FUNCTIONAL CONNECTIVITY IN CHILDREN WITH DEVELOPMENTAL COORDINATION DISORDER:
AN EXPLORATORY STUDY

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Abstract

Developmental Coordination Disorder (DCD) is a neurodevelopmental disorder that affects a child’s ability to learn motor skills and participate in self-care, educational, and leisure activities. The cause of DCD is unknown, but evidence suggests that children with DCD have atypical brain structure and function. Resting-state MRI assesses functional connectivity by identifying brain regions that have correlated activation during rest. As only a few studies have examined functional connectivity in this population, our objective was to compare whole-brain resting-state functional connectivity of children with DCD and typically-developing children, and examine the correlation of functional connectivity with behavioural measures of motor function and ADHD symptoms.

Children 8-12 years old were classified as DCD if they scored ≤16th percentile on the Movement Assessment Battery for Children - 2nd edition (MABC-2) and scored in the suspected or indicative range on the DCD Questionnaire (N=35). The control group included children with a score ≥25th percentile on the MABC-2 (N=23). Children were excluded if they were born preterm (<38 weeks), were diagnosed with other conditions that could affect their motor proficiency, or had high levels of motion during scans.

We used Independent Component Analysis (ICA) to identify resting-state networks. We compared functional connectivity between children with DCD and typically-developing children across 19 networks, controlling for age and sex. Using Pearson’s r, we examined the correlation of functional connectivity and behavioural measurements.

Children with DCD demonstrate altered functional connectivity between the sensorimotor network and the posterior cingulate cortex (PCC), and the posterior middle temporal gyrus (pMTG) (p<0.0001). The functional connectivity between the sensorimotor network and the
PCC ($r=0.47$, $p<0.0001$) and right precuneus ($r=0.46$, $p=0.003$) was positively correlated with motor function, regardless of diagnosis.

Previous evidence suggests the PCC acts as a link between functionally distinct networks. Our results indicate that ineffective communication between the sensorimotor network and the PCC might play a role in inefficient motor learning seen in DCD. The pMTG acts as hub for action-related information and processing, and its involvement could explain some of the functional difficulties seen in DCD. This first-of-its-kind study increases our understanding of the neurological differences that characterize DCD.
Lay Summary

Developmental Coordination Disorder (DCD) affects 1–2 children in every classroom in Canada. Children with DCD have difficulty in performing and learning motor tasks, such as getting dressed, catching a ball, and writing. Evidence suggests that there are differences in brain structure and brain activity in children with DCD compared to typically-developing children. An imaging method called resting-state magnetic reasoning imaging provides a full picture of brain activity during rest. It investigates which areas of the brain are active together.

In this study, we compared the functional connectivity of children with DCD and typically-developing children, and examined the association between motor skills and functional connectivity. Our results show that brain regions that coordinate activity across the brain are less connected to regions responsible for motor and sensory function. These results promote our understanding of the brain differences associated with DCD and potentially why these children struggle to learn motor skills.
Preface

This thesis contains the work of a research study conducted by the candidate, Shie Rinat, under the supervision of Dr. Jill G. Zwicker, with guidance from Dr. Lara Boyd and Dr. Liisa Holsti. This is part of a larger study, in which the design and conception are the work of Dr. Jill Zwicker. Data collection was conducted by the candidate, Shie Rinat, PhD candidates Sara Izadi-Najafabadi and Kamaldeep Gill, and MSc graduate, Meisan Brown-Lum. Data analysis plan, data processing and interpretation, and documentation are the work of the candidate.

The research project was approved by UBC Children's and Women's Research Ethics Board, certificate #H14-00397.

This research work was presented at scientific conferences, as follows (presenting author(s) underlined):


Finding from this research will be prepared for future publication in a peer-reviewed journal.
# Table of Contents

Abstract .......................................................................................................................... iii

Lay Summary .................................................................................................................... v

Preface .............................................................................................................................. vi

Table of Contents .......................................................................................................... vii

List of Tables ................................................................................................................... x

List of Figures .................................................................................................................. xi

List of Abbreviations ...................................................................................................... xii

Acknowledgements ........................................................................................................ xiv

Chapter 1: Introduction ................................................................................................. 1

1.1 Developmental Coordination Disorder ................................................................. 1

1.1.1 Definition and Diagnosis of DCD ............................................................... 2

1.1.2 Epidemiology, Etiology, and Comorbidity ................................................... 2

1.1.3 Comorbidity with ADHD ........................................................................... 4

1.2 Neuroimaging Studies in DCD ............................................................................ 5

1.2.1 Neural Correlates of DCD ......................................................................... 6

1.2.2 Limitations of Current Evidence .................................................................. 8

1.3 Resting-state MRI ............................................................................................... 8

1.3.1 Resting-state Networks .............................................................................. 9

1.3.2 Resting-state MRI Studies in DCD ............................................................. 10

1.3.3 Independent Component Analysis .............................................................. 11

1.4 Purpose of Study .................................................................................................. 12

Chapter 2: Methods ..................................................................................................... 13

vii
2.1 Study Design .................................................................................................................. 13
2.2 Participants ..................................................................................................................... 13
  2.2.1 Inclusion Criteria ........................................................................................................ 13
  2.2.2 Exclusion Criteria ....................................................................................................... 14
2.3 Procedure ...................................................................................................................... 14
2.4 Clinical Measurement ................................................................................................... 14
  2.4.1 Movement Assessment Battery for Children - 2nd Edition (MABC-2) .................. 14
  2.4.2 Developmental Coordination Disorder Questionnaire (DCDQ) ......................... 15
  2.4.3 Conners 3 ADHD Index (Conners 3 AI) ................................................................. 16
  2.4.4 Socio-demographic Questionnaire ........................................................................... 16
2.5 MRI Data Acquisition .................................................................................................... 17
2.6 Preprocessing and Denoising ....................................................................................... 17
2.7 Identification of Resting-state Networks ..................................................................... 21
2.8 Statistical Analysis ....................................................................................................... 23
  2.8.1 Descriptive Data Analysis ......................................................................................... 23
  2.8.2 Resting-state Data Analysis ..................................................................................... 23

Chapter 3: Results ............................................................................................................. 24
3.1 Cohort Characteristics ................................................................................................... 24
3.2 Head Motion Parameters .............................................................................................. 26
3.3 Research Aim 1 - Group Differences in Functional Connectivity ............................ 27
3.4 Research Aim 2 - Correlation with Behavioural Parameters ................................... 30
  3.4.1 Motor Function and Functional Connectivity .......................................................... 30
  3.4.2 ADHD Symptoms and Functional Connectivity ....................................................... 31
Chapter 4: Discussion ............................................................................................................ 33

4.1 Functional Connectivity in DCD ..................................................................................... 33

4.2 The Sensorimotor Network ......................................................................................... 33

4.3 The Posterior Cingulate Cortex and the Precuneus ....................................................... 35

4.3.1 Structural and Functional Connectivity of the PCC and Precuneus ......................... 36

4.3.2 Functional Roles of the PCC ..................................................................................... 37

4.3.3 Functional Roles of the precuneus .......................................................................... 38

4.3.4 PCC and Precuneus in DCD .................................................................................. 39

4.4 Left Posterior Middle Temporal Gyrus ...................................................................... 41

4.4.1 Temporal Involvement and Praxis in DCD ............................................................... 42

4.5 Theoretical Implications ............................................................................................. 43

4.6 ADHD Symptoms and Functional Connectivity .......................................................... 45

4.7 Discrepancy with Past Results .................................................................................... 45

4.8 Clinical Implications .................................................................................................... 47

4.9 Limitations and Future Directions ............................................................................... 47

4.10 Conclusions .................................................................................................................. 48

References ........................................................................................................................ 50
List of Tables

Table 1. Participant characteristics ........................................................................................................... 24
Table 2. Motion parameters ......................................................................................................................... 27
Table 3. Group differences in functional connectivity ............................................................................... 28
Table 4. Correlation between functional connectivity and motor function .............................................. 30
Table 5. Correlation between functional connectivity and ADHD symptoms ......................................... 32
List of Figures

Figure 1. QC-FC plot before and after denoising ................................................................. 20
Figure 2. Identified resting state-networks .............................................................................. 22
Figure 3. Participant enrolment and exclusion chart ............................................................... 25
Figure 4. Group differences in functional connectivity ........................................................... 29
Figure 5. Correlation between functional connectivity and motor function ......................... 31
Figure 6. The sensorimotor cortex ......................................................................................... 34
List of Abbreviations

ACC - Anterior Cingulate Cortex
ADHD - Attention Deficit Hyperactivity Disorder
ASD - Autism Spectrum Disorder
BA - Brodmann Area
BCCH - BC Children’s Hospital
BOLD - Blood Oxygen Level Dependent
Conners 3 AI - Conners 3 ADHD Index
DCD - Developmental Coordination Disorder
DCDQ - Developmental Coordination Disorder Questionnaire
DMN - Default Mode Network
DTI - Diffusion Tensor Imaging
FD - Framewise Displacement
fMRI - Functional Magnetic Resonance Imaging
FSL - FMRIB Software Library
FWE - Family Wise Error
ICA - Independent Component Analysis
MABC-2 - Movement Assessment Battery for Children - 2nd edition
MNI - Montreal Neurological Institute
mPFC - Medial Prefrontal Cortex
MR - Magnetic Resonance

MRI - Magnetic Resonance Imaging

M1 - Primary Motor Cortex

PALM - Permutation Analysis of Linear Models

PCC - Posterior Cingulate Cortex

RMS - Root Mean Square of intensity differences

rsMRI - Resting-state Magnetic Resonance Imaging

SD - Standard deviation

S1 - Primary sensory cortex

SMA - Supplementary Motor Area

TD - Typical Development

tDOF - Temporal Degrees of Freedom

TFCE - Threshold Free Cluster Enhancement

UBC - University of British Columbia

WM - White Matter
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Chapter 1: Introduction

1.1 Developmental Coordination Disorder

Neurodevelopmental disorders are a wide group of disorders in which there is an impairment of the growth, development, or function of the central nervous system. These impairments are expressed in different forms, such as impaired learning, motor function, cognition, or communication.[1,2] Developmental Coordination Disorder (DCD) is a neurodevelopmental disorder characterized by difficulty in performing and learning coordinated motor skills, which significantly impacts the performance of daily life activities. DCD is one of the most common motor disorders in childhood, affecting 400,000 children in Canada, or 1-2 children in each classroom.[3] Children with DCD have impairment in the performance and acquisition of motor tasks (e.g., getting dressed, tying their shoes, writing or printing, riding a bicycle). Children with DCD have lower academic achievement, as well as reduced participation in self-care, social, and leisure activities.[4,5] These difficulties often persist into adulthood,[6] and are related to a more sedentary lifestyle, higher rates of obesity,[7,8] low self-esteem, social isolation,[4,9] anxiety, and depression.[10-12]

Children with DCD also show higher levels of anxiety and depression compared to typically-developing children.[13-15] Other emotional problems include emotional distress, frustration, and anger.[16] Research has shown that motor skills in early childhood can predict mental-health outcomes of school-age children.[17] These comorbidities are associated with higher rates of educational and social problems that progress with time and last throughout adolescence and adulthood.[10,12,18,19]


1.1.1 Definition and Diagnosis of DCD

According to the Diagnostic Statistical Manual – 5th ed. (DSM-5),[1] a child must meet four criteria to be diagnosed with DCD. First, performance and learning of motor skills are significantly lower than expected based on their chronological age, and the opportunities they have had to learn and use those motor skills (criterion A). In addition, the level of their motor skills significantly and consistently interferes with their participation in daily life activities across different domains, such as play, self-care, and academic activities (criterion B). The symptoms are present from early in development (criterion C), and are not explained by any other condition, such as intellectual disability, visual impairment, or other neurological condition (criterion D).

The International Classification of Diseases (ICD-11) offers a similar definition under the diagnosis of Developmental Motor Coordination Disorder.[2] Other terms that have been used in the literature to describe this disorder include developmental dyspraxia or apraxia, clumsy child syndrome, specific developmental disorder of motor function (SDDMF), or deficits in attention, motor control, and perception (DAMP).[20, 21] The term DCD is widely accepted as the preferred term.[20-22] Both the ICD-11 and the DSM-5 agree that DCD is a neurodevelopmental disorder, suggesting an underlying neural dysfunction as the cause for the motor impairment.

1.1.2 Epidemiology, Etiology, and Comorbidity

The prevalence of DCD depends on the clinical population and the diagnostic criteria being used, and varies from 1.7 percent [23] and up to 10 percent.[24] However, most reviews agree that the
prevalence of DCD is approximately 5-6 percent.[1,20,25-27] DCD is 2 to 7 times more common in males compared to females,[23,25] as in many other neurodevelopmental disorders.[28,29] However, while prevalence in clinical samples indicate significant prevalence in boys, population-based studies indicate that a referral bias exists and that more girls have DCD than suggested by clinically referred samples.[30] Up to 50 percent of children with DCD are either left-handed or ambidextrous, compared to only 10 percent in the general population.[31-33] Due to this high prevalence, which is similar to other neurodevelopmental disorders, it has been suggested that incomplete cerebral lateralization may play a role in DCD.[32]

While the etiology of DCD is unknown, several risk factors have been identified in the literature. DCD is 6 to 8 times more common in children born preterm or with low birth-weight, compared to children born in full term or normal birth weight.[34] Prematurity is known to be related to disruption to the myelination process and to white matter (WM) disorganization, which in turn is associated with motor and other neurodevelopmental difficulties.[35] Other risk factors for DCD includes fetal distress and older maternal age.[36] DCD is thought to have a genetic component, as some studies report high heritability for probable DCD.[37-41]

Many children with DCD have at least one other diagnosis, so a single diagnosis is almost the exception rather than the rule.[20] Common comorbidities include Attention Deficit Hyperactivity Disorder (ADHD),[39,42,43] Autism Spectrum Disorder (ASD), learning disorders, and specific language impairment.[42] Other comorbidities reported in the literature, although not as often, include epilepsy and joint hypermobility syndrome.[44,45]
1.1.3 Comorbidity with ADHD

ADHD is the most common comorbidity of DCD, with up to 50% of children with DCD having co-occurring ADHD,[25, 46, 47] which further intensifies their functional difficulties.[48] With a prevalence of 5% to 9.5% among school-age children, ADHD is characterized by deficits in attention, executive functions, hyperactivity, and impulsivity.[1,49] Similar to DCD and other neurodevelopmental disorders, the prevalence in males is 3-8 times higher compared to prevalence in females,[1,50] although this difference is thought to be partially due to referral bias.[50] Although ADHD is known to have a strong genetic component, etiology of the disorder is believed to be multifactorial, with interaction of genetic, psychosocial and environmental factors.[51,52]

Since motor impairments are so common in ADHD, it has been suggested that the motor impairments in ADHD are due to inattention or lack of inhibition.[53,54] However, evidence shows that motor impairments in children with dual diagnosis are persistent, and that treatment with stimulant medication is not sufficient to resolve their motor difficulties;[55-57] these findings indicate that motor impairments are not solely the result of ADHD symptoms. It has been suggested that both disorders share the same etiology, due to the similarities in prevalence, onset age, long-term course, and high co-occurrence.[37,54,58] These hypotheses, however, have not been confirmed.[59] Co-occurrence of DCD and ADHD is related to poorer outcomes in adulthood compared to a single diagnosis.[60] These may include higher rates of alcohol abuse, criminal offences, antisocial disorder, and low educational level.[60-62]

Neuroimaging studies in ADHD have found abnormalities in many brain regions and functional networks. Structural studies report reduction in overall grey matter volume in ADHD, as well as
local reduction in grey matter volume in frontal regions and in the parietal and occipital lobes.[63-65] WM differences were investigated using Diffusion Tensor Imaging (DTI), and revealed elevated fractional anisotropy in the cingulum, posterior corpus callosum, and left inferior fronto-occipital fasciculus, and lower fractional anisotropy in anterior cingulate cortex (ACC) and orbitofrontal regions compared to controls.[66] Functional neuroimaging studies have found many brain regions with atypical activation in ADHD, including frontal regions (mostly in the prefrontal cortex), basal ganglia, insula, cingulate cortex, inferior parietal lobe, and cerebellum.[67-71] Brain regions that were found to be implicated in ADHD consistently, across different studies and modalities, include the frontal lobe (specifically the prefrontal cortex), basal ganglia, and corpus callosum.[70,72]

1.2 Neuroimaging Studies in DCD

While the neural correlates of other neurodevelopmental disorders have been investigated extensively in the last decades, the evidence regarding neural correlates of DCD remains relatively scarce. Most neuroimaging studies in DCD to date have used the different modalities of Magnetic Resonance Imaging (MRI), and most commonly functional MRI (fMRI). fMRI enables the indirect measurement of brain activity by assessing fluctuations in blood oxygen level. Blood Oxygen Level Dependent (BOLD) signals use the different magnetic properties of oxygenated and deoxygenated hemoglobin. Due to these properties, deoxygenated hemoglobin suppresses the MR signal, while the oxygenated hemoglobin does not. The cerebral blood flow to active brain areas increases the oxygenated hemoglobin levels, and thus changes the MR signal in the active brain area and allows its localization.[73] The efforts to identify the neural characteristics of DCD continue, and although the etiology for DCD appears to be multifactorial,[37,39,43] some brain
regions have been identified consistently as atypical in children DCD compared to typically-developing children.

1.2.1 Neural Correlates of DCD

Structural and functional neuroimaging studies in DCD report on the involvement of the parietal lobe.\cite{73-82} Many of these studies report atypical function of the inferior parietal lobules, precuneus, and parts of the superior parietal lobules.\cite{75,82,83} The parietal lobe is associated with visuospatial abilities,\cite{85} which are known to be poorer among children with DCD compared to typically-developing children.\cite{86-89} The parietal lobe also has an important role in motor imagery,\cite{90} a cognitive process that stimulates an internal representation of movement, and is an important component in performing and learning motor tasks.\cite{91-93} Previous studies found that children with DCD show reduced ability to engage in motor imagery,\cite{83,94-97} and it was suggested that this deficit may be the cause for the motor difficulties seen in DCD.\cite{98-100} However, this hypothesis alone cannot explain the complexity of the disorder.\cite{101}

The involvement of the frontal lobe in DCD is also reported frequently,\cite{74,75,78-81,102-108} and includes regions in the prefrontal cortex and the motor cortex. Atypical activation of the primary and secondary motor areas in DCD is to be expected, since motor difficulties are the essence of DCD. Many studies have found the medial prefrontal cortex (mPFC) to be implicated in DCD.\cite{74,75,79-81,104-106} The roles of the mPFC are varied; it is involved in several high cognitive functions, such as executive control, decision-making, error detection, reward-guided learning and memory,\cite{109-111} and it is a part of the default mode network (DMN).\cite{112} It is known that children with DCD have poorer executive functions compared to typically-developing children,\cite{113-115} and that they struggle with online control and error correction.\cite{116-119}
Other regions that have been identified as neural correlates of DCD, including the basal ganglia [77,81,120] and the posterior cingulate cortex (PCC). [74,76,80,83,103] The basal ganglia involve and mediate numerous goal-directed behaviors, including sensorimotor, emotional, and cognitive processes. In the motor system, the basal ganglia have an important role in motor planning and motor learning, motor and postural control, automatic movement, and sensorimotor integration. [121] Motor characteristics of DCD, such as impaired motor planning, difficulty learning motor tasks, [122,123] poor postural control, [124,125] and reduced ability to automate motor skills [103,126] might be explained by the involvement of the basal ganglia. The basal ganglia are a key node in several functional networks, including the DMN, the dorsal attention network, and the fronto-parietal network. [127,128] However, there is no consensus yet regarding the role of the PCC. It was suggested that the PCC has an important role in direction of internally-guided cognition and in the regulation of the focus of attention. [128] The PCC is also involved in working and long-term memory, visuospatial functions, navigation and body orientation in space, and premotor functions. [129] These cognitive functions are also reported to be compromised among children with DCD. [88,130,131]

Results of neuroimaging studies also indicate that the cerebellum is implicated in DCD. [75,78-81,84,103,106,107] The role of the cerebellum in coordination, postural control, motor execution, and motor learning, as well as its involvement in visuospatial processes, working memory, and executive functions, [132-134] all suggest that the cerebellum is implicated in DCD. The motor behaviour that characterizes children with DCD includes effortful and inaccurate movement, difficulty learning motor tasks, [122,123] reduced ability to automate motor skills, [126] and higher variability in both novel and familiar motor tasks, [135-137] which can be explained by
cerebellar involvement in DCD. Other brain regions, such as the medial temporal lobe, [76,79] the lingual gyrus [77,79,80] and the insular cortex [77,80] were also reported to show atypical activation in DCD compared to typically-developing controls, although not as frequently.

1.2.2 Limitations of Current Evidence

It is clear that significant progress has been made in the field of neuroimaging studies of DCD in the last decade. Yet, several common limitations need to be addressed in order to reach a more definite conclusion regarding the neural correlates of DCD. First, the majority of neuroimaging studies included a very small sample size (with an average sample size of 10 participants in the DCD group).[138] Such a small sample size may lead to a bias in study results, making it harder to replicate findings. Second, although some of the studies also included a wide age range (8-17 years),[77,107] most studies did not control for this potential confounder. Moreover, most neuroimaging studies in this field are task-based MRI studies. These studies are rarely replicated due to the high variance in task and study parameters, and the inferences that can be made are limited to the specific task conditions under investigation. A potential solution for these limitations can be found in a well-conducted, large-scale resting-state MRI study.

1.3 Resting-state MRI

Resting-state MRI (rsMRI) assesses brain activity during rest, allowing the study of functional connectivity between spatially-distinct brain regions. Functional networks share a common temporal pattern of low-frequency spontaneous neural activation that reflect the functional communication between those brain regions.[139] Using BOLD signal, rsMRI can be used to identify temporal correlations in activity across the brain.[140,141] The use of rsMRI enables the
investigation of functional networks without the constraint of a specific task. Resting-state networks are highly reproducible,[142] and are found consistently across participants and groups, in different developmental stages, and even in other species.[127,139,142,143] Another advantage of rsMRI is the relative simplicity of data collection due to minimal compliance demands, making it a perfect candidate for investigation of neural differences in pediatric and clinical populations.[144] Indeed, rsMRI has been applied to study development and aging,[145,146] neurological, psychiatric and neurodevelopmental disorders,[147-151] and neural changes following intervention.[152-154]

1.3.1 Resting-state Networks

In the last two decades, a growing body of evidence reports on the existence of several functional networks that are activated during specific type of tasks,[155] and that are also highly detectable from neural signals at rest.[128,139,145] These include the sensorimotor network, dorsal attention network, fronto-parietal control network, cerebellar network, visual network, auditory network, and language network.[156-158] Another network that was consistently found is known as the DMN. The DMN shows decreased activation during the performance of a wide range of tasks, and includes the ventral and dorsal prefrontal cortex, mPFC, precuneus and PCC, inferior parietal lobules, medial temporal lobes.[127,145] There is no consensus regarding the roles of the DMN, but it has been hypothesized to be linked to spontaneous cognition, self-referential cognitive processing, support of emotional processing, and monitoring the environment.[127,139,159,160] The spatial overlap between regions implicated in DCD and the DMN led to the hypothesis that the DMN will show atypical pattern of activation in DCD.[161] Considering the behavioural characteristics and the neural correlates of DCD, other networks
that might be implicated in DCD include the sensorimotor network, the dorsal attention networks, the fronto-parietal network, and the cerebellar network.

1.3.2 Resting-state MRI Studies in DCD

Only two studies have used rsMRI to assess the functional connectivity in children with DCD and with co-occurring DCD and ADHD.[77,107] Both studies used a seed-based analysis, in which brain connectivity is investigated as the measure of correlation between a pre-defined seed (i.e., voxel or a brain region) and all other voxels in the brain. Both studies analyzed the same data to examine functional connectivity within the motor system. The first study used the primary motor cortex (M1) as a seed for the analysis.[77] Their results indicate that, compared to typically-developing children, children with DCD with/without co-occurring ADHD exhibit atypical functional connectivity between M1 and the inferior frontal gyri, supramarginal gyrus, and the basal ganglia. The second study used the left and right primary motor and sensory cortices as seeds, and found altered functional connectivity of primary sensory and motor regions and regions within the cerebellum and the basal ganglia, within and between the hemispheres.[107]

However, the method that was used in both these studies, seed-based analysis, has several limitations. First, it requires a priori selection of a seed or seeds to be used as a regressor for the analysis. This limits the potential results to the pre-defined network or networks, and disregards all other information available in the data. Seed selection also imposes a risk for inherent noise within the seeds that may lead to bias in network identification and interpretation.[157] Differences in seed selection across participants or studies can lead to high variability in the recognized networks, or to presentation of smaller sub-systems instead of a distinct
network.[112,157] In addition, seed-based analysis is very sensitive to noise in the rsMRI data, such as due to head motions during scans, which can lead to false-positive results and to overestimation of group differences.[157,162,163] Application of whole-brain independent component analysis (ICA) can overcome these limitations.

1.3.3 Independent Component Analysis

ICA is a data-driven approach that uses blind source separation to decompose the data into different components, thus allowing the separation of noise (due to head motion, respiratory, scanner artifacts, etc.) from neural signal (functional networks).[164] This approach is less prone to artifacts due to noise compared to other methods, such as seed-based analysis.[166] Since the pediatric population is prone to motion during scans, and even more so in case of children with neurodevelopmental disorders,[163,165,166] this is an important consideration for rsMRI analysis. Moreover, ICA does not require a priori assumption about the findings. Since the evidence regarding neural correlates of DCD is limited, and even more so regarding functional connectivity in this population, such an exploratory method is favorable. The use of ICA allows investigation of whole-brain functional connectivity, while reducing the risk for bias results due to artifacts.
1.4 Purpose of Study

In order to overcome the limitations of previous research and bridge the gap in knowledge regarding functional connectivity in children with DCD, the purpose of our study was to determine if whole-brain functional connectivity is altered in DCD. Thus, our specific aims are:

1. To assess the differences in functional connectivity during rest between children with DCD (with or without co-occurring ADHD) and typically-developing children.

2. To assess if continuous behavioural measurements of motor function and ADHD symptoms correlate with functional connectivity in the whole cohort, regardless of group assignment.
Chapter 2: Methods

2.1 Study Design

A cross-sectional study was conducted to evaluate differences in functional connectivity between children with DCD and typically-developing children. This study is part of a larger randomized controlled trial in which an intervention effect will be investigated (ClinicalTrials.gov ID: NCT02597751). Therefore, a sample size calculation was conducted for the entire study, which yielded a sample of 30 participants per group to allow power of 80% to detect clinically significant group differences in the main outcome measures of the larger study. Such a sample size is considered large for neuroimaging studies and should allow for sufficient reliability of the neuroimaging data analysis.[167-169]

2.2 Participants

Using a convenience sample, we recruited children 8- to 12-years old with DCD+/-ADHD from Dr. Zwicker’s research-integrated DCD Clinic at Sunny Hill, BC Children’s Hospital (BCCH) ADHD Clinic, and from the community in the Greater Vancouver area. Typically-developing children were recruited through advertisements posted at the bulletin boards at BCCH, UBC, Vancouver schools, and the community.

2.2.1 Inclusion Criteria

Children were diagnosed with DCD according to the DSM-5 criteria [1] as follows: (1) score at or below the 16th percentile on the Movement Assessment Battery for Children - 2nd edition (MABC-2);[170] (2) score in the suspected or indicative range on the DCD Questionnaire
(DCDQ);[171] (3) parent-reported motor difficulties from a young age; and (4) no other medical condition that could explain motor difficulties as per parent-report, clinical reports, and/or medical exam. The control group included 8- to 12-year-old typically-developing children with no history of motor difficulties and a score $\geq 25^{\text{th}}$ percentile on the MABC-2.

2.2.2 Exclusion Criteria

Children were excluded from the study if they were: (1) born pre-term ($<38$ weeks gestational age); and (2) diagnosed with ASD or intellectual disability. Children assigned to the typically-developing group were excluded if they were diagnosed with ADHD.

2.3 Procedure

After screening and recruitment, all parents or legal guardians provided written consent and children assented to participate in the study. In the beginning of the session, children participated in an MRI safety screening and were informed about the MRI procedure. Prior to MRI scanning, children participated in an MRI simulator session, to familiarize themselves with the sights and sounds of the MRI environment and to alleviate their anxiety, after which children went through an MRI scanning session. Total scanning time was approximately 60 minutes.

2.4 Clinical Measurement

2.4.1 Movement Assessment Battery for Children - 2nd Edition (MABC-2)

The MABC-2 [170] performance test is designed to assess severity and extent of motor impairments in children 3- to 16-years old. This is one of the most common assessment tools for motor impairments in children, in both research and clinical settings.[20,21,172] The MABC-2
performance test assesses the child's performance in a series of gross and fine motor tasks, which are scored and rated in three areas of motor performance: (1) manual dexterity, (2) aiming and catching, and (3) balance,[170,173] and takes approximately 30 minutes for administration. The raw scores are converted to age-specific normative percentile scores for each subscale and for total performance. The MABC-2 has good internal consistency (α= 0.9) and excellent test-retest reliability (ICC=0.97).[174] The MABC-2 was also found to have good factorial and construct validity [172,173,175] and is widely acceptable as a valid and reliable measure to assess motor impairments in children.[172-175]

In this study, a cut-off score at or below the 16th percentile on the MABC-2 was used to determine if children met criterion A of the DSM-5 diagnostic criteria, as suggested by International Clinical Practice Recommendations for DCD.[21] Children who scored at or above the 25th percentile were classified as the control group of typically-developing children. The MABC was administered prior to group assignment by an occupational therapist or by trained graduate students.

2.4.2 Developmental Coordination Disorder Questionnaire (DCDQ)

The DCDQ [171,176] is a parent questionnaire designed to be used as a screening tool for identification of motor impairments in children 5- to 15-years old. Parents are asked to compare their child's performance in various every-day tasks to the performance of their typically-developing peers. The DCDQ encompasses 15 activities which are rated on a 5-point scale, and are grouped into three different factors: (1) control during movement; (2) fine motor/handwriting; and (3) general coordination.[30] The DCDQ takes parents 5 to 10 minutes to complete. The scores are summed to a total score between 15 and 75, with higher scores
indicating better motor coordination. In this study, we used the age specific cut-off scores as specified in the manual. The DCDQ has high internal consistency (α= 0.94), as well as adequate sensitivity (85%), and good validity and reliability.[171,176,177] The DCDQ is widely used worldwide,[178-182] and is the recommended screening tool for DCD according to the international guidelines for identification of children with DCD.[20,21]

2.4.3 Conners 3 ADHD Index (Conners 3 AI)

The Conners 3 AI parent form [183] was used to assess for ADHD symptoms. This short questionnaire can distinguish between children with and without ADHD.[183,184] A score above 70 is considered clinically significant. The Conners 3 norms are based on a large normative, North-American sample.[184] It is one of the most commonly used screening tool to assess ADHD worldwide, both in research and in clinical settings.[185-188] The Conners 3 AI has high internal consistency (mean α = 0.90), high predictive value, and mean test-retest reliability of 0.83.[183,184] Since children with DCD are more likely to have ADHD compared to typically-developing children,[21,37] the Conners score was used as a measure of attentional difficulties.

2.4.4 Socio-demographic Questionnaire

A socio-demographic questionnaire was used to collect information regarding participants’ demographics, such as age, sex, and additional diagnoses.
2.5 MRI Data Acquisition

All brain imaging was performed on a 3-Tesla General-Electric Discovery MR 750 scanner. An echo-planar imaging was conducted to acquire resting state functional MR data (TE: 30ms, TR: 3000ms, slice thickness: 3mm, FOV: 288, matrix: 128x128). Resting-state functional data was acquired for six minutes while participants rested in the scanner. A high-resolution 3D T1 anatomical image was collected for co-registration and anatomic localization (3D SPGR, TE: 3.2ms, TR: 8.1ms, slice thickness: 1mm, FOV: 256 mm, matrix: 256x256, scan time: 5 minutes). Anatomical and functional MRI imaging were acquired and reconstructed on the scanner console, and then transferred to an independent workstation for preprocessing and data analysis.

2.6 Preprocessing and Denoising

Data were converted from DICOM to Nifti format using the dcm2nii tool from MRIcron (https://www.nitrc.org/projects/mricron).[189] Structural images were visually inspected for motion artifacts, and low-quality scans were excluded from the data (n=12). Brain extraction was done using FreeSurfer (v5.3.0).[190] Initial preprocessing of functional data was done using FSL (FMRIB Software Library, 5.0.10, Oxford, UK).[191-193] Our pre-processing pipeline included the following steps: (1) motion correction using MCFLIRT [194] with six parameters (three translations: left-right; up-down; front-back; and three rotations: pitch; yaw; roll); (2) slice timing correction (TR=3s; interleaved); and (3) high-pass filtering (cut-off frequency of 0.01 Hz) using FEAT [195] to remove noise due to scanner drifts at the voxel level without removal of neural signal.[196]
Head motion during scans is known to be a serious confounder that can easily influence functional connectivity estimation. Although application of denoising methods, as described below, can eliminate some of the effect that head motion has on the data, group differences in head-motion levels can systematically bias the results.[197] This is a particular problem when investigating pediatric or clinical populations, where motion is often correlated with the independent variable (such as diagnosis, symptoms severity, or age).[162,198] To ensure minimal effect of motion on estimation of functional connectivity, we excluded participants from further analysis if they had high levels of head motion during rsMRI scan, exceeding mean framewise displacement (FD) of 0.5 (n=13).[199]

Evidence from recent publications indicate the high importance of implementing participant-level denoising methods to alleviate the effect of motion on functional connectivity and to increase reliability of rsMRI.[162,197,198] However, many methods used for denoising have high costs in terms of statistical power. For example, censoring or scrubbing methods can be effective in removing motion effect, but using these methods will lead to significant reduction in temporal degrees of freedom (tDOF) [198] and reduced statistical power. ICA-based denoising offers a good trade-off between effective removal of motion effect and other noise from the data, and moderate tDOF loss.[197,198,200] In addition, ICA denoising is one of the most reliable methods for denoising rsMRI data and for identification of group differences in functional connectivity.[197] Since pediatric and clinical population tend to have higher levels of motion,[162] we included additional steps in the denoising process.

First, we performed single-subject ICA analysis using MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components).[191,201] This method allows
decomposition of BOLD signal changes and separation of signal that is assumed to be the result of a neural activity from noise (e.g., due to head motion, physiological noise, scanner artifacts). We used FIX (FMRIB's ICA-based Xnoiseifier) to automate the components classification process. FIX uses machine-learning to identify over 40 characteristics of components (such as spatial distribution, cluster size, power spectra, and time series) and to classify the different components as either noise or signal.

Components of a selective sample (n=20), including participants from both groups, with different levels of head motion, were hand-classified according to the guidelines suggested by Griffanti. Each component was visually inspected for its spatial characteristics (such as location, size, and number of clusters), power spectra, and time-series. Two independent assessors (S.R. and S.I.) classified and labelled each component as either noise, signal, or unknown. Level of agreement was high (85.2%), and consensus was reached regarding all other components. The labels were then fed into FIX to allow the automated classification process. Then, FIX was used to classify components of all other participants, using soft clean-up, which further separates the ‘noise’ components to noise-related characteristics that are removed from the data, and signal-related characteristics that are retained in the data.

Following single-subject ICA denoising, functional and structural scans were further analyzed using CONN functional connectivity toolbox v18a and MATLAB (R2018a). All functional scans were registered to 152MNI standard space and segmented using an indirect two-step process. The structural scans were registered and segmented and the same transformation was applied to the functional images, followed by spatial smoothing using 6mm FWHM (full-
width-half-maximum) to improve signal-to-noise ratio (SNR). To further control for motion effect on functional connectivity, we included global signal regression, as recommended by Parkes [197] for ICA denoising, followed by regression of cerebral spinal fluid mean signal and three WM components using the CompCor method.[208] CompCor is a component-based noise correction method that further removes motion effect by regressing out signal from WM and cerebral spinal fluid from the data set. QC-FC (quality control / functional connectivity) plots graphically present the level of association between functional connectivity and average participant’s motion, and the physical distance between these voxels; it can be used to assess variability in the shape of the functional connectivity distributions between participants.[199] We used QC-FC and estimated the residual relationship between motion and connectivity following denoising, as it quantifies the effect of motion of functional connectivity and evaluates the denoising process prior to group analysis (Figure 1).[162,199]

![QC-FC plot before and after denoising](image)

**Figure 1. QC-FC plot before and after denoising**
2.7 Identification of Resting-state Networks

We performed group-level ICA using MELODIC [191,201,209] to identify group-level resting-state networks, with dimension reduction to 30 components. Next, we performed a dual regression to obtain individual networks that corresponded to each of the group networks. At the first phase, each spatial component was regressed out of each individual functional scan and a representative time-series for each component and each participant was determined. In the second phase, these time-series were used as regressors in a second regression to obtain an individual-level spatial map for each group network, which we used for the group comparison. We used FSLutils tool fslcc, a cross-correlation to statistically compare the group-level networks with a pediatric resting-state networks template.[210] Only networks with significant spatial correlation ($r > 0.35$) were carried over to the group comparison to allow later interpretation, as suggested by Reineberg et al.[211] We excluded networks that were classified as visual networks (i.e., visual, anterior visual, and lateral-visual networks) from the group comparison as we were unable to control for participants’ visual stimulus during scans (whether they had their eyes open or closed during scans). Overall, 19 networks were carried over for group comparison (Figure 2).
Figure 2. Identified resting state-networks
Coordinates are in MNI space; Threshold Z > 5; Network classification based on spatial correlation with pediatric template [210]
2.8 Statistical Analysis

2.8.1 Descriptive Data Analysis

We used RStudio (Version 1.1.463) for analysis of behavioural data. To compare the distribution of sex between groups, we used the Chi-square test. To compare group differences in age, ADHD symptoms as measured by Conners 3AI, and motion parameters, we used the student’s t-test. To compare MABC-2, we used Welch’s t, since Levene’s test indicated violation of the assumptions of equal variance.

2.8.2 Resting-state Data Analysis

We used PALM (Permutation Analysis of Linear Models) for statistical analysis of rsMRI data.[212-214] PALM allows statistical inference for neuroimaging data using permutation methods that do not require assumptions regarding data distribution. We used Threshold Free Cluster Enhancement (TFCE), a voxel-wise statistical method in which each voxel’s value represents the cluster-like spatial support, to integrate spatial neighbourhood information. TFCE enhances sensitivity and detectability of neural signal, without enforcing assumptions regarding cluster size, thus improving the results’ stability compared to cluster thresholding.[212]

To address Aim 1 and assess group differences in functional connectivity, we performed t-tests, across all 19 functional networks, while controlling for the effect of age and sex. To address Aim 2 and assess the correlation between behavioural measurements of motor function (as measured by the MABC-2) and ADHD symptoms (as measured by the Conners 3 AI), we used Pearson’s r across 19 functional networks, while controlling for the effect of age and sex using the general linear model.
Chapter 3: Results

3.1 Cohort Characteristics

This study is part of a larger, on-going study. The overall cohort included 105 children recruited between September 2014 and January 2019, from which 58 participants met the criteria and were included in this study. Figure 3 describes participant enrolment and exclusion process. Table 1 presents demographics and behavioural characteristics of the sample. The DCD group included 35 children, and the TD group included 23 children. Co-occurring ADHD was diagnosed in 17 participants in the DCD group (48%), which is similar to the co-occurrence rate reported in the literature.[46,54,62] There were no group differences in age or sex between the groups. A higher proportion of males in the sample was expected, as DCD is more common in males compared to females.[1]

<table>
<thead>
<tr>
<th></th>
<th>DCD (N=35)</th>
<th>TD (N=23)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%) or Mean (SD)</td>
<td>N (%) or Mean (SD)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>27 (77)</td>
<td>15 (65)</td>
<td>0.32</td>
</tr>
<tr>
<td>Age (years)</td>
<td>9.77 (1.6)</td>
<td>9.91 (1.4)</td>
<td>0.73</td>
</tr>
<tr>
<td>MABC-2 (percentile)</td>
<td>3.78 (4.5)</td>
<td>64.8 (22.2)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Conner’s 3 AI (t-scores)</td>
<td>81.9 (13.0)</td>
<td>54.3 (12.3)</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

AI, ADHD (Attention Deficit Hyperactivity Disorder) Index; DCD, Developmental Coordination Disorder; MABC-2, Movement Assessment Battery for Children – 2nd ed; TD, typically-developing children.
Figure 3. Participant enrolment and exclusion chart
DCD, Developmental Coordination Disorder; FD, Framewise displacement; MABC-2, Movement Assessment Battery for Children – 2nd ed; TD, typically-developing children.
3.2 Head Motion Parameters

Head movement during scans can cause bias in estimation of functional connectivity, and therefore it is essential to ensure minimal differences in head motion during scans.[197] There are several parameters used to measure head motion during scans, each of which measures different aspects of motion including framewise displacement, root mean square, absolute displacement, and relative displacement. Framewise displacement (FD) measures movement of the head between two consecutive volumes, and is calculated from the sum of the absolute values of realignment estimation (rotation and translation) in millimeters at each time point.[200,215] RMS (root mean square) measures the intensity differences of each volume and the reference volume, and is not based on motion correction estimation.[198] Since motion correction estimation can be inaccurate when scan include high levels of motion, it is important to use both methods.[216] Absolute displacement measures the absolute change in head position from origin position to each time point, and relative displacement measures the average voxel-wise displacement between two consecutive time points as measured relatively to the center of gravity of the brain.[196,216] A summary comparison of group level motion parameters is presented in Table 2. There were no significant group differences in head motion between the groups in any of the motion parameters.
Table 2. Motion parameters

<table>
<thead>
<tr>
<th></th>
<th>DCD Mean (SD)</th>
<th>TD Mean (SD)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Framewise displacement</td>
<td>0.20 (0.10)</td>
<td>0.18 (0.13)</td>
<td>0.56</td>
<td>0.57</td>
</tr>
<tr>
<td>Root mean square</td>
<td>0.34 (0.25)</td>
<td>0.30 (0.27)</td>
<td>0.62</td>
<td>0.53</td>
</tr>
<tr>
<td>Relative displacement</td>
<td>0.11 (0.06)</td>
<td>0.10 (0.08)</td>
<td>0.31</td>
<td>0.75</td>
</tr>
<tr>
<td>Absolute displacement</td>
<td>0.46 (0.37)</td>
<td>0.59 (0.73)</td>
<td>0.82</td>
<td>0.42</td>
</tr>
</tbody>
</table>

3.3 Research Aim 1 - Group Differences in Functional Connectivity

Significant group differences in functional connectivity between children with DCD and typically developing children were found in the sensorimotor network (Table 3; Figure 4). The DCD group showed significantly less functional connectivity between the sensorimotor network and a cluster located at the PCC and precuneus bilaterally. A second cluster was found in the posterior division of the left middle temporal gyrus.

Following correction of p-value to adjust to the multiple comparisons across all functional networks and contrasts, the difference between the two groups’ functional connectivity in the sensorimotor network remained significant only in the right PCC (p=0.006). There were no significant group differences in other functional networks.

While initially we planned to compare functional connectivity between three groups (DCD, DCD+ADHD, TD), we had limited power due to a smaller sample size than anticipated. Therefore, we decided to combine both DCD and DCD+ADHD groups for the analysis. Due to high correlation between motor function as measured by MABC-2 scores and ADHD symptoms,
as measured by Conners 3 AI scores (Spearman’s r=-0.62, p<0.001), we could not control for this potential confounder in our analysis. However, we did not find significant correlation between ADHD symptoms as measured by Conners 3 AI and functional connectivity in the sensorimotor network.

Table 3. Group differences in functional connectivity

<table>
<thead>
<tr>
<th>Network</th>
<th>Region</th>
<th>MNI-space</th>
<th>t</th>
<th>Cluster p</th>
<th>Cluster size</th>
<th>Cohen’s d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensorimotor</td>
<td>PCC</td>
<td>2 -26 57</td>
<td>3.62</td>
<td>&lt;0.0001</td>
<td>317</td>
<td>1.24</td>
</tr>
<tr>
<td>Sensorimotor</td>
<td>L middle</td>
<td>49 -40 1</td>
<td>4.16</td>
<td>&lt;0.0001</td>
<td>32</td>
<td>1.11</td>
</tr>
</tbody>
</table>

Effects are shown at a threshold of p<0.05 (FWE corrected, with TFCE), before correction for multiple comparisons across networks, and a minimum cluster size of 5 voxels. Effects in bold survived correction for multiple comparisons.

Number of voxels (voxel size = 2 mm).
Figure 4. Group differences in functional connectivity
A. The posterior cingulate cortex and precuneus (in blue) show significantly less functional connectivity with the sensorimotor network (in red) in DCD compared to TD (<0.0001).
B. Posterior middle temporal gyrus (in blue) show significantly less functional connectivity with the sensorimotor network (in red) in DCD compared to TD (p<0.0001).
3.4 Research Aim 2 - Correlation with Behavioural Parameters

3.4.1 Motor Function and Functional Connectivity

Motor function, as measured by the MABC-2, was positively correlated with functional connectivity between the sensorimotor network and bilateral PCC, after controlling for the effect of age and sex (Figure 5). The functional connectivity between the sensorimotor network and the right precuneus was also positively correlated with motor function (Table 4).

Table 4. Correlation between functional connectivity and motor functiona

<table>
<thead>
<tr>
<th>Network</th>
<th>Region</th>
<th>MNI-space</th>
<th>r</th>
<th>Cluster p</th>
<th>Cluster size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensorimotor</td>
<td>R + L PCC</td>
<td>x -34 y 44</td>
<td>0.47</td>
<td>&lt;0.0001</td>
<td>25</td>
</tr>
<tr>
<td>Sensorimotor</td>
<td>R Precuneus</td>
<td>1 -40 z 51</td>
<td>0.46</td>
<td>0.003</td>
<td>7</td>
</tr>
</tbody>
</table>

a Effects are shown at a threshold of p<0.05 (FWE corrected, with TFCE), before correction for multiple comparisons across networks, and a minimum cluster size of 5 voxels.
b Correlation coefficient between functional connectivity and MABC-2 scores.
c Number of voxels (voxel size = 2 mm).
Figure 5. Correlation between functional connectivity and motor function
Functional connectivity between the sensorimotor network (in red) and the PCC (in blue) was significantly correlated with MABC-2 scores (r=0.47; p<0.0001)

3.4.2 ADHD Symptoms and Functional Connectivity

We did not find any correlation between ADHD symptoms as measured by Conners-AI scores and functional connectivity in any of the networks included over the threshold used in this study (p < 0.05, FWE corrected using TFCE, cluster size > 5). However, we identified a trend, correlating Conners scores and functional connectivity between the inferior frontal network and the left occipital pole, and between the executive control network and the right frontal pole (Table 5). Since these results have not reached significance threshold (due to the small cluster size), they should be interpreted with caution.
### Table 5. Correlation between functional connectivity and ADHD symptoms

<table>
<thead>
<tr>
<th>Network</th>
<th>Region</th>
<th>MNI-space</th>
<th>r</th>
<th>Cluster p</th>
<th>Cluster size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Harvard-Oxford Atlas)</td>
<td>x</td>
<td>y</td>
<td>z</td>
<td></td>
</tr>
<tr>
<td>Inferior frontal</td>
<td>L occipital pole</td>
<td>-26</td>
<td>-92</td>
<td>6</td>
<td>-0.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Executive control</td>
<td>R frontal pole</td>
<td>24</td>
<td>43</td>
<td>42</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

*Effects are shown at a threshold of p<0.05 (FWE corrected, with TFCE), before correction for multiple comparisons across networks.*

*b Correlation coefficient between functional connectivity and Conners 3 AI scores.*

*c Number of voxels (voxel size = 2 mm).*
Chapter 4: Discussion

4.1 Functional Connectivity in DCD

The aim of this study was to investigate functional connectivity among children with DCD, with and without co-occurring ADHD. This is the first study to investigate whole-brain functional connectivity in children with DCD. Our results indicate that functional connectivity between the sensorimotor network and bilateral PCC and the left pMTG, was disrupted in children with DCD compared to children with typical motor development. Furthermore, the connectivity between the sensorimotor network and the precuneus and PCC was correlated with motor function regardless of diagnosis; better motor skills were associated with stronger functional connectivity between the sensorimotor network and the right precuneus as well as between the sensorimotor network and the PCC.

4.2 The Sensorimotor Network

The sensorimotor network consists of the primary motor cortex (M1), supplementary motor area (SMA), premotor cortex, primary somatosensory cortex (S1), and the somatosensory association cortex, all of which are regions associated with motor and sensory function (Figure 6). The primary motor cortex, located in the precentral gyrus (Brodmann Area [BA] 4), contains a somatotopic representation of the body, and it controls voluntary movement. It receives input from several regions, including the premotor cortex and the posterior parietal cortex, and has a reciprocal connection with the somatosensory cortex. [217] Output of M1 is also projected to the basal ganglia, the cerebellum, brainstem and spinal cord, all of which have a role in motor control.
Figure 6. The sensorimotor cortex

(adapted from: http://thebrain.mcgill.ca/flash/i/i_06/i_06_cr/i_06_cr_mou/i_06_cr_mou.html)

The SMA is located in the dorsomedial frontal cortex, in the medial portion of BA 6, and is involved in planning, initiation of movement, sequential movement, and motor imagery.[218-220] It receives input from the parietal lobe, and projects output to M1, the spinal cord, and the reticular formation. The premotor area, located in the lateral portion of BA 6, receives information from sensory regions in the parietal cortex, and similarly to the SMA, projects its output to M1, the spinal cord, and the reticular formation. It has a role in preparation for motor action, such as re-orientation of the limbs or body, and in postural adjustments and control.[219,220] Both the premotor cortex and the SMA have somatotopic organization, although not as detailed as in M1.[220]
The primary somatosensory cortex includes BA 1, BA 2 and BA 3, and is located at the postcentral gyrus. These three subdivisions are structurally connected to each other, BA 2 and 3 are also connected to M1, and BA 2 is connected to the premotor cortex as well.[85] Input originates from different receptors in the skin, joints, and muscles (including temperature, pain, vibration pressure and proprioceptive). The main function of the primary somatosensory cortex is to detect and characterize sensory information from the body, locate it (via somatotopic representation of the body), and transmit the information onward, including to the somatosensory association regions and motor regions.

The somatosensory association cortex, located in the posterior parietal cortex, collects and synthesizes sensory information from several sensory modalities, including vestibular and somatosensory input (BA 5) and visual and spatial information (BA 7) to allow movement in space or interaction with objects. Input origin includes the primary somatosensory cortex, M1, and the thalamus, as well as interconnections within the somatosensory association cortex. Output is projected to many regions, including premotor and motor cortices, but also to the insular cortex, cingulate, basal ganglia, amygdala, and hippocampus.[85,220]

Motor learning and motor performance depend on coordination and integration of information from all regions of the sensorimotor network, alongside the cerebellum, basal ganglia, thalamus, and brainstem.

4.3 The Posterior Cingulate Cortex and the Precuneus

Our results indicate disruption in the functional connectivity of the sensorimotor network in DCD, including weaker connectivity with the PCC and precuneus. The PCC (BA 23 and 31) and
the precuneus (BA 7 and 31) are located on the posterior medial surface of the hemispheres, above the corpus callosum. While the PCC is considered to be part of the limbic lobe, the precuneus is part of the parietal lobe. Both the PCC and the precuneus present complex and rich structural and functional connectivity.[221,222]

The energy consumption of the PCC and the precuneus is one of the highest in the brain, and its metabolic rate is estimated to be about 40% higher compared to other regions in the brain.[128,223,224] Similar to other components of the DMN, the PCC and precuneus present a decrease in activation during task performance when attention is directed externally, but it is estimated to only cause a 6 percent reduction in blood flow, and therefore the overall metabolic rate is still higher than most brain regions.[223]

4.3.1 Structural and Functional Connectivity of the PCC and Precuneus

The PCC is structurally connected to the precuneus, ACC, medial temporal lobes, ventromedial prefrontal cortex, superior frontal gyrus, to the contralateral hemisphere through the corpus callosum as well as local parcellations.[128,225] Functionally, it is connected to many regions across the brain, and it is a component of several functional networks, including the DMN, dorsal attention network, right and left fronto-parietal networks, the salience network, and the sensorimotor network.[226,227]

The precuneus presents complex connections both structurally and functionally. It is a component of the DMN, ventral and dorsal attention networks, and the left and right frontoparietal networks.[228,229] The precuneus is functionally connected to many structures across the brain, including the PCC and ACC, superior parietal cortex, inferior and superior
frontal gyri, SMA, M1, cerebellum, amygdala, and hippocampus.[222,228-230] Structurally, the precuneus is connected to the PCC and retrosplenial cortex reciprocally, and shows structural connections with other parietal regions (including the caudal parietal operculum, superior and inferior parietal lobules), with the ACC, superior frontal gyrus, medial prefrontal cortex, thalamus, premotor area, and the SMA.[222,231]

4.3.2 Functional Roles of the PCC

Task-based fMRI studies show PCC involvement in many different functions, including higher levels of visual processing,[232,233] visuospatial navigation and body orientation,[234] decision-making and outcomes evaluation,[235-237] working memory involving processing images of body or places,[225] memory retrieval and emotion processing,[225,234] and in motor performance, including some somatotopic organization within the cingulate cortex.[232,238]

While its exact role remains unclear, it is increasingly recognized that the PCC is not a single-function or a monolithic structure.[225,234]

The characteristics of the PCC - including high and complex structural connectivity,[221,225] extensive functional connectivity with multiple functional networks,[226] very high metabolic rate,[223] and its involvement in variety of tasks, have supported the assumption that the PCC has a key role in cognitive function.[128,221,225-227,235,239,240] Due to these characteristics, it has been suggested that the PCC acts as a hub for information processing, integrating information flow across the brain, and coordinating activation of different functional networks.[128,221,226,227,240,241] Leech and Sharp [128] proposed a multi-dimensional model to capture the varied functions of the PCC, in which, the PCC function concerns the level
of arousal and awareness, balance between external and internal foci of attention, and the breadth of attention (narrow vs. broad).

4.3.3 Functional Roles of the precuneus

As implicated by its rich and complex structural and functional connectivity, the precuneus cannot be considered a single-function region. It is active in self-related processes such as during autobiographical [242,243] and episodic memory,[244-247] and during visuospatial processing,[248,249] including visual and spatial imagery,[222,250-252] navigation,[248] visuospatial search,[248,2489,253] and motor imagery.[254] Due to its involvement in various tasks and extensive structural and functional connectivity, it has been suggested that the precuneus has a key role as an associative memory region.[249]

Several studies suggest that sub-division of the precuneus will allow better understanding of its complex functions and connections.[229-231] In a recent study, Zhiguo et al.[229] suggested such parcellation. They divided the precuneus into six symmetrical subregions that are associated with visuospatial, sensorimotor, and attentional functions, and interact with the DMN, the frontoparietal, and the dorsal and ventral attention networks.[229] The subregion corresponding to the group differences found in this study (BA 31) is suggested to be an associative region, and it shows the strongest connectivity to the DMN within the precuneus subregions.[229] Other studies suggest that this subregion is a transitional zone between medial parietal areas and the PCC, and its main roles involve memory retrieval and self-related processing.[230,255]
4.3.4 PCC and Precuneus in DCD

The results of our study indicate there is a disruption in functional connectivity between the PCC and precuneus and the sensorimotor network in DCD. These results are in line with several neuroimaging studies that showed atypical activation of the PCC and precuneus in DCD.[76,80,83,126,161] In a task-based fMRI study by Zwicker et al.,[81,162] children with DCD showed significantly more activation of the PCC during fine motor task performance compared to the control group, while the control group showed increased activation in the precuneus during task performance. Following practice, the control group showed a significant reduction in PCC activation, but no such change was observed in the DCD group. In addition, activation of the PCC was negatively correlated with task performance in the control group, so better motor performance was associated with less activation of the PCC. If we consider the PCC role as a mediator between functional networks,[128] an increased activation could indicate an attempt to recruit more brain regions or attentional resources to complete the motor task. The reduced activation in PCC following practice in the control group, and the negative correlation between PCC activation during task and the motor performance seen in this study,[161] supports this assumption.

In two studies conducted by Reynolds and colleagues,[76,83] decreased activation of the PCC and precuneus was found in the DCD group compared to control during a finger sequencing task, and a negative correlation between PCC and precuneus activation and performance of a praxis imitation task.[76] A potential explanation to these contradicting results might be different involvement of the PCC in different levels of task complexity: while Zwicker’s study evaluated novel and complex fine motor task, Reynolds’ studies involved finger sequencing tasks, which
could require different cognitive resources. In a structural study, Reynolds et al.[74] found a positive correlation between PCC and precuneus grey-matter volume and motor performance in the DCD and the control groups.[74] Lastly, another study reported differences in PCC activation in children with DCD compared to children with developmental dyslexia. However, the lack of a typically-developing control group in this study prevents a comparison to typical activation patterns.[103]

The weak functional connectivity between the PCC, precuneus, and the sensorimotor network could potentially be a key to understanding the neural nature of DCD. Many studies, as detailed above, indicate precuneus involvement in different aspects of visuospatial processing. Visuospatial abilities were reported to be implicated in DCD,[88,89,256] and specifically visuospatial memory.[257] While this is a potential explanation to the precuneus involvement in DCD, other studies suggest that the precuneus subregion located within BA 31 is associated mainly with memory retrieval and self-relating processing, and propose it is a transitional zone between medial parietal regions and the PCC.[230,255] Most neuroimaging studies that found precuneus involvement in DCD, report similar involvement of the PCC.[74,765,83,103] If the activity of both structures is inter-dependent, as the strong structural and functional connections indicate, it could explain these results.

Considering the multi-dimensional role of the PCC according to the Arousal, Balance and Breadth of Attention model,[128] we can assume that the disrupted connectivity between the PCC and the sensorimotor network indicates an inability to allocate the appropriate attentional resources for sensorimotor tasks, that lead to a deficit in motor learning and motor performance. Such an interpretation suits the high level of inattention symptoms in DCD, both with and
without co-occurring ADHD, and their association with level of motor impairment.[130,258,259] That is, in DCD, the deficit in attention is related to sensorimotor function at the neural level. If we consider the role of PCC as a functional hub for information processing, that links and coordinates the activation of functional networks across the brain, altered functional connectivity between the PCC and the sensorimotor network might indicate an inability to integrate information from other networks to sensorimotor processing at the neural level.

4.4 Left Posterior Middle Temporal Gyrus

The role of the posterior middle temporal gyrus (pMTG, BA 21) is not well understood. The pMTG is active in language processing and semantic retrieval, especially in presence of action-or tool-related concepts, when interpretation is embodied in sensorimotor experiences.[260-265] It is also active in action observation,[266] in naming tasks that involve tools and actions,[267,268] in knowledge and memories retrieval about actions and tools,[265,269] or following acquisition of knowledge regarding use of a new tool.[270,271] The pMTG was also found to be involved in interpretation of gestures,[272,273] and meaningful actions.[266,273-275] Focal damage to the pMTG could result in deficit in action recognition, gesture imitation, or even apraxia.[273-275] It was suggested that the left pMTG acts as a semantic hub, an interface between the lingual and semantic representations of tools and actions, and the sensorimotor representation to which these refer.[276-278]

In a recent study, van Kemenade and colleagues [279] suggested that the pMTG role is detecting mismatches between predicted and actual sensory feedback, or in presence of conflicted intersensory input.[279] Indeed, several other studies also found MTG activity following sensory input that was different than predicted.[280-286] However, this role alone is not enough to
explain its involvement in the semantic and lingual system. Combining the different evidence regarding the roles of pMTG in semantic action recognition,[273] action representation,[264,287] action monitoring during performance,[279] and comparison of sensory input to sensory prediction,[280-286] makes it reasonable to conclude that the pMTG has a key role in organization and interpretation of action-related knowledge, linking together semantic and sensorimotor knowledge about meaningful actions.[288,289]

4.4.1 Temporal Involvement and Praxis in DCD

We found altered functional connectivity between pMTG and the sensorimotor network in DCD compared to typically-developing children. These results are in agreement with several other studies that have identified abnormalities in temporal regions.[76-80,103,104,290] In a task-based fMRI study by Reynolds et al.,[76] children participated in finger sequencing tasks. During the action observation phase, children with DCD had significantly less activation of left pMTG compared to the control group. These results are in line with previous work that investigated imitation, gestures, and tool use in DCD.[291-298]

Many studies found that children with DCD demonstrate lower performance on gesture production tasks, and show overall slower, less accurate, and more variable patterns in this type of task.[291-298] The few studies that evaluated action- or tool-related knowledge in DCD found no significant group differences,[295-298] and concluded that DCD does not involve a semantic knowledge problem in relation to action, gestures, or tool use.[298]

Models of apraxia often distinguish between the conceptual knowledge system and the production system.[299-302] The conceptual system holds knowledge that supports the internal
mental representation of an action (such as semantic knowledge about relevant tools, the actions for which they are used, and the relevant context); in some models, the conceptual system includes the body movements that are associated with the action as well.[301,302] The production system makes the necessary adaptations that allow appropriate execution of an action in a given context and environment.[302-304]

Our results indicate that disrupted connectivity between regions associated with action-related knowledge and the sensorimotor network is present in DCD. Such a disruption could explain the praxis problem associated with DCD - while the conceptual action-related knowledge exists, and even while the sensorimotor knowledge is intact,[280] the communication of this knowledge to the production system (i.e., the sensorimotor network) is disrupted, potentially preventing efficient use of this knowledge and impairing motor learning. Our results could explain the behavioural evidence indicating that performance of gestures is better with visual instruction (e.g., imitation) compared to verbal instructions in DCD,[291-293,295,296,298] given the pMTG role in lingual and semantic aspects of action knowledge, and the weak connectivity to the sensorimotor system.

4.5 Theoretical Implications

Our results also can be viewed in relation to several theories regarding DCD. The mirror neuron dysfunction theory suggests that the origin for the motor impairment in DCD is in abnormal function of the mirror neuron system.[76, 83, 87, 305] The mirror neuron system refers to clusters of neurons that are active when observing another person in action. The mirror neuron dysfunction theory suggests that impairment in a network of mirror neurons in frontal-parietal regions (which are activated during imitation of motor tasks) is implicated in DCD.[305] The
mirror neuron system has a role in visual-motor learning, and therefore its involvement could explain motor deficit seen in DCD.[76] The main regions associated with the mirror neurons fronto-parietal network include the inferior frontal gyrus, ventral premotor cortex, and the inferior parietal lobe.[76, 305] Regions identified in this study with atypical functional connectivity in DCD include the PCC, precuneus and pMTG, all of which are not considered to be part of the mirror neuron system; therefore, our results do not support this theory. These conclusions are in line with past studies who asked to evaluate the involvement of the mirror neuron system in DCD and found results similar to ours.[76,83]

Another theory suggests that the motor dysfunction in DCD is due to deficit in internal models of movement.[94-100] According to the Internal Modeling Deficit hypothesis, children with DCD have a deficit in the ability to produce internal forward models of movement.[97-99] Internal models of movement are neural representations of motor movement that are used to predict and adjust movement.[92,93] Internal models of movement are often assessed using motor imagery tasks, especially in neuroimaging studies, as it is assumed to capture internal representation of movement without any overt movement.[92,253]

Brain regions that are active during motor imagery tasks include M1, premotor area, SMA, posterior parietal cortex, cingulate cortex, lingual gyrus, and the cerebellum.[92,253] The results of our study indicate weak functional connectivity between sensorimotor network and PCC, precuneus, and the pMTG. Although our results cannot prove or disprove this theory, these weak connections complement the internal model deficit theory, and suggest it is a possible that this mechanism is implicated in DCD. Yet, other evidence indicates that the internal model deficit theory cannot explain the complexity of DCD.[101] Further research is needed to determine the
role of internal models in DCD, and clarify the underlying mechanism of motor learning impairment in this disorder.

4.6 ADHD Symptoms and Functional Connectivity

We did not find a significant correlation between ADHD symptoms, as measured by Conners 3 AI, and functional connectivity in the current study. This could be due to insufficient power, or due to relatively small sample of participants with ADHD and high levels of ADHD symptoms. We identified a trend, linking functional connectivity in two networks, the inferior frontal network and the executive control networks, to ADHD symptoms. While these results did not reach the statistical significance threshold, and should be interpreted cautiously, they are in agreement to previous findings reported in the literature. Inferior-frontal regions, associated with inhibitory control,[306,307] show reduced activation and atypical patterns of connectivity in ADHD.[308-311] Similarly, the executive control networks, which, as implied by its name, are associated with executive functions, have been implicated in ADHD.[312-317] These results reassure other findings of the study – although we were unable to control for ADHD symptoms in the analysis due to the correlation between motor function and ADHD symptoms, the group differences identified correspond to regions correlated with motor function, while ADHD symptoms tend to correlate with functional connectivity in regions associated with executive function.

4.7 Discrepancy with Past Results

The results of our study do not agree with the previous study investigated functional connectivity in DCD.[77,107] There are several potential explanations for this disagreement. First, as
discussed in the literature review, there are significant methodological differences. McLeod and colleagues used seed-based analysis, a method that is highly prone to false-positive results, especially in presence of motion in the rsMRI data.[157,216] Moreover, they did not perform any denoising steps to remove the effect of motion from the rsMRI data, which is recommended to mitigate its effect.[162,197-200] In addition, correction for multiple comparisons was not conducted [77] or data was only corrected at the cluster level and not the voxel level.[107] While this is not uncommon practice in neuroimaging studies, this results in inflation of alpha level, which dramatically increases the risk for false positive results. Since McLeod and colleagues did not include the exact p value of each cluster, or an effect size, it is not possible to further evaluate their results. The authors [107] also used AlphaSim, an analysis package of the AFNI, which has recently been shown to have low validity, inflating the rate for false positive results from the nominal 5% up to 70%.[318] Furthermore, a close examination of their results indicates that some of the clusters reported in McLeod’s articles are located outside the brain or in WM regions. Since this is not physiologically possible, it puts into question the validity of these studies and their results. Other methodological weaknesses include wide age range of participants (8-17 years) and no control for age and sex in their analyses. The relatively small sample size (7 participants with DCD; 18 participants with DCD+ADHD) increases the risk for an unrepresentative sample.

The results of our study show large effect size (Cohen’s d >1 for both clusters) and high statistical significance (p<0.0001) for the group differences in functional connectivity. We have included relatively large sample, used rigorous denoising steps and an analysis method that is less sensitive to motion, which provide support to the accuracy of this analysis.
4.8 Clinical Implications

The results of this study join the growing body of evidence that indicate that neural impairment underpins the motor deficit in DCD. Our results further support the understanding that children with DCD cannot learn and perform motor tasks in the same way as typically developing children. These results may help therapists explain to parents why children with DCD struggle to learn motor skills. Furthermore, the theoretical implications discussed earlier should inform researcher and clinicians, and can guide future research and development of new interventions.

4.9 Limitations and Future Directions

The first limitation of this study is inherent to rsMRI- while it allows us to study neural characteristics across the entire brain, the association of the results to risk factors, clinical symptoms, and function is limited and indirect. In addition, since this is a cross-sectional study, it investigates functional connectivity at one given time-point, and so it does not allow for causal inference. Future studies could try and overcome this limitation by using a longitudinal cohort design, and potentially evaluate neural risk factors even prior to diagnosis.

Second, the results of our study do not agree with previous studies on functional connectivity in DCD. While methodological differences, as discussed above, could be the reason for this discrepancy, more research is needed to replicate our results and determine the neural characteristics of DCD.

The final sample size in our study was smaller than anticipated. The reduced power prevented the planned comparison between children with DCD and children with co-occurring DCD and ADHD. In addition, it is possible that other, more subtle differences in functional connectivity
exist in DCD, but require more statistical power to detect. This smaller sample size is partially due to high rate of participants’ exclusion from the study. While this limitation should be acknowledged, similar exclusion rates are quite common in resting-state MRI studies in pediatric populations, especially those with neurodevelopmental disorders.[163,166] In addition, while longer scans produce more data points and, therefore, considered more reliable, longer scans can also increase the risk for high levels of head motions, especially in pediatric population, which can result in high exclusion rate.[162, 189] Another limitation is due to our inability to control for participants visual stimulus during scans (whether their eyes were open or closed), which prevented analysis of visual networks. Future studies should address this unanswered question.

Due to the high correlation between motor function and ADHD symptoms, we were unable to control for ADHD symptoms in our analysis. The rate of cooccurrence DCD and ADHD in the sample is similar to rates reported in the literature, and therefore is representative of children with DCD. In addition, while the correlation analysis of Conners-3 AI scores with functional connectivity did not yield significant results, the identified trend involving attention-related networks makes sense clinically; however, future studies should examine functional connectivity in DCD with and without co-occurring ADHD, and investigate differences between the two groups, as we initially planned to do. Similarly, while we were able to exclude participants with major neurodevelopmental disorders, such as CP and ASD, future studies should address other common known comorbidities of DCD, such as developmental dyslexia and learning disorders.

4.10 Conclusions

We used rsMRI to study functional connectivity across the brain in children with DCD compared to typically-developing peers. Our results indicate a disruption in functional connectivity in DCD
in two main regions connected to the sensorimotor network: one is located at the PCC, extending to the precuneus, and the other is located in pMTG. These results suggest that the neural impairment seen in DCD is predominantly in the sensorimotor network. Disrupted functional connectivity between the PCC and the sensorimotor network could indicate a deficit in allocation of attentional and neural resources to the sensorimotor system, or impaired coordination in activation between the sensorimotor network and other functional networks. The involvement of the pMTG could indicate a disruption between the semantic system that holds the mental representation of meaningful actions, and the sensorimotor network that produce these actions.

Our study included a well phenotyped sample, extensive denoising steps in our analysis, and large group differences (Cohen’s d > 1) for both clusters. The group differences identified in PCC and precuneus remain significant even following the most stringent correction for multiple testing. These results are robust compared to most neuroimaging studies in the field, and improved our understanding of the neuro-deficit associated with DCD. Future research should build upon our study results and extend our understanding of neural impairment seen in DCD, as well as the effect of treatment on functional connectivity in this population.
References


