occipital pole network; V3 = lateral visual/parietal network; DM = default-mode network; SM = sensorimotor network; AN = auditory/language network; SA = salience network; FPNL/R = left/right frontoparietal networks, Wei Gao et al. Functional Network Development During the First Year: Relative Sequence and Socioeconomic Correlations, Cerebral Cortex, Volume 25, Issue 9, September 2015, Pages 2919–2928, https://doi.org/10.1093/cercor/bhu088 by permission of Oxford University Press.

Different maturational patterns for different resting state networks suggest different developmental trajectories for different networks (Gao et al., 2016), thereby providing an avenue for understanding how potential early life experiences affect brain function. For example, higher socioeconomic status during the first year of life was positively correlated with higher connectivity within the default mode network and the sensorimotor network, an indication of
higher maturation of these networks (Gao et al., 2015a). Moreover, evidence linking RSN
dysfunction with different neuropathologies, such as ASD (Cerliani et al., 2015) and depression
in adulthood (Sheline et al., 2010; Wang et al., 2012), warrants a close examination of brain
functional connectivity very early in life, and raises questions regarding whether the origin of
some of these neuropathologies can possibly be traced back to the neonatal period (Fransson et
al., 2009; Gao et al., 2016).

To summarize, there are multiple approaches to study how functional connectivity
develops in infancy. One approach is to use traditional methods and examine how the brain
functions during a specific task. This provides specific information on the functional networks
that support the performance of the task. Another approach is to study how the brain functions at
rest, providing valuable information about intrinsic brain activity, which is believed to maintain
and preserve the functional connections in an active state and improve performance and
efficiency whenever functional connectivity is needed. The latter option is specifically beneficial
in very young infants when task-based studies are rather limited. In this thesis, I examine the
functional connectivity in the typically developing infant brain during an emotional task (using
EEG) and during rest (using fMRI). I also examine how functional connectivity changes
following prenatal exposure to maternal depression and/or SSRI antidepressant drugs.

1.2.2.1 Analysis of resting-state functional connectivity using fMRI

There are multiple approaches to quantifying resting-state functional connectivity, which
can be broadly divided into two classes: model-dependent and model free methods (van den
Heuvel and Hulshoff Pol, 2010). Within the model dependent class, the seed-based approach is
the most common one. According to this approach, correlations between a pre-selected region and any other regions are measured by comparing resting state time series of the pre-selected brain region against resting state time series of all the other brain regions (van den Heuvel and Hulshoff Pol, 2010). This yields a functional connectivity map of this specific brain region, revealing which other brain regions are highly correlated in their spontaneous activity patterns to this region (van den Heuvel and Hulshoff Pol, 2010). Thus, the seed-based approach requires a \textit{a priori} selection of a region of interest.

On the other hand, model-free approaches do not require an initial choice of a specific region and are advantageous in cases when an \textit{a priori} assumption is not available. One such method is the clustering analysis. With this approach, voxels showing a high degree of similarity are grouped together into a cluster, while different clusters have a low degree of similarity to other clusters (van den Heuvel et al., 2008). Thus, clustering analysis results in a direct reflection of how different brain regions are functionally connected (van den Heuvel and Hulshoff Pol, 2010). However, one drawback of this method relates to its inability to directly test for group comparison analysis and the requirement of additional analysis steps similar to seed selection. Another model-free method for analyzing resting-state data is ICA, which allows for a data-driven (non-biased) approach to examining resting-state functional networks (Beckmann et al., 2009; Gao et al., 2015b). The ICA approach can decompose the whole-brain voxel-wise data into a mixture of underlying sources (components). This enables an easy separation of the components of different resting networks from noise-related components of signal variations that occur as a result of motion artifacts such as cardiac pulsation or respiration and head motions (Beckmann and Smith, 2004; Damoiseaux et al., 2006). Thus, noise-related components can be
easily eliminated from the data, while resting-state components can be selected for further analysis (Damoiseaux et al., 2006). ICA analysis provides a powerful non-biased, data driven approach. In Chapter 6, ICA analysis was used as a non-biased approach to examine the RSN in the neonatal brain and to compare the RSNs between newborn infants of healthy control mothers, newborn infants of depressed mothers, and newborn infants of depressed-SSRI treated mothers.

While these approaches for analysis are different, the results are remarkably comparable and consistently support the existence of multiple RSNs (Beckmann, DeLuca, Devlin, & Smith, 2005; Damoiseaux et al., 2006; van den Heuvel et al., 2008).

1.3 Thesis overview and objectives

The following experiments described in this thesis investigate the functional networks of the neonatal brain, while observing emotional faces and while at rest, during the first year of life. Further, associations between these functional networks and prenatal exposure to maternal mood disturbances, with and without SSRI treatment, were examined. Maternal mood measures were evaluated both prenatally, in the third trimester of pregnancy, and postnatally. RSNs of typically developing infants, and of infants of depressed mothers with and without exposure to SSRIs, were probed by rs-fMRI scans postnatally at day-6. Emotion perception networks were studied by EEG during a task of emotional observation at the age of 8-10-months. The overarching hypothesis of this thesis is that infants with exposure to prenatal maternal mood disturbances without SSRI exposure, will exhibit different functional connectivity organization compared to infants with prenatal SSRI exposure, both during an emotional task and during sleep.
Chapter 2: To discuss the graph theoretical framework, and explain how it can add to the current knowledge and increase our understanding of functional networks.

Chapter 3: To compare the functional network properties for processing emotional facial expressions between 8-10 month old infants and adults at the global level and regional levels, and to determine whether network organization differ between positive and negative emotions in both infants and adults.

I hypothesized that the global and regional network characteristics underlying the perception of emotional facial expressions would be similar in infants and adults. In addition, I hypothesize that each emotional expression would be characterized by a distinct network organization.

Chapter 4: To overview emerging findings from neuroimaging studies reflecting early brain functional and structural development associated with prenatal exposure to maternal mood and SSRI antidepressants, and to explain why neuroimaging methods offer novel insights into central processes that might elucidate the neural correlates of fetal programming.

Chapter 5: To determine association between prenatal maternal depression and in utero SSRIs exposure and the network organization underlying perception of emotional facial expressions in young infants.

I hypothesized that in utero SSRI exposure would be associated with alterations in the organization of functional networks for perceiving emotions in infants, independent of exposure
to prenatal or postnatal depression. Further, I hypothesized that prenatal depression and stress will be associated with functional organization alterations especially for negative emotions. I also anticipated that postnatal maternal mood would be associated with less efficient processing, reflected by a reduction in frontal hub values.

Chapter 6: To examine functional RSNs and functional organization in typically developing neonates and to determine associations between maternal mood disturbances and in utero exposure to SSRIs and alterations to the functional RSNs in the neonatal brain.

I hypothesized that basic sensory functional resting state network will exhibit in the neonatal brain. Further, I hypothesized that prenatal maternal depression and in utero SSRI-exposed infants will have altered functional RSNs. Since current findings are sparse I did not make any preliminary assumption on the direction of the results and used a data driven un-biased approach (ICA) to test my hypothesis.

Chapter 7: To examine the topological organization of RSNs in typically developing neonates, and to determine associations between prenatal exposure to maternal depression with or without SSRI exposure and alterations of the topological organization of RSNs.

I hypothesized that prenatal maternal depression and SSRI exposure will be differently associated with alterations in the RSNs. Further, I hypothesized that prenatal depression would be associated with increased hub values of key stress-related regions such as the amygdala, insula, and anterior-cingulate compared to controls. Based on the results from Chapter 6, I also
hypothesized that prenatal SSRI-exposure would be associated with higher hub values of regions related to auditory function.
Chapter 2: **Graph theory analysis**

Since the 1990s, advances in the understanding of the physical properties of complex systems have led to increased interest in network science (Strogatz, 2001). Graph theory is a mathematical tool to analyze complex networks. A network is defined as a set of nodes, which are connected to each other by edges (Bullmore and Sporns, 2009). It is now well-established that different complex systems or networks, behave similarly at the macroscopic level (Bullmore & Sporns, 2009). Namely, complex networks (even from different domains) share key organizational properties, which can be quantified using similar parameters (Bullmore & Sporns, 2009). Complex networks are different from random graphs on account of different topological characteristics, such as high level of connectedness, highly connected hubs, and modular organization (stability of sub-networks) (Bassett & Bullmore, 2009; Bullmore & Sporns, 2009). Moreover, complex networks possess “small world” network qualities, such that nodes are densely connected, with a small number of long distance connections mostly between hubs, to create an efficient organization (Sporns, 2004). Watts and Strogatz (Watts and Strogatz, 1998) suggested that small-world networks, which balance between local specialization and global integration, are optimal for information processing. Several real-life networks, including the internet and social networks, possess small-world features (Bullmore & Sporns, 2012; Watts & Strogatz, 1998). Over the past two decades, there has been a growing interest in applying the graph theory framework to study the brain (Bathelt et al., 2013; Khundrakpam et al., 2012; Rotem-Kohavi et al., 2014; Sporns, 2004). Using the graph theory approach, the brain is studied as a network or a graph, such that brain regions are defined as nodes and the connections between them (structural or functional) are defined as the edges (Bullmore & Sporns, 2009).
The graph theory analysis applied on fMRI or EEG data is used to characterize the topological organization and efficiency of the brain’s functional connectivity (Sporns, 2004). Graph theory analysis, applied on functional connectivity data, builds from the information provided by functional connectivity methods and provides an additional layer of information regarding the properties of the interaction between the components constituting the various functions of the brain (Sporns, 2004). Graph theory analysis broadens our understanding of how the different elements of the brain network are organized to support different behaviors and cognitive processes (van den Heuvel and Hulshoff Pol, 2010). Graph theory analysis has been increasingly used in translational neuroscience to study how the brain’s functional organization is altered under different neuropathologies (De Vico Fallani et al., 2014), such as autism disorder (Keown et al., 2017; Sadeghi et al., 2017), Alzheimer disease (Khazaee et al., 2015), concussion and traumatic brain injuries (Virji-Babul et al., 2014), and depression (Gong and He, 2015).

More recently, graph theory analysis has been used to study the functional brain architecture in the developing brain (Fransson et al., 2010; Gao et al., 2015b; Vogel et al., 2010). Fransson et al. (2010) were the first to investigate the functional architecture of the infant brain using rs-fMRI. They reported that the observed functional RSNs demonstrate small-world topology and that the cortical hubs in the newborn brain are predominantly confined to the primary sensory and motor regions of the brain. The authors suggest that the functional networks are already present, even if in rudimentary form, at birth and are organized primarily to support sensorimotor development. In a previous study from our lab, graph theory analysis has been used to investigate changes in network organization during infancy using EEG recordings. It shows
that the influence of motor experience on functional brain organization is characterized by a shift toward a more efficient organization (Rotem-Kohavi et al., 2014). Moreover, two recent studies have examined the development of hubs in the fetal period (van den Heuvel et al., 2018), and during the first year of life (Wen et al., 2018). It was reported that key hub regions are emerging even before birth and, consistent with earlier reports on infants (Fransson et al., 2010), they are located in primary sensory and motor regions and in the cerebellum (van den Heuvel et al., 2018). Other hub regions were identified in the inferior temporal lobe, which develop into the fusiform facial area, and also in the left angular gyrus, which develop into Wernicke’s area, a region important for language development, thereby emphasizing the importance of regions related to language and facial perception in the developing brain (van den Heuvel et al., 2018). Moreover, it was shown that during the first year of life, modular organization of the brain stabilizes and gradually generates additional modules, with the enhancement of both functional segregation and integration (Wei Gao et al., 2011; Wen et al., 2018); however, others have shown a different pattern of increased segregation and decreased integration (Gao et al., 2015b; Thomason et al., 2014). During the first year of life, connector hubs—which connect different modules—increase, while provincial hubs—which connect regions within the module—decrease, possibly leading to a more efficient organization (Wen et al., 2018). This emphasizes that early brain development is very dynamic and possibly also very fragile and sensitive to early adverse exposures. Further, it is suggested that hub regions play a special role in facilitating information transfer; however, this comes with a price as they may also be more vulnerable to the adversities (van den Heuvel et al., 2018).
To summarize, graph theory is increasingly being used to study the brain organization across the lifespan. It provides a unique opportunity to change the perspective from focusing on a single brain area to a more complete view of the brain (De Vico Fallani et al., 2014). This approach enables the examination of how brain topology supports function and cognition, involving intricate communication between local and distant brain regions. Graph theory analysis was used in this thesis to examine the brain functional organization in typically developing infants and in infants exposed to prenatal depression and SSRIs both during an emotional task and during natural sleep.

2.1.1.1 Graph construction

In order to construct the graph, first the nodes must be defined and then the association between the nodes must be evaluated (Bullmore & Sporns, 2009). To generate an association matrix, all possible associations between the nodes must be identified to produce an adjacency matrix, which indicates the number of edges between each pair of nodes (Bullmore & Sporns, 2009). After constructing the graph, different measures can be used to characterize different properties of the graph both at the global level and at the nodal or local level (Mears and Pollard, 2016); see Figure 2-1).
Figure 2-1 Graph construction

To create the graph, first the nodes need to be defined, typically anatomical regions or in the case of EEG electrode positions (1), next the connection probabilities between any two nodes need to be estimated, which in the case of fMRI and EEG is the functional connectivity, or structural connections of white matter tracts for structural brain networks (2), then association matrix needs to be produced which compiles all association between each two nodes to produce association matrix (by either applying a threshold or not) (3). Last, graph theory analysis can be applied on the matrix (4). Reprinted by permission from Springer Nature, Nature Reviews Neuroscience, Complex brain networks: graph theoretical analysis of structural and functional systems, Ed Bullmore, Olaf Sporns, Copyright Clearance Center, 2010.
Chapter 3: Infants and adults have similar regional functional brain organization for the perception of emotions

3.1 Introduction

Recognizing and understanding the actions and emotions of others is an essential prerequisite for learning, social cognition and social interactions. Adults make critical inferences about the emotional state of others by observing facial expressions and use emotional cues to respond under different social contexts (Frith, 2009). For non-verbal infants, perception of emotional faces is of great importance as it may provide valuable information for interpreting the immediate environment, and offer clues on how they should act on different objects and in different social contexts (Sorce et al., 1985). How these abilities develop from early infancy is still unclear. It is well established that infants are attracted to and selectively respond to faces early in life (Farroni et al., 2013). Young infants are sensitive to emotional information and are able to discriminate between facial expressions of different emotions including happiness, anger, fear, sadness, and surprise (Caron et al., 1988; Nelson, 1987; Serrano et al., 1992). A fundamental question in developmental cognitive neuroscience is how the neural networks underlying the perception of emotional facial expressions emerge and how the characteristics of

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these networks develop over time. From a behavioral perspective, there are clear developmental changes in response to the processing of emotional facial expression during the first year of life (Grossmann et al., 2007). At three months of age, infants are able to discriminate between happy and angry faces (Barrera and Maurer, 1981). By 7 months of age, infants are able to discriminate fearful faces in comparison with happy/neutral faces (Leppänen et al., 2007). By the end of the first year of life, infants are able to use the facial expressions of others for guiding them in ambiguous situations (Sorce et al., 1985).

Neuroimaging studies in adults suggest that emotional facial processing does not occur only in a specific brain region, but involves different brain regions (including the amygdala, temporal gyrus, frontal, parietal and occipital cortices (Paolo Fusar-Poli et al., 2009) that are associated with different aspects of facial and emotional processing (Haxby et al., 2000). These brain circuits use processing strategies by which faces are viewed more holistically (rather than focusing on the features of the face) in comparison to objects (Simion and Giorgio, 2015). EEG studies are commonly used to study developmental changes of facial processing in infancy. Studies of the event related potentials (ERP) to emotional faces have shown that between 3 and 12 months of age there is an increased specificity of the evoked components (N290 and P400 similar to the adults N170 – the face sensitive component) for upright human faces in comparison to non-human faces. This suggests that infant’s cortical response to human faces exhibit adult-like specificity toward the end of the first year of life (Halit et al., 2003). For example, 7-month-old infants show a larger amplitude of the negative central (Nc) ERP component (an indicator for allocation of attention towards salient stimulations) over the fronto-central scalp regions in response to fearful expressions compared with happy expressions, similar
to adults (Nelson and Dolgin, 1985). Seven month old infants also show a distinct evoked response to different categories of emotions (happy and sad) such that the Nc component is similar when infant observed faces from the same category (happy expressions) while they exhibit a differentiated response when they observed faces from different categories (different happy and sad emotions (Leppänen et al., 2009)). Together, the results from ERP studies suggest that infants in the second half of their first year of life have the basic ERP components for processing human emotional faces. However, processing emotional faces involves several brain regions that are connected with each other and it is becoming increasingly clear that a network perspective is needed to better understand how the brain processes emotional faces (Varela et al., 2001).

One approach that uses a network perspective analysis is graph theory, which has emerged in recent years as a promising tool for understanding both anatomical and functional brain networks (Bullmore and Sporns, 2009; Rubinov and Sporns, 2010). Brain networks consist of spatially distributed brain regions that are functionally connected. The functional interactions between local and distant brain regions can be evaluated using functional connectivity which refers to the statistical interdependencies between time series recorded from different brain regions (Friston and Price, 2001). In this approach, the brain is characterized as a network that contains nodes and edges. Nodes represent different brain regions and edges represent the connecting pathways between those regions (Sporns et al., 2004). When describing functional connectivity, the edges represent functional connections between different brain regions (nodes) rather than structural (anatomical) connections. The relationship between nodes and edges provides information about the functional organization and efficiency of the network (Sporns et
al., 2004). Information processing is proposed to be optimal in “small-world” networks that balance local specialization and global integration (Watts and Strogatz, 1998).

Fransson and colleagues (Fransson et al., 2010) were the first to investigate the functional architecture in sleeping infants and showed that a small-world topology exists at birth with cortical hubs primarily in the sensory and motor regions of the brain. More recently, De Asis-Cruz reported that the neonatal brain has highly-connected hubs (De Asis-Cruz et al., 2015) suggesting that infants exhibit hub organization that is more similar to the adult configuration than previously suggested. While there are a number of researchers that have used a network perspective to understand network organization in sleeping infants, to our knowledge, there has been only one study that has used this approach to study task based responses in infants (Rotem-Kohavi et al., 2014). In particular, there have been no studies using this network perspective to understand facial emotion processing in infants. Since infants between 8-to-10-months of age typically show selective responses to happy and sad expressions (Leppänen et al., 2009; Parker and Nelson, 2005), we used these basic expressions to compare the network organization underlying emotion processing in infants and adults. We ask: (1) Do infants and adults share common network properties for processing emotional facial expressions at the global level and regional levels? And 2) does the network organization differ between positive and negative emotions in both infants and adults? Based on recent results showing similarities in network organization between adults and infants in the resting state and in specific tasks related to action perception, we hypothesized that the global and regional network characteristics underlying the perception of emotional facial expressions would be similar in infants and adults. In addition, based on the literature showing emotion specific responses in both infants and adults (Leppänen
et al., 2009; Nelson and Dolgin, 1985), we hypothesize that each emotional expression would be characterized by a distinct network organization.

3.2 Methods

3.2.1 Participants

The Human Ethics Review Board at the University of British Columbia approved all experiments. All adults and parents provided written consent according to the guidelines of the Human Ethics Review Board at the University of British Columbia.

A total of 43 infants participated in this study. Data from 19 infants was excluded from the analysis due to excessive motion or insufficient artifact-free trials for each emotional expression. These exclusion rates are similar to exclusion rates reported previously for similar studies with infants in this age range (de Haan et al., 2004; Leppänen et al., 2007). A total of 24 infants between the ages of 8 and 10 months (mean age: 8.95 months, SD = 0.87, 13 males, 11 females) were included in the final analysis. Parents completed a developmental and social-emotional questionnaire (Ages & Stages) to confirm each infant’s developmental stage (Squires et al., 1995).

Twenty adults between the ages of 21 and 37 years (mean age: 28.4 years, SD = 6.02, ten females, ten males) were also included in the analysis. All adult subjects reported normal or corrected-to-normal vision.

3.2.2 Stimuli

Given the importance of keeping infants engaged during the task, we used short video
clips depicting dynamic facial expressions as opposed to static photographs. Previous work in adults has shown that dynamic displays are associated with more accurate performance in recognizing emotions in comparison with static photographs (Harwood et al., 1999). Dynamic facial expressions are more realistic and attractive and infants are more attentive to such stimuli in comparison with static photographs (Missana et al., 2014).

We used stimuli developed and validated by Simon et.al (2008). These stimuli have recently been used to study infant responses (Missana et al., 2014). The stimuli consisted of color video clips of two different facial expressions (happy and sad) displayed by two different models (Simon et al., 2008). Each emotional expression began with a neutral face and progressed to a full expression depicting happiness, sadness. The time from the neutral expression to the full expression was completed in one second. A total of 60 videos (30 happy and 30 sad) were included in each experiment (examples of dynamic emotional stimuli are presented in Figure 3-1), and were shown in a randomized order using Presentation software (Neurobehavioral Systems, Presentation 17.0 01.14.14.Ink).

![Figure 3-1 Examples of the emotional dynamic expression stimuli](image)
This figure shows representative examples of the stimuli. Single video frames of facial expressions of happy (top row) sad (bottom row) for one actress are shown (with permission from Simon and colleagues (Simon et al., 2008)).

A grey screen followed each video clip depicting each emotion. The total viewing time was approximately two minutes. All adults and infants were shown the same number of videos. The minimum criterion for inclusion of an infant’s data was 5 artifact free trials per condition, and for adults the minimum criterion was set to 10 artifact free trials per condition. Infants observed an average of 11 trials for the happy face, and 10 trials for the sad face. Adults observed an average of 26 trials for the two emotions. All participants viewed one of two possible female models performing both facial expressions. The presentation of the models was counterbalanced.

### 3.2.3 Experimental setup and procedures

All participants sat comfortably in a dimly lit, sound-attenuated room in front of a computer monitor. All adult participants were seated on a chair facing a 24 cm (diagonal length) monitor at a viewing distance of approximately 80 cm. Infants were seated on their parent’s lap who was seated on a chair at a distance of approximately 80 cm from the monitor. The stimuli were presented in the middle of the monitor over a black background. The circumference of the faces presented on the screen was 46 cm. An experimenter sat beside each parent/infant to monitor the infant’s attention to the screen. A gentle tap on the screen was used to draw the infant's attention to the screen if needed. A video camera was positioned above the monitor screen to record each participant’s eye and limb motion. All videos were examined prior to
analysis. Trials where participants were not looking at the monitor, made excessive limb
movement or trials in which infant became fussy and distracted were removed from the analysis.

### 3.2.4 EEG recordings

EEG was recorded using a 64-channel HydroCel Geodesic SensorNet (EGI, Eugene, OR). An infant sized 64-channel HydroCel Geodesic SensorNet was used for infants. EEG was recorded and amplified using Net Amps 300 amplifier, at a sampling rate of 250 Hz. Scalp electrode impedances were generally under 50 kΩ. The signal was referenced to the vertex (Cz) and filtered from 4 to 40Hz. A notch filtered at 60 Hz was applied. The EEG signals were analyzed offline using Brain Electrical Source Analysis (BESA) (MEGIS Software GmbH). An automated artifact scan available by BESA was performed for extracting motion and excessive eye movement artifacts. Data were epoched from 0 to 1000ms at the beginning of each video clip. BESA brain source montage was used to convert the EEG activity obtained from all the 64 scalp channels into predicted contributions of a set of 15 different brain source spaces.

### 3.2.5 Graph Theoretical Analysis

We used the “Brain Connectivity Toolbox” (Rubinov and Sporns, 2010) running Matlab (Natick, MA) to carry out the graph theoretical based analysis. We used traditional global graph theoretical calculations to characterize different features of the network of interest such as density and modularity. Density (cost) is determined by the ratio of the present connections to all possible connections which can imply on the efficiency of the network (Achard and Bullmore, 2007). Modularity of the network is used to measure the degree to which the network tends to divide into modules or sub-unit within the network and implies on the stability of sub-networks.
within the global network (Bullmore and Sporns, 2009). Clustering coefficient is used to measure the overall clustering levels in the network (Bullmore and Sporns, 2009).

Recently, there has been increasing interest regarding the relative importance of regional nodes in task based conditions (Widen, 2013). In the present study we focused on one such feature betweenness centrality, which calculates the ratio of the number of shortest paths from all of the nodes to all others that pass through that node, and indicates on the significance of a specific node in the network (Sporns, 2004). Higher levels of betweenness centrality for a specific node indicate that this node has higher impact on the information flow and can serve as a “hub” within the network (Beauchamp, 1965; Wilke et al., 2011).

### 3.2.6 Construction of Functional Connectivity matrix

In this study, we constructed the brain functional connectivity networks using the preselected EEG signals and an error-rate controlled network learning algorithm. Based on the learned connectivity networks, the graph measures were further calculated to extract the functional network features. Surface EEG signals were transformed into 15 brain source-locations using BESA’s Source brain montage. EEG time series from these 15 source locations were used to construct the brain connectivity networks with each channel representing one brain region in the network. The connectivity network graphs were then computed for each individual subject and for each emotional expression using false discovery rate controlled PC (PCFDR) algorithm, which is a statistical model that tests the conditional dependence/independence between any two regions based on all other brain regions (Li and Wang, 2009). We used partial correlation to evaluate the conditional independence, which estimates the directed interactions
between any two brain regions after removing the effects of all other brain areas. The PC algorithm starts from a complete graph and tests for conditional independence in an efficient way. The PCFDR algorithm enables to asymptotically control the false discovery rate (FDR) below the predefined levels which evaluates the proportion between the connections that are falsely detected to all those detected. Compared to the traditional Type-1 and Type-2 error rates, FDR has more conservative error rate criteria for modeling brain connectivity due to its direct relation to the uncertainty of the networks of interest. The PCFDR algorithm and pseudo-code are described in details in (Li and Wang, 2009). The FDR threshold was set at the 5% level. The learned connectivity networks are binary undirected graph with the inferred functional connections at the 5% significance level. Figure 3-2 represents the average functional connectivity for each group. Dots represent the brain sources (nodes), while the size of the dot indicates the value of betweenness centrality for that source, and the thickness of the line connecting two separate dots indicates how common this connection is at the group level.
Figure 3-2 Group average functional connectivity graphs.

Group average functional connectivity graphs of the infant group (top), and the adult group (bottom), in an axial view, for the observation of happy and sad facial expressions. EEG signals recorded from 64 channels were converted into 15 brain sources (nodes- represented as dots). The size of the dot represents the value of betweenness centrality for that node. A threshold was applied on the connections between different nodes were such that only connections which were observed in more than half of the subjects in that group (i.e more than 10 for adults, or 12 infant groups respectively), and connection that were more common at the group level appears thicker in the figure. Note that qualitatively, size of frontal and parietal nodes appears similar between groups, while the infant’s graph appears to have denser connections than the adult’s graph. (FL=frontal left, PL=parietal left, TAL= temporal anterior left, TPL= temporal posterior left, OpM=occipito polar cortex medial).
3.2.7 Statistical analysis

As a first step we performed a mixed ANOVA to analyze the overall differences in the global measures of modularity, clustering coefficient and density. We used emotion (happy, sad) as within subjects’ factor and group as between subject factor (Adults, infants). Next, we performed a mixed ANOVA to analyze the regional differences in betweenness values between the groups. For the nodal measures we focused the analysis on frontal, parietal, occipital and temporal (anterior and posterior) brain sources. We used a region (frontal, parietal, occipital, temporal anterior and temporal posterior) * expression (happy vs sad) and between subject factor was group (adults, infants) (i.e. 5*2*2). This was followed by a separate mixed ANOVA for each region and included hemisphere in addition to emotion as within subject factor (2*2*2). All pair-wise t-tests were corrected for multiple comparisons using a Bonferroni correction.

3.3 Results

3.3.1 Infant Development

Maternal reports based on The Ages and Stages (Squires et al., 1995) indicated that all infants were developing within the typical range for motor and social development. In addition, none of the mothers reported concerns regarding their child’s development.

3.3.2 Global measures

A group average graph for the functional network for adults and infants for observation of happy and sad faces is shown in Figure 3-2. Qualitatively, it is apparent that in comparison to
the adult graph, the infant’s graph is characterized by denser connections. Graph theoretical analysis using a mixed ANOVA showed a statistically significant main effect of group with higher density for infants in comparison with adults ($F (1, 42) =6.206, P=0.017, \eta_p^2=0.129$). There were no significant differences for the global network properties of clustering coefficient and modularity between infants and adults or within each of the groups ($p>0.2$). There was no effect of emotion or an emotion*group interaction. Group differences for global measures are shown in Figure 3-3.

![Figure 3-3 Group differences in global measures](image)

Bar graphs showing group differences in global measures for density, modularity and clustering coefficient measures between infants and adults for the observation of emotional expressions (happy and sad are combined together). Error bars represent 1 standard error. (*, $P <0.05$).

### 3.3.3 Regional measures

A mixed ANOVA (region *emotion*group) revealed no main effect of group or emotion ($p>0.4$), but a significant effect of region ($F (4, 168) =9.1777, p<0.001, \eta_p^2=0.179$). In both infants and adults, betweenness levels were significantly higher for parietal, occipital and temporal posterior nodes in comparison with frontal and temporal anterior nodes (frontal $M=.082\pm.006$, parietal $M=.118\pm.005$, occipital $M=.11\pm.011$, temporal anterior $M=.074\pm.006$)
and temporal posterior M=1.24±.007) (See Figure 3-4). In particular, parietal and posterior temporal nodes had highest values. Additional analysis was performed on each region separately.
Figure 3-4 Group differences in betweenness centrality

Bar graph showing differences in betweenness centrality between groups for left and right hemispheres for happy and sad expressions: upper: Frontal nodes middle: Parietal nodes bottom: Temporal posterior nodes. For temporal posterior regions, a significant interaction exist between hemisphere and group p=0.011, stemming from a significant hemispheric effect for the adults group p=0.008, but not for the infant group p>0.1. Error bars represent 1 standard error.
For frontal brain nodes, there was no main effect of group for betweenness (p>0.5). There was a significant interaction of hemisphere and emotion ($F(1,42) = 7.189$, $P = 0.01$, $\eta^2_p = 0.146$). Post hoc testing for each hemisphere separately showed an emotion effect in the left hemisphere only ($F(1,42) = 5.391$, $p = 0.025$, $\eta^2_p = 0.114$) (see Figure 3-4).

For the parietal brain nodes there were no significant group differences ($p>0.6$). We did find an effect of emotion ($F(1,42) = 4.476$, $p = 0.04$, $\eta^2_p = 0.096$), with higher betweenness values for the sad emotion (see Figure 3-4).

For the temporal posterior brain nodes there was no significant effect of group. There was a significant interaction between hemisphere and group ($F(1,42) = 7.031$, $p = 0.011$, $\eta^2_p = 0.143$) resulting from a significant difference between groups in the left hemisphere ($F(1,42) = 5.06$, $p = 0.03$, $\eta^2_p = 0.108$). The adults showed hemispheric differences $F(1,19) = 8.739$, $p = 0.008$, $\eta^2_p = 0.315$) with higher betweenness values for both emotions in the left hemisphere. In contrast, there were no hemispheric differences in the infants $p>0.1$ (Figure 3-4).

To evaluate whether the number of trials substantially influenced the observed findings, both the infant and the adult groups were divided into two groups according to the median value of the number of trials for each of the emotions (i.e. “poor” for number of trials that were lower than median and “well preforming” for number of trials that were median and above). The average number of valid trials was $7.85 \pm 0.38$ versus $12.17 \pm 4.4$ for happy facial expressions, and $7.3 \pm 1.06$ versus $11.9 \pm 3.3$ for sad facial expressions and for the adult group $24.7 \pm 2.5$ versus $28.8 \pm 1.02$ for happy facial expressions, and $24.12 \pm 2.74$ versus $28.5 \pm 2.8$ for sad facial expressions. Independent samples t-tests between the well performing and the poorly performing
groups showed no significant differences (p>0.05).

3.4 Discussion

3.4.1 Global measures

In this study, we applied graph theory analysis to EEG recordings to compare the functional connectivity underlying the perception of positive and negative dynamic facial expressions in infants and in adults. In contrast to most studies examining infants’ response to emotional faces (de Haan et al., 2004; Grossmann et al., 2007; Nelson and de Haan, 1996) we used dynamic emotional faces as opposed to static faces which are more ecologically valid. Our results show at the global level, the infant network is characterized by a higher connectivity in comparison with the adult network. Global measures reflect the characteristics of the whole network and provides information about long and short-distance functional interactions (Achard and Bullmore, 2007). A more efficient network will favor high density of short connections which are balanced with a few long connection that can mediate efficient information flow (Achard and Bullmore, 2007). High global density or global cost is associated with disproportionate short and long connections resulting in less efficient networks. Our findings provide a glimpse of one stage of the dynamic and evolving changes in brain function in the infant brain in relation to emotional face processing.

We have previously used the same approach of combing EEG based functional connectivity with graph theory analysis to evaluate the impact of motor experience on functional network organization in the context of perceptual-motor development (Rotem-Kohavi et al., 2014). In comparison with adults, we found that global density was higher in combination with
lower modularity in infants only in response to observation of an action that was not within their motor repertoire (i.e., independent walking). When infants observed object motion or actions that were within their motor repertoire, we found no differences in these global measures suggesting that motor experience may be the impetus for a shift towards a more efficient network organization.

While the relationship between structural and functional brain networks are yet to be completely elucidated, there is evidence that structural networks constrain functional networks (Honey et al., 2007). The decreased density of functional connections in the adult brain may reflect changes that occur at a structural neural level during which inefficient or excess connections are pruned (Boersma et al., 2011) to maximize integration in local brain regions to conserve energy (Bullmore and Sporns, 2009).

This has been demonstrated by Gao and colleges (Gao et al., 2009) who examined developmental changes in functional connectivity in the default mode network (DMN) over the first two years of life. They found that the infant DMN includes additional brain regions in comparison with the adult DMN. This implies that throughout development, functional networks are sculpted by removing redundant connections, possibly improving efficiency and reducing the energy cost (Gao et al., 2009). Our findings provide additional support for the differences in the specialization of the functional organization between infants and adults for emotional facial observation.
3.4.2 Regional measures

While global measures provide information about the network as a whole, nodal measures provide information on the functional characteristics of specific nodes in the network. In our analysis we focused on the measure of betweenness centrality which measures the influence of the node through the number of short connections going through it and reflects the importance of the node as integrator of information flow in the network (Sporns et al., 2004). Comparing the betweenness values of the different nodes first highlights that the processing of emotional faces occurs in multiple brain regions. Second, it appears that the hierarchy for nodal importance in integrating information was similar between infants and adults such that betweenness values for posterior temporal and parietal were significantly higher than in frontal and anterior temporal regions. Most interestingly, parietal nodes which are usually not considered in the core emotional facial processing network seem to have a high impact on information flow. This might be attributed to the dynamic aspect of the stimulations used in this study. In support of this interpretation, Goldberg and colleagues showed that brain areas related to the dorsal visual stream show the highest activation in a task involving observation of emotional dynamic video clips of short movies (Goldberg et al., 2014).

An additional significant finding in our current study was the similarity in betweenness centrality values between infants and adults for frontal and parietal brain sources. Our results suggest that the foundations for the functional topography of higher order information integration crossing through those brain sources may already be in place at this stage. These similarities in the functional networks underlying viewing emotional faces are expected considering that infants
are oriented to the human face almost from birth (de Haan et al., 2002; Farroni et al., 2013; Nelson and de Haan, 1996; Nelson and Dolgin, 1985). This observation is consistent with results from prior ERP studies showing that 7-months-old infants have a similar pattern of response to adults when observing facial expressions of happiness and fear (Leppänen et al., 2007; Nelson and Dolgin, 1985), and that 12-month-old infants show an “adult-like” cortical pattern of response to upright faces versus inverted faces (Halit et al., 2003). Our results go beyond the evoked response of specific brain regions by providing additional insights on the cortical functional organization for viewing emotional faces, showing similarities between infants and adults in the organization of parietal and frontal regions in the overall information flow.

Furthermore, Deen and colleagues examined the functional organization of 4-6-month-old infants while observing faces, and found that the spatial organization for viewing faces is very similar to that observed in adults and includes cortical regions such as temporal, parietal and frontal regions in addition to occipital regions (Deen et al., 2017).

Interestingly, our results suggest a distinct functional organization at the regional level for happy versus sad emotions. Specifically, we found that both infants and adults showed higher betweenness centrality values for sad versus happy emotions. This difference in the functional organization has also been reported recently by Zhang et al. (Zhang et al., 2015). Using graph theoretical analysis they reported that betweenness centrality values in adults differ in response to positive versus negative information and that this organization is very dynamic and changes in relation to task demands (Zhang et al., 2015). However Zhang and his group did not specifically use emotional faces in the study and thus the question of how emotional valence is represented in the brain requires further investigation. It may be possible that the higher betweenness values
and higher functional connectivity that we observed in parietal regions may be related to increased attention to sad stimuli (Corbetta and Shulman, 2002). We do not have enough data from this study to interpret these results in relation to valence. Further studies with a range of emotions would provide more information about the functional role of the parietal areas in processing of emotional faces.

Another interesting finding in our results is the lateralization in the organization of processing emotional faces. For parietal regions higher betweenness values for right versus left exist for both adults and infants independent of the emotional valance. In contrast, in temporal posterior nodes, only adults showed lateralization in the functional organization, such that higher betweenness values were observed for left compared to right side for both sad and happy emotions, suggesting larger lateralization in the response for adults’ versus infants. This observation suggests that subtle differences do exist in the functional architecture of brain networks for viewing emotional faces that are mostly related to hemispheric differences that are not fully established in infants. The question of lateralization in processing emotions and regarding the role of each hemisphere in this process is still debated in the literature (P. Fusar-Poli et al., 2009; Meng et al., 2012). While some studies support the hypothesis of right hemispheric dominance in processing emotions (Joseph, 1988) and increased right hemispheric processing for negative emotions (i.e fear) (de Haan et al., 2004), others assert that emotional facial processing is mostly bilateral (P. Fusar-Poli et al., 2009). Our results do support hemispheric differences, which were not associated with a specific emotional valance. Moreover, our results show that lateralization increases from infancy to adulthood. Additional studies are needed to further elucidate lateralization effect in emotional processing throughout development.
Overall our results show that the foundations for the global characteristics functional connectivity for emotional processing are in place in infancy, however the infant network has an excess of connections suggesting that additional pruning occurs later in development. In addition, it appears that processing of emotional faces involves multiple brain regions, and the topography of nodes involved in emotional face processing as characterized by the betweenness values of core source cortical regions throughout the brain is already established at this stage. Specifically, the key nodes for both infants and adults seem to be in parietal and temporal posterior regions. Fine-tuning of the network connections, particularly in temporal posterior regions most likely occurs in response to maturation and experience.

From a developmental perspective it has been suggested that initially infants categorize faces according to the tone (positive vs negative) and gradually learn to distinguish discrete facial expressions (i.e. fear vs. sadness vs. anger) (Widen, 2013). A reasonable assumption is that the core functional network organization for processing of basic emotion is already in place in young infants at the age of 8-to-10-months; processing more ambiguous emotions and higher order processing of different levels of emotional contents and in more complex social situations may be developing over time.

To our knowledge it is the first study that used graph theory analysis measures to characterize the functional networks involved in emotional facial processing in infancy. We have provided new evidence for the topographical characteristics of the functional networks for observation of negative and positive facial expression in both infants and adults. Our preliminary data show one stage in the developmental organization of the functional network organization underlying emotional face processing in infancy.
The novel approach presented here provides opportunities for future studies to examine the neural correlates of emotion perception development in infants who experience a range of parental and environmental experiences. For example, it has been suggested that infants of mothers who are clinically depressed may show alterations in the perception of facial emotions (Hernandez-Reif et al., 2006). Using a combination of dynamic emotional stimuli with graph theory analysis may provide important insights into how maternal mood disturbances may impact the functional brain development of these infants.

3.4.3 Limitations

One limitation of our study is that our analysis depicts an average response of one second. The response to faces is very dynamic and starts as early as 130 to 170 ms post stimulus. Future studies should analyze shorter time windows to depict the dynamic changes occur following emotional facial observation.

Another limitation of this study relates to the properties of EEG recordings. EEG is able to record brain response only in the cortex, while the activation of deeper brain structure such as the amygdala which plays a role in emotional processing cannot be detected. The sub-cortical regional organization might exhibit different patterns of functional organization than what we have observed for the cortical nodes. Future studies that include other imaging techniques such as fMRI in combination with sophisticated analysis tools are clearly warranted.

Finally, our study had a smaller number of trials for the infants in comparison with the adults due to the nature of conducting EEG studies in very young infants. Given the nature of studying infants who tend to be fussy and cannot pay attention to stimuli for long periods, future
studies should find methods to increase the number of trials or to develop better signal processing methods to analyze information from single trials.
Chapter 4: Advanced neuroimaging: A window into the neural correlates of fetal programming related to prenatal exposure to maternal mood disturbances and SSRIs²

4.1 Introduction

The notion of fetal programming has been widely applied to understanding associations between prenatal stress in the context of maternal mood disturbances and adverse infant and child development (Barker, 2000; Davis et al., 2007; Dipietro et al., 2010; Gluckman et al., 2007; Lundy et al., 1996; Rai et al., 2013; Zijlmans et al., 2017). While prenatal depression has been widely linked to biological and behavioral risks (Stein et al., 2014) for decreased birth weight, increased rates of preterm birth (Orr and Miller, 1995), elevated levels of cortisol and norepinephrine (Diego et al., 2004), and emotional (Hayes et al., 2013; Zhu et al., 2014), social (Field, 2011), and cognitive disturbances (Barker et al., 2013; Stein et al., 2014; Zhu et al., 2014), not all outcomes reflect adverse developmental programming (Dipietro et al., 2006; Weikum et al., 2013b). We often wonder why some but not all children appear to be differentially susceptible to early adversity. To further understand variations in developmental

² Naama Rotem-Kohavi, Lynne J. Williams, Tim F. Oberlander (2019, accepted for publication). Advanced neuroimaging: A window into the neural correlates of fetal programming related to prenatal exposure to maternal mood disturbances and SSRIs, seminars in perinatology.
phenotypes associated with prenatal stress, Pluess and Belsky (2011) proposed that prenatal stress “programs” postnatal developmental plasticity (Pluess and Belsky, 2011), in a “for better and for worse” manner. According to this model, individuals exposed to prenatal stress might be differentially susceptible to early adversity of either negative but also positive postnatal environment, leading to either negative or positive outcomes. While substantial observational and experimental evidence supports individual differences in sensitivity to life circumstances, the neural correlates of developmental plasticity remain to be established. Recent advances in neuroimaging using MR and EEG offer novel insights into central processes that help uncover the neural correlates of fetal programming related to prenatal stress. This review focuses on findings emerging from neuroimaging studies reflecting early brain functional and structural development associated with prenatal exposure to maternal mood and SSRI antidepressants.

Using a fetal programing perspective this review will examine early brain structural, microstructural and functional alterations in the context of prenatal maternal exposure to mood disturbances and SSRIs (listed in Table 4-1). We will show that empiric evidence based on neuroimaging studies is supporting specific neural imaging correlates that link prenatal maternal mood disturbances and antidepressant exposure with both positive and negative developmental outcomes reflecting variations in a susceptibility to pre and postnatal environment. Suggestions for future research directions are offered whereby neuroimaging could be used as a ‘window’ into advancing our understanding of the early origins of postnatal developmental plasticity.
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13. Marroun et al., 2015 654 6 to 10 years old BSI MRI Prenatal maternal depressive symptoms associated with a reduced cortical thickness of superior frontal cortex in the left hemisphere

14. Rifkin Graboi et al, 2015 54 5–17 days old newborns SATI DTI Increased levels of anxiety associated with decreased FA of the insula and PFC, PCC and angular cortex

2. In utero SSRIs

1. Videman et al., 2016 22 SSRI-exposed 62 control newborn infants 22 SSRI-exposed SCID-I EEG EEG Associations between in utero SSRI exposure and reduced hemispheric coupling, reduced frontal activation and reduced subcortical and cortical layers synchronization. A separate analysis which combined SSRI-exposed and controls together showed that subtle differences could also be explained by prenatal maternal mood.

2. Jha et al., 2016 1 month old Medical records DTI and MRI Infants prenatally exposed to SSRIs had decreased FA across multiple fiber bundles compared with
1:27 SSRI-exposed and 54 controls

Cohort 2: 41 depressed-only and 82 controls

No differences were found for global, regional or grey matter volume between groups.

No differences were observed for Depressed-only and matched controls

3. Podrebarac et al., 177 preterm infants, 14 of them SSRI-exposed, scanned at 2 weeks and again at term equivalent

No assessment of depressive symptoms

SSRI exposure associated with increased FA in the superior white matter. An opposite pattern observed for basal ganglia and thalamus.

4. Lugo-Candelas et al., 98 3 and a half weeks old newborns

Center for Epidemiologic Studies

MRI and DTI

Infants exposed to SSRIs in utero had an increase amygdaLa’s and insula’s volume concurrent with increased white matter connections of these regions,
compared with healthy control infants and with infants of non-pharmacologically treated depressed mothers. Higher volume of superior frontal gyrus was also observed in the SSRI exposed versus the depressed only group.

Table 4-1 Summary of neuroimaging studies.

Symptom Checklist (SCL-90; (Arrindell and Ettema, 1981)); mindfulness using the Dutch short version of the Freiburg Mindfulness Inventory (FMIs-14 (Walach et al., 2006)); Brief Symptom Inventory (BSI); Center for Epidemiologic Studies Depression Scale (CES-D); Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) interview; State-Trait Anxiety Inventory (STAI); Edinburgh Postnatal Depression Scale (EPDS) (Cox et al., 1987); prefrontal cortex (PFC); independent component analysis (ICA); beck depression inventory (BDI); Hamilton Depression Rating Scale (HAM-D); pregnancy experiences scale (PES).
4.2 Prenatal maternal mood, SSRI antidepressant drugs and developmental fetal programming

Depression and anxiety during pregnancy affect an estimated 7% and 20% women worldwide (Biaggi et al., 2016) and at least 10% are treated with a Selective Serotonin Reuptake Inhibitor (SSRI) antidepressant (Cohen et al., 2004; Payne and Meltzer-Brody, 2009; Strauss et al., 2014; Vigod et al., 2016a). SSRIs block the reuptake of the serotonin transporter and increase intrasynaptic levels of the developmental neurotransmitter serotonin (5HT) (Gaspar et al., 2003). SSRIs readily cross the placenta and the fetal blood-brain barrier, thereby altering 5HT signaling during early brain development (Rampono et al., 2009). 5HT is a widely distributed neurotransmitter throughout key brain regions involved in cognition, emotion and learning (for review (Brummelte et al., 2017)). 5HT plays a central role in the development and regulation of the hypothalamic-pituitary-adrenal (HPA) stress response system, which, may underlie the psychopathology of depression and anxiety (Brummelte et al., 2017). Importantly, long before it takes on its role regulating mood and cognition, 5HT serves as a trophic factor, regulating various aspects of fundamental developmental processes such as cell growth, differentiation, migration, myelination, synaptogenesis and pruning (Gaspar et al., 2003). Therefore it is not surprising that fetal alterations in 5HT signaling could be associated with early brain development.

In utero exposure to SSRIs has been associated with a wide variety of physiological and behavioral disturbances that include poor neonatal adaption, including respiratory distress,
unstable temperature regulation, irritability, sleep difficulties and jitteriness (Olivier et al., 2013), as well as socio-emotional, mood and behavioral difficulties in childhood and adolescence (Casper et al., 2011; Hanley and Oberlander, 2014; Malm et al., 2016; Moses-kolko et al., 2005). Distinguishing the effects of drug exposure from the effects of maternal mental mood for which the SSRIs were prescribed, and from other genetic and environmental factors remains challenging (i.e., confounding by indication (Brown et al., 2017; Vigod et al., 2016b)) . A case in point is variations in outcomes investigating risk for autism spectrum disorder (ASD) in children with prenatal SSRI exposure (Boukhris et al., 2015; Hviid et al., 2013; Kobayashi et al., 2016). Specifically, Kobayashi et al. (Kobayashi et al., 2016) reported that when comparing an SSRI exposed group and non-pharmacologically treated group of mothers with mood disturbances, maternal SSRI exposure was not associated with increased risks of ASD. In contrast to these findings, others have reported on an increased risk for ASD in prenatally SSRI exposed infants even when controlling for prenatal mood measures (Boukhris et al., 2015), while Hviid and colleagues (Hviid et al., 2013) found no increase in risk of ASD in prenatally SSRI-exposed children. Collectively, these reports suggest inconsistencies and emphasizing the challenges in distinguishing the effects of the drug from the effects of maternal mood disturbances (Oberlander and Zwaigenbaum, 2017).

Many of these findings are consistent with a fetal programming framework (Barker, 2000) – the notion that the intra-uterine environment is impacted by both intrinsic (such as maternal mood) and extrinsic exposures (such as SSRIs), factors that play a pivotal role in shaping the environment of the fetus in preparation for the postnatal world ahead.
The prenatal environment results in fetal developmental changes and in long-term consequences (Barker, 2000; Dipietro et al., 2006; Gluckman and Hanson, 2004). Evidence shows that in utero malnutrition may lead to cardiovascular and metabolic adaptive mechanisms in the fetus, which may be associated with long term risks for the offspring, thereby increasing the risk for cardiovascular disease and Type 2 diabetes in adulthood (Barker, 2000). While evidence supporting fetal programing has been demonstrated in behavioral studies showing correlations between prenatal mood disturbances and an increased risk for physiological, and socio-emotional problems later in childhood (Barker et al., 2002; Conroy et al., 2012; Feldman et al., 2009; Oberlander et al., 2007), evidence for brain developmental effects are emerging (Posner et al., 2016; A. Qiu et al., 2017). Findings of subtle physiological effects, especially those related to brain development, are of great importance, as they can advance our understanding of the origins of some of the behavioral effects observed. In rodents, manipulations to 5HT signaling during early stages of development result in disturbances in electrophysiological patterns, including alterations in raphe circuitry, and in tonotopic organization of the primary auditory cortex in adult rats (Khatri et al., 2014; Simpson et al., 2011). While SSRI-related brain fetal programming may be evident in animal models (Maciag et al., 2006; Simpson et al., 2011), the neural correlates of SSRI related fetal programming in humans is slowly becoming apparent.

4.3 Neuroimaging and early brain development

Advances in neuroimaging techniques have yielded critical insights into structural-functional relationships elucidating the nature of early brain development associated with prenatal maternal exposure to mood disturbances and to in utero SSRIs. Neuroimaging techniques, such as electroencephalography (EEG), magnetic resonance imaging (MRI),
diffusion tensor imaging (DTI), and resting-state functional (rs)fMRI, each target a different aspect of brain development. Structural MRI provides high spatial resolution of grey and white matter used to measure volume and surface of brain regions (Qiu et al., 2013). DTI measures diffusion of water molecules to reveal a microscopic view of white matter or fractional anisotropy (FA) – a measure of the extent to which water diffuses in a given direction, with higher values suggesting increased maturation (Rifkin-Graboi et al., 2013). While MRI and DTI provide static anatomical information, fMRI and EEG provide dynamic physiological information (Symms et al., 2004). EEG measures the electrical activity of synchronized postsynaptic potentials generated by pyramidal cells of cortical brain regions with millisecond temporal resolution (Kirschstein and Köhling, 2009). EEG research mostly focuses on the event related potentials (ERP) and frontal asymmetry, which measures differences in power of right versus left frontal regions. Frontal asymmetry has been extensively studied in this context, as right frontal asymmetry has been associated with behavioral inhibition and avoidance in depressed adults (Wen et al., 2017b). fMRI measures small changes in blood flow which is referred to as the Blood Oxygen Level Dependent (BOLD) signal, that are coupled with brain activity. The detection of changes in BOLD signals are possible based on two different factors: first, the amount of blood flowing to activated neurons is larger than the amount needed for metabolism, and second, oxygenated and deoxygenated hemoglobin have different magnetic properties (Birdwell and Calhoun, 2014). Both of these factors contribute to the phenomena that increase in brain activity will result in a stronger BOLD signal (Birdwell and Calhoun, 2014). Thus, compared to EEG, fMRI uses an indirect measure (hemodynamic response) of brain function, however both EEG and fMRI explore how different brain regions are functionally connected with each other, either at rest or during a task (Lee et al., 2013). These imaging
methods are non-invasive tools, complementing each other to get a closer view on different aspects of early brain development of brain structure, microstructure (white matter) and functional connectivity, even very early in development. Using neuroimaging tools, we can better understand the mechanisms involved in later behavioral alterations and different psychopathologies that could be traced back to the fetal period. Importantly, findings from imaging studies have tremendous potential for guiding the development of objective measures or biomarkers for the origin of psychopathologies even at early stages of development (Posner et al., 2016).

These tools will be beneficial for driving early preventative strategies and interventions to improve short and long-term outcomes of the offspring exposed to depression or SSRIs. Postnatal factors in the context of maternal mood disturbances, especially the character of the environment where a child lives, also shape brain development.

4.4 Maternal mood and neuroimaging

At the core of examining SSRI effects is the impact of maternal mood disturbances which independently have widely reported impacts on early development (Baibazarova et al., 2013; Barker et al., 2013; DiPietro, 2013; Rai et al., 2012). Newborn infants of a depressed mother have lower scores on the Brazelton neonatal behavior assessment scale, show more irritability and stress behaviors (Abrams et al., 1995), and have higher risk for later social and emotional problems such as restless/disruptive temperament, disorganized attachment (Hayes et al., 2013), and externalizing problems (Glover, 2011; Gutteling et al., 2005). Increased risk for internalizing and behavioral inhibition were also suggested (Park et al., 2014), and these are associated with
increased risk for anxiety (Muris et al., 2011). In a study examining fetal heart rate (FHR) as an indicator for fetal well-being, fetuses of depressed mothers showed increased FHR at baseline, with prolonged habituation following a stimulation thought to reflect maternal hormonal disturbances, possibly via changes in the stress hormone cortisol or catecholamines (Allister et al., 2001). While acute or moderate stress might have some beneficial effects on the offspring (Monaghan and Haussmann, 2015), prolonged stress and extremely high cortisol levels cross the placenta and change the activity of neurotransmitters and hormone secretion (Wyrwoll and Holmes, 2012). Evidence from developmental studies show that cortisol controls oligodendrocyte development via regulation of proteins important for myelin and glial cells production (Howell et al., 2013), thereby possibly affecting brain structure and function. This further highlights the importance of neuroimaging to advance our understanding of mechanisms that underlie the developmental effects of prenatal maternal mood disturbances.

Frontal asymmetry measured with EEG has been associated with negative affect and mood disturbances, and with difficulties with emotion regulation, and has also been used as a biomarker for mood disturbances (Field et al., 2006). Left frontal asymmetry scores indicating of propensities for approaching behaviors, or positive emotion positively correlated between prenatally depressed mothers and their neonates. These left frontal asymmetry scores also positively correlated with urine levels of prenatal serotonin levels, and were negatively correlated with prenatal depressive and anxiety symptoms (Field et al., 2004). Associations between neonatal frontal EEG asymmetry and prenatal depression were also reported by Field and colleagues, and Gustafsson and colleagues (Soe et al., 2016) found that prenatal mood symptoms alone, using the Edinburgh Postnatal Depression
Scale (EPDS) questionnaire (Cox et al., 1987), were not sufficient to predict infant frontal asymmetry; rather, worsening in symptoms from the prenatal to the postnatal period predicted frontal asymmetry in 6-month-old infants. These results are consistent with the predictive adaptive hypothesis (Gluckman et al., 2007) which is an extension of the fetal programming hypothesis. The predictive adaptive hypothesis asserts that a mismatch between the conditions in utero and the conditions outside the womb, also have an impact on development. Whether a specific individual is able to adapt to the new condition is critical and may lead to either vulnerability or resiliency (Gluckman et al., 2007). Together, these studies emphasize the importance of both the prenatal environment and how congruency between the pre and postnatal environments shape neurobehavioral trajectories. Interestingly, another EEG study using event related potentials (ERP) to compare prenatal anxiety as a negative prenatal exposure with prenatal mindfulness as a positive prenatal exposure evaluated whether positive exposures could benefit the infant (van den Heuvel et al., 2015). While increased levels of anxiety during pregnancy correlated with higher attention to standard, irrelevant sounds, infants exposed to increased prenatal maternal mindfulness showed reduced attention to these sounds. These results highlight how positive traits during pregnancy may program the offspring’s’ cognitive neurodevelopment in a positive way. Future studies should focus not only on negative physiological states but also on positive ones.

The limbic and para-limbic systems are known key players in mood disorders and several studies have targeted these brain regions in their analysis as a priori selected regions (Qiu et al., 2015; Rifkin-Graboi et al., 2013; Scheinost et al., 2016). Using structural MRI, Qiu and colleagues (Qiu et al., 2013) showed that increased anxiety symptoms during pregnancy,
were associated with right infant hippocampal growth, but not with total hippocampal volume, during the first 6 months controlling for postnatal symptoms. As the hippocampus is critical for stress regulation and has been related to anxiety and stress related disorders in adults, these findings support the possibility of an early neurodevelopmental signature for later anxiety-related pathologies associated with prenatal exposure to mood disturbances. Associations between the volume of the right hippocampus seem to be moderated by genetic variations in in FKBP5 (regulates the HPA axis), such that infants with low genetic risk have increased right hippocampal volume with increased maternal depressive symptoms while infants with high genetic risk show decreased volume with increased maternal symptoms (Wang et al., 2018). Additional support to alterations in hippocampal structure has also been demonstrated in animal models of juvenile Rhesus monkeys exposed to prenatal stress (Coe et al., 2003).

The amygdala, is also a known key player in mood disturbances (Godlewska et al., 2012) that also has been targeted as a region of interest in studies examining associations between prenatal mood disturbances and their influence on the offspring neurodevelopment. Using DTI, higher prenatal mood symptoms (EPDS) were associated with lower white matter microstructure of the right amygdala in neonates (Rifkin-Graboi et al., 2013) and also with higher grey matter volume of the right amygdala of preschool children, but only in girls (Wen et al., 2017a). Using rs-fMRI, Qiu and colleagues (Qiu et al., 2015) showed that 6-month-old infants exhibited increased functional connectivity of the amygdala with the left temporal cortex, insula and anterior cingulate with exposure to higher levels of prenatal depressive symptoms (EPDS) controlling for postnatal depression at 3 months, while Scheinost and colleagues showed that prenatal stress additionally altered the amygdala’s functional connectivity patterns above
alterations already seen in extremely premature neonates (Scheinost et al., 2016). Others have reported disturbances of the functional connectivity patterns of the amygdala, especially with the dorsal prefrontal cortex (Posner et al., 2016).

Mood disturbances in adults have also been associated with aberrant connectivity between the amygdala and prefrontal cortex (Ramasubbu et al., 2014), a pattern which was also apparent in infants exposed to prenatal maternal mood disturbances (El Marroun et al., 2018). In a population-based prospective neuroimaging study (n= 690) in 6 to 10 years old children using DTI, Marroun and colleagues (El Marroun et al., 2018) demonstrated associations between maternal depressive symptoms during pregnancy and reduced FA in the uncinate fasciculus, a white matter fiber tracts connecting between limbic regions such as the hippocampus and amygdala with frontal regions. However, these findings are limited by potential confounding effects such as paternal depressive symptoms, which were also related to lower FA. This suggests possible links to a genetic predisposition and shared familial traits. Earlier, the same group examined morphological structural characteristics in the same data set, and found that prenatal depressive symptoms correlated with thinner superior frontal cortex, while no associations were found between maternal depressive symptoms nor paternal depressive symptoms or any morphological alterations at 3 years of age (El Marroun et al., 2016a).

In a study of early microstructure, increased levels of maternal anxiety and decreased fractional anisotropy (FA) of regions related to stress regulation (e.g., insula and prefrontal cortex (PFC)) were demonstrated. Associations between decreased FA and anxiety symptoms were also observed in the posterior cingulate, parahippocampus, and angular cortex, regions
related to socio-emotional processing as well as in the middle occipital region related to visual perception-related region (Rifkin-Graboi et al., 2015). These results again emphasize the involvement of cortico-limbic and structures associated with emotion regulation in the cross-generational transmission of mood disturbances.

To summarize, increasing interest in recent years provides evidence linking alterations in brain structure, microstructure and functional connectivity with exposure to maternal mood disturbances during pregnancy. While many of the studies targeted the amygdala and hippocampus, others using a whole brain approach found alteration in other regions such as dorsolateral, PFC, insula, and visual cortex, suggesting that brain alterations related to exposure to prenatal maternal mood disturbances are not specific to one brain region. Researchers found similar alterations in structure and function between adults with mood disturbances and infants exposed to maternal mood disturbances, suggesting that the origin of the disease can be traced back to the neonatal/fetal period. However, genetic variants and epigenetic changes may also contribute to infant outcomes, and could not be ruled out in these studies.

4.5 In utero SSRI exposure and neuroimaging

Although SSRIs are prescribed with the expectation that they will alleviate the mother’s mood symptoms and by extension improve her and her infant’s overall well-being, evidence for adverse outcomes in the offspring are accumulating (Oberlander et al., 2009; Pawluski et al., 2012; Zeskind and Stephens, 2004),( for review (Brummelte et al., 2017; Rotem-Kohavi and Oberlander, 2017)). Alterations in fetal and neonatal patterns of rapid eye movement (REM) during sleep (Mulder et al., 2011; Olivier et al., 2013), decreased birth weight and increased
prematurity, alterations in processing painful stimulations, and changes in 5HT metabolite profile (Laine et al., 2003; Oberlander et al., 2002) have been reported.

Some of these findings have long term consequences. The level of disturbances in neonatal adaptation was linked to the level of difficulties in engaging in playful interactions and imitation, and in demonstrating age-appropriate independence in everyday activities (Klinger et al., 2011). However, concurrent mood may also play a role in shaping behavior. Three-year-old children demonstrated increased internalizing behaviors, which were predicted by prenatal SSRI exposure, but also by concurrent maternal mood symptoms (Oberlander et al., 2010). To overcome possible confounding effects of genetic variations, Brandlistuen and colleagues (Brandlistuen et al., 2015) conducted a population-based study in Norway of more than 14,000 siblings and their mothers and reported an association between in utero antidepressant exposure and greater risk for internalizing behaviors and anxiety at 3-years of age. Differences remained significant even after controlling for shared environmental and genetic factors and for pre and postnatal maternal mood symptoms. Overall, these results suggest that while maternal mood disturbances are associated with infant outcomes, in utero SSRI exposure independently shapes the infant’s socio-emotional development. However some outcomes reflect no effect (Hviid et al., 2013) or even a development benefit (Dipietro et al., 2006; Frankenhuis and de Weerth, 2013; Weikum et al., 2013b). An interactions between genetic variations (Weikum et al., 2013a), and everyday household environment long after fetal development has ended also has been demonstrated (Dhaliwal et al., 2017). Together these findings should help move from a perspective whereby prenatal mood disturbances and antidepressant exposure are invariably linked to behavioral and biological developmental dysfunction. In this way prenatal stress (or
antidepressant exposure) may shape a susceptibility to both positive and negative environments, and may account for variations in developmental outcomes, thereby reflecting the hypothesis proposed by Pluess and Belsky (2011) (Pluess and Belsky, 2011) that prenatal exposures program postnatal plasticity (see (Hartman and Belsky, 2018) for review).

4.5.1 Fetal Imaging

Even before birth SSRI exposure is already evident. Rurak and colleagues (Rurak et al., 2011) reported that SSRI exposed fetuses at 36 weeks gestation exhibited reduced fetal heart rate (FHR) variability. Additionally, in utero SSRI exposure was associated with reduced fetal middle cerebral artery (MCA) flow resistance and decreased MCA cross sectional area (Rurak et al., 2011) which suggests changes in fetal brain blood flow. This reduction was demonstrated prior to and following SSRI administration, signifying a persistent effect. Thus, changes in fetal brain blood flow may alter microstructure and brain functional development and offer another possible link to fetal programming of brain development via prenatal SSRI exposure. Importantly, these findings underscore the need for in-depth investigation using neuroimaging tools to further understand possible links between prenatal SSRI exposure and brain structural development.

4.5.2 Neonatal Imaging

To date, evidence supporting associations between alterations in brain structure, microstructure and function in infants prenatally exposed to SSRIs (Jha et al., 2016; Lugo-Candelas et al., 2018; Podrebarac et al., 2017) remains limited. Videman and colleagues (Videman et al., 2016) in an EEG study to assessing local and global characteristics of cortical
function in the neonatal brain showed associations between in utero SSRI exposure and reduced hemispheric coupling. Reduced frontal activation at 5.7 Hz, and reduced subcortical and cortical layer synchronization were present in SSRI exposed infants. In a supplemental analysis, where SSRI exposed infants and control infants were pooled together, it was shown that subtle, yet significant, differences could be explained by maternal mood (Videman et al., 2016). Thus, the authors concluded that the alterations found in connectivity are attributed mostly to the exposure to SSRIs but could not rule out an interaction between maternal mood and SSRI exposure.

Using DTI, Jha and colleagues (Jha et al., 2016) reported that infants prenatally exposed to SSRIs (n= 27) had decreased white matter microstructure (FA) across multiple fiber bundles compared with matched control infants (n=54) at 1 month of age, while no significant differences were found for global, regional or grey matter volume. In a separate cohort, infants of depressed mothers who were not treated with antidepressant drugs have not shown differences in white matter microstructure or with grey matter compared with control infants (Jha et al., 2016). However, these results are limited by the lack of direct comparison between the SSRI exposed group and the depressed only group.

In contrast to Jha and colleagues Podrebarac and colleagues showed that prenatally SSRI exposed infants had increased FA values compared to non SSRI exposed infants (Podrebarac et al., 2017); however, the infants who participated in this cohort were born very preterm, so it could not be determined whether the results were confounded by the degree of prematurity. Additional support for these latter findings was recently reported by a study combining both structural and microstructural imaging of 3.5 weeks old infants. Lugo-Candelas and colleagues showed that infants exposed to SSRIs in utero had an increase in amygdala and insula volume
that correlated with an increase of the white matter connections of these regions, compared with healthy control infants and with infants of non-pharmacologically treated depressed mothers (Lugo-Candelas et al., 2018). Higher gray matter volume of the superior frontal gyrus was also observed in the SSRI exposed versus the depressed only group. Alterations of the amygdala circuitry are also supported by evidence from animal models showing an increased spine density on basolateral amygdala in rats lacking the serotonin transporter (Wellman et al., 2007). The authors hypothesized that these alterations might be related to transient changes of the 5HT transporter during sensitive developmental period in the corticolimbic system that may lead to the observed alterations. Thus, these finding suggest that exposure to SSRI independent from the exposure to prenatal mood disturbances also has an impact on fetal brain development, which especially related to brain regions important to emotional processing.

To summarize, extensive evidence from behavioral and physiological studies have recognized the importance of the pregnant mother’s well-being for the developing offspring, and how mood disturbances can negatively affect both short and long term outcomes (Field et al., 1985; Talge et al., 2007). Antidepressant drugs, especially SSRIs, initially prescribed to improve mothers mood with the hope that the offspring will benefit as well, have proven to carry their own risks to the infant (Rotem-Kohavi and Oberlander, 2017). While different hypotheses have been suggested to explain the underlying behavioral outcomes, our understanding of these mechanisms is still poor. With non-invasive neuroimaging techniques becoming more accessible, research in this field enters a new era that compels re-examination of the proposed mechanisms at a finer scale.
This chapter reviewed emerging findings of fetal programming in the context of maternal depression and in utero SSRIs, using different neuroimaging methods. Overall the existing studies demonstrate that prenatal depression is associated with structural, microstructural and functional alterations related (illustrated in Error! Reference source not found.). Neuroimaging studies examining the effects of in utero SSRIs on infant’s development are emerging, though, at this point, are very limited and show structural and microstructural alterations. Most study designs are still lacking sufficient control groups to eliminate confounding effects of mother’s mood disturbances.

Importantly, the question whether prenatal SSRI exposure program the functional connectivity of the infant has not been answered yet and is directly addressed in this thesis in Chapters 5, 6 and 7.

The following chapter will examine prenatal effects of maternal depression and SSRI on emotion perception development using graph theory analysis of the functional networks during observation of emotional faces.
Chapter 5: **Maternal depression and prenatal exposure to SSRIs differentially associate with the functional connectivity organization underlying emotion perception in 8-to-10-month-old infants**

5.1 Introduction

The social-emotional development of infants emerges within the context of the mother-infant relationship and within this context, maternal disposition is a key factor in shaping development (Feldman et al., 2004). Maternal mood disturbances are strongly associated with alterations in infants’ emotion perception (A. Porto et al., 2016). Infants of depressed mothers take longer to habituate to their mother’s face (Hernandez-Reif et al., 2002), and show suppressed activity in frontal regions during happy interactions in comparison to control infants (Jones et al., 2001), suggesting that maternal depression has a significant impact on infants’ emotion perception.

The influence of maternal mood may occur early during fetal development (Barker, 2000). High levels of prenatal maternal anxiety (correlated with depression) during the first trimester, are associated with larger evoked responses in fronto-central electrodes in response to

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fearful visual and vocal stimuli in 9-months-old infants (Otte et al., 2015). This increased brain activity may be due to increased perceptual-attention to fearful stimuli (Otte et al., 2015), also observed in anxious adults (Leppänen and Nelson, 2012).

Similarly, a perceptual bias towards negative stimuli, is one of the characteristics of depression (Fu et al., 2004). Studies in adults suggest that selective serotonin reuptake inhibitors (SSRIs) antidepressants may “correct” this bias, possibly by changing the serotonin circuitry to produce a perceptual shift toward reduction of negative emotions processing, parallel with positive emotions processing enhancement (Harmer and Cowen, 2013); however SSRIs are also associated with emotional blunting and apathy in adults (Sansone and Sansone, 2010) and children (Reinblatt and Riddle, 2006). As SSRIs are commonly used to treat depression during pregnancy, and readily cross the placenta (Rampono et al., 2009), intriguing questions about whether and how prenatal SSRI exposure shapes emotional processing during infancy arise. Prenatal SSRIs have been associated with socio-emotional, behavioral and cognitive developmental risks in infancy and childhood (Brummelte et al., 2017; Hanley et al., 2015; Rotem-Kohavi and Oberlander, 2017; Talge et al., 2007). Alterations in intrinsic brain functional-connectivity have been recently demonstrated, such that infants prenatally exposed to SSRIs showed reduced hemispheric functional correlation and reduced frontal activity, independent of exposure to prenatal maternal depression (Videman et al., 2016). We have also shown an association between in utero SSRIs and alterations in functional connectivity in resting state networks in newborns (Rotem-Kohavi et al., 2019b). However, the impact on emotional processing during infancy in the context of prenatal SSRI-exposure has not been extensively studied. In addition to serotonin’s (5HT) role as a neurotransmitter regulation mood and social
behavior, in early stages of development 5HT serves as a trophic factor, shaping the neural circuitry (Gaspar et al., 2003). Thus, it is conceivable that SSRIs may affect early neurodevelopment and influence perception of emotional faces via early SSRI-related alterations in central 5HT signaling.

5.1.1 **Taking a network perspective**

To date, studies examining infant emotion perception have typically used the event related potentials (ERP) which provide information on the electrical brain response at different brain regions (de Haan et al., 2004; Leppänen et al., 2007). A growing number of studies are investigating brain functional connectivity from a complex network perspective (Sporns, 2004). According to this view, the brain is modelled as a network while different brain regions are defined as nodes and the functional connections between them are defined as edges (Bullmore and Sporns, 2009). Graph theory analysis (GTA) is used to characterize the organization of the brain’s functional connectivity both at a global and at the local level (Sporns, 2004). Specifically, traditional global graph theoretical calculations characterize different features of the network such as density and modularity. Density is determined by the ratio of the present connections to all possible connections, to assess the functional efficiency or load of the global organization. Modularity of the network is used to measure the stability of sub-networks within the global network (Newman, 2004). It has been suggested that higher loads of cognition and effortful processes are associated with lower levels of network modularity, and with increased long-distance synchronization between brain regions (Kitzbichler et al., 2011). An increasing interest in the field of GTA is in exploring hubs in the brain (van den Heuvel et al., 2018; van den Heuvel and Sporns, 2013). Hub regions are thought to serve a more important role related to
information traffic in the brain, and play a critical role in facilitating effective neuronal communication (Bullmore and Sporns, 2012). Hubs are hypothesized to underlie cognitive processes by taking part in many connections thus playing an important role in integrating information (Bullmore & Sporns, 2009). We have recently showed that while the global organizational features for viewing sad and happy emotions are still developing, the basic functional organization of hubs are in place at 8-to-10-months (Rotem-Kohavi et al., 2017).

In the current study our main focus was to examine the association between prenatal maternal SSRI exposure and the network organization underlying perception of emotional facial expressions in young infants. Similar to our previous study (Rotem-Kohavi et al., 2017), infants viewed short video clips of happy and sad faces. We also included pain as an additional expression due to evidence relating SSRIs to apathy (Reinblatt and Riddle, 2006; Sansone and Sansone, 2010). We expected that SSRI exposure would be associated with global alterations in the organization of functional networks for perceiving emotions in infants, independent of exposure to prenatal or postnatal depression. We had two possible hypotheses: infants exposed to SSRIs will have a more blunted or indifferent response to all emotions, reflected by increased modularity (less cognitive demand), and lower density (Kitzbichler et al., 2011) in network measures, compared to non-SSRI exposed infants. Another possibility is that SSRI would have a “corrective” effect reflected by a reduced modularity and increased density in response to viewing happy emotions. In addition, we anticipated that both pre and postnatal exposure to maternal depressed mood would be associated with alterations in functional organization for perceiving emotional faces. We hypothesized that prenatal depression and stress will be associated with reduced modularity, and increased density (more cognitive demand) especially
for negative emotions (pain and sadness). We also anticipated that postnatal maternal mood would be associated with less efficient processing, reflected by a reduction in frontal hub values.

5.2 Methods

5.2.1 Participants

This study was approved by the Human Ethics Review Board at the University of British Columbia. Parents provided written consent for participating in the study. Mothers were recruited for this study during their second trimester of pregnancy, from Reproductive Mental Health Clinic, midwifery-services, through ads on social media, and family-care clinics in metropolitan Vancouver, Canada. Non-SSRI-treated pregnant women and pregnant women depressed and treated with SSRIs were recruited.

Thirty-five mother-infant dyads participated in this study comprising 12 prenatally SSRI-treated mothers, and 23 non-SSRI-treated mothers. All infants were between 8 -10 months of age at the time of testing.

5.2.2 Maternal mood

We assessed maternal mood during the third trimester (between 26 weeks and 36 weeks of gestation) using the clinician rated Hamilton Rating Scales for Depression (HAM-D) (Hamilton, 1960). A pregnancy specific questionnaire for the appraisal of positive and negative experiences during pregnancy (Pregnancy Experiences Scale (PES)) (DiPietro et al., 2004) and the Beck depression inventory (BDI) (Beck et al., 1961) were also administered at the time of the study visit.
5.2.3 Stimuli

The stimuli protocol were based on methods used in our previous study (Rotem-Kohavi et al., 2017). Dynamic displays (Simon et al., 2008), which are more realistic than static photos, were used to increase attractiveness and attentiveness to the stimulation (Missana et al., 2014).

The stimuli comprised of color video clips of different facial expressions (happy, sad and pain) displayed by two different models (Simon et al., 2008). Each one second video, started with a neutral face and progressed to a full expression depicting happiness, sadness or pain, followed by 1100ms - 1400ms of a black screen (see Error! Reference source not found.).

![Figure 5-1 Emotion perception protocol](image)

This figure illustrates a representative example of the stimulation protocol. A screenshot of a single video frame of facial expressions of happy (top row), sad (middle), and pain (bottom row) for one actress are shown (Simon et al., 2008).
In addition to the emotional faces, a picture of a teddy bear was also included (see Figure A-2). A total of 120 videos (30 of each expression) were included in each experiment, in randomized order (Neurobehavioral Systems, Presentation 17.0 01.14.14.Ink). The total viewing time was approximately four minutes. Infants who did not have a minimum of 5 artifact free trials per condition were excluded from further analysis. Thus, data from 5 infants ((n=4) non-exposed, (n=1) exposed) were excluded from further analysis due to insufficient artifact-free trials for each emotional expression. A total of 30 infants were included in the final analysis (See Table 5-1 for demographics).

<table>
<thead>
<tr>
<th>Maternal characteristics</th>
<th>Non-exposed (n=19)</th>
<th>SSRI-exposed (n = 11)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (Year), Mean (SD)(^a)</td>
<td>32.16±2.45</td>
<td>35.35±5.305</td>
<td>0.047</td>
</tr>
<tr>
<td>Maternal education (Year), Mean (SD)</td>
<td>17.20±3.78</td>
<td>17.55±2.66</td>
<td>0.771</td>
</tr>
<tr>
<td>Smoking per pregnancy (# cigarettes), Mean (SD)</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>-</td>
</tr>
<tr>
<td>Alcohol per pregnancy (#single drinks), Mean (SD)</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.65</td>
</tr>
<tr>
<td>Prenatal HAMD 3rd trimester Average, Mean (SD)(^a)</td>
<td>7.40±3.08</td>
<td>10.63±5.00</td>
<td>0.036</td>
</tr>
<tr>
<td>Prenatal PES HASS frequency 3rd trimester Average, Mean (SD)</td>
<td>7.25±2.19</td>
<td>7.4±1.92</td>
<td>0.845</td>
</tr>
<tr>
<td>Prenatal PES Uplift frequency 3rd trimester Average, Mean (SD)</td>
<td>8.97±1.02</td>
<td>9.09±0.99</td>
<td>0.762</td>
</tr>
<tr>
<td>Prenatal PES Uplift intensity 3rd trimester Average, Mean (SD)</td>
<td>2.19±0.44</td>
<td>2.21±0.56</td>
<td>0.964</td>
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<tr>
<td>Prenatal PES HASS intensity 3rd trimester Average, Mean (SD)(^a)</td>
<td>1.46±0.32</td>
<td>1.79±0.39</td>
<td>0.016</td>
</tr>
<tr>
<td>BDI, EEG study visit, Mean (SD)</td>
<td>10.26±8.88</td>
<td>11.45±9.50</td>
<td>0.73</td>
</tr>
</tbody>
</table>

**Infant characteristics**

Type of delivery (vaginal/C-section) 13/4 5/6 0.101
<table>
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<tr>
<th>Demographic</th>
<th>Mean (SD)</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Birth GA (weeks)</td>
<td>40.08±1.65</td>
<td>0.059</td>
</tr>
<tr>
<td>Age at the EEG (months)</td>
<td>9.6±1.03</td>
<td>0.098</td>
</tr>
<tr>
<td>Sex male/female</td>
<td>8/11</td>
<td>0.527</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>3.62±0.44</td>
<td>0.195</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>52.28±1.83</td>
<td>0.033</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>35.11±1.32</td>
<td>0.44</td>
</tr>
<tr>
<td>Apgar score 1 min</td>
<td>8.00±1.54</td>
<td>0.029</td>
</tr>
<tr>
<td>Apgar score 5 min</td>
<td>8.94±0.243</td>
<td>0.035</td>
</tr>
</tbody>
</table>

Table 5.1 Demographics

*Significant difference using one way ANOVA p<0.05

Demographics for mother-infant diads. PES, Pregnancy Experiences Scale; HAM-D, Hamilton Rating Scales for Depression; BDI, Beck Depression Inventory, GA gestational age.

Non-exposed infants observed an average of 9.53±3.13 happy, 9.95±3.1 sad and 9.32+3.05 painful faces, and exposed infants observed an average of 9.09±3.17 happy, 9.27±2.9 sad and 9.36+3.6 painful faces. No significant differences were found between groups (ANOVA p>.56). Each infant viewed one of two possible females performing all three expressions. The presentation of the models was counterbalanced.

5.2.4 Experimental setup and procedures

Infants were seated on their parent’s lap approximately 80 cm from the monitor. Faces presented were 46cm long (circumference). To attract the infant’s attention to the stimuli, an experimenter gently tapped on the screen if needed. A video camera located above the monitor screen recorded participant’s eye and limb motion. Prior to analysis, videos were manually examined to exclude parts were participants were not looking at the monitor, or moving limbs, or
parts in which infant was fussy and distracted.

5.2.5 EEG recordings

An infant sized 60-channel HydroCel Geodesic SensorNet was used for recording. EEG was recorded and amplified using Net Amps 300 amplifier, at a sampling rate of 250 Hz and signals were online referenced to Cz. At the beginning of data acquisition scalp electrode impedances were less than 50 kΩ. The EEG signals were analyzed offline using Brain Electrical Source Analysis (BESA) (MEGIS Software GmbH). The signal was filtered from 4 to 40Hz with a notch filtered at 60 Hz and manually scanned for artifacts. Following, an automated artifact scan (BESA) was applied on the data to remove additional motion and excessive eye movement artifacts. Data were epoched from 0 to 1000ms at the beginning of each video clip. BESA brain source montage was applied to convert the EEG surface activity recorded from 60 electrodes into a set of 15 different brain source spaces.

5.2.6 Graph Theoretical Analysis

Graph theory analysis (GTA) was conducted using “Brain Connectivity Toolbox” (Rubinov and Sporns, 2010) running Matlab (Natick, MA). We used both global and local graph measures to characterize associations between prenatal SSRI drug exposure and different features of the functional network for viewing emotional faces.

For global measures we used modularity, to evaluate the stability of sub-modules within the overall network, and density which measures the ratio of the present connections to all possible connections (Achard and Bullmore, 2007; Bullmore and Sporns, 2009). For local
measures, we focused on the measure of betweenness centrality, which evaluates the number of times for which a specific node is included in the shortest paths that pass from every node to all of the other nodes, indicating on the ability of a specific node to serve as a hub in the network (Sporns, 2004; van den Heuvel and Sporns, 2013).

5.2.6.1 Construction of Functional Connectivity matrix

The construction of the brain functional connectivity matrix was done using a network learning algorithm controlling for error-rates. EEG time-series of source locations were used to create the brain connectivity matrix with each node representing one brain source in the connectivity matrix (Appendix A).

5.2.7 Statistical analysis

For analysis, we have compared connectivity measures between SSRI-exposed infants and non-SSRI-exposed infants. Maternal mood measures (pre and post) were used as covariates in our statistical model. First, we performed a general linear model (GLM) of mixed ANOVA to determine group differences for the global measures of modularity and density. Emotion (happy, sad and pain) was the within subjects’ factor and SSRI-exposure was the between subject factor (non-exposed, exposed). HAM-D (prenatal depression) and BDI (postnatal depression) were used as covariates. We included mean PES scores (DiPietro et al., 2010) focusing on measures that differed between groups (Table 5-1). To evaluate possible associations between prenatal SSRI exposure and hubs of the functional network for emotional face perception, we focused solely on frontal nodes (i.e. frontal left (FL), frontal midline (FM) and frontal right (FR)) as functional connectivity in these regions have been shown to be altered in prenatally SSRI-
exposed infants (Videman et al., 2016). These nodes were included in a GLM mixed ANOVA, using HAM-D, PES and BDI as covariates. Bonferroni correction was used to correct for multiple comparisons.

5.3 Results

5.3.1 Participant characteristics

Maternal and infants characteristics for non-exposed (n=19) and for SSRI-exposed group (n=11) are shown in Table 5-1. Independent sample t-tests revealed no group differences in maternal education, alcohol use, or smoking (p > 0.65). SSRI treated mothers had significantly higher HAM-D scores and higher PES hassle intensity scores compared to non-treated mothers. No group differences were found in birth method, gestational age or head circumference (p > .05). However, SSRI-exposed infants had lower birth length, and Apgar scores at 1 and 5 minute.

5.3.2 Graph Analysis

5.3.2.1 Global measures

A group average graph for the functional network for non-exposed and for SSRI-exposed infants for observation of happy, sad and pain faces is shown in Figure 5-2.
Group average functional connectivity graphs. Represents the average functional connectivity graph for the exposed and non-exposed groups for happy, sad and pain. Dots represent the brain sources (nodes), while the size of the dot indicates the value of betweenness centrality for that source. Lines in the graph represent the functional connection between two separate dots, and the thickness indicates how its prevalence at the group level. Visualized with the BrainNet Viewer http://www.nitrc.org/projects/bnv/.

Qualitatively, the SSRI-exposed graphs appeared more modular than graphs from non-SSRI exposed graphs. A mixed ANOVA showed a statistically significant main effect of exposure group with higher modularity observed among SSRI-exposed infants compared to non-
exposed infants ($F(1, 25) = 5.49, P = .03, \eta^2_p = .18$) (see Figure 5-3). A multivariate analysis revealed that differences in modularity were specific to the emotional stimuli, whereas no significant differences were observed between group for the non-emotional stimulation (teddy bear, see A.2, and Figure A-2).

![Modularity Graph](image)

**Figure 5-3 Main effect of drug exposure.**
Bar graphs showing group differences for modularity between SSRI-exposed and non-exposed infants for the observation of all emotional expressions combined together. Error bars represent 1 standard error. (*, $P < .05$) (Using average of HAM-D, maternal PES scores for intensity of hassles in the third trimester of pregnancy, and maternal BDI at the time of the study).

HAM-D also had an impact ($F(1, 25) = 5.12, P = .03, \eta^2_p = .17$), namely increased levels of maternal depressed symptoms (HAM-D) during pregnancy, were associated with lower levels of modularity among non-exposed infants ($r = -.614, p = .005$). This correlation was not observed in the SSRI-exposed group ($r = -.376, p = .255$) (see Figure 5-4.).
Figure 5-4 Correlation between HAM-D and modularity

Scatter plot showing the correlation between modularity and the z-score of maternal HAM-D during the 3rd trimester, for the observation of all emotional expressions combined together, by group: (red represents SSRI-exposed (n=11) (r=-.376, p=.255), blue represents non-exposed group (n=19), (r=-.614, p=.005).

In addition, there was a main effect of PES intensity of hassles (F (1, 25) = 4.715, P = .040, η_p^2 = .159), such that higher levels of hassles’ intensity during pregnancy were associated with lower levels of modularity among non-exposed infants (r=-.724, p<.001 see Figure 5-5.).
PES intensity of hassles

Figure 5-5 Correlations between intensity of pregnancy hassles and modularity

Scatter plot showing the correlation between modularity and the pregnancy experiences scale for intensity of Hassles during the 3rd trimester (all emotions are combined together), by group (red represents SSRI-exposed (n=11) (r=-.459, p=.156), blue represents non-exposed group (n=19) (r=-.724, p<.001).

There was also a trend for an interaction between PES and emotion, suggesting that higher levels of PES were associated with lower levels of modularity mostly for pain and sad, while for happy an opposite pattern was observed (see Appendix A and Error! Reference source not found.). No associations were observed among SSRI-exposed group (r=-.459, p=.156) (see Figure 5-5.). Similar results were observed when sex was added as a covariate (group effect p=.27, HAM-D p=.037, PES p=.045).

No group differences were found for global density, although there was a significant main effect of PES scores for intensity of hassles (F (2, 50) = 5.44, P = .028, $\eta^2_p = .178$), such
that increased levels of intensity of hassles was associated with increased levels of density in both non-exposed (r=.835, p<.001) and SSRI-exposed infants (r=.70, p=.016), (Figure 5-6). Adding sex as a covariate did not change the results.

**Density**

![Figure 5-6 Correlations between intensity of pregnancy hassles and density](image)

Figure 5-6 Correlations between intensity of pregnancy hassles and density

Scatter plot showing the correlation between density and the pregnancy experiences scale for intensity of Hassles during the 3rd trimester, by group (red represents SSRI-exposed (n=11) (r=,70, p=.016). blue represents non-exposed group (n=19) (r=.835, p<.001).

5.3.2.2 Local measures

Our analysis for differences in betweeness centrality levels, a measure reflecting the
ability of a specific node to serve as a hub in the network, were focused only on frontal nodes frontal left (FL), frontal midline (FM) and frontal right (FR).

There was no main effect of exposure group, however, there was a main effect of prenatal intensity of hassles (F (1, 25) = 5.94, P = .022, $\eta^2_p = .19$) such that infants exposed to higher levels of prenatal hassles intensity showed lower levels of betweenness centrality for the frontal regions (r=-.793, p<.001, non-exposed, r=-.884, p<.001 (Figure 5-7).

**Betweenness centrality**

![Betweenness centrality](image)

**Figure 5-7 Correlation between betweenness centrality and the intensity of pregnancy hassles**
Scatter plot showing the correlation between betweenness centrality and the pregnancy experiences scale for intensity of Hassles during the 3rd trimester, by group (red represents SSRI-exposed (n=11) (r=.70, p=.016)., blue represents non-exposed group (n=19) (non-exposed: r=-.793, p<.001, SSRI-exposed: r=-.884, p<.001).
There was a three-way interaction of Emotion X Region X BDI (F (4, 100) = 2.678, P = .036, \( \eta_p^2 = .097 \)). To better understand this interaction we divided infants from both groups (n=30) to two sub groups of high BDI levels (n=15) and low BDI levels (n=15) according to the median levels of BDI (median = 8.5). A multivariate GLM was performed using BDI levels (high/low) as the fixed factor for the predicted values of the different regions, separately for each emotion (see Figure 5-8.).

**Betweenness centrality:**

**Emotion X Region X BDI**

Figure 5-8 Betweenness centrality BDI interaction with region and emotion

Bar graph showing interaction between BDI X Emotion X Region. Error bars represent 1 standard error. (*, P < .05) (Maternal Beck Depression inventory (BDI) was measured at the time of the study).

In response to happy emotions, in right frontal regions, infants of mothers with lower
postnatal depressed mood had significantly higher levels of betweenness centrality compared with infants of more symptomatic mothers (F (1, 28) = 11.35, P = .002, $\eta_p^2 = .288$).

In response to sad emotions, for both left and midline frontal regions, infants of mothers with the lower depressed symptoms levels exhibited higher betweenness centrality levels (for left frontal: F (1, 28) = 4.84, P = .036, $\eta_p^2 = .148$, and for midline frontal: (1, 28) = 20.355, P < .001, $\eta_p^2 = .421$).

In contrast, for perception of pain, infants of more depressed mothers exhibited high levels of betweenness centrality for midline frontal regions compared to infants of less depressed mothers (1, 28) = 9.813, P = .004, $\eta_p^2 = .260$) see Figure 5-8. Adding sex as a covariate did not change the results.

5.4 Discussion

In this study we examined the association between in utero SSRI exposure and infant functional brain network organization in response to observing happy, sad and painful emotions. We used a network perspective utilizing graph theory to evaluate changes in functional organization between exposed and non-exposed infants. We found a main effect for drug exposure associated with higher modularity for SSRI-exposed infants compared with non-exposed for all of the emotions vignettes, reflecting increased segregation and decreased integration between the sub-modules. It has been suggested that in adults, levels of modularity have been negatively associated with processing dynamics, or task demand (Kitzbichler et al., 2011). Greater cognitive demands were associated with less clustered and less segregated brain network organization, allowing a dynamic integration between more distant brain regions (Bola
and Sabel, 2015; Cole et al., 2014). Interestingly, Kinnision et al (2012), have shown that participants exhibited decreased network modularity associated with an emotional task while the participants anticipated a safe or a threat condition (Kinnison et al., 2012). In line with this, our results suggest that infants exposed to SSRIs compared to non-exposed infants may be devoting less cognitive resources to process emotional faces, and may reflect a more passive processing of emotions. We cautiously interpret this functional pattern of SSRI exposed infants to suggest on a more blunted or indifferent response for viewing emotional faces. Compared to our hypothesis, increased modularity was evident for all of the emotions. It is possible that exposure to SSRs in utero predisposes emotional perception, via altered functional connectivity organization, leading to less engagement and more blunted response to viewing emotional faces in infancy.

Additionally, our results showed that higher levels of depression and hassles during pregnancy were only correlated with modularity in infants not exposed to SSRIs. These correlations were not evident for SSRI-exposed infants, raising the possibility of an SSRI-related “protective” effect in response to life with a depressed mother. Thus, SSRIs might play a dual role by blunting the overall response to emotions, as well as masking part of the effects of in utero exposure to prenatal depression and pregnancy related stress. In support of our interpretation, studies in both adults and pediatric population have been previously suggested that SSRIs could result in an overall decrease in the intensity or experience of both positive and negative emotions (Price et al., 2009). Together, these novel findings suggest that prenatal exposure to SSRIs and maternal depression appear have a differential impact shaping the subsequent functional network organization for perceiving emotional faces during infancy.

Pre and postnatal mood also appeared to play a role in shaping infant functional
architecture for perceiving emotional faces. Increased prenatal depression symptoms were negatively associated with modularity. Similarly, pregnancy related hassles were also negatively associated with modularity, and were positively associated with density. This could suggest that infants exposed to heightened prenatal mood and pregnancy-related stress require more cognitive effort, and activating more connections to perceive emotional faces. These findings again point towards possible prenatal programing, suggesting that the higher levels of depression and stress during pregnancy may prepare the fetus to living outside the womb (Barker, 2000) with a depressed mother. It has been suggested that increased cortisol and lower levels of serotonin and dopamine exhibited in newborns of prenatally depressed mothers (Hilli et al., 2009), might be associated with higher arousal levels and with decreased discrimination abilities of different stimuli, indicating suboptimal perceptual performance in newborns exposed to prenatal depression (Field et al., 2009). Decreased modularity and higher density, which reflect higher cognitive effort (Kitzbichler et al., 2011), in infants prenatally exposed to maternal depression and stress could be explained by increased motivation to extract as many emotional cues as possible from emotional faces. As we expected, this was evident only for negative expressions of sad and painful expression, but not in response to happy expressions (Appendix A). Our results are in line with previous studies showing associations between prenatal maternal anxiety and heightened attention to fearful faces (Otte et al., 2015). Our results add to the existing literature that the global organization of the functional networks supports an increased allocation of cognitive resources to perceiving sad and painful expressions. In adults, in an analogous negative valence setting (for sad and pain), a heightened response to threat-related stimuli has been associated with greater anxiety symptoms (Leppänen and Nelson, 2012) and depression (Fu et al., 2004), suggesting that the origin of altered emotional processing could be traced to the in
Pregnancy-related stress was also associated with reduced levels of hub values reflected by reduced levels of betweenness centrality. This suggests an association between prenatal stress and alterations in frontal processing of emotional faces possibly related to decreased efficiency of processing emotions in frontal hubs. Previous studies have reported on associations between prenatal mood symptoms and greater relative frontal asymmetry during rest (Chichlowski, 2015; Field et al., 2006; Gustafsson et al., 2018). Here, we provide additional support for functional alterations in frontal regions in relation to prenatal stress, in the context of emotion perception. We also found that increased levels of postnatal depression were associated with reduced hub values for happy and sad emotions. Surprisingly, the opposite pattern was observed for painful emotions but only in the medial frontal pole. Although, we do not fully understand this latter observation, increased activity of medial frontal cortex has been positively associated with maternal depression scores of BDI in response to the mother’s own infant cry (Swain et al., 2008). It is possible that higher levels hub levels of the medial frontal hub in response to painful expressions resemble the hyper connectivity of medial prefrontal in relation to perception of distress of others observed in depressed mothers.

There are several limitations of this study. The final sample size in the current study is small, reflecting the challenges of recruiting depressed mothers and their infants for a longitudinal study. Moreover, our study had a smaller number of trials (an average of 9.4 trials per emotion) due to increased fussiness and reduced attention to stimuli for long periods in the infant population. Though the number of trials did not affect the results (Appendix A), future
studies should replicate our results with increased number of trials with larger sample size. Moreover, mothers treated with SSRIs might be inherently different from mothers not treated with SSRI. While we adjusted for prenatal maternal mood symptoms, the effect of residual confounding related to maternal mood disturbances (e.g. illness severity, and genetic factors inherent to mood disorders) could not be ruled out.

In the current study we focused on maternal factors, however, infant temperament is also associated with alterations in emotion perception, and could interact with maternal mood (Rajhans et al., 2015). Future studies should include measures of the infant emotion regulation and temperament, to evaluate interactions between functional connectivity organization, maternal depression, and SSRI-exposure and emotion perception. This would help shed light on the environmental factors associated with social-emotional development.

In summary, we examined associations between prenatal exposure to SSRIs and functional brain organizational responses to viewing emotional salient faces in infants. We used a data-driven network approach applied to EEG recordings in 8-to-10-month old infants of mothers who were either depressed-SSRI treated, or not SSRI-treated. We have found that prenatal exposure to SSRIs, and pre and postnatal maternal mood were both associated with distinct patterns of functional organization in response to viewing dynamic happy, sad and painful facial expressions. These findings reflect the impact of both prenatal programming and postnatal environment in shaping the infants’ brain organization in response to emotional demands typically occurring during infancy. This influence may have important implications for developmental trajectories of social-emotional development in infants of depressed and SSRI treated mothers.
Chapter 6: **Alterations in resting state networks following in utero SSRI exposure in the neonatal brain**

### 6.1 Introduction

Mood disorders during pregnancy are common and carry risks for maternal health and infant social, behavioral and cognitive development in infancy and childhood. (Barker et al., 2013; Copper et al., 1996; Field et al., 1988; Hayes et al., 2013; Lebel et al., 2016; Luoma et al., 2001; Rifkin-Graboi et al., 2013; Sandman et al., 2012; Talge et al., 2007). Increasing use of Selective Serotonin Reuptake Inhibitor (SSRIs) antidepressants used to treat perinatal mood disorders (Vigod et al., 2016b) adds risk for fetal and early neonatal development (Brummelte et al., 2017). SSRIs act primarily by the inhibition of serotonin 5HT transporter at the presynaptic neuron increasing extracellular intrasynaptic serotonin levels (Gaspar et al., 2003). SSRIs freely cross the placenta (Rampono et al., 2009) and have been shown to modulate fetal serotonin (5HT) levels in animals (Rampono et al., 2009) and

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in human infants’ cord blood (Davidson et al., 2009; Hilli et al., 2009; Laine et al., 2003). 5HT is widely distributed throughout the brain and is prevalent in regions involved in cognition,
emotion regulation, and learning (reviewed in (Brummelte et al., 2017; Lucki, 1998)). Beyond its role as a neurotransmitter, 5HT also plays a role as a trophic factor early in development, regulating aspects of neuronal differentiation, migration, myelination and synaptogenesis (Gaspar et al., 2003). Therefore, early alterations of 5HT signaling may have downstream consequences for serotonergic related brain development and function.

In rodent models, early SSRI exposure during the period akin to the human 3rd trimester was associated with increased depressive-like and anxiety-like behavior, together with a decrease in exploratory tendencies (Ansorge et al., 2004; Hansen et al., 1997). Other animal studies have shown altered sensory (Lee, 2009) and auditory cortex functional properties (Simpson et al., 2011). Early SSRI exposure was also associated with altered axonal development, raphe and callosal connectivity in adult rats, with play behavior disruption and with an exaggerated response to novel sounds in young rats (Simpson et al., 2011).

Long before birth, the impact of SSRI exposure is apparent. In human fetuses at 36 weeks gestation, SSRI exposure was associated with reduced cerebral blood flow and reduced fetal heart rate (fHR) variability (Rurak et al., 2011). In addition, SSRI-exposed fetuses showed greater sensitivity to consonant voice onset time than non-exposed fetuses (Weikum et al., 2012). In the newborn period, prenatal SSRI exposure has been associated with an increased rate of premature birth, decreased birth weight, increased risk for congenital malformations, and poor neonatal behavioral adaptation (Chambers et al., 2006; Oberlander et al., 2008; Olivier et al., 2013). Beyond the newborn period, early sensory and perceptual alterations have been reported (Oberlander et al., 2005, 2002), and neurodevelopmental and behavioral effects persist across
the first years of life (Olivier et al., 2013), possibly reflecting early altered brain development. Of particular note, shifts in language development during late fetal development and infancy that differentiate the impacts of SSRI exposure and maternal depression have been reported (Weikum et al., 2012).

Reports examining the neural correlates of associations between prenatal SSRI exposure and neurodevelopment are emerging, suggesting that SSRI-exposed neonates have changes in white matter (WM) microstructure (Jha et al., 2016; Lugo-Candelas et al., 2018; Podrebarac et al., 2017), and grey matter volume (Lugo-Candelas et al., 2018). While Jah et al., (Jha et al., 2016) showed fractional anisotropy (FA) decrease and mean diffusivity (MD) increase (indicative of maturation) in widespread tracts in SSRI-exposed relative to controls, Podrebarac and colleagues (Podrebarac et al., 2017) showed opposite patterns in the superior white matter. It remains unclear whether these microstructural connections alterations are confounded by the severity of prenatal maternal depressive symptoms (Jha et al., 2016) or, in the case of very preterm infants antenatally exposed to SSRIs, the degree of prematurity (Podrebarac et al., 2017). In addition, Lugo-Candelas and colleagues (2018) has shown increased amygdalar and insular grey matter volume together with increased amygdalar-insular white matter connectivity in prenatally SSRI-exposed infants compared to infants of depressed non-pharmacologically-treated mothers and control infants. Using electroencephalograph (EEG), Videman and colleagues (Videman et al., 2016) showed prenatal SSRI exposure associations with reduced inter-hemispheric network connectivity, reduced frontal activation at slower frequencies, and reduced subcortical and cortical layers’ coupling, suggesting both local and global effects of prenatal SSRIs on neonate brain activity.
In adults, distinct functional brain networks have been identified at rest (i.e., resting-state-networks (RSNs)), including default mode (thought to support self-referential processes (Raichle, 2015)), as well as visual-like and auditory-like RSNs. In early infancy, similar proto-RSNs are evident for some sensory systems (e.g., visual, auditory). This suggests that auditory and visual networks are functionally synchronized from birth, whereas networks associated with higher order cognitive functions are established later (Dennis L. E. and Thompson M. P., 2014; Fransson et al., 2007; Gao et al., 2016, 2015b), and therefore have different developmental trajectories (Doria et al., 2010; Fransson et al., 2009; Gao et al., 2015a).

Inherent to examining the potential effects of SSRI exposure is the effect of maternal mood disturbances on early brain development (Rifkin-Graboi et al., 2015; Soe et al., 2016). Comparing infants of non-pharmacologically-treated depressed mothers with infants of non-depressed mothers, Qiu and colleagues (Qiu et al., 2015) showed that increased amygdala connectivity with the left temporal cortex, insula, anterior cingulate and ventromedial frontal cortices was positively correlated with increased maternal prenatal depressive symptoms (Qiu et al., 2015). Such functional connectivity alterations of the amygdala are consistent with alterations observed in adult patients with major depressive disorders, reflecting possible developmental origins of neural circuits associated with an increased risk for depression. Functional connectivity alterations of the amygdala in infants of depressed mothers have also been reported by Posner and colleagues (Posner et al., 2016).
In the current study, we used newborns resting-state fMRI data to examine the effect of in utero exposure to SSRIs and maternal mood disturbances on functional connectivity organization and RSNs development in the neonatal brain. Given the previous findings associating functional alterations with prenatal SSRI exposure (Jha et al., 2016; Lugo-Candelas et al., 2018; Videman et al., 2016), and neonatal neurobehavioral disturbances associated with prenatal SSRI exposure (Olivier et al., 2013), we hypothesized that in utero SSRI-exposed infants will have altered RSN functional organization compared with infants of depressed mothers not SSRI-treated and with control infants. However, since current findings are sparse and this is the first study examining RSN organization in this population we did not want to make any preliminary assumption and used a data driven un-biased approach to test our hypothesis.

6.2 Methods

6.2.1 Participants

This study was approved by the University of British Columbia Clinical Research Ethics Board and the BC Women’s Hospital Research Review Committee. Informed consent was obtained from mothers recruited during their second trimester of pregnancy, from a Reproductive Mental Health Clinic, midwifery services, and family physician clinics in metropolitan Vancouver, Canada. Healthy non-SSRI treated, non-depressed pregnant women and depressed pregnant women non-pharmacologically-treated and also depressed SSRI treated were recruited to the study; (see additional information in Appendix B). All pregnant women treated with SSRIs had been diagnosed with a mood disorder and were prescribed SSRI based on their clinical need.
6.2.2 Maternal mood

Prenatal maternal mood was assessed at 26 weeks and 36 weeks of gestation using the clinician rated Hamilton Rating Scales for Depression (HAM-D) (Hamilton, 1960). Given that psychological experience related to pregnancy is not necessarily negative, in addition to HAM-D, we used a pregnancy specific questionnaire for maternal appraisal of positive and negative experiences during pregnancy (Pregnancy Experiences Scale (PES)) (DiPietro et al., 2004), to assess another distinct dimension of maternal psychological experience related to pregnancy. HAM-D depressive symptoms were used to form 3 maternal prenatal exposure groups; control infants with maternal HAM-D <8, infants of depressed mother HAM-D≥8 with no drug exposure, and infants exposed to in utero SSRIs. PES score targets pregnancy-related maternal uplifts and hassles to specifically address how pregnant women experience their pregnancy, and was used as a covariate in our statistical model.

6.2.3 MR image acquisition

A total of 96 pregnant women were recruited to the study. Of the 96 women, 12 women decided not to undergo the imaging component, so did not contribute to the analyses reported below (see S1). At postnatal day 6, 84 Infants underwent MR scanning during natural sleep (n = 31 control, n=24 depressed-non-SSR-exposed, n = 29 SSRI-exposed infants). Images were acquired on a pediatric-dedicated 3T MRI scanner (GE 750 Discovery, Milwaukee, WI USA) at the BC Children’s Hospital MRI Research Facility in Vancouver, BC Canada. Infants were placed in an MR-compatible neonatal incubator (Advanced Imaging Research, Inc dba SREE MEDICAL SYSTEMS, Cleveland, OH, USA) that eliminated the need for sedation.
This study was part of larger scan series that included structural, microstructural, resting-state functional and metabolic imaging. A 6 minute 20 second resting-state fMRI scan was acquired using echo planar image (EPI) sequence (Oblique axial, TR = 3000 ms; TE = 18.4 ms, flip angle = 90°, 39 interleaved slices, 2 mm isotropic, no gap). For anatomical co-registration, T1 structural images were acquired using a 3D fast spoiled gradient echo scan (TE = 2.95 ms, TR = 7.7 ms), with a voxel size of $1 \times 1 \times 1 \text{ mm}^3$ and reconstructed to 0.4mm.

6.2.4 Image preprocessing

Functional image analysis was carried out using fMRIB Software Library (FSL, www.fmrib.ox.ac.uk/fsl). Preprocessing was done using FEAT (FMRI Expert Analysis Tool) v6.0 and included motion correction with MCFLIRT (Jenkinson et al., 2002), non-brain structure removal with BET (S M Smith, 2002), slice timing-correction for interleaved acquisition, spatial smoothing using Gaussian kernel 3-mm FWHM, grand-mean intensity normalization of the entire 4D dataset, and high-pass temporal filtering (sigma 50 s). Additional motion artifact correction was performed using FSL Melodic (Beckmann and Smith, 2004) and AFNI 3dDespike (Cox, 1996). Registration to high resolution structural and/or standard space (T1-weighted MR image of 40 weeks gestation neonatal template (Serag et al., 2012)) images was carried out using FLIRT (Jenkinson et al., 2002; Jenkinson and Smith, 2001). In total, data from 53 infants were included in the analysis. Data from 21 infants were excluded due to excessive motion (sustained motion n=3, and infant woke up during the scan n=5), susceptibility (n=6) and ghost (n=1) artefacts, and technical error (n=6). An additional 10 infants did not have resting-state functional data and were excluded from the study (n=5 SSRI-exposed infants, n=3 depressed-only). Excluded infants were similar in all demographic measures to infants included
in the final analysis ($p > 0.07$, Table B-1). To evaluate motion parameters in our data we measured two different parameters using MCFLIRT (Jenkinson et al., 2002), in accordance with recent guidelines for analyzing neonatal (rs)fMRI (Mongerson, Chandler et al., 2017); absolute displacement (AD) which compares the transformation matrix at time-point N with that of the reference time-point, and relative displacement (RD) which compares two subsequent time-points, N and N+1. For the majority of our subjects (n=48), motion was minimal throughout the whole scanning session with a maximal AD of less than 1mm with mean absolute displacement of $0.282 \pm 0.20$ mm, and relative displacement of $0.092 \pm 0.05$ mm. An additional five subjects (n=3 SSRI-exposed, n=1 depressed-only) exhibited one or two episodes of motion in which the infants tilted their head away from the original position for 10–30 s and then titled their head back close to the original head position, and had higher levels of AD (ranged from 1 to 2.94 mm). For these five infants, following denoising with MCFLIRT (Beckmann and Smith, 2004) and AFNI 3dDespike (Cox, 1996) AD ranged between 0.02 to 0.06. Overall, for all of our 53 subjects AD ranged between 0.05 mm to 2.94 mm (mean = $0.433 \pm 0.55$) and relative displacement (RD) ranged from 0.03 to 0.69 (mean = $0.11 \pm 0.1$). No significant group differences were detected ($p > 0.1$, using one-way ANOVA). Following denoising, values for AD were reduced and ranged between 0.001 to 0.15 (mean = $0.032 \pm 0.025$) and RD ranged from 0.001 to 0.04 (mean = $0.019 \pm 0.01$), with no group differences ($p > 0.7$ using one-way ANOVA).

### 6.2.5 Statistical Analyses

Model-free probabilistic Independent component analysis (ICA)-based exploratory data analysis was carried out using Multivariate Exploratory Linear Decomposition into Independent Components (MELODIC) Version 3.14 (Beckmann and Smith, 2004), applied to each individual
infant’s BOLD preprocessed time series. ICA was used to investigate the possible presence of unexpected artifacts or spatiotemporal resting-state patterns of synchronization (see Appendix B). To assess the consistency of independent components across all infants at the group level, ICA analysis was then carried out using temporal concatenation approach with MELODIC (Beckmann and Smith, 2004). This yielded group independent component maps that were thresholded at p < 0.05 to control for the false discovery rate (FDR) for each RSN map (Beckmann and Smith, 2004). First, we created an averaged RSN template from 30 infants (10 randomly selected infants from each of the 3 groups, using random number generator), which unbiasedly represents all of the groups in our sample to serve as a reference for group comparison, which was carried out using dual regression. In short, the spatial maps from the group-average analysis were used to produce subject-specific versions of the group’s spatial maps, and associated time-series, using dual regression (Beckmann et al., 2009), resulting in a set of subject-specific spatial maps, one per group-level spatial map.

We then tested between group differences to compare between control, depressed-only and SSRI-exposed infants. As a first step we ran the analysis with no control variables. Next, to reduce the need for additional correction for multiple networks, based on the results from the first analysis the second analysis was focused only on networks showing differences between groups (Qiu et al., 2015). To calculate between group differences, we used FSL’s randomize permutation-testing tool with a general linear model (GLM) (Beckmann et al., 2009) adjusted for postmenstrual age at the MRI scan and sex. In addition we included mean PES scores (DiPietro et al., 2004) focusing on measures that differed between groups (Table 6-1).
6.3 Results

6.3.1 Participant characteristics

Table 6-1 presents both the maternal and neonatal characteristics for the whole group (n = 53), for controls (n=17), depressed-only (n = 16) and SSRI-exposed group (n = 20). Independent sample t-tests revealed no group differences in maternal education, alcohol use, or smoking (p > 0.085). SSRI treated mothers and depressed non-pharmacologically treated mothers had significantly higher HAM-D scores and higher PES hassle intensity scores compared to control mothers. No group differences were found in birth weight, gestational age or head circumference (p > 0.05). However, SSRI-exposed infants had lower birth length, and Apgar scores at 1 minute compared to the other groups. In addition, measures of irritability using Neurobehavioral Assessment of the Preterm Infant (NAPI (Constantinou and Korner, 1993)) did not differ between groups (p=0.548).
Entire sample (n = 53) | Control (n=17) | Depressed (n = 16) | SSRI (n = 20) | P value
---|---|---|---|---
Maternal age (Year), Mean (SD)<sup>a</sup> | 34.62±3.75 | 32.92±2.79 | 33.79±2.02 | 36.75±4.55 | 0.003
SSRI duration (Days), Mean (SD) | - | - | - | 258.9±33.8 | -
SSRI dose at 36 weeks gestation (mg) | - | - | - | 41.31±50.84 | -
Maternal education (Year), Mean (SD) | 17.79±3.02 | 18.71±3.01 | 17.56±3.34 | 17.2±2.7 | 0.305
Smoking per pregnancy (# cigarettes), Mean (SD) | 0.02±0.14 | 0.00±0.00 | 0.00±0.00 | 0.05±0.24 | 0.447
Alcohol per pregnancy (#single drinks), Mean (SD) | 0.83±3.16 | 0.59±2.45 | 0.44±1.31 | 1.35±4.53 | 0.65
Prenatal HAMD 3rd trimester Average, Mean (SD)<sup>a</sup> | 9.00±4.39 | 4.91±1.82 | 10.68±3.17 | 11.12±4.47 | -
PES HASS frequency 3rd trimester Average, Mean (SD) | 7.69±1.96 | 6.76±2.27 | 8.21±1.85 | 7.8±1.76 | 0.098
PES Uplift frequency 3rd trimester Average, Mean (SD) | 9.07±1.21 | 9.29±0.86 | 9.5±0.68 | 8.8±1.18 | 0.085
PES Uplift intensity 3rd trimester Average, Mean (SD) | 2.15±0.40 | 2.16±0.47 | 2.15±0.35 | 2.14±0.39 | 0.982
PES HASS intensity 3rd trimester Average, Mean (SD)<sup>a</sup> | 1.62±0.39 | 1.37±0.33 | 1.61±0.32 | 1.84±0.38 | 0.001

Neonatal characteristics

Type of delivery (vaginal/C-section) | 36/17 | 14/3 | 12/4 | 10/10 | 0.087
Birth GA (weeks), Mean (SD) | 39.53±1.67 | 39.9±1.81 | 39.84±1.60 | 38.97±1.53 | 0.165
Age at the MRI (hours) | 251.4±193.95 | 199.85±161.0 | 292.58±217.94 | 262.53±199.2 | 0.378
Sex male/female | 30/23 | 12/5 | 10/6 | 8/12 | 0.154
Birth weight (kg), Mean (SD) | 3.43±0.49 | 3.48±0.36 | 3.55±0.45 | 3.29±0.59 | 0.272
Birth length (cm), Mean (SD)<sup>a</sup> | 51.1±2.37 | 51.89±1.46 | 52.09±2.28 | 49.62±2.41 | 0.001
Head circumference (cm), Mean (SD) | 34.66±1.55 | 34.78±1.77 | 34.79±1.62 | 34.42±1.44 | 0.706
Apgar score 1 min, Mean (SD)<sup>a</sup> | 7.58±2.10 | 8.18±1.5 | 8.19±1.94 | 6.6±2.37 | 0.027
Apgar score 5 min, Mean (SD) | 8.66±1.02 | 9.00±0.0 | 8.81±0.91 | 8.25±1.37 | 0.061
NAPI irritability scores. Mean (SD)<sup>b</sup> | 66.938±18.32 | 68.28±17.8 | 69.96±18.27 | 62.363±17.8 | 0.548
Mental health family history (maternal report) - 2\textsuperscript{nd} generation (% reporting positive responses)

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<td>Alcohol/substance abuse</td>
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<td>Anxiety/panic attacks</td>
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Table 6-1 Demographics

\(\text{PES, Pregnancy Experiences Scale; HAM-D, Hamilton Rating Scales for Depression}^{a}\text{NAPI, Neurobehavioral Assessment of the Preterm Infant, data was available for 41 infants (control:15, depressed only:13, SSRI:13).}\)
6.3.2 Between groups differences (control vs. depressed vs. SSRI-exposed infants)

We used dual regression GLM (Beckmann et al., 2009) (Table 6-2) to compare between the control, depressed-only, and SSRI-exposed groups. First we ran the analysis using no confounder variables. Significant group differences were evident in a RSN similar to the putative auditory network (Figure 6-1, and see Appendix B, Error! Reference source not found. for RSN template from 30 randomly selected infants).
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<th>Estimated regions*</th>
<th>**P-value</th>
<th>Effect Size</th>
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### Dual regression, GLM with covariates, (Figure 6-3.A)

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### SSRI VS. Depressed only

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Table 6-2 Dual regression GLM results
The size of the cluster is given using a threshold of 0.05. All of the clusters were found for the auditory RSN (Figure 6-1).

*Edinburgh Neonatal Atlas (ENA33)(Cabez et al., 2016) was used to identify the brain regions.

** corrected for multiple comparisons

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<tr>
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<th>X1</th>
<th>Y1</th>
<th>Z1</th>
<th>Color</th>
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<th>T-score</th>
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(Figure 6-3.B)

**SSRI VS. Depressed only**

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Conservatively, effect sizes are computed using Cohen’s D (calculator #5 from https://www.psychometrica.de/effect_size.html) assuming independency. Effect sizes would have been higher if we would have assumed dependency.
Figure 6-1 Putative auditory network

ICA component in putative auditory network in neonatal template space (Serag et al., 2012) realigned to the anterior commissure - posterior commissure line (AC-PC) plane. (A) Glass brain showing ICA network thresholded at 2.9 (typical threshold for ICA networks). (B) The same ICA network more stringently thresholded at 4. (C) The same network shown on the neonatal template image thresholded at 4. The grids represent Talairach slices as outlined in reference (Talairach and Tournoux, 1988) and highlight the primary auditory cortex. Note that the correlated ICA synchronizations are centred in the superior temporal gyrus. (D) Anatomical underlay for the ICA synchronization.
Note that the synchronization is centred on Heshl’s gyrus bilaterally (arrows). This anatomical localization of data-driven ICA components in primary auditory cortex strongly suggests that this network represents an auditory network.

**Error! Reference source not found.** (top) shows clusters where significant hyperconnectivity was observed for the main effect of SSRI exposure group for the auditory network compared with control (**Error! Reference source not found..A**), and compared with infants of depressed mothers not treated with SSRIs (**Error! Reference source not found..B**), while other group comparisons (i.e. control vs depressed) were not significantly different (p>0.1) (see color codes in Table 6-2). Overall, the SSRI-exposed group showed hyperconnectivity within the auditory network, and in regions that were co-activated with the network.

![Figure 6-2](image)

**Figure 6-2 Dual regression for IC component representing the auditory network with no covariates**

SSRI-exposed infants (n=20) show greater BOLD signal compared to depressed (n=16) and control infants (n=17). Dual regression results registered to a neonatal template space (Serag et al., 2012) of 3 groups comparison (SSRI-
exposed vs. depressed-only vs. control) using a general linear model relative to the RSN template (Figure 6-1.).

*Edinburgh Neonatal Atlas (ENA33)* (Cabez et al., 2016) was used to identify the brain regions. Colored clusters in sagittal, axial and coronal views (see color codes in Table 6-2) show significant hyperconnectivity in regions co-activated with the auditory network (corrected for multiple comparisons). A: Dual regression results for IC component representing the auditory network with no covariates comparing SSRI-exposed infants with control infants; (voxel dimension 4mm isotropic). Differences are shown in regions that are both within the auditory network, such as the left angular cortex and also in non-auditory regions such as the left occipital inferior gyrus with positive, albeit weak, associations with the auditory network that were larger for the SSRI-exposed group compared with the control group ($p = 0.01$). B: Dual regression results for IC component representing the auditory network with no covariates comparing SSRI-exposed infants with infants of depressed non-pharmacologically treated mothers; (voxel dimension 4mm isotropic). Differences are shown in regions that are both within the auditory network, such as the left Heschl gyrus and also in non-auditory regions such as the left insula with positive, albeit weak, associations with the auditory network that were larger for the SSRI-exposed group compared with the depressed group (Panel B; $p = 0.013$), see also Table 6-2.

Building on the results of the first analysis, we then ran a second analysis to examine the specific effects of SSRI exposure on the putative auditory network, accounting for sex, age at the MRI scan and maternal PES scores. Results revealed significant hyperconnectivity in SSRI-exposed compared to control ($p = 0.02$, see 3.A) and depressed-only ($p = 0.01$ see 3.B) groups, corrected for multiple comparisons in the auditory network (Figure 6-1). Figure 6-3 (see also Table 6-2 bottom) shows the clusters where significant BOLD signal differences were observed, stemming from larger BOLD signal in regions in the temporal cortex, frontal cortex, angular and occipital cortex. We also analyzed the data using gestational age at birth instead of age at the MRI and found similar results (SSRI vs control $p=0.018$, SSRI vs depressed-only $p=0.012$).
However, since infants in our cohort were born full-term, we used age at the MRI in the analysis as a more critical reflection of brain functional growth, inherent to postconceptual age.

![Figure 6-3](image)

**Figure 6-3 Dual regression for IC component representing the auditory network with covariates.**

The following covariates were included: postmenstrual age at MRI, sex of the infant, and average of maternal PES scores for intensity of hassles in the third trimester of pregnancy. A: Dual regression results for IC component representing the auditory network comparing SSRI-exposed infants with control infants; (voxel dimension 4mm isotropic). Differences are shown in regions that are both within the auditory network such as the superior temporal cortex and the left angular gyrus and also in regions outside of the auditory network such as the left occipital inferior gyrus with positive, albeit weak, associations with the auditory network that were larger for the SSRI-exposed group compared with the control group (Panel A; $p = 0.022$). B: Dual regression results for IC component representing the auditory network comparing SSRI-exposed infants with infants of depressed-non-pharmacologically-treated mothers; (voxel dimension 4mm isotropic). Differences are shown in regions that are both within the auditory network such as the superior temporal cortex and also in regions outside of the auditory network such as the right
fusiform gyrus with positive, albeit weak, associations with the auditory network that were larger for the SSRI-exposed group compared with the depressed-only group (Panel A; \( p = 0.01 \)) (see also Table 6-2.)

6.4 Discussion

In this study we found an association between prenatal SSRI exposure and increased functional connectivity synchronization or hyperconnectivity of RSNs in the newborn infant, relative to control infants and to infants of non-pharmacologically-treated-depressed mothers. Using a data driven approach, we studied overall MR derived resting-state functional effects without limiting ourselves to specific brain regions or networks. Newborns exposed to SSRIs \textit{in utero} exhibited differences in the pattern of greater functional connectivity of a component which is qualitatively similar to an auditory network previously shown in similar populations using ICA analysis of resting-state fMRI data (Doria et al., 2010; Fransson et al., 2009) and an auditory task based fMRI approach (66). These differences remained even when we adjusted for mother’s pregnancy-related negative experiences and for sex and infants’ age at the scan. These differences stemmed from hyperconnectivity in regions within the auditory network in the temporal cortex and parietal lobe. Group differences were also seen in non-auditory regions with positive, albeit weak, associations with the auditory network in frontal and visual regions. Interestingly, in a recent neuroimaging study of functional connectivity in preterm infants, Thomason and colleagues (Thomason et al., 2017) demonstrated that fetuses who were eventually born preterm had lower levels of functional connectivity synchronization in regions associated with the auditory network, which is considered a pre-lingual network hypothesized to develop into language processing regions with maturation. In contrast, our results showed hyperactivation of regions functionally associated with the auditory network in SSRI-exposed
infants, suggesting a special sensitivity of these regions to different in utero experiences. Consistent with this interpretation, Gao and colleagues (Gao et al., 2015a) demonstrated that the auditory network is one of the earliest networks to develop: functional connections within the network are already synchronized at birth and resemble “adult like” patterns of synchronization. Moreover, studies exploring developmental trajectories of the auditory network and other early networks showed that the auditory network, compared to other networks such as the dorsal attention and default mode networks, exhibits only minimal maturational changes in the first year of life (Doria et al., 2010; Fransson et al., 2009; Gao et al., 2015a), reflected by reduced connectivity strength (specialization) thought to be related to pruning processes (Gao et al., 2015a). Therefore, it is reasonable to speculate that the auditory network might be more susceptible to the intrauterine environment as a significant amount of its maturation occurs during prenatal development. Indeed negative experiences during pregnancy as assessed by the PES questionnaire of intensity of hassles, are positively correlated with neurological maturation as reflected in shorter brainstem auditory evoked potential latencies (BAEP) (Dipietro et al., 2010).

Our findings are consistent with functional organization alterations reported in animal studies (Bonnin et al., 2012; Simpson et al., 2011). In rodents, early 5HT manipulations have been associated with altered raphe and callosal connections, sensory processing, and myelin sheath formation (Bonnin et al., 2012; Simpson et al., 2011). However, it is not clear whether these central 5HT-signaling changes, which reflect constrained downstream neural development, are also present in humans.
Although the functional consequences of increased functional connectivity synchronization in the auditory networks is yet to be fully understood, a previous study in our lab on a different cohort, reported on shifts in language perception in SSRI-exposed infants possibly reflecting accelerated language development compared to both healthy control infants and infants of symptomatic non-SSRI-treated-depressed mothers during the first year of life (Weikum et al., 2012). This study indicated that even before birth, SSRI related accelerated development of language (speech sound) perception is already evident. Weikum and colleagues (Weikum et al., 2012) reported that at 36 weeks gestation, fetuses exposed to SSRIs could discriminate not only similar sounding vowels (as has previously been reported in utero (Zimmer et al., 1993)) but that they could also discriminate between similar sounding consonants (e.g. ta/vs/da). Control fetuses not exposed to SSRIs were able to discriminate only between similar sounding vowels but not between similar sounding consonants as expected for this developmental stage. These findings were taken as evidence of an SSRI-related acceleration in auditory-perceptual development (Weikum et al., 2012). Differences between the SSRI-exposed infants and the non-exposed infants were also apparent following birth. Young infants show perceptual sensitivities that enable them to discriminate speech sound differences in both their native and in unfamiliar languages, but as they establish the speech sound categories used in their native language, they stop discriminating non-native speech sound differences. In the Weikum, and colleagues study, the control infants showed this pattern. However, the SSRI-exposed infants performed at 6-months like typically developing infants do at 10-months, and were already discriminating only native speech sound differences while infants of non-pharmacologically-treated-depressed mothers responded to non-native language only at 10 months (Weikum et al., 2012). Such developmental shifts in language development, may reflect an acceleration in the
closure of a critical period “window” for early language discrimination associated with prenatal SSRI exposure (Weikum et al., 2012). Although we cannot infer a causal relationship, it might be that hyperconnectivity within the auditory network in newborn infants could help explain the accelerated speech perception development seen in prenatally SSRI-exposed fetuses and infants.

While it may seem that accelerated language perception in the first year of life (Weikum et al., 2012) or hyperconnectivity in the auditory network could result in neurodevelopmental benefits for the infant that might be advantageous for subsequent language development, in fact it is more often the case that asynchrony in developing systems is detrimental to long term development. If the auditory network has developed prior to the infant accruing sufficient exposure to their native language and prior to the development of the cognitive skills to begin learning language, then later deficits could emerge. And indeed, a number of studies have suggested long term negative associations between prenatal SSRI exposure and expressive language development (Johnson et al., 2016), possible associations between prenatal SSRI exposure and poorer capabilities of verbal fluency (El Marroun et al., 2016b). Others have reported on increased risk of speech and language disorders, such as expressive language disorder and receptive language disorder (Brown et al., 2016), and risk for autism spectrum disorder (Man et al., 2015). Importantly, the long term effects of the prenatal environment, and the postnatal environment also play a role in shaping neurodevelopment (Oberlander et al., 2007). The in utero environment “prepares” the fetus for life outside the womb (Barker, 2000). According to the predictive adaptive hypothesis, in cases where these expectations are not met, it is at least to some extent, the capacity of an individual to cope with these mismatches between the in utero environment and the postnatal environment that may put an infant at risk for future
psychopathology (Gluckman et al., 2007). Longitudinal studies examining both pre and postnatal environmental aspects, using both functional-connectivity imaging and evaluation of language perception and proficiency development might be able to better address these questions.

Surprisingly, infants of non-pharmacologically-treated-depressed mothers did not show a significant alteration compared to control infants. It might be that although there were substantial clinical differences in mood symptom severity in our cohort, namely, mild symptoms were observed (HAM-D: 10.68±3.1 depressed, 4.91±1.82 controls, 11.31±4.49 SSRI-treated, see Table 6-1). Thus, such symptoms might not have been sufficiently severe to alter the functional connectivity patterns in the auditory network. Subsequent studies are warranted to test whether increased mood symptoms might be associated with alterations of the functional organization of RSNs.

 Mothers treated with an SSRI might be inherently different from depressed mothers not treated with an SSRI for a mood disturbance during pregnancy. While we included both a SSRI treated and non-SSRI-treated-depressed women and adjusted for pregnancy-related experiences, the effect of residual confounding related to maternal mood disturbances, such as illness severity, and genetic factors inherent to mood disorders, (ie. confounding by indication) could not be completely ruled out. Due to the challenges inherent to studying mothers and their neonates in this setting (ie maternal drop out due to unpredictable nature of perinatal depression, success rate per scan), our final sample size was ultimately reduced. Future studies are needed to replicate these findings with larger cohorts and a wider range of depressive and stress symptoms to allow us to account for yet unmeasured potential confounders.
Additional limitation of our study is that our sample size did not allow us to further investigate dose related effects or to subdivide the SSRI-exposed group to the different types of SSRIs. Larger cohorts of infants should address the questions whether different type/dosage of SSRI has an effect on the infant’s resting-state network patterns of synchronization.

In summary, in this study we used a non-biased approach to examine possible associations between in utero exposure to SSRIs and development of functional organization of RSNs in neonates. We found that SSRI exposure was associated with hyperconnectivity in the co-activation of the auditory network compared to control and to infants of non-pharmacologically-treated-depressed mothers. In light of previous findings showing accelerated auditory capacity of language perception’ effect of in utero exposure to SSRIs, our results might serve to advance our understanding of a possible developmental acceleration associated with prenatal SSRI exposure. Further, to control for different aspects of the maternal prenatal in utero environment, we used an analytic model that included a depressed-non-pharmacologically-treated group, and also accounted for the intensity of pregnancy related negative experiences which differed between groups. Our study is the first to show an association of in utero SSRI exposure on functional connectivity patterns in the auditory network of the newborn infant. The implication of these positive associations is yet to be determined.
7.1 Introduction

Depression during pregnancy affects between 14 to 23% of women, shaping offspring’s development (Gentile, 2015; Stein et al., 2014). Importantly, both depressed mood and Selective Serotonin Reuptake Inhibitor (SSRI) antidepressants used as treatment (Vigod et al., 2016b) are associated with socio-emotional, behavioral and cognitive developmental risks across infancy and childhood (Brummelte et al., 2017; Hanley et al., 2015; Rotem-Kohavi and Oberlander, 2017; Talge et al., 2007). Distinguishing effects of these two key gestational exposures is challenging, but critical for understanding the differential mechanistic pathways of each and for helping clinicians and mothers decide the best treatment option.

Recent studies using MR imaging have now begun to shed light on early developmental alterations related to prenatal exposure to maternal mood. Rifkin-Graboi and colleagues (Rifkin-Graboi et al., 2013) reported that prenatal depression is associated with reduced microstructure, but not with the volume of the right amygdala in newborn infants. Qiu and colleagues (Qiu et al., 2015) reported hyperconnectivity of the amygdala with the left temporal cortex, insula, anterior

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cingulate with ventromedial frontal cortices with higher levels of pregnancy-related maternal depressive symptoms (Qiu et al., 2015). Altered functional connectivity of the amygdala was also reported by Posner and colleagues (Posner et al., 2016).

Associations between prenatal SSRI exposure and brain function and structure during early infancy are also emerging (Jha et al., 2016; Lugo-Candelas et al., 2018; Podrebarac et al., 2017). Lugo-Candelas and colleagues (Lugo-Candelas et al., 2018) reported that infants exposed to SSRIs in utero had concomitant increases in amygdala and insula volume correlated with increases in white matter connectivity relative to infants of untreated depressed mothers and control infants (Lugo-Candelas et al., 2018). Jha and colleagues (Jha et al., 2016) reported widespread microstructure reduction in prenatally SSRI-exposed compared to control infants, while Videman and colleagues (Videman et al., 2016), using electroencephalography (EEG), demonstrated alterations of connectivity patterns associated with prenatal exposure to SSRIs. In addition, we have reported that prenatal SSRI exposure is associated with hyperconnectivity in the putative auditory network relative to control infants and to infants of non-pharmacologically-treated depressed mothers (Rotem-Kohavi et al., 2019b). Together, these findings suggest that prenatal depression and prenatal SSRI-exposure are associated with microstructural and functional alterations mostly in the insula, anterior-cingulate and the amygdala. However, to date a network perspective has not been used to study the impact of prenatal depression and SSRI exposure.

Graph theory analysis (GTA) views the brain as a network, where nodes are brain regions and edges are the pathways (structural or functional) connecting them (Bullmore and Sporns,
The relationship between nodes and edges offers insight into the topological properties of functional organization and efficiency of brain networks (Sporns et al., 2004), with optimal communication in “small-world” networks, comprising both local segregation and global integration (Watts and Strogatz, 1998). GTA applied to rs-fMRI has been used to characterize the functional topography of the human brain from the fetal period through to adulthood (Bathelt et al., 2013; De Asis-Cruz et al., 2015; Gao et al., 2015b; Thomason et al., 2017; van den Heuvel et al., 2018).

Wen and colleagues (Wen et al., 2018) have shown that during the first year of life the modular organization of the brain functional network is progressively increasing and subdividing into an increasing number of functional modules together with stabilizing intra-modular connection and clustering and inter-modular connections. Patterns of gradual increase in inter-modular connectivity have also been demonstrated in fetuses with increasing gestational age (Thomason et al., 2014).

Using GTA, studies have reported the existence of highly connected brain regions, called “hubs”, in both adults (Bullmore and Sporns, 2009), infants (Gao et al., 2015b) and fetuses (van den Heuvel et al., 2018). Hub nodes have a greater impact on information traffic within the network and are critical in facilitating efficient neuronal communication (Bullmore and Sporns, 2012). It is hypothesized that hubs underlie complex cognitive processes through their high connectedness and ability to integrate information (Bullmore and Sporns, 2009; Fransson et al., 2010). However, hub regions are more vulnerable to pathology (Bullmore and Sporns, 2012). For example, the insula, anterior and posterior cingulate and dorsolateral prefrontal cortex—all hub regions—are considered “at-risk” in depressed adults, suggesting that hubs are more
susceptible than regions with lower impact on information flow (reviewed in Gong & He., 2015).

While evidence on the effects of in utero influences on brain function (Qiu et al., 2015; Rifkin-Graboi et al., 2013; Salzwedel et al., 2015) and behavior (Hanley et al., 2015; Oberlander et al., 2007; Weikum et al., 2013b) have been well demonstrated, studies using measures of brain function to predict behavioral outcomes are limited. Recently, Yoshida and colleagues (Yoshida et al., 2017) showed that in adults positive correlations between the default mode network and superior frontal gyrus were predictive of depressive symptoms. Moreover, Rifkin-Graboi and colleagues (Rifkin-Graboi et al., 2015) in a prospective, birth cohort study found that lower levels of microstructure of the right insula, middle occipital and temporal regions of newborn infants may predict externalizing behaviors at one year, but findings were not significant after correction for multiple comparisons.

In the present study, we applied GTA to characterize organization of the resting state networks (RSNs) in the neonatal brain, to the data previously reported by Rotem-Kohavi et al (Rotem-Kohavi et al., 2019b). Our first aim was to describe network alterations associated with prenatal exposure to maternal depression and/or SSRI exposure compared with control infants. Given previous functional connectivity findings (Posner et al., 2016; Qiu et al., 2015) we expected the impact of prenatal maternal depression would differ from SSRI exposure, however based on the specificity of our previous results showing a localized effect (Rotem-Kohavi et al., 2019b), we did not expect significant differences at the global level. We hypothesized that prenatal depression would associate with increased hub values of key stress-related regions such
as the amygdala, insula, and anterior-cingulate compared to controls. However, prenatal SSRI-exposure would be associated only with higher hub values of regions related to auditory function (Rotem-Kohavi et al., 2019b). Secondly, we aimed to test whether hub measures defined by GTA at 6 days, predicted mothers’ rating of infant temperament at 6 months of age. Due to the critical role hubs play in cognitive function (Bullmore and Sporns, 2012), we expected that prenatal exposures would shape associations between newborns’ hub values and infants’ behavior at 6 months. Since no other studies have examined links between GTA measures with infant temperament in this context, we chose a non-biased approach for analysis.

7.2 Method

7.2.1 Participants

With informed maternal consent and approval from the University of British Columbia Clinical Research Ethics Board and the BC Women’s Hospital Research Review Committee, women were recruited at their second trimester of pregnancy, from family physician clinics, Reproductive Mental Health Clinic, and Midwifery Services from Vancouver, Canada. Healthy pregnant women, depressed pregnant women with no pharmacological treatment, and depressed pregnant women treated with SSRIs were recruited. Upon enrolment, the Mini-Neuropsychiatric Interview (MINI) (Lecrubier et al., 1997) was used to determine depression diagnoses. If the participant met the criteria for a unipolar depressive mood disorder per DSM-5 clinical criteria and was previously diagnosed by a qualified physician, the participant was included in the depressed-only group; mothers that did not meet these criteria were included in the control group. The SSRI group comprised women who had been diagnosed with a unipolar mood disorder, were treated with SSRIs for a minimum of 75 days during the third trimester of
pregnancy based on their clinical need. Women treated with SSRIs (fluoxetine, paroxetine, sertraline, citalopram, citalopram), and also with selective-norepinephrine reuptake inhibitors (SNRI) (desvenlafaxine, duloxetine, and venlafaxine) were included. For reasons of simplicity, we will not differentiate between these two pharmacological treatments. Only healthy, term pregnancies with one fetus were included in the study. Mothers with substance abuse, bipolar disorder, and those with significant medical, obstetrical, or fetal conditions were excluded from the study.

7.2.2 Maternal mood symptoms

We assessed prenatal maternal mood at 26 weeks and 36 weeks gestation using the Hamilton Rating Scales for Depression (HAM-D) (Hamilton, 1960). HAM-D depressive symptom scores served to create 3 groups (Zimmerman et al., 2013): infants with maternal HAM-D below 8 (control), infants of depressed mother with HAM-D ≥8 with no SSRI exposure (depressed-only), and infants exposed to in utero SSRIs (SSRI). To assess an additional distinct dimension of pregnancy-related maternal emotional experience, we also used the Pregnancy Experiences Scale (PES) (DiPietro et al., 2004). PES assesses pregnancy-related maternal uplifts and stressors to evaluate how women experience their pregnancy and was included in the GTA as a covariate.

7.2.3 Infant temperament at 6 months

At 6 months of age, mothers reported on their infant’s ability to regulate stress using the Infant Behavior Questionnaire (IBQ) (Rothbart, 1981) which is a widely used caregiver-report questionnaire consists 94 questions tapping on specific infant behaviors. These questions
produce six scales assessing temperament dimensions of: activity Level (Level of gross motor activity) smiling & laughing (as indicators of arousal under safe conditions) distress Latency (defined as acceptance or rejection of new objects or persons), distress Limitation (persistence and goal orientation), soothability (adaptability, how easily the baby is able to soothe), duration of orientation (measure both attention span and distractibility). Indicators of internal consistency for the IBQ have ranged from .67 to .84 (Rothbart, 1981). Parent-report temperament measures are recognized for their ability to provide data on infant behavior across settings (Gartstein et al., 2012), with IBQ items constructed to minimize “global judgments” of infant behaviors that may introduce bias (Gartstein and Rothbart, 2003; Rothbart, 1981).

7.2.4 MR image acquisition

All scans were performed at the BC Children’s Hospital MRI Research Facility in Vancouver, BC Canada. Infants were fed, swaddled and positioned in an MR-compatible neonatal incubator (Advanced Imaging Research, Inc dba SREE MEDICAL SYSTEMS, Cleveland, OH, USA) cushioned with pillows. Ear protectors and ear muffs were used to reduce noise from the MRI. Physiologic measures of heart rate and oxygen saturation were monitored by a registered pediatric nurse during the study. Infants underwent structural, microstructural, resting-state functional and metabolic imaging at 40.9 weeks (postmenstrual age). We recruited 95 women to the study, 11 woman/infant dyads did not complete the imaging component of the study and were excluded (see S1), 84 infants underwent MR scanning during natural sleep (n = 31 control, n = 24 depressed-only, n = 29 SSRI) without sedation using methods described previously (Rotem-Kohavi et al., 2019b).
Resting-state fMRI scan was acquired: oblique axial EPI, TR = 3000 ms, TE = 18.4 ms, flip angle = 90°, 39 interleaved slices, 2 mm isotropic, no gap (Rotem-Kohavi et al., 2019b). T1 structural images were acquired using a 3D fast spoiled gradient echo scan (TE = 2.95 ms, TR = 7.7 ms), with a voxel size of $1 \times 1 \times 1 \text{ mm}^3$ and reconstructed to 0.4 mm and were used to align the functional images.

### 7.2.5 Image preprocessing

We preprocessed rs-fMRI as described in (Rotem-Kohavi et al., 2019b). In short, fMRIB Software Library was used for preprocessing (FSL, www.fmrib.ox.ac.uk/fsl). FEAT (FMRI Expert Analysis Tool) v6.0, was used to remove non-brain structures with BET (Stephen M. Smith, 2002), correct for slice timing, spatial smoothing (3-mm FWHM), intensity normalization, and high-pass temporal filtering (sigma 50 s). FSL Melodic (Beckmann and Smith, 2004) and AFNI 3dDespike (Cox, 1996) were used for motion correction. FLIRT was used to register functional images to standard space (T1-weighted MR image of 40 weeks gestation neonatal template (Jenkinson and Smith, 2001; Serag et al., 2012). Data from 53 infants were included in the final analysis. For an overview of the inclusion and exclusion of mother/infant dyads, see the flowchart illustrated in Figure 7-1.
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Figure 7-1 Study flow chart.

An overview of the inclusion and exclusion of mother/infant dyads in the study

Functional images from 21 infants were excluded due to motion (sustained motion n=3, and infant woke up during the scan n=5), susceptibility (n=6) and ghost (n=1) artefacts, and errors in data acquisition (n=6). 10 additional infants did not go through resting-state functional imaging and were excluded from the study (n=5 SSRI-exposed infants, n=3 depressed-only). Excluded infants were similar in all demographic measures to infants included in the final
analysis ($p > 0.07$, Table B-1 Demographics of the entire sample, and infants excluded from the study). To evaluate motion parameters in our data we followed recent guidelines for the analysis of neonatal (rs)fMRI (Mongerson, Chandler et al., 2017). We used two motion parameters using MCFLIRT (Jenkinson et al., 2002); absolute displacement (AD) which compares the transformation matrix at time-point N with a reference time-point, and relative displacement (RD) which compares two subsequent time-points. For most subjects ($n=48$), only minimal motion was exist throughout the scan with a maximum values of AD of less than 1mm with mean absolute displacement of $0.282 \pm 0.20$ mm, and relative displacement of $0.092 \pm 0.05$ mm. Additional five subjects ($n=3$ SSRI-exposed, $n=1$ depressed-only) had one or two brief episodes of large (ranged from 1 to 2.94 mm). However, following denoising with MCFLIRT (Jenkinson et al., 2002)and AFNI 3dDespike (Cox, 1996) AD for these 5 infants ranged between 0.02 to 0.06. Overall, all 53 subjects AD ranged between 0.05 mm to 2.94 mm (mean = $0.433 \pm 0.55$) and relative displacement (RD) ranged from 0.03 to 0.69 (mean = $0.11 \pm 0.1$). No significant group differences were detected ($p > 0.1$, using one-way ANOVA). Following denoising, AD values were reduced and ranged between 0.001 to 0.15 (mean = $0.032 \pm 0.025$) and RD ranged from 0.001 to 0.04 (mean = $0.019 \pm 0.01$), with no group differences ($p > 0.7$ using one-way ANOVA).

Following preprocessing, we defined 90 cortical and subcortical regions according to the Automated Anatomical Labeling (AAL) (Tzourio-Mazoyer et al., 2002) algorithm of the Edinburgh Neonatal Atlas ENA33 segmentation tool (Cabez et al., 2016). According to Cabez and colleagues the ENA33 tool was transformed from an adult atlas, so it is consistent with adult label protocols, and the size of each brain region in the atlas corresponds to its actual size in the neonatal brain. In addition, ROIs in the left and right hemisphere were symmetric. The 90
regions were then individually mapped onto the residual fMRI time series coregistered to a 40 weeks gestation neonatal template (Serag et al., 2012). To generate the individual subject graphs, data were extracted from all of the voxels within the 90 regions to create a time by voxel matrix. For each graph, voxels within each of the 90 regions were averaged to create a correlation matrix between each of the 90 regions or nodes. This yielded a $90 \times 90$ undirected weighted correlation matrix which was then normalized using Fisher's $z$-transformation in Matlab (MathWorks Inc., Natick, MA).

#### 7.2.6 Graph metrics

We used the Brain Connectivity Toolbox (Rubinov and Sporns, 2010) for the computation of graph metrics. Our focus was on better understanding the global network organization of the newborns’ brains as well as the role of single nodes in this network. To this end, we chose the GTA parameters “Modularity” which evaluates how well the brain is divided into sub-modules, and to which sub-module each region belongs to (Girvan and Newman, 2002). We also measured “Global cluster coefficient” which informs us on the degree to which different nodes tends to connect to their neighboring nodes, and how well the neighbors tend to connect to each other on the global level (Achard and Bullmore, 2007). At the nodal level, we were interested to quantify the importance of each single node for the communication between the modules (“Participation coefficient” or connector hub), as well as its role in the communication within its own module/subnetwork (“Within-module degree z-score” or provincial hub) (Fan et al., 2011; Guimera and Amaral, 2005). Higher connector or provincial hub values indicate a node’s “hubbiness” and ability to play a greater role in the inter/intra-module communication. Additional graph measures examined are described in Appendix C.
7.2.7 Statistical analysis

To examine group difference in global GTA measures, we used GLM, with sex, infant age at the MRI, and PES scores as covariates in the model. To determine whether a node could be considered a hub region, we examined the difference in the group average of positive and negative weights to delineate quartiles in our data. Only nodes at the seventy-fifth (75) percentile and above were considered as hub regions and were included in a multivariate GLM model (Chan et al., 2017). For GLM analyses, Bonferonni-Holm methods (Abdi, n.d.) were used to correct for multiple comparisons.

7.2.8 Behavioral measures prediction at 6 months of age

PLSR (projection to latent structures regression) was performed using the methods outlined in Abdi (Abdi, 2010). PLSR is a latent variable approach to modeling the covariance structure between predictor (\(X\)) and predicted (\(Y\)) variables, whereby the latent variables of \(X\) best explain the variance in \(Y\). PLSR can be used in cases where there is multicollinearity in \(X\)—a case when standard multivariate regression will fail. In the current case, the provincial and connector hub values were included as the predictors with group treatment contrasts added. The predictor set was then used to predict mothers’ ratings of their infant’s behavior at 6 months of age using the IBQ (Gartstein and Rothbart, 2003).

7.3 Result
7.3.1 Participant characteristics

Maternal and neonatal characteristics are summarized in Table 6-1. Mothers treated with SSRIs and mothers belonging to the depressed-only group had significantly higher PES hassle intensity scores compared to control mothers.
At age 6 months, infant IBQ measures were available for 48 infants (summarized in Table 7-1). No group differences were observed ($p > .085$).

<table>
<thead>
<tr>
<th></th>
<th>Control (n=17)</th>
<th>SSRI (n=17)</th>
<th>Depressed-Only (n=14)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity Level</td>
<td>4.04 ± 0.771</td>
<td>4.00 ± 0.748</td>
<td>4.38 ± 0.665</td>
<td>0.307</td>
</tr>
<tr>
<td>Smiling &amp; Laughing</td>
<td>4.56 ± 0.624</td>
<td>5.01 ± 0.663</td>
<td>5.03 ± 0.703</td>
<td>0.085</td>
</tr>
<tr>
<td>Distress Latency</td>
<td>2.47 ± 0.66</td>
<td>2.29 ± 0.48</td>
<td>2.55 ± 0.67</td>
<td>0.469</td>
</tr>
<tr>
<td>Distress Limitation</td>
<td>3.23 ± 0.731</td>
<td>3.70 ± 0.77</td>
<td>3.52 ± 0.665</td>
<td>0.176</td>
</tr>
<tr>
<td>Soothability</td>
<td>5.21 ± 0.71</td>
<td>5.21 ± 0.744</td>
<td>5.27 ± 0.674</td>
<td>0.967</td>
</tr>
<tr>
<td>Duration of Orientation</td>
<td>3.86 ± 0.706</td>
<td>4.11 ± 1.10</td>
<td>3.56 ± 0.898</td>
<td>0.254</td>
</tr>
</tbody>
</table>

Table 7-1 IBQ-Measures at 6 months

All measures are showing mean values ± Std.Deviation, P values are corrected for multiple comparisons (Bonferroni)

### 7.3.2 Graph measures

Mean connectivity matrices for each group are shown in Figure 7-2 (left) and revealed no anti-correlations but showed qualitative differences between the control group and the SSRI and depressed-only groups. We found no significant group differences in global clustering coefficient measures ($p > 0.5$, univariate GLM). Using the Louvain algorithm (Blondel et al., 2008), we identified three modules in each of the groups (see Figure 7-2, right). Module 1 comprised mostly primary, frontal regions and the insula. Module 2 comprised mostly limbic, occipital and parietal regions. Module 3 comprised basal ganglia, auditory and temporal regions.
No significant differences were found in modularity levels between groups ($p > .80$, univariate general linear model). However, subtle, qualitative differences were observed in the community structure of infants from the depressed-only group compared to control and SSRI groups. While module 3 in the depressed-only group included the hippocampus and parahippocampal regions, those regions were included in module 2 in the control and SSRI groups.

**Figure 7-2 Group connectivity matrices.**

Left: The averaged unthresholded correlation matrix shows subtle differences between groups. In these correlation matrices, the blue color represents lower correlations, and red color represents higher correlations between 90 subcortical and cortical regions (Cabez et al. 2016). Brain connectivity toolbox was used to create the matrices (Rubinov & Sporns 2010). Right: Using the Louvain algorithm, we identified 3 modules in each group. Sub-modules within the network, averaged for control (upper), depressed-only (middle), SSRI (lower) groups. Green,
blue and red represent the different sub-modules within the network. Modularity level did not differ between groups ($p > .800$, general linear model). Subtle differences (red circles) were observed in the community structure of the depressed-only compared to control and SSRI groups.

To evaluate the ability of a node to serve as a hub within the network, two measures were considered: the participation coefficient, which evaluates the ability of a node to serve as a connector (inter-modular) hub; and within module degree, which evaluates the ability of a node to serve as a provincial (intra-modular) hub. Connector hubs included the Heschel's gyrus bilaterally, the rectus, insula, anterior cingulate, superior temporal caudate, putamen, pallidum, right thalamus, and right operculum (Table C-2). Provincial hubs included the left Heschel’s gyrus, bilateral olfactory, caudate, putamen and pallidum, right angular gyrus, right fusiform gyrus, left rectus, bilateral posterior cingulate, left superior occipital gyrus, right cuneus, right and middle anterior cingulate gyri, bilateral amygdala, and left superior and middle orbital gyri (Table C-3).

For connector hub values, a multivariate GLM analysis corrected for sex, age at the MRI and PES scores revealed a significant group effect in the left insula ($F(5, 47) = 3.826, p = .029$, $\eta^2_p = .140$), left anterior cingulate ($F(5,47) = 5.874, p = .005$, $\eta^2_p = .200$), left caudate ($F(5,47) = 5.207, p = .009$, $\eta^2_p = .181$) adjusted p values). Pairwise comparisons showed group differences in the left caudate, anterior cingulate, and insula, stemming from significantly higher values in infants of the depressed group compared to the control group, while differences between the SSRI and control or the depressed group were not significant (see Error! Reference source not found., Table C-4 and Table C-5).
A multivariate GLM analysis corrected for sex, age at the MRI, and PES revealed group differences between Depressed-only and Control groups, Bonferroni-corrected for multiple comparisons, also see Table C-5.

For provincial hubs, a multivariate GLM analysis corrected for sex, age at the MRI and PES scores revealed a significant group effect in the right medial frontal orbital $F(5,47) = 3.494, p = .038, \eta_p^2 = .129$, left amygdala $F(5,47) = 3.192, p = .050, \eta_p^2 = .120$, and left Heschl's gyrus $F(5,47) = 5.345, p = .008, \eta_p^2 = .185$, (Bonferroni-corrected). Pairwise comparisons showed that differences in the left amygdala stemmed from higher provincial hub levels in infants of the depressed group compared to the control group, while for left Heschl's gyrus and the right medial frontal orbital gyrus, infants in the SSRI group had significantly higher levels compared to the depressed group (see Figure 7-4, Table C-6, and Table C-7).
A multivariate GLM analysis corrected for sex, age at the MRI, and PES revealed group differences between Depressed-only and Control groups for the Amygdala, and between SSRI and Depressed-only for the right frontal orbital and left Heschl gyrus corrected for multiple comparisons, Bonferroni, also see Table C-6, and Table C-7)

### 7.3.3 PLSR

PLS regression was applied to the 90 provincial and 90 connector hub values. This analysis revealed that hub values representing the 75th percentile of provincial and connector hubs best predicted mothers’ ratings of their infants’ temperament at 6 months of age. Thus, with the goal of reducing the number of variables in the prediction model, only 44 regions of interest (22 provincial hubs and 22 connector hubs) were used as predictors in subsequent analyses (see Table C-2 and Table C-3).
To determine the number of factors to take into account, we used the ratio of the residual sums of squares and predicted residual sum of squares (Abdi, 2010). Factors with values above a threshold of 0.0975 were considered for further interpretation (Abdi, 2010), resulting in a prediction model based on a single factor. This single factor model predicted 11% of the variance in mothers’ ratings using all subscale behavioral measures (activity level, smiling and laughter, distress latency, distress of limitation, soothing and duration of orientation; see Table 7-1). Provincial hubs, including the left superior temporal gyrus and Heschl’s gyrus bilaterally, best predicted orientation ratings, whereas lower connector hub levels in the right fusiform and right mid-cingulate regions best predicted ratings of smiling behaviour, activity levels and distress to limitations subscales in a way that is more closely related to the SSRI and depressed-only groups compared to the control group (see Figure 7-5.). Together, these findings suggest that early local connections of provincial hubs in the auditory regions, associated with prenatal exposure to depressed mood and SSRI, predict later orienting behaviour, while more long-range connections of connector hubs of visual and frontal regions are associated with subsequent smiling and reaching behavior.
Figure 7-5 PLSR results

PLSR results - Y (predicted) in the space of W (X variables). Dark green circles represent provincial hubs, and red circles represent connector hubs. Blue circles of a, b, and c represent depressed, SSR and control groups respectively. This model predicted 11% of the variance in mothers' ratings on the IBQ (in light green: activity level, smiling and laughter, distress latency, distress of limitation, soothing and duration of orientation, see Table 7-1).
7.4 Discussion

This study examined the impact of prenatal exposure to maternal depression and SSRI antidepressants on the characteristics of newborn’s functional networks. We found that each exposure is associated with different characteristics of the RSN topology extracted using a graph computational approach, applied on rs-fMRI data of infants at 6 days of age. Differences between groups were not evident at the global level; both global clustering coefficient and the overall division of the functional network into sub-modules were similar between groups. In particular, the general network community structure reflected by modularity and clustering coefficient, was not associated with prenatal maternal exposure to either depression or SSRIs, suggesting no global differences between the groups. Similarly, in adults, major depression did not correlate with clustering coefficient (Zhang et al., 2011), but in contrast to our results, major depression did correlate with increased network modularity compared to healthy controls (Ye et al., 2015). Also, symptoms severity (reported using HAMD) was positively correlated with modularity (Ye et al., 2015). As modularity has been shown to gradually increase in the first year of life (Wen et al., 2018), it might be that alterations in global modularity do not exist in the neonatal brain but might develop over time. Also, mothers in our cohort showed only mild depressive symptoms (See Table 1). Thus, it could be that symptoms severity was not adequate to alter global network modularity.

When the regional characteristics—which provide information at the nodal level—were examined, a different picture appeared. At first, we identified both connector hubs, which are thought to play an important role in integrating information from one module to other modules in the network (Rubinov and Sporns, 2010) and provincial hubs, hypothesized to serve as a relay.
station for information flow, exerting their influence mostly within their own sub-module. Our data showed that the identified connector and provincial hubs largely overlap with regions previously described as hubs in the neonatal and fetal brain (De Asis-Cruz et al., 2015; Gao et al., 2011; van den Heuvel et al., 2018) and also in the adult brain (Gong and He, 2015; He et al., 2009).

Importantly, our analysis revealed increased connector hub values in the left caudate, insula, and anterior cingulate in infants in the depressed-only group, relative to the control group while the SSRI group did not differ from the other groups. In addition, exposure to maternal depression was also associated with higher provincial hub values in the amygdala. Independently, prenatal SSRI exposure had an impact. Provincial hub values were significantly higher in Heschl's gyrus and the right medial frontal orbital gyrus, compared to the depressed group. Together, these findings appear to reflect a regional specific differential susceptibility to maternal depression and SSRI exposure. Similarities between control and SSRI groups in connector hubs suggest that antidepressants may have a ‘corrective’ effect on early brain development that occurs with exposure to prenatal maternal depressed mood. Whereas, differences in provincial hub connectedness between SSRI exposed and depressed non-exposed infants may reflect a direct impact of SSRI exposure.

Using PLSR on a subset of infants, we have shown that hub values of connector and provincial hubs extracted from resting-state fMRI at 6 days of age accounted for more than 10 percent of the variance in temperament between infant groups at 6 months of age. Importantly, the regions that best predicted temperament subscales are critical to early orientation and face
recognition. Namely decreased connector “hubbiness” in the right fusiform and right midcingulate regions predicted smiling behaviour at 6 months, while auditory region connectedness levels were the best predictors of orienting behavior, in a pattern which is more closely associated with the SSRI and depressed-only groups than the control group. These findings support the importance of early brain connectivity in creating foundations for later cognitive and social-cognitive functions (Bullmore and Sporns, 2012), and highlights possible differing developmental trajectories between SSRI exposed and depressed-only, and control infants (Hanley et al., 2013; Weikum et al., 2013b). Nonetheless, the small predictive value of hub regions in relation to subsequent behavior suggests that additional biological factors and ongoing environmental influences also play a role in shaping infants’ social-emotional development.

Our findings are consistent with reports from animal studies (Bonnin et al., 2012; Simpson et al., 2011). Prenatal stress is shown to be associated with increased numbers of neurons in the anterior cingulate which were labeled with c-Fos (a marker for neuronal activation), suggesting a link between prenatal stress and hyperconnectivity of the anterior cingulate (Rosene et al., 2004). In addition, larger numbers of amygdalar glial cells and neurons have been reported in prenatally stressed adult rats (Salm et al., 2004). In animal models of prenatal exposure to SSRIs, alterations of the premature 5HT circuitry were correlated with the aberrant connectivity of the raphe and callosal, alterations in processing patterns of sensory and auditory information, and in the development of myelin (Bonnin et al., 2012; Simpson et al., 2011). However, the relationship between the alterations in 5HT circuitry demonstrated in animal models and the neurodevelopmental consequences in humans are still not clear. Importantly, our findings are consistent with the adult literature related to depression (Connolly
et al., 2013; Meng et al., 2014; Ramasubbu et al., 2014) showing altered connectivity architecture of the ACC, amygdala, insula, and basal ganglia, which are all stress regulatory brain regions. Thus, the intrauterine environment associated with depressed but non-pharmacologically treated mothers may shape the development of the neural circuits in a way that might increase their vulnerability for future depression.

Our findings are consistent with previously reported associations between elevated prenatal maternal depressive symptoms and greater connectivity of the left amygdala with the ACC and left insula in 6-month-old infants of mothers with depression during pregnancy (J. Qiu et al., 2017). Our results suggest that these brain regions are hyperconnected, and that such connectedness is organized in ways that enable increased efficiency in supporting neuronal communications in the resting-state network of infants of depressed mothers. Further, resting-state hyperconnectivity might be linked to a disproportionally heightened response of the amygdala and insula to negative valance expressions such as fearful, angry, or sad faces which have been demonstrated (Surguladze et al., 2005). Such negative biases processing emotions and information are critical to the etiology and maintenance of depression (Beck et al., 1979). Our findings, coupled with previous reports (Qiu et al., 2015; Rifkin-Graboi et al., 2013), as similar patterns of hyperconnectivity were found in the fetal period - may shed light on the fetal origins of these psychological disturbances. Our evidence of the impact of fetal exposure to maternal depression confirms that in-utero exposures shape functional brain development (Belsky and Pluess, 2009) in ways that may increase risk for subsequent psychopathology (Qiu et al., 2015).
Our results also point to a SSRI fetal programming effect that might help elucidate the developmental impact of SSRI exposure. Using a data driven analytical approach, we found higher hub values in Heschl’s gyrus among SSRI group compared to the depressed-only group, consistent with our previous report of hyperconnectivity in the auditory network with prenatal SSRI exposure (Rotem-Kohavi et al., 2019b). These findings suggest a more efficient flow of information in local regions connected to Heschl’s gyrus in SSRI exposed infants and support the accelerated auditory language perception development Weikum and colleagues (Weikum et al., 2012) observed in SSRI-exposed infants, which was already evident at 36 weeks gestation (Weikum et al., 2012). While a causal relationship cannot be determined, hyperconnectivity within the auditory network, together with higher hub values in Heschl's gyrus with SSRI exposure, reveals functional connectivity change that could potentially reflect the accelerated onset of speech perception observed in fetuses and infants exposed to SSRIs. Though, it is not clear yet whether this language perception shift is beneficial or detrimental to language development.

The hub values differences we found are lateralized, and were demonstrated only for the left hemisphere. Previous reports have reported lateralized differences in neonatal functional connectivity following prenatal exposures. Left lateralization of language-networks has been reported in newborn infants (Dehaene-Lambertz et al., 2002), suggesting higher susceptibility of left lateralized auditory regions. However, while Qiu and colleagues (Qiu et al., 2015) reported increased vulnerability of the left side, others have reported alterations on the right side (Posner et al., 2016). Further investigations are needed to understand the nature of these inconsistencies.
Importantly, mothers treated with SSRI could have inherently different characteristics compared to mothers with depression not to be pharmacologically treated. Although we used two different control groups for the SSRI-treated group (depressed only non-SSRI treated and non-depressed) we could not rule out the effect of residual confounding, such as variations in depressive symptoms, and genetic variations related to mood disturbances which also confer developmental risk for the child. Our study provides a glimpse into functional topology of early brain development, but it is limited by measurements at only one time point. Additional studies with longitudinal designs are needed to determine whether these in utero exposures have long term associations with functional topology.

Conclusion

Our findings support the hypothesis that the environment inside the womb has a critical role in preparing the fetus for life following birth (Barker, 2000). We report differences in the functional network topology for the different exposures, which implies that different in utero conditions might set a path to different developmental trajectories that may increase or reduce developmental risk.

At present, it remains unclear how – at a neural level - the impact of SSRIs in the context of maternal depression during gestation differs from the impact of depression alone. Both prenatal factors lead to similar behavioral disturbances, presumably reflecting altered central serotonin signaling. SSRIs readily cross the placenta (Rampono et al., 2009) and the blood-brain barrier, and have been shown both in animal models (Rampono et al., 2009) and in human studies (Laine et al. 2003; Hilli et al. 2009; Davidson et al. 2009) to alter 5HT signaling in the
fetus. It has been hypothesized that acute in utero SSRI exposure leads to higher levels of serotonin in the fetal brain, and in the long term leads, via negative feedback, to constrained development of serotonin circuitry, reduced serotonergic tone and lower effective levels of serotonin in the brain during development (Oberlander et al., 2009). Moreover, given 5HT’s role as a trophic factor regulating various aspects of fundamental developmental processes such as cell growth, differentiation, migration, myelination, synaptogenesis and pruning (Gaspar et al., 2003), it is conceivable that changes in the levels of 5HT as a result from exposure to SSRI in those critical periods of brain development before birth might influence developmental pathways differently from the in utero exposure to depression. Differences in behavioral outcomes between depressed only and SSRI exposed infants and children have been reported (Skurtveit et al., 2014; Weikum et al., 2013b, 2012), however, the neural correlates reflecting these outcomes and clinical implications still need to be determined. Further, using PLSR, our findings provide support for associations between region-specific hubs in the newborn brain and subsequent differences in developmental pathways for specific infant behaviors in the context of prenatal maternal depression and SSRI antidepressants. Whether these paths lead to particular long term developmental outcomes warrants additional investigation.
Chapter 8: Discussion

8.1 Overview of experimental findings

In this dissertation, I examined the functional connectivity of the typically developing infant brain in the first year of life and studied how functional brain connectivity is altered as a function of exposure to depression in mothers who were not pharmacologically treated (for the sake of the discussion, I will refer to them as “depressed only”) or treated with antidepressants (“SSRI”). Specifically, I compared the brain’s response during the observation of emotional facial expressions in typically developing 8-to-10-month-old infants and healthy adults using graph theory analysis applied to EEG recordings. I also characterized the associations between exposure to depression only or exposure to SSRI and alterations in the brain response to the observation of emotional facial expressions in the infant. In addition, I characterized resting-state functional connectivity in the neonatal brain using rs-fMRI and graph theory analysis in typically developing newborns and in newborn infants exposed to depression only or to SSRI during pregnancy. The following novel and key findings emerged from these studies:

1. Typically developing 8-to-10-month-old infants have similar network characteristics as adults in response to observing basic emotions. Infants exhibited similar global features such as clustering and modular organization to adults, and the functional characteristics of the frontal and parietal nodes were similar between infants and adults. However, infants had higher network density as compared to adults (Rotem-Kohavi et al., 2017).

2. Newborn infants prenatally exposed to SSRIs exhibited hyperconnectivity in the putative auditory network compared to newborns of depressed only mothers and newborns of healthy
mothers (Chapter 6, Rotem-Kohavi et al., 2019) and higher provincial hub values in Heschl’s gyrus compared to the depressed-only group (Chapter 7, Rotem-Kohavi et al., 2019).

3. Newborn infants of depressed only mothers exhibited higher connector hub values in the left anterior-cingulate, insula, and caudate as well as higher provincial hub values in the amygdala compared to the non-exposed control group (Chapter 7, Rotem-Kohavi et al., 2019).

4. Pre and postnatal exposure to maternal mood was differently associated with alterations in functional connectivity in infants in response to viewing dynamic emotional faces. An SSRI drug-exposure effect was associated with higher modularity of the brain network in response to observing both negative and positive emotional faces. Increased symptoms of prenatal maternal depression were associated with reduced modularity for negative emotions (sadness and pain), but only in non SSRI-exposed infants. Further, increased postnatal mood was associated with changes in frontal hub values, which were emotion-dependent (Chapter 5, Rotem-Kohavi et al., under review).

Overall, these data indicate that typically developing infants are born with basic sensory networks that are functionally connected at birth, thereby confirming previous findings (Fransson et al., 2009; Gao et al., 2015a). 8-to-10-month-old infants appear to have in place the core functional brain organization associated with perceiving happy and sad expressions. Maternal depression and prenatal exposure to SSRI are associated with different functional RSNs and brain functional topology of the newborn. In addition, the data suggest that individual variability in prenatal exposures may have an impact on shaping emotional perception networks. Specifically, these data suggest that exposure to depression both prenatally and postnatally and
prenatal SSRI exposure may differentially shape the functional networks that support emotional perception during infancy.

8.2 8-to-10-month-old infants have similar network characteristics as adults in response to observation of basic emotions

The findings of this thesis suggest that the general organization of the functional networks for observing basic emotional faces of happy and sad expressions is already established in 8-to-10-month-old infants. Several key similarities were observed in the functional brain organization in response to happy and sad expressions between infants and adults. This is consistent with previous reports by Grossmann et al. (2007), who found that 12-month-old infants have enhanced early posterior negativity (EPN) response (reflecting early processing of emotional stimuli) to angry faces, which is similar to adults (Schupp et al., 2004). Using a network perspective, I show that infants and adults have similar characteristics in the functional network organization related to observing happy and sad expressions. Specifically, global modularity or stability of sub-networks and the level of network connectedness was similar between infants and adults. This suggests that at this stage, infants perceive basic emotions in a manner similar to adults.

Deen et al. (2017) reported on results from an fMRI study that compared the functional connectivity of the cortical brain response to faces between 4-to-6-month-old infants and adults. They found that the cortical response of infants has a spatial organization pattern, similar to the pattern of activation exhibited in adults. In agreement with the results reported by Deen et al. (2007), the results from this thesis show that similar to adults, infants show distributed activation
in multiple brain regions including the occipital, temporal, parietal and frontal regions. The results from this thesis add a network perspective such that hub distribution—prioritized parietal and posterior temporal nodes for integrating information—is similar between infants and adults.

Together, these findings suggest that from very early stages of development, basic features of functional organization underlying the perception of emotional faces are present across the cortex. Moreover, graph theory analysis reveals that the distribution of functional processing of emotional faces is similar between infants and adults.

The observation that the functional organization associated with specific emotional expressions is in place early in development raises the question of whether typically developing infants are able to discriminate and recognize basic emotional expressions by 8-to-10 months of age. Behaviourally, there is evidence that young infants can discriminate between static photos of different facial expressions—including happy and sad emotions—at the age of five months (Caron et al., 1988), and at 7 months, they can respond emotionally to both positive and negative emotions (Soussignan et al., 2018; Termine and Izard, 1988). Similarly, an ERP study revealed that 7-month-old infants were able to distinguish different sad faces from different happy faces (i.e., between-category), such that the Nc component—reflecting attention or sensitivity—had higher amplitude for a novel facial expression compared to the familiar facial expression from different categories (Leppänen et al., 2009). The results from this thesis provide additional network-perspective insights into the organization of the functional networks supporting observation of happy and sad faces by showing that these differences are subtle and stem from differences at the regional level, not at the global level. Together, based on current
literature and on the results from this thesis, I suggest that 8-to-10-month-old infants can recognize and discriminate between sad and happy emotions.

One feature of the functional networks that was different is the density of the number of actual connections to overall connections, which was higher for infants compared to adults. One interpretation for higher density is that the perceptual processing of emotional faces requires higher cognitive demand or effort in infants than in adults (Kitzbichler et al., 2011). It is possible that the visual processing of emotional faces in infants is less efficient, thereby activating more connections as compared to those in adults. During development, with increasing exposure to emotional faces, the functional network becomes more fine-tuned and efficient in perceiving emotional faces (Macchi Cassia et al., 2014). Thus, the results from this thesis support the proposed model of the development of emotion perception mechanisms suggested by Leppänen & Nelson (2009), according to which the emotional network undergoes refinement through stabilizing and preserving more frequently used connections while pruning less relevant, infrequently used connections (Macchi Cassia et al., 2014). Building on this view, this thesis reveals that infants still have excess connections that are expected to be eliminated during development to create a more efficient network for perceiving emotional faces.

Given that 8-to-10-month-old infants show a basic functional network organization related to emotion perception, this suggests that these networks are shaped in very early stages of development. This raises the question of whether the “clues” for emotion perception network can be found in earlier stages of development. This raises additional key questions regarding how environmental factors shape functional networks in infants, both prenatally and postnatally.
8.3 Characteristics of newborn RSNs

Using a data-driven approach, I studied overall MR-derived functional RSNs without limiting the analysis to specific brain regions or networks (Chapter 2: 6). As a first step, I created a neonatal functional template representing infants in this study and found that visual, sensorimotor, and auditory networks are already functionally synchronized at birth, which was a similar finding to previous reports of similar populations (Doria et al., 2010; Fransson et al., 2009; Gao et al., 2015a). This is believed to reflect an evolutionary optimization so that basic sensory functions—such as visual, auditory, and sensorimotor functions—begin functioning very early in life, as they are critical for survival (Gao et al., 2015a).

Interestingly, using graph theory analysis enabled the identification of hubs in regions such as the right fusiform gyrus, superior temporal sulcus, bilateral amygdala, and left superior and orbital gyri—all of which are regions suggested to form the emotion perception network (Leppänen and Nelson, 2009; Powell et al., 2018; Reynolds and Roth, 2018). This suggests that the neonatal brain is organized in a manner that prioritizes these regions for integrating information. This finding offers intriguing possible support for extensive behavioral research, thereby suggesting that infants are tuned to faces from birth (Farroni et al., 2013; Leppänen and Nelson, 2009; Reynolds and Roth, 2018) by providing evidence that the neonatal brain’s functional connectivity organization is primed for perceiving emotions. A model of non-human primate macaque monkeys showed that the structural anatomy of the amygdala and orbitofrontal regions emerges relatively early in development (Machado and Bachevalier, 2003). While Grimaldi, Saleem, & Tsao (2016) find that subcortical regions such as the amygdala have strong
connections with different parts of the temporal cortex related to face perception, Livingstone et al. (2017) did not find increased activation of the amygdala, but did find early functional organization of the STS for viewing faces by one month of age. Thus, although the current evidence is not consistent, models of non-human primates support the existence of key anatomical correlates associated with emotion perception in early stages of development, while a more complex wiring pattern develops over a longer period of time (Grimaldi et al., 2016; Leppänen and Nelson, 2009; Livingstone et al., 2017; Machado and Bachevalier, 2003). van den Heuvel et al. (2018) found hubs in the inferior temporal gyrus of the fetal human brain, which is in close proximity to the fusiform facial area, further emphasizing the importance of these regions as a relay station for information flow even before birth (van den Heuvel et al., 2018). This again suggests that the core organization for perceiving emotional faces is in place at early stages of development. An interesting question that I was unable to address in this thesis due to lack of statistical power is whether these hubs related to emotion perception in the neonatal brain would be able to predict alterations in emotion perception of 8-to-10-month-old infants. Future research with sufficient sample sizes could possibly address this question.

To summarize, this data suggests that basic sensory RSNs and key hubs for the emotion perception network already exist in the newborn phase. Thus, it is reasonable to speculate that the functional connectivity organization associated with these networks might be susceptible to the intrauterine environment, as a significant amount of its maturation occurs prenatally.
8.4 Prenatal maternal depression and prenatal SSRI program the infants functional network organization

Although hub regions are highly connected and serve as central building blocks for early brain development, they are also more vulnerable to pathology (Bullmore & Sporns, 2012). The results from this thesis suggest that prenatal exposure to maternal depression was associated with prenatal programming of hubs in functional RSNs of newborn infants (Rotem-Kohavi et al., 2019a). Specifically, I found that infants from the depressed only group showed higher connector hub values in the left anterior-cingulate, insula, and caudate as well as higher provincial hub values in the amygdala compared to the control group. These findings are in agreement with reports from animal studies, revealing that prenatal stress resulted in increased number of neurons in the anterior cingulate and suggesting a link between prenatal stress and hyperconnectivity of the anterior cingulate (Rosene et al., 2004). An increased number of amygdalar neurons have been reported in adult rats with prenatal stress (Salm et al., 2004). However, it is important to mention that the relationship between animal models of prenatal stress and possible implications in humans are still not well understood. Nonetheless, these findings show associations between exposure to prenatal mood disturbances and alteration in the functional connectivity organization of the ACC, amygdala, insula, and basal ganglia—all functioning as emotion and stress regulators (Gu et al., 2013; LeDoux, 2003; Stevens et al., 2011). Importantly, studies in depressed adults have demonstrated that all these regions play a key role in depression (Connolly et al., 2013; Meng et al., 2014; Ramasubbu et al., 2014). These findings support possible prenatal programming, related to the prenatal environment of non-pharmacologically treated depressed mothers that may shape the development of neural circuits in a manner that might pose higher risks or vulnerability for future depression. The findings from
this thesis are also consistent with the report by Qiu et al. (2015) on associations between elevated prenatal maternal depressive symptoms and greater connectivity of the left amygdala with the ACC and left insula in six-month-old infants. The current study adds to the existing findings and suggests that these brain regions are not only hyperconnected but that this connectedness is organized in a manner that enables increased efficiency in supporting neuronal communication in the RSNs of infants of depressed mothers. Intriguingly, the hub values of these regions did not significantly differ between infants exposed to prenatal SSRIs compared to control infants, thereby implying a possible “corrective” effect of exposure to SSRIs.

However, prenatal SSRI exposure is also associated with prenatal programming of the organization of functional RSNs. Newborn infants exposed to prenatal SSRI had increased hub values in Heschel’s gyrus, thereby suggesting increased ability to transfer information to other regions compared with the depressed only group. Moreover, the results from graph theory analysis provided additional support to the results from the first analysis using ICA. Specifically, I found that infants exposed to prenatal SSRI exhibited hyperconnectivity (i.e., increased functional connectivity) in the putative auditory network in regions that are part of the auditory network, such as the superior temporal cortex and parietal lobe. Hyperconnectivity was also exhibited in regions that are not part of the auditory network but have positive, though weak, associations with the auditory network, such as frontal and visual regions. The putative auditory network (which includes Heschel’s gyrus) is assumed to function as a pre-lingual network that, with maturation, develops into language processing regions. These results are in agreement with results from animal models of prenatal exposure to SSRIs, showing that alterations in the 5HT circuitry resulted in abnormal connectivity of the raphe and callosal and in abnormal processing
patterns of sensory and auditory information (Bonnin et al., 2012; Simpson et al., 2011). These results are particularly intriguing when considered in the context of findings from a previous study by Weikum et al. (2012) that shows that, prenatally SSRI exposed fetuses and infants had accelerated language perception compared to the control and depressed only groups. I suggest that hyperconnectivity and increased “hubinness” of the auditory network observed with rs-fMRI could offer at least a partial explanation to these accelerated language perception behaviours. It is not clear whether accelerated language perception in the first year of life (Weikum et al., 2012) or hyperconnectivity and increased “hubinness” of the auditory network reflect neurodevelopmental benefits that might be “advantageous” for subsequent language development in childhood. A number of studies have suggested long-term negative associations between prenatal SSRI exposure and language abilities (El Marroun et al., 2016; Johnson et al., 2016). For example, it was reported that prenatal SSRI exposure was negatively correlated with expressive language development (Johnson et al., 2016), poorer capabilities of verbal fluency (El Marroun et al., 2016), and increased risk of speech and language disorders, such as expressive language disorder and receptive language disorder (Brown et al., 2016). Longitudinal studies using both functional connectivity imaging and evaluation of language perception development and proficiency might be able to better address these questions. To summarize, although it remains unclear whether hyperactivation of the auditory network has potential beneficial implications, SSRI exposure does appear to mask at least part of the effects of in utero exposure to prenatal depression.

It is important to emphasize that the results from these two different types of analysis using ICA and graph theory analysis provide knowledge on different aspects of functional
connectivity organization. While I first used ICA as an explorative approach, which help to uncover the nontrivial components of interest (McKeown et al., 2003), graph theory analysis served as a complementary method to further explore the relationship and hierarchy between various brain regions. The results from this thesis could help in guiding future studies in generating new hypotheses regarding brain regions and networks that might be more vulnerable to prenatal depression and SSRIs.

In addition, the results from PLSR showed that hub values extracted from resting-state fMRI at six days of age accounted for over 10% of the variance in infant temperament at six months of age. This highlights the importance of early brain functional organization in shaping subsequent cognitive and social-cognitive functions (Bullmore & Sporns, 2012). It is important to mention that regions that were best at predicting temperament subscales are key regions involved in early orientation and face processing. Specifically, decreased connector “hubbiness” in the right fusiform and right mid-cingulate regions predicted smiling behaviour at six months, while connectedness levels in auditory regions were best at predicting orienting behavior and were more closely associated with the SSRI and depressed-only groups compared to the control group. This suggests possible differing developmental trajectories between SSRI exposed and depressed-only and control infants (Hanley et al., 2013; Weikum et al., 2013).

These findings suggest that postnatal maternal depression and prenatal exposure to maternal depression and maternal SSRI shape the functional networks of emotional observation as well. These results suggest that environmental exposure is distinctively associated with different patterns of functional organization. As hypothesized, a mother’s concurrent, postnatal
mood is associated with functional network organization. These results showed lower levels of betweenness centrality of frontal nodes for viewing happy and sad emotions, which reflects lower involvement of frontal regions in information transfer among infants of mothers with more depressive symptoms compared with infants of mothers with lesser symptoms. Based on previous evidence showing that infants of depressed mothers have more difficulties in discriminating between emotions (Bornstein et al., 2011; Hernandez-Reif et al., 2002), it is possible that lower frontal betweenness centrality indicates reduced frontal efficiency in processing emotion. Surprisingly, this pattern was reversed for painful emotions. Although the explanation to this reversal pattern is not clear, evidence from adult literature suggests that low mood is associated with reports of enhanced response to other’s pain (Cao et al., 2017). A heightened frontal synchronization is also found to be positively associated with maternal depression scores in response to the mother’s own infant cry (Swain et al., 2008). One interpretation could be that infants of depressed mothers show patterns of synchronization that resemble those in adults. However, although infants at this age have been shown to discriminate pain from another negative emotion—anger (Missana et al., 2014)—neither this thesis nor current literature can determine if at this stage infants can recognize distinct painful expressions.

The effects of the postnatal environment, particularly in the context of maternal personality and mood symptoms, on the development of emotion perception have been extensively studied. As infants mostly interact with their mothers in the early postnatal period, it is hypothesized that maternal personality and maternal mood symptoms may be closely related to the amount of exposure to different emotional faces in the day-to-day environment (Haan et al., 2004; Taylor-Colls & Pasco Fearon, 2015). For example, depressed mothers have been shown to
express more sad emotional faces versus happy faces when interacting with their infants (Cohn et al., 1990).

To a lesser extent, the effects of prenatal environmental exposures have been studied in relation to emotion perception (A. Porto et al., 2016). The results of this thesis suggest that an infant’s functional networks for emotion perception are not only shaped by the postnatal environment but that the prenatal environment also plays a role in emotion perception development. Prenatal SSRI exposure was associated with increased modularity organization, which was independent of the emotion observed, thereby suggesting a general effect rather than an emotion-specific effect. Further, decreased network modularity (less segregation) of infants not exposed to SSRI suggests that they invest more cognitive load while processing emotions as compared to SSRI exposed infants. This is supported by studies of depressed adults that report that SSRI treatment is associated with the experience of emotional blunting or “emotional constraint”, by which emotional responses to both enjoyable and aversive experiences are lessened (McCabe et al., 2010; Opbroek et al., 2002). Building on these findings, it could be that SSRI-exposed infants in the current study exhibited a more blunted general response to both positive and negative emotional faces. While facial perception cannot be directly studied in mice, experimental animal studies suggest that 5HT alters different types of social behaviors, such as aggression, avoidance, and reactivity to reward and sensory stimulation (Zald and Depue, 2001). It was suggested that 5HT influences these behaviours by inhibiting the neural processes of both positive and negative effects (Zald and Depue, 2001). Interestingly, in a social approach task in a mouse model, it was shown that mice with prolonged exposure to prenatal SSRI had decreased social contacts with an unfamiliar mouse and did not prefer to explore a social stimulus over an
empty cup (Maloney et al., 2018), which could suggest altered social communication. Moreover, the levels of prenatal depression symptoms were also associated with network modularity, such that increased depressive symptoms were associated with reduced modularity for negative emotions and increased modularity for positive emotions. This indicates that infants exposed to higher maternal depression and stress during pregnancy devoted increased cognitive effort to processing negative emotions and decreased cognitive effort to processing happy emotions. The phenomena of biased enhanced perception toward negative emotions and reduced perception of positive emotions is common in depressed adults and is believed to be part of the characteristics of depression (Leppänen, 2006). It was also shown that a lack of maternal empathetic behaviors (Rajhans et al., 2015) and higher maternal depressive symptoms (Forssman et al., 2014) were associated with infants’ increased allocation of attention to fearful compared to happy emotional faces.

Stress and depression during pregnancy have been proposed to be related to fetal programming through imbalances of the hypothalamic-pituitary-adrenocortical (HPA) system (Jones, Brooks, & Challis, 1989). Increased depressed mood symptoms during pregnancy have been associated with sustained increased levels of cortisol, which may ultimately alter the developing fetal HPA system, and result in long-term alterations in infant perception and behaviour (A. Porto et al., 2016). However, the results from studies reported in this thesis cannot directly support this proposed mechanism.

Interestingly, infants exposed to in utero SSRI did not exhibit the same pattern and in some way were “protected” from the detrimental effect of the mother’s mood symptoms of
depression and related maternal stress. The “protective” effects of SSRI have also been observed in animal models of prenatal exposure to SSRI (Rayen et al., 2011). Rayen et al. (2011) have reported that rat offspring of prenatally stressed rat dams showed depression-like behavior, while prenatal administration of fluoxetine (SSRI) reversed these behaviors (Rayen et al., 2011).

Overall, these results are in agreement with the prenatal programming theory, according to which exposures during the fetal period shape subsequent infant neurodevelopment as a preparation or forecasting for life outside the womb. These findings suggest that in utero exposure to SSRI might predispose the functional organization of emotion perception networks of the infant in a manner that supports a more blunted response to emotions compared to infants not exposed to SSRI, independent of maternal mood symptoms. Moreover, increased depressive mood symptoms during pregnancy may be associated with different predispositions that shape emotion perception in a manner that promotes processing of negative emotions. Independent of prenatal exposures, the postnatal environment is also found to be associated with alterations in the pattern of emotional network organization of reduced frontal efficiency of processing sad and happy emotions. Thus, it appears that each of these exposures, prenatally and postnatally, associate differently with the emotional development of infants. These influences may have important implications for the developmental trajectories of social-emotional development in infants of depressed and SSRI-treated mothers.
8.5 Limitations and future directions

While use of neuroimaging tools brings us a step further in providing a more detailed profile of the offspring outcomes, in this context, it is important to realize that they reflect a dynamic interaction between maternal mood, SSRI exposure, and genetic and epigenetic factors (Booij et al., 2013) as well as other environmental factors.

Studies in this thesis have small sample sizes and do not have sufficient power for testing these complex relations with mediating or confounding factors; as such, they should be replicated with larger sample groups. Population-based studies using neuroimaging tools with data on prenatal mood, drug exposure, and genetic variations are needed to disentangle the complicated interactions influencing outcomes.

The studies in this thesis report on findings from the first year of life, while long-term longitudinal neuroimaging studies combining structural, microstructural, and functional outcomes have not been reported. Neuroimaging studies that follow the offspring from the stage of pregnancy through adolescent years are needed to understand developmental consequences, trajectories, and long-term outcomes.

The long-term impact of prenatal SSRI exposure is far from reflecting invariant neurodevelopmental outcomes. For some, as in this thesis, there is a “main effect” that reflects an association with prenatal SSRI exposure, or with prenatal depression. Such outcomes are evident in the newborn period; however, over time, the influence of postnatal maternal mood symptoms becomes increasingly more prominent as well as the impact of interactions between childhood

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environment, familial support, genetics, and developmental pharmacology (Rotem-Kohavi and Oberlander, 2017). Further, and importantly, the outcomes in this thesis on prenatal programing are constrained by key methodological limitations that are inherent in perinatal pharmacology (i.e., confounding by indication) and factors associated with maternal illness that lead to prenatal SSRI exposure in the first place. In addition, the outcomes are also constrained by the ability to account for variations in the severity of maternal mental illness during pregnancy as well as ongoing maternal mental illness postnatally, which often remains unclear (Oberlander & Vigod, 2016). Importantly, depression is a chronic illness (50% of those with one episode are likely to have another and 80% of these become chronic), and women who take medication are likely to have more severe and chronic illness and greater risk of relapse upon the discontinuation of medication (Yonkers et al., 2011). While randomized controlled trial designs may help sort out the impact of maternal mood from SSRI exposure, such methods have not been used due to ethical reasons (Oberlander & Vigod, 2016). Even if randomization of exposure (which would be ethically and logistically challenging) could have been used, there are key differences related to maternal illness—whereby women who use antidepressant medications may be inherently different, for example, in terms of illness severity and SES from women who do not take the medication (El Marroun et al. 2016). This highlights the importance of accounting for symptom severity, which may influence the decision of whether to continue or discontinue medication use when considering the possible risks to the developing fetus (Clements et al., 2015). Secondary health characteristics associated with depression—such as smoking, alcohol consumption, and variations in educational achievement (Nulman et al., 1997; Pedersen et al., 2013)—did not differ between the exposure groups in the current study and were not included in the statistical
model. However, future studies with a larger sample size should also account for these factors, as they may interact with the outcomes (Hermansen et al., 2016; Hermansen and Melinder, 2015).

Another limitation of this study, and of other studies in this field, is that behavioral questionnaires rely on maternal reports, thereby raising key questions regarding reporter biases (El Marroun et al., 2016; Galbally et al., 2015). In this thesis, biases could have potentially affected both maternal self-reports as well as maternal reports on their infant’s temperament. In addition to maternal reports, future studies should include other measures, such as an objective assessment of infant temperament during a live situation or during mother-infant interaction.

On a general note, it is important to mention that current literature studying the effects of exposure to SSRI still lacks sufficient evidence using 5HT-related biomarkers that indicate that central 5HT has indeed been altered by prenatal SSRI exposure. While early changes in 5HT secondary to serotonergic antidepressant medication exposure may contribute to developmental risk, prenatal SSRI exposure cannot by itself account for variations in long term developmental outcomes. The timing of the exposure and life experiences after birth are also critical influences and emphasize that accounting for factors that set the central serotonergic tone are essential for healthy brain development (Rotem-Kohavi and Oberlander, 2017). More importantly, it is not merely a question of changes in 5HT levels (either higher or lower) during critical developmental periods but rather of identifying possible impacts of finely tuning the serotonergic system within a specific social context (Hanley and Oberlander, 2012). It may be that an incongruence in
stressors across prenatal and postnatal environments confers a heightened risk for adverse outcomes for the child or vice versa (Davis et al., 2007; Sandman et al., 2012).

Ultimately, perinatal circumstances influence neurodevelopment in ways that are more complex than can be addressed under experimental laboratory conditions or even with the use of population-level data. Distinguishing the effects of the mother’s mental illness from SSRI treatment remains a key challenge. Developmental processes and child outcomes are not always predicted by prenatal exposure status and development can reflect both vulnerability and resiliency. Identifying mothers who can benefit from prenatal pharmacotherapy remains a critical yet unanswered question. Well-designed longitudinal studies following both mothers and their children could help in identifying patterns of risk and interactions with concurrent genetic and environmental (maternal and child) factors that promote or reduce developmental vulnerability (Hanley and Oberlander, 2012). There are no risk-free options for the management of perinatal mood disorders, and the risks and potential benefits of untreated maternal mood disturbances cannot be ignored. Treatment goals should identify modifiable risk factors related to both the mother’s mental condition and to the SSRIs used to treat the mother’s disorders. Deciding whether to treat with antidepressants requires an examination of the risks against the benefits, considering the mother and her child as part of familial and community supportive-systems. While researchers continue to attempt to identify the specific developmental impact of SSRI exposure, it is critical to recognize that children of mothers with depression remain at increased risk for developmental disturbances. Bearing this in mind, there is a need to identify how maternal mood and related genetic and environmental factors also shape developmental risk in the context of prenatal SSRI exposure. By doing so, there can be a shift from studying the impact
of antidepressant medications alone toward examining interactions between risk factors and identifying whether some mothers and their children might actually benefit from prenatal maternal antidepressant treatment. Further, it should be noted that not all children with antidepressant exposure experience developmental adversity and identifying why some children do remains a pressing question. With emerging knowledge of how antidepressant use in pregnancy may shape development, reflecting both drug effects and risks inherent to maternal mental health, it is important to continue to focus on how best to promote optimal healthy child development in the context of maternal perinatal mood disturbances.

Finally, perinatal pharmacotherapy has been initiated with the expectation that it will improve maternal mental health during and after pregnancy, by extension improving the child’s developmental outcomes (Oberlander et al., 2010; Weikum et al., 2013a). However, mothers treated with SSRI recruited for the studies conducted in this thesis reported experiencing depressive symptoms even after drug treatment (Oberlander et al., 2010, 2007), which has implications both for the mother as well as for her infant/child. This highlights an urgent need to identify other supportive prenatal and postnatal systems to promote mental health to ensure optimal developmental outcomes for the child (Hanley and Oberlander, 2012).

Summary

In conclusion, future neuroimaging tools combined with an understanding of the function of genetic factors (Qiu et al., 2017) and susceptibility to the postnatal environment should help to
identify *who* might be at risk and *why*. Understanding neural and molecular correlates could ultimately enable identification of factors that will predict successful interventions tailored to individual mothers and their infants. In this manner, neuroimaging may be regarded as a “window” into advancing our understanding of the early origins of developmental brain plasticity.
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Appendix A  Maternal depression and prenatal exposure to SSRIs differentially associate with the functional connectivity organization underlying emotion perception in 8-to-10-month-old infants – Supplemental data

A.1 Construction of Functional Connectivity matrix

We used the false discovery rate controlled partial correlation (PCFDR) algorithm, as a statistical model which tests the ratio between the conditional dependence to independence between any two nodes based on all other nodes (Li and Wang, 2009), to compute connectivity matrices, for each subject, and for the different emotional faces. Partial correlation was used to calculate the conditional independence, to assess the directed interactions between any two nodes following the elimination of the effects from other nodes.

The PC algorithm begins from a complete graph which then efficiently tests for conditional independence. The PCFDR algorithm allows to asymptotically control the false discovery rate (FDR) lower than the predefined levels which estimates the ratio of all the falsely detected connections to all those detected. FDR uses a more conservative error rate criterion than the traditional Type-1 and Type-2 error, for modeling brain connectivity due to its direct relation to the uncertainty of the graph (see Li & Wang, 2009 for details). The FDR threshold was set at
the 5% level. The connectivity matrices of interest are binary, undirected graphs with the inferred functional connections at the 5% significance level.

A.2 Results

Global measures

To evaluate whether differences in modularity between the SSRI-exposed group compared to the non-exposed group were specific to the emotional content of the stimulation, a multivariate analysis comparing between modularity levels averaged across all of the emotions (i.e. emotional faces of happy, sad and painful faces) and modularity values for non-emotional face (teddy bear see Error! Reference source not found.) was conducted. The results revealed a between subject effect of

Figure A-1 Static photo of Teddy bear.

A static photo of a teddy bear was included in the stimulation protocol, presented for 1 second as part of the stimulation protocol.

group for emotions (F (1, 25) = 6.41, P = 0.018, ηp² = 0.204), while no significant differences were observed for the non-emotional condition (F (1, 25) = 0.52, P = 0.82, ηp² = 0.002). (see Error! Reference source not found.)
Multivariate analysis showing differences between SSRI-exposed (Exposed dark blue) compared to non-exposed group (light blue), for modularity values for the emotional stimuli (averaged across happy, sad, and painful faces) vs. non-emotional stimuli (bear).

In addition to main effect of PES on modularity, there was a close to significant interaction between Emotion and PES scores ($F(2, 50) = 3.13, P = 0.052, \eta_p^2 = 0.11$). These interaction stems from different pattern of interaction for the different emotions (see Error! Reference source not found.). For happy emotions, a positive correlation between increased levels of intensity of hassles and increased modularity was present for the non-exposed group ($r=0.648, p=0.003$) while the SSRI group did not show this correlation ($r=0.269, p=0.424$). For sad emotions, both groups showed a correlation between higher levels of intensity and lower levels of modularity (non-exposed: $r=-0.779, p<0.001$, SSRI-exposed: $r=-0.730, p=0.011$). For pain emotions, a negative correlation between increased levels of intensity of hassles and lower levels of modularity was present for the non-exposed ($r=-0.486, p=0.035$) group while the SSRI group did not show this pattern ($r=0.001, p=0.997$). Similar results were observed when sex was
added as a covariate (group effect $p=0.27$, PES X Emotion $p=0.054$, main effect of HAM-D $p=0.037$).

To examine whether the number of valid trials significantly influenced our findings, we divided each group into two sub-groups of performance using a median split based on the number of valid trials for each of the emotions (i.e. “Low” for number of trials that were lower than median and “High” for number of trials that were equal or above median). In the non-exposed group the average number of valid trials was $6.75 \pm 1.1$ versus $11.55 \pm 2.4$ for happy facial expressions, $6.83 \pm 1.4$ versus $11.38 \pm 2.5$ for sad facial expressions and $6.50 \pm 1.2$ versus $11.36 \pm 2.2$ for painful facial expressions for the exposed group $6.25 \pm 0.957$ versus $10.71\pm 2.812$ for happy facial expressions, $6.8 \pm 1.1$ versus $11.33 \pm 2.25$ for sad faces, and $6.2 \pm 0.5$ versus $12.0 \pm 2.75$ for painful expressions. Independent samples t-tests between the high performance sub-groups and low-performance sub-groups were not significantly differences ($p > 0.165$).
Figure A.3 Association between intensity of Hassles during pregnancy and network modularity for the different emotional faces.

Scatter plot showing the correlation between modularity and the pregnancy experiences scale for intensity of Hassles during the 3rd trimester for each of the emotions (Happy upper, Sad middle, Pain bottom), divided by group (red represents SSRI-exposed (n=11), blue represents non-exposed group (n=19).
Appendix B  Alterations in Resting State Networks Following in Utero Selective Serotonin Reuptake Inhibitor Exposure in the Neonatal Brain - Supplemental Information

B.1 Supplemental Methods

Participants

In the SSRI-treated group we included women treated with SSRI for at least 75 days during their pregnancies including the 3rd trimester. We included women treated with SSRIs (fluoxetine, paroxetine, sertraline, citalopram, escitalopram), and also with selective norepinephrine reuptake inhibitors (SNRIs) (desvenlafaxine, duloxetine and venlafaxine). For the sake of simplicity, we will not distinguish between these two types of exposures and will refer them all as SSRI-treated. At the time of recruitment, women were screened using the Mini-Neuropsychiatric Interview (MINI) (Lecrubier et al., 1997) to determine diagnoses. Mothers in the ‘depressed’ group met the criteria for a unipolar depressive mood disorder, according to DSM-5 clinical criteria (i.e. Major Depressive episode, peripartum onset or Major Depressive Disorder, etc.) as per treating psychiatrist/family physician; mothers in the control group did not meet these criteria. Only singleton healthy pregnancies were included in the study. Exclusion criteria included maternal substance abuse, bipolar disorder, and significant medical, obstetrical, or fetal conditions.

Statistical analysis

Independent component analysis (ICA) was used to investigate the possible presence of unexpected artifacts or spatiotemporal resting state patterns of activation. The anatomical
location was used to eliminate components that exhibited activity located in known major blood vessels. Similar to component elimination methods used in previous studies (Fransson et al., 2009; Kelly et al., 2010) components showing a “ring”-like activation pattern which is typically motion related were not considered for further analysis. Components that exhibited more than 50% of the activity in gray matter regions were included for analysis. Furthermore, only components for which the majority of the signal variance was below 0.1 Hz were considered relevant. This generated a series of 16 to 74 (mean = 36.92±13.23) independent components for each infant.
Figure B-1 RSN template of 30 randomly selected infants (10 randomly selected infants from each of the 3 groups).

RSNs are superimposed on a T1-weighted MR image of 40 weeks gestation neonatal template (4). Note that left side of the image corresponds to the left side of the brain.
<table>
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<th>Excluded (n = 31)</th>
<th>P value</th>
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<tr>
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<td>Smoking per pregnancy (# cigarettes), Mean (SD)</td>
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<td>Alcohol per pregnancy (#single drinks), Mean (SD)</td>
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<td>Prenatal HAM-D 3rd trimester Average, Mean (SD)</td>
<td>9.00±4.39</td>
<td>9.38±5.21</td>
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<td>Prenatal PES HASS frequency 3rd trimester Average, Mean (SD)</td>
<td>7.69±1.96</td>
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<td>Prenatal PES Uplift frequency 3rd trimester Average, Mean (SD)</td>
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<td>Prenatal PES Uplift intensity 3rd trimester Average, Mean (SD)</td>
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<td>Age at the MRI (hours)</td>
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<td>Sex male/female</td>
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<td>Birth weight (kg), Mean (SD)</td>
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<td>66.938±18.32</td>
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Table B-1 Demographics of the entire sample, and infants excluded from the study

PES, Pregnancy Experiences Scale; HAM-D, Hamilton Rating Scale for Depression.

aNAPI, Neurobehavioral Assessment of the Preterm Infant, data was available for 41 infants (entire sample) and 26 (excluded).
### Maternal characteristics

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<td>Maternal education (Year), Mean (SD)</td>
<td>17.79±3.02</td>
<td>18.91±3.44</td>
<td>0.280</td>
</tr>
<tr>
<td>Smoking per pregnancy (# cigarettes), Mean (SD)</td>
<td>0.02±0.14</td>
<td>0.00±0.00</td>
<td>0.322</td>
</tr>
<tr>
<td>Alcohol per pregnancy (# single drinks), Mean (SD)</td>
<td>0.83±3.16</td>
<td>1.82±6.03</td>
<td>0.607</td>
</tr>
<tr>
<td>Prenatal HAM-D 3rd trimester Average, Mean (SD)</td>
<td>9.00±4.39</td>
<td>10.40±4.99</td>
<td>0.348</td>
</tr>
<tr>
<td>Prenatal PES HASS frequency 3rd trimester Average, Mean (SD)</td>
<td>7.69±1.96</td>
<td>6.90±1.89</td>
<td>0.233</td>
</tr>
<tr>
<td>Prenatal PES Uplift frequency 3rd trimester Average, Mean (SD)</td>
<td>9.07±1.21</td>
<td>9.00±1.56</td>
<td>0.859</td>
</tr>
<tr>
<td>Prenatal PES Uplift intensity 3rd trimester Average, Mean (SD)</td>
<td>2.15±0.40</td>
<td>2.12±0.44</td>
<td>0.862</td>
</tr>
<tr>
<td>Prenatal PES HASS intensity 3rd trimester Average, Mean (SD)</td>
<td>1.62±0.39</td>
<td>1.57±0.38</td>
<td>0.688</td>
</tr>
</tbody>
</table>

### Neonatal characteristics

<table>
<thead>
<tr>
<th></th>
<th>Entire sample</th>
<th>Not scanned</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of delivery (vaginal/C-section)</td>
<td>36/17</td>
<td>4/5</td>
<td>0.048</td>
</tr>
<tr>
<td>Birth GA (weeks), Mean (SD)</td>
<td>39.53±1.67</td>
<td>39.64±1.43</td>
<td>0.851</td>
</tr>
<tr>
<td>Sex male/female</td>
<td>30/23</td>
<td>5/4</td>
<td>0.954</td>
</tr>
<tr>
<td>Birth weight (kg), Mean (SD)</td>
<td>3.43±0.49</td>
<td>3.56±0.41</td>
<td>0.446</td>
</tr>
<tr>
<td>Birth length (cm), Mean (SD)</td>
<td>51.1±2.37</td>
<td>51.21±1.83</td>
<td>0.893</td>
</tr>
<tr>
<td>Head circumference (cm), Mean (SD)</td>
<td>34.66±1.55</td>
<td>35.18±1.25</td>
<td>0.334</td>
</tr>
<tr>
<td>Apgar score 1 min, Mean (SD)</td>
<td>7.58±2.10</td>
<td>8.56±1.014</td>
<td>0.182</td>
</tr>
<tr>
<td>Apgar score 5 min, Mean (SD)</td>
<td>8.66±1.02</td>
<td>8.66±1.018</td>
<td>0.324</td>
</tr>
<tr>
<td>NAPI irritability scores, Mean (SD)²</td>
<td>66.938±18.32</td>
<td>70.71±20.10</td>
<td>0.668</td>
</tr>
</tbody>
</table>

Table B-2 Demographics of the entire sample, and infants not scanned

PES, Pregnancy Experiences Scale; HAM-D, Hamilton Rating Scale for Depression. ²NAPI, Neurobehavioral Assessment of the Preterm Infant, data was available for 41 infants (entire sample) and 5 (not-scanned).

An additional mother withdrew from the study after the first visit – thus data for this mother and her infant are missing.
Appendix C  Hub distribution of the brain functional networks of newborns prenatally exposed to maternal depression and SSRI anti-depressant use - Supplemental data

C.1 Methods

Participants

Upon enrolment, the Mini-Neuropsychiatric Interview (MINI) (Lecrubier et al., 1997) was used to determine depression diagnoses. If the participant met the criteria for a unipolar depressive mood disorder per DSM-5 clinical criteria and was previously diagnosed by a qualified physician, the participant was included in the depressed-only group; mothers that did not meet these criteria were included in the control group. The SSRI group comprised women who had been diagnosed with a unipolar mood disorder, were treated with SSRIs for a minimum of 75 days during the third trimester of pregnancy based on their clinical need. Women treated with SSRIs (fluoxetine, paroxetine, sertraline, citalopram, citalopram), and also with selective-norepinephrine reuptake inhibitors (SNRI) (desvenlafaxine, duloxetine, and venlafaxine) were included. For reasons of simplicity, we will not differentiate between these two pharmacological treatments. Only healthy, term pregnancies with one fetus were included in the study. Mothers with substance abuse, bipolar disorder, and those with significant medical, obstetrical, or fetal conditions were excluded from the study.

MRI data acquisition

All scans were performed at the BC Children’s Hospital MRI Research Facility in Vancouver, BC Canada. Infants were fed, swaddled and positioned in an MR-compatible neonatal incubator (Advanced Imaging Research, Inc dba SREE MEDICAL SYSTEMS,
Cleveland, OH, USA) cushioned with pillows. Ear protectors and ear muffs were used to reduce noise from the MRI. Physiologic measures of heart rate and oxygen saturation were monitored by a registered pediatric nurse during the study.

<table>
<thead>
<tr>
<th>Maternal characteristics</th>
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<th>Not scanned</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
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<td>34.62±3.75</td>
<td>33.90±4.87</td>
<td>0.609</td>
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<tr>
<td>Maternal education (Year), Mean (SD)</td>
<td>17.79±3.02</td>
<td>18.91±3.44</td>
<td>0.280</td>
</tr>
<tr>
<td>Smoking per pregnancy (# cigarettes), Mean (SD)</td>
<td>0.02±0.14</td>
<td>0.00±0.00</td>
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</tr>
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<td>0.83±3.16</td>
<td>1.82±6.03</td>
<td>0.607</td>
</tr>
<tr>
<td>Prenatal HAMD 3rd trimester Average, Mean (SD)</td>
<td>9.00±4.39</td>
<td>10.40±4.99</td>
<td>0.348</td>
</tr>
<tr>
<td>Prenatal PES HASS frequency 3rd trimester Average, Mean</td>
<td>7.69±1.96</td>
<td>6.90±1.89</td>
<td>0.233</td>
</tr>
<tr>
<td>Prenatal PES Uplift frequency 3rd trimester Average, Mean</td>
<td>9.07±1.21</td>
<td>9.00±1.56</td>
<td>0.859</td>
</tr>
<tr>
<td>Prenatal PES Uplift intensity 3rd trimester</td>
<td>2.15±0.40</td>
<td>2.12±0.44</td>
<td>0.862</td>
</tr>
</tbody>
</table>
Average, Mean (SD)

Prenatal PES HASS intensity  3rd trimester

<table>
<thead>
<tr>
<th></th>
<th>Average, Mean (SD)</th>
<th>1.62±0.39</th>
<th>1.57±0.38</th>
<th>0.688</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prenatal SSRI exposure</td>
<td>0.38±0.489</td>
<td>0.18±0.40</td>
<td>0.220</td>
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</tbody>
</table>

**Neonatal characteristic**

<table>
<thead>
<tr>
<th>Neonatal characteristic</th>
<th>Type of delivery (vaginal/C-section)</th>
<th>36/17</th>
<th>4/5</th>
<th>0.048</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth GA (weeks), Mean (SD)</td>
<td>39.53±1.67</td>
<td>39.64±1.43</td>
<td>0.851</td>
<td></td>
</tr>
<tr>
<td>Sex male/female</td>
<td>30/23</td>
<td>5/4</td>
<td>0.954</td>
<td></td>
</tr>
<tr>
<td>Birth weight (kg), Mean (SD)</td>
<td>3.43±0.49</td>
<td>3.56±0.41</td>
<td>0.446</td>
<td></td>
</tr>
<tr>
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<td>51.21±1.83</td>
<td>0.893</td>
<td></td>
</tr>
<tr>
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<td>34.66±1.55</td>
<td>35.18±1.25</td>
<td>0.334</td>
<td></td>
</tr>
<tr>
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<td>8.56±1.014</td>
<td>0.182</td>
<td></td>
</tr>
<tr>
<td>Apgar score 5 min, Mean (SD)</td>
<td>8.66±1.02</td>
<td>8.66±1.018</td>
<td>0.324</td>
<td></td>
</tr>
<tr>
<td>NAPI irritability scores. Mean (SD)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66.938±18.32</td>
<td>70.71±20.10</td>
<td>0.668</td>
<td></td>
</tr>
</tbody>
</table>

---

**Table C-1 Demographics**

PES, Pregnancy Experiences Scale; HAM-D, Hamilton Rating Scales for Depression

<sup>b</sup> NAPI, Neurobehavioral Assessment of the Preterm Infant, data was available for 41 infants (entire sample) and 5 (not-scanned).
<table>
<thead>
<tr>
<th>Connector hubs</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rolandic_Oper_R</td>
<td>53</td>
<td>0.62717599</td>
<td>0.08282276</td>
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<tr>
<td>Rectus_L</td>
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<td>0.62818686</td>
<td>0.07345548</td>
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<tr>
<td>Rectus_R</td>
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<tr>
<td>Insula_L</td>
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<tr>
<td>Cingulum_Ant_L</td>
<td>53</td>
<td>0.63232154</td>
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<tr>
<td>Cingulum_Ant_R</td>
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<td>0.01131385</td>
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<tr>
<td>Cingulum_Mid_L</td>
<td>53</td>
<td>0.62726909</td>
<td>0.02984264</td>
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<tr>
<td>Paracentral_Lobule_R</td>
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<td>0.62754288</td>
<td>0.0620302</td>
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<tr>
<td>Caudate_L</td>
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<td>0.01334706</td>
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<td>Caudate_R</td>
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<td>0.64268281</td>
<td>0.03985943</td>
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<td>Putamen_L</td>
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<td>0.04233503</td>
</tr>
<tr>
<td>Putamen_R</td>
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<td>Pallidum_L</td>
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<td>0.04933238</td>
</tr>
<tr>
<td>Pallidum_R</td>
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<td>0.63614859</td>
<td>0.06061012</td>
</tr>
<tr>
<td>Thalamus_R</td>
<td>53</td>
<td>0.63542586</td>
<td>0.05699693</td>
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<tr>
<td>Heschl_L</td>
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<td>0.01806142</td>
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<td>Heschl_R</td>
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<td>Temporal_Sup_L</td>
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<tr>
<td>Temporal_Sup_R</td>
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</tr>
<tr>
<td>Temporal_Pole_Sup_L</td>
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<td>0.01249237</td>
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<tr>
<td>---------------------</td>
<td>----</td>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td>Temporal_Mid_L</td>
<td>53</td>
<td>0.63517724</td>
<td>0.1032587</td>
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</table>

Table C-2 Connector hubs

Group average connector hubs values

<table>
<thead>
<tr>
<th>Provincial hubs</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal_Sup_Orb_L</td>
<td>53</td>
<td>1.007486</td>
<td>0.70436089</td>
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<tr>
<td>Frontal_Mid_Orb_R</td>
<td>53</td>
<td>0.509511</td>
<td>0.777622425</td>
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<tr>
<td>Olfactory_L</td>
<td>53</td>
<td>0.575797</td>
<td>0.712091422</td>
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<tr>
<td>Olfactory_R</td>
<td>53</td>
<td>0.646059</td>
<td>0.503961833</td>
</tr>
<tr>
<td>Rectus_L</td>
<td>53</td>
<td>0.516323</td>
<td>0.639081934</td>
</tr>
<tr>
<td>Insula_R</td>
<td>53</td>
<td>0.49907</td>
<td>0.542804339</td>
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<tr>
<td>Cingulum_Ant_R</td>
<td>53</td>
<td>0.468823</td>
<td>0.724723793</td>
</tr>
<tr>
<td>Cingulum_Mid_R</td>
<td>53</td>
<td>0.43357</td>
<td>0.638627709</td>
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<tr>
<td>Cingulum_Post_L</td>
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<td>1.11702</td>
<td>0.636035844</td>
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<td>Cingulum_Post_R</td>
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<td>Amygdala_L</td>
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<tr>
<td>Cuneus_R</td>
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<tr>
<td>Occipital_Sup_L</td>
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<td>Fusiform_R</td>
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<td>Angular_R</td>
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<td>0.707684994</td>
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<tr>
<td>Regional Hub</td>
<td>Sample Size</td>
<td>Group 1 Mean</td>
<td>Group 1 Std. Error</td>
</tr>
<tr>
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<td>-------------</td>
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<td>-------------------</td>
</tr>
<tr>
<td>Caudate_L</td>
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<td>Pallidum_L</td>
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<td>Heschl_L</td>
<td>53</td>
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</table>

Table C-3 Provincial hubs

Group average provincial hubs values

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<thead>
<tr>
<th>Dependent Variable</th>
<th>Group</th>
<th>Mean</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insula Left</td>
<td>Control</td>
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<td>.004</td>
</tr>
<tr>
<td></td>
<td>SSRI</td>
<td>.641a</td>
<td>.003</td>
</tr>
<tr>
<td></td>
<td>Depressed</td>
<td>.648a</td>
<td>.003</td>
</tr>
<tr>
<td>Cingulum Anterior Left</td>
<td>Control</td>
<td>.632a</td>
<td>.003</td>
</tr>
<tr>
<td></td>
<td>SSRI</td>
<td>.636a</td>
<td>.003</td>
</tr>
<tr>
<td></td>
<td>Depressed</td>
<td>.645a</td>
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<td>Control</td>
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<td></td>
<td>SSRI</td>
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<td>.002</td>
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<tr>
<td></td>
<td>Depressed</td>
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<td>.002</td>
</tr>
</tbody>
</table>

Table C-4 Estimates Connector hubs (Participation coefficient positive weights)

Based on estimated marginal means
* The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.
<table>
<thead>
<tr>
<th>Region</th>
<th>(I) Group</th>
<th>(J) Group</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insula Left</td>
<td>Control</td>
<td>SSRI</td>
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<td>.005</td>
<td>.655</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Depressed</td>
<td>-.014*</td>
<td>.005</td>
<td>.026</td>
</tr>
<tr>
<td></td>
<td>SSRI</td>
<td>Control</td>
<td>.007</td>
<td>.005</td>
<td>.655</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Depressed</td>
<td>-.007</td>
<td>.005</td>
<td>.450</td>
</tr>
<tr>
<td></td>
<td>Depressed</td>
<td>Control</td>
<td>.014*</td>
<td>.005</td>
<td>.026</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SSRI</td>
<td>.007</td>
<td>.005</td>
<td>.450</td>
</tr>
<tr>
<td>Cingulum anterior Left</td>
<td>Control</td>
<td>SSRI</td>
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<td>.004</td>
<td>1.000</td>
</tr>
<tr>
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<td>-.013*</td>
<td>.004</td>
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</tr>
<tr>
<td></td>
<td>SSRI</td>
<td>Control</td>
<td>.004</td>
<td>.004</td>
<td>1.000</td>
</tr>
<tr>
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<td></td>
<td>Depressed</td>
<td>-.009</td>
<td>.004</td>
<td>.066</td>
</tr>
<tr>
<td></td>
<td>Depressed</td>
<td>Control</td>
<td>.013*</td>
<td>.004</td>
<td>.007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SSRI</td>
<td>.009</td>
<td>.004</td>
<td>.066</td>
</tr>
<tr>
<td>Caudate Left</td>
<td>Control</td>
<td>SSRI</td>
<td>-.004</td>
<td>.003</td>
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</tr>
<tr>
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<td>Depressed</td>
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<td>.003</td>
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</tr>
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<td></td>
<td>SSRI</td>
<td>Control</td>
<td>.004</td>
<td>.003</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Depressed</td>
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<td>.003</td>
<td>.198</td>
</tr>
</tbody>
</table>
### Table C-5 Connector hubs (Participation coefficient: positive weight)

Based on estimated marginal means

* The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Group</th>
<th>Mean</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
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### Table C-6 Estimates Provincial hubs

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Table C-7 Estimates Provincial hubs
Based on estimated marginal means
*The mean difference is significant at the .05 level. b. Adjustment for multiple comparisons: Bonferroni