4-21-2017 12:00 AM

Functional Connectivity in the Motor Network Largely Matures Before Motor Function

Jordynne L V Ropat, *The University of Western Ontario*

Supervisor: Dr. Rhodri Cusack, *The University of Western Ontario*

A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Neuroscience

Recommended Citation

Ropat, Jordynne L V, "Functional Connectivity in the Motor Network Largely Matures Before Motor Function" (2017). *Electronic Thesis and Dissertation Repository*. 4539.
https://ir.lib.uwo.ca/etd/4539

Abstract

The brain changes in many ways in the first year. It is not known which of these changes are most critical for the development of cognitive functions. According to the Interactive Specialization Theory, developments in behaviour result from changes in brain connectivity. We tested this using functional connectivity magnetic resonance imaging (fcMRI) of the motor system. fcMRI was acquired at three and nine months – two time-points between which motor behaviour develops enormously. Infants were additionally compared with adults. Subjects were scanned with a 3T MRI scanner, yielding BOLD signal time-courses that were correlated with one another. Our results do not support the Interactive Specialization Theory, as connectivity did not change with motor development and instead was adult-like in the youngest infants. fcMRI has enabled deeper exploration of network connectivity patterns and continues to emerge as a leading method in infant neuroscience.

Keywords

Keywords: functional magnetic resonance imaging, functional connectivity, motor network, infant neuroimaging, neurodevelopment

Co-Authorship Statement

Chapter two is presented in the form of a manuscript. The infants were recruited by DSCL and VKH; data was acquired by ACL, CJW, HD, and CH prior to my arrival. I analyzed the data and wrote the manuscript together with my supervisor, RC.

Acknowledgments

I would like to sincerely thank my supervisor, Rhodri Cusack, for his help and support throughout my Master's degree. Thank you for the guidance you have given me, over the past two-and-a-half years but especially throughout the final drafts of my thesis. I appreciate having had the opportunity to work with functional MRI as well as with infants, both of which have helped me to develop invaluable transferable skills. Thank you also for giving me the opportunity to travel to present my research. I am very grateful for the experiences I have had.

I would also like to thank all of the postdoctoral fellows in my lab who collected the data for my thesis and who were instrumental in guiding me through this degree.

Finally, I would like to thank my family and close friends who have supported me through everything. Thank you especially to my father, Michael, and my partner, Dante, for your unconditional love and support. I love you.

Table of Contents

List of Tables

List of Figures

Chapter 1

1 Introduction and Literature Review

"The passage from the limited motor repertoire of the newborn to the complex locomotor and manipulatory skills of the toddler stands among the most visible and dramatic transformations in the human life cycle."

(Thelen, Kelso, & Fogel, 1987)

1.1 Motor Behaviour Changes Dramatically Throughout Infancy

Motor behaviour changes from birth through childhood and beyond as new skills are learned and mastered. However, the most rapid and some of the most impressive changes occur during in the first year of life **(**Figure 1). Infants enter the world virtually helpless and – in the span of just a year – become active players in their own lives and the lives of others.

Figure 1. Chronologic progression of gross motor development in infants from zero to six months of age.

Using cartoon infants, this image illustrates the monthly milestones that many infants reach. The image has been removed due to copyright restrictions.To view this image, please see Figure 2 in the following manuscript: Johnson, C., & Blasco, P. (1997). Infant growth and development. *Pediatrics in review / American Academy of Pediatrics, 18* (7), 224-242. Adapted from Piper, M. C. (1994). Motor assessment of the developing infant. WB Saunders Company. Illustrations by Marcia Smith.

Though vastly limited in their motor abilities, most neonates (infants under four weeks of age) consistently exhibit a number of actions often referred to as reflexes. Although originally thought to be simplistic, stereotyped behaviours, reflexes may be more complex than originally believed. For example, when an infant's cheek is touched, she will move her mouth there in search of milk – this is called rooting. However, when she touches her own cheek, or if she is not hungry, she won't display this behaviour, suggesting that she has some voluntary control over this action (Von Hofsten, 2004). An especially interesting example is the Asymmetric Tonic Neck Reflex; this reflex is observed when an infant lies on his back with his head turned to one side and he extends his arm on the side that his head is turned. van der Meer and colleagues hypothesized that this “reflex” was actually an attempt by the infant to see his arm, so they placed infants into an apparatus which could pull both arms down using strings tied to the infant’s wrists (van der Meer, van der Weel, & Lee, 1995). When both arms were pulled down, the infant attempted to maintain extension on the side that his head was turned (the ipsilateral arm) but did not resist the pull on the arm that he couldn't see (the contralateral arm) (Von Hofsten, 2004) (van der Meer, van der Weel, & Lee, 1995). The researchers then occluded the infant’s view of both his arms, but allowed him to see the contralateral arm in a mirror; when both arms were pulled down, he resisted the pull on his contralateral arm (Figure 2). Finally, when both arms were occluded from the infant’s view, he did not resist the pull on either arm (van der Meer, van der Weel, & Lee, 1995) (Von Hofsten, 2004).

Figure 2. Infants will resist a pull on their visible arm.

This image is a black-and-white sketch of the infant in the arm-pulling apparatus. In this particular sketch, the infant’s head is turned to the right and they are looking into a mirror. In the mirror, they can see their left hand. The apparatus easily pulls down the infant’s right hand; however, since the left hand is visible in the mirror, the infant resists the pull. The image has been removed due to copyright restrictions. To view this image, please see Figure 1 in the following manuscript: Von Hofsten, C. (2004). An action perspective on motor development. *Trends in Cognitive Sciences, 8*(6), 266-272.

This research suggests there may be hidden complexity in infant motor behaviour as early as just a few weeks of age (Von Hofsten, 2004) (van der Meer, van der Weel, & Lee, 1995), which marks the first steps in a childhood of increasingly complex motor behaviour.

Though an infant spends the first few months of life either lying down or fully supported by adults, they nevertheless steadily gain more control over their bodies and more understanding of their surroundings beyond the womb. Muscle tone in the newborn is poor, and an infant can only lift her head for a few seconds at a time; however, by 8 weeks of age, she is able to lift her head while prone and maintain this position at will for a number of minutes by using her arms and torso for support. (Law, Lee, Hulse, & Tomassetti, 2011). Observing others' actions plays a crucial role in future motor control and is likely one of the most important motor behaviours in the first months. One of the first motor behaviours infants display in their first month are saccades, rapid eye movements that change the point of eye fixation (Law, Lee, Hulse, & Tomassetti, 2011) (Purves, et al., 2001). Infants make increasingly more saccades over the first two months as they learn to focus their attention on objects and visually track moving stimuli (Law, Lee, Hulse, & Tomassetti, 2011). Gross motor skills develop, beginning with uncoordinated, jerky arm and leg movements and progressing to prereaching – i.e. imprecise swiping motions in the direction of stimuli (von Hofsten, 1984) (Law, Lee, Hulse, & Tomassetti, 2011). By the third month, the infant is able to use the head and eyes in tandem to track moving objects (Law, Lee, Hulse, & Tomassetti, 2011). Infants have also developed the fine motor skills required to grasp objects that have been placed in their hands, but are not yet able to reach for and successfully grasp an object themselves (Law, Lee, Hulse, & Tomassetti, 2011).

About a third of the way into their first year, infants gain more opportunities to observe and interact with the world as they can now sit up, though they still need support (White, Castle, & Held, 1964). Infants also have greater control of their arms and become much more skilled at reaching and intercepting objects smoothly (White, Castle, & Held, 1964). Successful coordination of these goal-directed movements depend in part on smooth pursuit, the ability to accurately track moving objects with the eyes. Infants with the skills necessary for smooth pursuit also often show the ability to visually predict external events: when watching an object moving on a path, where the object is at one point obstructed from view, infants will shift their gaze to the object's final location before the object reappears (Von Hofsten, 2004). By five months of age, infants can roll from back to front, pull themselves to sitting, and move their head to look for an object (Gerber, Wilks, & Erdie-Lalena, 2010) (White, Castle, & Held, 1964). Impressive fine motor developments also emerge by this age, such as successful reaching and grasping while seated, transferring objects between hands, and one-hand grasping (Law, Lee, Hulse, & Tomassetti, 2011). Many infants have also developed sufficiently adequate balance to allow for reaching up for dangling objects, and down for dropped objects while seated (Law, Lee, Hulse, & Tomassetti, 2011).

The transition from six months to one year is especially striking, as developments in motor behaviour are frequent and rapid. Atun-Einy and colleagues studied 27 infants at two time points within the first year: seven months and 12 months of age (Atun-Einy, Berger, & Scher, 2013). At the beginning of the study, none of the seven month old infants could crawl; however, at the conclusion of the study, all infants could crawl, pull themselves to standing, and walk with support, and a quarter of the infants had begun to walk unsupported (Atun-Einy, Berger, & Scher, 2013). Though at six months of age most infants still need some pelvic support to stay seated, many seven month olds can sit unsupported steadily and have even learned to put their arms out for balance (Gerber, Wilks, & Erdie-Lalena, 2010). Infants at this age also have sufficient muscle tone and control in their leg muscles to bounce while held (Gerber, Wilks, & Erdie-Lalena, 2010). By eight and nine months of age, infants are remarkably autonomous. The infant can successfully move around on her own accord for the first time by crawling on her stomach (Gerber, Wilks, & Erdie-Lalena, 2010) (Law, Lee, Hulse, & Tomassetti, 2011). Infants can also pull themselves to standing and use their arms and legs to get into a sitting position (Gerber, Wilks, & Erdie-Lalena, 2010) (Law, Lee, Hulse, & Tomassetti, 2011). Infants have now developed the fine motor skills to pincer-grasp objects, take an object out of a container, hold a bottle on their own, and feed themselves small items (Gerber, Wilks, & Erdie-Lalena, 2010) (Law, Lee, Hulse, & Tomassetti, 2011). By ten months, many infants have become experts at creeping (crawling with hands, knees, and belly) and some are able to move around by “bear-walking” (crawling with their arms and legs straight) (Gerber, Wilks, & Erdie-Lalena, 2010) (Law, Lee, Hulse, & Tomassetti, 2011). Infants are also able to walk with both hands holding onto furniture and stand using only one hand (Gerber, Wilks, & Erdie-Lalena, 2010) (Law, Lee, Hulse, & Tomassetti, 2011). At 11 and 12 months, fine motor skills have developed tremendously. Infants begin to use a pincer grip to hold objects using the thumb and index finger, throw objects, hold a crayon, attempt to stack two cubes, and begin to show handedness (Gerber, Wilks, & Erdie-Lalena, 2010) (Law, Lee, Hulse, & Tomassetti, 2011). Gross motor development is equally notable as many infants are able to cruise with only one hand holding onto furniture and most can crawl confidently (Gerber, Wilks, & Erdie-Lalena, 2010) (Law, Lee, Hulse, & Tomassetti, 2011). Some are able to stand only for a few seconds; others can stand up confidently for longer by holding their arms out for balance; and a few may be able to take their first steps independently (Gerber, Wilks, & Erdie-Lalena, 2010) (Law, Lee, Hulse, & Tomassetti, 2011).

Although there are time points at which many infants reach a milestone, there are of course innumerable individual differences between infants. For example, many infants achieve the ability to sit independently, pull themselves up to standing, and crawl at around eight months of age. However, some infants learn to do so as early as six months of age or as late as 11 (Atun-Einy, Berger, & Scher, 2013). Rates of growth and milestone achievement are not stable even within individual infants; that is, an infant that has reached all major milestones at three months of age may be late to develop at six months. Darrah and colleagues followed 45 infants from two weeks until they could walk independently and assessed their gross motor skills monthly using the Alberta Infant Motor Scale (AIMS) (Darrah, Redfern, Maguire, Beaulne, & Watt, 1998). A paediatrician assessed these infants at 18 months and all were typically developing; however, one third of the infants, at some point in their assessment, received a score below the 10th percentile (Darrah, Redfern, Maguire, Beaulne, & Watt, 1998). An individual may be precocious in fine motor skills but take 15 months to walk; another may progress from crawling to walking quickly and easily. However, it is important to note that both may still grow up to be typically-developing. In fact, (Hadders-Algra, 2002) argues that variability indicates adaptability and is in fact a feature of a healthy nervous system.

An infant’s motivation to move can also influence their acquisition of motor milestones; as expected, infants who were more motivated to move reached their motor milestones earlier (Atun-Einy, Berger, & Scher, 2013). In addition, infants’ motivation increased shortly, but not immediately, after gaining a new skill (Atun-Einy, Berger, & Scher, 2013). Researchers interpreted that infants became more motivated partly because of feedback based on experiences gained via their newly acquired skill, highlighting the interplay between motor behaviour, social interaction, and other sensory and cognitive abilities (Atun-Einy, Berger, & Scher, 2013).

It’s clear that an infant’s ability to perform complex motor actions, as well as her confidence, motivation, and skill, change remarkably during the first year of life. It is therefore logical to infer that major changes in the structure, connectivity, and efficiency of the brain and nervous system may underlie these changes.

1.2 The Adult Motor System: A Look into the Brain

Before discussing the infant motor network, I will survey the location, function and connectivity of the areas in the adult brain that are typically related to motor functioning and/or motor activity. Using neurosynth.org, an open-source database used for large-scale meta-analysis of functional magnetic resonance imaging data, I identified brain regions that are activated more often in studies that use the keyword “motor” in their abstracts (Yarkoni, 2011). A meta-analysis of 2081 studies displays a map with all activated brain regions (all regions with a positive z-score) in coronal, sagittal, and axial planes (Yarkoni, 2011) (Figure 3). Scrolling through the slices in each plane, one can see activation in a number of motor-related regions; however, for brevity, only the major regions, as selected in Chapter Two, will be described. The regions activated include the supplementary motor area and the left and right precentral gyrus, rolandic operculum, thalamus, pallidum, and cerebellum. In addition to these regions, a few other notable regions will be discussed: the primary motor cortex, which is located on the precentral gyrus, and the rolandic operculum, which is posterior to the precentral gyrus and is part of the premotor cortex. Though this list is not exhaustive, it represents a diverse group of regions that are activated during motor activity. These regions will be discussed briefly to provide some context for when they are mentioned in the experimental chapter (Chapter Two).

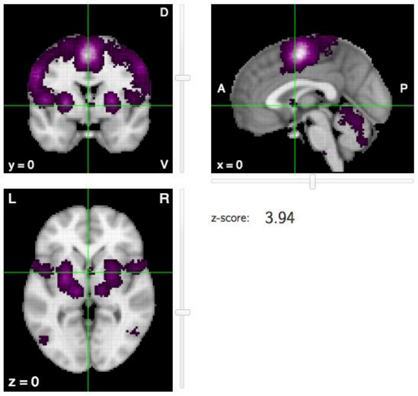


Figure 3. Results of neurosynth.org meta-analysis.

The above figure illustrates the results of an automated meta-analysis of 2081 studies which have the keyword “motor” in their abstract and show increased activation in motor areas.

Frontal brain regions – such as the SMA, precentral gyrus, and rolandic operculum – are thought to be involved in coordinating voluntary movements. The supplementary motor area (SMA) has been shown to activate in response to both planning (Roland, Larsen, Lassen, & Skinhøj, 1980) and executing (Roland, Larsen, Lassen, & Skinhøj, 1980) (Shibasaki, et al., 1993) (Orgogozo & Larsen, 1979) complex voluntary movements, such as touching the thumb to each finger in rapid succession in a particular learned order. In addition, stimulation of the SMA results in a number of diverse motor behaviours, from vocalizations and head movements to finger, hand, and leg movements (Penfield & Welch, 1949). Both premotor and primary motor cortices were also activated during a finger movement task (Baraldi, et al., 1999); additionally, researchers have located an area of the precentral gyrus that appears to be related to hand movement (Jasper & Penfield, 1949). Observing or imagining motor activity (Szameitat, McNamara, Shen, & Sterr, 2012) can also activate the premotor cortex; this is thought to represent the mirror neuron system within the brain (Culham, 2015). Finally, the rolandic operculum is activated in response to mouth, tongue, and larynx coordination – i.e. during language production (Tonkonogy & Goodglass, 1981) (Brown, Ngan, & Liotti, 2008).

While frontal regions control voluntary movements, more so-called primordial brain regions – the thalamus, cerebellum and pons, and subcortical structures such as the basal ganglia (which includes the pallidum, putamen, and striatum) – regulate the involuntary or automatic components of motor control. Doyon and colleagues studied the basal ganglia and cerebellum, from early skill learning to automaticity, to tease apart their roles in the learning process (Doyon, Penhune, & Ungerleider, 2003). They found the cerebellum to be most active during early skill development, its likely role being interpreting sensory input in order to moderate motor output (Doyon, Penhune, & Ungerleider, 2003). In contrast, basal ganglia structures are more active later in learning, when movement is more automatic and routine (Doyon, Penhune, & Ungerleider, 2003) (Graybiel, 2005). However, the cerebellum and basal ganglia play many more roles in complex motor skill development. For example, the cortico-striatal system in the basal ganglia, including the SMA and premotor area (Roland, Larsen, Lassen, & Skinhøj, 1980), also influences action planning just prior to performance (Doyon, et al., 2009); part of this involves deciding upon the desired activity and inhibiting undesired motion (Mink, 1996). Additionally, extensive studies implicate the cerebellum in the encoding, consolidation, and long-term memory storage of routine motor activity (Doyon, et al., 2009). The cerebellum, basal ganglia, and cortex connect to the thalamus via a variety of projections, and the thalamus plays a major role in the circuits that control the development and execution of motor activity (Haber & Calzavara, 2009). The thalamus serves as a relay centre that receives and sends out projections and integrates information from many other brain regions (Haber & Calzavara, 2009).

These subcortical regions connect to areas in the cortex via circuits, such as the cortico-basal ganglia-thalamocortical loop, that allow them to work together to control movement (Middleton & Strick, 2000) (Parent & Hazrati, 1994). One such loop is the closed motor circuit described by Joel and Weiner, where the circuit originates in motor and premotor cortical areas, leads to the basal ganglia and thalamus, and returns to the original cortical areas (Joel & Weiner, 1994). These circuits are thought to be crucial to the processes involved in learning complex motor skills (Doyon, Penhune, & Ungerleider, 2003) as well as performing simple actions – e.g. basal ganglia- and cerebello-thalamocortical projections innervate the motor and premotor cortices to support hand and arm movements (Nakano, 2000).

Together, these regions provide adult humans with the ability to perform a variety of complex motor behaviours with ease. However, as discussed in Section A, infants' motor abilities are much less impressive. A variety of techniques have been used to study fetal and infant brains to gain a better understanding of motor development at birth and throughout the first year of life. The remainder of Section B will introduce and examine structural and microstructural brain development in infants as well as insights from functional imaging. Finally, disrupted development, and its important contributions to our understanding of typical development, will be discussed.

1.3 Functional MRI in Brief, and its Challenges and Opportunities for Infant Neuroimaging

1.3.1 Background

Since its first use in 1991, functional MRI (fMRI) has made a tremendous contribution to neuroscience research (Bandettini, 2012). Functional MRI records changes in the BOLD (blood oxygen level-dependent) signal within the brain (Uludag, Dubowitz, & Buxton, 2005) using T2* weighted MRI. Haemoglobin, a group of proteins present in red blood cells, transports oxygen within the body (National Institutes of Health - National Cancer Institute, n.d.). In particular, deoxygenated haemoglobin molecules (those that are not carrying oxygen) have magnetic properties that change the local MR signal; since these molecules are paramagnetic, they cause a decrease in the MR signal (Uludag, Dubowitz, & Buxton, 2005). When one is performing a task or using cognitive resources in some way, blood flow increases to the active areas of the brain in order to provide more oxygen (Uludag, Dubowitz, & Buxton, 2005). As a consequence of an oversupply in oxygenated blood and resulting increased oxygenation, the local MR signal will tend to increase (Uludag, Dubowitz, & Buxton, 2005). It is important to clarify that the BOLD signal does not directly measure blood flow; only oxygenation (or, more accurately, lack of deoxygenation) (Buxton, Uludag, & Dubowitz, 2004). The fMRI signal during a task is compared to baseline activity during rest (in the absence of an overt task) (Uludag, Dubowitz, & Buxton, 2005). This signal change is statistically evaluated and mapped onto a structural image of the brain to show which areas have significant activation (Uludag, Dubowitz, & Buxton, 2005).

1.3.2 MRI Acquisition

To conduct MRI scanning, the scanning protocol for both structural and functional scans must be decided. Most functional MRI is collected using an echo-planar imaging (EPI) pulse sequence (Uludag, Dubowitz, & Buxton, 2005). There are typical or standard parameters for different types of research (e.g. one may choose to use parameters exactly as described in a previous study); however, sometimes a particular population or type of analysis requires parameter changes. For example, studying infant populations poses challenges that will be discussed later in this section.

Researchers must also decide how, and in which order, they would like to collect slices of the brain; these slices will then be pieced together to provide functional data. Many researchers are starting to use multiband EPI acquisition, a relatively new method that allows multiple slices to be collected at once, as it results in a higher sampling rate, and reduced sensitivity to the effects of motion (Preibisch, Castrillón G., Bührer, & Riedl, 2015) (Feinberg, et al., 2010) (Xu, et al., 2013) (Cusack, Ball, Smyser, & Dehaene-Lambertz, 2016). Minimizing the amount of time the infant has to spend in the scanner increases the chance of the infant sleeping through the entire scan, greatly increasing the possibility of successful data acquisition.

1.3.3 Data Analysis

After image acquisition, the data is pre-processed and analyzed. One can pre-process data manually using an analysis program such as Statistical Parametric Mapping, or SPM (The FIL Methods group, 1991, 1994-2016); however, sophisticated analysis pipelines have been programmed to perform standard processing on data automatically (Cusack, et al., 2014). Pre-processing is necessary in order to “clean up” the data prior to functional connectivity analysis (Huettel, Song, & McCarthy, 2014). Standard pre-processing usually includes a number of well-established steps; a few are discussed below.

Though researchers try to minimize head motion as much as possible during the scan, motion correction is still a crucial part of analysis. Rigid-body realignment, to correct for motion, involves rotating and translating each slice so they align with one another (Huettel, Song, & McCarthy, 2014). High-pass filtering (for example, at a threshold of 120 seconds) removes signals slower than a cut-off frequency to reduce the effect of low-frequency noise; this includes scanner drift as well as physiological noise (such as the basal metabolic rate) (Huettel, Song, & McCarthy, 2014). Functional images are then co-registered to (spatially aligned with) the T1-weighted structural image and the structural images are warped so that they fit a brain template (Cusack, et al., 2014). Templates representing the average brain are available for adults [e.g. MNI (NeuroImaging and Surgical Technologies Lab (NIST), 2016)] and infants [e.g. the UNC Neonate Atlas (Shi, et al., 2011)]. Templates are important when comparing subjects as structural brain images first have to be normalized in relation to one another. When comparing adults to infants, the adults will first be normalized to the MNI template and infants to an infant template; then, the adult images are scaled to fit the infant images. After scaling and warping structural images, similar distortion is applied to the functional images until they fit the normalized structural images. Finally, images are smoothed [e.g. with an 8mm Gaussian kernel (Wylie, et al., 2014)] to increase signal-to-noise and make noise distributions Gaussian to allow parametric statistics. Smoothing is an especially important step in infant studies because infant data tends to be noisier and of lower-resolution (Molloy, Meyerand, & Birn, 2014).

1.3.4 Opportunities and Challenges of Infant fMRI

The field of infant functional MRI has seen a remarkable increase in published articles over the last decade, with 24 studies published since 2007. This new field, though still in its infancy, is proving to be an exciting one that is providing important insights on infant development. Impressively, over 2/3 of these articles have been published in the past 5 years, demonstrating just how quickly the field is changing. Despite this success, scanning even one infant is not an insignificant feat. Infant functional imaging (and, of course, infant research in general) is complicated and poses a number of difficulties (Almli, Rivkin, & McKinstry, 2007) (Raschle, 2012) (Dean, et al.) (Cusack, Ball, Smyser, & Dehaene-Lambertz, 2016).

One of the greatest challenges in infant imaging is the reduction of movement, and researchers have established and refined a number of methods to keep infants as still as possible (Cusack, Ball, Smyser, & Dehaene-Lambertz, 2016). In clinical settings, infants sometimes receive sedation to ensure efficient data collection. However, sedation has two disadvantages. First, although sedatives are thought to be safe, not all of their effects on the developing brain are currently understood (Dean, et al.). Second, sedatives are likely to have an effect on brain function, although exactly what is poorly understood. Thus, in many research settings, infants are not sedated prior to scans; instead, they are most often scanned while sleeping. The sights and sounds of a new environment can be distracting and exciting to an infant; it is therefore important to provide a comfortable, calm, and dimly lit environment to ensure that parents or caregivers are able to feed the infant and soothe them to sleep prior to scanning. Although infants tend to move less while sleeping than awake, motion still needs to be addressed and minimized. This is easier in younger infants as they can be wrapped in a vacuum immobilization bag (http://cfimedical.com/medvac/), which swaddles and soothes the infants and greatly reduces movement (Cusack, Ball, Smyser, & Dehaene-Lambertz, 2016). Older infants don’t usually like to be swaddled, and are more attentive to the new noises and shapes in the scanner room, so researchers should make efforts to ensure older infants are sleeping relatively deeply before the scan. Finally, an MRI scanner is noisy and infants must be protected with proper ear protection; infants are most commonly fitted with earplugs, Natus® mini muffs (Natus Medical Incorporated, 2016), and ear defenders, which in combination reduces the exposure to scanner noise by at least 30 decibels.

Additionally, ideal scanning parameters for infants are different than those for adults. For example, a longer TE is used in infants than in adults because infants have a higher T2* than adults (in other words, signal strength takes longer to relax after excitation) due to the increased water content of their brains (Rivkin, et al., 2004).

These changes in relaxation parameters change the contrast between tissues in structural MRI. Commonly in infant studies, both T1- and T2-weighted structural scans are acquired for functional imaging analysis because they provide different contrast between cortex, white matter, sulci and gyri, and so on, allowing researchers to better analyze structural differences (Kwon, Vasung, Ment, & Huppi, 2014). This is especially useful for infant scans because, as mentioned previously, infant data tends to be noisier and better contrast is quite useful in the analysis stages.

Finally, the BOLD signal hemodynamic response in infants is much different than that observed in adults and must be accounted for in both study design and in post-scan analysis (Cusack, Ball, Smyser, & Dehaene-Lambertz, 2016) (Cusack, Wild, Linke, Arichi, Lee, & Han, 2015).

Despite the challenges inherent in conducting developmental functional imaging research in infants, advances in methodology and technology continue to develop and the field continues to grow. Of all the innovations in functional imaging, especially promising is the ability to view functional networks within the brain.

1.4 Resting-State Functional Connectivity as a Tool to Examine the Infant Brain

In 1995, Bharat Biswal and colleagues discovered what they called “functional connectivity” between the left and right motor cortex (Biswal, Yetkin, Haughton, & Hyde, 1995). The experiment began with a rest period (resting-state fMRI), where participants were instructed to close their eyes, rest, and think about nothing in particular. Then, during the task phase of Biswal’s experiment, participants were instructed to perform finger tapping between each index finger and thumb; each 20-second period of finger tapping was followed by a 20-second period of rest (Biswal, Yetkin, Haughton, & Hyde, 1995). As expected, the motor cortex responded to the finger-tapping task (Figure 4).

Figure 4. fMRI task-based activation response to finger movement.

This image shows brain activation in the left and right motor cortex, as well as the supplementary motor area, superimposed onto an anatomical image. The activation is a result of bilateral left and right finger movement. The image has been removed due to copyright restrictions. To view this image, please see Figure 3a in the following manuscript: Biswal, B., Yetkin, F., Haughton, V., & Hyde, J. (1995). Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn Reson Med, 34*(9), 537-541.

Task-based activation using functional MRI was not novel; however, Biswal and colleagues were the first to discover spontaneous BOLD fluctuations in participants at rest (Biswal, Yetkin, Haughton, & Hyde, 1995). Specifically, separate brain regions within the motor cortex exhibited similar fluctuations, suggesting functional connectivity between those regions (Van Dijk, et al., 2010) (Figure 5). The analysis method used to first demonstrate resting-state functional connectivity, which is still used today, is as follows. Two regions of the brain are chosen and their fluctuation patterns through time are correlated and given an r-value ($-1.0 < r < 1.0$). An r-value of zero indicates zero correlation and one indicates perfect correlation. A significant correlation suggests that these two regions may be functionally connected (Figure 5).

Figure 5. A visual depiction of functional connectivity MRI.

In this case, the top graph shows two regions whose BOLD signal patterns are highly correlated (the left motor cortex and right motor cortex). The bottom graph illustrates the connectivity patterns of two regions with low similarity (the left motor cortex and left visual cortex). The image has been removed due to copyright restrictions. To view this image, please see Figure 1 in the following manuscript: Van Dijk, K. R., Hedden, T., Venkataraman, A., Evans, K. C., Lazar, S. W., & Buckner, R. L. (2010). Intrinsic functional connectivity as a tool for human connectomics: theory, properties, and optimization. *Journal of neurophysiology, 103*(1), 297-321.

Biswal's development of functional connectivity MRI (fcMRI) has been influential in the field of developmental neuroscience. Resting-state fcMRI is ideal for researchers studying infants and other nonverbal populations, as successful data collection does not depend on understanding a task, an overt action, or response to stimuli (Smyser, Snyder, & Neil, 2011) (Seghier & Hüppi, 2010). Resting-state fc-MRI is still quite novel to the field of infant research, having been successfully executed in infants only during the past decade; Peter Fransson and colleagues at the Karolinska Institute in Sweden were the first to identify functional connectivity resting-state networks in the infant brain (Figure 6) (Fransson, et al., 2007).

Figure 6. BOLD signal time courses.

This photo illustrates BOLD signal intensity time courses showing coherent spontaneous oscillations in a preterm infant during rest across the hemispheres in the left (green) and right (red) sensorimotor cortex (temporal correlation coefficient, 0.73). The image has been removed due to copyright restrictions. To view this image, please see Figure 2 in the following manuscript: Fransson, P., Skiöld, B., Horsch, S., Nordell, A., Blennow, M., Lagercrantz, H., & Aden, U. (2007). Resting-state networks in the infant brain. *Proceedings of the National Academy of Sciences of the United States of America, 104*(39), 15531-15536.

A number of research groups since then have taken on this challenge and produced exciting results; notably: Wei Gao and colleagues at the Cedars-Sinai Medical Centre in California, USA (previously at the University of North Carolina); A. David Edwards, Serena Counsell, Gareth Ball, and colleagues at Imperial and King's College in London, UK; and Christopher Smyser, Terrie Inder, and colleagues at Washington University in Missouri, USA.

To begin, it should be noted that there are a few ways to analyze resting-state functional connectivity data, and all have been represented in the developmental fcMRI research.

Independent component analysis is a data-driven connectivity analysis. This means that researchers can analyze the data without first coming up with a hypothesis on which regions should be connected or not. The algorithm groups together regions with correlated fluctuations into networks, and separates regions with independent fluctuations. Researchers can then suggest that these groups of brain regions are part of a functional network. See: (Fransson, et al., 2007); (Liu, Flax, & Benasich, 2008); (Fransson, et al., 2009); (Gao W. , Alcauter, Smith, Gilmore, & Lin, 2014); (Doria, et al., 2010).

Another method, originally introduced by Biswal (1995), is seed-based or ROI-based functional connectivity, which requires researchers to first have an idea of which brain regions they expect to be part of a network. "Seeds" are placed in particular brain regions

– for example, the left and right sensorimotor cortex (Lin, et al., 2008) – after researchers determine the precise location of these regions (e.g. via an online structural brain template). Temporal BOLD fluctuations in each of these brain regions are then determined and these fluctuations can be correlated with the other seeded brain regions. Researchers can then determine whether or not the correlation between regions is strong enough to suggest functional connectivity. See: Lin et al., 2008; Weinstein et al., 2016; Doria et al., 2010.

1.4.1 Age groups

Most of the current research has been conducted on full-term neonates scanned soon after birth or preterm infants scanned at term-equivalent age (TEA) or prior to TEA (Fransson, et al., 2007) (Fransson, et al., 2009) (Doria, et al., 2010) (Smyser, et al., 2010) (Fransson, Aden, Blennow, & Lagercrantz, 2011) (Smyser C. D., et al., 2013) (Lee, Morgan, Shroff, Sled, & Taylor, 2013) (van den Heuvel, 2014). Three research groups in the United States have scanned infants past term. Damaraju and colleagues studied four- and nine-month-old infants longitudinally (Damaraju, et al., 2014); Liu and colleagues studied infants just over 12 months of age (Liu, Flax, & Benasich, 2008); and Gao and colleagues conducted an impressive longitudinal/cross-sectional study of over 100 infants at term, 12 months, and 24 months of age. For the purposes of this thesis, only results pertaining to infants one year of age or younger will be discussed.

1.4.2 Overview of networks identified

Researchers have confirmed, in infants at term, four months, nine months, and 12 months, the existence of a bilateral, functionally- and interhemispherically-connected, predominantly unlateralized network in sensorimotor, somatomotor, and somatosensory cortices; typically, this network was mainly comprised of primary motor and sensory cortices, including the supplementary motor area and left and right M1 (Fransson, et al., Resting-state networks in the infant brain., 2007) (Lin, et al., 2008) (Fransson, et al., 2009) (Doria, et al., 2010) (Smyser, et al., 2010) (Fransson, Aden, Blennow, & Lagercrantz, 2011) (Gao, Shen, Zhu, & Lin, 2011) (Lee, Morgan, Shroff, Sled, & Taylor, 2013) (van den Heuvel, 2014) (Wylie, et al., 2014) (Alcauter, et al., 2014) (Damaraju, et al., 2014) (Gao W. , et al., 2014) (Gao W. , Alcauter, Smith, Gilmore, & Lin, 2014) (Smyser C. D., et al., 2014) (Arichi, et al., 2014) (Gao W. , Alcauter, Smith, Gilmore, & Lin, 2015) (Weinstein, et al., 2016). Two unilateral, intrahemispheric functional connectivity networks in the sensorimotor cortices have also been reported (Liu, Flax, & Benasich, 2008), though these results may be influenced by an inadequate sample size and differences in analysis (Fransson, et al., 2009). Some research groups have also identified regions outside of the primary sensorimotor cortex. Using independent component analysis, researchers identified networks within the following brain regions: basal ganglia (bilateral) (Fransson, et al., 2009); caudate (bilateral) (Gao, Shen, Zhu, & Lin, 2011); cerebellum (Doria, et al., 2010) (Smyser, et al., 2010) (Smyser C. D., et al., 2013); thalamus (Smyser, et al., 2010) (Smyser C. D., et al., 2013) (Alcauter, et al., 2014). Researchers also identified multi-region networks; notably, networks comprised of: the basal ganglia, peri-rolandic area, insula, operculum, thalamus, and SMA (Arichi, et al., 2014); the brainstem and thalamus (Doria, et al., 2010); and a subcortical network of the basal ganglia, thalamus, and brain stem (Damaraju, et al., 2013).

1.4.3 fcMRI of motor networks

fcMRI has greatly contributed to our understanding of the complexity of the infant motor system just after birth. Though infants were scanned at term, and as young as 29 weeks PMA, every study reported the existence of at least one motor network. This subsection will detail the pertinent findings from the 19 studies cited above.

In 2008, Lin and colleagues (Lin, et al., 2008) cross-sectionally studied neonates and one-year-olds. They chose regions of interest (ROI) in the left and right sensorimotor cortex and found that the areas that temporally correlated most strongly with their ROI were in the primary sensorimotor cortex. They also found that the strength of the functional connectivity between regions, the area of connectivity, and the volume of activation increased with age.

Using ICA, Liu and colleagues (Liu, Flax, & Benasich, 2008) compared one-year-old infants to adults. They found that the connectivity within their two unilateral sensorimotor networks was not adult-like at one year of age (except in two infants); however, when both unilateral networks were combined, the resulting network was adult-like.

Doria and colleagues (Doria, et al., 2010) studied infants at four time points: very preterm infants at birth (29 – 32 weeks PMA); late* preterm infants (33-36 weeks PMA, *though technically late preterm infants are born past 34 weeks); preterm infants at term-equivalent age; and a control group of healthy, term-born infants scanned at term. It is worthwhile to note that infants were sedated, which has the potential to affect the signal. These researchers selected ROI in the left motor cortex and in the left ventrolateral nucleus of the thalamus. Interestingly, researchers found that these two ROI were significantly correlated in the two older groups, but not in the early and late preterm groups. This sheds some light on the maturation of the motor network in utero and, in the case of preterm infants, prior to term equivalent age.

In a study of neonates, Smyser and colleagues (Smyser, et al., 2010) found the sensorimotor network to be better developed than the others; while most networks only exhibited within-network connectivity between homotopic regions, the sensorimotor network showed intrahemispheric connections to other ipsilateral supplementary motor regions. Preterm infants were scanned at between 26 and 40 weeks and compared with term-born controls scanned at term. Length and strength of sensorimotor network connectivity increased with age (particularly interhemispheric, rather than localized intrahemispheric, connections increased) and the preterm group, in general, had fewer and weaker connections. In addition, connections between sensorimotor cortex and thalamus were observed; however, they were limited in the preterm infant group when compared with the term-born controls.

Gao and colleagues (Gao, Shen, Zhu, & Lin, 2011) compared neonates to one-year-olds using graph theory and found that connectivity between primary sensorimotor cortex and caudate decreased with age. For example, path length between two regions tended to decrease. The researchers hypothesized that the infant brain decreases connectivity between simpler brain areas (such as primary motor cortex), thereby de-emphasizing the motor network, to better develop higher-order networks (such as the default mode network).

Smyser and colleagues (Smyser C. D., et al., 2013) studied preterm infants with white matter injury at term equivalent age. Specifically, they examined the right and left components of motor cortex, thalamus, and cerebellum. Researchers found that, compared with term-born controls, connectivity between homologous pairs (e.g. connectivity between left and right motor cortex) was significantly lower in the preterm infants; this was also true for correlations between the thalamus and other motor regions.

Additionally, the closer the motor cortex and thalamus were to the injury, the more apparent the decrease in connectivity.

In 2014, Wylie and colleagues (Wylie, et al., 2014) compared infants and adults and found that, in primary motor areas, adults had greater connectivity than infants; however, infants showed greater connectivity in areas outside of primary motor areas. The authors hypothesized that adults' connectivity was more restricted within primary motor areas because the adult motor network was generally much more specialized.

Damaraju and colleagues (Damaraju, et al., 2013) studied infants longitudinally at four and nine months of age and found that connectivity within local networks was generally strong but decreased with age while between-network connectivity increased with age (e.g. sensorimotor to frontal areas). The authors also mentioned that the infant networks showed some similarity to adult networks.

In a 2014 study, Gao and colleagues (Gao W. , et al., 2014) showed that the strength of sensorimotor network connectivity was lower in one-year-olds when compared with neonates.

In preterm infants scanned at term-equivalent age and term-born infants, Smyser and colleagues (Smyser C. D., et al., 2014) showed that connections within the motor cortex were high compared with other regions (e.g., frontal), providing support for the idea that the motor network is one of the earliest to develop. The sensorimotor network also correlated strongly with the thalamus and partially with the cerebellum.

Using a graph theory approach called betweenness centrality, Arichi and colleagues (Arichi, et al., 2014) studied six preterm infants (three of which had brain injury) and shed some light upon which regions of the motor network exert more control over other regions; for example, the basal ganglia appeared to exert more control than the other brain regions.

Gao and colleagues (Gao W. , Alcauter, Smith, Gilmore, & Lin, 2015) studied healthy paediatric subjects at term and one year of age and found that sensorimotor networks were quite adult-like in neonates; in addition, while other networks changed dramatically during the first year, connectivity within the sensorimotor network remained largely the same (and in some cases decreased). Interestingly, an infant that later had poor motor outcomes was found to have weak intrahemispheric connectivity between the insula, potentially indicating a relationship between motor ability and functional connectivity.

Finally, Weinstein and colleagues (Weinstein, et al., 2016) found that, in preterm infants scanned at term equivalent age, connectivity was strong between homologous primary sensory brain regions; additionally, researchers noted a relationship between this connectivity strength and integrity of the corpus callosum (as measured by diffusion-weighted imaging).

1.5 Interactive Specialization Theory and Gaps in Literature

In 2001, Mark Johnson postulated a developmental theory referred to as “interactive specialization”. The basic premise of this theory is that developments in behaviour are due to changes in connectivity within brain regions. He argues that despite the presence of structural connections between regions, and even some activity, development of new actions or skills are ultimately due to changing communication between these regions.

The structural neuroimaging research discussed above suggests that, in many ways, the infant motor system is quite mature at birth. A variety of structural connections within the brain are well established, especially within the motor cortex; even resting-state networks, representative of spontaneous activity fluctuations within motor regions of the brain, are present at birth and among the first to mature (Smyser & Neil, Use of resting-state functional MRI to study brain development and injury in neonates, 2015). At first glance, this evidence appears to be at odds with Johnson’s theory: if the brain is so incredibly mature at birth, why then is behaviour so immature? However, perhaps the theory and evidence are not so contradictory: although the motor network is present, with many of its structural connections formed early in development, it is possible that it is not yet fully mature. Doria and colleagues provided support for this idea; as discussed above, connections between the thalamus and motor cortex were only seen in infants at term or term equivalent age, and not in preterm infants scanned prior to term equivalent age. Perhaps other changes in connectivity during the first year of life account for the maturation of behaviour.

While previous research on infant functional connectivity has been informative, our study is unique in that we compared the infant motor network to that of adults by studying the regions most activated in adult fcMRI motor studies. Additionally, while the majority of research has centred on infants within the first weeks of life, we have examined infants at three and nine months of age (two time-points between which motor behaviour has developed enormously). With regards to this unique framework, two aspects of connectivity in the motor network have not been measured. First, in none of the previous studies has connectivity across the full range of cortical, cerebellar and thalamic regions in the adult motor system been examined. Second, there has been no quantitative examination of the relative strengths of connectivity within the motor network. For example, perhaps the balance of connectivity between cortical regions, versus between the cortex and thalamus, changes through the first year. I hypothesize that set of regions, and/or the balance of connectivity, will change through the first year.

1.6 Thesis Outline

In relation to the Interactive Specialization Theory and the present gaps in infant functional connectivity MRI literature, the following questions are asked:

- When do all of the brain regions that are part of the adult motor network become connected?
- When does the relative strength of connectivity in the motor network become mature?

Chapter two of this thesis contains the experimental chapter, which details the work done over the past two years in order to answer the above questions. Finally, chapter three concludes this thesis and discusses future directions for infant resting-state functional MRI research.

1.7 References

Alcauter, S., Lin, W., Smith, J. K., Short, S. J., Goldman, B. D., Reznick, J. S., . . . Gao, W. (2014). Development of thalamocortical connectivity during infancy and its cognitive correlations. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 34*(27), 9067-75.

Alexander, A. L., Lee, J. E., Lazar, M., & Field, A. S. (2007). Diffusion tensor imaging of the brain. *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics, 4*(3), 316-329.

Almli, C., Rivkin, M., & McKinstry, R. (2007). The NIH MRI study of normal brain development (Objective-2): newborns, infants, toddlers, and preschoolers. *NeuroImage, 35*(1), 308-325.

Alonso-Ortiz, E., Levesque, I. R., & Pike, G. B. (2015). MRI-based myelin water imaging: A technical review. *Magnetic Resonance in Medicine, 73*(1), 70-81.

Arichi, T., Counsell, S., Allievi, A., Chew, A., Martinez-Biarge, M., Mondi, V., . . . Edwards, A. (2014). The effects of hemorrhagic parenchymal infarction on the establishment of sensori-motor structural and functional connectivity in early infancy. *Neuroradiology, 56*(11), 985-994.

Atun-Einy, O., Berger, S. E., & Scher, A. (2013). Assessing motivation to move and its relationship to motor development in infancy. *Infant Behavior and Development, 36*(3), 457-469.

Ball, G., Boardman, J., Rueckert, D., Aljabar, P., Arichi, T., Merchant, N., . . . Counsell, S. (2012). The Effect of Preterm Birth on Thalamic and Cortical Development. *Cerebral Cortex, 22*, 1016-1024.

Ball, G., Srinivasan, L., Aljabar, P., Counsell, S. J., Durighel, G., Hajnal, J. V., . . . Edwards, A. D. (2013). Development of cortical microstructure in the preterm human brain. *Proceedings of the National Academy of Sciences of the United States of America, 110*(23), 9541-9546.

Bandettini, P. A. (2012). Twenty years of functional MRI: The science and the stories. *NeuroImage, 62*(2), 575-588.

Baraldi, P., Porro, C. A., Serafini, M., Pagnoni, G., Murari, C., Corazza, R., & Nichelli, P. (1999). Bilateral representation of sequential finger movements in human cortical areas. *Neuroscience Letters, 269*(2), 95-98.

Basser, P. J., & Pierpaoli, C. (1996). Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. *Journal of magnetic resonance, 213*(2), 560-570.

Belliveau, J., Kennedy, D., McKinstry, R., Buchbinder, B., Weisskoff, R., Cohen, M., . . . Rosen, B. (1991). Functional mapping of the human visual-cortex by magnetic-resonance-imaging. *Science, 254*, 716–719.

Berman, J. I., Mukhaerjee, P., Partridge, S. C., Miller, S. P., Ferriero, D. M., Barkovich, A. J., . . . Henry, R. G. (2005). Quantitative diffusion tensor MRI fiber tractography of sensorimotor white matter development in premature infants. *NeuroImage, 27*(4), 862-871.

Biswal, B., Yetkin, F., Haughton, V., & Hyde, J. (1995). Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn Reson Med*, *34*(9), 537-541.

Brant-Zawadzki, M., Gillian, G., & Nitz, W. (1992). MP RAGE: a three-dimensional, T1-weighted, gradient-echo sequence--initial experience in the brain. . *Radiology, 182*(3), 769-775.

Brown, S., Ngan, E., & Liotti, M. (2008). A Larynx Area in the Human Motor Cortex. *Cerebral Cortex*, *18*(4), 837-845.

Buxton, R., Uludag, K., & Dubowitz, D. (2004). Investigating the Physiology of Brain Activation with MRI. *SPIE Proceedings, 5369*, 207-218.

Chugani, H. (1998). A critical period of brain development: studies of cerebral glucose utilization with PET. *Preventive medicine*, *27*(2), 184-188.

Chugani, H., Phelps, M., & Mazziotta, J. (1987). Positron emission tomography study of human brain functional development. *Annals of neurology*, *22*(4), 487-497.

Culham, J. (2015). Cortical Areas Engaged in Movement: Neuroimaging Methods. In J. Wright (Ed.), *International Encyclopedia of the Social & Behavioral Sciences* (Second Edition ed.). Elsevier Ltd.

Cusack, R., Ball, G., Smyser, C. D., & Dehaene-Lambertz, G. (2016). A Neural Window on the Emergence of Cognition. *Annals Of The New York Academy Of Sciences, 1369*(1), 7-23.

Cusack, R., Vicente-Grabovetsky, A., Mitchell, D. J., Wild, C. J., Auer, T., Linke, A. C., & Peelle, J. E. (2014). Automatic analysis (aa): efficient neuroimaging workflows and parallel processing using Matlab and XML. *Frontiers in neuroinformatics, 8*, 90.

Cusack, R., Wild, C., Linke, A., Arichi, T., Lee, D., & Han, V. (2015). Optimizing stimulation and analysis protocols for neonatal fMRI. *PLoS One, 10*, e0120202.

Damaraju, E., Caprihan, A., Lowe, J., Allen, E., Calhoun, V., & Phillips, J. (2013). Functional connectivity in the developing brain: a longitudinal study from 4 to 9months of age. *NeuroImage, 84*, 169-80.

Damaraju, E., Caprihan, A., Lowe, J., Allen, E., Calhoun, V., & Phillips, J. (2014). Functional connectivity in the developing brain: a longitudinal study from 4 to 9months of age. *NeuroImage, 84*, 169-80.

Darrah, J., Redfern, L., Maguire, T. O., Beaulne, A. P., & Watt, J. (1998). Intra-individual stability of rate of gross motor development in full-term infants. *Early Human Development, 52*(2), 169-179.

Davidson, R. J., Jackson, D. C., & Larson, C. L. (2000). Chapter Two: Human Electroencephalography. In J. T. Cacioppo, L. G. Tassinary, & G. G. Berntson (Eds.), *Handbook of Psychophysiology* (2nd Edition ed., pp. 27-52). Cambridge University Press.

De Vries, L., Groenendaal, F., van Haastert, I., Eken, P., Rademaker, K., & Meiners, L. (1999). Asymmetrical myelination of the posterior limb of the internal capsule in infants with periventricular haemorrhagic infarction: an early predictor of hemiplegia. *Neuropediatrics, 30*(6), 314-319.

Dean, D., Dirks, H., O'Muircheartaigh, J. W., Jerskey, B., Lehman, K., Han, M., . . . Deoni, S. (n.d.).

Deoni, S. C., Dean, D. C., O'Muircheartaigh, J., Dirks, H., & Jerskey, B. A. (2012). Investigating white matter development in infancy and early childhood using myelin water faction and relaxation time mapping. *NeuroImage, 63*(3), 1038-1053.

Doria, V., Beckmann, C. F., Arichi, T., Merchant, N., Groppo, M., Turkheimer, F. E., . . . Edwards, A. D. (2010). Emergence of resting state networks in the preterm human brain. *Proceedings of the National Academy of Sciences of the United States of America, 107*(46), 20015-20.

Doyon, J., Bellec, P., Amsel, R., Penhune, V., Monchi, O., Carrier, J., . . . Benaly, H. (2009). Contributions of the basal ganglia and functionally related brain structures to motor learning. *Behavioural Brain Research, 199*, 61-75.

Doyon, J., Penhune, V., & Ungerleider, L. (2003). Distinct contribution of the cortico-striatal and cortico-cerebellar systems to motor skill learning. *Neuropsychologia, 41*, 252-262.

Drobyshevsky, A., Bregman, J., Storey, P., Meyer, J., & Prasad, P. V. (2007). Serial diffusion tensor imaging detects white matter changes that correlate with motor outcome in premature infants. *Developmental neuroscience, 29*(4-5), 289-301.

Eyre, J., Miller, S., Clowry, G., Conway, E., & Watts, C. (2000). Functional corticospinal projections are established prenatally in the human foetus permitting involvement in the development of spinal motor centres. *Brain : a journal of neurology, 123*, 51-64.

Feinberg, D. A., Moeller, S., Smith, S. M., Auerbach, E., Ramanna, S., Gunther, M., & … Yacoub, E. (2010). Multiplexed echo planar imaging for sub-second whole brain FMRI and fast diffusion imaging. *PloS One, 5*(12), e15710.

Fransson, P., Aden, U., Blennow, M., & Lagercrantz, H. (2011). The functional architecture of the infant brain as revealed by resting-state fMRI. *Cerebral cortex, 21*, 145-54.

Fransson, P., Skiöld, B., Engström, M., Hallberg, B., Mosskin, M., Aden, U., . . . Blennow, M. (2009). Spontaneous brain activity in the newborn brain during natural sleep--an fMRI study in infants born at full term. *Pediatric research, 66*(3), 301-5.

Fransson, P., Skiöld, B., Horsch, S., Nordell, A., Blennow, M., Lagercrantz, H., & Aden, U. (2007). Resting-state networks in the infant brain. *Proceedings of the National Academy of Sciences of the United States of America, 104*(39), 15531-15536.

Fransson, P., Skiöld, B., Horsch, S., Nordell, A., Blennow, M., Lagercrantz, H., & Aden, U. (2007). Resting-state networks in the infant brain. *Proceedings of the National Academy of Sciences of the United States of America, 104*(39), 15531-6.

Gao, W. G., Shen, D., Zhu, H., & Lin, W. (2011). Temporal and spatial evolution of brain network topology during the first two years of life. *PLoS ONE, 6*(9), e25278.

Gao, W., Alcauter, S., Elton, A., Hernandez-Castillo, C. R., Smith, J. K., Ramirez, J., & Lin, W. (2014). Functional Network Development During the First Year: Relative Sequence and Socioeconomic Correlations. *Cerebral cortex (New York, N.Y. : 1991)*, 1-10.

Gao, W., Alcauter, S., Smith, J. K., Gilmore, J. H., & Lin, W. (2014). Development of human brain cortical network architecture during infancy. *Brain structure & function*.

Gao, W., Alcauter, S., Smith, J., Gilmore, J., & Lin, W. (2015). Development of human brain cortical network architecture during infancy. *Brain Structure & Function, 220*, 1173-1186.

Gerber, R. J., Wilks, T., & Erdie-Lalena, C. (2010). Developmental milestones: motor development. *Pediatrics in review / American Academy of Pediatrics, 31*(7), 267-276.

Graybiel, A. M. (2005). The basal ganglia: Learning new tricks and loving it. *Current Opinion in Neurobiology, 15,* 638-644.

Grieve, S., Williams, L., Paul, R., Clark, C., & Gordon, E. (2007). Cognitive Aging, Executive Function, and Fractional Anisotropy: A Diffusion Tensor MR Imaging Study. *American Journal of Neuroradiology, 28*, 226-235.

Haber, S., & Calzavara, R. (2009). The cortico-basal ganglia integrative network: The role of the thalamus. *Brain Research Bulletin, 78*, 69-74.

Hadders-Algra, M. (2002). Variability in infant motor behavior: A hallmark of the healthy nervous system. *Infant Behavior and Development, 25*(4), 433-451.

Hermoye, L., Saint-Martin, C., Cosnard, G., Lee, S.-K., Kim, J., Nassogne, M.-C., . . . Mori, S. (2006). Pediatric diffusion tensor imaging: normal database and observation of the white matter maturation in early childhood. *NeuroImage, 29*(2), 493-504.

Hirai, M., & Hiraki, K. (2005). An event-related potentials study of biological motion perception in human infants. *Cognitive Brain Research, 22*(2), 301-304.

Hoff, G. A.-J., Van den Heuvel, M. P., Benders, M. J., Kersbergen, K. J., & De Vries, L. (2013). On development of functional brain connectivity in the young brain. *Frontiers in human neuroscience, 7,* 650.

Huang, H., Jeon, T., Sedmak, G., Pletikos, M., Vasung, L., Xu, X., . . . Mori, S. (2013). Coupling Diffusion Imaging with Histological and Gene Expression Analysis to Examine the Dynamics of Cortical Areas across the Fetal Period of Human Brain Development. *Cerebral Cortex, 23*(11), 2620-2631.

Huettel, S. A., Song, A. W., & McCarthy, G. (2014). *Functional Magnetic Resonance Imaging.* Sinauer Associates, Inc.

Hüppi, P. S., & Dubois, J. (2006). Diffusion tensor imaging of brain development. *Seminars in fetal & neonatal medicine, 11*(6), 489-497.

Hüppi, P. S., Maier, S. E., Peled, S., Zientara, G. P., Barnes, P. D., Jolesz, F. A., & Volpe, J. J. (1998). Microstructural Development of Human Newborn Cerebral White Matter Assessed in Vivo by Diffusion Tensor Magnetic Resonance Imaging. *Pediatric Research, 44,* 584-590.

Hüppi, P., Murphy, B., Maier, S., Zientara, G., Inder, T., Barnes, P., . . . Volpe, J. (2001). Microstructural Brain Development After Perinatal Cerebral White Matter Injury Assessed by Diffusion Tensor Magnetic Resonance Imaging. *Pediatrics, 107*(3), 455-460.

Huttenlocher, P. R., & Dabholkar, A. S. (1997). Regional differences in synaptogenesis in human cerebral cortex. *The Journal of Comparative Neurology, 387*(2), 167-178.

Inder, T., Warfield, S., Wang, H., Hüppi, P., & Volpe, J. (2005). Abnormal Cerebral Structure Is Present at Term in Premature Infants. *Pediatrics, 115*(2), 286-294.

Jasper, H., & Penfield, W. (1949). Electrocorticograms in man: Effect of voluntary movement upon the electrical activity of the precentral gyrus. *European Archives of Psychiatry and Clinical Neuroscience*, 163-174.

Joel, D., & Weiner, I. (1994). The organization of the basal ganglia-thalamocortical circuits: Open interconnected rather than closed segregated. *Neuroscience, 63*(2), 363-379.

Johnson, C., & Blasco, P. (1997). Infant growth and development. *Pediatrics in review / American Academy of Pediatrics, 18*(7), 224-242.

Kwon, S. H., Vasung, L., Ment, L. R., & Huppi, P. S. (2014). The Role of Neuroimaging in Predicting Neurodevelopmental Outcomes of Preterm Neonates. *Clinics in Perinatology, 41*(1), 257-283.

Law, J., Lee, M., Hulse, M., & Tomassetti, A. (2011). The infant development timeline and its application to robot shaping. *Adaptive Behavior, 19*(5), 335-358.

Lee, W., Morgan, B. R., Shroff, M. M., Sled, J. G., & Taylor, M. J. (2013). The development of regional functional connectivity in preterm infants into early childhood. *Neuroradiology, 55*, 105-11.

Lin, W., Zhu, Q., Gao, W., Chen, Y., Toh, C.-H., Styner, M., . . . Gilmore, J. (2008). Functional connectivity MR imaging reveals cortical functional connectivity in the developing brain. *AJNR. American journal of neuroradiology, 29*(10), 1883-9.

Liu, W.-C., Flax, J. F., & Benasich, A. A. (2008). Functional connectivity of the sensorimotor area in naturally sleeping infants. *Brain research, 1223*, 42-49.

Lowe, M., Horenstein, C., Hirsch, J., Marrie, R., Stone, L., Bhattacharyya, P., . . . Phillips, M. (2006). Functional pathway-defined MRI diffusion measures reveal increased transverse diffusivity of water in multiple sclerosis. *NeuroImage, 32*(3), 1127-1133.

Marshall, P. J., & Meltzoff, A. N. (2011). Neural Mirroring Systems: Exploring the EEG Mu Rhythm in Human Infancy. *Dev Cogn Neurosci. , 1*(2), 110-123.

Marshall, P. J., & Shipley, T. F. (2009). Event-related potentials to point-light displays of human actions in five-month-old infants. *Dev Neuropsychol, 34*(3), 368-377.

Mathew, P., Pannek, K., Snow, P., Acunto, M. G., Guzzetta, A., Rose, S. E., . . . Finnigan, S. (2013). Maturation of Corpus Callosum Anterior Midbody Is Associated with Neonatal Motor Function in Eight Preterm-Born Infants. *Neural Plasticity*, 7.

Matsumoto, J., Fujiwara, T., Takahashi, O., Liu, M., Kimura, A., & Ushiba, J. (2010). Modulation of mu rhythm desynchronization during motor imagery by transcranial direct current stimulation. *Journal of neuroengineering and rehabilitation, 7*, 27.

Middleton, F. A., & Strick, P. L. (2000). Basal ganglia and cerebellar loops: Motor and cognitive circuits. *Brain Research Reviews, 31*(2-3), 236-250.

Mink, J. (1996). The basal ganglia: Focused selection and inhibition of competing motor programs. *Progress in Neurobiology, 50*, 381-425.

Molloy, E. K., Meyerand, M. E., & Birn, R. M. (2014). The influence of spatial resolution and smoothing on the detectability of resting-state and task fMRI. *NeuroImage, 86*, 221-230.

Mukherjee, P., Miller, J., Shimony, J., Conturo, T., Lee, B., Almli, C., & McKinstry, R. (2001). Normal brain maturation during childhood: developmental trends characterized with diffusion-tensor MR imaging. *Radiology, 221*(2), 349-358.

Nakano, K. (2000). Neural circuits and topographic organization of the basal ganglia and related regions. *Brain & Development, 22*, S5-S16.

National Institutes of Health - National Cancer Institute. (n.d.). *Hemoglobin*. (National Center for Biotechnology Information) Retrieved June 13, 2016, from PubMed Health: http://www.ncbi.nlm.nih.gov/pubmedhealth/PMHT0022015/

Natus Medical Incorporated. (2016). *MiniMuffs® Neonatal Noise Attenuators*. (Natus Medical Incorporated) Retrieved June 13, 2016, from Natus: Newborn Care: http://www.natus.com/index.cfm?page=products_1&crid=199

NeuroImaging and Surgical Technologies Lab (NIST). (2016). Atlases. Montreal, QC, Canada: NIST.

Nyström, P. (2008). The infant mirror neuron system studied with high density EEG. *Social neuroscience, 3*(3-4), 334-347.

Nyström, P., Ljunghammar, T., Rosander, K., & Von Hofsten, C. (2011). Using mu rhythm desynchronization to measure mirror neuron activity in infants. *Developmental Science, 14*(2), 327-335.

Orgogozo, J., & Larsen, B. (1979). Activation of the supplementary motor area during voluntary movement in man suggests it works as a supramotor area. *Science, 206*(4420), 847-850.

Parent, A., & Hazrati, L.-N. (1994). Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamocortical loop. *Brain Research Review, 20,* 91-127.

Penfield, W., & Welch, K. (1949). The supplementary motor area of the cerebral cortex: A clinical and experimental study. *Seventy-Fourth Annual Meeting of the American Neurological Association,* (pp. 289-317). Atlantic City.

Penke, L., Maniega, S. M., Bastin, M., Valdes Hernandez, M., Murray, C., Royle, N., . . . Deary, I. .. (2012). Brain white matter tract integrity as a neural foundation for general intelligence. *Mol Psychiatry, 17*(10), 1026-1030.

Pfurtscheller, G., & Lopes, F. (1999). Event-related EEG / MEG synchronization and desynchronization : basic principles. *Clinical Neurophysiology, 110,* 1842-1857.

Piper, M. C. (1994). *Motor assessment of the developing infant.* WB Saunders Company.

Preibisch, C., Castrillón G., J. G., Bührer, M., & Riedl, V. (2015). Evaluation of Multiband EPI Acquisitions for Resting State fMRI. *PLoS One., 10*(9), e0136961.

Proverbio, A. M. (2012). Tool perception suppresses 10-12Hz μ rhythm of EEG over the somatosensory area. *Biological Psychology, 91*(1), 1-7.

Puig, J., Pedraza, S., Blasco, G., Daunis-i-Estadella, J., Prados, F., Remollo, S., . . . Counsell, S. J. (2011). Acute damage to the posterior limb of the internal capsule on diffusion tensor tractography as an early imaging predictor of motor outcome after stroke. *American Journal of Neuroradiology, 32*(5), 857-863.

Purves, D., Augustine, G. J., Fitzpatrick, D., Katz, L. C., LaMantia, A.-S., McNamara, J. O., & Williams, S. M. (Eds.). (2001). *Neuroscience* (2 ed.). Sunderland, MA, USA: Sinauer Associates.

Raschle, N. Z.-M. (2012). Pediatric neuroimaging in early childhood and infancy: challenges and practical guidelines. *Annals of the New York Academy of Sciences, 1252*, 43–50.

Regents of the University of California. (2016). *Structural MRI Imaging*. Retrieved June 12, 2016, from UC San Diego School of Medicine: Centre for Functional MRI (in the Department of Radiology): http://fmri.ucsd.edu/Howto/3T/structure.html

Reid, V. (2013). How infants detect information in biological motion. In V. Slaughter, & C. A. Brownell (Eds.), *Early Development of Body Representations* (pp. 146-162). Cambridge University Press.

Reid, V. M., Hoehl, S., & Striano, T. (2006). The perception of biological motion by infants: An event-related potential study. *Neuroscience Letters, 395*(3), 211-214.

Rivkin, M. J., Wolraich, D., Als, H., McAnulty, G., Butler, S., Conneman, N., & … Mulkern, R. (2004). Prolonged T*2 values in newborn versus adult brain: Implications for fMRI studies of newborns. *Magnetic Resonance in Medicine, 51*(6), 1287–1291.

Roland, P., Larsen, B., Lassen, N., & Skinhøj, E. (1980). Supplementary motor area and other cortical areas in organization of voluntary movements in man. *Journal of neurophysiology, 43*(1), 118-136.

Rose, J., Mirmiran, M., Butler, E. E., Lin, C. Y., Barnes, P. D., Kermoian, R., & Stevenson, D. K. (2007). Neonatal microstructural development of the internal capsule on diffusion tensor imaging correlates with severity of gait and motor deficits . *Developmental Medicine & Child Neurology , 49*(10).

Rugg, M. (2009). Event-Related Potentials (ERPs). In *Encyclopedia of Neuroscience* (pp. 7-12). Irvine, CA, U.S.A.: Academic Press.

Seghier, M. L., & Hüppi, P. S. (2010). The role of functional magnetic resonance imaging in the study of brain development, injury, and recovery in the newborn. *Seminars in Perinatology, 34*(1), 79-86.

Shi, F., Yap, P.-T., Wu, G., Jia, H., Gilmore, J. H., Lin, W., & Shen, D. (2011). Infant Brain Atlases from Neonates to 1- and 2-year-olds. *PLoS ONE, 6*(4), e18746.

Shibasaki, H., Sadato, N., Lyshkow, H., Yonekura, Y., Honda, M., Nagamine, T., . . . Miyazaki, M. (1993). Both primary motor cortex and supplementary motor area play an important role in complex finger movement. *Brain : a journal of neurology, 116 (pt. 6)*, 1387-1398.

Smyser, C. D., & Neil, J. J. (2015). Use of resting-state functional MRI to study brain development and injury in neonates. *Seminars in Perinatology, 39*(2), 130-140.

Smyser, C. D., Snyder, A. Z., & Neil, J. J. (2011). Functional connectivity MRI in infants: exploration of the functional organization of the developing brain. *NeuroImage, 56*(3), 1437-1452.

Smyser, C. D., Snyder, A. Z., Shimony, J. S., Blazey, T. M., Inder, T. E., & Neil, J. J. (2013). Effects of white matter injury on resting state fMRI measures in prematurely born infants. *PloS one, 8*(7), e68098.

Smyser, C. D., Snyder, A. Z., Shimony, J. S., Mitra, A., Inder, T. E., & Neil, J. J. (2014). Resting-State Network Complexity and Magnitude Are Reduced in Prematurely Born Infants. *Cerebral cortex (New York, N.Y. : 1991)*, 1-12.

Smyser, C., Inder, T., Shimony, J., Hill, J., Degnan, A., Snyder, A., & Neil, J. (2010). Longitudinal Analysis of Neural Network Development in Preterm Infants. *Cerebral Cortex, 20*, 2852-2862.

Southgate, V., Johnson, M. H., Karoui, I., & Csibra, G. (2010). Motor System Activation Reveals Infants' On-Line Prediction of Others' Goals. *Psychological Science, 21*(3), 355-359.

Southgate, V., Johnson, M. H., Osborne, T., & Csibra, G. (2009). Predictive motor activation during action observation in human infants. *Biology letters, 5*(6), 769-772.

Stapel, J. C., Hunnius, S., van Elk, M., & Bekkering, H. (2010). Motor activation during observation of unusual versus ordinary actions in infancy. *Social neuroscience, 5*(5-6), 451-460.

Szameitat, A. J., McNamara, A., Shen, S., & Sterr, A. (2012). Neural activation and functional connectivity during motor imagery of bimanual everyday actions. *PLoS ONE, 7*(6), e38506.

Takazawa, T., & MacDermott, A. B. (2010). Synaptic pathways and inhibitory gates in the spinal cord dorsal horn. *Annals of the New York Academy of Sciences, 1198*, 153-138.

The FIL Methods group. (1991, 1994-2016). Statistical Parametric Mapping. London, UK.

Thelen, E., Kelso, J. S., & Fogel, A. (1987). Self-organizing systems and infant motor development. *Developmental Review, 7*(1), 39-65.

Thompson, P. M., Sowell, E. R., Gogtay, N., Giedd, J. N., Vidal, C. N., Hayashi, K. M., . . . Toga, A. W. (2005). Structural MRI and Brain Development. *International Review of Neurobiology, 67*, 285-323.

Tonkonogy, J., & Goodglass, H. (1981). Language function, foot of the third frontal gyrus, and rolandic operculum. *Archives of Neurology, 38*(8), 486-490.

Travis, K., Ford, K., & Jacobs, B. (2005). Regional dendritic variation in neonatal human cortex: A quantitative Golgi study. *Developmental Neuroscience, 27*(5), 277-287.

Tymofiyeva, O., Hess, C. P., Ziv, E., Lee, P. N., Glass, H. C., Ferriero, D. M., . . . Xu, D. (2013). A DTI-based template-free cortical connectome study of brain maturation. *PloS one, 8*(5), e63310.

Udupa, K., & Chen, R. (2013). Central motor conduction time. In A. M. Lozano, & M. Hallett (Eds.), *Brain Stimulation (Handbook of Clinical Neurology)* (3 ed., Vol. 116, pp. 375-386). Elsevier.

Uludag, K., Dubowitz, D., & Buxton, R. (2005). Basic Principles of Functional MRI. In *Clinical MRI.*

van den Heuvel, M. P. (2014). The Neonatal Connectome During Preterm Brain Development. *Cerebral cortex, 25*(9), 3000-3013.

van der Meer, A., van der Weel, F., & Lee, D. (1995). The functional significance of arm movements in neonates. *Science, 267,* 693-695.

Van Dijk, K. R., Hedden, T., Venkataraman, A., Evans, K. C., Lazar, S. W., & Buckner, R. L. (2010). Intrinsic functional connectivity as a tool for human connectomics: theory, properties, and optimization. *Journal of neurophysiology, 103*(1), 297-321.

von Hofsten, C. (1984). Developmental changes in the organization of prereaching movements. *Developmental Psychology, 20*(3), 378-388.

Von Hofsten, C. (2004). An action perspective on motor development. *Trends in Cognitive Sciences, 8*(6), 266-272.

Warlop, N. P., Achten, E., Fieremans, E., Debruyne, J., & Vingerhoets, G. (2009). Transverse diffusivity of cerebral parenchyma predicts visual tracking performance in relapsing-remitting multiple sclerosis. *Brain and Cognition, 71*(3), 410-415.

Weinstein, M., Ben Sira, L., Moran, A., Berger, I., Marom, R., Geva, R., . . . Bashat, D. B. (2016). The motor and visual networks in preterm infants: an fMRI and DTI study. *Brain research, 1642,* 603-611.

White, B. L., Castle, P., & Held, R. (1964). Observations on the Development of Visually-Directed Reaching . *Society for Research in Child Development, 35*(2), 349-364.

Woodward, L. J., Anderson, P. J., Austin, N. C., Howard, K., & Inder, T. E. (2006). Neonatal MRI to Predict Neurodevelopmental Outcomes in Preterm Infants. *The New England Journal of Medicine, 355*(7), 685-694.

Wylie, K. P., Rojas, D. C., Ross, R. G., Hunter, S. K., Maharajh, K., Cornier, M. A., & Tregellas, J. R. (2014). Reduced brain resting-state network specificity in infants compared with adults. . *Neuropsychiatric Disease and Treatment, 10,* 1349-1359.

Xu, J., Moeller, S., Auerbach, E. J., Strupp, J., Smith, S. M., Feinberg, D., & … Uğurbil, K. (2013). Evaluation of slice accelerations using multiband echo planar imaging at 3 Tesla. . *NeuroImage, 83,* 991-1001.

Yarkoni, T. (2011). *motor*. Retrieved June 25, 2016, from neurosynth.org: http://neurosynth.org/analyses/terms/motor/

Chapter 2

2 Manuscript-Based Experimental Data

This chapter is presented in the form of a manuscript. The infants were recruited by DSCL and VKH; data was acquired by ACL, CJW, HD and CH prior to my arrival. I analyzed the data and wrote the manuscript together with my supervisor, RC.

Functional Connectivity Within the Motor System at 3 Months is Similar to Adults Although Motor Function is Primitive

Jordynne S. Ropat, Annika C. Linke, Conor J. Wild, Charlotte Herzmann, Hester Duffy, Victor K. Han, David S.C. Lee, Rhodri Cusack

2.1 Abstract

The brain changes in many ways in the first year. It is not known which of these changes are most critical for the development of cognitive functions. According to the Interactive Specialization Theory, developments in behaviour result from changes in brain connectivity. We tested this using functional connectivity magnetic resonance imaging (fcMRI) of the motor system. fcMRI was acquired at three and nine months – two time-points between which motor behaviour develops enormously. Infants were additionally compared with adults. Subjects were scanned with a 3T MRI scanner, yielding BOLD signal time-courses that were correlated with one another. Our results do not support the Interactive Specialization Theory, as connectivity did not change with motor development and instead was adult-like in the youngest infants. fcMRI has enabled deeper exploration of network connectivity patterns and continues to emerge as a leading method in infant neuroscience.

2.2 Introduction

During the first year of an infant’s life, motor function develops tremendously. Neonates enter the world with few motor abilities beyond simple reflexes, but by just half a year old, motor skills are rapidly emerging (Nogueira, Figueiredo, Gonçalves, & Mancini, 2015). By nine months of age, many infants begin crawling and some begin to walk before their first birthday. The brain undergoes many changes during this period, but it is not known which are critical to the development of motor skills. The interactive specialization framework (Johnson, 2001, 2002, 2011; Johnson & de Haan, 2015) proposes that cognitive functions develop due to the maturation of interactions between brain regions, which in turn drives the specialization of neural tuning within regions. A prediction of this account (Johnson, 2011) is that as a cognitive function matures, so will the connectivity of the neural system that supports it. This emphasis on connectivity stands in contrast to the predictions of two other accounts, the maturational account that functional development is driven by genetically driven changes within local brain regions (Johnson, 2011), and the skill learning account which emphasizes experience-driven learning within regions (Johnson, 2011). To distinguish between these accounts of development, It is critical to establish whether connectivity matures along with function.

Major tracts are present at birth and thalamic radiations develop between 34 and 41 weeks gestational age (Aeby et al., 2009), and corticospinal innervation can be observed even as early as 24 weeks post-conception age (Eyre, Miller, Clowry, Conway, & Watts, 2000) in utero. On a much smaller scale, even individual neurons migrate to their final locations within the cortex and cerebellum by the time an infant is born (Johnson, 2001). PET imaging measures glucose uptake in order to show which regions of the brain are more active, and can be used in infants to determine which areas may be experiencing greater developmental changes. Glucose uptake in infants younger than five weeks of age was found to be greatest in motor areas such as the sensorimotor cortex, thalamus, cerebellar vermis, and brainstem (Chugani, Phelps, & Mazziotta, 1987); this suggests that the motor network is most actively developing early in infancy.

Structural and diffusion magnetic resonance imaging (MRI) have been used to examine the structure of the motor network in infants and adults (Liu et al., 2010; Ratnarajah et al., 2013). Liu et al. (2010) studied the motor network using diffusion imaging and found structural asymmetries at birth in a population of preterm neonates, potentially indicating the development of handedness during the fetal period. However, functional magnetic resonance imaging (fMRI) has been particularly useful in observing the connectivity and broader organization of the brain (Seghier & Huppi, 2010), and may be able to elucidate more subtle developmental differences that are not able to be captured by the structural scan (Smyser, 2013). In addition, while structural methods allow us to see which regions are physically connected, fMRI provides information on how different brain regions interact with one another. Recently, there is some evidence from ICA and functional connectivity MRI (fc-MRI) for at least rudimentary motor networks at birth, when motor function is poorly developed (Doria et al., 2010; Damaraju et al., 2014; Gao, Alcauter, Smith, Gilmore, & Lin, 2014).

Collectively, this evidence suggests that the development of connectivity could precede the development of function, which would be more consistent with the maturational or skill learning accounts than the interactive specialization framework. However, there are two substantial limitations to current research. First, studies of connectivity within the motor network have investigated only a narrow part of this network, centred on M1. Functional development might be driven by the maturation of longer-range connections that comprise the broader motor network, including the thalamus, cerebellum and premotor regions. Second, there has been no attempt to quantitatively examine the pattern of connectivity within this network. It might be that the network is crudely initialised, and functional development results from a refinement of the pattern of connectivity, which would be very much consistent with interactive specialization framework. To address these limitations, we investigated the extent to which connectivity in the motor system is mature in infancy using functional neuroimaging. We identified a broad set of motor regions in the mature adult brain using a meta-analysis of functional neuroimaging studies (Yarkoni, 2010). We measured functional connectivity within this network in adults, by correlating the time-courses of activations between brain areas. Regions that were more tightly connected will show more similar time-courses and thus higher correlations. We then examined brain connectivity of three- and nine-month-old infants in order to determine if connectivity differs between two groups that show clear differences in motor abilities. Finally, we compared infant and adult connectivity in order to better understand how mature the motor network is in infancy.

2.3 Results

Sixteen adults, twenty-four 3-month old infants (3-4 months; M=3.33) and fifteen 9-month old (9-11 months; M=9.41) infants were recruited. One adult was rejected following the detection of an abnormal finding by the MR technologist (N=15). Two sessions of 7.5 minutes of fMRI were acquired on a 3T Siemens Prisma at the Robarts Research Institute using a simultaneous multi-slice EPI protocol (see methods). Not all infants could be persuaded to sleep, one 9-month old had poor coverage in inferior regions in the motor network, and following stringent exclusion for movement eighteen 3-month olds (yielding N=6) and nine 9-month olds were rejected (yielding N=6).

The motor network was defined using neurosynth.org, an open-source database of thousands of published functional MRI studies and a platform for large-scale meta-analysis of fMRI data (Yarkoni, 2011). The keyword "motor" generated an automated meta-analysis of 1748 studies that studied brain regions involved in motor function (Figure 7). For each voxel, a z-score was generated that provides the likelihood that activation at that point was caused by motor activity (i.e., reverse inference *p(Motor task|Activation)*) (Yarkoni, 2011). This map was then parcellated into separate regions using the lowest z-threshold that would separate each region it from neighbouring regions. The final network of R=11 regions comprised the midline supplementary motor area and five regions split into left and right components: the precentral gyri (i.e. motor cortex); rolandic opercula (i.e. premotor cortex); pallidum; thalamus, and cerebella (Figure 8). These regions-of-interest were mapped to the infant space using a two-stage transformation (adult-template -> infant-template; infant-template -> individual infant, see methods).

Figure 9 and Figure 10 show the functional connectivity between every pair of the 11 regions of the network in adults. Signal strength differs between adults and infants, as infants have smaller brains with a different water content and thus T2* relaxation in MRI

(Rivkin et al, 2004). Furthermore, they generate a weaker BOLD fMRI response (Arichi et al, 2012) and are more likely to move. We thus focused on the relative strength of connectivities in the motor network. The pattern of connectivity within the motor system was found to be consistent across the adults ($r=0.57$, $t(119)=37.5$, $p<0.0001$). This was not driven by the distance between nodes, as the results were similar if the distance between nodes was regressed out ($r=0.59$, $t(119)=39.1$, $p<0.0001$). A number of connectivity features were visible by eye, such as the supplementary motor area (SMA) being moderately to strongly correlated with all nodes except for the left pallidum. In particular, the SMA showed strong correlations with the other cortical nodes (the left and right precentral gyri and left and right rolandic opercula). Strong interhemispheric correlations between homologous regions were observed for the cerebella, thalami, and motor cortices.

There was strong consistency across subjects in the connectivity within the three-month group ($r=0.54$, $t(14)=20.3$, $p<0.0001$) and the nine-month group ($r=0.37$, $t(14)=4.88$, $p<0.0005$). Contrary to the prediction of immature connectivity prior to the development of function, both groups show a pattern of pairwise functional connectivity that is strikingly similar to the adults (Figure 9 & Figure 10). Each infant's connectivity pattern was similar to the adult mean, for both the three-month group ($r=0.70$, $t(5)=30.0$, $p<0.0001$) and the nine-month group ($r=0.58$, $t(5)=5.41$, $p<0.005$). This can be visualized in the tight relationship between connectivity strength in the adults and infants in Figure 11Figure 11Figure 11. In both three- and nine- month olds, the SMA was strongly connected to the other cortical nodes. The cortical nodes were also well-connected with one another, though again, slightly stronger in the three-month olds. Strong interhemispheric connectivity was observed between the left and right cerebella and precentral gyri in both three- and nine-month olds. Moderate interhemispheric connectivity was also observed between the left and right thalami in three-month olds and nine-month olds, respectively.

Although there was strong similarity between the infant groups and the adults, this might mask some consistent differences. To try to uncover these, for each infant we regressed out the mean adult connectivity pattern, and then tested whether the resulting residual patterns were consistent within each of the infant groups. We found no evidence that there was a unique immature signature of infant connectivity, once similarity to the adult group had been accounted for, in either the three-month group (r=0.097, t(14)=1.74, NS) or the nine-month group (r=0.11, t(14)=1.95, NS).

Finally, although the pairwise connectivity patterns were similar for the infants and adults, perhaps there is some higher-order structural difference that is difficult to identify from pairwise-connectivity measures. To investigate this, we used hierarchical clustering to group together nodes that are more connected to each other, for each of the three age groups. The results (Figure 12) showed that the higher-order structure of each of the infant groups and the adults was also strikingly similar.

As a further approach to try to capture any differences in higher order structure between the groups, we used multidimensional scaling to visualize which regions in the motor network had similar or different patterns of connectivity. The results (Figure 13) any provided no evidence of any difference in network structure across the groups. In each participant group, cortical areas (SMA, rolandic opercula, and precentral gyri) are clustered closely to one another. The thalami and pallida are clustered as well. The left and right cerebella are close to one another but are approximately equidistant to the other brain regions.

2.4 Discussion

Our study shows that functional connectivity between nodes of the infant motor network is adult-like at both three and nine months of age. Our results support the findings of previous studies that have used resting-state fMRI to demonstrate the presence of networks at birth in both preterm and term-born infants. For example, resting state networks are present in neonates; even in infants born as early as 29 weeks gestational age (Doria et al., 2010). Changes in network connectivity have also been examined using a seed-based functional connectivity method, and it was found that, visually, connections between the motor cortex and the rest of the brain remained consistent over the first year of life (Gao et al., 2014). While this method is useful, it is difficult to determine connections between other brain areas; for example, the motor cortex doesn’t appear to be connected with subcortical structures. Where our work differs is the level of detail with which we’ve examined a single cognitive system. Specifically, we sought to investigate pairwise connectivity between nodes of the motor network and how this relates to overall network structure.

The interactive specialization theory postulates that changes in functional connectivity underlie the development of complex motor behaviours (Johnson, 2001). We hypothesized that while the anatomical structure of the network does not change over the first year, connections between individual regions within the motor network should. However, here we have shown that functional connectivity is strong -- and exhibits a number of important, adult-like connections -- at just three months of age. Furthermore, and most notably, connectivity doesn’t change between three and nine months, a time when motor function develops tremendously. A three-month-old is just learning to hold his chin up and sit with support, while a nine-month-old is able to pull herself up to stand and perhaps even crawl -- so, it is perplexing that motor functional connectivity remains the same.

Although our research has not shown support for the interactive specialization theory, it is possible that connectivity is changing on a level unmeasurable by our fMRI methods. For example, we were not able to measure connectivity between individual neurons. We also were not able to measure multivariate pattern analysis (MVPA)-type fine representations between regions.

Additionally, methods with higher temporal resolution, such as electroencephalography (EEG), have shown promise in elucidating infant motor development. Event-related potentials (ERPs) appear to underlie many motor developmental changes. Infants and adults show mu and beta wave desynchronizations, patterns of suppressed brain activity observable on electroencephalography (EEG) recordings, which occur while they execute or observe an action (van Elk et al., 2008). It has been shown that mu frequencies increase during infancy and early childhood. At five months, frequencies peak between 5–6 Hz; at two years, they increase to 8 Hz; and at just four years of age, frequencies reach their adult level at 9–10 Hz (Marshall, Bar-Haim, Fox, 2002). In addition, infants may only begin to exhibit motor resonance (perceiving and understanding the goals of another's actions) after six months of age. Eight-month-old infants show mu desynchronizations in response to observation of grasping behaviour (Southgate, Johnson, Karoui, & Csibra, 2010); in contrast, six-month-olds did not (Nystrom et al., 2012). Infants also respond differentially to activities that they have performed versus those that they have observed. Ten-month-old infants were trained to perform a novel motor activity and observe a separate novel activity, both of which were associated with different sounds. Infants were then tested by presenting both previous sounds as well as a novel sound while associated neural activity was recorded. It was found that infants responded with stronger mu desynchronization to the sound associated with the performed activity than to the activity that was simply observed (Gerson, Bekkering, & Hunnius, 2015). Finally, infants perceive others' motor activity differently depending on their ability to perform said activity. While observing videos of other infants crawling and walking, 14- to 16-month-old infants exhibited greater mu desynchronization to the crawling videos; in addition, this effect was greater if the infant had more experience crawling. The existence and strength of mu wave desynchronizations change with age, depending on the activity that is performed or observed, and/or upon learning new motor skills. It is therefore possible that either an increase in mu frequencies aids in the development of new motor skills throughout infancy, or skill development helps to “activate” mirror neurons (leading to the onset of mu frequencies), or a combination of the two (Vanderwert, Fox, & Ferrari, 2012).

Additionally, throughout the first year of life there are notable changes in myelination, which may change the function of the motor network without changing patterns of connectivity. A recent study of three- to 11-month infants examined the development of myelination using a unique MRI technique and has shown that myelin develops relatively early in deep, motor-focused areas and gradually increases throughout the cortex over the first year. For example, myelination within the cerebellum and pons has begun to change by three months of age. Interestingly, the areas in which myelination develops early also tend to display slower myelin development, potentially alluding to its importance in facilitating the many changes that take place over the first year (Deoni et al., 2011).

Despite its limitations, the use of functional connectivity MRI in neonatal and infant developmental research has increased remarkably over the last decade, with over ⅔ of all published papers released in the past five years. Its high spatial resolution has allowed for more accurate identification of the brain regions most active in infancy, and multifarious analysis techniques have provided researchers with greater flexibility to explore increasingly inventive research questions. Future research should explore techniques, such as MVPA, which allow for more sensitive detection of developmental changes in infancy.

2.5 Methods

2.5.1 Participants

2.5.1.1 Demographics

Twelve infants were scanned (six three-month-olds and six nine-month-olds). Half of these infants were term-born and half were born preterm; ¼ were female. See Table 1 for more detailed subject demographics.

2.5.1.2 Neurodiagnoses

Four of the infants had a neurodiagnosis (most often related to preterm birth), and four infants exhibited slight white matter injury (Table 2). However, the structural scans of all infants were reviewed by a neonatal neurologist and the infants' brains were found to be structurally sound and healthy.

2.5.1.3 Motor ability

The infants who were also patients at Victoria Hospital (most of the preterm-born infants) also underwent regular physical examinations to determine their motor abilities. All infants assessed ranged from below average to normal (Table 3).

2.5.2 Data acquisition

Infants were scanned during natural sleep, without the use of sedation. A neonatal/perinatal nurse was present during the duration of the scan to monitor the infant's well-being and assist in the case of a medical emergency.

A Siemens Prisma 3T MRI scanner with a 12-channel head coil (Siemens, Erlangen Germany) was used to acquire two sessions (each 7.5 minutes long) of functional MRI (fMRI). Multiband acceleration (Feinberg et al., 2010; Xu et al., 2013; Nunes, Hajnal, Golay, & Larkman, 2006) was used to reduce sensitivity to movement (36 slices of 64x64 matrix size with 3x3 mm in-plane resolution, and slice thickness 3 mm, multiband factor 4). The echo-time was adjusted for the 3-month group (TE=40 ms) to reflect the longer T2* relaxation due to increased water content in brain tissue (Rivkin et al, 2004), but used a more typical value in the 9-month and adult group (TE=40 ms). There was slight variation in protocol between subjects, reflecting ongoing optimization (3 month: slice gap=0-0.3 mm, TR=776-861 ms. Adults and 9 month: slice gap=0-0.3 mm; TR=686-861 ms). First, both T1 and T2* weighted structural images were acquired (36 oblique slices of 3 mm thickness, 64 x 64 matrix, voxel size 3 x 3 x 3 mm^3, TR = 4000 ms, TE = 120 ms) at the beginning of the MRI testing.

2.5.3 Analysis

As discussed in Chapter 1, ROIs were selected based on data acquired from neurosynth.org (Yarkoni, 2011). Initial search for ROIs returned a number of brain regions (Table 4). Some of these brain regions were not selected as standalone ROIs because they were too small, and others because the search did not detect the particular region as a quantifiable cluster. The final selected ROIs include the supplementary motor area and the left and right precentral gyrus, rolandic operculum, thalamus, pallidum, and cerebellum (Table 5).

Data were analyzed with aa 4.2 (automatic analysis) (Cusack et al., 2015) and SPM 8 in Matlab (The MathWorks, 2017). The adult ROIs in MNI-152 space were transformed via an infant template to each individual infant's space, using their T1 or T2 image to derive normalisation transformations. The EPIs were motion corrected and high-pass filtered with a cutoff of 128s. Using non-linear normalisation as implemented in SPM 8's "segment and normalise", the transformation between the infant and adult space was calculated in a two-stage transformation. First, the transformation was calculated that would warp an individual infant's brain to match the UNC templates (Shi et al., 2011); the newborn template was used for the three-month group, and the one year template for the nine-month group. Then, the transformation from templates to the adult MNI-152 space was calculated. Using the inverse of these transformations, the adult ROIs (as derived from Neurosynth, see main text) (Yarkoni, 2011) could then be projected back into the space of individual infants. This allowed the timecourse of BOLD activity in each ROI to be extracted.

To quantify the functional connectivity between each pair of ROIs, we used Pearson correlation. For R ROIs, there are R*(R-1)/2 unique pairwise comparisons. As overall signal sensitivity is likely to differ between adults and infants, we did not use absolute *r* values but focussed on the relative strength of connectivity for different nodes in the motor network, by z transforming the set of pairwise correlations within each individual. For each connection, this will yield a measure whether it is stronger (+ve) or weaker (-ve) than the mean connection in the motor network, expressed in units of “standard deviations”.

To quantify the similarity of patterns of connectivity between different individuals, we used a second-order correlation between the R*(R-1)/2 unique z-transformed correlation values in each individual’s connectivity matrix and the corresponding R*(R-1)/2 values for every other individual. The resulting second-order correlations were then tested against zero with a one-sample t-test. Under the null hypothesis that the expected second-order correlation across subjects is zero, this set of correlations is independent (i.e., the covariance of the pairwise correlations is zero) ensuring the validity of the one-sample t-test.

2.6 References

Aeby, A., Liu, Y., De Tiège, X., Denolin, V., David, P., Balériaux, D., … Van Bogaert, P. (2009). Maturation of thalamic radiations between 34 and 41 weeks' gestation: A combined voxel-based study and probabilistic tractography with diffusion tensor imaging. *AJNR. American journal of neuroradiology, 30*(9), 1780-1786.

Alcauter, S., Lin, W., Smith, J. K., Short, S. J., Goldman, B. D., Reznick, J. S., et al. (2014). Development of thalamocortical connectivity during infancy and its cognitive correlations. *The Journal of neuroscience : the official journal of the Society for Neuroscience , 34* (27), 9067-75.

Arichi, T.; Counsell, S.J.; Allievi, A.G.; Chew, A.T.; Martinez-Biarge, M.; Mondi, V.; … Edwards, A.D. (2014). The effects of hemorrhagic parenchymal infarction on the establishment of sensori-motor structural and functional connectivity in early infancy. *Neuroradiology, 56*(11), 985-994.

Chugani, H.T.; Phelps, M.E.; Mazziotta, J.C. (1987). Positron emission tomography study of human brain functional development. *Annals of neurology, 22*(4), 487-497.

Cusack, R.; Wild, C.J.; Linke, A.C.; Arichi, T.; Lee, D.S.C.; Han, V.K. (2015). Optimizing stimulation and analysis protocols for neonatal fMRI. *PLoS One, 10,* e0120202.

Damaraju, E., Caprihan, A., Lowe, J.R., Allen, E.A., Calhoun, V.D., Phillips, J.P. (2014). Functional connectivity in the developing brain: a longitudinal study from 4 to 9 months of age. *Neuroimage, 84,* 169-180.

Deoni, S.C.L., Mercure, E., Blasi, A., Gasston, D., Thomson, A., Johnson, M., … Declan G. M. Murphy. Mapping Infant Brain Myelination with Magnetic Resonance Imaging. Journal of Neuroscience 12 January 2011, 31 (2) 784-791; DOI: https://doi-org.proxy1.lib.uwo.ca/10.1523/JNEUROSCI.2106-10.2011]

Doria, V., Beckmann, C.F., Arichi, T., Merchant, N., Groppo, M., Turkheimer, F.E., … Edwards, A.D. (2010). Emergence of resting state networks in the preterm human brain. *PNAS, 107*(46), 20015-20020.

Eyre, J.A., Miller, S., Clowry, G.J., Conway, E.A., & Watts, C. (2000). Functional corticospinal projections are established prenatally in the human foetus permitting involvement in the development of spinal motor centres. *Brain, 123*, 51-64.

Feinberg, D. a; Moeller, S.; Smith, S. M.; Auerbach, E.; Ramanna, S.; Gunther, M.; … Yacoub, E. (2010). Multiplexed echo planar imaging for sub-second whole brain FMRI and fast diffusion imaging. *PloS One, 5*(12), e15710.

Gao, W. Alcauter, S. Smith, J.K. Gilmore, J.H., Lin, W. (2014). Development of human brain cortical network architecture during infancy. *Brain structure & function, 220*(2), 1173-1186.

Gerson, S.A., Bekkering, H., & Hunnius, S. Short-term Motor Training, but Not Observational Training, Alters Neurocognitive Mechanisms of Action Processing in Infancy.

Johnson, M.H. (2001). Functional Brain Development in Humans. *Nature Reviews: Neuroscience. 2*, 475-483.

Johnson, M.H. (2002). Functional Brain Development in Infants: Elements of an Interactive Specialization Framework. *Child Development, 71*(1), 75-81.

Johnson, M.H. (2011). Interactive Specialization: A domain-general framework for human functional brain development? *Developmental Cognitive Neuroscience, 1*, 7-21.

Johnson, M.H., de Haan, M. (2015). *Developmental Cognitive Neuroscience: An Introduction*. West Sussex, United Kingdom: John Wiley & Sons, Ltd.

Liu, Y., Balériaux, D., Kavec, M., Metens, T., Absil, J., Denolin, … Aeby, A. (2010). Structural asymmetries in motor and language networks in a population of healthy preterm neonates at term equivalent age: A diffusion tensor imaging and probabilistic tractography study. *Neuroimage*, *51*(2), 783-788.

Marshall, P.J., Bar-Haim, Y., Fox. N.A. (2002). Development of the EEG from 5 months to 4 years of age. *Clinical Neurophysiology, 113*, 1199–1208.

Nogueira, S.F., Figueiredo, E.M., Gonçalves, R.V., & Mancini, M.C. (2015). Relation between hand function and gross motor function in full term infants aged 4 to 8 months. *Brazilian Journal of Physical Therapy*, *19*(1), 52-60. https://dx-doi-org.proxy1.lib.uwo.ca/10.1590/bjpt-rbf.2014.0070

Nunes, R.G., Hajnal J.V., Golay X., & Larkman, D.J. (2006). Simultaneous slice excitation and reconstruction for single shot EPI. *Proc. Intl. Soc. Mag. Reson. Med., 14*, 293.

Nystrom, P., T. Ljunghammar, K. Rosander, C. von Hofsten Using mu rhythm perturbations to measure mirror neuron activity in infants. Developmental Science, 14 (2012), 327–335.

Ratnarajah, N., Rifkin-Graboi, A., Fortier, M.V., Chong, Y.S., Kwek, K., Saw, S., … Qiu, A. (2013). Structural connectivity asymmetry in the neonatal brain. *Neuroimage, 75*, 187-194.

Rivkin, M. J.; Wolraich, D.; Als, H.; McAnulty, G.; Butler, S.; Conneman, N.; … Mulkern, R.V. (2004). Prolonged T*2 values in newborn versus adult brain: Implications for fMRI studies of newborns. Magnetic Resonance in Medicine, 51(6), 1287-1291.

Seghier, M. L., & Hüppi, P. S. (2010). The Role of Functional Magnetic Resonance Imaging in the Study of Brain Development, Injury, and Recovery in the Newborn. *Seminars in Perinatology, 34*(1), 79-86.

Shi, F.; Yap, P.-T.; Wu, G.; Jia, H.; Gilmore, J.H.; Lin, W.; Shen, D. Infant Brain Atlases from Neonates to 1- and 2-year-olds. (2011). *PLoS ONE, 6*(4), e18746.

Smyser, C. D., Snyder, A. Z., Shimony, J. S., Blazey, T. M., Inder, T. E., & Neil, J. J. (2013). Effects of White Matter Injury on Resting State fMRI Measures in Prematurely Born Infants. *PLoS ONE, 8*(7).

Southgate, V., Johnson, M.H., Karoui, I. E, Csibra, G. (2010). Motor system activation reveals infants' on-line prediction of others' goals. *Psychological Science, 21*, 355–359.

The MathWorks, I. (2017). MATLAB. Retrieved from MathWorks: https://www.mathworks.com/products/matlab.html

van Elk, M. van Schie, H.T. Hunnius, S. Vesper, C. Bekkering, H. (2008). You'll never crawl alone: Neurophysiological evidence for experience-dependent motor resonance in infancy. *NeuroImage, 43*(4), 808-814.

Vanderwert, Fox, Ferrari (2012). The mirror mechanism and mu rhythm in social development.

Xu, J.; Moeller, S.; Auerbach, E. J.; Strupp, J.; Smith, S. M.; Feinberg, D.A.; … Uğurbil, K. (2013). Evaluation of slice accelerations using multiband echo planar imaging at 3 Tesla. *Neuroimage, 83*, 991-1001.

Yarkoni, T. Poldrack, R.A. Van Essen, D.C. Wager, T.D. (2010). Cognitive neuroscience 2.0: building a cumulative science of human brain function. *Trends in Cognitive Sciences*, *14*(11), 489-496.

2.7 Tables

Table 1. Subject demographics.

Subject Number	Sex	GA at Birth (weeks). * indicates that GA data was unavailable but it is known that the infant was term-born, so 40 weeks is assumed.	Age at Scan (months)
3_1	M	29	4
3_2	F	28	4
3_3	F	40*	3
3_4	M	41	3
3_5	F	41	3
3_6	F	40*	3
9_1	M	40*	9
9_2	M	40*	9.5
9_3	M	27	9
9_4	M	27	9
9_5	M	29	9
9_6	M	25	11

Table 2. Subject neurodiagnosis and assessment of white and grey matter integrity.

A Woodward score between 0 and 6 is normal; increasing scores indicate injury. The acronym IVH stands for intraventricular haemorrhage; there are four grades of IVH, four being the most severe. Blacked-out rows indicate infants who were not at increased risk for brain injury, not given a neurodiagnosis, and for whom a Woodward score was not calculated.

Subject Number	Neurodiagnosis	Woodward White Matter (WM) Score	Woodward Grey Matter (GM) Score
3_1	None	Data unavailable	Data unavailable
3_2	None	8	4
3_3	N/A	N/A	N/A
3_4	Hypoxia, stroke, seizures	7	3
3_5	Stroke, WM haemorrhages	8	3
3_6	N/A	N/A	N/A
9_1	N/A	N/A	N/A
9_2	N/A	N/A	N/A
9_3	No	5	3
9_4	IVH grade II	Data unavailable	Data unavailable
9_5	No	Data unavailable	Data unavailable
9_6 1022	IVH grade II	8	3

Table 3. Rating of motor ability at four time points.

These ratings were obtained from three motor tests: Test of Infant Motor Performance (TIMP) at term-equivalent age; and Alberta Infant Motor Scale (AIMS) and Infant Neurological International Battery (INFANIB) at four, eight, and 12 months. Only infants that were patients at Victoria Hospital and at greater risk for motor impairment (as indicated by prematurity or neurodiagnosis) underwent these examinations. Blacked-out rows indicate infants who were not eligible to attend the Developmental Follow-Up Clinic at Victoria Hospital, because they were not at greater risk of brain injury or motor impairment, and therefore did not undergo these examinations.

		Age (months)		
Subject Number	Term-equivalent age	4	8	12
3_1	Normal	Below average-normal	Below average-normal	Below average-normal
3_2	Normal	Data unavailable	Data unavailable	Data unavailable
3_3	N/A	N/A	N/A	N/A
3_4	Below average	Data unavailable	Data unavailable	Data unavailable
3_5	Data unavailable	Normal	Data unavailable	Data unavailable
3_6	N/A	N/A	N/A	N/A
9_1	N/A	N/A	N/A	N/A
9_2	N/A	N/A	N/A	N/A
9_3	Normal	Normal	Normal	Normal
9_4	Data unavailable	Normal	Below average-normal	Below average-normal

9_5	Normal	Below average-normal	Below average-normal	Below average-normal
9_6	Below average	Below average	Below average	Data unavailable

Table 4. Results of preliminary ROI (regions of interest) search.

The regions labelled as either “small” or “very small” were not selected as standalone ROIs, but did overlap with selected ROIs. Though the left and right rolandic operculum were very small, they were separated from all other regions and did not overlap, so they were considered to be separate regions. The regions where a cluster was not found were too small to be detected and therefore should not be considered as overlapping with selected ROIs.

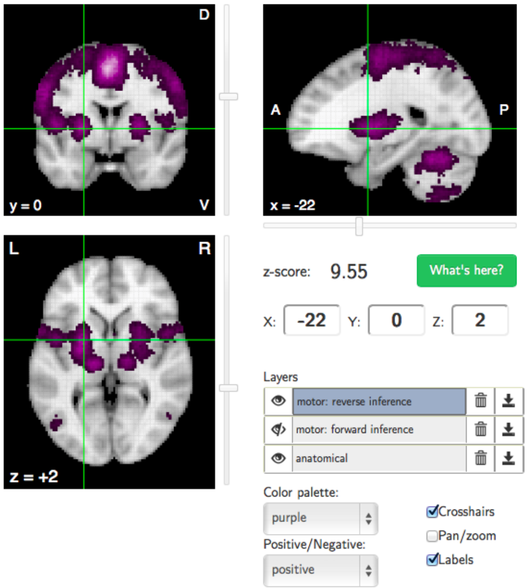
Abbreviated Label	**Coordinates of Centre of Mass**	**Full Name**	**Small?**	**Finds cluster?**
L-SMA	-7 x -4 x 60	Supp. motor area, Left		
R-SMA	6 x -4 x 58	Supp. motor area, Right		
L-CER6	-22 x -53 x -23	Cerebellum 6, Left		
R-CER6	19 x -53 x -23	Cerebellum 6, Right		
L-CER8	-24 x -61 x -52	Cerebellum 8, Left		
R-CER8	19 x -60 x -53	Cerebellum 8, Right		
L-SPG	-35 x -44 x 62	Superior parietal gyrus, Left		No
R-SPG	25 x -52 x 66	Superior parietal gyrus, Right		No
L-IPG	-37 x -42 x 54	Inferior parietal gyrus, Left		No
R-IPG	37 x -37 x 49	Inferior parietal gyrus, Right		No
L-PREG	-38 x -16 x 56	Precentral gyrus, Left		
R-PREG	39 x -20 x 56	Precentral gyrus, Right		

L-POSTG	-56 x 17 x 26	Postcentral gyrus, Left		No
R-POSTG	57 x -17 x 36	Postcentral gyrus, Right		No
L-ROC	-49 x 4 x 8	Rolandic operculum, Left	very small	
R-ROC	51 x 7 x 5	Rolandic operculum, Right	very small	
L-SMG	-55 x -21 x 35	Supramarginal gyrus, Left		No
R-SMG	57 x -20 x 38	Supramarginal gyrus, Right		No
L-PUT	-26 x -6 x 7	Putamen, Left	small	No
R-PUT	30 x -2 x 7	Putamen, Right	very small	No
L-PAL	-26 x -6 x 1	Pallidum, Left	small	
R-PAL	22 x -2 x 4	Pallidum, Right	very small	
L-CING	-6 x -4 x 45	Cingulate and paracingulate gyri, Left		No
R-CING	5 x -4 x 47	Cingulate and paracingulate gyri, Right		No
L-SFG	-28 x -6 x 61	Superior frontal gyrus, Left		No
R-SFG	29 x -6 x -61	Superior frontal gyrus, Right		No
L-MFG	-26 x -6 x 52	Medial frontal gyrus, Left	very small	No
R-MFG	39 x -6 x 58	Medial frontal gyrus, Right		No
L-TH	-15 x -18 x 4	Thalamus, Left		
R-TH	16 x -17 x 4	Thalamus, Right		
L-TEMPS	-54 x -33 x 19	Temporal sup l		No

Table 5. Final ROIs selected to utilize in analysis.

#	Main ROI label	Coordinates of Centre of Mass (Main)	Overlapping region (if applicable)	Coordinates of Centre of Mass (Overlapping)
1	Supplementary motor area (SMA); incl. both left and right	-1 x -2 x 56	N/A	N/A
2	Left precentral gyrus (L-PREG)	-37 x -22 x 62	Left postcentral gyrus (L-POSG)	-36 x -21 x 50
3	Right precentral gyrus (R-PREG)	35 x -21 x 60	Right postcentral gyrus (R-POSG)	43 x -21 x 52
4	Left rolandic operculum (L-ROC)	-48 x 4 x 8	Left insula (L-INS)	-43 x 4 x 5
5	Right rolandic operculum (R-ROC)	50 x 4 x 8	Right insula (R-INS)	47 x 4 x 5
6	Left pallidum (L-PAL)	-26 x -6 x 2	L-putamen (L-PUT)	-26 x 1 x 2
7	Right pallidum (R-PAL)	23 x 0 x 5	R-putamen (R-PUT)	26 x 7 x 5
8	Left cerebellum (L-CER); incl. lobule 6	-23 x 54 x -25	Left cerebellum lobules 4 and 8	-13 x 54 x -22
9	Right cerebellum (R-CER); incl. lobule 6	22 x -54 x -26	Right cerebellum lobules 4 and 8	12 x -54 x -18
10	Left thalamus (L-TH)	-14 x -15 x 3	N/A	N/A
11	Right thalamus (R-TH)	14 x -15 x 4	N/A	N/A

2.8 Figures



z-score: 9.55

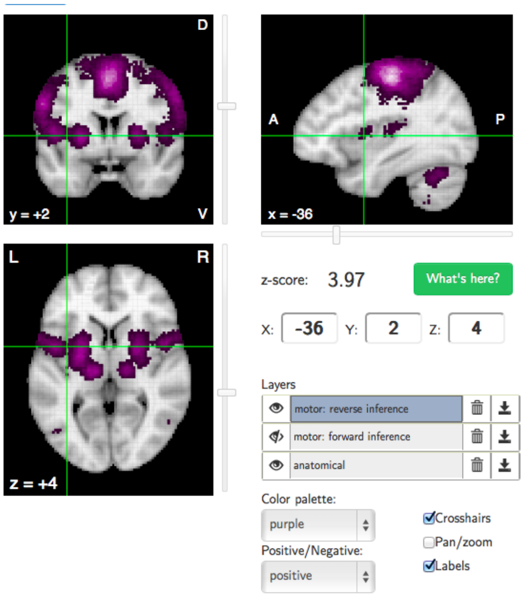
X: -22 Y: 0 Z: 2

Layers: motor: reverse inference; motor: forward inference; anatomical

Color palette: purple

Positive/Negative: positive

☑ Crosshairs
☐ Pan/zoom
☑ Labels



z-score: 3.97

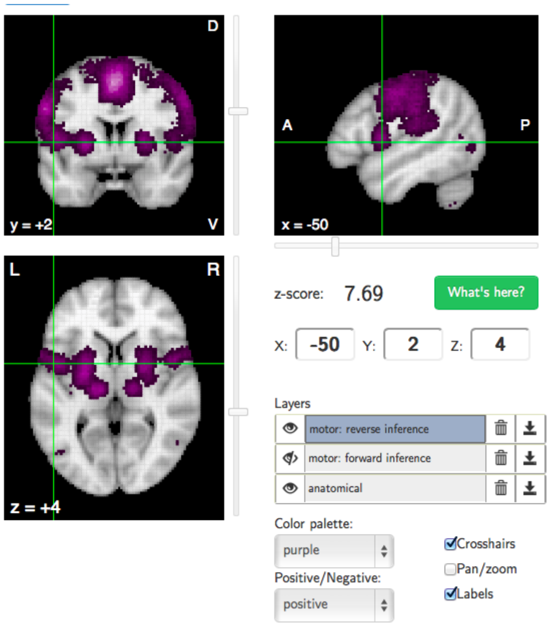
X: -36 Y: 2 Z: 4

Layers: motor: reverse inference; motor: forward inference; anatomical

Color palette: purple

Positive/Negative: positive

☑ Crosshairs
☐ Pan/zoom
☑ Labels



z-score: 7.69

X: -50 Y: 2 Z: 4

Layers: motor: reverse inference; motor: forward inference; anatomical

Color palette: purple

Positive/Negative: positive

☑ Crosshairs
☐ Pan/zoom
☑ Labels

Figure 7. Results of initial reverse inference search on neurosynth.org.

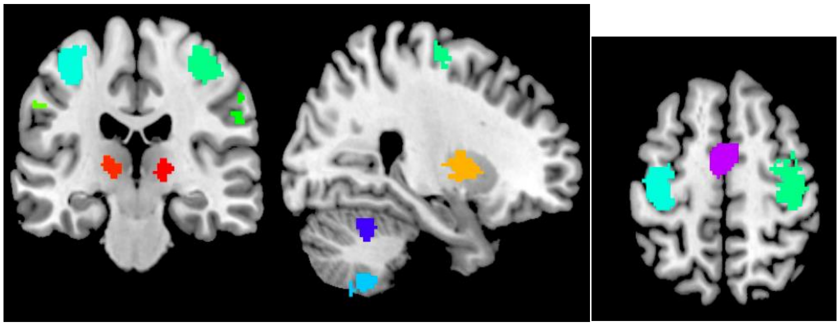


Figure 8. Three orthogonal slices illustrating the final selected ROIs.

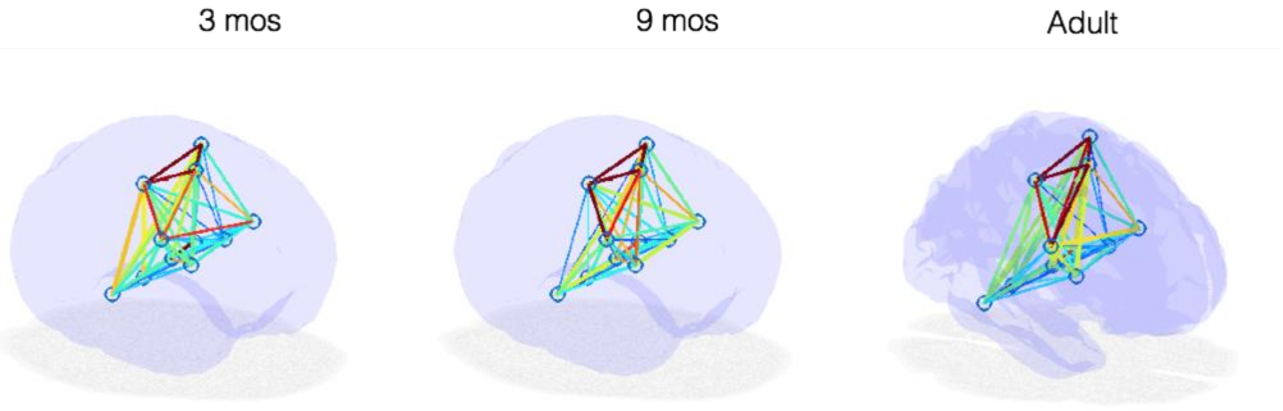


Figure 9. A three-dimensional rendering of the conn
brain regions, for each of the three age groups.

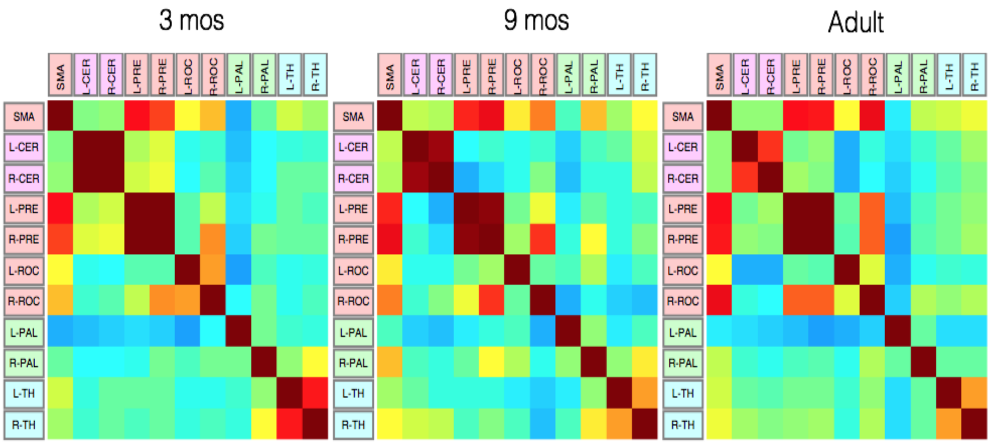


Figure 10. A matrix representation of the pairwise connectivities shown in Fig. 1.

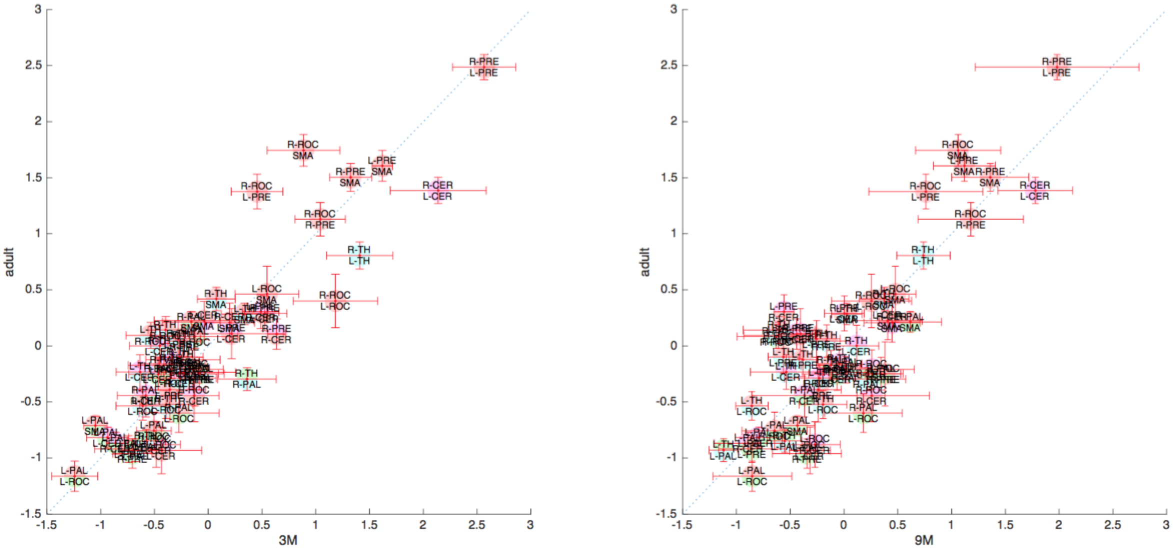


Figure 11. For region pair of brain regions, a comparison of the strength of connectivity in the infants and adults.

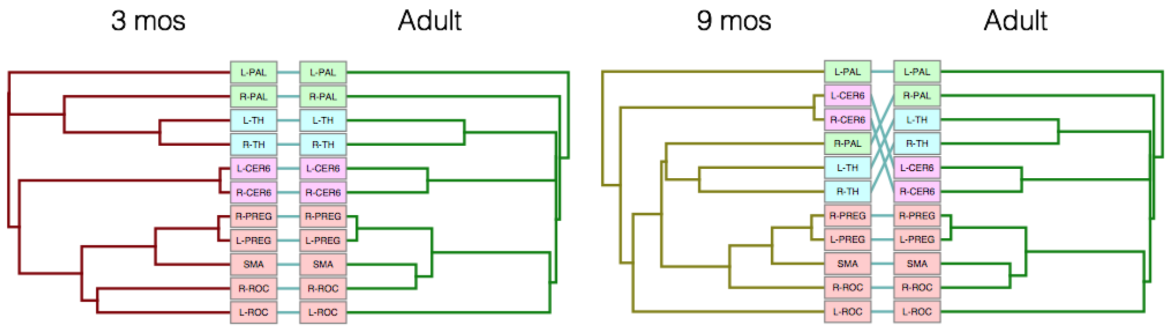


Figure 12. The higher-order structure in the connectivity, as revealed with hierarchical clustering, for each of the groups.

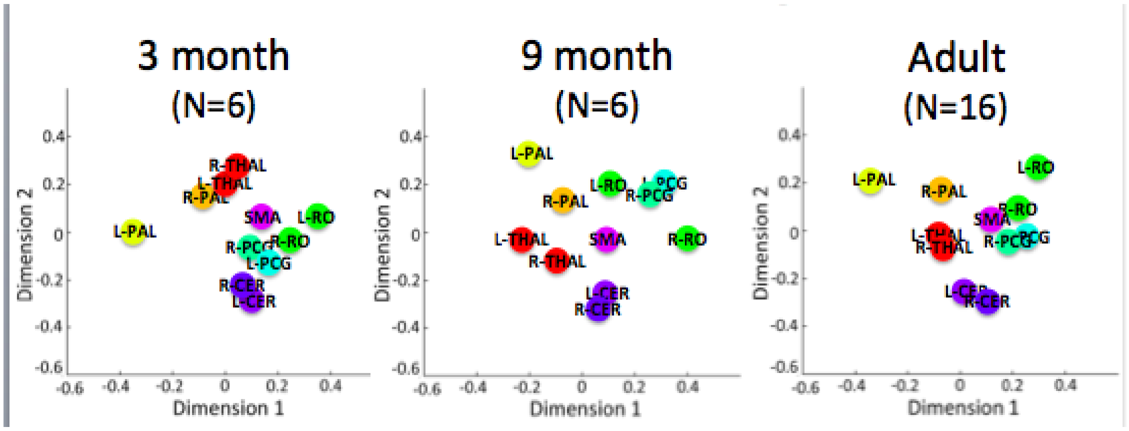


Figure 13. Multidimensional scaling plots illustrating connectivity within the brain as a whole.

n.b.: Regions that are closer together in the multidimensional scaling representation have more similar patterns of connectivity.

Chapter Three

3 Conclusion

3.1 Discussion

Using functional connectivity MRI, we were able to further elucidate the structure and connectivity patterns of the infant and adult motor networks. Based on the Interactive Specialization Theory, it was hypothesized that connectivity would strengthen from three to nine months, with strongest connectivity in adults. However, connectivity was found to be remarkably similar in all three age groups, and both infant groups' BOLD signal timecourses significantly correlated with those of the adults. We created a hierarchical clustering map that strongly illustrates the similarities between the nine-month-old group and adults. We computed pairwise correlations between brain regions in each group and strong interhemispheric connectivity was observed between the left and right cerebella, motor cortices, and thalami. Cortical regions - in particular, the supplementary motor area (SMA) and motor cortices - were strongly connected to one another as well. An examination of overall network connectivity patterns shows that brain regions can be grouped into three clusters based on the fluctuations in their BOLD signal timecourses: a cortical cluster of the SMA and left and right motor and premotor cortices; a subcortical cluster of the left and right thalami and pallida; and a cerebellar cluster of the left and right cerebella.

Though we did not observe motor network changes in typically-developing infants, unpublished data from our group indicates that injury to the motor network does indeed affect motor development (Linke et al., 2016). Linke et al. (2016) scanned neonates at term or term-equivalent age who were either born very early preterm (<29 weeks) or born >29 weeks but at heightened risk of brain injury. Researchers were able to identify functional networks in all of these infants and then compared network integrity with neurodevelopmental outcomes. Functional connectivity did not relate to information in the neonates’ medical reports at the time of discharge from the neonatal intensive care unit. At eight months of age, infants underwent a number of clinical motor examinations including the Alberta Infant Motor Scale (AIMS) and the Infant Neurological Battery (INFANIB). Interestingly, integrity of functional connectivity at term positively correlated with motor neurodevelopmental outcome; infants with more intact motor functional connectivity tended to have higher scores on the AIMS and INFANIB and more typical motor behaviour. In other words, while we did not find a change in connectivity despite a dramatic change in behaviour, Linke et al. (2016) did find a relationship between early connectivity and later behaviour. These findings are not actually inconsistent - we hypothesize that the motor network may be necessary but not sufficient for good function. An intact motor network at birth appears to be necessary for typical motor behaviour, but other changes throughout the first year of life must also influence the extent to which motor skills are developed. This study by Linke et al. (2016) also confirms that it is possible to detect behaviourally meaningful changes in networks data like ours.

Recent research shows that the brain can change with plasticity; learning a new motor skill (in this case, juggling) changes motor connectivity, as measured with diffusion-weighted imaging (DWI) (Scholz, Klein, Behrens, & Johansen-Berg, 2009). It is possible that fcMRI was unable to detect differences to the extent that DWI was; however, the differences they observed were small and may be the reason why similar differences not have been detected in our study.

Perhaps the most parsimonious model is that the network is set up from birth (innately) with the form that will be right for mature function. Theoretically, under typical development, the network won't change; however, it is plastic, so learning a new skill will lead to changes. Alternatively, if you injure or deprive the network, then it will change. For example, in congenitally blind people, the visual cortex has been co-opted to deal with language (Röder et al., 2002).

3.2 Limitations and Future Work

A major limitation of our work is that we can only conclude we found no evidence of any change with age - i.e., we can't reject our null hypothesis - and it is possible that there are smaller changes we couldn't detect in this study. Future work could increase N or length of scanning. Alternatively, there may be changes at a finer spatial scale than we observed. For example, one of the first infant imaging studies was conducted using positron emission tomography (PET). In 1987, Harry Chugani used PET to measure glucose uptake in various areas of the brain (e.g. sensorimotor cortex; parietal, temporal, and occipital cortices; frontal occipital regions; etc.) and found that during the neonatal period (infants under five weeks of age), glucose uptake was higher in the motor regions than any other regions in the brain (Chugani, Phelps, & Mazziotta, 1987). These motor regions included sensorimotor cortex, thalamus, brainstem, and cerebellar vermis (Chugani, Phelps, & Mazziotta, 1987). This increase in glucose uptake, representative of increased energy expenditure, is thought to be an indirect measure of development (particularly of synaptogenesis and pruning) (Chugani, Phelps, & Mazziotta, 1987) (Chugani, 1998). Indeed, many synapses are formed and eliminated during the first year of life as the brain changes and adapts rapidly (Huttenlocher, 1994). In addition, dendrites and axons grow rapidly during the third trimester and shortly postnatally (Huttenlocher, 1994). Changes on such a small scale are not detectable by our methods; however, higher-resolution MRI (for example, using multi-voxel pattern analysis, or MVPA) may detect these changes.

Additionally, we have only used one MRI-based method (resting state) and perhaps another - such as DTI - would reveal differences. On a smaller scale, neurotransmitter balances could be responsible for the differences, in which case using MR spectrometry may be useful.

It may also be worthwhile to conduct similar experiments with regards to other brain systems that are developing rapidly (e.g., language, executive function) to determine whether or not changes in connectivity precede changes in behaviour - for example, if changes in the language network precede an infant speaking their first word.

The use of functional connectivity MRI has increased exponentially since its first use in 1995, and as analysis techniques evolve further and more information is available to be extracted from functional scans, the utility of fcMRI in functional neuroscience research will continue to increase. Its impressive spatial resolution of MRI and versatility as a functional imaging method, as well as the ability to scan infants without an overt task (and while sleeping) further cement its place as a pioneering method to examine infant development.

3.3 References

Chugani, H., Phelps, M., & Mazziotta, J. (1987). Positron emission tomography study of human brain functional development. *Annals of neurology , 22* (4), 487-497.

Chugani, H. (1998). A critical period of brain development: studies of cerebral glucose utilization with PET. *Preventive medicine , 27* (2), 184-188.

Huttenlocher, P.R. & Dabholkar, A.S. (1997). Regional differences in synaptogenesis in human cerebral cortex. *The Journal of Comparative Neurology*, 167-178.

Linke, A.C., Wild, C., Zubiaurre-Elorza, L., Herzmann, C., Duffy, H., Ropat, J.L.V., Han, V.K., Lee, D.S.C., & Cusack, R. (2016). Functional connectivity networks in neonates with perinatal brain injury predict neurodevelopmental outcome at 8 months.

Röder, B., Stock, O., Bien, S., Neville, H., Rösler, F. (2002). Speech processing activates visual cortex in congenitally blind humans. *European Journal of Neuroscience*, *16,* 930-036.

Scholz, J., Klein, M.C., Behrens, T.E.J., Johansen-Berg, H. (2009). Training induces changes in white-matter architecture. *Nature Neuroscience*, *12*(11), 1370-1371.

4 Curriculum Vitae

VITA

Name:	Jordynne L.V. Ropat
Post-secondary Education and Degrees:	University of Windsor Windsor, Ontario, Canada 2010-2014 B.Sc. The University of Western Ontario London, Ontario, Canada 2014-2017 (expected) M.Sc.
Honours and Awards:	Canadian Society for Brain, Behaviour, and Cognitive Science Donald O. Hebb Award: Honourable Mention for Best Spoken Presentation by a Student 2015 Ontario Graduate Scholarship (Masters) 2015 Canadian Institutes for Health Research Canada Graduate Scholarship (Masters) 2015-2016 Canadian Institutes for Health Research Michael Smith Foreign Study Supplement 2016

Publications (submitted):

Ropat, J.L.V., Linke, A.C., Wild, C.J., Zubiaurre-Elorza, L., Herzmann, C., Duffy, H., Han, V.K., Lee, D.S.C., Cusack, R. (2017) Functional connectivity in the motor network largely matures before motor function develops.

Linke, A.C., Wild, C.J., Zubiaurre-Elorza, L., Herzmann, C., Duffy, H., **Ropat, J.L.V.**, Han, V., Lee, D.S.C., Cusack, R. (2017). Disruption to motor and executive networks in neonates with perinatal brain injury predicts motor skills at eight months.