METABOLITE CONCENTRATIONS IN THE ANTERIOR CINGULATE CORTEX: A STUDY OF CHILDREN WITH ATTENTION-DEFICIT HYPERACTIVITY DISORDER AND OBSESSIVE-COMPULSIVE DISORDER

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LAY ABSTRACT

The current study investigated concentrations of metabolites in the brains of children with neurodevelopmental disorders. We focused on a structure called the anterior cingulate cortex (ACC), which is largely responsible for attention and cognitive control, processes that are impaired in individuals with neurodevelopmental disorders. Participants with OCD (n = 24) and ADHD (n = 29) completed clinical testing and neuroimaging. We found differences in N-acetyl aspartate, a metabolite related to the number of neuronal connections in a given region, between the groups. Across groups, there were associations between metabolite concentrations and symptom dimensions, including anxiety/depression and affective problems. Overall, this study suggests that an interdisciplinary approach to studying children with neurodevelopmental disorders could reveal information about the underlying deficits common in all neurodevelopmental disorders.

ABSTRACT

The dorsal anterior cingulate cortex (ACC) plays an important role in attention and cognitive control (Bush et al., 2000; Margulies et al., 2007; Shackman et al., 2011). Individuals with neurodevelopmental disorders often struggle with inattention, hyperactivity, and repetitive behaviours, which is indicative of deficits in the ACC (Baribeau et al., 2015; Bush et al., 1999; Fitzgerald et al., 2005). This study investigated metabolite abnormalities in the ACC of two groups of children with neurodevelopmental disorders. Participants were children with obsessive-compulsive disorder (i.e., OCD; n = 24) and children with attention-deficit hyperactivity disorder (ADHD; n = 29). Participants completed cognitive and clinical testing and proton magnetic resonance imaging (^{1}H-MRS) of the ACC. Children with ADHD had higher concentrations of N-acetyl aspartate than those with OCD. In the OCD group, creatine concentrations were correlated with scores on the Children's Yale-Brown Obsessive-Compulsive Scale (CY-BOCS). Across diagnostic groups, concentrations of N-acetyl aspartate predicted scores on the anxious/depressed syndrome scale of the Child Behaviour Checklist (CBCL), and creatine, myo-inositol, and glutamate concentrations predicted scores on the affective problems syndrome scale of the CBCL. This study was the first to compare two groups of children with neurodevelopmental disorders using 1H-MRS, and the first to look the relationship between metabolites and symptom dimensions (i.e., CBCL syndrome scales) rather than the relationship between metabolites and symptom severity alone. Our findings support an interdisciplinary approach to studying neurodevelopmental disorders, and invite future investigations combining 1H-MRS with anatomical and functional imaging techniques.

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AUTHOR'S DECLARATION OF ORIGINALITY

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CHAPTER I

INTRODUCTION

Province of Ontario Neurodevelopmental Disorders Network

Over 300,000 children and youth in Ontario are affected by one or more neurodevelopmental disorders (POND, 2011). The National Institute of Mental Health (NIMH) has defined neurodevelopmental disorders as "mental disorders that have their onset during the developmental period" (Rumsey, 2008). Neurodevelopmental disorders are characterized by deficits in personal, social, academic, or occupational abilities (APA, 2013). A broad list of neurodevelopmental disorders includes attention deficit hyperactivity disorder (ADHD), autism spectrum disorder (ASD), pediatric bipolar disorder, obsessive-compulsive disorder (OCD), childhood-onset schizophrenia, Tourette's syndrome, and those disorders with a known genetic etiology such as Fragile X disorder and 22q deletion (Rumsey, 2008).

Neurodevelopmental disorders interfere with skill acquisition early in life, often triggering a cascade of events with negative consequences for development (Rumsey, 2008). Individuals with neurodevelopmental disorders demonstrate overlapping behavioral manifestations such as inattention, social deficits, hyperactivity, impaired emotion regulation, and repetitive behaviors (Baribeau et al., 2015).

Given the tremendous challenges associated with neurodevelopmental disorders, in 2007, the NIMH recognized the need for interdisciplinary research to help gain a comprehensive understanding of the path extending from etiology - to brain - to behavior in neurodevelopmental disorders. Importantly, they noted that there is a large amount of heterogeneity within individual neurodevelopmental disorders, and concluded that it is

important to develop treatment regimens that target impaired processes (e.g., attentional deficits, repetitive behaviors) that are common across disorders, rather than specific diagnostic categories (NIHM, 2007). An illustration of this approach can be found in early clinical trials of oxytocin, which found improvement in the social deficits of ASD and subsequent reductions in repetitive/compulsive behaviors, which are present in ASD, ADHD, and OCD (Andari et al., 2010; Hollander et al., 2003; NIHM, 2007) In 2010, the NIMH introduced its Research Domain Criteria (RDoC) project, as a framework for new ways of studying mental disorders (Insel et al., 2010). The RDoC framework focuses on incorporating data on pathophysiology from many domains (e.g., genomics, self-report), in order to ultimately provide a better match between research findings and clinical decision-making, and identify new targets for treatment development (Insel et al., 2010). The RDoC framework highlights the difference between neurological disorders with identifiable lesions and mental illnesses; where the latter are disorders of brain circuits (Insel et al., 2010).

Based on the objectives set by the NIMH, the Province of Ontario Neurodevelopmental Disorders (POND) Network was formed. The POND network is an interdisciplinary team of researchers, engineers, clinicians, and community members who have recognized the need for the study of those affected by neurodevelopmental disorders. The sub-studies within POND include clinical trials (i.e., first clinical trial network targeted at childhood neurodevelopmental disorders in Canada), behavioral phenotyping, epigenetics, family studies (high-risk infants), mouse and cell models, and neuroimaging. The current work focuses on the results of the neuroimaging sub-study.

The POND neuroimaging sub-study is ongoing at three imaging sites in Ontario,

Canada: Toronto (i.e., the Hospital for Sick Children), London (i.e., Lawson Health Research Institute), and Hamilton (i.e., St. Joseph's Healthcare Hamilton), with the help of key players at the Holland-Bloorview Rehabilitation Hospital and McMaster University.

My involvement with the POND neuroimaging sub-study was two-fold. First, I was responsible for the MRI scanning of children with ASD and OCD at the Hamilton imaging site. Second, I conducted the analysis of proton magnetic resonance spectroscopy (^{1}H-MRS) data, which was collected at the Toronto imaging site, for children with ADHD and OCD. I was the responsible for the POND imaging study in Hamilton for half of the first fiscal year (i.e., Sept. 2013 – Mar. 2014), the entire second fiscal year (i.e., Mar. 2014 – Mar. 2015), and half of the third fiscal year (i.e., Mar. 2015 – Sept. 2015. Below is a summary of my scanning progress (Table 1).

The POND study's intake procedure will be described in detail in the methods section of this thesis. However, it is important to note that in order to participate in the neuroimaging sub-study, children first must enroll in the POND study, and consent to and complete light and deep phenotyping, which includes clinical and cognitive testing. If and only if a child completed these preliminary steps and consented to learn more about neuroimaging, their contact information was passed to me.

My role in the collection of imaging data at the Hamilton imaging site involved initial telephone and/or email communication with all families interested in neuroimaging, in order to help them understand the imaging sub-study and gauge their child's ability to participate. If they remained interested, we scheduled a visit one (i.e., V1), during which consent was obtained and training in the mock scanner was completed

Table 1

Completed scans at Hamilton imaging site, by fiscal year and population.

Fiscal Year	ASD	OCD
1 (Apr 2013 – Mar 2014)	0*	8
2 (Apr 2014 – Mar 2015)	8	2
3 (Apr 2015 – Mar 2016)	2	7
	10	17

*Note: During the first fiscal year, recruitment for imaging in the ASD population had not yet begun.

with the child. Upon successful completion of V1, a second visit (i.e., V2) was booked for the actual MRI protocol (approximately 2.5 hours). For older children, or those whose parents had little apprehension about their ability to complete the scan protocol, the V1 and V2 were combined into one.

With regard to the OCD population, scanning success was quite high (81% success rate); only 4 of the 21 children who expressed interest and were eligible to partake in imaging were not scanned. One of these children declined, one was afraid in the MRI scanner (but contacted us hoping to try again in a year), one is currently wearing dental braces and will be scanned when they are removed, and the final family was unreachable. Success in the ASD population was lower (40% success rate), with 10 of 25 children being scanned. Fifteen children from the ASD population, who had expressed interest in imaging, were contacted but not scanned. Three families declined participation, one gave a tentative no but said they could be contacted in the future, five either cancelled last minute or did not show, two failed the mock scanner screen (i.e., were deemed unfit to complete the scanning protocol), one currently wears dental braces and will be re-contacted when they are removed, and three were unreachable via telephone or email.

The remainder of this thesis will focus on my second contribution to the POND study, which was the analysis of ^{1}H-MRS data collected at the Hospital for Sick Children. I will begin by introducing ADHD and OCD as neurodevelopmental disorders, discussing the cortical networks that are implicated in each disorder, and introducing the anterior cingulate cortex as the region of interest (ROI) of our ^{1}H-MRS study. Next, I will

introduce ^{1}H-MRS as an imaging technique and review the literature surrounding OCD and ADHD populations. Finally, I will present the current study and hypotheses.

CHAPTER II

REVIEW OF LITERATURE

Introduction to Attention Deficit/Hyperactivity Disorder

Attention deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder involving a persistent pattern of inattention, impulsivity, and hyperactivity in a number of settings (Polanczyk, Willcutt, Salum, Kieling, & Rohde, 2014). Criteria for inattention include: a lack of attention to detail, seemingly not listening when spoken to, struggling to sustain attention in tasks or daily activities, poor organizational skills, losing personal belongings, being forgetful in daily life, avoidance of tasks that require sustained mental effort, and being easily distracted by external stimuli (APA, 2013). Hyperactivity and impulsivity include: fidgeting, leaving a situation when it is not appropriate to do so, talking excessively, blurting answers to questions before being prompted to speak, interrupting or intruding on others, difficulty waiting for one's turn, an inability to play quietly, and running or climbing in situations where it's inappropriate (APA, 2013). To have inattention and/or hyperactivity/impulsivity that is diagnostic, a persistent pattern of each, which interferes with daily functioning or development, must be identified in two or more settings (e.g., home, school, etc.; APA, 2013). There are three sub-types of ADHD: ADHD-C (i.e., combined type), where both inattention and hyperactivity/impulsivity are present); ADHD-I, where criteria for inattention, but not hyperactivity/impulsivity, are met; and ADHD-HI, where criteria for hyperactivity/impulsivity, but not inattention, are met (APA, 2013). Symptoms of ADHD present in childhood, with problems being identified during the first few years of elementary school, which then lead to formal diagnosis and medical intervention

(Goldman, Genel, Bezman, & Slanetz, 1998). It is difficult to identify symptoms before the age of 4 years old due to overlap with typical developmentally appropriate behaviors before this age (APA, 2013).

Prevalence estimates of ADHD in school-aged children range from 2-14%, making it the most common childhood neurodevelopmental disorder (Polanczyk et al., 2014; Rowland, Lesesne, & Abramowitz, 2002; Scahill et al., 1999). A more conservative estimate is that, in most cultures, 5% of children and 2.5% of adults have ADHD (APA, 2013).

Due to the cross-cultural variation in the interpretation of children's behaviors, there are certain populations that tend to be diagnosed with ADHD more often than others; specifically Caucasian children are identified more often than African American or Latino children in the United States (APA, 2013; Kessler et al., 2006). Nonetheless, it is estimated that of US children between 8-16 years old, approximately 2.4 million meet criteria for ADHD, although less than half will ever receive a formal diagnosis or regular treatment (Froehlich et al., 2007). Males are diagnosed with ADHD about four times as often as females; this has been suggested to reflect an actual sex difference that is perhaps exacerbated by a stronger tendency to refer males and the under-diagnosis of females with less identifiable symptoms (e.g., females with inattentive sub-type; Castle, Aubert, Verbrugge, Khalid, & Epstein, 2007; Rowland et al., 2002).

The symptoms of ADHD are often associated with language, motor, and social deficits and an ADHD diagnosis is associated with impaired academic performance in school-aged individuals (APA, 2013). Youth with ADHD are at an increased risk for engaging in risky sexual behavior (e.g., multiple partners, lack of contraceptive use),

smoking, and alcohol and other substance abuse (Rowland et al., 2002). Approximately 50% of youth with ADHD-C will receive a diagnosis of either oppositional defiant disorder (ODD) or conduct disorder (i.e., CD) in their lifetime (APA, 2013). ADHD also has significant comorbidity rates with anxiety disorders (47.1%), mood disorders (38.3%), and impulse-control disorders such as intermittent explosive disorder (19.6%; Kessler et al., 2006).

Neural networks involved in ADHD

Impairments in fronto-striatal circuitry are thought to underlie ADHD symptomology (Durston et al., 2003; MacMaster, Carrey, Sparkes, & Kusumakar, 2003; Perlov et al., 2010). In the late 90s, a "direct" and "indirect" model of the cortico-striatal-thalamo-cortical pathway was proposed, based on which part of the globus pallidus the caudate nucleus synapses on (Castellanos, 1997). The direct pathway (i.e., caudate → medial globus pallidus → thalamus) corresponds to behavior changes in response engagement, while the indirect pathway (i.e., caudate → lateral globus pallidus ←→ subthalamic nucleus → medial globus pallidus → thalamus) is associated with alterations in response inhibition (Castellanos, 1997). Excessive activation via the direct pathway and/or inadequate inhibition via the indirect pathway, were suggested to contribute to pathology in a number of disorders including ADHD, OCD, and Tourette's Syndrome (Castellanos, 1997).

A number of functional imaging studies have provided support for abnormalities in fronto-striatal circuitry among youth with ADHD. For example, an early study examining alterations in neurocircuitry in ADHD found that children with ADHD made more errors on a go-nogo task, activated the prefrontal cortex and ACC less, and

activated other regions in the brain more, areas not typically associated with cognitive control, than healthy controls (i.e., parietal cortex, occipital cortex; Durston et al., 2003). This suggested a deviation from normal development and a potential compensatory mechanism in those with ADHD (Durston et al., 2003). Adolescents with ADHD show reduced engagement of the right orbitofrontal and inferior prefrontal cortex, putamen, and caudate, compared to healthy controls on successful inhibition trials during the performance of a stop task (Rubia, Smith, Brammer, Toone, & Taylor, 2005). Likewise, during unsuccessful inhibition trials on the stop task, healthy controls displayed greater activation in the posterior cingulate cortex and precuneus, relative to adolescents with ADHD (Rubia et al., 2005). Together, these studies provide support for functional abnormalities in the fronto-striatal circuitry of youth with ADHD.

Fronto-striatal abnormalities have been identified in adults with ADHD as well. Cubillo and colleagues (2010) recruited a sample of adults who'd had childhood ADHD, and found that the ADHD adults showed decreased activation in the frontal and premotor cortices, caudate and putamen (striatum), right thalamus, and anterior and posterior cingulate cortex, relative to healthy controls, during a Stop task (exploits inhibition of preprogrammed response). Another study using a continuous performance test (CPT) reported decreased activity during the no-go condition in the right frontal cortex and right caudate, but increased activation in the left and right occipital cortex, in adults with ADHD relative to healthy controls (Schneider et al., 2010). These findings mirror those done in youth with ADHD, where frontal-striatal activation is impaired and other regions in the brain, perhaps for compensatory purposes, are activated more.

In addition to functional imaging studies, studies looking at functional connectivity have suggested impairment in frontal-striatal circuitry in ADHD as well. In a study utilizing resting-state low-frequency fluctuations to deduce functional connectivity, it was found that adolescents with ADHD exhibit greater bilateral resting-state functional connectivity between the dACC and thalamus, cerebellum, insula, and brainstem (Tian et al., 2006). A study of parent-child dyads with ADHD found that fractional anisotropy (i.e., FA) in the prefrontal cortex is associated with activity in frontostriatal areas (i.e., inferior frontal gyrus and caudate nucleus) and performance on a go-nogo task in ADHD parents and children; with lower levels of activation in frontostriatal regions predicting lower FA and poorer cognitive control (Casey et al., 2007).

Introduction to Obsessive-Compulsive Disorder

Obsessive-Compulsive Disorder (OCD) is another neurodevelopmental disorder, and is characterized by obsessions, which are recurrent, unwanted thoughts, images or impulses; and/or compulsions, which are repetitive behaviors or mental acts performed to alleviate anxiety or distress caused by obsessions (Bloch et al., 2006). Obsessions and compulsions can take many forms, but commonly center around cleaning (e.g., contamination obsessions, cleaning compulsions), symmetry (e.g., symmetry obsessions, counting/ordering compulsions), forbidden/taboo thoughts (e.g., aggressive, sexual, or religions obsessions paired with related compulsions), and harm (e.g., fear of harm to self or others, checking compulsions; APA, 2013). The mean age of onset of OCD in the United States is 19.5 years old, with 25% of cases beginning by age 14 and very few new cases emerging past 30 years of age (APA, 2013; Ruscio, Stein, Chiu, & Kessler, 2010).

It has been identified that there is a bimodal age distribution in the age of onset of OCD, with the first wave of new cases peaking at around 10 years of age and a second peak occurring at around 21 years old (Geller et al., 1998; Maia, Cooney, & Peterson, 2008). Overall, the twelve-month prevalence rate of OCD is approximately 1.1-1.8% internationally; over a lifetime, prevalence rates are approximately 1-2.5% (APA, 2013; Weber et al., 2014). OCD is commonly comorbid with a number of disorders, such as anxiety disorders (75.8%), mood disorders (63.3%), and impulse-control disorders (55.9%; Ruscio et al., 2010).

Previous research has indicated that OCD occurs in males and female at roughly even rates (Lensi et al., 1996). However, gender differences have since been found: males make up the majority of early onset (i.e., diagnosis before 10 years old) cases, and in adulthood there are a greater number of females affected by OCD (Labad et al., 2008; Ruscio et al., 2010). Males with OCD are more likely to have comorbid tic disorders, whereas females are more likely to have a comorbid major depressive disorder. Additionally, there are differences with regard to the content of obsessions and compulsions across sexes; namely, females are more likely to have cleaning/contamination obsessions/compulsions, whereas males are more likely to have sexual/religious obsessions/compulsions (APA, 2013; Labad et al., 2008).

Individuals with OCD face substantial challenges in their work, relationships, home management, and social functioning (Ruscio et al., 2010). In fact, obsessive-compulsive disorder is the tenth-leading cause of disability in the world (Bloch et al., 2006). OCD has a chronic course, with waxing and waning symptoms and a low remission rate if left untreated (APA, 2013). In general, 40% of childhood cases of OCD

persist into adulthood, and this number increases to 60% if sub-threshold cases are included (Maia et al., 2008). About 25% of individuals with OCD make a suicide attempt at some point in their lives, and an alarming 50% experience suicidal thoughts (APA, 2013).

Neural networks involved in OCD

Over-activity in the cortico-striato-thalamo-cortical (CSTC) circuit has been put forward as the underlying neurofunctional alteration responsible for impairment in individuals with OCD, with considerable agreement across researchers (Beucke et al., 2013; Posner et al., 2014; Ting & Feng, 2011). There are generally thought to be three divisions of the CSTC loop, including the sensorimotor division (involving sensorimotor cortices), the associative/cognitive division (involving dorsolateral prefrontal cortex), and the limbic division (involving orbitofrontal cortex, and anterior cingulate cortex, ACC), which modulates affect and motivation (Posner et al., 2014). The limbic division and its associated areas are thought to contribute to OCD, with over-activity identified in the orbitofrontal cortex, ACC, anterior thalamus, and the basal ganglia in several studies (encompassing the dorsal striatum, ventral striatum, and sub-thalamic nucleus; Beucke et al., 2013; Maia et al., 2008; Pittenger, Bloch, & Williams, 2011; Posner et al., 2014). Some researchers have found that over-activity in the ventral striatum, orbitofrontal cortex, and ACC (latter two project to ventral striatum) lessens with successful treatment of OCD symptoms (Maia et al., 2008; Posner et al., 2014). Additionally, the head of caudate, which is part of the dorsal striatum, the orbitofrontal cortex, and the ACC, demonstrate increased activity during OCD symptom provocation (Maia et al., 2008). Overall, the OFC, ACC, and striatum are likely to be implicated in OCD symptomology.

Morphometric imaging studies have identified increased bilateral grey matter volume in the lenticular nuclei extending to the caudate nuclei in OCD groups, compared to healthy controls and those with panic disorder or post-traumatic stress disorder (Pittenger et al., 2011; Radua, van den Heuvel, Surguladze, & Mataix-Cols, 2010). Individuals with OCD also have decreased grey matter volume in the dorsomedial frontal gyrus and the anterior cingulate gyrus, relative to controls (Radua et al., 2010). Radua and colleagues (2010) attributed abnormal grey matter volume in the anterior cingulate gyrus to the presence of anxiety in general, as reduced grey matter volume was present in individuals with panic disorder and post-traumatic disorder as well, while suggesting that increased grey matter volume in the caudate is likely linked to the unique repetitive nature of compulsions in OCD.

In addition to the functional and anatomical abnormalities associated with the brain regions of the CSTC circuit in OCD, functional connectivity has been explored. Studies of functional connectivity have produced compelling evidence of increased functional connectivity in the orbitofrontal cortex, ACC, and caudate nucleus (part of dorsal striatum in the basal ganglia; Maia et al., 2008). In a study comparing un-medicated adults with OCD to those being treated with antidepressants for their OCD symptoms, the un-medicated adults had greater long-range connectivity in the orbitofrontal cortex and sub-thalamic nucleus (part of basal ganglia) and greater short-range connectivity in the orbitofrontal cortex and putamen (component of the dorsal striatum in the basal ganglia; Beucke et al., 2013). Long-range connectivity of the orbitofrontal cortex and putamen was positively associated with symptom severity (measured by Yale-Brown Obsessive-Compulsive Scale (Y-BOCS); Beucke et al., 2013).

Medicated adults had decreased short-range connectivity of the striatum, which was offered as preliminary support for the finding by McCabe and Mosher (2011) that taking antidepressants reduces resting-state functional connectivity (i.e., between prefrontal cortex and amygdala, and orbitofrontal cortex and striatum; Beucke et al., 2013). Contrary to the commonly reported finding of increased activity and functional connectivity associated with limbic CSTC circuitry in OCD, a study of un-medicated adults with OCD showed decreased functional connectivity of the left ventral striatum with the left ACC, and the ventral striatum with the left orbitofrontal cortex (Posner et al., 2014). There was also a negative relationship between functional connectivity of the orbitofrontal cortex and ventral striatum and Y-BOCS scores, with increased symptom severity associated with lower degrees of functional connectivity (Posner et al., 2014). Additionally, there was reduced connectivity within the sensorimotor CSTC loop and greater connectivity within the cognitive CSTC circuitry, relative to controls (Posner et al., 2014). Posner and colleagues pointed out that only one other resting-state functional connectivity study of *un-medicated adults* with OCD has been conducted (i.e., Beucke et al., 2013); noting differences in methodology, comorbidities, and time since exposure to medication in controls that make it difficult to compare the two (Posner et al., 2014).

Based on the literature presented above, it is evident that the neural circuits implicated in ADHD and OCD are similar, with involvement of the frontal cortices, striatum, and thalamus common to both populations. In the OCD literature, the CSTC circuitry is likely responsible for pathology, based on findings of over-activity in the orbitofrontal cortex, anterior cingulate cortex, striatum, and thalamus, greater local and distant connectivity between regions of the CSTC, and increased grey matter volume in

the caudate nuclei (Beucke et al., 2013; Pittenger et al., 2011; Radua et al., 2010). In the ADHD literature, impairment in frontal-striatal activation, compensatory mechanisms by other brain regions (e.g., occipital cortex), and associations between frontostriatal activation, FA in frontal cortices, and cognitive control have been identified (Casey et al., 2007; Durston et al., 2003; Rubia et al., 2005). One brain area that has been implicated in both disorders is the anterior cingulate cortex, which is the region of interest (ROI) for the current thesis. What follows is an overview of the ACC and further evidence for its involvement in neuropathology of OCD and ADHD.

The anterior cingulate cortex

The anterior cingulate cortex (ACC) is intricately involved in the cortical pathways implicated in studies of both ADHD and OCD. The ACC is a structurally and functionally heterogeneous area in the brain, and has projections to all major parts of the striatum, including the caudate nucleus, nucleus accumbens, and putamen (Devinsky, Morrell, & Vogt, 1995; Margulies et al., 2007). The ACC is functionally divided into two regions: the ventral division, which is largely involved in affective processing, and the dorsal division, which plays a role in attention and cognitive control (Bush, Luu, & Posner, 2000; Margulies et al., 2007; Shackman et al., 2011).

While cognitive control is modulated in the brain by the anterior cingulate cortex (particularly the monitoring component of cognitive control), the dorsolateral prefrontal cortex also plays a role in the top-down support and maintenance of attentional demands (a regulative component of cognitive control; MacDonald, Cohen, Stenger, & Carter, 2000; Shenhav, Botvinick, & Cohen, 2013). Projections from other brain regions to the anterior cingulate cortex and to the dorsolateral prefrontal cortex are similar, but

typically, the cortico-cortico connections are more widespread for the ACC (e.g., receives additional input from regions of the OFC, temporal and parietal cortices, and insula; Shenhav et al., 2013).

The cognitive division of the ACC (ACCcd, i.e., dorsal) is specifically involved in focusing attention, execution of control, action and response selection, performance monitoring, and reward processing (Bush et al., 1999; Shenhav et al., 2013). The ACCcd is thought to play a vital role in the proper functioning of frontostriatal attention networks (Bush et al., 1999). In contrast, the affective division of the anterior cingulate cortex (i.e., ventral) plays a role in emotional processing, induced sadness, and symptom provocation (i.e. anxiety disorders, OCD; Bush et al., 2000).

Dysfunction in the ACC may lead to increased ADHD and OCD symptomology. Inattention, hyperactivity, and impaired emotion regulation are hallmarks of all neurodevelopmental disorders, and these are closely linked to the function of the ACCcd (Baribeau et al., 2015; Bush et al., 1999; Shenhav et al., 2013). In a study of cognitive control, adults with ADHD displayed activation in the fronto-striatal circuitry, but not the ACCcd, during a counting Stroop task, suggesting that the inattentive symptomology of ADHD can be attributed to dysfunction in the ACC (rather than under-activity in frontostriatal areas discussed above; Bush et al., 1999). In an fMRI study, a "flanker-interference" task was designed, with increasing levels of interference, to elicit errors of commission in participants in order to assess the effect of over-activity in the ACC in individuals with OCD (i.e., cognitive control; Fitzgerald et al., 2005). Adults with OCD exhibited greater error-related activation in the ACCcd than controls, and activity in this

region was positively correlated to symptom severity (measured by the Y-BOCS; Fitzgerald et al., 2005).

These studies suggest that the ACC plays a role in the impairments associated with both ADHD and OCD. Accordingly, the ACC was selected as the ROI for the current thesis. In order to further understand the role of metabolite concentrations in ACC impairment in our sample, we collected data on the concentration of metabolites in the ACC via proton magnetic resonance spectroscopy (^{1}H-MRS). What follows is an introduction to this imaging technique, and an overview of ADHD and OCD studies that have utilized it.

Introduction to proton magnetic resonance spectroscopy

Magnetic resonance spectroscopy (MRS) uses a standard MR signal to extract chemical information about tissues in the body (Soares & Law, 2009). MRS studies of the brain are practical and reliable, due to the brain's fairly homogenous tissue composition and limited motion artifacts (Gujar, Maheshwari, Bjorkman-Burtscher, & Sundgren, 2005). MRS can be used to examine a single voxel (i.e., single-voxel spectroscopy, SVS), or multiple voxels (i.e., chemical shift imaging) in the brain. Although it would be ideal to sample from more than one voxel, considerations regarding the total length of time in the scanner impose limits on the number of brain regions studied during a single scan session. Thus, SVS is often used. It is done by prescribing a 3-dimensional region (i.e., a voxel), typically 2.0 cm x 2.0 cm x 2.0 cm, in an anatomically defined region of interest in the brain (Stagg, Bachtiar, & Johansen-Berg, 2011). It is common to acquire MR spectra from the nucleus of hydrogen atoms, a technique known as proton magnetic resonance spectroscopy (i.e., ^{1}H-MRS), owing to

hydrogen's high magnetic sensitivity and natural abundance in organic tissue (Soares & Law, 2009).

Unlike standard imaging techniques, which provide an image of the brain for interpretation, the output of ^{1}H-MRS is in the form of a line spectrum, based on resonant frequencies of a finite list of particular compounds and neurotransmitters in the brain (Maddock & Buonocore, 2012). The X-axis of an MR spectrum increases from right to left and represents the chemical shift position of a nucleus (in parts per million, or ppm, which is the preferable unit because it is independent of scanner field strength), otherwise known as the *resonance frequency* for a given metabolite (Bertholdo, Watcharakorn, & Castillo, 2013). The Y-axis corresponds to peak amplitude (Bertholdo et al., 2013). See Figure 1 for a sample ^{1}H-MRS spectrum.

The metabolites containing protons that are measured by ^{1}H-MRS include glutamate, creatine, N-acetyl aspartate, choline, and myo-inositol (Gujar et al., 2005; Posse et al., 2007). Glutamate, the carboxylate anion of glutamic acid, is the principle excitatory neurotransmitter in the brain (Govindaraju, Young, & Maudsley, 2000; Pittenger et al., 2011). Glutamate neural projections from the cerebral cortex, thalamus, and hippocampus target the GABAergic medium spiny neurons of the striatum, which is the primary input to the

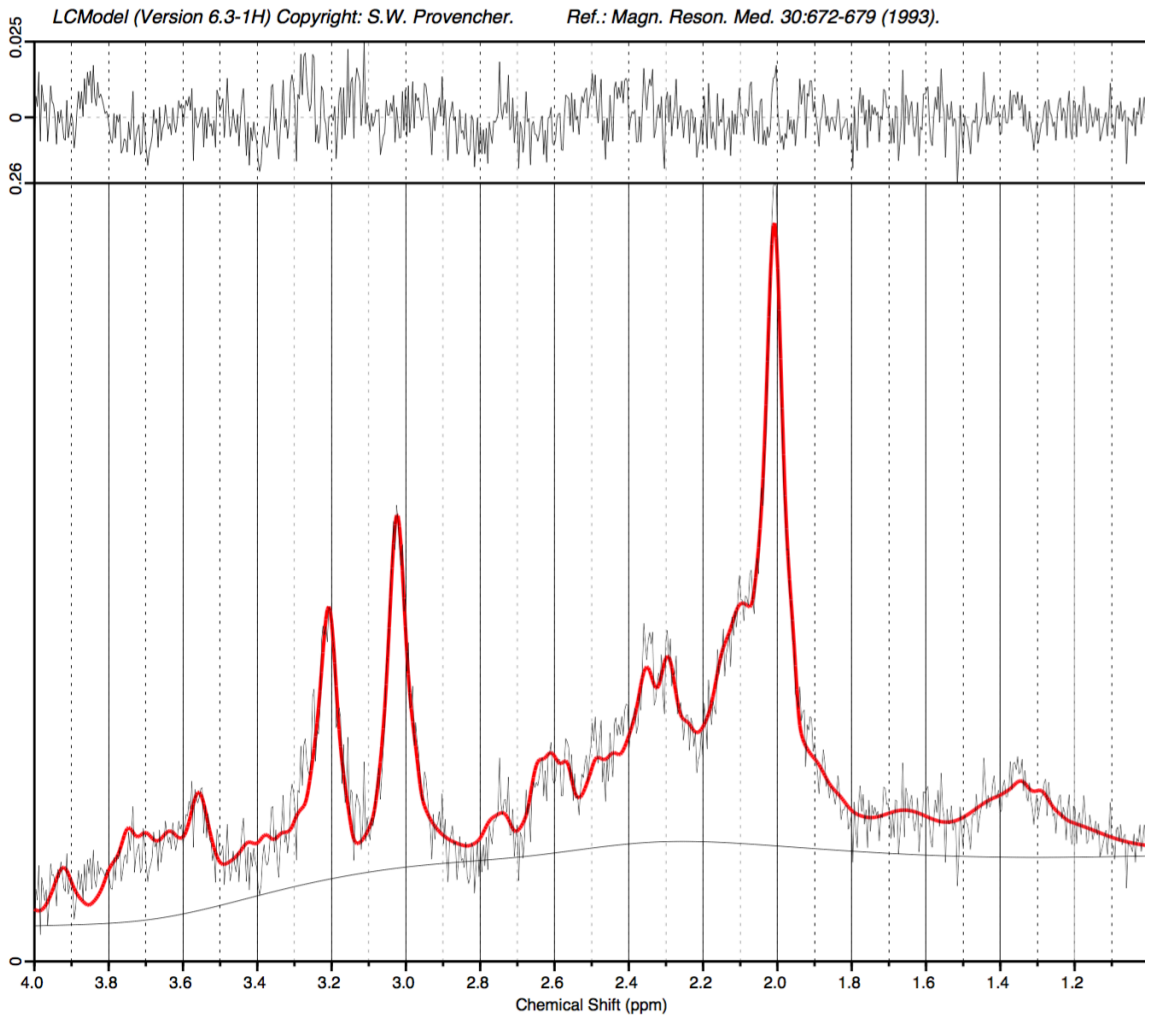

Figure 1. ^{1}H-MRS spectrum from ACC of a 14-year-old female control participant.

basal ganglia (Pittenger et al., 2011). Creatine serves as a carrier for high-energy phosphate ions that are used to create adenosine triphosphate, and is traditionally thought to remain relatively constant in the brain (Drost, Riddle, & Clarke, 2002; Starck et al., 2008). N-acetyl aspartate is a marker of neuronal integrity, is involved in neuronal osmoregulation and axon-glial signalling, and is only present in neurons (Drost et al., 2002; Gujar et al., 2005; Starck et al., 2008). Importantly, changes in N-acetyl aspartate may reflect reversible changes in metabolism rather than permanent changes in neuronal integrity or density (Drost et al., 2002). Choline is associated with glial cell membrane turnover (i.e., synthesis and degradation of the phospholipid bilayer), and it's projections play an important role in modulating selective attention (Bertholdo et al., 2013; Gujar et al., 2005; Perry, Walker, Grace, & Perry, 1999; Starck et al., 2008). Myo-inositol is related to cell growth and considered a marker for glial cell loss, as it is produced in astrocytes and located in glial cells primarily (Bertholdo et al., 2013; Gujar et al., 2005; Starck et al., 2008).

As mentioned, SVS is often used in spectroscopy studies to limit the total acquisition time required. The voxel of interest is thus examined in isolation, rather than in the context of surrounding tissue/brain structures, which imposes limits on the conclusions that can be drawn from such studies. Another important consideration is the placement of the voxel during scanning. The MR technologist visualizes and places the voxel in the same region, consistently across subjects being scanned, using a predefined protocol and detailed anatomic reference points. Although there may be efforts to ensure some standardization in voxel placement, individual heterogeneity in brain morphology can introduce variability in the voxel placement. As a result, the data for each individual

will vary with regard to the composition of grey matter, white matter, and cerebrospinal fluid (CSF) in the voxel. This is important because metabolite differences across individuals may potentially become amplified or masked by variations in voxel tissue composition. As a consequence, there are several things that can be done in the post-processing stage to address this. Glutamate, choline, myo-inositol, creatine, and N-acetyl aspartate have been well studied in the literature such that T1 and T2 relaxation correction values can be reliably used, in conjunction with grey matter/white matter/cerebrospinal fluid segmentation, to calculate absolute metabolite concentrations in each voxel (Gasparovic et al., 2006; Posse et al., 2007; Weber et al., 2014). Other ways to control for the composition of voxels include setting restrictions for acceptable data (e.g., only use data with proportion of white matter in voxel greater than 50%, if ROI is a white matter structure), or the use of white matter/grey matter/cerebrospinal fluid proportions as covariates during statistical analyses. To date, most ^{1}H-MRS studies have not taken into account the relative contribution of grey matter, white matter, and CSF in their voxels, which presents as a limitation in the interpretation of their findings (e.g., Carrey et al., 2003; Rosenberg et al., 2000).

There are other methodological limitations of proton magnetic resonance spectroscopy that are worth noting. First, it provides lower sensitivity and molecular specificity than neurochemical analysis of the CSF (e.g., Lumbar puncture; Pittenger et al., 2011). However, this concern is most relevant for the many previous proton magnetic resonance spectroscopy studies carried out on clinical scanners with relatively low magnetic field strength (i.e., 1.5 T; MacMaster et al., 2003; Perlov et al., 2007, 2010; Rosenberg et al., 2004). Another issue, particularly with earlier ^{1}H-MRS studies, is the

use of long-echo (vs. short-echo) protocols, which have poor resolution of glutamate/glutamine resonance (MacMaster et al., 2003). A final issue is that of movement during MRS acquisition. The MRS voxel is placed into the ROI before acquisition begins, and it is not observed during the acquisition. As a result, even slight movement by a participant in the scanner can result in the collection of MRS data from the wrong location in the brain. This can be addressed, though not fully resolved, by examining images of voxel placement in anatomic scans acquired before and after 1H-MRS acquisition to confirm the voxel has remained in the correct location.

^{1}H-MRS studies OCD populations

There have been several studies implicating glutamate in OCD pathology. In a lumbar puncture study of adult OCD patients and healthy controls, a significant main effect of diagnosis on glutamate levels in the cerebrospinal fluid (CSF) was identified, with increased glutamate in the OCD pro-band group (Chakrabarty, Bhattacharyya, Christopher, & Khanna, 2005). There was no relationship between CSF glutamate levels and symptom severity, as measured by the Yale-Brown Obsessive Compulsive Scale (Y-BOCS) and the Clinical Global Impression – Severity Scale (CGI-S; Chakrabarty et al., 2005). It is important to note that as a neurotransmitter, glutamate makes up a minority of total brain glutamate; glutamic acid is a basic amino acid present in many proteins in the body, and glutamate is a precursor for other neurotransmitters, including GABA and glutamine (Pittenger et al., 2011). Thus, an excess CSF glutamate is not necessarily indicative of increased glutamate activity, but rather taken to reflect a complex interplay between various neurotransmitter dysfunctions (Chakrabarty et al., 2005). This study highlights the usefulness of ^{1}H-MRS; while lumbar puncture allows for the quantification

of metabolites directly from CSF, ^{1}H-MRS considers the proportion of CSF, grey matter, and white matter in each voxel studied. In this way, only when a voxel contains primarily grey matter (or white matter, if the study focuses on that) can one conclude that an excess or shortage of a given metabolite is a result of increased or decreased activity, respectively.

Glutamate abnormalities have been explored using ^{1}H-MRS techniques in children with OCD. One of the early studies looking at pediatric OCD explored the effect of paroxetine on psychotropic-naïve children by examining the left caudate before and after paroxetine, a selective serotonin reuptake inhibitor (SSRI) medication (Rosenberg et al., 2000). Levels of glutamate were elevated in the OCD group, but decreased significantly post-paroxetine treatment, and post-treatment decreases in glutamate were associated with post-treatment decreases in CY-BOCS score (Rosenberg et al., 2000). Rosenberg and colleagues (2000) suggested that these findings could be explained by SSRIs causing altered serotonergic transmission within the frontal cortex, which, in this case, influenced fronto-striatal glutamate projections and led to decreased glutamate post-treatment. Rosenberg and associates (2004) drew on reports in the literature of elevated glutamate concentrations in OCD and decreased glutamate concentrations in MDD and, in keeping with the the RDoC framework, proposed that glutamate levels could be examined as a potential biomarker that spans both disorders. They found significantly greater levels of ACC glutamate in controls compared to both clinical groups, with no differences in metabolites between children with OCD and MDD (Rosenberg et al., 2004).

Another metabolite highlighted in the OCD literature is N-acetyl aspartate. Jang and colleagues (2006) examined voxels in prefrontal white matter and grey matter, parietal white matter and grey matter, anterior cingulate cortex, and posterior cingulate cortex before and after a 12-week citalopram regimen in previously un-medicated adults with OCD. Prior to treatment, there were reduced levels of N-acetyl aspartate, relative to controls, in the prefrontal white matter, prefrontal grey matter, and anterior cingulate of adults with OCD. None of these values correlated to Y-BOCS score pre or post-citalopram treatment, but all concentrations increased post-treatment, and looked more similar to the control population's levels (Jang et al., 2006). In another adult study, voxels in the head of caudate and orbitofrontal white matter were examined, with a priori hypotheses of increased glutamate and decreased N-acetyl aspartate in the head of caudate in OCD patients (Whiteside, Abramowitz, & Port, 2012). There were no differences in N-acetyl aspartate or glutamate in the head of caudate in the OCD vs. control population, and decreased N-acetyl aspartate and creatine in the right orbitofrontal white matter in patients with OCD relative to controls (Whiteside et al., 2012). Bilateral orbitofrontal white matter concentrations of N-acetyl aspartate were negatively associated with anxiety (measured by the State-Trait Anxiety Inventory-Trait Version, STAI-T; Whiteside et al., 2012). N-acetyl aspartate, a marker of neuronal viability, can be used to reference neuronal health, viability, and number; thus lower N-acetyl aspartate may represent decreased function and number of neurons in the orbitofrontal white matter, and a putative structure-function association to symptoms of anxiety (Whiteside et al., 2012). An interesting study that combined ^{1}H-MRS (bilateral voxels in dACC) with fMRI tasks designed to activate the medial frontal cortex

(encompasses dACC and supplementary motor area) reported decreased N-acetyl aspartate concentrations in the dACC of OCD participants relative to controls (Yucel et al., 2007). The fMRI results in this study revealed that the OCD group recruited a broader region of MFC structures, and exhibited heightened activation, during the conflict condition of the fMRI task (Yucel et al., 2007). Additionally, in the OCD sample, there was a negative relationship between N-acetyl aspartate and BOLD signal change in the MFC, such that lower levels of N-acetyl aspartate predicted a larger bold signal change in the MFC network (i.e., hyper-activity; Yucel et al., 2007). This finding is consistent with that of Whiteside and colleagues (2012) described above, whereby decreased levels of N-acetyl aspartate represent some reduction in neuronal function, which, in this case, may have lead to a compensatory mechanism (i.e., broader recruitment of brain regions and hyperactivity during conflict condition; Yucel et al., 2007).

More recently, Weber and colleagues (2014) revealed N-acetyl aspartate differences between medication-naïve children with OCD and controls, in the right and left prefrontal white matter (Weber et al., 2014). The authors found increased choline and N-acetyl aspartate in the right prefrontal white matter of OCD children versus controls, with creatine, myo-inositol, and N-acetyl aspartate in this region positively associated with total CY-BOCS score (Weber et al., 2014). Importantly, this finding is not consistent with the adult studies above, which identified *lower* levels of N-acetyl aspartate in OCD samples. It is difficult to compare because the studies noting lower N-acetyl aspartate were done in adults, while the study by Weber and colleagues (2014) was done in children. The increased concentration of N-acetyl aspartate could perhaps be related to increased number and metabolism of neurons in the prefrontal white matter of

children with OCD (due to less maturational pruning or less consolidated neural networks in the younger demographic), suggesting more direct involvement in symptomology than the explanation for lower N-acetyl aspartate above.

Similarly, other research has noted differences between the metabolite abnormalities of children and adults with OCD. Starck and colleagues (2008) used a multivariate approach to compare metabolites in the caudate nucleus, anterior cingulate, and occipital cortex (the latter was included as control region) of adults with OCD and controls. There was no difference in mean metabolite levels between the groups, but levels of creatine, glutamate, and choline in the caudate, and myo-inositol in the occipital cortices, were positively related to Y-BOCS scores (Starck et al., 2008). This contrasts with Rosenberg's earlier work in OCD, where there were glutamate differences in the caudate and ACC of children with OCD and controls. However, it is difficult to compare directly since Rosenberg's studies involved children who had no prior exposure to pharmacotherapy, and thus had higher mean CY-BOCS scores (i.e., greater severity of symptoms) than the adults in Starck and colleagues' work.

The thalamus is another region of the CSTC circuitry that has been implicated in OCD. An interesting study of psychotropic-naïve children with OCD found that compared to controls, those with OCD had decreased N-acetyl aspartate/choline + creatine and N-acetyl aspartate/choline in the medial thalamus, with the latter negatively related to obsessive symptoms measured by the CY-BOCS (Fitzgerald, Moore, Paulson, Stewart, & Rosenberg, 2000). There were greater concentrations of N-acetyl aspartate/creatine in the left lateral thalamus as well (Fitzgerald et al., 2000). It was noted that greater dysfunction in the medial rather than lateral thalamus is expected for OCD

populations, as the former is involved in affective and motivational processes, which are impaired in OCD, and the latter largely in motor function (Fitzgerald et al., 2000).

It is possible that factors other than diagnosis alone, such as the presence of specific behaviours or personality dimensions, are involved in the relationship between metabolite levels and OCD symptomology. This is especially important within the context of neurodevelopmental disorders described by the NIHM above, and the POND framework, which focuses on the underlying dimensions contributing to various neurodevelopmental disorders. Whiteside and colleagues (2006) explored the role that anxiety plays in metabolite abnormalities in OCD. Adults with OCD, when compared with healthy controls, displayed decreased levels of myo-inositol in the head of caudate (bilateral), and increased N-acetyl aspartate and glutamate in the orbitofrontal white matter (right; Whiteside, Port, Deacon, & Abramowitz, 2006). However, after controlling for state anxiety during the MRI procedure, the majority of differences between the OCD and control group no longer reached significance; the only remaining difference was elevated myo-inositol in right head of caudate for OCD (Whiteside et al., 2006). This suggests that the differences in metabolite concentration between the OCD group and controls were related to higher state anxiety, as observed during the MRI procedure, in OCD participants. These findings point out the importance of pre-training children for MRI (e.g., with the use of a mock scanner) and collecting measures on the subjective state of participants during scanning (de Bie et al., 2010).

Metabolite abnormalities in adults and children with OCD have been identified in structures of the CSTC circuitry, albeit with disparate findings. It is important that future research aim to build on such studies in order to accurately determine if metabolite

differences exist between individuals with OCD and controls, and to assess the role of other factors, such as personality dimensions, in such differences.

^{1}H-MRS studies in ADHD populations

Brain metabolite abnormalities have been explored in adults with ADHD. Historically, neurochemical models of ADHD suggested that dopamine alone drove pathology, whereas current models have begun to integrate dopamine's role with glutamatergic dysfunction (Perlov et al., 2007). One hypothesis suggests that dopaminergic neurons terminating on glutamatergic neurons fail to modulate glutamate signal transmission, resulting in abnormal glutamate function in those with ADHD (Moore et al., 2006; Perlov et al., 2007). In a ^{1}H-MRS study looking at metabolite concentrations in the ACC, adults with ADHD, compared to healthy controls, had decreased glutamate in the right ACC (Perlov et al., 2007). In another study, adults with ADHD had lower glutamate, choline, creatine, and N-acetyl aspartate in the basal ganglia, and lower glutamate, creatine, and N-acetyl aspartate in the dorsolateral prefrontal cortex, than healthy controls (Maltezos et al., 2014). This suggests reduced metabolism in brain regions associated with the frontostriatal circuitry in ADHD. Perhaps this is indicative of decreased activity in frontostriatal areas of the brain in ADHD, which would be consistent with functional imaging studies reporting decreased activation in areas associated with cognitive control in ADHD (e.g., prefrontal cortex, orbitofrontal cortex, ACC) and increased activation in other, typically un-associated brain regions (e.g., occipital cortex, parietal cortex; Durston et al., 2003; Rubia et al., 2005).

Many ^{1}H-MRS studies in children with ADHD have measured metabolite concentrations from voxels in the frontal cortex. In one study, smaller left dorsolateral

frontal cortex volumes, and poorer performance on a Continuous Performance Task (CPT) were identified in an ADHD group (relative to controls; Yeo et al., 2003). Despite this, Yeo et al. (2003) found no difference in frontal metabolite concentrations between children with ADHD and healthy controls, although girls with ADHD had lower metabolite concentrations across the board. MacMaster and colleagues (2003) examined the prefrontal cortex and striatum of medication-free children with ADHD. In the ADHD group, there were significantly higher levels of glutamate/creatine in the right prefrontal cortex, and a trend towards higher glutamate/creatine in the left striatum, relative to healthy controls (MacMaster et al., 2003). In an interesting ^{1}H-MRS study, consistent with the RDoC framework and the value of cross-disorder investigations emphasized by the NIMH, children with ADHD and children with ADHD and comorbid bipolar disorder were compared (Moore et al., 2006). The children with ADHD had higher glutamate/creatine in the ACC, compared to those with ADHD + bipolar disorder and healthy controls (Moore et al., 2006). The authors suggested that glutamate/myo-inositol ratios offered a way to differentiate between children with ADHD and ADHD + bipolar disorder (based on greater myo-inositol/creatine in bipolar disorder, which decreases glutamate/myo-inositol in those with ADHD + bipolar disorder; Moore et al., 2006). The latter two studies provide support for frontal cortex glutamatergic abnormalities in ADHD pathology. One possible reason that glutamate is elevated in ADHD is due to reduced levels of dopamine in the nucleus accumbens among individuals with ADHD (Moore et al., 2006; Perlov et al., 2007). Specifically, since dopamine release is stimulated by glutamate, decreased levels of dopamine may indirectly result in a build-up of glutamate in the frontal cortices (MacMaster et al., 2003; Moore et al., 2006). This can

be understood in the context of a negative feedback system in the brain: if glutamate stimulates release of dopamine but dopamine levels never get high enough, the negative feedback system is not intact and consequently, glutamate release is never inhibited (i.e., glutamate concentrations continue to rise). Thus, perhaps the reduced levels of dopamine associated with ADHD contribute to elevated glutamate in the frontal cortex in ADHD.

The striatum has also received attention in the ^{1}H-MRS literature due to its involvement in the functional deficits of ADHD. One of the first ^{1}H-MRS studies of pediatric ADHD targeted the globus pallidus of psychotropic-drug naïve boys, before and after one oral dose of methylphenidate (Ritalin 10 mg; Jin, Zang, Zeng, Zhang, & Wang, 2001). Relative to the control population, boys with ADHD had lower N-acetyl aspartate/creatine bilaterally, with no significant changes after pharmacotherapy (Jin et al., 2001). Decreased N-acetyl aspartate, which reflects neuronal integrity, is consistent with functional imaging studies reporting reduced recruitment of frontostriatal areas in ADHD (Rubia et al., 2005); and perhaps, a single, relatively low dose of methylphenidate is not enough to produce robust changes in metabolite concentrations. Another study, which examined the effects of methylphenidate using a longer treatment period, involved an 8-week regimen in treatment-naïve male children with ADHD (Carrey, MacMaster, Gaudet, & Schmidt, 2007). At baseline, the ADHD group had higher glutamate in the striatum, relative to healthy controls (Carrey et al., 2007). Post-treatment, the ADHD children no longer met criteria for ADHD, but still had elevated glutamate levels (Carrey et al., 2007). These studies explored the striatal deficits of lower N-acetyl aspartate/creatine, and increased glutamate in ADHD, while shedding light on the effects of medical intervention. The findings are consistent with the negative feedback system

between glutamate and dopamine discussed above, which ultimately results in elevated glutamate concentrations in ADHD. What these studies failed to find is support for reduced glutamate concentrations, which would be expected following administration of medications like methylphenidate (i.e., inhibited reuptake of dopamine as a result of methylphenidate would increase dopamine levels, thereby reducing glutamate; Moore et al., 2006).

There have been a number of studies providing support for reduced glutamate following methylphenidate treatment. Hammerness and colleagues (2012) administered a 6-week methylphenidate regimen in adolescents with ADHD, who initially had higher concentrations of glutamate than control participants. Post-treatment, glutamate concentrations in the ADHD group were comparable to that of controls, though not statistically significant (Hammerness, Biederman, Petty, Henin, & Moore, 2012; Wiguna, Guerrero, Wibisono, & Sastroasmoro, 2012). In a study of medicated children with ADHD, children were briefly taken off their medication to participate in a "medication-free" scan, then put back on their regimen and scanned again (Carrey et al., 2003). The ADHD group initially had higher glutamate/creatine than controls in the prefrontal cortex and striatum (Carrey et al., 2003). Post-treatment, there was a significant reduction in glutamate/creatine in the striatum, but not the prefrontal cortex (Carrey et al., 2003). The authors considered the decrease in striatal glutamate as an indication that glutamate metabolism had returned to normal levels in the ADHD group, attributable to successful pharmacological intervention of their symptoms (Carrey et al., 2003).

Individuals with ADHD may present with different metabolite abnormalities according to the sub-type of ADHD they have. Sun and colleagues (2005) compared

metabolite concentrations in the lenticular nucleus (i.e., putamen and globus pallidus) of boys with ADHD-I (i.e., inattentive type; no hyperactivity/impulsivity), ADHD-C (i.e., combined type; both inattention and hyperactivity/impulsivity present), and healthy controls. N-acetyl aspartate/creatine was significantly lower in the right lenticular nucleus for the ADHD-C group compared to both the ADHD-I and control groups, and lower in the left lenticular nucleus relative to controls only (Sun et al., 2005). In an adult study, metabolite concentrations in the left dorsolateral prefrontal cortex and left striatum were measured in males, with or without hyperactivity/impulsivity (Hesslinger, Thiel, van Elst, Hennig, & Ebert, 2001). Those with hyperactivity had significantly lower N-acetyl aspartate in the left dorsolateral prefrontal cortex, and there was no difference between controls and those without hyperactivity (i.e., those with ADHD-I; Hesslinger et al., 2001). These studies suggest that individuals with different sub-types of ADHD may vary in their metabolite makeup, though further investigation is required to clarify the role that diagnosis plays.

Overall, there has been ample exploration of the metabolite abnormalities in the CSTC and fronto-striatal circuitry, both of which the ACC interacts with, in OCD and ADHD populations, respectively (Bush et al., 1999; Devinsky et al., 1995; Margulies et al., 2007). Most consistently, abnormalities in glutamate and N-acetyl aspartate have been reported in these groups. Children with OCD have elevated glutamate (in caudate, ACC) and elevated N-acetyl aspartate (PFWM), relative to controls (Rosenberg et al., 2000; Rosenberg et al., 2004; Weber et al., 2014). Children with ADHD typically display elevated glutamate (in PFC, ACC) and reduced N-acetyl aspartate (in striatum), compared to controls (MacMaster et al., 2003; Moore et al., 2006; Jin et al., 2001).

PURPOSE OF PRESENT STUDY

Consistent with the RDoC framework and the NIMH emphasis on studying neurodevelopmental disorders collectively, the POND imaging study involves scanning children with ASD, OCD, and ADHD utilizing MRI, fMRI, DTI, and ^{1}H-MRS techniques (Insel et al., 2010; NIHM, 2007; Rumsey, 2008). The current ^{1}H-MRS investigation is a sub-study within the POND imaging study, contrasting the metabolite concentrations of children with OCD and ADHD. Given its involvement in CSTC circuitry and fronto-striatal connections, we chose the ACC as the region of interest for the current study (Bush et al., 1999, 2000; Fitzgerald et al., 2005; Shenhav et al., 2013). Our goal was to use ^{1}H-MRS as a tool to identify the subtle biochemical differences that exist between children with ADHD and OCD, by comparing their metabolite concentrations in a between-group analysis. The ultimate purpose is to uncover biomarkers that may be used in the future to identify and differentiate between neurodevelopmental disorders in young children. We also explored the relationship of metabolites in the ACC with indicators of symptom severity using a within-group analysis. Finally, based on the similarity of behaviors in children with neurodevelopmental disorders, we collapsed across groups to determine if metabolite concentrations can be used to predict behavioural problems common to both OCD and ADHD (Baribeau et al., 2015). To our knowledge, this is the first ^{1}H-MRS study directly contrasting pediatric OCD and ADHD populations, and one of the first to explore the relationship between metabolites and symptom dimensions, rather than the relationship between metabolites and symptom severity alone. Thus, this study is considered largely exploratory.

Research Questions and Hypotheses

Research Question #1: Is there a difference in mean metabolite levels between children with ADHD and OCD?

Hypothesis #1: Due to the relative consistency with which they are cited in the ^{1}H-MRS literature for both groups, we predicted that glutamate and N-acetyl aspartate would vary between children with ADHD and OCD (Jin et al., 2001; Weber et al., 2014; Moore et al., 2006; Rosenberg et al., 2004). Specifically, we predicted that N-acetyl aspartate would be higher in children with OCD versus ADHD, and did not make a prediction about the directionality of glutamate differences, siunce glutamate is elevated in children with both disorders.

Research Question #2: Within diagnostic group, what is the relationship between symptom severity and metabolite concentrations in the anterior cingulate cortex?

Hypothesis #2: We predicted that N-acetyl acetate and glutamate would be associated with severity of symptoms in each group.

Research Question #3: Across diagnostic groups, what is the relationship between metabolite concentrations and behavioural symptoms that are common to both populations?

Hypothesis #3: Since we are the first to look at ADHD and OCD together, and the first to look at the relationship between metabolites and symptom dimensions (i.e., not just severity of symptoms) in children with neurodevelopmental disorders, this final question is largely exploratory and, as such, concrete hypotheses were not made.

CHAPTER III

METHODS

Participants and Procedure

Participants of any age were recruited to join the Province of Ontario Neurodevelopmental Disorders (POND) study, up to a maximum age of 21 years, 11 months. The POND Network is a multi-site collaboration between the Hospital for Sick Children in Toronto, Holland Bloorview Kids Rehabilitation Hospital in Toronto, McMaster University and the Offord Centre for Child Studies in Hamilton, and Lawson Health Research Institute in London. Recruitment for the POND study is done through many avenues within Ontario, including postings in doctor's offices, partner organizations, and mail-outs to previously treated families. To enroll in the POND study, one must have a formal DSM-5 diagnosis of a neurodevelopmental disorder (i.e., attention deficit hyperactivity disorder (ADHD), autism spectrum disorder (ASD), pediatric bipolar disorder, obsessive-compulsive disorder (OCD), childhood-onset schizophrenia, Tourette's syndrome, or those disorders with a known genetic etiology such as Fragile X disorder and 22q deletion (Rumsey, 2008). The POND study involves non-restrictive exclusion and inclusion criteria in hopes that this will result in a database representative of children with neurodevelopmental disorders in Ontario. Aside from having a formal diagnosis, the only requirement is that participants agree to contribute a genetic sample to the Ontario Brain Institute (OBI), which is made available to the POND research community, and it is preferred that the parent and child are English-speaking. Participants were recruited for the current imaging sub-study exclusively at the Hospital for Sick Children. After being seen in an ADHD or OCD clinic, children given a

formal diagnosis were invited to join the POND study. Metal in the body, such as dental braces or implanted medical devices, or other MRI contraindications served as additional exclusion criteria for imaging specifically. The Hospital for Sick Children Research Ethics Board [and the Holland Bloorview Research Ethics Board] approved this study. In accordance with the laws surrounding consent in Ontario, each participant's capacity to provide consent was assessed. If a child was deemed capable of providing consent, they were asked to provide written and informed consent. If a child was deemed incapable of providing informed consent, they provided written assent. In this case, written and informed consent was obtained from their legal guardian.

POND Intake. All participants completed four stages associated with POND enrolment before they were eligible to engage in any of the sub-studies. Stages 1 and 2 involved collection of parent measures; stage 3 included IQ testing, language testing, and administration of clinical/diagnostic measures; and stage 4 involved cognitive testing, involving games such as the Stop Signal Task. In stage 3 of the POND intake procedure, DSM-5 diagnoses were verified with the Kiddie-Schedule for Affective Disorders (K-SADS; Kaufman, Birmaher, Rao, & Ryan, 1996) and the Parent Interview for Child Symptoms-6 (The Hospital for Sick Children, 2013) for ADHD, and the K-SADS and Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS; Goodman et al., 1989) for OCD. Verification of DSM-5 diagnosis was completed by trained research personnel (i.e., research assistant or research coordinator). All research personnel received training under the supervision of a licensed clinical psychologist. After completing intake stages 1-4, families were invited to learn more about POND sub-studies and contacted by the imaging team if they expressed interest in the neuroimaging sub-study.

Current 1H-MRS Study. As of January 15, 2016, 98 children had participated in ^{1}H-MRS scanning as part of the ongoing POND imaging study. After data cleaning, which is explained in detail below, the final sample contained 53 participants (14 females, 39 males). The mean age of participants was 11.42 years (SD = 2. 78, range = 6.7-17.1). The average proportion of grey matter collected from the ACC voxel was 59.2% (SD = 0.087, range = 0.402-0.775). As indicated in Table 2, the current study sample was not equally distributed with regard to sex and age. The ADHD group contained 24 males and 5 females (83% males), whereas the OCD group contained 15 males and 9 females (63% males). The ADHD sample was, on average, younger than the OCD sample; mean age 10.1 (SD = 2.20, range = 6.7-14.7) versus mean age 13.0 years (SD = 2.7, range = 8.3-17.1), respectively. This is attributable, perhaps, to the difference in age at first onset between the disorders, with ADHD generally being identified and diagnosed earlier in life than OCD (i.e., 7 versus 10 years old, respectively, APA, 2013; Geller et al., 1998; Maia, Cooney, & Peterson, 2008).

Table 2

Study Design (N = 53)

	ADHD (n = 29; 24 males)		OCD (n = 24; 15 males)	
	M (range)	SD	M (range)	SD
Demographics				
Age	10.1 (6.7-14.7)	2.2	13.0 (8.3-17.1)	2.7
Acquisition parameters				
FWHM	.046 (.024-.095)	.018	.045 (.028-.079)	.014
SNR	14.9 (5.0-23)	4.2	14.8 (5.0-21)	3.6
fgm	.592 (.402-.775)	.096	.591 (.407-.705)	.077
Metabolites				
Cr	4.92 (2.93-6.12)	.781	4.74 (2.94-5.74)	.558
mI	3.15 (1.92-3.87)	.455	3.11 (2.07-3.58)	.368
Cho	1.40 (1.05-2.08)	.237	1.41 (1.00-1.80)	.186
NAA	6.94 (4.97-9.38)	.880	6.70 (4.90-7.78)	.648
Glx	7.97 (4.88-10.4)	1.35	7.50 (3.13-8.91)	1.20

Neuroimaging

All scanning was done on a Siemens 3T MAGNETOM Trio, a Tim (total imaging matrix) MRI system, using a twelve-channel head coil (Siemens Healthcare, Erlangen, Germany). Each scanning session involved a localizer scan (40 seconds), a high-resolution 3D magnetization prepared rapid gradient echo (MP-RAGE) sagittal T1-weighted imaging sequence (TR/TE/TI = 2300/2.96/900 ms, flip angle = 9 degrees, FOV/Res = 192x240x256, 1mm ISO voxels, 192 acquired slices; duration 5:03 min), and a turbo spin echo (TSE) axial T2-weighted imaging sequence (TR/TE = 9000/104 ms, flipangle = 120degrees, FOV = 230x230x154, Res = 192 x 192, 1.2mm ISO voxels, 128 acquired slices; duration 3:20 min). In vivo ^{1}H-MRS data were collected from 20 mm ISO voxels, prescribed to the anterior cingulate cortex by a trained MRI technician based on the localizer sequence conducted prior to MRS scanning. See Figure 2 for an image of the ACC voxel used. Each ^{1}H-MRS acquisition involved first-order shimming with stimulated echo acquisition mode (STEAM) pulse sequence (TR/TE = 3000/5 ms, acquisition duration = 1024 ms, 2000-Hz spectral bandwidth, duration 5:00 min). In addition, an unsuppressed water reference signal was acquired for each participant, to be used as a control parameter in LC Model (i.e., used to estimate absolute metabolite concentrations; Provencher, 2014).

Post-Processing

Quantification of the ^{1}H-MRS data was done using LC Model, a robust, user-independent fitting technique that eliminates the subjectivity associated with interpreting MRS data (S. W. Provencher, 1993). LC Model allows for the measurement of spectral signals by comparing against external standards from phantoms containing known

concentrations of the compounds being measured; this a priori database enables the quantification of metabolites with overlapping signals (S. W. Provencher, 1993). Spectra were analyzed between 4.0 and 0.2 ppm. The LC Model analysis was done blinded to diagnosis until completion of LC Model processing and all stages of quality control (see below).

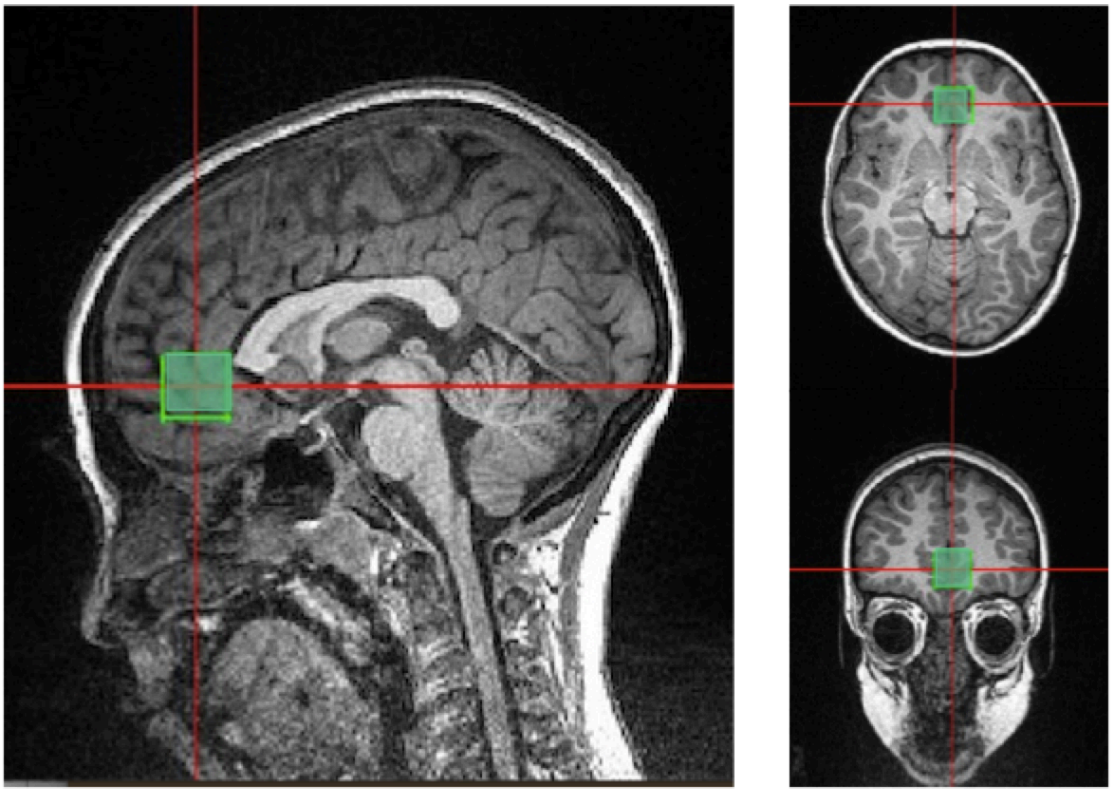

Figure 2. Data-driven image of the ACC voxel prescribed during scanning

Voxel segmentation

The proportion of grey matter, white matter, and cerebrospinal fluid in each voxel was calculated on T_1 images using CIVET (Version 2.0.0, McConnell Brain Imaging Centre). Resulting partial volume effects (PVE) maps were then transformed back into native space for all T_1 and T_2-weighted images acquired. The MRS voxel location was extracted from raw MRS data and the PVEs within this voxel were summed. The resulting PVEs were assessed during data cleaning, to ensure that we only included participants with a sufficient proportion of grey matter in our analyses (i.e., greater than 40%). In addition, screen captures collected close in time to the MRS acquisition were inspected during data cleaning, to ensure that the MRS voxel was placed in the anterior cingulate. This was an important step as inter-scan movement could result in data collection from regions proximal to the ACC.

Measures

The clinical measures used in our study include the Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS; Goodman et al., 1989) for severity of OCD symptoms, the Parent Interview for Child Symptoms 6 (PICS-6; The Hospital for Sick Children, Department of Psychiatry, 2013) for severity of ADHD symptoms, and the Child Behaviour Checklist (CBCL; Achenbach, T.M., & Rescorla, L.A., 2000-2001) for the assessment of behavioural problems in all participants.

CY-BOCS. Symptoms of OCD were measured using the CY-BOCS. The CY-BOCS contains 10 items. The items can be used to form a total OCD symptom severity score, or can be broken down into subscales, providing separate "obsessions" and "compulsions" scores. Each item is scored on a 5-point Likert scale (1 = *strongly*

disagree, 5 = *strongly agree*). A total OCD score is obtained by summing the items, such that higher score are indicative of more severe symptomology. Similarly, subscale scores are obtained by summing the items corresponding to "obsessions" or "compulsions" alone, as indicated in the CY-BOCS administration guidelines.

Past research has indicated adequate internal consistency for the total OCD score (Cronbach's alpha = .90), as well as for the obsessions and compulsions subscales (Cronbach's alpha = .80 and .82, respectively; Storch et al., 2004). In the current study, internal consistency for total OCD score was .92, and was .74 and .91 for obsessions and compulsions, respectively, indicating adequate internal consistency.

PICS-6. The PICS-6 is a semi-structured diagnostic interview used to evaluate and diagnose disruptive behavior disorders including ADHD, oppositional defiant disorder (ODD), and conduct disorder (CD). The PICS contains 63 items. Based on their discussion with the child's parent or guardian, the interviewer rates severity of the child's symptoms on a 4-point Likert-type scale (0 = *absent*, 3 = *marked abnormality*; and 9 = *not known or unable to rate*). The PICS-6 provides an inattentive (ADHD-I), hyperactivity/impulsivity (ADHD-HI), ODD, and CD score. The PICS-6 is a fairly new measure and as such, there are no known studies confirming its internal consistency.

CBCL. The CBCL is a checklist used to detect problem behaviours in children. It is typically completed by the parent/guardian who spends the most time with the child. There are two versions of the CBCL. The preschool-aged checklist, for children 1.5 – 5 years, contains 99 items. The school-aged checklist, for children 6 – 18 years, contains 113 items. Each item is scored on a 3-point Likert-type scale (0 = *not true [as far as you know]*, 2 = *very true or often true*). During scoring of the CBCL, similar items are

grouped together into syndromes and DSM-oriented categories, and summed to produce a score for that syndrome or category. The syndrome scales include: emotionally reactive, anxious/depressed, somatic complaints, withdrawn, attention problems, aggressive behavior, sleep problems. The DSM-oriented scales include: affective problems, anxiety problems, pervasive developmental problems, attention-deficit/hyperactivity problems, stress problems, autism spectrum problems, and oppositional defiant problems.

Statistical analyses

All statistical analyses were completed in IBM Statistical Package for the Social Sciences (SPSS) for Mac (Version 21.0). Preliminary analyses were conducted in order to account for missing, incomplete, or unreliable data. We identified covariates for each analysis based on the correlations between predictor variables and outcome variables, and by conducting t-tests to identify which predictors differ between groups (latter done for research question #1 only). To answer our first research question regarding the difference in creatine, choline, myo-inositol, N-acetyl aspartate, and glutamate between groups, we conducted five one-way ANCOVAs. To assess the association between symptom severity and metabolites within the ADHD and OCD sample separately (i.e., research question #2), we used Pearson and partial correlations. To answer our final research question, we collapsed across groups, and used linear and hierarchical regression to evaluate the extent to which metabolite concentrations predicted behavioural problems.

CHAPTER IV

RESULTS

Preliminary analyses

Data cleaning. Visual inspection of spectra, water peaks, and voxel placement for each participant was completed in order to ensure quality data acquisition. During this stage, five participants were removed from the analysis due to poor acquisition of data (e.g., poor shim, doublets in water peak, misplaced voxel). Full-width at half-maximum (FWHM) was used as a criterion for spectral quality, with 0.1 parts per million (ppm) used as a cut-off (i.e., larger values considered too broad; Kreis, 2004; S. Provencher, personal communication, March 15, 2016). We excluded all spectra with Cramer-Rao lower bound values for creatine, Cr, (i.e., Cr + PCr as measured in LC Model); choline, Cho, (i.e., GPC + PCh); myo-inositol, mI; N-acetyl aspartate, NAA, (i.e., NAA +NAAG); and glutamate, Glx, (i.e., Glu + Gln), higher than 20%, as estimates below 20% are generally used as a criterion for acceptable reliability (estimates greater than 20% indicate that only changes greater than 40% can be detected with reliability; Provencher, 1993). To account for differences in the GM/WM/CSF composition of each voxel, and because our aim was to collect data from the anterior cingulate, a cortical grey matter region, we only used voxels with a proportion of grey matter greater than 40% in our analyses. During this latter stage of quality control, another five participants, those with grey matter partial volumes below 40%, were removed. Finally, one participant was removed from the analyses based on extremely high concentrations of all metabolites, as indicated by observation of their MR spectrum and visualization of boxplots representing all the collected data. Although this could perhaps be attributable to severity of diagnosis,

this participant was removed because the consistency with which metabolite concentrations were elevated led us to believe that movement or improper voxel placement could be responsible for the elevated concentrations. After data cleaning was complete, we removed a total of 11 participants from our analysis, resulting in a final sample of 53.

Missing data. Though our final count of ^{1}H-MRS spectra was 53, not all of these participants completed the clinical measures used in the assessment of research questions #2 and #3. There were 9 participants missing CBCL data, 7 missing CY-BOCS data, and 1 missing PICS-6 data, leaving a total sample with clinical measures of 44, 17 OCD participants (of 24 total), and 28 ADHD participants (of 29 total). The participants with missing clinical measures were included in the analyses to answer research question #1, but not included during the analyses for research questions #2 and #3.

Covariates. We identified covariates separately for the analysis of each research question. This is because the sample being considered, as well as the variables used as predictor and outcome measures, changed based on the analyses being conducted.

Main analyses

Research Question #1: Is there a difference in mean metabolite levels between children with ADHD and OCD, after controlling for potential covariates?

Covariates. In order to determine which measures to control for in our statistical analyses, we assessed the correlations between key predictor variables (i.e., diagnosis, age, gender, FWHM, signal to noise ratio (SNR), and fgm) and our metabolites (i.e., Cr, Cho, mI, NAA, and Glx; see Table 3). Creatine was associated with SNR; Cho with fgm; mI with SNR and fgm; and NAA with FWHM. Thus, each time Cr, Cho, mI, or NAA

Table 3

Zero order correlations of predictor variables with creatine, myo-inositol, choline, N-acetyl aspartate, and glutamate.

	Cr	mI	Cho	NAA	Glx
Pearson's R					
Age	.143	.110	.186	.172	.059
FWHM	.054	.170	-.015	.343*	.130
SNR	.342*	.297*	.191	-.024	.111
fgm	.136	.378**	.279*	.193	.196
Spearman's rho					
Diagnosis	-.218	-.067	.092	-.079	-.151
Sex	.215	-.078	.158	.148	-.003

* $p < .05$, ** $p < .01$

was included in an analysis, the corresponding predictor variable was included as a covariate in the model. There were no associations between Glx and any of the predictor variables. We also conducted independent samples t-tests to assess differences between groups in age, FWHM, SNR, and fgm. There were no differences between groups in FWHM, $t(51) = 0.299$, $p = 0.766$; SNR, $t(51) = 0.089$, $p = 0.929$; or fgm, $t(51) = 0.058$, $p = 0.954$. There was a statistically significant difference between groups with respect to age; the ADHD group was significantly younger than the OCD group, mean difference, M = -2.837, 95% CI [-4.173, -1.501], $t(51) = -4.264$, $p < 0.001$. Since the groups differed in age, we controlled for age in all analyses for research question #1.

Between-group analyses. In order to determine whether groups differed in their ACC concentrations of creatine, choline, myo-inositol, glutamate, and N-acetyl aspartate, we conducted five one-way ANCOVAs. Diagnosis was entered as the independent variable and metabolite concentration was entered as the dependent variable. Each ANCOVA included age as a covariate, as well as any predictors that were statistically associated with the metabolite being considered (see Table 3). There was a statistically significant difference between groups for N-acetyl aspartate, $F(1,49) = 6.086$, $p = 0.017$, partial eta squared = 0.110. The ADHD group (M = 6.94, SD = .880) had higher concentrations of NAA than the OCD group (M = 6.70, SD = .648). The difference in glutamate between groups was not statistically significant, but there was a trend towards significance, $F(1,50) = 3.351$, $p = 0.073$, partial eta squared = 0.06. The ADHD group (M = 7.97, SD = 1.35) had higher concentrations of Glx than the OCD group (M = 7.50, SD = 1.20). There were no differences between groups for creatine, $F(1,49) = 2.45$, $p =$

0.124, partial eta squared = 0.039; mI, $F(1,48) = 0.302$, $p = 0.585$, partial eta squared = 0.006; or choline, $F(1,49) = 0.165$, $p = 0.687$, partial eta squared = 0.003.

Research question 1: Summary of results. There was a statistically significant difference in ACC NAA concentrations, and a trend towards significance for Glx concentrations, between our diagnostic groups. Both metabolites were present in higher concentrations in the ADHD group compared to the OCD group.

Research Question #2: Within groups, what is the relationship between symptom severity and metabolite concentrations in the anterior cingulate cortex?

In order to answer this question, we looked at the OCD and ADHD groups separately. We began by assessing the relationship between predictor variables (i.e., demographics, imaging parameters) and metabolites, and the relationship between demographics and indicators of symptom severity. Where there were significant associations, we controlled for them by including the predictor variable as a control in the subsequent analyses. We conducted Pearson correlations and partial correlations to assess the associations between our metabolites of interest and measures of symptom severity.

Covariates for OCD. We assessed the correlation between our predictor variables (i.e., age, gender, full-width at half maximum, signal-to-noise ratio, fraction of gray matter) and metabolites (i.e., Cr, mI, Cho, NAA, Glx), in order to determine which variables to control for in our analyses (see Table 4). Only associations with demographic variables (i.e., age, gender) were considered for the CY-BOCS outcome measures (i.e., total CY-BOCS score, obsessions score, compulsions score) because there would be no reason to suspect associations between outcome measures and FWHM, SNR, or fgm. We then used Pearson correlations and partial correlations to assess the relationship between

Table 4

Zero order correlations of predictor variables with creatine, myo-inositol, choline, N-acetyl aspartate, glutamate, total CY-BOCS score, obsessions, and compulsions.

	Cr	mI	Cho	NAA	Glx	CYB	OB	CM
Pearson's R								
Age	.348	.182	.247	.275	.301	-.303	-.126	-.272
FWHM	.015	.072	.212	.263	.224			
SNR	.492*	.324	.183	.131	.247			
fgm	.183	.165	.311	.130	.137			
Spearman's rho								
Sex	.242	-.031	.255	.093	-.106	-.538*	-.445	-.332

* $p < .05$, ** $p < .01$

metabolites and measures of OCD symptom severity based on CY-BOCS scores. There was a significant association between anterior cingulate Cr and SNR. The four other metabolites were not associated with any predictor variables. Thus for correlations involving Cr, we controlled for SNR, conducting partial correlational analyses. Total CY-BOCS score was associated with sex, so all correlations between metabolites and total CY-BOCS score were conducted as partial correlations, controlling for sex.

OCD symptom severity. The results of our Pearson and partial correlations are displayed in Table 5. We used total CY-BOCS score, CY-BOCS obsessions score, and CY-BOCS compulsions score as indicators of symptom severity. Creatine was strongly significantly associated with total CY-BOCS score, $r(13) = -.617$, $p = .014$; obsessions, $r(14) = -.534$, $p = .033$; and compulsions, $r(13) = -.626$, $p = .013$. The associations between the remaining metabolites (i.e., mI, Cho, NAA, Glx) and indicators of symptom severity were not statistically significant.

Covariates for ADHD. We assessed the correlation between our predictor variables (i.e., age, gender, FWHM, SNR, fgm) and metabolites (i.e., Cr, mI, Cho, NAA, Glx,) within the ADHD group in order to determine which variables to control for in our analyses (Table 6). Only associations with demographic variables (i.e., age, gender) were considered for the PICS-6 outcome measures (i.e., inattentiveness, hyperactivity) because there would be no reason to suspect associations between these measures and FWHM, SNR, or fgm. There was a significant relationship between anterior cingulate mI and fgm, and between NAA and FWHM. The other metabolites (i.e., Cr, Cho, and Glx) were not significantly associated with any predictor variables. There was also a significant relationship between hyperactivity and age. We thus used Pearson correlation to assess

Table 5

Pearson and partial correlations between metabolites and total CY-BOCS score, obsessions, and compulsions, in children with OCD.

	Cr	mI	Cho	NAA	Glx
CYB	-.617*	-.079	-.336	-.252	-.402
OB	-.534*	-.252	-.291	-.340	-.353
CM	-.626*	-.251	-.374	-.236	-.340

Note: All correlations involving Cr and CYB are partial correlations. For Cr, SNR was included as a control. For CYB, gender was included as a control.
* $p < .05$, ** $p < .01$

Table 6

Zero order correlations of predictor variables with creatine, myo-inositol, choline, N-acetyl aspartate, glutamate, inattentiveness, and hyperactivity.

	Cr	mI	Cho	NAA	Glx	IA	HY
Pearson's R							
Age	.191	.147	.168	.333	.081	.160	-.464*
FWHM	.063	-.060	.153	.376*	.072		
SNR	.271	.281	.196	-.108	.025		
fgm	.114	.493**	.262	.226	.235		
Spearman's rho							
Sex	.175	-.164	.136	.098	.011	-.065	.193

* $p < .05$, ** $p < .01$

the relationship between inattentiveness and Cr, Cho, and Glx. For all correlations involving mI and NAA, we used partial correlation, controlling for fgm and FWHM, respectively. As well, for all correlations between metabolites and hyperactivity, we used partial correlation, controlling for age.

ADHD symptom severity. The results of the Pearson correlations and partial correlations conducted are in Table 7. For the ADHD sample, PICS-6 measures of inattentiveness and hyperactivity were used as markers of symptom severity. We included covariates, and thus conducted partial correlation, for whichever outcome measures were associated with predictor variables (see above explanation). There was a significant moderate correlation between creatine and inattentiveness, $r(26) = -0.401$, $p = 0.034$. The association between choline and inattentiveness, although not statistically significant, suggested a trend towards significance, $r(26) = -0.345$, $p = 0.072$. None of the [five] metabolites were significantly associated with scores on the hyperactivity scale, though the partial correlation between myo-inositol and hyperactivity, controlling for proportion of grey matter and age, suggested a trend towards significance, $r(24) = -.341$, $p = 0.088$.

Research question 2: Summary of results. In the OCD sample, creatine was negatively correlated with CY-BOCS total score, obsessions, and compulsions. None of the remaining metabolites included were significantly related to CY-BOCS measures of symptom severity. In the ADHD sample, creatine was associated with PICS-6 score for inattentiveness. The correlations between choline and inattentiveness, and myo-inositol and hyperactivity, suggested a trend towards significance.

Table 7

Pearson and partial correlations between metabolites, hyperactivity, and inattentiveness in children with ADHD.

	Cr	mI	Cho	NAA	Glx
IA	-.401*	-.241	-.345	-.143	-.034
HY	-.200	-.341	.015	-.040	-.220

Note: All correlations involving HY, mI, and NAA are partial correlations. For HY, age was included as a control. For mI, fgm was included as a control. For NAA, FWHM was included as a control.
* $p < .05$, ** $p < .01$

Research Question #3: Across groups, what is the relationship between metabolites and behavioural problems?

Controls/Covariates. To answer this research question, we began by assessing the relationship between demographic variables (i.e., age, diagnosis, and gender) and CBCL outcome measures (anxious/depressed T score, ADt attention problems T score, ATt; and affective problems T score, APt; Table 8).

There was a significant relationship between attention problems and age, attention problems and diagnosis, and no relationship between anxious/depressed problems or affective problems and the predictor variables. We thus controlled for age and diagnosis in regression analyses assessing the variance in attention problems explained by our metabolites, resulting in a hierarchical regression. For predicting anxious/depressed problems and affective problems, we used linear regression analyses.

Creatine. As shown in Table 9, we conducted three regression analyses to assess creatine concentrations' contribution to the variability in anxiety/depression, affective problems, and attention problems in our sample. We used linear regression to assess whether anterior cingulate levels of Cr could predict anxious/depressed problems scores. Creatine concentrations did not significantly predict anxious/depressed score, $F(1,42) = 2.96$, $p = .093$, and only accounted for 6.6% of the variability in anxious/depressed score. We also used linear regression to assess whether Cr predicted affective problems. Creatine concentration significantly predicted affective problems, $F(1,42) = 7.26$, $p = 0.01$, and accounted for 14.7% of the variability in affective problems scores. To determine if Cr levels could explain variability in attention problems, we used hierarchical regression with age and diagnosis as the first set of predictors, and Cr as the

Table 8

Zero order correlations of demographic variables with anxiety/depression, affective problems, and attention problems.

	ADt	APt	ATt
Pearson's R			
Age	.219	.255	-.453**
Spearman's rho			
Dx	.140	.059	-.622**
Gender	-.039	-.178	.165

* $p < .05$, ** $p < .01$

Table 9

Summary of regression analyses for creatine predicting anxiety/depression, affective problems, and attention problems.

Variable	B	SE B	β	R^2	Δ R^2
Linear Regression					
ADt					
	3.89	2.26	.257	.066	--
APt					
	4.17	1.55	.384**	.147**	--
Hierarchical Regression					
ATt					
Step 1				.427**	--
Age	-.707	.570	-.174		
Dx	-12.2	3.06	-.556**		
Step 2				.431	.004
Cr	-1.05	1.91	-.068		

* $p < .05$, ** $p < .01$

second. The model was significant and accounted for 43.1% of the variance in attention problems, $F(3,40) = 10.1$, $p < 0.001$. In step 1, diagnosis and age accounted for 42.7% of the variability in attention problems, $F(2,41) = 15.26$, $p < 0.001$. Therefore, it can be concluded that the overall model was significant largely due to the inclusion of diagnosis and age as covariates and not due to the metabolite of interest (i.e., creatine). The addition of Cr in step 2 did not significantly change the model, $F_{change}(1,40) = .304$, $p = .584$. An analysis of the standardized beta weights in model 2 indicated that diagnosis significantly added to the prediction of attention problems, $\beta = -.556$, $t(3,40) = -3.97$, $p < 0.001$; though age, $\beta = -.174$, $t(3,40) = -1.24$, $p = .222$; and Cr, $\beta = -.068$, $t(3,40) = -.552$, $p = .584$, did not.

Myo-inositol. The regression analyses with mI as a predictor for anxious/depressed problems, affective problems, and attention problems are shown in Table 10. For anxious/depressed problems and affective problems, we used linear regression, with myo-inositol as the predictor variable. For attention problems, we used hierarchical regression, controlling for the effects of age and diagnosis first, then entering mI into the model. Myo-inositol did not significantly predict the variability in anxious/depressed problems, $F(1,42) = .944$, $p = 0.337$, accounting for just 2.2% of the variability in anxiety/depression scores. The linear regression for affective problems was significant, $F(1,42) = 5.087$, $p = 0.029$; mI accounted for 10.8% of the variance in affective problems scores. Finally, the hierarchical regression was significant, accounting for 43.5% of the variance in attention problems, $F(3,40) = 10.27$, $p < 0.001$. However, in step 1, diagnosis and age accounted for 42.7% of the variance, $F(2,41) = 15.26$, $p < 0.001$. Therefore, the overall model was likely significant as a result of our covariates

Table 10

Summary of regression analyses for myo-inositol predicting anxiety/depression, affective problems, and attention problems.

Variable	B	SE B	β	R^2	Δ R^2
Linear Regression					
ADt					
	3.84	3.95	.148	.022	--
APt					
	6.09	2.70	.329*	.108*	--
Hierarchical Regression					
ATt					
Step 1				.427**	--
Age	-.718	.559	-.176		
Dx	-12.1	2.30	-.552**		
Step 2				.435	.008
mI	-2.45	3.18	-.093		

* $p < .05$, ** $p < .01$

(i.e., diagnosis and age), rather than myo-inositol. The addition of mI increased the fit of the model by 8%, which was not significant, $F_{change}(1,40) = 0.595$, $p = 0.445$. An analysis of the standardized beta weights in model 2 indicated that diagnosis significantly contributed to the prediction of attention problems, $\beta = -.552$, $t(3,40) = -4.03$, $p < 0.001$; though age, $\beta = -.176$, $t(3,40) = -1.28$, $p = .207$, and mI, $\beta = -.093$, $t(3,40) = -.771$, $p = .445$, did not.

Choline. The regression analyses conducted to assess the contribution of Cho to anxious/depressed problems, affective problems, and attention problems are in Table 11. Choline did not significantly predict variance in anxious/depressed problems, $F(1,42) = 3.41$, $p = 0.072$, or affective problems, $F(1,42) = 1.67$, $p = 0.204$. We conducted a hierarchical regression with attention problems as the dependent variable, age and diagnosis as the first set of predictors, and Cho as the second. The model was significant, $F(3,40) = 9.94$, $p < 0.001$, accounting for 42.7% of the variance in attention problems. However, like the hierarchical regressions presented above, choline was not responsible for this result, $F_{change}(1,40) = 0.021$, $p = 0.885$. Comparisons of the beta weights in model 2 confirm this, with diagnosis having a significant contribution to predicting attention problems, $\beta = -.541$, $t(3,40) = -3.92$, $p < 0.001$, but not age, $\beta = -.187$, $t(3,40) = -1.34$, $p = .187$ or Cho, $\beta = -.018$, $t(3,40) = -.145$, $p = .885$.

N-acetyl aspartate. The results of the linear regressions and hierarchical regression conducted, with NAA as the predictor variable, are in Table 12. N-acetyl aspartate significantly predicted the variance in anxious/depressed problems, $F(1,42) = 4.88$, $p = 0.03$, but not affective problems, $F(1,42) = 1.91$, $p = .175$. The outcome of the

Table 11

Summary of regression analyses for choline predicting anxiety/depression, affective problems, and attention problems.

Variable	B	SE B	β	R^2	Δ R^2
Linear Regression					
ADt					
	13.7	7.42	.274	.075	--
APt					
	6.99	5.42	.195	.038	--
Hierarchical Regression					
ATt					
Step 1				.427**	--
Age	-.761	.567	-.187		
Dx	-11.8	3.02	-.541**		
Step 2				.427	.000
Cho	-.901	6.21	-.018		

* $p < .05$, ** $p < .01$

Table 12

Summary of regression analyses for N-acetyl aspartate predicting anxiety/depression, affective problems, and attention problems.

Variable	B	SE B	β	R^2	Δ R^2
Linear Regression					
ADt					
	4.18	1.89	.323*	.104*	--
APt					
	1.93	1.40	.208	.043	--
Hierarchical Regression					
ATt					
Step 1				.427**	--
Age	-.672	.603	-.165		
Dx	-12.2	3.12	-.557**		
Step 2				.430	.003
NAA	-.768	1.72	-.058		

* $p < .05$, ** $p < .01$

hierarchical regression used to predict variance in attention problems follows the same trend as the metabolites discussed above. The model was significant, $F(3,40) = 10.04$, $p < 0.01$, but the addition of NAA to the model was not responsible, $F_{change}(1,40) = .199$, $p = .658$ for this. Analysis of the beta weights for diagnosis, $\beta = -.557$, $t(3,40) = -3.91$, $p < 0.001$, age, $\beta = -.165$, $t(3,40) = -1.12$, $p = .272$, and NAA, $\beta = -.058$, $t(3,40) = -.447$, $p = .658$, reveal that only diagnosis contributed significantly to the prediction of attention problems.

Glutamate. The results of the linear regressions and hierarchical regression using Glx as a predictor for anxious/depressed problems, affective problems, and attention problems are in Table 13. Concentrations of cingulate Glutamate did not significantly predict the variance in anxious/depressed problems, $F(1,42) = 2.38$, $p = 0.13$, but significantly predicted variance in affective problems, $F(1,42) = 10.27$, $p = 0.003$. The hierarchical regression for attention problems, with predictors diagnosis and age in the first step, and then Glx in the second step, was significant, $F(3,40) = 11.45$, $p < 0.001$. Again, this was not attributable to the addition of Glx to the model, $F_{change}(1,40) = 2.62$, $p = 0.113$. This was confirmed with analysis of the beta weights for diagnosis, $\beta = -.588$, $t(3,40) = -4.32$, $p < 0.001$; age, $\beta = -.149$, $t(3,40) = -1.10$, $p = .277$; and Glx, $\beta = -.193$, $t(3,40) = -1.62$, $p = .113$; for which only diagnosis was significant.

Research question 3: Summary of results. Creatine, myo-inositol, and glutamate explained significant variance in scores on the affective problems syndrome scale of the CBCL. N-acetyl aspartate predicted scores on the anxious/depressed syndrome scale of the CBCL.

Table 13

Summary of regression analyses for glutamate predicting anxiety/depression, affective problems, and attention problems.

Variable	B	SE B	β	R^2	Δ R^2
Linear Regression					
ADt					
	2.03	1.31	.232	.054	--
APt					
	2.78	.866	.443**	.196**	--
Hierarchical Regression					
ATt					
Step 1				.427**	
Age	-.607	.551	-.149		
Dx	-12.9	2.98	-.588**		
Step 2				.462	.035
Glx	1.73	1.07	-.193		

* $p < .05$, ** $p < .01$

Although the hierarchical regressions for creatine, myo-inositol, choline, N-acetyl aspartate, and glutamate predicting scores on the attention problems syndrome scale were all significant, this was attributable to the inclusion of diagnosis as a predictor in the models. The metabolites themselves did not significantly contribute to the prediction of scores on the attention problems syndrome scale, as confirmed by examination of their beta weights.

CHAPTER V

DISCUSSION

Review of Results

The purpose of the present study was to investigate similarities and differences, particularly in the metabolite concentrations in the anterior cingulate cortex, in two groups of children with neurodevelopmental disorders through the use of ^{1}H-MRS imaging techniques. First, this study assessed difference in the concentration of metabolites in the anterior cingulate of children with ADHD and OCD. Second, we examined the relationship between metabolites concentrations and symptom severity in each of these groups individually, which has been studied in previous literature with disparate results. Finally, we explored the relationship between symptom dimensions and metabolites in children with OCD and ADHD. This research controlled for factors such as spectral quality/acquisition, proportion of grey matter in the ACC voxel, and age, in each of the statistical analyses conducted.

Metabolite differences between groups. To our knowledge, this is the first ^{1}H-MRS study to look at children with ADHD and OCD together. Specifically, this study responds to the call for interdisciplinary research of neurodevelopmental disorders by the NIMH (NIHM, 2007). The current study revealed statistically significant differences in N-acetyl aspartate concentration, and a trend towards statistical significance for differences in glutamate, between children with ADHD and OCD. The ADHD group had higher concentrations of both N-acetyl aspartate and glutamate, relative to the OCD group. NAA is a marker of neuronal integrity, so perhaps this difference suggests a relative increase in neuronal integrity in the ADHD group, compared to those with OCD.

As mentioned, our ^{1}H-MRS study is the first to examine ADHD and OCD directly, making it difficult to interpret our findings in the context of the existing literature. Each group has been compared with healthy controls independently, and, though the findings are still preliminary, children with ADHD and OCD are generally found to have higher metabolite concentrations than controls in the frontal cortices. In childhood ADHD, prefrontal cortex glutamate/creatine ratios are higher than controls (Carrey et al., 2003; MacMaster et al., 2003). Higher NAA in the PFWM and decreased glutamate in the ACC have been reported in studies comparing children with OCD and healthy controls (Rosenberg et al., 2004; Weber et al., 2014). Thus our findings may represent metabolite differences along a continuum. If controls were included in our analysis, perhaps we would find higher NAA and glutamate concentrations for ADHD versus controls and OCD versus controls, with concentrations in the ADHD group higher than both the OCD and control group (i.e., ADHD > OCD > controls). Of course, this is merely speculative and requires further work to be understood.

Future research could combine 1H-MRS with diffusion tensor imaging (DTI) and resting-state fMRI (to elucidate functional connectivity) methods, to determine whether the relatively higher neuronal integrity (as indicated by higher NAA concentrations) in ADHD is associated with greater structural and/or functional connectivity in the ACC. Such a framework was employed in a study comparing fractional anisotropy (FA) and metabolite concentrations in the medial temporal white matter, in patients with schizophrenia (Tang et al., 2007). Tang and colleagues (2007) found reduced FA in the right and left medial temporal white matter, reduced NAA in the right and left medial temporal white matter voxels, and a correlation between FA and N-acetyl aspartate on the

left side. Based on the positive correlation between NAA and FA in schizophrenia, higher N-acetyl aspartate concentrations in ADHD may suggest greater white matter connectivity. However, this is notion is inconsistent with some functional imaging studies in children with ADHD, which have generally reported decreased activation of areas involved in cognitive control; including the anterior cingulate gyrus (Bush et al., 1999; Durston et al., 2003; Rubia et al., 2005). In contrast, another study that examined resting-state functional connectivity of the default mode network, children with ADHD had lower functional connectivity in the ACC, posterior cingulate cortex, and lateral prefrontal cortex; but *higher* functional connectivity in the posterior medial frontal cortex (Qiu et al., 2011). The medial frontal cortex includes the dorsal ACC (i.e., cognitive division of the ACC), and thus it is may be reasonable to suggest our ACC voxel would be associated with higher functional connectivity (Yucel et al., 2007). A review of DTI studies in OCD reported that pediatric OCD populations generally display increased FA in a number of regions, including the cingulum, corpus callosum, corticospinal tract, and frontal-occipital fasciculus, relative to healthy controls (Koch, Reeß, Rus, Zimmer, & Zaudig, 2014). Koch and colleagues emphasized that the limited number of DTI studies in pediatric OCD do not yet allow one to draw substantial conclusions about the white matter alterations in this population. These preliminary DTI studies are, however, consistent with the above hypothesis that N-acetyl aspartate concentrations in the ACC (and thus, white matter connectivity) decrease from ADHD to OCD to healthy control populations.

Correlations between metabolites and symptom severity. In the OCD group, ACC creatine levels were associated with total CY-BOCS score, CY-BOCS obsessions

score, and CY-BOCS compulsions score. The other metabolites we examined were not associated with CY-BOCS scores in OCD. In the ADHD group, creatine was associated with PICS-6 score for inattentiveness, and the correlations between choline and inattentiveness, and myo-inositol and hyperactivity, approached significance. It is important to remember that the sample size in each investigation was low (n = 17 for OCD, n = 24 for ADHD) and our analyses were, in turn, underpowered. Additionally, we used the PICS-6 as a measure of inattentiveness and hyperactivity in ADHD, which is a new scale that is still in the process of being validated (A. Iaboni, personal communication, March 28, 2016).

The correlation between creatine and CY-BOCS scores in OCD is interesting; creatine levels generally remain constant, which is why many ^{1}H-MRS studies report metabolite concentrations as a ratio of creatine (Bertholdo et al., 2013; Soares & Law, 2009). At least one other ^{1}H-MRS study has reported creatine levels that were not constant across participants. In a follow-up study to their investigation of ACC metabolites in OCD, MDD, and controls, Mirza and colleagues (2006) reported higher creatine concentrations in the thalamus in OCD, relative to both comparison groups. More recently, Weber and colleagues (2014) reported positive associations between CY-BOCS scores and creatine, myo-inositol, and N-acetyl aspartate in the prefrontal white matter. The current study revealed *negative* associations between ACC creatine and CY-BOCS scores; meaning that lower creatine was associated with higher severity of OCD symptoms in our sample. Creatine is a reservoir for phosphates used in the generation of ATP, the body's main source of energy, such that creatine concentration is akin to "energy metabolism" (Drost et al., 2002; Soares & Law, 2009). Although it is unclear

why the directionality of the creatine X CY-BOCS association is opposite to that of the aforementioned studies, which suggest greater creatine with worsening OCD symptoms, perhaps our findings represent a strain on regular energy metabolism due to the repetitive nature of thought patterns and behaviour in individuals with OCD.

Similarly, it is difficult to understand the association between creatine and PICS-6 inattentiveness in the ADHD group. A previous study reported elevated creatine, glutamate, and glutamine + glutamate concentrations in children with ADHD, relative to controls, and decreases in creatine concentration following successful treatment of ADHD symptoms (Carrey et al., 2007). The negative association between creatine and inattentiveness in our study suggests that children with greater inattentive symptoms actually have lower concentrations of creatine. This, again, is difficult to interpret in comparison to other studies, due to our lack of control population. As mentioned previously, the dACC is essential for the proper functioning of fronto-straital attention networks (Bush et al., 1999). A functional imaging study of children with ADHD reported lower recruitment of the prefrontal cortex and ACC, and greater recruitment of the parietal and occipital cortices, during a go-no-go task (Durston et al., 2003). Therefore, perhaps the children in our study who had greater attention deficits (i.e., higher inattention, as indicated by CBCL score) also have problems recruiting the ACC for tasks requiring attention, and thus, have lower energy demands and lower creatine concentrations in this region, explaining the negative association.

Contribution of metabolites to variance in symptom dimensions. Creatine, myo-inositol, and glutamate concentrations explained variance in scores on the affective problems syndrome scale of the CBCL. N-acetyl aspartate significantly predicted scores

on the anxious/depressed syndrome scale of the CBCL. ^{1}H-MRS studies of adults and children with major depressive disorder have reported alterations in N-acetyl aspartate relative to controls, with reductions in the medial frontal cortex and anterior cingulate cortex (Gonul et al., 2006; Olvera et al., 2010). The regression model with N-acetyl aspartate as a predictor for scores on the anxious/depressed subscale of the CBCL had an R^2 value of 0.104, which suggest a positive relationship between metabolite concentrations and severity of anxious/depressed symptoms. This seems inconsistent with the finding that N-acetyl aspartate is reduced in depressed versus non-depressed individuals, though it is difficult to compare because our measure includes anxiety problems, and we do not have data on controls for comparison. Similarly, Rosenberg and colleagues (2004) reported reduced glutamate concentrations in the ACC of their OCD and MDD sample, while we found a positive association between glutamate and scores on the affective problems scale of the CBCL.

Strengths, Limitations, and Implications

There were several strengths associated with the current study. Notably, this study was the first to compare metabolite concentrations in the ACC between two different groups of children with neurodevelopmental disorders. This study was also the first to look at the relationship between metabolite concentrations and symptom dimensions, where previous literature only focused on the relationship between metabolites and symptom severity in one clinical population. The current study was designed as a response to the call for interdisciplinary research to be conducted on individuals with neurodevelopmental disorders by the NIMH and the RDoC framework (Insel et al., 2010; NIHM, 2007). By studying children with ADHD and OCD together, we found support for similarities,

across groups, in the relationship between metabolites in the ACC and underlying behaviour problems (i.e., research question #3). Our findings, though preliminary, suggest that a complex interplay between brain and behaviour contributes to the heterogeneous nature of neurodevelopmental disorders. This re-iterates the need for treatment regimens that target impaired processes, rather than just diagnostic categories, in those with neurodevelopmental disorders (NIHM, 2007).

Despite the strengths of the present study, it is important to discuss its limitations. First, because there were so many variables included and controlled for in our analyses, it would have been useful to have a larger sample size in order to increase our statistical power. Many of the participants in our study were missing scores on some of the clinical measures used in our analyses. This was probably related to the multi-step nature of the POND intake procedure, with participants completing the questionnaires, interviews, and imaging at different points in time (i.e., not done questionnaires when we collected imaging data). It is important to note that the POND imaging study is ongoing and children in both populations are still being actively recruited. This will likely result in a larger and more robust sample size in the future, and elicit further investigation that will, ideally, confirm the findings of this thesis (and turn trends into significant findings).

Another limitation is that our demographic groups varied with regard to age and gender distribution. The difference in age between groups was significant, with the OCD sample older than the ADHD sample; thus age-related changes in metabolite concentrations could have influenced our findings. However, while significant age-related changes exist throughout the first year of life, by age 2 spectral patterns mimic that of adults and by age 4, adult metabolite concentrations have been established

(Bertholdo et al., 2013). So it is unlikely that the disparities between OCD and ADHD were attributable to differences in brain maturity alone.

Future Directions

This study opens to the door for many future investigations. One possibility for future work is to include a more diverse sample of patients with neurodevelopmental disorders, to be studied in conjunction with our ADHD and OCD groups. This could help elucidate more complex sub-categories of neurodevelopmental disorders, based on their metabolite abnormalities.

In the ^{1}H-MRS literature, there is often a disparity between the findings of adult versus child investigations. This is, at least partly, due to the ongoing maturation of the brain throughout childhood. In order to understand the changes in metabolite concentrations, and their relationship to behavior, over time, it would be very useful to conduct a longitudinal ^{1}H-MRS study following children with neurodevelopmental disorders into adulthood. A longitudinal study would glean information not just on the metabolite changes associated with brain maturity, but also the effects of medication treatment over time.

It would also be valuable to incorporate additional imaging methods in order to elucidate more information about the metabolite abnormalities we observed. Particularly, resting-state fMRI and DTI could be used to understand the relationship between metabolite abnormalities and functional and structural connectivity in the brain. Consistent with the RDoC framework, this would further enhance our understanding of the path from etiology to brain to behaviour in neurodevelopmental disorders (Insel et al., 2010; NIHM, 2007).

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