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Maturation of default mode network functional connectivity strength in utero and the association with subcortical macrostructure: mapping brain ontogeny supporting early cognitive processing

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Abstract

The third trimester of gestation is scarcely studied despite this being a key window for the development of learning and memory systems. In a prospective cohort study, 30 pregnant women participated in one or two fetal MR imaging sessions between 28- and 39 weeks of gestational age. Activation time courses were extracted from the default mode (DMN), medial temporal lobe (MTL), and thalamocortical (TCN) networks of the fetal brain. Generalized estimating equations were used to examine the association between the DMN-MTL, DMN-TCV connectivity strength, and subcortical volumes. Increased functional connectivity strength between the DMN-MTL networks was negatively associated with smaller hippocampal volumes. In contrast, increased functional connectivity strength in DMN-TCN was associated with smaller thalamic volumes. No associations between DMN connectivity strength were seen with cerebellar volumes. These associations indicate the emergence of strong short-range connectivity in the third trimester. Developing fetal MRI biomarkers facilitates the formation of a normative model of brain networks involved in cognitive processes.

Keywords

Fetal functional connectivity, Memory networks, Default mode network, Medial temporal lobe network, Thalamocortical network, Fetal neurodevelopment, Fetal subcortical development.

Summary for Lay Audience

The functional and structural formation of brain networks during the gestational period supports the development of learning and memory systems. These brain networks are present while the brain is at rest and start to develop in the fetal brain starting in the second trimester of pregnancy. Critical brain networks are the default mode network (DMN), the medial temporal lobe network (MTL), and the thalamocortical network (TCN) and play a critical role in learning and memory. Disruptions in the maturation of the networks during the fetal period may influence learning and memory difficulties later in life. In turn, better characterization of the networks and how they develop can improve our understanding of healthy brain development and aid in the identification of potential learning and memory difficulties early on in life, particularly in fetuses who may be at risk. This thesis aimed to analyze the association between subcortical structures and the DMN, MTL, and TCN connectivity strength during the third trimester of gestation. To achieve this aim, we acquired fetal magnetic resonance imaging (MRI) data during the third trimester of pregnancy. We obtained the volumes of brain regions involved in learning and memory, including the hippocampus, thalamus, cerebellum, amygdala, and basal ganglia. We also extracted the time courses of activation of the DMN, MTL, and TCN to study the strength of their connectivity. Finally, we performed a statistical analysis to examine the associations of the DMN-MTL, DMN-TCN, and subcortical volume size respectively.

We found negative associations between the hippocampus with the DMN-MTL networks. We also found negative associations between the Thalamus and the DMN-TCN networks. These results suggest that memory networks can be detected during the gestational period and are associated with the size of subcortical structures. Characterizing the development of brain networks in the fetus can aid in studying learning and memory abilities early in life.

Co-Authorship Statement

Chapter 2 was adapted from the manuscript title “Default Mode Network Functional Connectivity Strength *in utero* and the association with fetal subcortical development” currently under review at: *Cerebral Cortex*. The co-authors of this paper are Dr. Emily S. Nichols, Megan E. Mueller, Dr. Barbra de Vrijer, Dr. Roy Eagleson, Dr. Charles A. McKenzie, Dr. Sandrine de Ribaupierre, and Dr. Emma G. Duerden.

Drs. Barbra de Vrijer, Roy Eagleson, Charles A. McKenzie, Sandrine de Ribaupierre, and Emma G. Duerden were engaged in the design and conception of the study. Megan E. Mueller was involved during the pre-processing stages of the Magnetic Resonance Imaging data. Dr. Emily S. Nichols was involved in collecting and pre-processing the Magnetic Resonance Imaging data. I participated in collecting, analyzing, and interpreting the data and writing the complete paper.

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List of Abbreviations

Abbreviation	Meaning
DMN	Default Mode Network
fMRI	Functional Magnetic Resonance Imaging
FSE	Fast Spin Echo
GA	Gestational Age
HASTE	Half- Fourier Single Shot Turbo Spin Echo
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
MTL	Medial Temporal Lobe Network
PTV	Patch To Volume
SSFSE	Single Shot Fast Spin Echo
SVR	Slice to Volume Reconstruction
TCN	Thalamo-Cortical Network
TR	Repetition Time

Chapter 1

1 Introduction

The use of functional magnetic resonance imaging (fMRI) to detect resting-state networks has provided a new window into the understanding of the intrinsic processes underlying the formation of neural connectomes during gestation. Evidence has demonstrated that sensorimotor, language, and higher-order cognitive networks can be detected and studied prenatally (Canini et al., 2020; Turk et al., 2019). Development of these networks becomes evident as early as the second trimester, activation begins before birth, and maturation continues throughout the lifespan. Although the use of fMRI in the detection of resting-state networks has provided novel knowledge to the development of the neuronal circuitry, there remains uncertainty about the interdependence between functional networks supporting cognition and subcortical brain regions when they develop.

Functional networks such as the default mode network (DMN), medial temporal lobe network (MTL), and the thalamocortical network (TCN) are known to play a critical role in learning and memory systems. They can be identified in the fetal brain during the third trimester of gestation using resting-state fMRI. These networks have been highly characterized in child and adult brains. Subcortical areas such as the hippocampus, thalamus, and basal ganglia are crucial for the structural connectivity of these functional networks (Alves et al., 2019; Brandt & Dieterich, 2019). Until now, the study of brain structural and functional connectivity *in utero* has been limited due to the challenges associated with imaging the moving fetus. In turn, less is known about the development of the DMN and its functional and structural connectivity during gestation, despite this being a sensitive window for forming networks underlying cognition. Improved characterization of the development of the DMN and its short- and long-range functional and structural connectivity can aid in understanding the origins of brain systems involved in learning and memory in typical and atypical development.

1.1 Brain ontogeny

Understanding brain ontogeny is complemented through the study of the fetal connectome. Better characterization of how emerging brain regions begin to 'connect' can improve our understanding of the neural basis for cognition and behavior in children. Previous animal and human studies have demonstrated that functional growth pattern formation is dependent of neurohormonal and neurotransmitter regulations in the fetal brain (De Miguel et al., 2022; Hansen et al., 2022; Herlenius & Lagercrantz, 2001). These growth patterns are also known to begin regionally and later synchronize globally. Evidence suggests that all mammals' brains present an all-around pattern of circuitry formation in which neural efficiency is distributed equally across networks as the fetal brain matures (Assaf et al., 2020; De Miguel et al., 2022). During the early stages of cerebral ontogenesis, genetic influences also induce different processes of maturation (Pletikos et al., 2014). However, as the gestational period progresses, brain maturity is overtaken by both genetics and the external environment. The cerebral ontogeny and development process has been mainly understood through animal and *ex-utero* research (De Miguel et al., 2022). However, with the advances in neuroimaging tools, new research windows have opened to analyze the emergence of networks supporting cognitive function *in-utero.*

1.2 Embryonic and fetal brain development: formation of subcortical and functional structures

The development of the fetal brain begins during the third week of gestation during the embryonic stage. By week 3, cell proliferation and migration rapidly occur, and the neural tube closes and is then divided into three subdivisions, the hindbrain, midbrain, and forebrain (Govaert et al., 2020). At around 15 weeks of gestation, subcortical cortical connectivity starts to be active, and the brain starts to control motor movements of the limbs and immature chest movements (Huang et al., 2009; Joseph, 2000). During the following weeks, rapid development and differentiation of subcortical and cortical regions occur. At the cortical level, the longitudinal fissure between hemispheres becomes visible early in the trimester, and all significant cortical

subdivisions can be detected. By 19 and 2 weeks of gestation, the development of sulci and gyri has already occurred (Govaert et al., 2020).

At a subcortical level, specific structure development starts to occur during the embryonic period followed by multiple interconnected mechanisms that happen simultaneously driving subcortical maturation (Huang et al., 2009). For example, the hippocampus develops from the ventricular zone in an “inside out” manner beginning with the maturation of the Cornu Ammonis (CA) 1 followed by CA4 and the dentate gyrus (Prayer et al., 2006). By week 15 of gestation an immature structure of the hippocampus can be detected (Prayer et al., 2006).

The thalamus develops in the posterior area of the forebrain, and between 10- and 14 weeks of gestational age (GA), approximately differentiation of thalamic neurons begins (Mojsilović & Zečević, 1991; Prayer et al., 2006). The basal ganglia derive from the telencephalon in the forebrain. The caudate and putamen develop from neuroblasts at the base of the telencephalon (Prayer et al., 2006). Subsequent subcortical brain regions follow similar processes of formation and maturation during the first half of the gestational period. Neurogenetic events such as cell proliferation migration and synaptogenesis continue to occur throughout the pregnancy (Govaert et al., 2020; Kostović et al., 2021). By the third trimester of gestation, the fetal brain is structurally and functionally active (Govaert et al., 2020). During this last stage of gestation, the cortical and subcortical regions undergo further structural growth and maturation (Clouchoux et al., 2012).

Dramatic developmental changes characterize the third trimester of gestation. Limited research on fetal brain structures has demonstrated of accelerated volumetric growth in cortical and subcortical brain regions during this period (Fogliarini et al., 2005; Limperopoulos et al., 2010; Molnár, 2011). Because of these rapid growth trajectories, hemispheric asymmetry, for example, has been demonstrated to happen during the fetal period and the early years of life (Kivilevitch et al., 2010). Postmortem studies have also comprehensively presented evidence of structural growth trajectories during pregnancy (Kang et al., 2019; Zhang et al., 2011). Numerous studies done in animals have also

delineated early human brain development (Liu et al., 2020; Rees & Harding, 2004; Workman et al., 2013); however, given the physical differences between animals and humans, these imaging studies are frequently nearly impossible to be translated into human models. Although animal and postmortem studies are vital bodies of evidence that corroborate properties of brain maturation, research performed *in utero* is of the utmost importance to understand fetal brain development. Given the rapid development of these structures in such a short period, subcortical structures present increased susceptibility to abnormal development.

1.3 MRI protocols to study fetal grey and white matter structural development.

The architecture of the fetal brain has been elucidated largely through post-mortem studies. However structural imaging of the fetal brain using MRI has offered key insight into its complex developmental processes *in vivo*. The first MRI study of the fetus was reported in the early 1980s (Smith et al., 1983). However, this technique during the following years was rarely used because of fetal motion and the extensive time to acquire the images. In 1991 fMRI was introduced as a technique to demonstrate regional metabolic changes across time (Glover, 2011). Since then, this technique's popularity has increased, providing a better comprehension of structural organization supporting high order cognitive processes (Alsharif et al., 2021; andettini, 2007). Over the last 4 decades, the use of fetal MRI in research settings has become more accessible. With the introduction of MRI techniques such as single- shot- fast- spin echo (SSFSE) and Half-Fourier Angle Shot Turbo Spin Echo (HASTE) popularity increased to utilize this imaging technique in fetal populations (Gagoski et al., 2021; Huppert et al., 1999; Yamashita et al., 1997). However, fetal motion presents a significant challenge to obtaining high quality images that can subsequently be used for cortical and subcortical segmentation as well as functional imaging.

1.3.1 MRI protocols and techniques

Motion is an issue for all human neuroimaging studies; however, fetal neuroimaging provides a unique context. Fetuses can move significantly during a single MRI acquisition for several reasons, including fetal head, limb, and torso movement, yawns,

and maternal breathing and bowel movement (Malamateniou et al., 2013). Motion during neuroimaging acquisition produces significant artifacts in the data; anywhere from individual volumes to full acquisitions make the data unusable (Ferrazzi et al., 2014). Several procedures and techniques have been developed to prevent, minimize, and correct fetal motion, with varying degrees of success (Robinson & Ederies, 2018).

Although the ideal way to address motion is to prevent it from happening in the first place, there are few reliable methods for doing so during fetal MRI (Malamateniou et al., 2013; Robinson & Ederies, 2018). Ensuring the comfort of the mother, minimizing maternal stress or claustrophobic feelings, coaching on the necessity of staying still during the scan, and practicing breath-holding are all effective techniques for minimizing maternal movement, which lowers levels of motion artifacts in the data. In terms of the fetus however, there are few effective strategies for preventing motion. Early research suggests that maternal fasting and minimizing caffeine intake prior to the scan reduces fetal movement (Devoe et al., 1987; Mirghani et al., 2003). More recent research however has been mixed; for example, the effect of caffeine on fetal movement may depend on how much caffeine the mother regularly consumes (Mulder et al., 2010), while other studies have found no effect of short-term caffeine consumption on fetal movement at all (Estrin et al., 2012; Yen et al., 2019). Similar results have been described for maternal fasting, with reports of limiting food and drink consumption prior to scanning reducing (Abd-El-Aal et al., 2009; Mirghani et al., 2003), increasing (Bradford & Maude, 2014), and having no effect on fetal motion (Tug et al., 2011; Yen et al., 2019).

Maternal sedation has been used in the past; however, this is not a widely practiced technique (Malamateniou et al., 2013; Robinson & Ederies, 2018) and may not reduce fetal motion (Meyers et al., 2017). Also, there is conflicting evidence regarding whether fetuses move less in the morning compared to the afternoon. (Avitan et al., 2018; Patrick et al., 1982). A final method for preventing fetal motion is to scan at later gestational ages; research has generally agreed that fetal movement decreases modestly with gestational age (Koshida et al., 2019; Estrin et al., 2012; Pearson & Weaver, 1976; Sadovsky & Yaffe, 1973), although some studies have found no effect (Connors et al., 1988; Valentin & Marŝál, 1986). It is clear from the large variability in results that well-

controlled studies are required to determine what measures should be taken to prevent fetal motion during scanning.

As efforts to prevent fetal motion from creating distortions in MR data are not always successful, specific imaging protocols can be used to minimize the effect of motion on data quality. These adjustments mostly focus on reducing the time it takes to acquire a scan, as faster acquisitions provide less time for motion to occur. The imaging protocol most used in fetal MRI is fast spin echo (FSE). In a typical spin echo sequence, one echo is measured per repetition time (TR). In FSE, multiple echoes are measured per TR, reducing the number of TRs, and therefore the length of the scan, to collect a full volume. In the case of fetal imaging, a full volume can be acquired in a single TR, in a sequence called HASTE or single-shot FSE (Prayer et al., 2004; Robinson & Ederies, 2018). As fetal white matter contains more water than infant, child, or adult brains, and the long echo time (TE) of T2-weighted sequences best captures this contrast with grey matter. Despite requiring a longer TE than T1-weighted sequences, they are still shorter than traditional 3D volumes acquired in children and adults, minimizing the time to complete a scan and therefore the possibility of motion corruption. Images are acquired in a single plane at a high resolution (Malamateniou et al., 2013).

The high-resolution in-plane images acquired in three different planes can be merged using several different techniques. A common technique is slice-to-volume reconstruction (SVR; Rousseau et al., 2006). Using these methods, multiple high-resolution 2D SSFSE images can be combined to form a single, high-resolution 3D image. In SVR, each slice is re-aligned to the high-resolution volume, forming a motion-corrected 3D volume. Open-source toolboxes for SVR toolkit (Deprez et al., 2012), and the newly developed eloped NiftyMIC (Ebner et al., 2020) software, are available. The NiftyMIC toolbox builds upon the SVR method; however, it includes an algorithm to better localize the fetal brain.

1.4 Resting state memory networks

1.4.1 Default mode network

The DMN is a network comprised of dispersed brain areas in the parietal, temporal, and frontal cortices (Kaefer et al., 2022; Smallwood et al., 2021). This resting state network presents higher activation during various complex cognitive processes, including memory and abstract reasoning (Kaefer et al., 2022). It also displays decreased activation during attention-demanding tasks (Fan et al., 2021). The DMN includes cortical areas such as the medial prefrontal cortex, lateral temporal cortex, cingulate cortex, and the inferior parietal lobule (Mak et al., 2017; Smallwood et al., 2021). However, previous research has demonstrated that subcortical structures such as the hippocampus, basal ganglia, precuneus, and thalamus are also associated with the proper functioning of the network (Cunningham et al., 2017; Harrison et al., 2021). Numerous studies have explored how, at different age groups, the DMN develops. In adults, previous studies have demonstrated that the DMN is related to instances of memory recollection and consolidation, reflection of the self, and semantic memory (Fan et al., 2021; Kaefer et al., 2022; ak et al., 2017). During childhood and adolescence, the DMN develops into a more dynamic architecture strengthening functional areas where the connectivity is more active (Sato et al., 2014). In preterm infants, previous research has shown the presence of an immature form of the DMN that resembles the functional network of an adult. Onofrj et al. (2022) suggested that in fetuses, the DMN can be detected as early as the second trimester of gestation and can also be used as a metric to determine the gestational age of preterm newborns. Given the dynamic nature of this network across the human lifespan, the DMN is highly susceptible to environmental factors altering its proper functioning and development. Additionally, the role of this network and subcortical structures in cognitive functions across the lifespan reflects the persistent interest of researchers in understanding its complex development. However, there is no clear understanding of the intrinsic dependency between this network with subcortical macrostructures during gestation.

1.4.2 Medial temporal lobe network

The MTL is a group of interdependent brain structures that are known to be involved in episodic and memory consolidation (Zaiser et al., 2022). This network comprises

subcortical and cortical structures such as the hippocampus and the cortex. The MTL network is also considered a subsystem of the DMN because of its neural synchronization with regions such as the medial prefrontal cortex, the precuneus, and the posterior cingulate cortex (Fan et al., 2021). It has been widely studied the connectivity of this network with other cerebral circuits. Cognitive studies have demonstrated that this network synchronizes activation with the salient, frontal, basal ganglia, and visual networks (Ruiz-Rizzo et al., 2020). These findings demonstrate the importance of this network in the overall functional organization of the human brain. Neuroanatomical studies have reported the early development of the MTL during gestation (Bajic et al., 2012). Although the MTL is present before birth, maturation of the network causes memory development with the presence of external stimuli (Ghetti et al., 2010; Townsend et al., 2010). However, the presence of harmful factors can also deteriorate the function of the MTL in cognition. For example, previous cognitive studies have shown that in older adults, impairment to the MTL has been linked to early stages of Alzheimer's Disease, mild cognitive impairments, and overall cognitive decline (Berron et al., 2020). Despite the vital execution of this network in memory development, scarce studies have researched the role of the MTL in early cognitive systems. Given the strikingly few studies done in the functional properties of the MTL during the gestational period, much remains to be understood.

1.4.3 Thalamocortical network

The thalamus, a subcortical structure located between the midbrain and the cerebral cortex, plays a role in higher order cognitive processes and the integration of peripheral sensory stimuli into the cortex (Alcauter et al., 2014). This subcortical structure also acts as an integrative hub for higher-order multimodal brain networks (Hwang et al., 2017). The third trimester of gestation is a period of development characterized by the proliferation of neurons and the rapid specialization of the TCN (Cai et al., 2017). These functional pathways are vital for the proper development of cortical systems. Thalamic neurons are particularly susceptible to external factors. Previous studies suggest that alterations in establishing thalamocortical connections increases the risk of neurodevelopmental disorders such as autism and schizophrenia (Nair et al., 2021; Yao et

al., 2020). Evidence also suggests that impairment to the thalamus causes dysfunction of language and cognitive function (Stockert et al., 2022). Despite the critical role of the thalamus for cortico- subcortical integration and centralization of cognitive circuitries during gestation and early years of life, relevant research about the functional and structural normative properties of this network remains limited.

1.5 Structure-Function Connections in the Fetal Brain

The understanding of resting state *functional* network development can be complemented by studying the correlates with the structural maturation of subcortical structures. High-order cognitive networks present modular properties between cortical and subcortical structures (Tao et al., 2015). The hippocampus, thalamus, basal ganglia, and cerebellum are functionally and structurally associated with the DMN and MTL in adults (Alves et al., 2019; Li et al., 2021; Tao et al., 2015). The thalamus also provides the subcortical infrastructure of a stimulus expectant TCN (Shepherd & Yamawaki, 2021). The involvement of these brain regions in functional connectivity underlying cognition indicates that subcortical structures play a crucial role in memory retrieval and network integration. Moreover, Favaretto et al. (2022) demonstrated that lesions to the hippocampus and thalamus can provoke deficits in the dynamics of cortical and subcortical interactions of the DMN and other task-positive networks.

Limited research to date has focused on the dynamic codependence of resting state networks and subcortical structures in the fetal brain. Canini et al. (2020) presented the first cognitive development blueprint in the fetal brain and suggested that subcortical cortical maturation of cognitive networks begins prenatally. The role of subcortical structures in the functioning of resting state networks has been widely characterized in adults, and fetal research methodologies are persistently opening new areas of research. Thus, we can better characterize local connectivity of resting state networks accounting for the involvement of subcortical structures in memory systems during the gestational period.

1.5.1 Learning and memory systems

The fetal brain is highly receptive to environmental stimuli. Previous research has demonstrated the influence of maternal stress, anxiety, alcohol and drug consumption and even socioeconomic status in the structural and functional development of the fetal brain (Lin et al., 2017; Mattson et al., 2019; Ross et al., 2014). The presence of these environmental factors can alter the typical development of the brain more precisely the maturation of functional networks vital for cognitive function. For example, exposure to alcohol prenatally decreases the typical development of the connectome involved in cognitive functioning in utero (Wozniak et al., 2017).

Better characterization of early brain networks can aid in understanding alterations in systems supporting learning and memory processes. In a typical classroom, it is estimated that 14% of children struggle with recurrent learning and memory issues caused by cognitive skill deficiencies (Spencer et al., 2014). Approximately 5 to 9% of children suffer from dyslexia (Shaywitz et al., 2021). 3-5% of students struggle with reading comprehension (Spencer et al., 2014). Up to 15% of school children also struggle with becoming proficient in language and mathematics (Holmes et al., 2019). These various cognitive difficulties typically co-occur with each other and with deficits in social skills and behavioral disorders (Pratt & Patel, 2007). Moreover, some of these cognitive impairments arise from the abnormal development of memory networks during the gestational period (Holmes et al., 2020). Many studies have demonstrated the high vulnerability of the fetal brain to external factors providing evidence that cognitive deficits can emerge prenatally.

1.6 The current study:

Understanding the aspects of functional networks in the fetal brain that support cognitive functions is of specific interest due to increasing awareness concerning environmental influences that may alter the typical development of cognitive systems. The current study examined the associations between the functional connectivity strength of the DMN with the MTL and TCN and the association with subcortical macrostructure during the third trimester of gestation. Our first objective was to examine functional connectivity strength

of the DMN with the MTL and the association with the development of the hippocampus, the basal ganglia, thalamus, and the cerebellum. The second objective was to examine DMN connectivity strength with the TCN and its association with the maturation of subcortical structures during the third trimester of pregnancy. Based on previous research in the adult brain, suggesting that the DMN, MTL and TCN networks have extensive local connectivity with subcortical structures *within* their networks (e.g., hippocampus) and long-range structural connectivity with subcortical structures *outside* their networks (e.g., cerebellum), we hypothesized that in the fetal brain during the third trimester, the DMN, MTL and TCN functional connectivity strength would be strongly associated with the subcortical structural development.

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Chapter 2

2 Experiment: Default Mode Network Functional Connectivity Strength *in utero* and the association with fetal subcortical development

2.1 Introduction

Approximately 6 to 10% of school-aged children experience learning and memory difficulties due to deficiency in cognitive skills (Jacob et al., 2011). Some persistent memory related difficulties have their origin in utero (Barker et al., 2002). Development of multiple functional connectivity networks is a major predictor of cognitive function. The default-mode network (DMN) is the largest network and is essential for cognitive function. Macrostructural development of subcortical structures including the hippocampus and basal ganglia also support early learning and memory systems (Duerden et al., 2016, Overfeld et al., 2020, Loh et al., 2017). In adults, the DMN shows extensive connections with these subcortical structures including the thalamus, hippocampus, and amygdala (Alves et al., 2019). Early fetal functional and structural brain development represents a key maturational window and a potential avenue for future biomarker identification for adverse learning and memory outcomes. However, the timeline associated with the emergence of these early functional networks, and their association with macrostructural development of subcortical structures, remains unknown.

Recent advances in Magnetic Resonance Imaging (MRI) pregnancy research have provided new insights into typical fetal brain development (Van Den Heuvel & Thomason, 2016), and may allow us to detect the early origins of learning and memory difficulties. Resting-state MRI functional-connectivity studies have identified and monitored in vivo the development of motor, visual, language, and temporal functional connectivity networks in the fetal brain (Thomason et al., 2015; Turk et al., 2019). The functional architecture of the brain dynamically develops before birth, progressing from primary sensory functions to higher order cognitive processes (Turk et al., 2019). The emergence of subcortico-cortical connections and gyrification occurs during late second trimester of pregnancy and maturation of these networks continues during pregnancy

(Jakab et al., 2014; Schmitt et al., 2022). Functional resting state networks such as the DMN and thalami-cortical network (TCN) and others come online during the third trimester of pregnancy (Thomason et al., 2015).

The TCN is a highly connected network that supports many brain processes. It is known as an integrative hub for information filtering within the brain's modular structure, mediating sensorimotor, affective, and cognitive functions such as language processing and memory retrieval (Brandt & Dieterich, 2019; Shepherd & Grillner, 2018). Research has shown that during the first two post-natal years connectivity between the thalamus and the sensorimotor, salience, and default mode networks are present (Alcauter et al., 2014). The development of the TCN network in the prenatal period has not been studied extensively, although thalamocortical axons are actively forming during the third trimester (Kostović & Jovanov-Milošević, 2006). Subsequent cortical function development depends on the proper establishment of thalamocortical connections, and alterations to this vital process can lead to developmental disorders later in life (Cai et al., 2017). Consequently, the TCN is a key network for broad cognitive processes and is dependent on functional and structural connectivity in the developing brain.

To better understand the origins of the fetal connectome in the human brain, especially the organization of networks underlying learning and memory systems, studies characterizing the development of cortical and subcortical structure-function relationships are needed. Given the significant role of the DMN in supporting cognitive development, understanding its functional and structural connectivity early in life in a normative sample can provide a reference for atypically developing fetuses. In this study, we aimed to explore the interdependence of functional networks with subcortical macrostructural development during the third trimester of pregnancy with a primary focus on the association between the DMN, TCN, and medial temporal lobe network (MTL) with the relevant subcortical structures. Pregnant women participated in one or two fetal MRI scans during which anatomical and resting-state MRI data was acquired. As the development of these cognitive networks begins early during the second trimester of pregnancy (Thomason et al., 2015), we hypothesized that the connectivity strength between the DMN, MTL, and TCN networks would be strongly associated with

subcortical structural development, particularly the hippocampus, basal ganglia, and thalamus. Analyzing the association between DMN, MTL and TCN networks and subcortical volumes will provide additional insight into the origin of neural mechanisms that support infant and child cognitive processes. These biological metrics will also provide a baseline to compare across clinical cohorts.

2.2 Methods

2.2.1 Participants

Thirty- six healthy women with singleton pregnancies in the third trimester of pregnancy (28-40 weeks) were recruited to participate in one or two MRI sessions. The second scan was conducted 2-8 weeks after the first scan for those participants who were able to return for a second scan. Participants were recruited through the community through advertisements. Participants were included in the study if the fetus presented normal growth, were older than 18 years of age, and reported a singleton pregnancy. Participants were excluded from the study if they presented contraindications to safely undergo the non-contrast MRI, presence of a congenital anomaly and, if the participant reported concomitant substance use. The Health Sciences Research Ethics Board at Western University approved the study and all individuals provided written informed consent prior to participating.

2.2.2 MRI protocol

The MRI scans were performed at two sites and the study procedures were maintained at both locations. Most of the scans (n=34, 85%) were carried out at the Translational Imaging Research Facility (TIRF) at Robarts Research Institute, Western University, London, Canada using a 3T (General Electric [GE], Milwaukee, WI; MR7500) MRI scanner and a 32-channel torso coil. The other five participants were scanned on a 70 cm bore 1.5 T (GE, MR450w) with a GEM posterior and anterior array coil at the London Health Sciences Centre, London, Canada.

Anatomical images were acquired using a T2-weighted sequence that consisted of 2D low resolution stacks of images. Participants were scanned to acquire at least three single shot fast spin echo (SSFSE) images (TR > 1,000 ms, TE = 80 ms, field of view

(FOV) = 38-44 mm, 0.74 x 0.74 (coronal and sagittal) or 0.86 x 0.86 (axial) matrix, slice thickness = 5 mm, 19 – 25 slices), with one in each x, y, and z plane.

Resting-state fMRI was acquired using an echo planar imaging sequence (TR: 2 sec, TE: 45-60 msec [3T] / 60 msec [1.5T], flip angle 70°, voxel size 3.75*3.75*4 mm3, and 26 slices per volume, field of view 24 cm). A total of 110 volumes were acquired for each participant.

2.2.3 Image preprocessing and analysis

Figure 1 illustrates a schematic layout of the preprocessing and analysis procedure for both the anatomical and functional MRI data. The stacks of T2-weighted images were corrected for motion and bias field inhomogeneities, and super-resolution reconstruction was performed using NiftyMIC (Ebner et al., 2020), resulting in 3D volumes resampled to 1 mm isotropic resolution according to previously published methods (Wang et al., 2022). To localize and segment the subcortical regions of interest we used a semi-automated pipeline (Wang et al., 2022) that uses machine learning-based segmentation algorithms for MRI data obtained during the third trimester of pregnancy.

As the brain in fetal fMRI data is surrounded by other tissue such as the placenta and the mother's organs, it must first be isolated within each volume. To do this, we created 3D brain masks using funcmasker-flex, an automated BIDS-app brain masking toolbox for fetal fMRI data (Nichols et al., 2023), and subsequently applied the masks to their corresponding volume. The open-source advanced normalization tools (ANTs) package was used for motion correction (Avants et al., 2011), in which an average of the first 10 volumes is first created, and each volume is then registered to this average using an affine transformation. The data were then checked to ensure proper realignment and orientation labels. Each segment was then despiked. To register the echo planar imaging (EPI) data to template, the anatomical data was first registered to the EPI scans, then the EPI-aligned anatomical data was registered to a 36-week gestational age fetal brain atlas (Gholipour et al., 2017). This transform matrix was then applied to the EPI data. Finally, we smoothed the data to 5mm and calculated percent signal change.

To obtain the resting-state network maps, we performed group Independent Components Analysis (ICA) using Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC, v3.0) as part of the suite of tools in FMRIB Software Library v6.0 (FSL, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/MELODIC). Based on the 30 independent components that were generated, we identified the three networks of interest (DMN, MTL, TCN). Two independent raters from the research team identified the resting state networks of interest with unanimous agreement. Further analysis was not performed in the remaining independent components. We then binarized each component of interest, creating masks of the DMN, MTL, and TCN networks. These masks were then applied to the preprocessed EPI data. We extracted the mean time series from each participant's EPI data within each mask using FSL's fslmeants, a command line tool commonly used within the FSL software that outputs the individual time series for a group of voxels of Nifti format data sets. A visual inspection was performed on the time series of each network with the goal of assessing data quality and the detection of spikes in the data. To calculate network strength between regions, the correlation coefficients between each network's time series were calculated. The values ranged from 0-1, with values of 0 indicating no correlation between two regions and a value of 1 indicating perfect correlation.

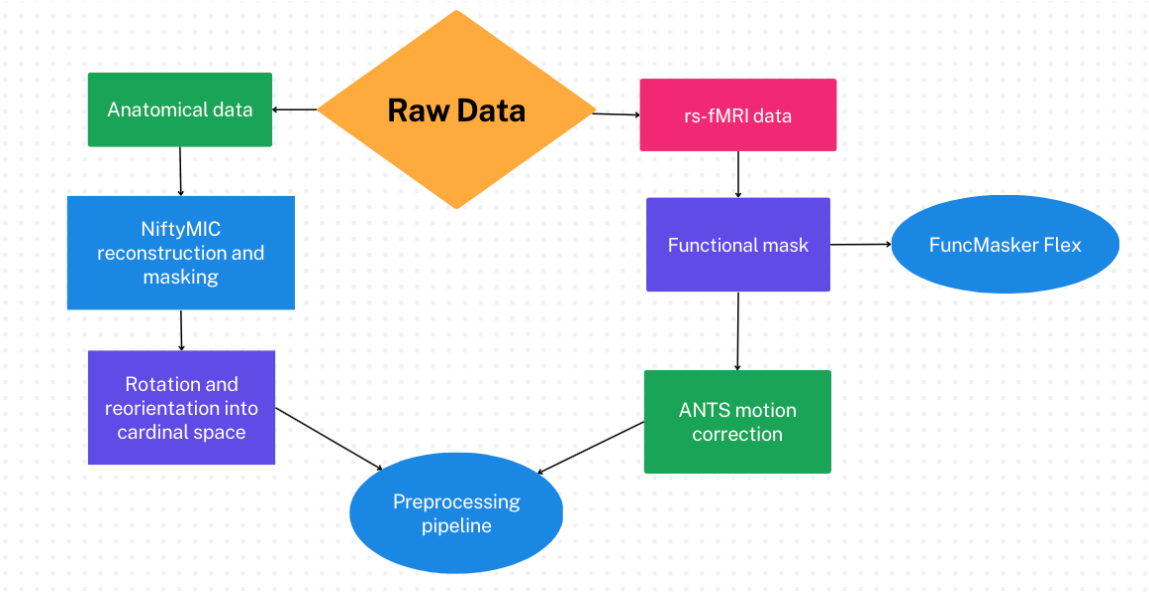

Figure 2.1 Single subject processing pipeline from raw fMRI data to subject-specific functional connectivity map.

2.2.4 Statistical analysis

Statistical analyses were performed using the IBM SPSS statistics software package (v 28, Statistical Package for the Social Sciences, IBM, Armonk, NY).

The first aim of this study was to examine the association between key resting-state networks involved in cortical-subcortical integration, and memory broadly. To examine this aim, we performed a generalized estimating equation to account for repeated measures, using an identity link function, with the volumes of subcortical structures (hippocampus, amygdala, thalamus, putamen, globus pallidus and cerebellum) as the independent variables and the functional connectivity strength of resting-state networks (MTL-DMN) as the dependent variable, adjusting for gestational age, fetal sex, and maternal age in the model. The second aim was to examine the association between the TCN-DMN connectivity and subcortical structures. We investigated this aim by performing a second generalized estimating equation, with subcortical structures (hippocampus, amygdala, thalamus, putamen, globus pallidus, and cerebellum) as independent variables and the functional connectivity strength between the DMN and TCN as the dependent variable, again adjusting for gestational age, fetal sex, and

maternal age in the model. In both models, scan was entered as a within-subjects factor. As we had two hypotheses, and two models were run to assess the association of functional connectivity strength (DMN-MTL, DMN-TCN) with subcortical volumes, we corrected for multiple comparisons using the Bonferroni method (p=0.05/2, p=0.03).

2.3 Results

2.3.1 Participants

Our sample consisted of healthy fetuses between 27.6 and 37.3 weeks of gestational age (mean gestational age= 33.4). Of the 36 participants, 13 (36%) participants returned for an optional second scan (median time between scans = 3 weeks). As a result, a total of 49 scans were acquired. After preprocessing the data, 6 participants were excluded from further analysis due to motion artifacts affecting the quality of the scans. A total of 30 pregnant women with 43 fetal resting state scans were included in the final analysis (Table 1).

Table 2.1 Maternal and fetal characteristics

Characteristic	Total (n=30) Scan 1	Total (n=13) Scan 2
Fetal gestational age, Median weeks (IQR)	33.6 (31 – 36.4)	34.35 (32.5 – 37.1)
Fetus biological sex, n (%)	Female 17 (56.7) Male 12 (40) Unknown 1 (3.3)	Female 8 (61.5) Male 4 (30.7) Unknown 1 (7.8)
Maternal Age, Median years (IQR)	32.5 (29 – 36.3)	--

Fetal gestational ages (weeks' gestation) and maternal ages (years), IQR, interquartile range (25%ile-75%ile).

2.3.2 Subcortical brain macrostructures in association with gestational age

In an exploratory analysis, we examined the association of subcortical volumes with fetal gestational age. Using Pearson's R correlation, positive relationships between gestational age and volumes across all subcortical macrostructures were identified (Fig 2; all p <

.001, corrected for multiple comparisons using a False Discovery Rate[FDR] set at q 0.05). The results were maintained when adjusting for site (all p<0.001), indicating findings were consistent across the two sites of data collection.

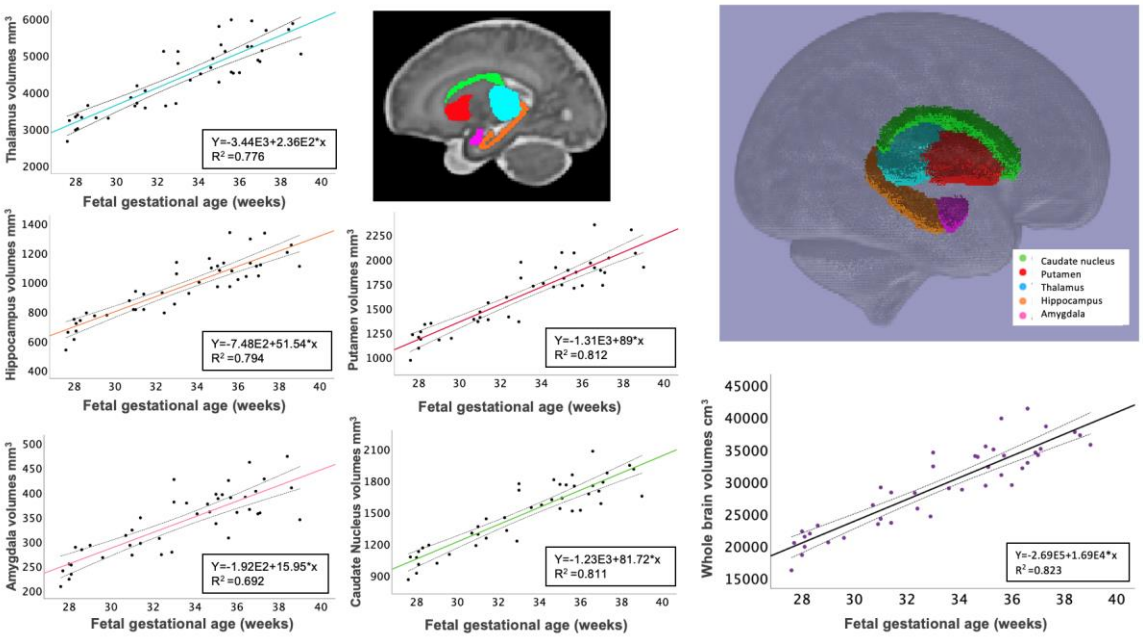

Figure 2.2 The association of fetal subcortical volumes with gestational age.

Thalamus, hippocampus, amygdala, putamen, caudate nucleus, and whole brain (From top to bottom, left to right). All subcortical regions were positively associated with gestational age based on Pearson's r correlation analyses (all, p < .001, FDR corrected).

2.3.3 Default mode and medial temporal lobe networks: connectivity strength and subcortical brain macrostructures

Our first aim was to assess whether the DMN-MTL connectivity strength was associated with subcortical structure volume (left and right hippocampus, thalamus, amygdala, caudate nucleus, putamen, and cerebellum). Results indicated that increased DMN-MTL connectivity strength was associated with smaller right hippocampal (B=-0.001, p= .012, Fig. 3a), left thalamic volumes (B=-0.001, p= .027, Fig 3b), and right caudate nucleus (B=-0.001, p= .016, Fig 3c), and larger volumes of the left caudate (B=0.001, p=.004). DMN-MTL network strength was positively associated with GA (B=0.054, p<.001),

however there was no significant relationship with maternal age or the biological sex of the fetus.

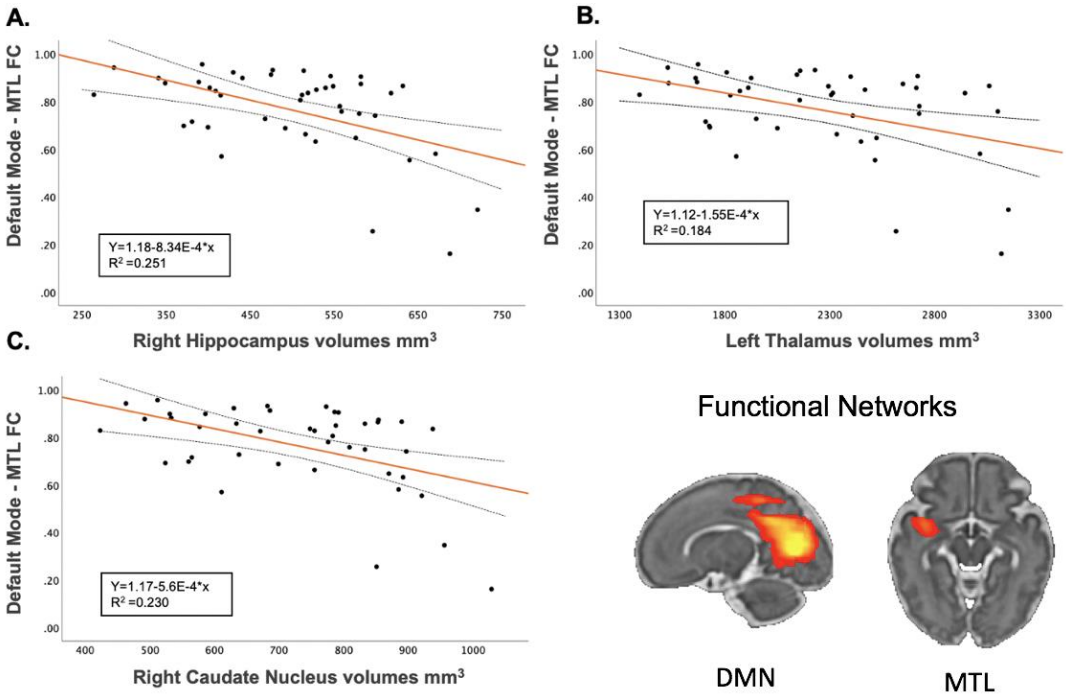

Figure 2.3 Functional connectivity between the DMN and MTL networks in third-trimester fetuses and the association with subcortical volumes. Plots show the relationship between DMN-MTL connectivity strength and A) right hippocampal volumes, B) left thalamus volumes, and C) and right caudate nucleus volumes.

2.3.4 Default mode and thalamocortical network: connectivity strength and subcortical brain macrostructures

Our second aim was to assess whether connectivity strength between the DMN-TCN was associated with the volume size of subcortical structures. DMN-TCN connectivity strength was significantly associated with left thalamus volumes (B= -0.000469 p= .022, Fig 4). A trend was seen for the left putamen (B=-0.0002, p= .032) but this association did not survive correction. GA, maternal age, and sex were not associated with FC strength.

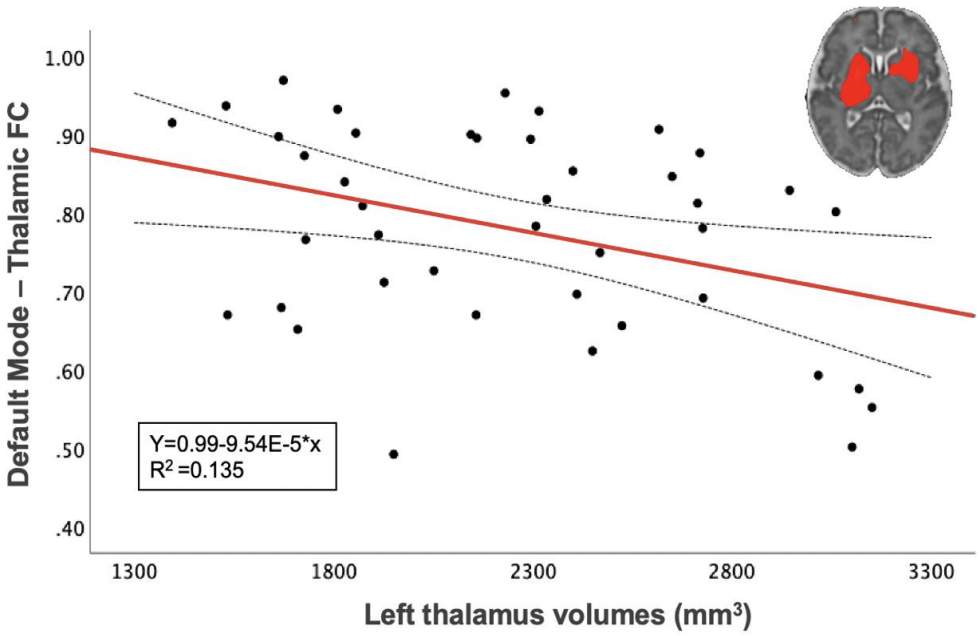

Figure 2.4 Functional connectivity between the default mode and thalamic networks in third trimester fetuses and the association with left thalamic volumes. Increased connectivity strength between the DMN and thalamic network was associated with smaller left thalamic volumes (B = -0.000469 P = .022). Dashed lines represent 95% confidence intervals.

2.4 Discussion

In this study, we assessed the association of key resting state networks involved in cortical-subcortical integration with subcortical macrostructural development. We provide evidence that during the third trimester of pregnancy, higher order cognitive resting-state networks are detectable, indicating the existence of the maturing connectome prenatally. As part of an exploratory aim, the volumetric maturation of subcortical areas including the hippocampus, basal ganglia, thalamus, and total cerebral volumes was examined in relation to gestational age. All volumes showed strong linear associations with gestational age from 28 to 39 weeks in agreement with previous volumetric studies of fetal brain developmental trajectories (Grossmean et al., 2006; Ren et al., 2022; Scott et al., 2011, Andescavage et al., 2016). We further demonstrated an association between the DMN-MTL network and subcortical volume size in the right

hippocampus, left thalamus and bilateral caudate nuclei. Additionally, increased DMN-TCN connectivity strength was negatively associated with left thalamus volumes. Key findings were that functional connectivity strength was associated with subcortical macrostructure during the third trimester of gestation, and the DMN, MTL and TCN are strongly associated, particularly with the hippocampus, the thalamus, and parts of the basal ganglia. These negative associations were seen among the DMN-MTL, and DMN-TCN with subcortical volumes after adjusting for gestational age, maternal age and sex. Based on these findings, we report a novel characterization of cortico-subcortical relationships for the DMN which may support early learning and memory systems. These brain-based biomarkers can aid in serving as a normative baseline to better characterize the fetal connectome.

2.4.1 DMN, MTL and TCN networks and the association with subcortical macrostructure in the fetal brain

Our findings showed strong associations among functional connectivity strength of the DMN and MTL, and the DMN and TCN, and subcortical macrostructure. This relationship suggests that networks involved in the cognitive-functional architecture of the fetal brain develop alongside densely connected subcortical regions.

The DMN is the most studied resting-state network and is involved in many cognitive functions, including declarative memory, theory of mind introspective thought processing (Buckner et al., 2008; Gusnard and Raichle, 2001; Salomon et al., 2009; Callard and Margulies, 2014). Many of these cognitive processes are thought to come online later in childhood or young adulthood, indicating that these networks undergo strong maturational changes throughout development. It has been established that the DMN includes strong functional connections with networks in the medial prefrontal cortex (mPFC), posterior parietal cortex (PPC) and MTL (Supekar et al., 2010). The subcortical components of the DMN have also been characterized (Alves et al., 2019); whole-brain network organizational studies have shown that subregions of the cerebellum and striatum are functionally connected with the cortical regions of the DMN (Stoodley et al., 2009; Choi et al., 2012). Seed-based functional connectivity studies further demonstrate additional DMN-specific connectivity to several subcortical structures,

including the amygdala (Bzdok et al., 2012) and striatum (Di Martino et al., 2008). In contrast, the structural connectivity of the DMN is less studied. In adults, the thalamus is both structurally and functionally connected to DMN regions (Cunnigham et al., 2016; Fransson et al., 2005). Evidence from diffusion tensor imaging (DTI) indicates that fibre densities and mean fractional anisotropy (FA) values of the PCC-mPFC tracts and the PCC-left MTL tracts were lower in children compared to adults (Supekar et al., 2010). The DMN also demonstrates strong structural connectivity with the hippocampus, as revealed using DTI (Tao et al., 2015).

The present results demonstrating an association between DMN-MTL connectivity strength and volumes of the right hippocampus, left thalamus and bilateral caudate nuclei aligns with previous work in adults. Functional networks known as “hubs” have been reported to be critical for neural efficiency and for the formation of functional connectomics prenatally (Oldham & Fornito, 2019; Turk et al., 2019; Van Den Heuvel et al., 2018). In the fetal brain, evidence suggests that the earliest formation of visual, motor, and cognitive processes is associated with functional hubs found in the motor and primary sensory regions (Van Den Heuvel et al., 2018). The thalamus in particular serves as crucial hub in high-order cognitive circuits and as a center for relaying sensory information to the cortex (Cai et al., 2017, Ball et al., 2015). Disruptions to the establishment of appropriate subcortical-cortical connections may increase the risk of developing learning disorders or even neurodevelopmental disorders (Ball et al., 2015; Nair et al., 2013; Nair et al., 2021)

The DMN, MTL, and TCN, extracted using ICA, were bilaterally activated, consistent with previous evidence in preterm born babies demonstrating the emergence of bilateral networks in the time corresponding to the third trimester of pregnancy (Doria et al., 2010). Interestingly, our results revealed significant associations between bilateral functional connectivity strength in several networks and largely unilateral subcortical maturation. The variability between the connectivity strength in the bilateral functional networks and the association with unilateral subcortical macrostructure could be explained by a variety of factors. Previous cross-sectional studies reported asymmetrical growth between contralateral subcortical regions during the third trimester of fetal life,

giving rise to heterogeneity in functional connectivity strength (Andescavage et al., 2016b; Thomason et al., 2013). Previous studies have shown that hemispheric asymmetry occurs during the third trimester of pregnancy, with evidence suggesting that left hemisphere structures are larger than in the right hemisphere (Andescavage et al., 2016; Cara et al., 2022). Whether functional networks are dependent upon macrostructural growth in unilateral regions, or whether enhanced functional connectivity strength is associated with enhanced macrostructural growth in the fetal brain, can only be assessed in longitudinal studies. In the current work, only a subset of our data was longitudinal, and the directionality of the effects cannot be inferred. However, our results presented an evidence-based account of significant association between functional connectivity strength with subcortical macrostructures located in the left and right hemispheres.

The variability seen in our findings might also be influenced by the formation of short- and long-range cortical networks. The second and third trimester of pregnancy are critical for the typical development of early synchronized network activity and for the consolidation of neuronal circuits (Jakab et al., 2014). Previous research has also demonstrated that the development of short-range connections occurs as early as the second trimester of pregnancy and long range and cross-hemispheric connections are detected later in the pregnancy. The formation of default mode and thalamocortical connections occurs during the second trimester (Seshamani et al., 2016; Taymourtash et al., 2022). However, during the third trimester of pregnancy maturation of local networks and synaptic pruning occurs to ensure the proper development of the neuronal architecture (Smyser et al., 2010). Taken together with the current findings, this suggests that the connectivity strength of networks underlying memory processes is highest during the early stages of the third trimester (Jakab et al., 2014; Thomason et al., 2013). At the same time, evidence demonstrates that maturation of the fetal brain exhibits patterns of functional consolidation and compartmentalization of brain regions and memory-related resting-state networks, a process that continues well into adulthood (Jakab et al., 2014). Previous studies have demonstrated that in childhood and adulthood a process of short-range functional connection pruning occurs as resting state networks mature (Fair et al., 2007; Fan et al., 2021). In children, a decrease of functional connectivity strength occurs in the DMN and MTL networks due to an increase in functional segregation during

development (Fan et al., 2021). During the third trimester of pregnancy, the process of functional segregation may occur in the DMN, MTL, and TCN, however, more knowledge on the specifics of these processes remains to be understood.

This work contributes to a body of evidence that functional networks may be associated with the development of subcortical macrostructures late in fetal life. Our findings suggest a prominent role of subcortical brain regions in the detection of early brain networks that support cognitive systems. The use of fetal MRI renders the opportunity to explore and detect early the presence of alterations in brain development, which may underlie later learning and memory difficulties. The understanding of the formation of structure-function associations in the fetal brain in the presence of abnormal development remains scarcely studied. Future fetal fMRI studies could focus on possible risk factors for altered brain structural-functional connectivity including exposure to teratogens or maternal distress, and nutrition that could alter the typical development of these neural circuits.

A limitation to consider in our study is the small sample size, which may have affected our ability to detect functional connectivity strength. Future studies using a larger number of healthy participants are needed to further explore structure-function relationships during fetal development. Another limitation of our study is the potential influence of fetal motion or maternal physiological artifacts that may have unduly influenced image quality. External components, even mild, such as maternal breathing, can lessen the quality of the data. Previous research has demonstrated that the presence of artifacts can decrease the detection of the functional strength of the DMN (Sobotka et al., 2022; Van Dijk et al., 2012). However, with the robust preprocessing and analysis that were implement for our data, the primary driving factor of our results were not related to fetal motion or maternal artifacts. Although the data from 6 participants were excluded because of fetal motion, the data used for our analysis fell under the expected standards reported by previous fMRI research studies with fetal populations (Sobotka et al., 2022; Wang et al., 2022) . Lastly a final limitation to our study was that a few participants were scanned on a different MRI scanner. While scanner effects were examined in our

statistical analysis and no significant effects were evident, there is the potential that differences in scanner strength influenced both the functional and structural imaging data. Our findings demonstrating an association between functional networks and macrostructural development in the fetal brain could be examined in larger scale, single site studies.

2.5 Conclusions

We observed two main patterns of association between the connectivity strength of the DMN and associated networks and subcortical macrostructures in fetuses. We report an increase in between-network functional connectivity strength in relation to smaller volume size in subcortical regions that are densely functionally connected areas. This pattern was observed in regionally specific areas. A positive association was observed in the left caudate, suggesting some variability in network development in late fetal life. The results provide a normative characterization of early brain networks.

2.6 Acknowledgments

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Chapter 3

3 Discussion

The functional and structural properties of the DMN were assessed in a heterogeneous sample of fetuses during the third trimester of pregnancy. The primary goal of our study was to examine the short- and long-range connectivity of the DMN, both within its network (i.e., MTL) and outside of the network (i.e., TCN), whether it was associated with subcortical maturation in the fetal brain. We observed a linear increase in subcortical volume size with increasing gestational age. We further demonstrated the association between the connectivity strength of the DMN-MTL and DMN-TCN and subcortical structures in the fetal brain from 28-39 weeks' gestation. Based on the generalized estimating equations analysis, increased connectivity strength between the DMN-MTL was associated with smaller volumes of the right hippocampus, left thalamus and right caudate nucleus. In turn these networks also presented positive associations with the left caudate nucleus. The DMN-TCN connectivity strength also yielded significant negative associations with left thalamus volumes; no association with basal ganglia, cerebellum, amygdala, or hippocampal volumes were evident. Factors such as gestational age, maternal age and fetal sex were accounted for on all our models. Only gestational age presented significant associations with the DMN-MTL network. Currently is it possible to study the developing connectome using MRI and fMRI in the fetuses. Our findings of a strong association amongst functional networks and subcortical development in between the 28^{th} and 39^{th} week of gestation can provide a blueprint for brain networks that may support early cognitive development.

3. 1 Implications

The current study provides insights into the interdependence of functional networks and subcortical structures, as well as evidence of subcortical growth trajectories in the third trimester of gestation. Here we demonstrated the significance of studying resting state networks during the gestational period in populations with unsuspected abnormal development. These valuable insights will provide a guideline of functional and structural metrics to compare with in instances of suspected abnormal development. Alterations to

the typical development of resting state functional networks *in utero* can have serious consequences later in life.

Our results are in line with previous research that suggest that subcortical brain structures are vital for the functioning of resting state networks (Canini et al., 2020; Faull et al., 2021; Li et al., 2021). They also highlight the importance of taking into consideration subcortical structures when studying functional networks (Faull et al., 2021). As previously mentioned, in adults the hippocampus and basal ganglia are important for the activation of the DMN (Ezama et al., 2021; Vatansever et al., 2016). Subcortical structures such as the thalamus are also developed early during the gestational period with the goal of forming a hub rich in functional activation (Hwang et al., 2017). Considering that in our results we discovered significant associations with these subcortical structures we interpret this as a demonstration of the strong associations between subcortical and functional properties in the development of brain networks during gestation.

Previous literature has demonstrated asymmetric development of subcortical structures during the fetal period and the first years of life (Kasprian et al., 2011; T. Liu et al., 2021). We divided the subcortical regions by location within the left and right hemisphere. After performing generalized estimated equations models, we found significant results in subcortical structures located in either the right hemisphere or the left hemisphere. This pattern perhaps suggests the asymmetrical maturation of subcortical areas may be the underlie the findings. Future prospective studies examining growth trajectories in subcortical structures are needed to determine the timing of these patterns.

In our analysis we also found negative associations between the functional connectivity strength in the DMN-MTL and DMN-TCN with subcortical structures. The formation of short- and long-range networks is a rapid process that occurs during the second trimester of gestation and continues until the first few months of life (Jakab et al., 2014b). This process occurs as a developmental measure of network consolidation. In adults the literature suggest that functional segregation is constantly occurring due to patterns of development and environmental influences (Rosenberg et al., 2020). Our

study provides some support that this process also occurs during the gestational period. Our focus was limited to the DMN and the TCN; however future research should explore these associations with broader networks outside the DMN.

3.2 Future directions

Characterization of the associations of functional networks and subcortical structures in the healthy fetus can serve as a model to compare with fetuses who are atypically developing. A future area of research could examine structural and functional network development of fetuses with suspected intrauterine growth restriction, or fetuses exposed to teratogens.

Intrauterine growth restriction results from placental insufficiency and the fetus experiences a significant reduction in cerebral oxygen delivery. In turn, decreased blood flow may impact the development of the fetal brain and reduce the formation of long-range networks during gestation (Batalle et al., 2012; Miller et al., 2016). Exposure to teratogens including alcohol and illicit drugs or even cortisol from maternal stress can alter the typical development of the fetal brain (Brady et al., 2022; Turk et al., 2019). Previous research demonstrated altered hippocampal (Wu et al., 2022) and cerebellum (Lu et al., 2022) volumes due to exposure to maternal stress during pregnancy. Cortical alterations were also reported in the same cohort demonstrating a delay in brain gyrification (Lu et al., 2022). Cognitive performance is also significantly affected in infants with exposure to maternal distress during the gestational period (Wu et al., 2022). Maternal anxiety in pregnancy has been linked to altered fetal functional connectivity development due to a persistent exposure to cortisol that causes permanent alterations in fetal programming of functional networks (De Asis-Cruz et al., 2020). The findings of these studies could advise the study of early neural networks that underlie cognitive processes, which may be influenced by these external factors.

The DMN is known to be active in instances of mind wondering and spontaneous cognition, however in the presence of external task-oriented performance, this network deactivates (Uddin et al., 2009). In adults the negative correlation, known as anticorrelation, between the DMN and task- positive networks has been widely studied

(Bauer et al., 2020; Esposito et al., 2018; Owens et al., 2020). Neurotypical adults demonstrate a more mature mechanism of functional connectivity activation between anticorrelated networks compared to children (Uddin et al., 2011). This maturation is likely to occur due to a strengthening in functional and structural associations that enhances the dynamic switching between networks (Menon & Uddin, 2010). These competitive interactions between cognitive networks are influenced by regional specificity. While previous studies demonstrated that structural development of subcortical regions plays a key role in the formation of effective associations between anticorrelated networks in the adult brain (Menon & Uddin, 2010; Uddin et al., 2011), no previous research has focused on the development of this intrinsic functional relationship in the fetal brain. Further, alterations in the ability to switch from the DMN to the central executive networks is thought to underlie difficulties in attention, set-shifting, and attentional control in children with neurodevelopmental disorders. Future research should explore this competitive relationship between functional networks in the fetal brain using task-based approaches. Few investigators have attempted task-based protocols in the fetus. However, research suggests that the fetus can respond to auditory (Goldberg et al., 2020) and visual stimuli (Fulford et al., 2003) The use of task-based methodologies will allow researchers to examine the profile of activation and deactivation of significant cognitive networks in fetal populations.

3.3 Conclusions

We demonstrated an association between functional networks and subcortical structures in the fetal brain during the third trimester of pregnancy. We provided evidence of a negative association between functional connectivity strength of the DMN-MTL and DMN-TCN with regionally specific subcortical volumetric development in the third trimester of pregnancy. To our knowledge this is the first study analysing the functional and structural properties of the DMN during gestation. The fetal brain undergoes functional segregation during this rapid period of development to consolidate short- and long-range network maturation. Our study was limited by the sample size that was used for the analysis, potential site effects due to a small number of participants being scanned on a different MRI scanner, and the typical effects of fetal motion and maternal artifacts

that may have affected the quality of the data. Better understanding of the development of the fetal brain networks may serve as a normative model that could be used for future hypothesis-driven research in at-risk fetuses.

3.4 References

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