Spring 5-15-2019

Typical and atypical development of the brain's functional network architecture

Ashley Nicole Nielsen
Washington University in St. Louis

WASHINGTON UNIVERSITY IN ST. LOUIS

Division of Biology and Biomedical Sciences
Neurosciences

Dissertation Examination Committee:
Bradley Schlaggar, Chair
Deanna Barch
Deanna Greene
Jonathan Peelle
Steven Petersen
Rebecca Treiman

Typical and Atypical Development of the Brain's Functional Network Architecture

by
Ashley Nielsen

A dissertation presented to
The Graduate School
of Washington University in
partial fulfillment of the
requirements for the degree
of Doctor of Philosophy

May 2019
St. Louis, Missouri

Table of Contents

List of Figures

List of Tables

Acknowledgments

Just as the development of mature thinking requires the coordination of many neural components and cognitive capacities, the development of this thesis work has been supported by the coordinated efforts of many, many people who deserve to be acknowledged. Removing any of these people from my life would have greatly delayed my growth as a scientist, a teacher, and a happy human. Here, I attempt to convey how truly grateful I am for all the help and guidance I have received while at WashU.

First, I need to thank my thesis advisor, Brad Schlaggar. Brad was my very first interview for graduate school, but the primacy effect alone is not sufficient to explain why I decided to join his lab. It was clear that, in being Brad's graduate student, I would have the opportunity to study both clinical and basic scientific questions about the organization of the brain in a top-notch environment. Throughout my time in the lab, Brad has given me incredible freedom to follow my own curiosity (even sometimes into "rabbit holes") which was critical to the work in this thesis and my development as a scientist. Even though Brad was often busy, dividing his time between research and the clinic, his task-switching abilities are impressively effective; after not meeting for a couple weeks, Brad would remember exactly what had been previously discussed and have insightful questions or suggestions for new findings. As a mentor, Brad has always been on my side—even when I was wrong, he would walk through my thinking and gently point out my mistakes. During difficult times in my family life, Brad was unreservedly understanding. Brad's extraordinary scientific and leadership abilities did not go unnoticed, and he was selected to run the Kennedy Krieger Institute in Baltimore. While this change did concern me at first, I found that Brad's mentorship style had empowered

me to be largely independent and self-sufficient even after the move. I am so thankful for the opportunities and experience that Brad has provided me in pursuing my PhD.

While Brad is my official mentor, Deanna Greene has been my behind-the-scenes, day-to-day mentor, and without her, this thesis work would surely not have been completed. Deanna was a significant factor in my decision to join the lab; she filled in any gaps and was always available when I knocked on her door for a quick question. Because of these constant interactions, Deanna is often my interpreter for Brad and Steve, deciphering my unconsciously opaque ramblings. When my other projects ran into road blocks, she generously offered me the opportunity and necessary guidance to explore the data that she had arduously collected from children and adults with Tourette syndrome—this work is now a central portion of this thesis. As a scientist, Deanna is a fantastic writer and an even better editor; I have learned a lot about clear and concise writing from her such that each subsequent manuscript I have written has been a bit less painful. Deanna has spent a ridiculous amount of time mentoring me, especially considering that I am not her direct responsibility. She is a fantastic model of a female scientist balancing work and family. Her only flaw is her knack for picking the losing contestants in the lab's competitive Bachelor Fantasy League (see below). In all seriousness, I am so grateful that I have been able to have Deanna as both a mentor and a friend.

Mark Wheeler, my undergraduate mentor and one of Steve Petersen's previous mentees, told me that he couldn't put into words exactly what he had learned from Steve, but was certain that it made him a better scientist. Now, with my own experience, I can corroborate this statement. Even by simply observing Steve discuss science, I feel that I have learned a lot about evaluating and designing experiments. Steve is a careful

scientist—always skeptical at first, but also excitable when results pass muster and are truly “cool”. Steve is also a great storyteller, both inside and outside of science. He brings this storytelling to the classroom and effectively communicates complex information to freshman undergrads and worse, half-asleep first year graduate students. I have learned a lot from Steve about teaching strategies and developing detailed, but digestible lectures. When I began graduate school, my data visualization skills were fairly poor and often my figures did not communicate the message I was intending. Early on, Steve (loudly) let me know that this needed to change. Thanks to his instructions, my ability to visualize and communicate data has greatly improved. I am actually grateful that Steve provides his unfiltered opinion on my work; while the criticism stings at the time, eventually, I find that I agree and that the work is strengthened by his suggestions.

Beyond these key mentors, this thesis work has also been devotedly supported by my amazing thesis committee, Deanna Barch, Jonathan Peelle, and Rebecca Treiman. My committee has celebrated my successes and supported me during delays and detours. Deanna is uncontested the best thesis chair one could ask for; she often reminds *me* when we need to set up my next update meeting. She’s provided me lots of additional advice on manuscripts, post-docs, and the job market. Jonathan, a science twitter tycoon, an avid advocate for women in science, and a thoughtful neuroscientist, has provided helpful encouragement when challenges arise and ensured my successes were applauded. I’d like to thank Becky for sticking with me, even as the portion of my thesis devoted to language development, her area of expertise, dwindled away. I couldn’t have asked for a better group of people to help me achieve the goals in this thesis.

Beyond my thesis committee, there are many other members of the collective Petersen/Schlaggar/Dosenbach/Greene Labs that I would like to thank. First, my former officemate, Caterina Gratton, played a crucial role in developing many of the hypotheses and analyses in this thesis. Caterina has an amazing ability to patiently listen to the issues in a project that she isn't a part of and then provide insightful comments and suggestions; I took advantage of this ability often. She also was essential in breaking into the "boys club" in the lab which is something I'll always admire. While her replacement, Scott Marek, isn't nearly as awesome, he makes up for these shortcomings by being a great friend, Disney partner, Bachelor co-conspirator, and Lord of Lameness. Ben Seitzman, the little brother I never had, played an important part in my graduate school experience by annoying me with spoken lyrics and bad puns. I'd also like to thank Becky Coalson, my crafting idol, for the sunny attitude that she brings to everything she does and everyone she meets. While definitively not as sunny, Fran Miezen and Tunde Adeyamo were also vital in helping me get started on the technical side of research. Both current and former members of the lab including Haoxin Sun, Timothy Laumann, and Evan Gordon have shaped my thinking about the functional organization of the brain and how to study it. Lastly, administrators in and adjacent to the lab including Carmen Horn, Laura Williams, and Melissa O'Sullivan were vital to maintaining my happiness and well-being throughout my time in the lab.

Outside of the lab, there are many people in the Neuroscience program at WashU who facilitated my growth as a neuroscientist. While she may call me "Amy" by mistake, Sally Vogt has been a constant ray of sunshine in my graduate career. From the moment she called to offer me my acceptance to WashU to the moment I submitted my intent to

graduate, Sally has always been open and available to chat about St. Louis, siblings, cats, and life in general. I'd also like to thank Larry Snyder for mentoring me as a teaching assistant for the Neural Systems course. Teaching Neural Systems was challenging, but highly rewarding and is a highlight of my time at WashU. I learned a lot from Larry and the other professors in the course about how (and how not) to structure a lecture, exam, recitation, or discussion. Being able to play a part in developing the curriculum of a course will surely benefit me as I go on to design my own courses in the future.

Graduate school can sometimes get you down, and this thesis would not have been possible without a few distractions. From my very first week in St. Louis, I have been playing competitive (and some not so competitive) softball. Thanks to all my teammates on the Potential for Action and of course the two-time reigning champions, the Mighty Homunculi for all the good times in the St. Louis summer and the generally good throws to first base. Harry Potter Book Club also provided a necessary distraction; this group of graduate students re-read each book, discussed, played silly HP-related drinking games, and ended up in Harry Potter World at Universal Studios in Orlando. I'd like to thank all the founding HP nerds for indulging my nerdiness. On the other end of the nerdiness scale, the Bachelor Fantasy League co-created by Carmen Horn, Deanna Greene, Scott Marek, and myself, has provided me just the right amount of drama, sappy romance, and pointless competition. At the beginning of my fourth year of graduate school, research wasn't progressing as smoothly as I'd hoped. A few of my friends, Tabbetha Bohac and Allison Soung, convinced me to sign up for my first 5K—it was terrible, but I realized that I could make progress in running, something I needed when research was slow. We then started a "Running Club" racing at least a 5K every month. In the last 2.5 years, I've raced

~260 miles, culminating in my first full marathon earlier this year. To fund some of these ventures, I was fortunate to have many opportunities to donate my mind (and limbs) to science. Through experiments in the Dosenbach and Culver labs, my brain has been imaged over 200 times and I have spent *at least* 2 full days (48 hours) in a “resting-state”.

Finally, I want to thank the family and friends who were there for me throughout the highs and lows of graduate school. First, I was fortunate to have an incredible cohort of Neuroscience graduate students; the “super six”, consisting of Krissy Sakers, Alex Russo, Amy Clippinger, Wade Self, Andrew Fishell, and myself, were an important group of people that I could always count on when I needed a break from lab. A notable standout is Andrew Fishell. I feel so lucky that Andrew and I (a.k.a. “SYSTEMS”, a beautiful 70’s couple, NOT twins) became friends. Through grad school, we’ve shared so many laughs, secrets, tears, awkward moments, and angry tyraids over coffee at Kaldi’s or drinks at the Pagan. I can’t imagine how I would have survived the insanity that is graduate school without him. I also would be remiss if I didn’t acknowledge that my happiness as a graduate student would have been suboptimal without the snuggles and company of my cat, Callie (short for Calcarine). I want to thank my sisters Amanda Nielsen and Sarah Nielsen for checking up on me, visiting me, and laughing with me throughout graduate school; while I don’t think they understand what I do (teaching? talking to children? magnets?) or how it contributes to society, they pretend they do, which I appreciate. Even though my father passed away while I was in graduate school, I know that the completion of my PhD would have been one of his “proud papa” moments and he would have bragged about these accomplishments to anyone that would hear them. Last but not least, I want to thank my mom for everything. She’s the first one I call when I have had a

bad day in lab and she humors me when I describe, in detail, the difficulties in calculating frame-wise displacement or something equally as boring. I would never have been able to complete this thesis work if not for her emotional support. I am so grateful for all the people in my life who made my graduate school experience feel shorter, easier, and more manageable.

Ashley Nielsen

Washington University

May 2019

ABSTRACT OF THE DISSERTATION

Typical and Atypical Development of the Brain's Functional Network Architecture

by

Ashley Nicole Nielsen

Doctor of Philosophy in Biology and Biomedical Sciences

Neurosciences

Washington University in St. Louis, 2019

Professor Bradley Schlaggar, Chairperson

The human brain is a complex organ that gives rise to many behaviors. Specialized neural regions cooperate as functional networks that form an intricate functional architecture. Development provides a unique window into how brain functioning and human thinking are affected if the necessary neural features and connections are not fully formed. Similarly, developmental disorders can shed light on atypical trajectories of neural systems that may lead to or be a consequence of symptomatic behavior. A description of the typical and atypical development of functional networks is essential to identify the features of brain organization critical for mature human thinking and to provide better diagnosis, treatment, and prognosis in neurodevelopmental disorders. Recently, resting-state functional MRI has been found to illuminate functionally related regions, giving access to functional networks and the organization of brain's functional architecture. This thesis aims to harness resting-state functional connectivity to explore how functional networks coordinate over the course of development. First, I present our work investigating the organizing principles of typical developmental patterns in functional

networks (Chapter 2). Then, I apply these approaches to the atypical development of functional networks in Tourette syndrome (TS), a developmental disorder characterized by motor and vocal tics. In this work, we tested whether the patterns in functional networks that distinguish individuals with TS from controls differ between children and adults and alter the typical developmental pattern of functional networks (Chapter 3). Lastly, I present our work to identify and describe the coordination of specific functional networks that develop atypically in TS (Chapter 4).

Chapter 1: Introduction

1.1 Why study development?

Humans have incredible potential. Individuals can design breathtaking skyscrapers, predict the future to minimize financial risk, observe and comprehend the movement of the tiniest of particles, or perform incredible physical feats (e.g., run a marathon). What gives individuals these incredible abilities? All humans begin life without these abilities as seemingly helpless and naive. Over a prolonged developmental course, human infants grow and hone many sets of abilities to perform specialized functions. Studying development and, specifically, developmental cognitive neuroscience is valuable for (1) answering basic science questions about human capabilities (How does a functioning human come to be? What are the necessary parts? How are those parts put together?) and (2) addressing clinical questions surrounding the diagnosis and treatment of developmental disorders (How can developmental processes go awry? How can the necessary parts be put back together?).

1.1.1 Brain development and the emergence of behavioral abilities

From a neuroscience perspective, complex human thinking is supported by the properties and organization of the nervous system. The brain can be investigated at many different levels of complexity: molecules, synapses, cells, circuits, areas, and systems (Churchland and Sejnowski, 1988). Each of these components exhibit distinct specialized properties that contribute to complex human thinking. Many of the neural precursors for the mature brain are established at birth, but change to varying extents across development.

While the birth and migration of most all the neurons present in the mature brain occurs prenatally (Spreen *et al.*, 1995; Eriksson *et al.*, 1998), the functional and morphological properties of these cells change over the course of development. Myelination of the axons of both projection and local circuit neurons continues into adolescence (Giedd *et al.*, 1999) potentially enabling more efficient communication. At birth, there is a rapid burst of synapse formation across cortex that lead to a density of synapses that surpasses the mature brain (Rakic *et al.*, 1986; Huttenlocher, 1990; Webster *et al.*, 2011). Synapses are then carefully pruned from birth to age 3-4 (Huttenlocher, 1990; Paolicelli *et al.*, 2011). The rate of synapse production and subsequent pruning varies by brain region (Gogtay *et al.*, 2004). Spontaneous and evoked activity are important for selecting the synaptic connections that persist to form specialized circuits and functional areas (Katz and Shatz, 1996; Bé and Markram, 2006). The strength of these synapses (mediated by vesicles, receptors, resting-potential, etc.) continues to turnover over the course of development (Puro *et al.*, 1977; Ruffolo *et al.*, 1978). For many functional areas, responses during behavioral tasks differ between childhood and adulthood (Johnson, 2011). However, how the functional systems of the brain change over the course of development remains poorly understood.

From a psychological perspective, complex human behavior is supported by many different cognitive processes including memory, attention, perception, action, language, executive function, and decision-making. These cognitive entities each contribute to mature human thinking but do not necessarily map directly onto brain systems and are rarely enlisted alone. Behaviors that are precursors of adult-like behavioral abilities can be observed shortly after birth. Extensive work in the field of developmental psychology

has been devoted to tracking the emergence and specialization of behavioral abilities from infancy to childhood.

Cleverly designed experiments in infants and toddlers have provided evidence that humans are endowed with a set of core capacities in many functional domains. Infants demonstrate the specialized abilities to distinguish different phonemes (Werker and Lalonde, 1988), discriminate faces (Pascalis *et al.*, 2002), and understand numerical magnitude (Xu and Spelke, 2000) before extensive experience with these stimuli. However, these abilities become more specialized over the course of development. At 6 months phoneme discrimination is best tuned to the infant's own language (Kuhl *et al.*, 1992), at 9 months face recognition is better for faces that are racially similar to the infant's caregivers' faces (Kelly *et al.*, 2007), and at 10 months infants can more precisely discriminate magnitude (1:2 at 6 months, 2:3 at 10 months, 7:8 in adulthood) (Feigenson *et al.*, 2004). Flexible skills and knowledge systems build upon these core foundations over the course of development. As an example, reading requires the coordination of several core capabilities such as attention, visual discrimination, and language skills and their refinement and specialization over the course of development (Schlaggar and McCandliss, 2007).

1.1.2 Theories of developmental cognitive neuroscience

The question of how such specialized neural circuitry and psychological functions arise is debated by both neuroscientists and psychologists. There are several hypotheses about how developmental differences in the brain might be related to developmental differences in the performance of different behaviors (for further detail see Johnson et al.

2001). These theories also have important implications for the etiology of aberrant behavioral abilities in developmental disorders.

Maturation. Some argue that the emergence of a behavioral ability can be attributed to the maturation of a new brain region (e.g., endogenous control of eye movements requires maturation of frontal areas (Johnson *et al.*, 1998), successful retrieval of a hidden object requires maturation of frontal lobes (Diamond and Goldman-Rakic, 1989)). Before this region matures, behavioral performance is poor and comparable to adults who acquire lesions to this region. According to this perspective, purely internal mechanisms of developmental change (e.g., genetics, spontaneous activity) drive the emergence of behavior.

Functional Specialization. Others argue that developmental differences in the brain involve a process of organizing the interactions among specialized regions through experience (e.g., face processing in the fusiform face area (Arcaro *et al.*, 2017), inhibitory control in the prefrontal cortex (Casey *et al.*, 1997)). Initially, the functional role of different regions is poorly defined, and regions are partially and inefficiently activated under many behavioral contexts. The onset of new behavioral competencies during development is thought to be associated with changes across several regions. In contrast to the maturational perspective, this viewpoint suggests that during development, the neural architecture underlying a behavioral task might differ or be more extensive than that observed in adulthood. According to this perspective, both intrinsic and experience-dependent mechanisms of developmental change drive the emergence of mature thinking.

Skill acquisition. Neuroimaging evidence from adults suggests that changes in the neural underpinnings of a behavior can result as a consequence of practice and acquiring expertise (e.g., “greeble” processing in the fusiform face area (Gauthier *et al.*, 1999; Gauthier and Nelson, 2001)). One hypothesis is that this type of skill acquisition occurs in development as well. In contrast to the functional specialization hypothesis, this perspective suggests that developmental differences in the brain are experience-dependent rather than experience-expectant. If true, developmental differences in the brain are not special and will mimic differences observed during skill learning in adults.

Each of these hypotheses have important implications for the study of atypical development. According to the maturational perspective, if a developmental disorder is associated with the disrupted maturation of a brain region, this atypicality leads to symptomatic behavior. Atypical functional specialization might arise from intrinsic and/or experience-dependent mechanisms and suggest that the neural underpinnings of an atypical behavior may differ from healthy controls even if performance is equivalent in a developmental disorder. Finally, atypical developmental differences in the brain might be a consequence of absent or augmented skill acquisition; experience with symptoms of the developmental disorder might produce compensatory or maladaptive changes to the functional architecture of the brain.

1.1.3 Motivation for this thesis work

A main objective of this thesis is to study the development of the functional systems of the brain. Currently, very little is known about brain development at the level and complexity of functional systems. As suggested above, the emergence of different

functions might rely upon interactions among specialized areas. Thus, understanding the development of functional systems might be the closest link to the development of complex behaviors (Johnson, 2001). In this thesis I aim to describe principles of the development of functional systems and to contextualize these developmental differences with 1) the brain development that occurs at other levels of complexity, 2) the ways in which behavioral abilities build upon each other, and 3) the theories of how these developmental differences might arise in the brain.

A second arm of this thesis applies our understanding of the typical development of functional systems to questions surrounding developmental disorders. Do developmental disorders disrupt typical developmental processes? To what extent? Can developmental status of the brain be useful for diagnosis or prognosis? While studies of typical development in isolation can detail how the developing brain contributes to mature thinking, studies of atypical development are also important and can illuminate the developmental changes necessary for the development of certain behaviors. Here, I investigate the development of functional systems in Tourette syndrome, a pediatric onset movement disorder characterized by motor and vocal tics, in order to understand the neural architecture supporting motor function and inhibitory control.

1.2 Using resting-state fMRI to study development of functional systems

1.2.1 Resting-state functional connectivity reflects the underlying functional architecture of the brain.

Neuroscientists have been mapping the functional systems of the human brain since the advent of neuroimaging. Areas that support similar functions (i.e., a functional system) can be identified by carefully designing tasks for participants to perform in a PET or MRI

scanner (e.g., Petersen *et al.*, 1988). In the seminal study by Biswal et al. (1995), participants performed finger tapping in the scanner and the regions associated with these movements (M1, S1, pre-motor cortex) could be identified by related fluctuations in the blood-oxygen level dependent (BOLD) signal during epochs of finger tapping. Interestingly, Biswal et al. observed that these same regions and other regions associated with the motor system (e.g., putamen, cerebellum) shared related fluctuations at rest, when participants were not moving. This correlated intrinsic activity, dubbed functional connectivity, is thought to illuminate functionally related regions in a task-free, "resting-state" (Biswal *et al.*, 1995).

By expanding this correlational approach to the whole brain, resting-state functional connectivity has been used to illuminate different features of the functional network architecture in the human brain. Functional areas, regions of the brain defined by similar functional, architectonic, connectivity, and topographic properties, can be localized by identifying pieces of cortex with relatively uniform functional connectivity that is distinct from adjacent pieces of cortex (Cohen *et al.*, 2008; Gordon *et al.*, 2016). Functional systems can be located using functional connectivity and graph theory, a branch of mathematics devoted to the study of complex networks. In graph theory, networks are typically represented as a set of well-defined nodes (here, functional areas) that are connected by edges (here, functional connections) that form densely interconnected communities (here, functional systems) (Petersen and Sporns, 2015). Community detection algorithms have been used to identify functional networks in resting-state functional connectivity data that resemble previously identified functional systems (e.g. visual, default mode) (Power *et al.*, 2011; Yeo *et al.*, 2011). Functional networks

identified with functional connectivity include “processing” networks that interface with the external world (somatomotor, auditory, visual), “control” networks that direct attention and perform different executive functions (fronto-parietal, cingulo-opercular, dorsal attention, ventral attention, salience), and “other” cortical association networks (default mode, parietal memory, context memory, reward). The basal ganglia, thalamus, and cerebellum also have non-uniform connections with different functional networks in cortex (Barnes *et al.*, 2010; Choi *et al.*, 2012; Greene *et al.*, 2014) and work is ongoing to parcellate and delineate the role of different subcortical structures in functional network organization.

Beyond parcellating the brain into areas and dividing the brain into functional networks, resting-state functional connectivity has revealed several additional properties of the organization of neural systems in the human brain. A region’s functional connectivity to the rest of the brain at rest can be predictive of how it will respond under different conditions (Gratton *et al.*, 2017). When sufficient quality data are collected, functional connectivity is stationary at rest (Laumann *et al.*, 2017) and is largely stable within an individual under various tasks and across sessions (Gratton *et al.*, 2018). Functional networks are organized such that certain regions (largely within control systems) act as between-network hubs (Power *et al.*, 2013). Hubs are commonly activated during tasks (Gratton *et al.*, 2016) and produce disastrous effects on cognitive functioning if lesioned (Warren *et al.*, 2014). As a whole, resting-state functional connectivity is a powerful tool to study brain organization at the level of functional systems. Whether and to what extent functional connectivity changes over the course of development remains to be established.

1.2.2 Development of functional networks before and after Power et al. 2012.

Early investigation of the development of functional systems in the human brain relied solely on task fMRI (e.g., word processing Schlaggar *et al.*, 2002). Carefully designed experiments were required to not only isolate cognitive processes but also equivalently engage children and adults (Church *et al.*, 2010). With the introduction of resting-state functional connectivity, it became apparent that there were many advantages of studying the development of neural systems with this complementary approach. First, as in adults, this technique theoretically enables the rapid and relatively easy assessment of many different functional systems from a single, simple scan. Second, the issues associated with probing developmental differences in the functional architecture of the brain with tasks such as performance burden and the imbalanced comparison "Task B" problem (Church *et al.*, 2010), are presumably avoided in a task-free design. Finally, the measured strength of functional connectivity is thought to reflect a history of co-activation across the lifespan (Lewis *et al.*, 2009) thus tracking the coordination of different functional systems across development.

Because of these advantages, many have studied the differences in functional architecture between school-age children and adults with resting-state functional connectivity. The studies prior to the identification of sub-millimeter motion-related artifacts revealed intriguing properties of the development of functional systems. Functional connections appeared to develop in distance-dependent manner: children had stronger local connections and adults had stronger connections distributed across cortex (Fair *et al.*, 2009). Functional connectivity within the default mode network appeared less integrated in children such that the homotopic connections were weaker, and the anterior

and posterior pieces were disconnected (Fair *et al.*, 2008). Patterns of developmental differences in functional connectivity were able to predict the maturity of single individuals (Dosenbach *et al.*, 2010). Studies of atypical development (in Tourette syndrome) with functional connectivity suggested that control systems important for inhibitory control were immature in patients when compared to controls (Church *et al.*, 2009).

Unfortunately, these studies, while using the “industry standard” for data quality control at the time, were conducted before the identification of sub-millimeter motion-related artifact in resting-state functional connectivity (Power *et al.*, 2012; Satterthwaite *et al.*, 2012; Van Dijk *et al.*, 2012). Head movement in the scanner produces spurious, but systematic effects on functional connectivity. Among other more global effects, head motion artificially alters functional connectivity in a distance-dependent manner: short-range connections are enhanced and long-range connections are weakened in high-motion subjects (Power *et al.*, 2012). As children, older adults, and patients tend to move more in the scanner than healthy, young adults, the observed “local-to-distributed” developmental differences in functional connectivity (Fair *et al.*, 2009), immaturity in Tourette syndrome (Church *et al.*, 2009) and other disorders, degradation of network architecture in aging (Andrews-Hanna *et al.*, 2007), and other results are likely confounded by movement-related differences. Fortunately, multiple groups, have developed approaches to reduce motion-related artifact (Macey *et al.*, 2004; Jo *et al.*, 2013; Yan *et al.*, 2013; Muschelli *et al.*, 2014; Power *et al.*, 2014) that have been externally benchmarked and validated (Ciric *et al.*, 2017).

After the discovery of motion-related effects on functional connectivity, it was necessary to determine the existence and/or extent of developmental changes in

functional networks that could be observed with functional connectivity after motion de-noising. Preliminary investigation of parietal cortex suggests that the parcellation of functional areas using functional connectivity does not differ between children and adults (Barnes *et al.*, 2012). Further, the previously observed developmental differences in the functional network definition are mitigated after adequately addressing motion-related artifacts (Power *et al.*, 2012). Additionally, the role of regions in overall network organization (e.g., hubs) appears similar in children and adults (Hwang *et al.*, 2013). However, there are subtle, yet reliable developmental differences in functional connectivity that remain after reducing motion-related artifact (Satterthwaite *et al.*, 2013; Marek *et al.*, 2015).

Measurable developmental differences in functional connectivity that do not correspond to changes in overall functional network organization may still reflect the refinement and heightening of cognitive abilities from school-age to adulthood. A more pessimistic view point is that these differences reflect residual developmental differences in motion artifact. In Chapter 2, I present our efforts to assess the existence of residual motion-related artifact in developmental data in addition to our efforts to determine organizing principles by which functional networks change over the course of development. If independent of head motion, identifying principles that characterize developmental differences in functional connectivity might shed light on the mechanisms underlying the development of functional systems.

1.2.3 Multivariate machine learning methods can detect complex developmental patterns.

The brain is enormously complex. It stands to reason that characterizing its development poses an extraordinarily complex problem. Behaviorally, many functional processes are inter-dependently modified and honed and, neurobiologically, components at many levels of organization are associated with these changes. Theoretically, even a small perturbation associated with a developmental disorder might produce complex effects on brain function and behavior. Univariate statistical approaches have been standardly used to study the development of functional networks in health and disease (see above). While these approaches are useful and informative, by nature, univariate statistical approaches are not sufficient to fully encompass the complex changes across the whole brain (Lessov-Schlaggar *et al.*, 2016). Multivariate pattern analysis can be better suited to address the complex problems in brain development as these approaches identify patterns of developmental changes.

Multivariate approaches applied to the development of functional connectivity combine developmental information across many functional connections. Univariately, a single functional connection may weakly differ between children and adults, but, when combined with a second functional connection, the pattern of variance across the two connections may strongly distinguish children and adults. Support vector machine learning, a type of multivariate pattern analysis, can be used to weight specific patterns of functional connections that best separate a group of children and adults. This approach can be extended to continuous developmental data to identify patterns in functional networks that vary according to age. This model can then be cross-validated and used for age prediction.

Multivariate machine learning approaches promise the ability to predict maturity in single subjects and to interrogate the features informing age prediction. Being able to detect whether the brain of a single individual appears developmentally delayed with respect to healthy controls would be extremely valuable to clinicians, particularly if these single-subject predictions of maturity were also informative of future prognosis. Another advantage of multivariate machine learning approaches is the ability to interrogate which features (here, functional connections) most inform the prediction of maturity in an attempt to better understand the underlying neurobiology. We, and others, have applied multivariate machine learning to the study of the development of functional networks (Dosenbach *et al.*, 2010; Fair *et al.*, 2013; Satterthwaite *et al.*, 2013) as well as the atypical functional networks in psychiatric disorders like ADHD (Fair *et al.*, 2013), autism (Uddin *et al.*, 2013; Emerson *et al.*, 2017), and Tourette syndrome (Greene *et al.*, 2016).

In Chapters 2 and 3, I present our work using multivariate machine learning approaches to study the typical and atypical development of functional networks. In Chapter 2, we evaluate the sets of functional connections that are most useful for age prediction and test whether these multivariate techniques are susceptible to residual individual differences in head motion. Chapter 3 applies these multivariate machine learning approaches to Tourette syndrome in order to determine whether and to what extent developmental patterns are disrupted in developmental disorders. Chapter 4 complements these approaches by applying univariate statistics to the study of the development of functional networks in Tourette syndrome and in healthy controls.

1.3 A study in atypical development: Tourette syndrome

While studies of typical development in isolation can detail how functional networks and their coordination contribute to the development of mature thinking, studies of atypical development are also important and can illuminate the developmental changes necessary for certain behaviors. Tourette syndrome, a neurodevelopmental movement disorder, is aptly suited for the study of the typical and atypical development of functional networks responsible for motor function and inhibitory control. As detailed below, understanding, diagnosing, and treating the neurobiology underlying TS will likely be benefited by investigation of the functional networks contributing to TS in addition to attention to the developmental changes to these abnormalities.

1.3.1 Characteristics of Tourette syndrome: a neurodevelopmental disorder

Tourette syndrome (TS) is a developmental neuropsychiatric disorder that affects 1-3% of children (Khalifa and Knorring, n.d.; Scahill *et al.*, 2009; Cubo *et al.*, 2011) and is characterized by motor and vocal tics (Leckman *et al.*, 2014). Tics are brief, unwanted, repetitive movements or noises that can be intrusive in daily life. Common tics include eye blinking, eye brow raising, nose twitching, sniffing, and throat clearing. More complex tics have also been observed (e.g., echopraxia, tapping, gestures, echolalia, utterance of words, coprolalia). Though often described as involuntary, tics have a semi-voluntary quality, as individuals with TS can often suppress their tics; yet this suppression is time-limited. Preceding tics, many individuals with TS experience a premonitory urge, which is a perceived sensation of discomfort that is relieved by the tic (Leckman *et al.*, 1993).

While tics are the characteristic symptom of the disorder, TS can often be accompanied by a myriad of other symptoms and consequences. Many children with TS also have comorbid diagnoses of other neurodevelopmental disorders including ADHD (60%) or OCD (30%) (Freeman *et al.*, 2000). In fact, only about 10% of the TS population only has tic symptoms (Freeman *et al.*, 2000), suggesting that issues with executive function and attention, even outside of the motor context might be a central symptom of TS. Other psychiatric disorders including depression, anxiety, and sleep disorders often also accompany TS (Conelea *et al.*, 2013). Further, TS is associated with impaired quality of life assessments (Cavanna *et al.*, 2008), increased family stress (Stewart *et al.*, 2015), and lasting psychosocial effects (Conelea *et al.*, 2013).

1.3.2 Atypical brain structure and function in Tourette syndrome

Many cortical and subcortical functional systems likely support the initiation, production, and suppression of tics and the other symptoms associated with TS.

The most prominent theory in TS is that disruption of cortico-striato-thalamo-cortical loops leads to the production of tics, as other movement disorders like Parkinson's or Huntington's disease involve aberrant activity in the subcortex (DeLong, 1990). Loops between different pieces of the cortex and the subcortex appear devoted to different functions (e.g., motor, control) (Haber, 2003) and these associations can be observed using resting-state functional connectivity (Choi *et al.*, 2012; Greene *et al.*, 2014). Mink et al. (2001) proposed that in TS, activity in the striatum propagates through these loops and leads to the disinhibition of unwanted motor plans and the production of tics. Several results support this hypothesis. First, microstimulation and biculine injections into the

basal ganglia yield tic-like movements in non-human primates (Alexander and DeLong, 1985; McCairn *et al.*, 2009). Second, regions in the basal ganglia, thalamus, sensorimotor cortex and cerebellum are consistently activated at the time of tic action in patients with TS (Bohlhalter *et al.*, 2006; Wang *et al.*, 2011; Neuner *et al.*, 2014). Further, reduced caudate volume has been consistently observed in children and adults with TS (Peterson *et al.*, 1993; but see Greene *et al.*, 2017), and smaller caudate and putamen volumes have been linked to more severe tics (Bloch *et al.*, 2005).

Cortical and subcortical regions involved in inhibitory motor control have also been shown to exhibit altered structure and function in TS. For example, thinning of areas within frontal and prefrontal cortex has been observed in adults with TS (Sowell *et al.*, 2008). Preceding tics, a period likely related to premonitory urges (and potentially tic suppression), regions including the anterior cingulate, insula, parietal operculum, and supplementary motor area are activated in TS (Bohlhalter *et al.*, 2006; Wang *et al.*, 2011; Neuner *et al.*, 2014). When directly instructed to suppress eye blinks, children and adults with TS activate the middle frontal gyrus, dorsal anterior cingulate cortex, middle temporal gyrus and superior temporal gyrus and deactivate the superior frontal gyrus more strongly than healthy controls (Mazzone *et al.*, 2010). Further evidence for the involvement of inhibitory control systems in TS beyond activity related to tic suppression include the high co-morbidity of TS with other disorders of inhibitory control like ADHD and OCD (Freeman *et al.*, 2000) and atypical control signals in frontal and other associated regions during the performance of a semantic judgment task in adolescents with TS (Church *et al.*, 2009).

Less is known about the neurobiology supporting the initiation of tics. In TS, tics can often be associated with environmental triggers (e.g., school vs. home setting).

Further, tic frequency can increase under stress, different emotion contexts, or different social situations and decrease when attention is allocated elsewhere (for review see Conelea and Woods, 2008). Therefore, the functional systems responsible for processing and orienting to these external triggers and their interactions with cortico-striatal-thalamo-cortical circuitry might play an important role in the initiation of tics.

While previous neuroimaging work has provided a valuable description of the neural abnormalities in TS at the level of brain regions, studying the network organization of the brain in TS may yield a more complete understanding of tics and the other symptoms in TS. Many of the advantages of using resting-state functional connectivity to study the development of functional systems apply to the study of TS including the rapid assessment of many different functional systems implicated in the initiation, production, and suppression of tics and avoidance of confounding task-related problems such as the performance confound and the “Task B” problem (Church *et al.*, 2010). Additionally, the measured strength of functional connectivity is thought to reflect a history of co-activation across the lifespan (Lewis *et al.*, 2009) thus tracking the atypical coordination (or lack of coordination) of different functional systems in TS.

Further, placing the observed neural differences in TS in a context of functional networks facilitates more specific and more powerful interpretations of these abnormalities. For example, differences observed in frontal cortex can be difficult to interpret as many functional systems reside in frontal cortex (e.g. fronto-parietal, cingulo-opercular, default mode, ventral attention, salience). Delineating the specific functional systems that are affected in TS would facilitate interpretations that leverage the extensive work elucidating the functional properties of functional systems in healthy controls. For

example, if differences observed in frontal cortex in TS are associated with the regions belonging to the cingulo-opercular system, then these differences might suggest atypical executive control signals related to task-set maintenance or the detection of errors (Dosenbach *et al.*, 2007; Neta *et al.*, 2014) in TS. Whereas if differences in frontal cortex in TS are associated with the neighboring ventral attention system, then these differences might suggest atypical stimulus-oriented attention (Corbetta and Shulman, 2002).

Previously, we demonstrated that patterns of resting-state functional connectivity across the whole brain contain information that can distinguish individuals with TS from controls (Greene *et al.*, 2016). However, the specific functional networks and connections that are altered in TS remains unknown. Uncovering how specific functional networks are altered may yield a more complete understanding of tics and the other symptoms in TS. In Chapter 3 and 4, we use resting-state functional connectivity to identify a history of atypical coordination of functional systems in children and adults with TS. Chapter 4 attempts to identify the specific functional networks and connections that are altered in TS and are involved in the initiation, production, and suppression of tics and other symptoms. In this chapter, we also aim to bridge previous studies of atypical brain function in TS with existing knowledge of the role of different functional networks in behavior and cognition.

1.3.3 Development and Tourette syndrome

TS is considered a neurodevelopmental disorder not only because tics emerge in childhood, but also because symptoms change through adolescence and early adulthood. Tic onset typically occurs at age 5-7 years, with tic severity peaking during late

childhood/early adolescence (10-12 years). The specific mode of tics often changes over the course of development (e.g., eye blink to eyebrow raising) (Leckman *et al.*, 1989) and reports of experiencing a premonitory urge, the sensation preceding tics, increase in adulthood TS (Leckman *et al.*, 1993). Tics usually continue into adulthood, but with marked improvement or even remission after adolescence (Erenberg *et al.*, 1987; Leckman *et al.*, 1998; Peterson *et al.*, 2001a; Bloch *et al.*, 2006; Hassan and Cavanna, 2012). However, this symptom progression varies substantially across individuals, with a sizeable subgroup of patients (~60%) experiencing moderate to severe tics that persist into adulthood (Leckman *et al.*, 1998; Pappert *et al.*, 2003).

Most neuroimaging studies of TS treat it as a singular disorder, unchanging across development, by grouping together a wide range of patients or focusing on a single age cohort. However, as symptoms vary by age, there is evidence that differences in brain structure and function in TS also vary by age. Some cortical regions (dorsal prefrontal, orbitofrontal, parieto-occipital cortex) exhibit distinct, even sometimes opposing, volumetric differences in children and adults with TS (Peterson *et al.*, 2001b). Previous research has also shown that motor excitability is selectively altered in children with TS (Pépés *et al.*, 2016) and atypical development of fronto-striatal self-regulatory signals only emerges in adulthood TS (Raz *et al.*, 2009). Comparing the brain differences observed in children and adults with TS is necessary to reveal effects that are present in both age groups (i.e., “age-invariant” TS effects) as well as effects that differ between age groups (i.e., “age-specific” TS effects).

Critically, a more complete understanding of the differences observed in children or adults with TS also requires taking into account typical maturational changes in the

brain. Given a context of typical development, one can determine whether brain differences reflect atypically shifted development (e.g., accelerated or delayed maturation) or an anomalous difference not observed in typical development, potentially providing clues into etiology. While several TS neuroimaging studies have interpreted their findings in the context of brain maturity (Muellner *et al.*, n.d.; Peterson *et al.*, 2001c; Raz *et al.*, 2009; Worbe *et al.*, 2012; Pépés *et al.*, 2016), few have included typical developmental comparisons to contextualize the differences observed in TS (Marsh *et al.*, 2007; Church *et al.*, 2009b; Debes *et al.*, 2015).

In this thesis I apply a developmental perspective (and lessons learned from the study of typical development in Chapter 2) to the study of the neurobiology underlying TS. Chapters 3 and 4 describe our investigation of the atypical development of functional networks in TS. In Chapter 3, we test whether the atypical functional connectivity in TS differs between children and adults and place these atypicalities in a developmental context. In Chapter 4, we focus on the developmental trajectories of specific functional systems that are atypical in TS. By combining a developmental and network-level approach, we aim to better understand the neurobiology underlying TS and utilize this knowledge to better diagnose and treat the disorder.

1.4 References

Alexander GE, DeLong MR. Microstimulation of the primate neostriatum. II. Somatotopic organization of striatal microexcitable zones and their relation to neuronal response properties. J. Neurophysiol. 1985; 53: 1417–1430.

Andrews-Hanna JR, Snyder AZ, Vincent JL, Lustig C, Head D, Raichle ME, et al. Disruption of Large-Scale Brain Systems in Advanced Aging. Neuron 2007; 56: 924–935.

Arcaro MJ, Schade PF, Vincent JL, Ponce CR, Livingstone MS. Seeing faces is necessary for face-domain formation. Nat. Neurosci. 2017; 20: 1404–1412.

Barnes KA, Cohen AL, Power JD, Nelson SM, Dosenbach YBL, Miezin FM, et al. Identifying basal ganglia divisions in individuals using resting-state functional connectivity MRI [Internet]. Front. Syst. Neurosci. 2010; 4[cited 2019 Jan 9] Available from: https://www.frontiersin.org/articles/10.3389/fnsys.2010.00018/full

Barnes KA, Nelson SM, Cohen AL, Power JD, Coalson RS, Miezin FM, et al. Parcellation in Left Lateral Parietal Cortex Is Similar in Adults and Children. Cereb. Cortex 2012; 22: 1148–1158.

Bé J-VL, Markram H. Spontaneous and evoked synaptic rewiring in the neonatal neocortex. Proc. Natl. Acad. Sci. 2006; 103: 13214–13219.

Biswal B, Yetkin FZ, Haughton VM, Hyde JS. Functional connectivity in the motor cortex of resting human brain using echo-planar mri. Magn. Reson. Med. 1995; 34: 537–541.

Bloch MH, Leckman JF, Zhu H, Peterson BS. Caudate volumes in childhood predict symptom severity in adults with Tourette syndrome. Neurology 2005; 65: 1253.

Bloch MH, Peterson BS, Scahill L, Otka J, Katsovich L, Zhang H, et al. Adulthood outcome of tic and obsessive-compulsive symptom severity in children with Tourette syndrome. Arch. Pediatr. Adolesc. Med. 2006; 160: 65–69.

Bohlhalter S, Goldfine A, Matteson S, Garraux G, Hanakawa T, Kansaku K, et al. Neural correlates of tic generation in Tourette syndrome: an event-related functional MRI study. Brain 2006; 129: 2029–2037.

Casey BJ, Trainor RJ, Orendi JL, Schubert AB, Nystrom LE, Giedd JN, et al. A Developmental Functional MRI Study of Prefrontal Activation during Performance of a Go-No-Go Task. J. Cogn. Neurosci. 1997; 9: 835–847.

Cavanna AE, Schrag A, Morley D, Orth M, Robertson MM, Joyce E, et al. The Gilles de la Tourette Syndrome–Quality of Life Scale (GTS-QOL). Neurology 2008; 71: 1410.

Choi EY, Yeo BTT, Buckner RL. The organization of the human striatum estimated by intrinsic functional connectivity. J. Neurophysiol. 2012; 108: 2242–2263.

Church JA, Fair DA, Dosenbach NUF, Cohen AL, Miezin FM, Petersen SE, et al. Control networks in paediatric Tourette syndrome show immature and anomalous patterns of functional connectivity. Brain 2009a; 132: 225–238.

Church JA, Petersen SE, Schlaggar BL. The “Task B problem” and other considerations in developmental functional neuroimaging. Hum. Brain Mapp. 2010; 31: 852–862.

Church JA, Wenger KK, Dosenbach NUF, Miezin FM, Petersen SE, Schlaggar BL. Task control signals in pediatric Tourette syndrome show evidence of immature and anomalous

functional activity [Internet]. Front. Hum. Neurosci. 2009b; 3[cited 2018 Jun 19] Available from: https://www.frontiersin.org/articles/10.3389/neuro.09.038.2009/full

Churchland PS, Sejnowski TJ. Perspectives on cognitive neuroscience. Science 1988; 242: 741–745.

Ciric R, Wolf DH, Power JD, Roalf DR, Baum GL, Ruparel K, et al. Benchmarking of participant-level confound regression strategies for the control of motion artifact in studies of functional connectivity. NeuroImage 2017; 154: 174–187.

Cohen AL, Fair DA, Dosenbach NUF, Miezin FM, Dierker D, Van Essen DC, et al. Defining Functional Areas in Individual Human Brains using Resting Functional Connectivity MRI. NeuroImage 2008; 41: 45–57.

Conelea CA, Woods DW. The influence of contextual factors on tic expression in Tourette's syndrome: A review. J. Psychosom. Res. 2008; 65: 487–496.

Conelea CA, Woods DW, Zinner SH, Budman CL, Murphy TK, Scahill LD, et al. The Impact of Tourette Syndrome in Adults: Results from the Tourette Syndrome Impact Survey. Community Ment. Health J. 2013; 49: 110–120.

Corbetta M, Shulman GL. Control of goal-directed and stimulus-driven attention in the brain. Nat. Rev. Neurosci. 2002; 3: 201–215.

Cubo E, Galán JMTG y, Villaverde VA, Velasco SS, Benito VD, Macarrón JV, et al. Prevalence of Tics in Schoolchildren in Central Spain: A Population-Based Study. Pediatr. Neurol. 2011; 45: 100–108.

Debes N, Jeppesen S, Raghava JM, Groth C, Rostrup E, Skov L. Longitudinal Magnetic Resonance Imaging (MRI) Analysis of the Developmental Changes of Tourette Syndrome Reveal Reduced Diffusion in the Cortico-Striato-Thalamo-Cortical Pathways. J. Child Neurol. 2015; 30: 1315–1326.

DeLong MR. Primate models of movement disorders of basal ganglia origin. Trends Neurosci. 1990; 13: 281–285.

Diamond A, Goldman-Rakic PS. Comparison of human infants and rhesus monkeys on Piaget's AB task: evidence for dependence on dorsolateral prefrontal cortex. Exp. Brain Res. 1989; 74: 24–40.

Dosenbach NUF, Fair DA, Miezin FM, Cohen AL, Wenger KK, Dosenbach RAT, et al. Distinct brain networks for adaptive and stable task control in humans. Proc. Natl. Acad. Sci. 2007; 104: 11073–11078.

Dosenbach NUF, Nardos B, Cohen AL, Fair DA, Power JD, Church JA, et al. Prediction of Individual Brain Maturity Using fMRI. Science 2010; 329: 1358–1361.

Emerson RW, Adams C, Nishino T, Hazlett HC, Wolff JJ, Zwaigenbaum L, et al. Functional neuroimaging of high-risk 6-month-old infants predicts a diagnosis of autism at 24 months of age. Sci. Transl. Med. 2017; 9: eaag2882.

Erenberg G, Cruse RP, Rothner AD. The natural history of Tourette syndrome: a follow-up study. Ann. Neurol. 1987; 22: 383–385.

Eriksson PS, Perfilieva E, Björk-Eriksson T, Alborn A-M, Nordborg C, Peterson DA, et al. Neurogenesis in the adult human hippocampus. Nat. Med. 1998; 4: 1313–1317.

Fair D, Nigg JT, Iyer S, Bathula D, Mills KL, Dosenbach NU, et al. Distinct neural signatures detected for ADHD subtypes after controlling for micro-movements in resting state functional connectivity MRI data [Internet]. Front. Syst. Neurosci. 2013; 6[cited 2018 Jun 29] Available from: https://www.frontiersin.org/articles/10.3389/fnsys.2012.00080/full

Fair DA, Cohen AL, Dosenbach NUF, Church JA, Miezin FM, Barch DM, et al. The maturing architecture of the brain's default network. Proc. Natl. Acad. Sci. 2008; 105: 4028–4032.

Fair DA, Cohen AL, Power JD, Dosenbach NUF, Church JA, Miezin FM, et al. Functional Brain Networks Develop from a "Local to Distributed" Organization. PLOS Comput. Biol. 2009; 5: e1000381.

Feigenson L, Dehaene S, Spelke E. Core systems of number. Trends Cogn. Sci. 2004; 8: 307–314.

Freeman RD, Fast DK, Burd L, Kerbeshian J, Robertson MM, Sandor P. An international perspective on Tourette syndrome: selected findings from 3500 individuals in 22 countries. Dev. Med. Child Neurol. 2000; 42: 436–447.

Gauthier I, Nelson CA. The development of face expertise. Curr. Opin. Neurobiol. 2001; 11: 219–224.

Gauthier I, Tarr MJ, Anderson AW, Skudlarski P, Gore JC. Activation of the middle fusiform 'face area' increases with expertise in recognizing novel objects. Nat. Neurosci. 1999; 2: 568–573.

Giedd JN, Blumenthal J, Jeffries NO, Castellanos FX, Liu H, Zijdenbos A, et al. Brain development during childhood and adolescence: a longitudinal MRI study. Nat. Neurosci. 1999; 2: 861–863.

Gogtay N, Giedd JN, Lusk L, Hayashi KM, Greenstein D, Vaituzis AC, et al. Dynamic mapping of human cortical development during childhood through early adulthood. Proc. Natl. Acad. Sci. 2004; 101: 8174–8179.

Gordon EM, Laumann TO, Adeyemo B, Huckins JF, Kelley WM, Petersen SE. Generation and Evaluation of a Cortical Area Parcellation from Resting-State Correlations. Cereb. Cortex 2016; 26: 288–303.

Gratton C, Laumann TO, Gordon EM, Adeyemo B, Petersen SE. Evidence for Two Independent Factors that Modify Brain Networks to Meet Task Goals. Cell Rep. 2016; 17: 1276–1288.

Gratton C, Laumann TO, Nielsen AN, Greene DJ, Gordon EM, Gilmore AW, et al. Functional Brain Networks Are Dominated by Stable Group and Individual Factors, Not Cognitive or Daily Variation. Neuron 2018; 98: 439-452.e5.

Gratton C, Neta M, Sun H, Ploran EJ, Schlaggar BL, Wheeler ME, et al. Distinct Stages of Moment-to-Moment Processing in the Cinguloopercular and Frontoparietal Networks. Cereb. Cortex 2017; 27: 2403–2417.

Greene DJ, Church JA, Dosenbach NUF, Nielsen AN, Adeyemo B, Nardos B, et al. Multivariate pattern classification of pediatric Tourette syndrome using functional connectivity MRI. Dev. Sci. 2016; 19: 581–598.

Greene DJ, Laumann TO, Dubis JW, Ihnen SK, Neta M, Power JD, et al. Developmental Changes in the Organization of Functional Connections between the Basal Ganglia and Cerebral Cortex. J. Neurosci. 2014; 34: 5842–5854.

Greene DJ, Williams Iii AC, Koller JM, Schlaggar BL, Black KJ. Brain structure in pediatric Tourette syndrome. Mol. Psychiatry 2017; 22: 972–980.

Haber SN. The primate basal ganglia: parallel and integrative networks. J. Chem. Neuroanat. 2003; 26: 317–330.

Hassan N, Cavanna AE. The prognosis of Tourette syndrome: implications for clinical practice. Funct. Neurol. 2012; 27: 23–27.

Huttenlocher PR. Morphometric study of human cerebral cortex development. Neuropsychologia 1990; 28: 517–527.

Hwang K, Hallquist MN, Luna B. The Development of Hub Architecture in the Human Functional Brain Network. Cereb. Cortex 2013; 23: 2380–2393.

Jo HJ, Gotts SJ, Reynolds RC, Bandettini PA, Martin A, Cox RW, et al. Effective Preprocessing Procedures Virtually Eliminate Distance-Dependent Motion Artifacts in Resting State FMRI [Internet]. J. Appl. Math. 2013[cited 2019 Jan 9] Available from: https://www.hindawi.com/journals/jam/2013/935154/abs/

Johnson MH. Functional brain development in humans. Nat. Rev. Neurosci. 2001; 2: 475–483.

Johnson MH. Interactive Specialization: A domain-general framework for human functional brain development? Dev. Cogn. Neurosci. 2011; 1: 7–21.

Johnson MH, Tucker LA, Stiles J, Trauner D. Visual attention in infants with perinatal brain damage: Evidence of the importance of anterior lesions. Dev. Sci. 1998; 1: 53–58.

Katz LC, Shatz CJ. Synaptic Activity and the Construction of Cortical Circuits. Science 1996; 274: 1133–1138.

Kelly DJ, Quinn PC, Slater AM, Lee K, Ge L, Pascalis O. The Other-Race Effect Develops During Infancy: Evidence of Perceptual Narrowing. Psychol. Sci. 2007; 18: 1084–1089.

Khalifa N, Knorring A-L von. Prevalence of tic disorders and Tourette syndrome in a Swedish school population. Dev. Med. Child Neurol.; 45: 315–319.

Kuhl PK, Williams KA, Lacerda F, Stevens KN, Lindblom B. Linguistic experience alters phonetic perception in infants by 6 months of age. Science 1992; 255: 606–608.

Laumann TO, Snyder AZ, Mitra A, Gordon EM, Gratton C, Adeyemo B, et al. On the Stability of BOLD fMRI Correlations. Cereb. Cortex 2017; 27: 4719–4732.

Leckman JF, King RA, Bloch MH. Clinical features of Tourette syndrome and tic disorders. J. Obsessive-Compuls. Relat. Disord. 2014; 3: 372–379.

Leckman JF, Riddle MA, Hardin MT, Ort SI, Swartz KL, Stevenson J, et al. The Yale Global Tic Severity Scale: Initial Testing of a Clinician-Rated Scale of Tic Severity. J. Am. Acad. Child Adolesc. Psychiatry 1989; 28: 566–573.

Leckman JF, Walker DE, Cohen DJ. Premonitory urges in Tourette's syndrome. Am. J. Psychiatry 1993; 150: 98.

Leckman JF, Zhang H, Vitale A, Lahnin F, Lynch K, Bondi C, et al. Course of Tic Severity in Tourette Syndrome: The First Two Decades. Pediatrics 1998; 102: 14–19.

Lessov-Schlaggar CN, Rubin JB, Schlaggar BL. The Fallacy of Univariate Solutions to Complex Systems Problems [Internet]. Front. Neurosci. 2016; 10[cited 2019 Jan 9] Available from: https://www.frontiersin.org/articles/10.3389/fnins.2016.00267/full

Lewis CM, Baldassarre A, Committeri G, Romani GL, Corbetta M. Learning sculpts the spontaneous activity of the resting human brain. Proc. Natl. Acad. Sci. 2009: pnas.0902455106.

Macey PM, Macey KE, Kumar R, Harper RM. A method for removal of global effects from fMRI time series. NeuroImage 2004; 22: 360–366.

Marek S, Hwang K, Foran W, Hallquist MN, Luna B. The Contribution of Network Organization and Integration to the Development of Cognitive Control. PLOS Biol. 2015; 13: e1002328.

Marsh R, Zhu H, Wang Z, Skudlarski P, Peterson BS. A Developmental fMRI Study of Self-Regulatory Control in Tourette's Syndrome. Am. J. Psychiatry 2007; 164: 955–966.

Mazzone L, Yu S, Blair C, Gunter BC, Wang Z, Marsh R, et al. An fMRI Study of Frontostriatal Circuits During the Inhibition of Eye Blinking in Persons With Tourette Syndrome. Am. J. Psychiatry 2010; 167: 341–349.

McCairn KW, Bronfeld M, Belelovsky K, Bar-Gad I. The neurophysiological correlates of motor tics following focal striatal disinhibition. Brain 2009; 132: 2125–2138.

Muellner J, Delmaire C, Valabrégue R, Schüpbach M, Mangin J-F, Vidailhet M, et al. Altered structure of cortical sulci in gilles de la Tourette syndrome: Further support for abnormal brain development. Mov. Disord.; 30: 655–661.

Muschelli J, Nebel MB, Caffo BS, Barber AD, Pekar JJ, Mostofsky SH. Reduction of motion-related artifacts in resting state fMRI using aCompCor. NeuroImage 2014; 96: 22–35.

Neta M, Schlaggar BL, Petersen SE. Separable responses to error, ambiguity, and reaction time in cingulo-opercular task control regions. NeuroImage 2014; 99: 59–68.

Neuner I, Werner CJ, Arrubla J, Stöcker T, Ehlen C, Wegener HP, et al. Imaging the where and when of tic generation and resting state networks in adult Tourette patients [Internet]. Front. Hum. Neurosci. 2014; 8[cited 2019 Jan 19] Available from: https://www.frontiersin.org/articles/10.3389/fnhum.2014.00362/full

Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, et al. Synaptic Pruning by Microglia Is Necessary for Normal Brain Development. Science 2011; 333: 1456–1458.

Pappert EJ, Goetz CG, Louis ED, Blasucci L, Leurgans S. Objective assessments of longitudinal outcome in Gilles de la Tourette's syndrome. Neurology 2003; 61: 936–940.

Pascalis O, Haan M de, Nelson CA. Is Face Processing Species-Specific During the First Year of Life? Science 2002; 296: 1321–1323.

Pépés SE, Draper A, Jackson GM, Jackson SR. Effects of age on motor excitability measures from children and adolescents with Tourette syndrome. Dev. Cogn. Neurosci. 2016; 19: 78–86.

Petersen SE, Fox PT, Posner MI, Mintun M, Raichle ME. Positron emission tomographic studies of the cortical anatomy of single-word processing. Nature 1988; 331: 585–589.

Petersen SE, Sporns O. Brain Networks and Cognitive Architectures. Neuron 2015; 88: 207–219.

Peterson B, Riddle MA, Cohen DJ, Katz LD, Smith JC, Hardin MT, et al. Reduced basal ganglia volumes in Tourette's syndrome using three-dimensional reconstruction techniques from magnetic resonance images. Neurology 1993; 43: 941.

Peterson BS, Pine DS, Cohen P, Brook JS. Prospective, longitudinal study of tic, obsessive-compulsive, and attention-deficit/hyperactivity disorders in an epidemiological sample. J. Am. Acad. Child Adolesc. Psychiatry 2001a; 40: 685–695.

Peterson BS, Staib L, Scahill L, Zhang H, Anderson C, Leckman JF, et al. Regional Brain and Ventricular Volumes in Tourette Syndrome. Arch. Gen. Psychiatry 2001b; 58: 427–440.

Peterson BS, Staib L, Scahill L, Zhang H, Anderson C, Leckman JF, et al. Regional Brain and Ventricular Volumes in Tourette Syndrome. Arch. Gen. Psychiatry 2001c; 58: 427–440.

Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. NeuroImage 2012; 59: 2142–2154.

Power JD, Cohen AL, Nelson SM, Wig GS, Barnes KA, Church JA, et al. Functional Network Organization of the Human Brain. Neuron 2011; 72: 665–678.

Power JD, Mitra A, Laumann TO, Snyder AZ, Schlaggar BL, Petersen SE. Methods to detect, characterize, and remove motion artifact in resting state fMRI. NeuroImage 2014; 84: 320–341.

Power JD, Schlaggar BL, Lessov-Schlaggar CN, Petersen SE. Evidence for Hubs in Human Functional Brain Networks. Neuron 2013; 79: 798–813.

Puro DG, Mello FGD, Nirenberg M. Synapse turnover: the formation and termination of transient synapses. Proc. Natl. Acad. Sci. 1977; 74: 4977–4981.

Rakic P, Bourgeois JP, Eckenhoff MF, Zecevic N, Goldman-Rakic PS. Concurrent overproduction of synapses in diverse regions of the primate cerebral cortex. Science 1986; 232: 232–235.

Raz A, Zhu H, Yu S, Bansal R, Wang Z, Alexander GM, et al. Neural Substrates of Self-Regulatory Control in Children and Adults with Tourette Syndrome , Neural Substrates of Self-Regulatory Control in Children and Adults with Tourette Syndrome. Can. J. Psychiatry 2009; 54: 579–588.

Ruffolo RR, Eisenbarth GS, Thompson JM, Nirenberg M. Synapse turnover: a mechanism for acquiring synaptic specificity. Proc. Natl. Acad. Sci. 1978; 75: 2281–2285.

Satterthwaite TD, Wolf DH, Loughead J, Ruparel K, Elliott MA, Hakonarson H, et al. Impact of in-scanner head motion on multiple measures of functional connectivity: Relevance for studies of neurodevelopment in youth. NeuroImage 2012; 60: 623–632.

Satterthwaite TD, Wolf DH, Ruparel K, Erus G, Elliott MA, Eickhoff SB, et al. Heterogeneous impact of motion on fundamental patterns of developmental changes in functional connectivity during youth. NeuroImage 2013; 83: 45–57.

Scahill L, Bitsko RH, Visser SN, Blumberg SJ. Prevalence of diagnosed tourette syndrome in persons aged 6-17 years - United States, 2007. Morb. Mortal. Wkly. Rep. 2009; 58: 581–585.

Schlaggar BL, Brown TT, Lugar HM, Visscher KM, Miezin FM, Petersen SE. Functional Neuroanatomical Differences Between Adults and School-Age Children in the Processing of Single Words. Science 2002; 296: 1476–1479.

Schlaggar BL, McCandliss BD. Development of Neural Systems for Reading. Annu. Rev. Neurosci. 2007; 30: 475–503.

Sowell ER, Kan E, Yoshii J, Thompson PM, Bansal R, Xu D, et al. Thinning of sensorimotor cortices in children with Tourette syndrome. Nat. Neurosci. 2008; 11: 637–639.

Spreen O, Risser AH, Edgell D. Developmental Neuropsychology. Oxford University Press; 1995.

Stewart SB, Greene DJ, Lessov-Schlaggar CN, Church JA, Schlaggar BL. Clinical Correlates of Parenting Stress in Children with Tourette Syndrome and in Typically Developing Children. J. Pediatr. 2015; 166: 1297-1302.e3.

Uddin LQ, Supekar K, Lynch CJ, Khouzam A, Phillips J, Feinstein C, et al. Salience Network–Based Classification and Prediction of Symptom Severity in Children With Autism. JAMA Psychiatry 2013; 70: 869–879.

Van Dijk KRA, Sabuncu MR, Buckner RL. The influence of head motion on intrinsic functional connectivity MRI. NeuroImage 2012; 59: 431–438.

Wang Z, Maia TV, Marsh R, Colibazzi T, Gerber A, Peterson BS. The Neural Circuits That Generate Tics in Tourette's Syndrome [Internet]. Am. J. Psychiatry 2011[cited 2019 Jan 19] Available from: https://ajp.psychiatryonline.org/doi/abs/10.1176/appi.ajp.2011.09111692

Warren DE, Power JD, Bruss J, Denburg NL, Waldron EJ, Sun H, et al. Network measures predict neuropsychological outcome after brain injury. Proc. Natl. Acad. Sci. 2014; 111: 14247–14252.

Webster MJ, Elashoff M, Weickert CS. Molecular evidence that cortical synaptic growth predominates during the first decade of life in humans. Int. J. Dev. Neurosci. 2011; 29: 225–236.

Werker JF, Lalonde CE. Cross-language speech perception: Initial capabilities and developmental change. Dev. Psychol. 1988; 24: 672–683.

Worbe Y, Malherbe C, Hartmann A, Pélégrini-Issac M, Messé A, Vidailhet M, et al. Functional immaturity of cortico-basal ganglia networks in Gilles de la Tourette syndrome. Brain 2012; 135: 1937–1946.

Xu F, Spelke ES. Large number discrimination in 6-month-old infants. Cognition 2000; 74: B1–B11.

Yan C-G, Cheung B, Kelly C, Colcombe S, Craddock RC, Di Martino A, et al. A comprehensive assessment of regional variation in the impact of head micromovements on functional connectomics. NeuroImage 2013; 76: 183–201.

Yeo BTT, Krienen FM, Sepulcre J, Sabuncu MR, Roffman JL, Smoller JW, et al. The organization of the human cerebral cortex estimated by intrinsic functional connectivity. J. Neurophysiol. 2011; 106: 1125–1165.

Chapter 2: Evaluating the prediction of brain maturity from functional connectivity after motion artifact de-noising

This chapter has been published as a journal article. The citation is:

Nielsen, Ashley N., Deanna J. Greene, Caterina Gratton, Nico UF Dosenbach, Steven E. Petersen, and Bradley L. Schlaggar. "Evaluating the Prediction of Brain Maturity From Functional Connectivity After Motion Artifact Denoising." Cerebral Cortex (2018).

2.1 Abstract

The ability to make individual-level predictions from neuroanatomy has the potential to be particularly useful in child development. Previously, resting-state functional connectivity (RSFC) MRI has been used to successfully predict maturity and diagnosis of typically and atypically developing individuals. Unfortunately, submillimeter head motion in the scanner produces systematic, distance-dependent differences in RSFC and may contaminate, and potentially facilitate, these predictions. Here, we evaluated individual age prediction with RSFC after stringent motion de-noising. Using multivariate machine learning, we found that 57% of the variance in individual RSFC after motion artifact de-noising was explained by age, while 4% was explained by residual effects of head motion. When RSFC data were not adequately de-noised, 50% of the variance was explained by motion. Reducing motion-related artifact also revealed that prediction did not depend upon characteristics of functional connections previously hypothesized to mediate development (e.g., connection distance). Instead, successful age prediction relied upon sampling functional connections across multiple functional systems with strong, reliable RSFC within an individual. Our results demonstrate that RSFC across the brain is sufficiently robust to make individual-level predictions of maturity in typical development,

and hence, may have clinical utility for the diagnosis and prognosis of individuals with atypical developmental trajectories.

2.2 Introduction

Individual-level prediction about brain maturity has the potential to be useful for the assessment of developmental progress. The ability to identify an individual with an atypical developmental trajectory might facilitate more accurate diagnoses and prognoses of developmental disorders and lead to earlier and individualized treatment (Emerson *et al.*, 2017; Hazlett *et al.*, 2017). Clinically useful neurobiological measurements should be sufficiently robust to make an accurate prediction of the maturity of typically developing individuals and be closely related to the dysfunction in developmental disorders. Multivariate descriptions of these measurements, based on patterns of information, may be best equipped to make such robust and accurate predictions about an individual child (Bray *et al.*, 2009; Jimura and Poldrack, 2012; Sundermann *et al.*, 2014). Measurements of functional connectivity may be more closely linked to behavior/cognition and more likely disrupted in developmental disorders. Resting-state functional connectivity (RSFC) MRI, the temporal correlation between spontaneous fluctuations in blood oxygen level-dependent signals across the brain (Biswal *et al.*, 1995), has been proposed to reflect the statistical history of co-activation across an individual's lifespan (Fox and Raichle, 2007; Dosenbach *et al.*, 2008). In addition, RSFC is thought to be disrupted in individuals with an atypical developmental trajectory (Fox and Greicius, 2010). Whether or not differences in functionally relevant neurobiology measured with RSFC carry multivariate information germane to make predictions about the health and maturity of an individual child is an important question.

Previously, Dosenbach and colleagues (2010) demonstrated successful prediction of the maturity of individuals based on RSFC using multivariate machine learning (Dosenbach *et al.*, 2010). Using a set of features (i.e. functional connections), they created a multivariate model relating age and RSFC in a training dataset and used this model to successfully predict the age of test individuals. Since then, others have also used machine learning to show that RSFC can make predictions about age (Supekar *et al.*, 2009; Meier *et al.*, 2012; Vergun *et al.*, 2013) as well as various other qualities of individuals, including sex (Casanova *et al.*, 2012) and IQ (Santarnecchi *et al.*, 2014). Additionally, multivariate machine learning approaches have shown that there is information in RSFC to classify healthy individuals from clinical populations including ADHD (Liang *et al.*, 2012), schizophrenia (Fan *et al.*, 2011; Bassett *et al.*, 2012; Du *et al.*, 2012), mild cognitive impairment/Alzheimer's Disease (Koch *et al.*, 2012; Wee *et al.*, 2012), major depressive disorder (Craddock *et al.*, 2009), and autism (Nielsen *et al.*, 2013; Chen *et al.*, 2016). Taken together, these results suggest that differences in RSFC carry information important to representing and making predictions about the individual.

Unfortunately, the success of many previous RSFC studies using machine learning to make predictions about individuals may be contaminated by (even submillimeter level) subject head motion in the scanner. Small amplitude movements in the scanner have been shown to have systematic effects on observed resting-state correlations; this motion-related artifact is distance-dependent, such that correlations are increased for short-range connections and decreased for long-range connections, with specific sets of functional connections being more affected than others (Power *et al.*, 2012, 2014; Van Dijk *et al.*, 2012; Satterthwaite *et al.*, 2013a; Ciric *et al.*, 2017). Motion-related artifact is

problematic for machine learning approaches because head motion is often correlated to the characteristics being predicted (e.g., age, disease status, IQ) (Siegel *et al.*, 2016). Fortunately, we and others have developed methods to reduce the adverse effects of motion-related artifact and other sources of physiological noise on functional MRI data (Power *et al.*, 2014; Ciric *et al.*, 2017). With these de-noising approaches as well as approaches that preemptively reduce head movements (Dosenbach *et al.*, 2017; Greene *et al.*, 2018), many have worked to validate previous machine learning results using RSFC after attempting to correct for individual differences in head motion (Fair *et al.*, 2013; Greene *et al.*, 2014, 2016b; Pruett *et al.*, 2015; Emerson *et al.*, 2017). Specifically, there is growing evidence that after reducing artifactual differences in RSFC related to movement, including signal processing and strict subject matching/selection (Fair *et al.*, 2013; Satterthwaite *et al.*, 2013b; Greene *et al.*, 2016a), RSFC can still be used to successfully predict an individual's age.

The present work has two major aims related to evaluating the prediction of age from RSFC after motion de-noising. First, we aimed to evaluate whether or not there are lingering *multivariate* effects of head motion on resting-state correlations that contribute to age prediction. We tested whether patterns of RSFC can be used to predict an individual's age and an individual's in-scanner head movement using machine learning before and after reducing motion-related artifact. Ensuring that head motion cannot be predicted from RSFC after motion de-noising using machine learning is important for assessing the viability of RSFC as an indicator of developmental progress rather than confounding transient characteristics of individuals. Second, we were interested in evaluating the specific functional connections that facilitate age prediction after reducing

motion-related artifact. Previously, Dosenbach et al. identified a set of functional connections thought to best predict age using a fairly straightforward data-driven, feature selection scheme (i.e. ranking the functional connections most correlated with age) (Dosenbach *et al.*, 2010). Of these top ranked functional connections, many were short-range and long-range connections, in accordance with the "local-to-distributed" theory of RSFC development (short-range became weaker and long-range became stronger with maturity) (Fair *et al.*, 2009; Supekar *et al.*, 2009). However, developmental differences in head motion produce differences in RSFC that reproduce this pattern (i.e., with less subject head motion, short-range functional connections become weaker while long-range functional connections become stronger). Thus, we aimed to identify the functional connections that best predict age and test the "local-to-distributed" hypothesis of RSFC development after reducing motion-related artifact. More recently, investigators have used feature selection to experimentally manipulate the information available for prediction and compare the resulting predictive performance. Whether prediction with RSFC depends upon a hypothesized, organizing principle (e.g., functional systems (Du *et al.*, 2012; Koch *et al.*, 2012; Uddin *et al.*, 2013; Greene *et al.*, 2016b), RSFC strength (Bassett *et al.*, 2012; Santarnecchi *et al.*, 2014)), can be assessed by selecting and testing a set of features with specific properties. Therefore, we also sought to determine whether other organizing principles (e.g. functional systems, RSFC strength) facilitate age prediction with hypothesis-driven feature selection.

2.3 Materials and Methods

2.3.1 Participants

A group of 122 healthy children and adults (ages 7-31 years old, 66 males) were selected from an extant database of participants (n = 487, ages 6-35 years old, 206 males) on the basis of having at least 120 data frames (~5 min) of usable resting-state fMRI data (as defined below). Participants were recruited from the Washington University campus and the surrounding community. All participants were native English speakers, right-handed, and reported no history of neurological or psychiatric disease or a current prescription of psychotropic medications (parental report for child participants). All adult participants, and a parent or guardian for each child participant, gave informed consent, and all children assented to data collection. All participants were compensated for their participation. The Washington University Human Research Protection Office approved all studies.

2.3.2 Image Processing

Image Acquisition

Data were collected on a Siemens 3T MAGNETOM Trio scanner with a Siemens 12-channel Head Matrix Coil. To help stabilize head position, each subject was fitted with a thermoplastic mask fastened to holders on the head coil. A T1-weighted sagittal MP-RAGE structural image (slice time echo, 3.06 ms; TR 2.4 s; inversion time, 1 s; flip angle, 8º; 127 slices; 1 x 1 x 1 mm voxels) in the same anatomical plane as the BOLD images were obtained to improve alignment to an atlas. Functional images were acquired using a BOLD contrast-sensitive echo planar sequence (TE, 27 ms; flip angle, 90º, in-plane resolution, 4 x 4 mm; volume TR 2.5 s). Whole-brain coverage was obtained with 32 contiguous interleaved 4 mm axial slices. Steady-state magnetization was assumed after four volumes. The total number of resting-state functional volumes acquired ranged from 184-780. The length of each resting-state run ranged from 5-30 minutes.

During the resting-state scans, participants viewed a centrally presented white crosshair (subtending <1º visual angle) on a black background. Participants were instructed to relax, “keep an eye on the plus sign”, and hold as still as possible.

Image Analysis

Functional images from each participant were preprocessed to reduce artifacts (Shulman *et al.*, 2010). These steps included: (i) temporal sinc interpolation of all slices to the temporal midpoint of the first slice, accounting for differences in the acquisition time of each individual slice, (ii) correction for head movement within and across runs, and (iii) intensity normalization of the functional data was computed for each individual via the MP-RAGE T1-weighted scans. Each run was then resampled in atlas space on an isotropic 3 mm grid combining movement correction and atlas transformation in a single interpolation. The target atlas was created from thirteen 7-9 year old children and twelve 21-30 year old adults using validated methods (Black *et al.*, 2004). The atlas was constructed to conform to the Talairach atlas space.

Several additional pre-processing steps were applied to reduce spurious variance unlikely to reflect neuronal activity (Fox *et al.*, 2009). These RSFC pre-processing steps included: (i) demeaning and detrending each run, (ii) multiple regression of nuisance variables, (iii) frame censoring (discussed below) and interpolation of data within each run, (iv) temporal band-pass filtering ($0.009\ Hz < f < 0.08\ Hz$), and (v) spatial smoothing (6 mm full width at half maximum). Nuisance variables included motion regressors (e.g. original motion estimates, motion derivatives, and Volterra expansion of motion estimates), an average of the signal across the whole brain (global signal), individualized ventricular and white matter signals, and the derivatives of these signals.

Reducing head motion-related artifact

We applied a procedure determined and validated to best reduce artifacts related to head motion (Power *et al.*, 2014; Ciric *et al.*, 2017). With this approach to reducing motion-related artifact, we can reevaluate whether patterns of RSFC can predict an individual's age, but not age-related head movement.

Specifically, frame-by-frame head displacement (FD) was calculated from preprocessing realignment estimates, and frames with FD > 0.2 mm were removed. An FD threshold of 0.2 mm was chosen because it best reduced the distance-dependence related to individual differences in head motion (estimated with mean FD and six motion parameters) in this developmental dataset, as assessed using procedures from Power et al. (2012) and Ciric et al. (2017) (see Supplemental Material A). Data were considered usable only in contiguous sets of at least 3 frames with FD < 0.2 and a minimum of 50 frames within a functional run. 'Bad' frames were censored from the continuous, processed resting-state time series before computing resting-state correlations. Notably, the global signal was included as a nuisance regressor (mentioned above) in order to further reduce global, motion-related spikes in BOLD data (Power *et al.*, 2014; Ciric *et al.*, 2017). To avoid motion-related differences in the amount of data used to calculate resting-state correlations across participants, 120 randomly selected 'good' frames of usable data (i.e., frames surviving motion censoring) from each participant were included in further analysis.

To quantify how motion censoring and global signal regression affect multivariate prediction with RSFC, we performed additional analyses with (1) no motion de-noising (no global signal regression + no frame censoring) and (2) partial motion de-noising

(global signal regression + no frame censoring and no global signal regression + frame censoring).

Resting-state functional connectivity network construction

For each participant, resting-state time courses were extracted from a set of 264 previously defined regions of interest (ROIs) covering much of the brain shown in Figure 2-1 A (Power *et al.*, 2011). A weighted correlation matrix representing an individual's RSFC was constructed by calculating the correlation between time-courses from each

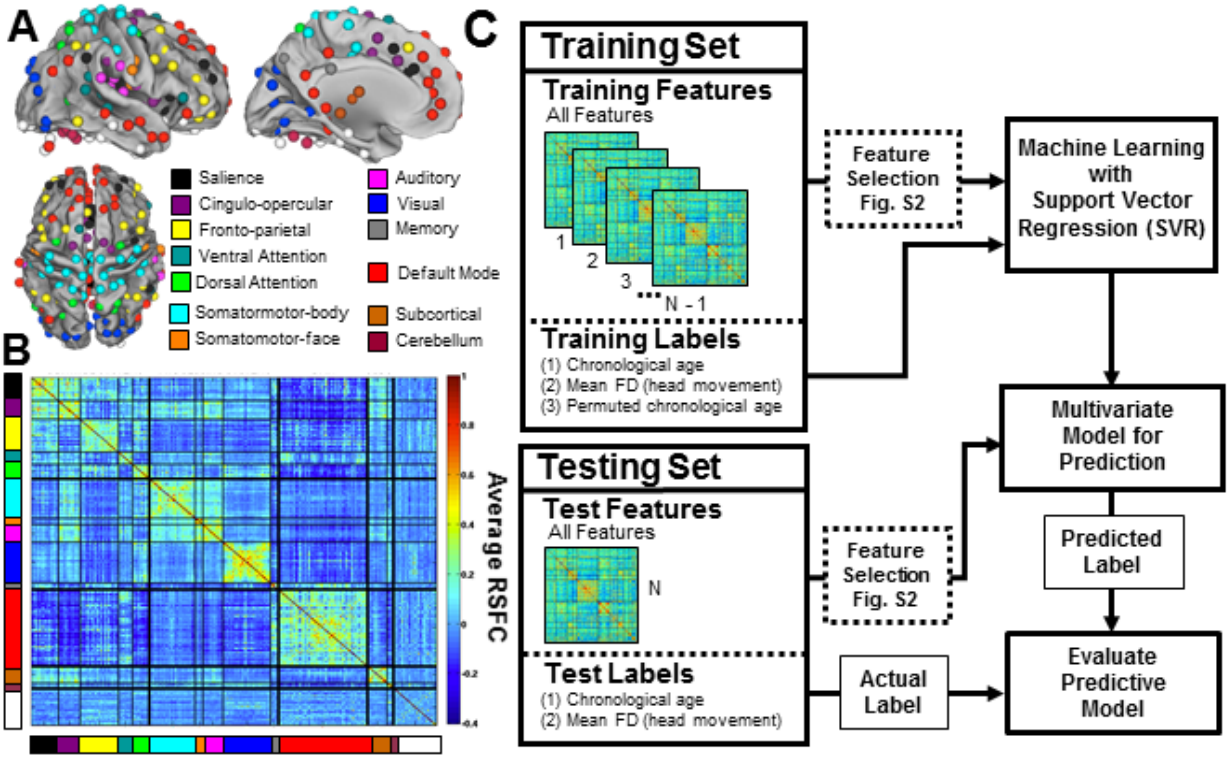

Figure 2-1. ***Overview of support vector machine learning with RSFC*****. (A)** Regions of interest (n = 264), defined in Power et al. 2011, used to create RSFC correlation matrices. Resting-state time courses were extracted from each of these regions. **(B)** Average resting-state functional connectivity across all participants. Correlations between the resting-state time courses of all pairs of regions from (A) were sorted according to functional system and average across all subjects included in this analysis. **(C)** Support vector regression was used to determine a multivariate model for prediction in a training set and this predictive model was evaluated by comparing the predicted labels and actual labels of individuals in a separate testing set. Different training labels (e.g., age, mean FD) were used to create multivariate models to predict different characteristics of individuals using RSFC. In some cases, feature selection was applied before training and testing (for specifics, see Fig. 2-S2).

pair of ROIs and normalizing these values with a Fisher transform. The group average correlation matrix for this developmental dataset is shown in Figure 2-1 B. The RSFC between these 264 ROIs reveals the organization of separable functional systems (e.g. default-mode, fronto-parietal, visual, etc.) in both children and adults (Power *et al.*, 2011; Yeo *et al.*, 2011).

2.3.3 Support Vector Regression

Support vector machine (SVM) learning was used to determine how well an individual's chronological age can be predicted from that individual's pattern of RSFC. We used the Spider Machine Learning Toolbox implemented in Matlab for SVM training and testing. Commonly, SVM is used to test whether patterns of RSFC can classify an individual as a part of a group, a binary label. This approach can be extended to the prediction of continuous labels (e.g., chronological age) using support vector machine regression (SVR). Briefly, SVR extracts the multivariate relationship between features (here, functional connections) and labels (here, age) from a training set of individuals with known labels. Further description of the parameters employed from multivariate machine learning is provided in Supplemental Material B.

We used a ten-fold cross-validation (ten-fold CV) procedure in which 10% of the participants were removed from the training set, a multivariate model was generated from the remaining participants (90% of the participants), and the left out participants were tested on the SVR-derived model. For each fold of CV, a different set of 10% of participants were removed from the training set and tested on the SVR-derived model. We tested the robustness of the SVR-derived models with three iterations of ten-fold CV (two iterations are shown in Supplemental Material E, Figure 2-S3). We also used a leave-

one-out cross-validation (LOOCV) procedure for consistency with Dosenbach et al. 2010 and to test the robustness of the results across cross-validation techniques. We found minimal differences between ten-fold CV and LOOCV (LOOCV results are provided in Supplemental Material F, Figure 2-S4).

The extent to which this derived model explains the label-related variance can be determined by applying the SVR-derived model to the features from a test individual outside of the training set and comparing the test individual's SVR predicted label and actual label. Previously, Dosenbach et al. 2010 compared several models in order to best fit the relationship between the predicted ages and actual ages of individuals. Here, we chose to use a simple, linear model in order to compare predictive performance across a variety of SVR-models built to predict different labels and built from different sets of features. A schematic of the training and testing in SVR is shown in Figure 2-1 C.

Predicting an individual's age

We used SVR to predict the age of each participant and determine whether there are age-related differences in individual patterns of RSFC. Using ten-fold CV, participants were removed from the training set and a multivariate model describing the relationship between RSFC and age was generated in the remaining participants. The left-out participants were then tested on this SVR-derived model yielding a SVR-predicted age for each participant. This process was repeated, resulting in a predicted age for every subject. Predicted ages were then compared to the true ages for each participant.

In order to identify the noise floor for prediction, we permuted the age labels of each participant in the training set. We used the same machine learning approach to assess how well SVR can use patterns of RSFC with fabricated relationships with age. We used

the same ten-fold CV procedure as described above, but trained on the permuted age labels rather than the actual ages.

Predicting an individual's head motion

Because of the issue of subject motion contaminating developmental neuroimaging data (Power *et al.*, 2012; Satterthwaite *et al.*, 2013a), we took a conservative approach to identifying potentially lurking, motion-related differences in RSFC that might spuriously enhance our ability to predict age. We used the same machine learning approach to determine whether patterns of RSFC could predict measurements of an individual participant's head movement. Using ten-fold CV, a multivariate model describing the relationship between RSFC and head motion – measured as mean FD – was generated and the left out participants were then tested on this SVR-derived model. Specifically, mean FD was calculated on the pre-frame censored data, thus quantifying the amount of movement during the entirety of the runs included for each participant. This process was repeated to predict each individual's mean FD. The predicted mean FD was then compared to the true mean FD for that participant. Similar analyses were also conducted using mean FD calculated on the post-frame censored data, which measures the residual head motion after de-noising (Supplemental Material C). To assess the impact of motion de-noising on RSFC, multivariate models describing the relationship between mean FD and RSFC that did not undergo motion de-noising (GSR + frame censoring) were also generated and tested.

Prediction across Feature Numbers

We aimed to explore how the number of features used to create the multivariate model affects the ability to predict age and head motion. We randomly selected functional

connections from the entire correlation matrix, sampling between 100 and 19,000 features (out of the possible 34,716) in logarithmic increments. Twenty-five random feature sets were generated for each of the forty-five feature numbers sampled. With these feature sets, we tested how well SVR can identify patterns of RSFC related to age, head motion, and permuted age labels in order to make predictions about individuals. Using ten-fold CV, a multivariate model describing the relationship between these labels and RSFC in randomly selected functional connections was generated and the left out participants were then tested on this SVR-derived model.

2.3.4 Feature Selection

Feature selection is a standard approach in the field of machine learning whose objective is to remove irrelevant features to reduce computational burden, avoid overfitting, and potentially improve predictive performance (Guyon and Elisseeff, 2003). Many investigators have interrogated the features derived from feature selection – in the case of RSFC, functional connections – facilitating prediction. The identified, reduced set of functional connections has often been interpreted as meaningful to the mechanism underlying the predicted characteristic (e.g., maturation, disease). We used feature selection to investigate which functional connections carry information useful for age prediction using both data-driven (features defined in a training set) and hypothesis-driven (features defined a priori) approaches. Before interpreting these identified features as meaningful to the mechanism(s) underlying typical development, we compared the performance of selected features to a null model built from a matched set of randomly selected features. Supplemental Material Figure 2-S2 summarizes the types of feature selection used for age prediction.

Data-driven Feature Selection

Univariate Feature Ranking & Selection in a Training Set

As a simple approach to identify the best features to predict an individual's age, we ranked and selected features according to the univariate correlation between each functional connection and age across subjects, as in Dosenbach et al. (2010). For each fold of CV, features were ranked according to the strength of the correlation between RSFC and age in the remaining subjects in the training set (note: this approach is different than features ranked according to the *RSFC strength within an individual*; see RSFC Strength, below). We sampled between 100 and 19,000 top ranked features in logarithmic increments, generated a multivariate model describing the relationship between age and RSFC in these features, and tested the left out participants on the SVR-derived models.

Matched Feature Set & Null Model Comparison: We evaluated whether these functional connections with strong age relationships were the most useful for multivariate age prediction by contrasting them with a matched set of randomly selected features (see Prediction across Feature Numbers). We generated a multivariate model describing the relationship between age and RSFC in these randomly selected features, tested the left out participants on the SVR-derived models, and compared the performance of top ranked features with randomly selected features.

Hypothesis-driven Feature Selection

Beyond identifying a set of features most related to age as described above, we were also interested in experimentally manipulating the information available for age prediction. We aimed to test whether development relies upon organizing principles of RSFC such

as connection distance, the definition of functional systems, or the strength of correlations.

Connection Distance

Previously, Dosenbach et al. (2010) described evidence that connection distance might underlie the usefulness of functional connections for age prediction. To compare how functional connections of different connection distance contribute to age prediction, we divided the resting-state correlations into ten separate windows (3471 functional connections per window) based on the distance of the connections in template Talairach space (computed via Euclidean volumetric distance among group ROIs). Using ten-fold CV, a multivariate model describing the relationship between age and the RSFC in these functional connections of a particular length (e.g., short-range, long-range) was determined and the left out participants were then tested on this SVR-derived model.

Matched Feature Set & Null Model Comparison: We compared the SVR performance derived from features of a particular connection length with the SVR performance derived from randomly selected features to determine whether connection distance underlies age prediction with RSFC. Randomly selected feature sets were specifically matched to have the same number of features as the ten separate distance windows (3471 functional connections). Twenty-five randomly selected feature sets were generated. Using ten-fold CV, a multivariate model describing the relationship between age and the RSFC in these randomly selected connections was determined and the left out participants were then tested on this SVR-derived model.

Functional Systems

The brain is organized into functional systems (e.g., visual, default-mode, dorsal attention, fronto-parietal, etc.) that can be revealed with RSFC at the group (Power *et al.*, 2011; Yeo *et al.*, 2011) and individual (Laumann *et al.*, 2015; Gordon *et al.*, 2017b) levels. Previously, we and others have shown that SVM classification accuracy for distinguishing children with developmental disorders (e.g Tourette syndrome (Greene *et al.*, 2016b), Autism Spectrum Disorder (Uddin *et al.*, 2013)) from healthy controls varied by the functional system(s) used for SVM training. To compare how functional connections from different functional systems contribute to age prediction, we divided the resting-state correlations according to the thirteen functional systems defined in Power et al. 2011, including control systems (fronto-parietal, cingulo-opercular, salience, ventral attention, dorsal attention), processing systems (somatomotor-body, somatomotor-mouth, visual, auditory, memory), the default-mode system, a subcortical system, and a cerebellar system depicted in Figure 2-1 A (Power *et al.*, 2011). For each system-level comparison, functional connections within the system and functional connections between that system and the other systems were included. Using ten-fold CV, a multivariate model describing the relationship between age and the RSFC in connections associated with a particular functional system was determined and the left out participants were then tested on this SVR-derived model.

Matched Feature Set & Null Model Comparison: Performance with each system-selective model was then compared with SVR performance derived from randomly selected features matched to have the same number of features as each functional system (see Prediction across Feature Numbers). Using ten-fold CV, a multivariate model describing

the relationship between age and the RSFC in these randomly selected connections was determined and the left out participants were then tested on this SVR-derived model.

RSFC Strength

While strong positive resting-state correlations have dominated most RSFC studies, strong negative functional connections, as well as weakly positive or negative functional connections, might also change in development and be useful for age prediction. Previously, Bassett et al. (2012) observed that SVM classification accuracy for distinguishing patients with schizophrenia from healthy controls differed when separately including features with strong positive and weakly positive RSFC; weakly positive functional connections were more predictive than strongly positive or moderately positive functional connections. To separately consider how functional connections of different RSFC strength contribute to age prediction, we divided resting-state correlations within each individual into ten separate windows based on the strength of each connection (3471 functional connections per window). Specifically, features were sorted by RSFC strength within each individual and a window of 10% of these functional connections were selected (note: this is distinct from features ranked according strength of correlation *between RSFC and age*; see Univariate Feature Ranking & Selection in Training Set, above). For example, connections with the strongest positive RSFC per individual, regardless of the actual correlation value, were included in the top 10% strong positive window (i.e., 1 if present or 0 if not present). Importantly, the actual functional connections selected for each window depended upon each individual's correlation matrix and varied across individuals. The lack of correspondence in the location of these functional connections across individuals is the information used for age prediction. For example, a

functional connection that is in the top 10% strong positive window for one subject but not another would provide useful information for age prediction, while a functional connection that is in the top 10% strong positive window across all participants would not. Using ten-fold CV, a multivariate model describing the relationship between age and the functional connections of a particular correlation RSFC strength (e.g., strong positive, weak, strong negative) was determined and the left out participants were then tested on this SVR-derived model.

Matched Feature Set & Null Model Comparison: The performance of these correlation-magnitude models was compared to a null model of features matched in number but randomly sampled from the distribution of resting-state correlations. Specifically, features were ranked by correlation magnitude within each individual, as before, but a random set of 10% of these ranks were selected. Importantly, this random set of ranks was consistent across subjects. Twenty-five randomly selected feature sets were generated. Using ten-fold CV, a multivariate model describing the relationship between age and the location of these randomly selected connections of was determined and the left out participants were then tested on this SVR-derived model.

Inter-correlation among features in feature sets

The usefulness of a feature set can be reduced if there is a large amount of inter-correlation among features (Guyon and Elisseeff, 2003). Correlated features are likely to provide redundant information for multivariate machine learning, increasing the likelihood of suboptimal predictive performance. Thus, we tested whether the feature sets described above (i.e., data-driven and hypothesis-driven feature selection) were more inter-correlated than feature sets with randomly selected features. For each feature set, we

calculated the correlation between the RSFC values in each pair of functional connections across all individuals. Using a matched number of randomly selected functional connections, we calculated the inter-correlation in those feature sets as well. Because differences in both the mean (Figure 2-S6 B) and shape (Figure 2-S6 D) of this inter-correlation distribution indicate an increased number of inter-correlated features (see Supplemental Material H), we computed the proportion of feature pairs with an inter-correlation greater than r = 0.2 (2 standard deviations greater the mean of in the inter-correlation of features in the full correlation matrix) in order to quantify the amount of redundancy in each feature set. To further explore the impact of redundancy among functional connections on age prediction, we employed the Fast Correlation-Based Filter (Yu and Liu, 2004) that aims to reduce the number of collinear features. With this approach, features are iteratively removed from a feature set if correlated with other, stronger (more correlated with age) features above a pre-determined threshold. More details are provided in Supplemental Material H.

2.4 Results

2.4.1 After motion de-noising, individual head motion cannot be predicted from RSFC, while age can.

First, we aimed to determine whether there was information available to predict measurement of head movement (mean FD) in RSFC before and after motion de-noising. Motion-related artifact was minimized with GSR and conservative frame censoring (Power *et al.*, 2014; Ciric *et al.*, 2017). SVR using a ten-fold CV procedure was used to test the multivariate relationship between RSFC and head motion as well as the multivariate relationship between RSFC and age. As is shown in Figure 2-2 A and Figure

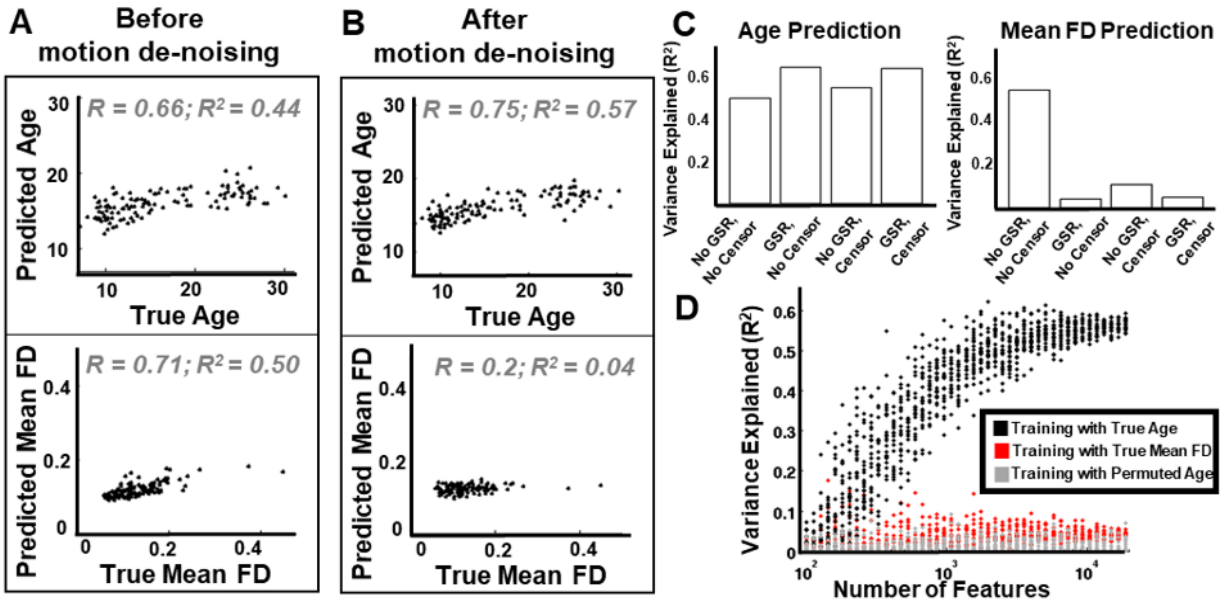

Figure 2-2. Motion de-noising affects whether *RSFC predicts head motion, but not age.* **(A)** Predicted age (top) and predicted mean FD (bottom) of individuals in the testing set compared to the true chronological age and true mean FD of each individual. Predictions were generated from RSFC before motion de-noising. **(B)** Predicted age (top) and predicted mean FD (bottom) of individuals in the testing set compared to the true chronological age and true mean FD of each individual. Predictions were generated from RSFC after motion de-noising. **(C)** Age prediction and mean FD prediction with RSFC that has undergone no motion de-noising, partial motion de-noising, and full motion de-noising. **(D)** Performance of SVR-derived models across feature sets with different number of features. Twenty-five feature sets were created by randomly selecting functional connections in forty-five logarithmic increments.

2-2 B, age was successfully and robustly predicted at the individual level in data with and without motion de-noising. In contrast, individual measurements of head motion could not be successfully predicted after reducing motion-related artifact. The amount of variance in RSFC explained by age or head motion can be quantified by comparing the true labels and SVR-predicted labels for each participant. Using the resting-state correlations between the full set of 264 ROIs, 57% of the variance in individual RSFC was explained by age with motion de-noising ($r = 0.75$, $p < 0.001$, $R^2 = 0.57$), while only 44% was explained by age without motion de-noising ($r = 0.66$, $p < 0.001$, $R^2 = 0.44$). Alternatively, 50% of the variance in RSFC was explained by individual head movement before reducing motion-related artifact ($r = 0.71$, $p < 0.001$, $R^2 = 0.50$), while only 4% was

explained by head motion after GSR and conservative frame censoring ($r = 0.2$, $p = 0.03$, $R^2 = 0.04$).

Additionally, after sufficient motion de-noising, SVR-predicted ages were less correlated with an individual's head movement. If individual head motion and age cannot be disentangled, predicted ages may still be confounded by motion-related variance in RSFC. Before motion de-noising, the ages predicted from the multivariate patterns in RSFC were negatively correlated with mean FD ($r = -0.44$, $p < 0.001$, $R^2 = 0.20$). After reducing motion-related artifact, the relationship between RSFC-predicted ages and individual mean FD was markedly reduced ($r = -0.32$, $p < 0.001$, $R^2 = 0.10$).

To determine the impact of different components of motion de-noising on the multivariate effects of head motion on RSFC, we tested how well patterns of partially de-noised RSFC (GSR alone, frame censoring alone) could be used to predict measurements of individual head movement. Of the steps that best remove systematic differences in RSFC, GSR alone eliminated most multivariate information related to an individual's head movement ($R^2 = 0.04$). Frame censoring alone also reduced multivariate effects of head motion as measured by mean FD across all data (pre-frame censoring mean FD, $R^2 = 0.10$). However, frame censoring alone was not sufficient to reduce the multivariate effects of residual head motion after frame censoring (post-frame censoring mean FD, $R^2 = 0.20$, Supplemental Material C). Figure 2-2 C shows that, while age information is preserved, information about individual-level head movement is drastically reduced after GSR or after frame censoring.

In order to further interrogate the robustness of multivariate information related to age and head motion in RSFC, we tested the multivariate prediction of age and mean FD

across many different feature sets. SVR performance for predicting age increased with the number of features (i.e. functional connections) included in training and testing as shown in Figure 2-2 D. As an experimental control, the multivariate relationship between RSFC and permuted age labels was derived with SVR in a training set and used to predict the age of test individuals. As expected, performance of this experimental control model was poor ($r = 0.08$, $p = 0.183$, $R^2 = 0.006$). While SVR performance for predicting age far surpassed this experimental control, the performance predicting mean FD with adequately de-noised RSFC did not outperform the experimental control.

2.4.2 Top ranked functional connections predict an individual's age, but not better than random functional connections.

Using data-driven feature selection, we aimed to determine a set of features that optimally predict age with SVR. Multivariate models were built with the functional connections with

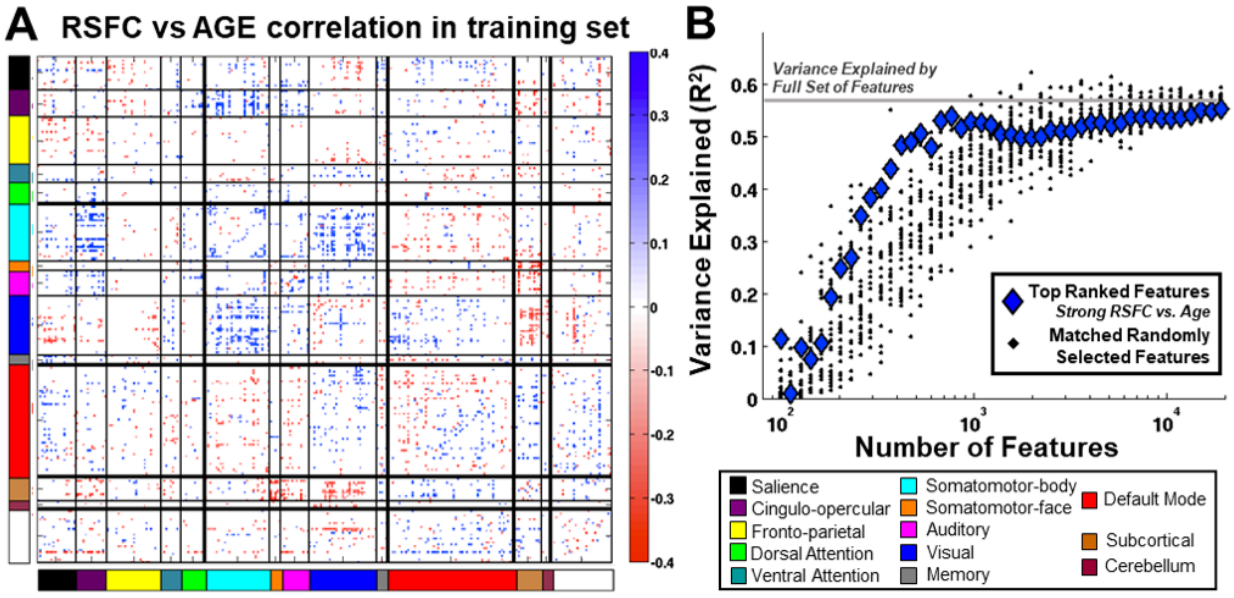

Figure 2-3. ***RSFC with strong, univariate age relationships predict age no better than randomly selected RSFC with multivariate SVR.*** **(A)** An example of the top ranked features (Consensus Features from 10%, 3471 features) across training sets. The correlation between RSFC and age was generated for these features and sorted according to functional systems. **(B)** Performance of SVR-derived models built with top ranked features and randomly selected features using different numbers of features. Feature sets were selected in logarithmic increments.

the strongest correlation with age within each training set (e.g., Figure 2-3 A: Consensus Features in Top Ranked 10%). Features with strong age relationships in the training set were able to predict the age of test individuals reasonably well, peaking at 57% of the variance explained. Figure 2-3 B shows how the amount of developmental variance explained in the testing set depends upon the number of features included in the model. Models built from a limited set of top ranked features matched, but never predicted age better than, the model build from the full correlation matrix (i.e., 57% variance explained) even though features weakly related to age were removed. Furthermore, the SVR performance of top ranked features was not significantly better than the performance of models built from randomly selected features of the same number, as shown in Figure 2-3 B. Some feature sets of intermediate number appear to produce marginally better age prediction than randomly selected features, suggesting that there might be a specific range of features which facilitate age prediction. However, further investigation of top ranked features with a different cross validation protocol (training set of 90 and testing set of 32, instead of ten-fold CV) indicates the performance of top ranked features does not differ from randomly selected features across feature numbers (see Supplemental Material G). Taken together, these different validation approaches indicate that the functional connections that are most correlated with age do not uniquely or especially facilitate age prediction.

2.4.3 After motion correction, connection length does not contribute to improved age prediction.

Given previous suggestions of a local-to-distributed development of brain networks (Fair *et al.*, 2009; Supekar *et al.*, 2009; Dosenbach *et al.*, 2010), we next aimed to compare how functional connections of different length (e.g., short-range, long-range) contribute

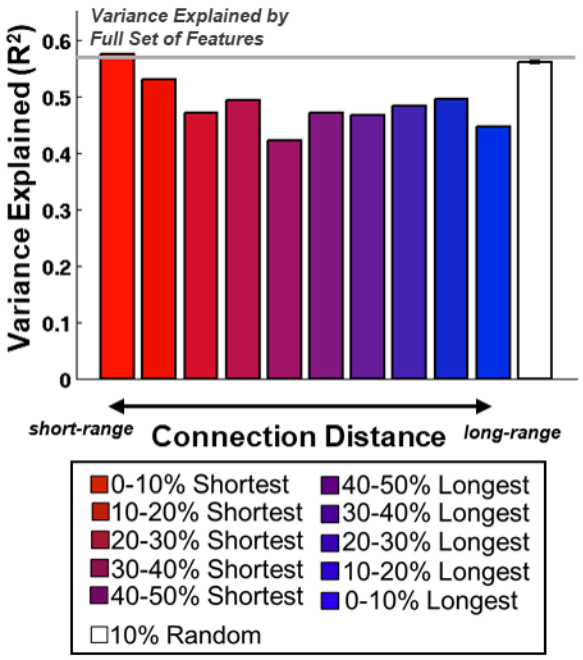

Figure 2-4. ***After motion correction, connection length does not contribute to age prediction.*** Performance of SVR-derived models built with features selected by connection length and features selected randomly (10%, 3471 features).

to age prediction. Multivariate models were built with features defined by connection distance. These models were able to predict the age of a left out individual well (R^2 = 0.49 ± 0.04; Figure 2-4). However, SVR performance of features selected by connection length was not better than the performance of models built from a matched set of randomly selected features. Additionally, prediction was uniform across different connection distances, with neither short- nor long-range connections facilitating age prediction in comparison to mid-range connections. Age prediction in these feature sets, while comparable to age prediction in randomly selected feature sets, did not depend on the length of the functional connections used to comprise the SVR-derived model.

2.4.4 Different functional systems can predict age, but poorer than distributed features.

We next aimed to compare how connections from different functional systems contribute to age prediction, given evidence that brain systems may develop at different rates (Gogtay *et al.*, 2004). Multivariate models were built by selecting features from each functional system individually. These models were able to predict age to some extent (Figure 2-5). However, prediction performance varied largely as a function of the number of features within each system. Notably, the SVR performance of features selected from each functional system was worse than the performance of models built from randomly selected features that were distributed across multiple functional systems. Thus, functional connections from individual functional systems carry less information to predict age than functional connections randomly distributed across the brain and the differences

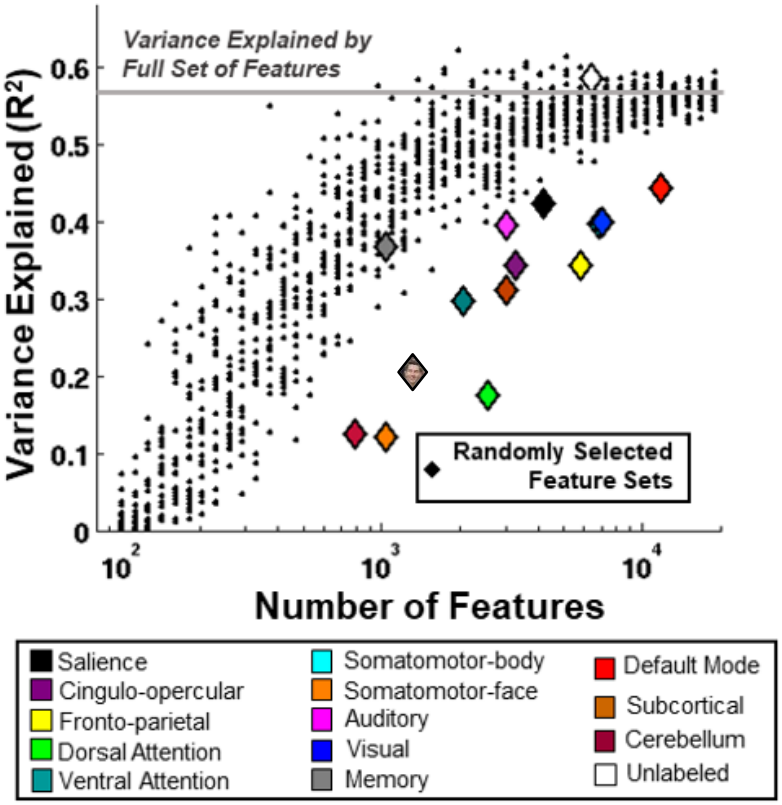

Figure 2-5. ***No single functional system predicts age better than randomly selected functional connections.*** Performance of SVR-derived models built with features selected from single functional systems and features selected randomly (matched by size).

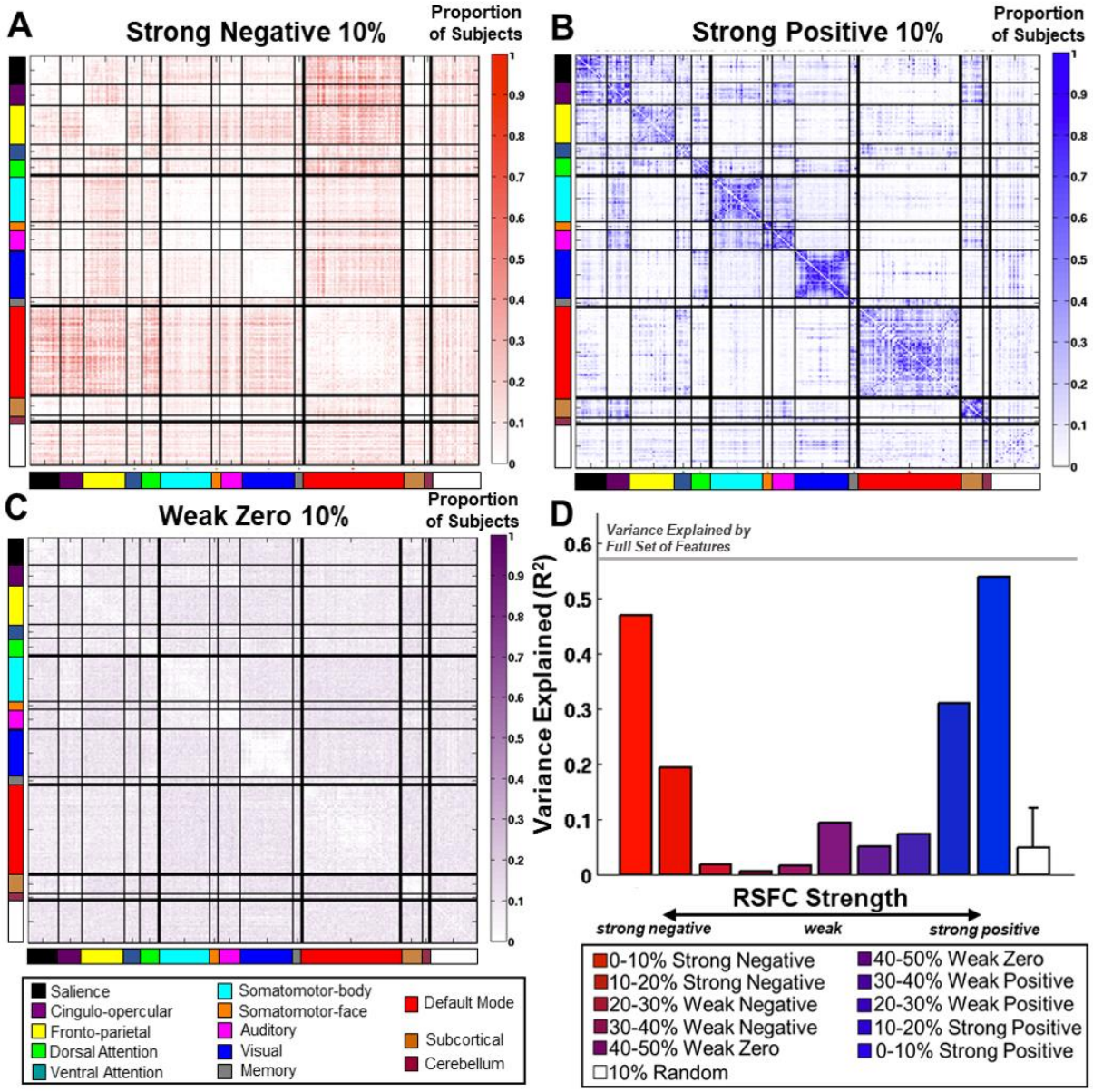

Figure 2-6*. RSFC strength contributes to age prediction.* **(A)** The distribution of strong negative resting-state correlations across all individuals in the developmental dataset. **(B)** The distribution of strong positive resting-state correlations across all individuals in the developmental dataset. **(C)** The distribution of weak zero resting-state correlations across all individuals in the developmental dataset. **(D)** Performance of SVR-derived models built with features selected by correlation strength and features selected randomly from the correlation distribution (10%, 3471 features).

in age prediction performance between different functional systems vary largely based on system size rather than system identity.

2.4.5 Strong positive and strong negative connections predicts age better than weak connections

Finally, we compared how connections from different parts of an individual's correlation distribution (i.e., strong positive, weak, strong negative) contribute to age prediction, given suggestions that even weak magnitude RSFC can improve prediction in disease states (Bassett *et al.*, 2012). The observed location of strongly-positive, weak, and strongly-negative RSFC across all individuals in the developmental dataset is shown in Figure 2-6 A-C. Strong negative RSFC was most frequently found between the DMN and other systems, and the strong positive RSFC was most frequently found within systems along the diagonal across all individuals. Weak RSFC was present in more variable locations across individuals. Multivariate models based on the location of strong positive and strong negative RSFC within an individual were able to predict age well (strong positive R^2 = 0.54; strong negative R^2 = 0.47). In contrast, multivariate models built from sets of features with weak functional connections were not able to predict age well as depicted in Figure 2-6 D. The SVR performance of features with strong positive and strong negative RSFC was better than the performance of models built from a matched set of randomly selected functional connections.

2.4.6 Some feature sets contain more redundant features than randomly-derived feature sets

Inter-correlated features may hinder multivariate age prediction because they may provide redundant information. Figure 2-7 compares the amount of inter-correlation among different feature sets and demonstrates that age-correlated functional connections

are consistently more inter-correlated across subjects than groups of randomly selected features. Additionally, functional systems, defined in part by the consistent RSFC relationships across individuals, contain features that are more inter-correlated than matched sets of randomly selected features, as might be expected. Thus, it is possible that inter-correlations among feature sets may reduce the power of age-correlated and functional system feature sets to predict age. For further characterization of the inter-correlation in these feature sets, see Supplemental Material H.

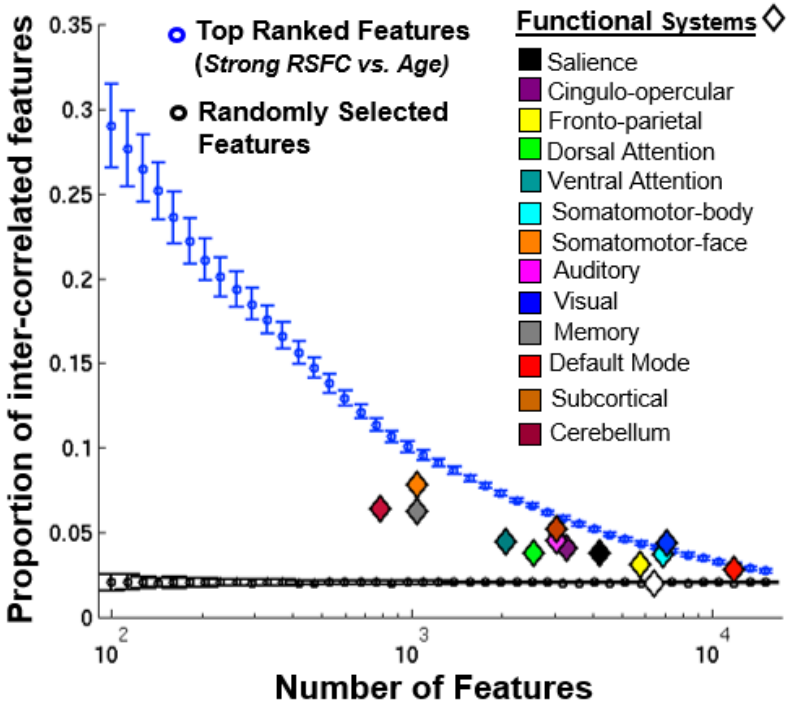

Figure 2-7. ***Proportion of inter-correlated features in the tested feature sets.*** Proportion of feature pairs in the tested feature set with inter-correlation greater than in the full correlation matrix (2 standard deviations greater than the mean; $r > 0.2$). The mean and 95% confidence interval of this measure of inter-correlation was generated for the top ranked features defined in each fold of ten-fold CV and for the randomly selected features across feature numbers. The inter-correlation was also generated for feature sets with functional connections from single functional systems.

2.5 Discussion

2.5.1 Motion de-noising eliminates the multivariate effects of head motion on RSFC, while preserving age information.

In this work, we have shown that de-noising methods to minimize motion artifact (Ciric et al., 2017) – including both global signal regression (GSR) and frame censoring - is necessary to remove multivariate effects of head motion on RSFC. Without motion de-noising, patterns of RSFC could be used to successfully predict measurements of head movement (Figure 2-2 A). After motion de-noising, we were unable predict individual variability in head movement with RSFC, while still successfully predicting age (Figure 2-2 B). Thus, even after reducing motion-related information, RSFC carries information relevant to typical development, validating previous claims (Dosenbach et al., 2010) and supporting more recent follow-ups (Fair *et al.*, 2013; Satterthwaite *et al.*, 2013b). While these previous studies have shown that age can still be predicted from RSFC after reducing motion-related artifact, our results extend such findings in a critical way by showing that there is limited lingering information about head movement as estimated with mean FD in RSFC after motion de-noising.

2.5.2 RSFC can predict an individual's age and may be a useful indicator of developmental progress.

In this work, we were able to well predict an individual's age from RSFC, explaining 57% of the developmental variance across participants. Our results are comparable to previous findings of age prediction with multivariate machine learning using other measurements of the developing brain such as voxel based morphometry of T1-weighted scans ((Franke *et al.*, 2012), $R = 0.93$, $R^2 = 86\%$), volume of grey matter, white matter, and lateral ventricles ((Erus *et al.*, 2015), $R = 0.89$, $R^2 = 79\%$), and regional cortical

thickness ((Khundrakpam *et al.*, 2015), R = 0.84, R^2 = 71%). Additionally, measurements of structural connectivity, such as fractional anisotropy and diffusivity obtained with diffusion tensor imaging ((Erus *et al.*, 2015), R = 0.89, R^2 = 79%), have also been used to successfully predict an individual's age with multivariate machine learning. Recently, task-related FC, a measurement of the transient changes in regional coherence during task performance, has been used to predict age with moderate accuracy, explaining 42% of variance related to age in a validation set (Rudolph *et al.*, 2017). Approaches that combine information from multiple imaging modalities (T1, T2, and diffusion weighted imaging, (Brown *et al.*, 2012), R = 0.96, R^2 = 92%) have been shown to achieve the highest prediction performance. However, there is increasing evidence that head motion in the scanner systematically affects measurements of cortical thickness, grey matter volume (Reuter *et al.*, 2015), and fractional anisotropy (Ling *et al.*, 2012; Yendiki *et al.*, 2014) as well as RSFC. Thus, the reported performance of multivariate age prediction with structural measurements may also be contaminated by head motion, and require additional validation.

While we (and others (Fair *et al.*, 2013; Satterthwaite *et al.*, 2013b)) have shown that RSFC carries substantial information about the development of an individual (R = 0.75; R^2 = 0.57), not all characteristics of individual brain maturity are likely, nor anticipated, to be captured in resting-state correlations. For example, we know that brain size changes systematically with age (Giedd and Rapoport, 2010). The distinctive utility of RSFC may lie in identifying the functional underpinnings of atypically developing individuals. RSFC, a measurement of the statistical history of co-activation across an individual's lifespan (Fox and Raichle, 2007; Dosenbach *et al.*, 2008), may be disrupted

in an abnormal developmental trajectory. Because RSFC is more closely related to function than measures of brain structure, differences in RSFC might be a particularly useful indicator of dysfunction in child brain development.

2.5.3 After reducing motion-related artifact, age prediction with RSFC does not support the local-to-distributed hypothesis of the development of RSFC.

Earlier studies of the development of RSFC organization suggested that as an individual matures, resting-state correlations shift from local, short-range connections to distributed, long-range connections. This evidence was appealing because it agreed with neurobiological evidence of the continued myelination of long-range pathways into adolescence and adulthood (Barnea-Goraly *et al.*, 2005). However, motion artifacts also amplify short-range RSFC and reduce long-range RSFC. While earlier attempts at age prediction with RSFC supported the local-to-distributed developmental hypothesis (Fair *et al.*, 2009; Supekar *et al.*, 2009; Dosenbach *et al.*, 2010), we did not find evidence for distance-dependence in predicting age after reducing motion-related artifact. Short-range and long-range connections predicted age similarly to mid-range connections and randomly selected functional connections (Figure 2-4). Other evidence based on network organization of RSFC also contradicts the local-to-distributed development of RSFC after correcting for individual head motion (Fair *et al.*, 2013; Marek *et al.*, 2015).

2.5.4 Age is best predicted by strong positive and strong negative RSFC within an individual.

Because the location of strong positive and strong negative RSFC is conserved across development (Figure 2-6 A, 2-6 B), these resting-state correlations likely represent important information about brain functioning in individuals. In most individuals in our sample, strong positive RSFC was between ROIs within functional systems and strong

negative RSFC was between functional systems involved in the engagement/disengagement from tasks (ex: DMN, FP, CO) (Fox *et al.*, 2005). Importantly, despite the fact that these connections appear highly conserved across individuals, individual differences in the location of strong RSFC predict age well (R^2 = 0.54 and 0.47) and better than weak/moderate RSFC or randomly selected connections. While the location of weak and moderate RSFC varies more across individuals than strong RSFC, inter-subject variance appears to show a negligible relationship with age (average R^2 = 0.043), and may reflect the noisy nature of these functional connections. The utility of strong-positive and strong-negative functional connections for age prediction might support previous contentions of network segregation in development (Fair *et al.*, 2007; Satterthwaite *et al.*, 2013b). Strong within-network and between-network connections may be modified over the course of development in order to refine functional network organization, yet further research is necessary to directly test such claims.

Using similar approaches, others have argued that the weak resting-state correlations contain information relevant for prediction of other characteristics of an individual, such as I.Q. and psychiatric diagnosis (Bassett *et al.*, 2012; Santarnecchi *et al.*, 2014). We contend that the disparity in these results is related to effectively addressing motion-related artifact using volume censoring and GSR. While GSR removes the great majority of the differences in RSFC related to head motion (Power *et al.*, 2014; Ciric *et al.*, 2017), this procedure also shifts an individual's resting-state correlation distribution so that it becomes zero-centered and necessarily increases the number of negatively correlated functional connections (Saad *et al.*, 2012; Power *et al.*, 2014). Thus, previously described weak (positive or negative) connections without GSR may be

equivalent to the strong negative resting-state correlations after GSR described here. In order to assess the importance of these connections in predicting an individual's age (or any characteristic), it is necessary to address motion-related artifact and to then demonstrate that the cleaned data are unable to predict that individual's head movement. As GSR eliminated most of the multivariate effects of head motion on RSFC, it is possible that weak connections without GSR could also predict measurements of head movement.

2.5.5 Broad sampling of functional connections yields better age prediction than directed sampling due to (1) the distributed nature of information and (2) the redundancy of relevant features.

Because RSFC was able to predict an individual's age with SVR after reducing motion-related artifact, we aimed to interrogate the specific functional connections facilitating age prediction to better understand the mechanisms underlying the development of RSFC. We attempted to interrogate the features relevant to age prediction with directed, data-driven (i.e., top ranked relationships with age) and hypothesis-driven (i.e., functional systems) feature selection schemes. Unexpectedly, we found that directed sampling of functional connections yielded age prediction that was no better or, in the case of functional systems, worse than that obtained with a broad sampling of functional connections (i.e., random feature selection) (see Figure 2-3 B and 2-5). We have found two related properties of this developmental dataset that may contribute to the poorer performance of directed sampling, addressed below.

Developmental differences in RSFC are distributed across many functional systems.

We found that information in RSFC related to age appears to be unevenly distributed in a structured way across functional systems (enriched in some blocks: e.g., many functional connections within somatomotor-visual have a strong positive correlation with

age), but resides in all functional systems. Because of the distributed nature of age-related RSFC, there may be many sets of features that are able to predict age well, even when randomly selected. Multivariate approaches are particularly well-suited to use patterns of features with variable age relationships to predict age (Jimura and Poldrack, 2012). Thus, in random feature selection, by chance, relevant features across multiple functional systems are often captured, which enables robust age prediction.

Adding to the evidence that developmental differences in RSFC are distributed across many functional systems, we found that each functional system predicted age worse than randomly selected features distributed across functional systems (Figure 2-5). Poorer performance of features associated with a single functional system suggests that information from multiple functional systems is necessary to achieve optimal age prediction. We did find that age prediction differed between functional systems; however, whether these differences are related to the usefulness of information from a given functional system or the number of features associated with that system remains unclear. If the mechanism by which RSFC develops is not system-dependent, then larger functional systems may be more likely to capture relevant information for age prediction by chance. Explanation-driven approaches beyond those employed in the present study may be better able to identify the specific brain systems or pieces of specific systems that change over the course of development.

While a significant portion of the extant developmental cognitive neuroscience literature has focused on the maturation of specific brain regions (e.g., the prefrontal cortex (Casey *et al.*, 2005)) or specific functional networks (e.g., the default mode (Supekar *et al.*, 2010)), the present results suggest that investigations of the maturation

of functional neuroanatomy might be more usefully addressed by a whole-brain or large-scale network approach. From a complex network perspective, the observation that developmental changes in functional connections are distributed across multiple systems may not be surprising. In the evolution of many complex networks, connections are modified across functional modules such that global communication is optimized and integrative hubs are created (Solé *et al.*, 2002). It is possible that the distributed nature of developmental differences in RSFC reflects a growth mechanism that optimizes global communication rather than enhancing a single functional system. The genetics literature offers an interesting analogy with the recently proposed "omnigenic" model for the inheritance of complex traits. In this model, signal associated with complex traits is spread out across the genome (Boyle *et al.*, 2017). Thus, one might predict that a complex characteristic of an individual, like maturity, could be supported by distributed changes in network functioning. An interesting future direction may be to determine whether more complex measures of network organization carry information useful for individual-level age prediction.

Many functional connections that are relevant to development provide redundant information for age prediction.

Although distributed across many functional systems, top ranked features (i.e., functional connections that are most strongly correlated with age) did not predict age better than randomly selected features with multivariate machine learning, as we had expected (Figure 2-3B). By definition, these functional connections have, on average, stronger relationships with age than randomly selected functional connections, but were no more useful for age prediction. We believe that the usefulness of top ranked features was limited by the inter-correlated information carried by these features. Even if two features

can each predict age well individually, there is little additional information contributed to facilitate age prediction if the pair of features are highly correlated, as they may use the same underlying information for age prediction (Guyon and Elisseeff, 2003). Given that the top ranked features were much more highly inter-correlated across participants than randomly selected features (Figure 2-7), this redundancy may explain why these features predicted age no better than randomly selected features. We tested this hypothesis by removing redundant features using a Fast Correlation-Based Filter (Yu and Liu, 2004) and found that age prediction performance decreased more slowly when removing redundant features than when randomly removing features (Figure 2-S7).

One likely source of redundancy is the network organization of RSFC. By definition, functional systems identified with RSFC are composed of regions with similar patterns of connectivity. The patterns of connectivity that define functional systems are largely conserved across individuals (Power *et al.*, 2011; Mueller *et al.*, 2013; Wang *et al.*, 2015; Gordon *et al.*, 2017a). The redundancy within systems may also explain why functional connections from a single system cannot predict age as well as randomly selected functional connections that sample multiple systems (Figure 2-5). The redundancy of features selected from functional systems is likely not unique to age prediction and might affect prediction of other characteristics of individuals with RSFC using multivariate machine learning.

While redundancy reduces the usefulness of a feature set for age prediction, it does not reduce the relevance of these features to the development of RSFC. Feature selection methods which identify orthogonal features (e.g., Partial Least Squares Regression, Principal Component Regression) might be able to produce a set of features

that is more useful for age prediction than randomly selected features, though it may be difficult to interpret the neurobiological principles underlying the importance of these features in a straightforward manner. We found that feature selection aimed at reducing collinearity (Fast Correlation-Based Filter) did not yield age prediction that was better than the full set of features (Figure 2-S7) indicating that removing redundant information does not improve performance. Furthermore, because of the redundancy present in this developmental dataset, there are likely many interchangeably and equally useful sets of features. While multivariate machine learning may not be the best approach for determining a single set of functional connections underlying the typical development of RSFC, we have shown that it is quite robust and powerful, predicting an individual's age well from many different sub-sets of functional connections.

2.5.6 Evaluating the utility of multivariate prediction with resting-state functional connectivity

Many researchers use multivariate machine learning in RSFC with the intent to make accurate predictions about individuals and to interrogate the neurobiological mechanism(s) underlying a predicted characteristic. We have shown that RSFC provides a robust neurobiological measurement of an individual, sufficient to make predictions about that individual's chronological age with relatively high accuracy even, notably, after correcting for systematic differences in RSFC related to subject head motion. This observation suggests that individual age prediction with RSFC could provide useful diagnostic information about the brain maturity of individuals with developmental delay or other developmental disorders—a feat that many group-level descriptions of brain development may not be able to provide. More generally, this observation demonstrates the capacity to make predictions about an individual based on patterns of RSFC.

However, we have also shown that our ability to interrogate the specific features facilitating prediction in the hopes of understanding the neural mechanisms underlying brain development is somewhat limited. Identifying a unique set of functional connections that carry information useful for age prediction with RSFC is difficult due to the inter-correlated nature of RSFC and the distributed nature of developmental differences in RSFC, as discussed above. Thus, both data-driven and hypothesis-driven feature selection were unable to reveal functional connections that predict age better than the full set of features; removing potentially irrelevant features did not boost predictive performance. Importantly, relative to other investigations, we evaluated the performance of selected features to a null model built from a matched set of randomly selected before interpreting features as meaningful to the mechanism underlying typical development. Here, most sets of selected features (excluding strong positive and strong negative RSFC; see Figure 2-6D) did not predict age better than the randomly selected null, indicating that these functional connections, while useful for prediction, are not exclusively meaningful nor indicative of a unique solution to age-prediction from RSFC. Our inability to identify specific features that predict age does not mean that machine learning approaches cannot be used to identify specific features that contribute to other group differences (e.g. disease status). However, the identified features should be tested against an appropriate null model before making claims about the unique utility of a set of features for prediction and inter-correlations among features should be carefully evaluated during interpretation.

Multivariate machine learning models are built to make predictions, and can only test hypotheses about neurobiological mechanisms indirectly. Both approaches that

make individual-level predictions and those that test group-level differences are important to our understanding of typical and atypical development. Multivariate prediction complemented by alternative approaches directed at more mechanistic questions (e.g., group-level studies, highly-sampled individuals, within-subject longitudinal studies) will likely yield the best mechanistic understanding of typically and atypically developing individuals. Here, we demonstrate that measurements of functional neuroanatomy with RSFC are sufficiently robust to make individual-level predictions of maturity in typical development and anticipate that these characterizations may have future clinical utility in making individual-level predictions about atypical development.

2.6 Acknowledgments

We thank Rebecca Coalson, Alecia Vogel, Jessica Church-Lang, John Pruett, Joe Dubis, Katie Ihnen-Zeller, Judy Lieu, Deanna Barch, and Tammy Hershey for assistance with original data collection. We also thank our study participants and their families. This project was supported by NIH K01MH104592 (DJG), NARSAD Young Investigator Award (DJG), NIH K23NS088590 (NUFD). Original data collection was supported by NIH R01HD057076 (BLS), NIH R01NS046424 (SEP), Simons Foundation Autism Research Initiative (SEP), NIH R21MH091512 (BLS), NIH R21 NS091635 (BLS), Tourette Association of America Neuroimaging Consortium Grant (BLS, DJG), NIH NINDS NRSA-F32 NS656492, American Hearing Research Foundation, NIH K23DC006638, P50 MH071616, P60 DK020579-31, and the McDonnell Foundation. Research reported in this publication was supported by the Eunice Kennedy Shriver National Institute Of Child Health & Human Development of the National Institutes of Health under Award Number U54 HD087011 to the Intellectual and Developmental Disabilities Research Center at

Washington University. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

2.7 Supplemental Material

A. Selecting an FD threshold to reduce distance-dependent motion-related artifacts in RSFC

Frames linked with head motion in the scanner produce distance-dependent artifacts in RSFC (Power *et al.*, 2012; Van Dijk *et al.*, 2012; Ciric *et al.*, 2017). Using the approach initially defined in (Power *et al.*, 2012; Ciric *et al.*, 2017), we aimed to identify a threshold of frame-wise displacement (FD) that, when applied, best reduces these distance-

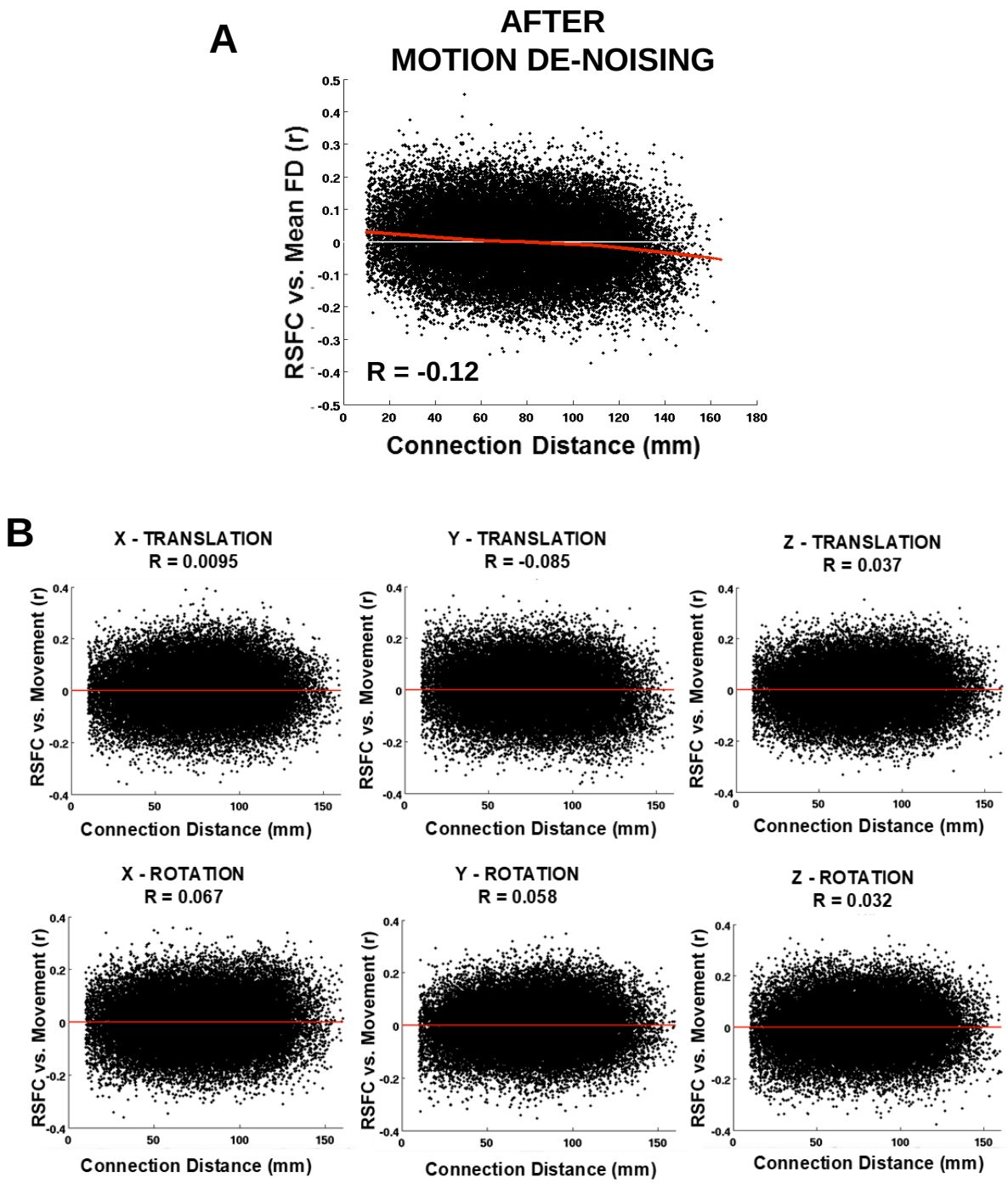

Figure 2-S1. ***Checking for distance-dependence in the relationship between RSFC and head motion.*** **(A)** For each functional connection, the correlation between the RSFC and mean FD across individuals is shown. These relationships did not strongly depend on connection distance. **(B)** For each functional connection, the correlation between the RSFC and the mean frame-wise change in six motion parameters is shown. These relationships did not strongly depend on connection distance.

dependent effects. For each subject, we created a set of resting-state time-series from all 264 ROIs with high-motion frames removed (here, high-motion frames were defined as having FD > 0.2 mm). Each individual resting-state time-series was trimmed to have to have 120 low-motion frames. RSFC correlation matrices (as discussed in Materials and Methods, resting-state functional connectivity network construction) were generated for each individual for each FD threshold. For each subject, we also calculated the mean FD before removing high-motion frames. Next, we calculated the correlation between RSFC and mean FD across all subjects. To assess whether the motion-related differences in RSFC are distant dependent, we plotted these correlations according to the Euclidean distance between the two ROIs involved in each functional connection. Distance-dependent motion-related artifact in RSFC presents as positive correlation with mean FD in short-range connections and a negative correlation with mean FD in long-range connections (Ciric *et al.*, 2017). An FD threshold which excluded frames with motion greater than 0.2 mm best reduced distance-dependent differences in RSFC related to head motion. Figure 2-S1 shows that the relationship between resting-state correlations and motion according to connection after frame censoring.

B. Support Vector Machine algorithm parameters

The parameters used for support vector regression (SVR) training were the same as those used in Dosenbach et al. 2010. SVR retains some of the main features of binary SVM classification. In SVM classification training, a penalty is incurred for misclassified data (points on the wrong side of the multivariate decision boundary). In SVR, a penalty is incurred for data that lie too far from the regression line in multivariate space. Epsilon-insensitive SVR defines a tube of width epsilon around the regression line in multivariate

space. Any data points (i.e., subjects) within this tube carry a loss of zero, meaning there is no penalty. In SVR, the C parameter controls the trade-off between how strongly subjects beyond the epsilon-insensitive tube are penalized and the flatness of the regression line (larger C allows the regression line to be less flat). All SVR predictions described in this article used epsilon-insensitive SVRs with the Spider Machine Learning Toolbox default setting of C = Infinity and epsilon = 0.00001.

C. Comparison of multivariate effects of head motion captured by pre- and post-frame censoring mean FD

In Figure 2-2, we used the average of the displacement of all collected frames across included runs to describe an individual's head movement. We found that after global signal regression and after frame censoring, patterns of RSFC could not be used to predict pre-scrubbing mean FD. We wanted to determine whether there were multivariate effects related to residual head motion in our RSFC data. Thus, with ten-fold cross-validation, we used SVR to build a multivariate model describing the relationship between RSFC and post-frame censoring mean FD and tested the left out participants on the resulting model. We found that predicted post-frame censoring mean FD was not related to participants' actual post-frame censoring mean FD ($R = 0.2$, $R^2 = 0.04$). Prediction of residual head motion was comparable to the prediction of total head motion with RSFC after motion de-noising.

To further assess the impact of frame censoring and global signal regression on the reduction of multivariate effects of head motion, we wanted to determine whether partially de-noised RSFC could be used predict residual head motion quantified by post-frame censoring FD. With ten-fold cross-validation, we used SVR to build a multivariate model

describing the relationship between partially de-noised RSFC data (frame censoring, but no global signal regression) and post-frame censoring mean FD and tested the left out participants on the resulting model. We found that patterns of partially de-noised RSFC data were able to predict residual head motion ($R = 0.44$, $R^2 = 0.20$). This suggests that frame censoring does play a role in removing multivariate effects of head motion on RSFC. However, global signal regression also eliminates motion-related effects in frames with very small amounts of motion (FD < 0.2 mm).

D. Illustration of Feature Selection

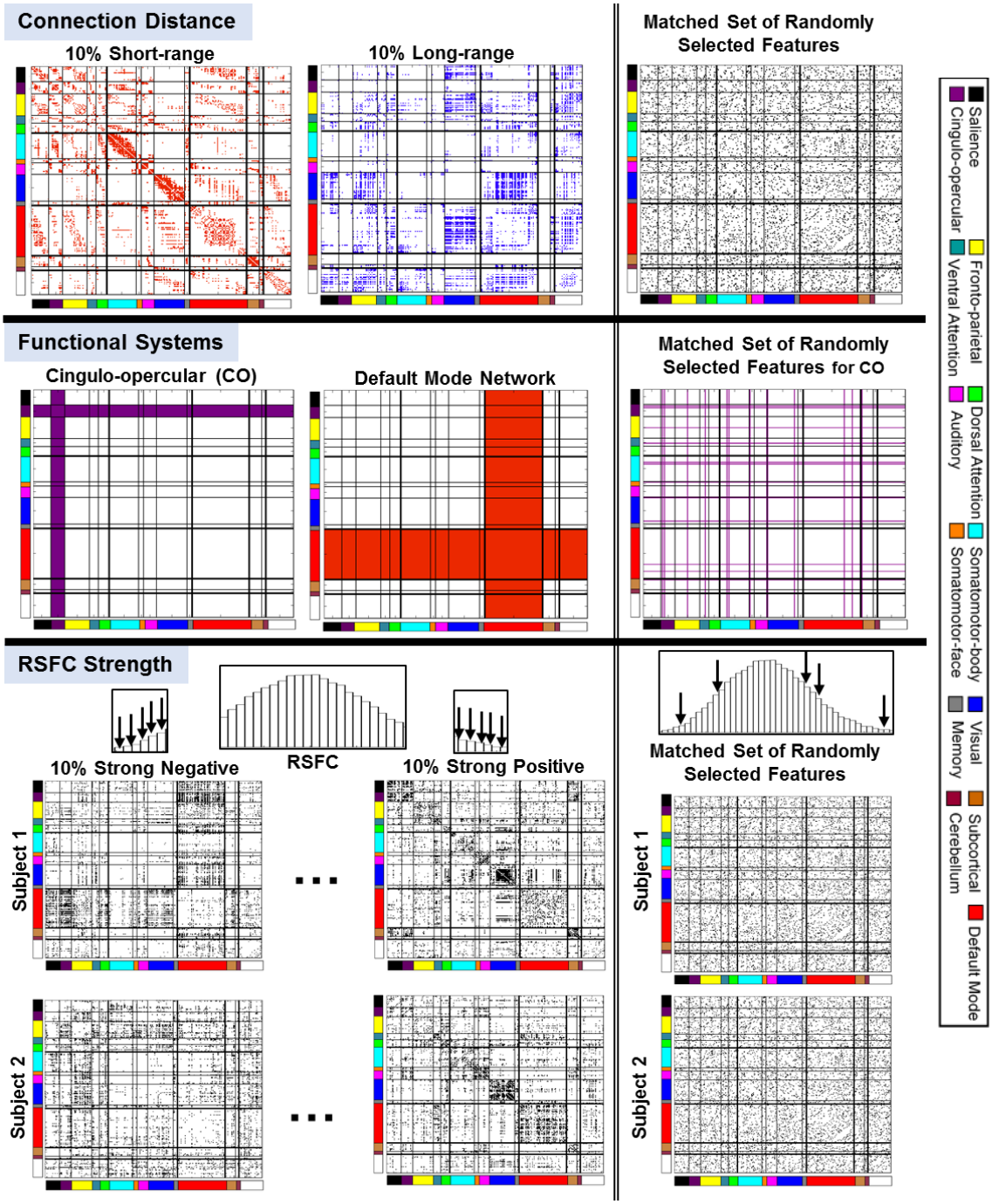

Figure 2-S2. ***Depiction of hypothesis-driven feature selection and the matched sets of randomly selected features.*** *Top:* Example sets of features selected by connection distance (l*eft*) and features selected randomly and matched by feature number (*right*). *Middle*: Example sets of features selected by functional system (l*eft)* and features selected randomly and matched by feature number (*left*). *Bottom*: Example sets of features selected by RSFC strength within the individual in two subjects (l*eft*) and features selected randomly by RSFC strength and matched by feature number (*right*).

E. Additional Iterations of Ten-Fold Cross-Validation

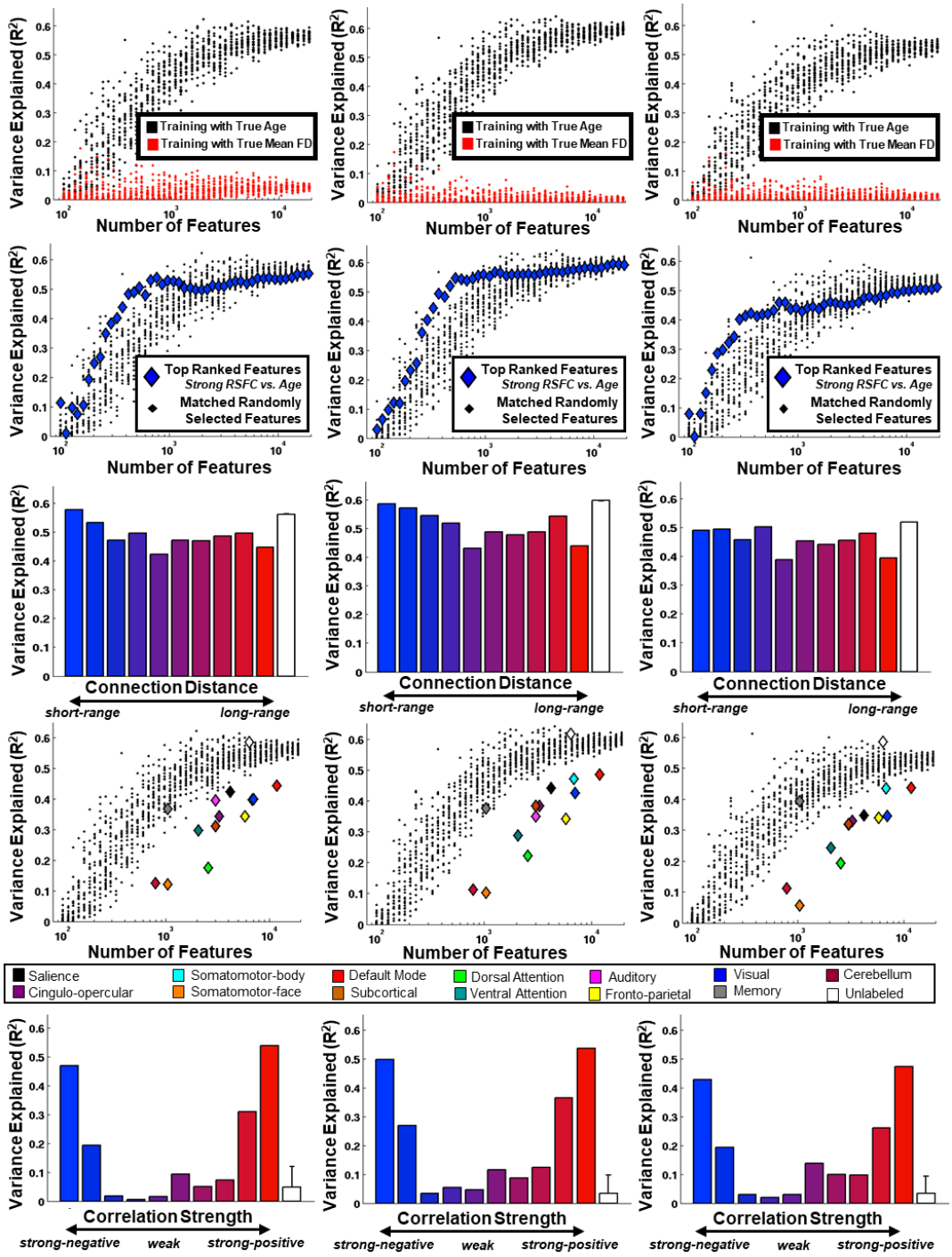

Figure 2-S3. Reported results across three different iterations of ten-fold cross-validation. Row 1: Prediction of age and the prediction of mean FD across many features sets. Row 2: Comparison of top ranked features and randomly selected features. Row 3: Comparison of long-range and short-range connections. Row 4: Comparison of features from functional systems and random features. Row 5: Comparison of strong RSFC features and weak RSFC features.

We conducted three iterations of ten-fold cross-validation to ensure that predictive performance did not depend on the specific grouping of individuals in the training and testing sets of each fold. Figure 2-S3 shows the results depicted in Figures 2-6 for all three iterations. All three iterations appear comparable.

F. Evaluation with Leave-One-Out Cross-Validation as in Dosenbach et al. 2010

In Dosenbach et al. 2010, leave-one-out cross-validation was used to evaluate the predictive model derived with SVR. Here we used both leave-one-out cross-validation and ten-fold cross-validation and found similar results. Figure 2-S4 compares the prediction of age and head motion before and after motion de-noising with ten-fold cross-validation and leave-one-out cross-validation.

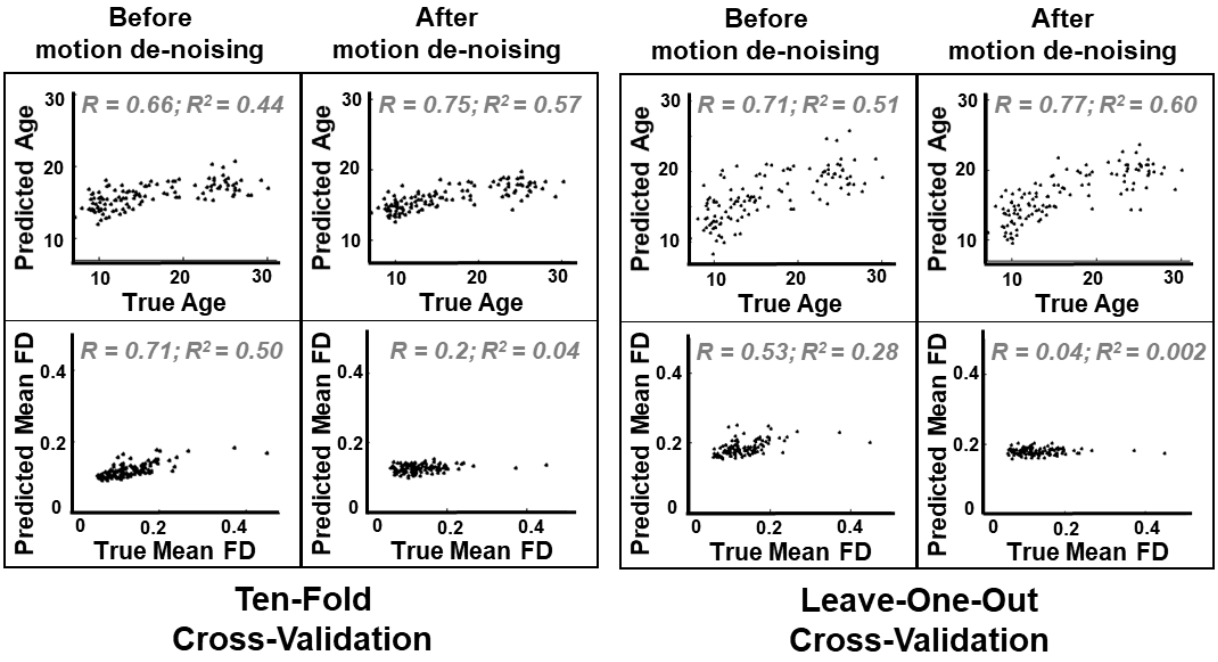

Figure 2-S4. ***Comparison of ten-fold cross-validation and leave-one-out cross-validation.*** Left: Evaluation of age prediction and mean FD prediction before and after motion de-noising with *ten-fold cross-validation*. Right: Evaluation of age prediction and mean FD prediction before and after motion de-noising with *leave-one-out cross-validation*.

G. Alternative Cross Validation approaches to evaluate Top Ranked Features

In evaluating whether top ranked features -- i.e., functional connections with strong, univariate relationships with age -- facilitate age prediction, we found through visual inspection of the relationship between variance explained and feature number (Figure 2-3) that an intermediate number of these features (~1000 features) might outperform randomly selected features. Using ten-fold cross-validation (ten-fold CV), top ranked features were identified in each fold of cross validation for each training set. While this cross validation approach maximizes the amount of samples for training with SVR, it also separately selects top ranked features for each training set; different sets of features are used to make predictions for each of the left out individuals. While there was a considerable amount of consistency across top ranked feature sets from different training sets, we tested whether these differences in feature sets might contribute to the apparent benefit of top ranked features of intermediate feature number.

We identified top ranked features in a training set of 90 randomly selected subjects from the total set of 122 (32 subjects left out for testing). We ranked and selected features according to the univariate correlation between the RSFC of each function connection and age. Top ranked feature sets were generated in fifty separate training sets of 90 randomly sampled subjects. We sampled between 300 and 1000 top ranked features in logarithmic increments, generated a multivariate model describing the relationship between age and RSFC in these features in the training set, and tested the remaining 32 participants on this SVR-derived model. This cross validation approach ensures that the same features are used to make predictions for the 32 left out individuals.

Matched Feature Set & Null Model Comparison: We evaluated whether these functional connections with strong age relationships were the most useful for multivariate age prediction by contrasting them with a matched set of randomly selected features. We randomly selected feature sets matched to have the same number of features as the top ranked features (300-1000). Twenty-five randomly selected features were generated for each of the fifty training sets and each of the ten feature numbers sampled. We generated a multivariate model describing the relationship between age and RSFC in these randomly selected features in the training set and tested the remaining 32 participants on this SVR-derived model.

We found that most feature sets containing top ranked features identified in the training set well predicted the age of the left out 32 individuals, with the variance explained in the test set averaging at about 48% of the variance. However, these features did not outperform the matched sets of randomly selected features as shown in Figure 2-S3. To compare the performance of top ranked features to the randomly selected features in each partition of the training set, we normalized the performance of the top ranked features to the mean and standard deviation of the performance of matched randomly selected features with the same training/testing set. This result, in combination with the performance of the top ranked features identified using ten-fold CV (reported in Figure 2-3), suggests that top ranked features identified by strong, univariate age relationships are no more useful than randomly selected features for multivariate age prediction.

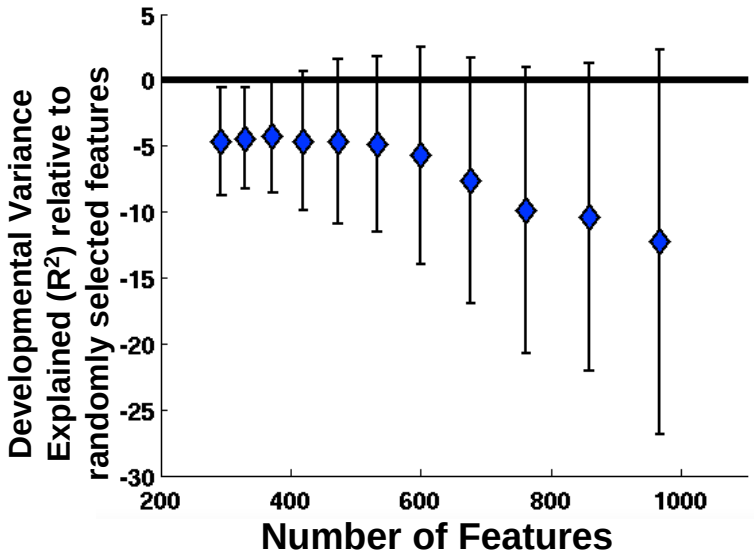

Figure 2-S5. ***RSFC with strong, univariate age relationships does not yield optimal age prediction with different cross validation approach.*** Age prediction in a testing set (N=32) of SVR-derived models built with top ranked features in a training set (N=90) and randomly selected features using different numbers of features. Performance is normalized to the performance of randomly selected features in the same training/testing sets.

H. Inter-correlation of Feature Sets

The usefulness of a feature set can be reduced if there is a large amount of inter-correlation among features (Guyon and Elisseeff, 2003). Correlated features are likely to provide redundant information for multivariate machine learning, increasing the likelihood of suboptimal predictive performance. Thus, we tested whether the feature sets used for age prediction were more inter-correlated than feature sets with randomly selected features. For each feature set, we calculated the correlation between the RSFC values (i.e., Fisher Z transformed *r* values) in each pair of functional connections across all individuals. We normalized the inter-correlation distribution by the total number of feature pairs to indicate the proportion of inter-correlated feature pairs in each feature set. The proportion of inter-correlated features are depicted for randomly selected feature sets, top

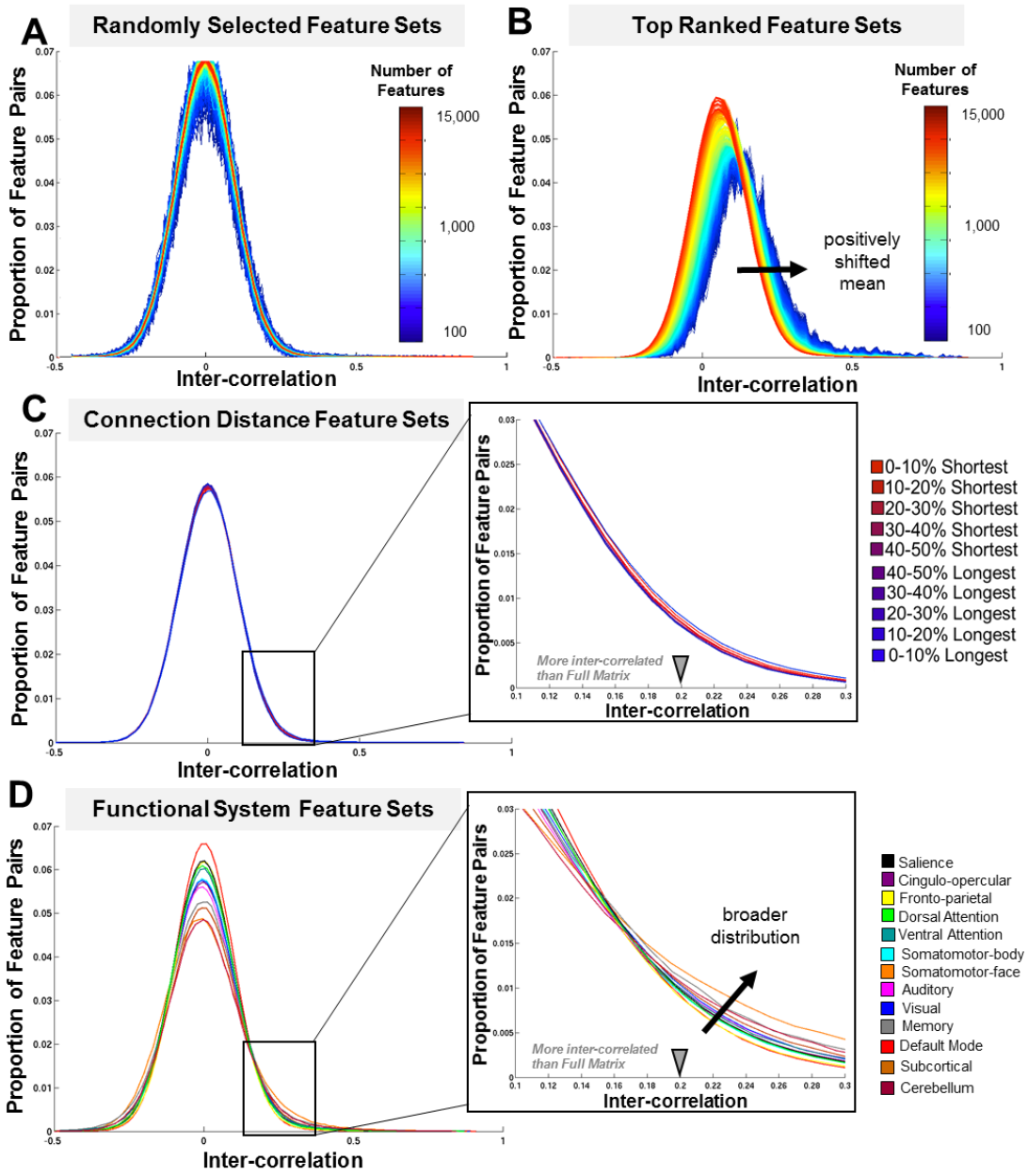

Figure 2-S6. ***Inter-correlation of tested feature sets.*** The proportion of inter-correlated feature pairs across individuals was generated for each set of features. (A) Inter-correlation of randomly selected feature sets of different feature number. (B) Inter-correlation of top ranked feature sets of difference feature number. (C) Inter-correlation of feature sets selected by connection distance. (D) Inter-correlation of feature sets selected by functional systems.

ranked feature sets, connection distance feature sets, and functional systems feature sets in Figure 2-S4. Because differences in both the mean (as in the Top Ranked Feature Sets in Figure 2-S6 B) and shape (as in the Function System Feature Sets in Figure 2-S6 D)

of this inter-correlation distribution might yield an increased number of inter-correlated features, we computed the proportion of feature pairs with an inter-correlation greater than r = 0.2 (2 standard deviations greater the mean of in the inter-correlation of features in the full correlation matrix) in order to quantify the amount of redundancy in each feature set. We found that top ranked features and features from functional systems were more inter-correlated than randomly selected feature sets (Figure 2-S6 A). Features selected by connection distance were slightly more inter-correlated than randomly selected features (broader distribution than randomly selected feature sets), but did not vary by connection distance.

After describing the inter-correlation among feature sets, we wanted to assess the impact of redundancy on age prediction with SVR. To remove redundant features from a feature set, we used the Fast Correlation-Based Filter (Yu and Liu, 2004). With this approach, a set of features are initially selected that have a strong, univariate relationship with the predicted label (here, age). These features are sorted by the strength of this relationship. The inter-correlation between pairs of features are calculated for all feature

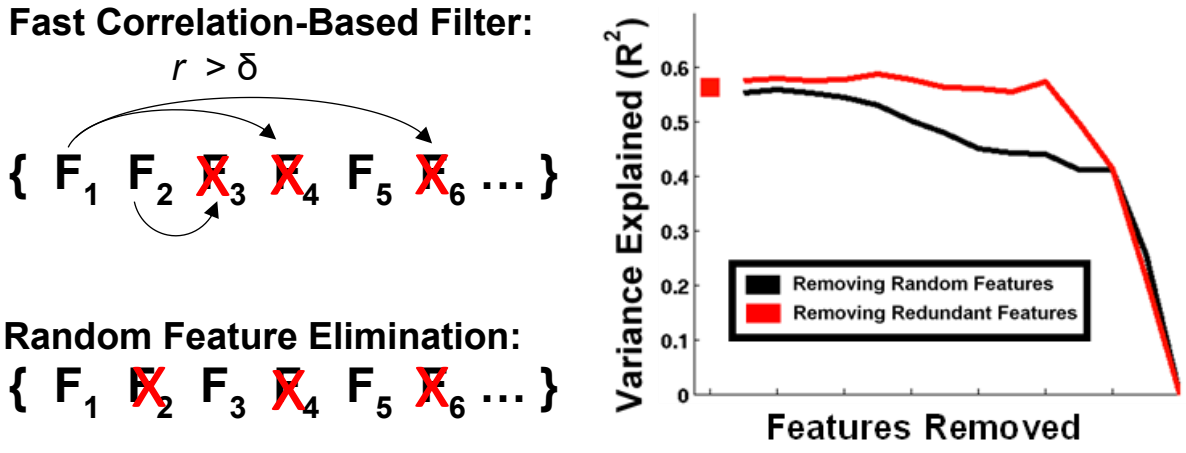

Figure 2-S7. ***Age prediction when removing redundant and random features.*** The variance explained when using different feature sets to predict an individual's age.

pairs. First, features are eliminated if they are strongly correlated (i.e. redundant) with the top ranked feature above a given threshold, δ. Features are iteratively eliminated if they are strongly correlated with the next top ranked feature. We started with a set of 3000 top ranked features and applied the Fast Correlation-Based filter at a range of threshold from r = 0.6 to r = 0.2. For comparison, we also randomly eliminated features to have the same number as those feature sets produced by the Fast Correlation-Based filter. The resulting feature sets were used to create a model describing the multivariate relationship between age and RSFC and tested with ten-fold CV. Figure 2-S7 shows that age prediction drops off more slowly when removing redundant features than when removing random features.

2.8 References

Barnea-Goraly N, Menon V, Eckert M, Tamm L, Bammer R, Karchemskiy A, et al. White Matter Development During Childhood and Adolescence: A Cross-sectional Diffusion Tensor Imaging Study. Cereb. Cortex 2005; 15: 1848–1854.

Bassett DS, Nelson BG, Mueller BA, Camchong J, Lim KO. Altered resting state complexity in schizophrenia. NeuroImage 2012; 59: 2196–2207.

Biswal B, Zerrin Yetkin F, Haughton VM, Hyde JS. Functional connectivity in the motor cortex of resting human brain using echo-planar mri. Magn. Reson. Med. 1995; 34: 537–541.

Black KJ, Koller JM, Snyder AZ, Perlmutter JS. Atlas Template Images for Nonhuman Primate Neuroimaging: Baboon and Macaque. Methods Enzymol. 2004; 385: 91–102.

Boyle EA, Li YI, Pritchard JK. An Expanded View of Complex Traits: From Polygenic to Omnigenic. Cell 2017; 169: 1177–1186.

Bray SL, Chang C, Hoeft F. Applications of multivariate pattern classification analyses in developmental neuroimaging of healthy and clinical populations [Internet]. Front. Hum. Neurosci. 2009; 3Available from: http://journal.frontiersin.org/article/10.3389/neuro.09.032.2009/full

Brown TT, Kuperman JM, Chung Y, Erhart M, McCabe C, Hagler Jr. DJ, et al. Neuroanatomical Assessment of Biological Maturity. Curr. Biol. 2012; 22: 1693–1698.

Casanova R, Whitlow C., Wagner B, Espeland M., Maldjian J. Combining Graph and Machine Learning Methods to Analyze Differences in Functional Connectivity Across Sex. Open Neuroimaging J. 2012; 6: 1–9.

Casey B, Galvan A, Hare TA. Changes in cerebral functional organization during cognitive development. Curr. Opin. Neurobiol. 2005; 15: 239–244.

Chen H, Duan X, Liu F, Lu F, Ma X, Zhang Y, et al. Multivariate classification of autism spectrum disorder using frequency-specific resting-state functional connectivity—A multi-center study. Prog. Neuropsychopharmacol. Biol. Psychiatry 2016; 64: 1–9.

Ciric R, Wolf DH, Power JD, Roalf DR, Baum GL, Ruparel K, et al. Benchmarking of participant-level confound regression strategies for the control of motion artifact in studies of functional connectivity. NeuroImage 2017; 154: 174–187.

Craddock RC, Holtzheimer PE, Hu XP, Mayberg HS. Disease state prediction from resting state functional connectivity. Magn. Reson. Med. 2009; 62: 1619–1628.

Dosenbach NUF, Fair DA, Cohen AL, Schlaggar BL, Petersen SE. A dual-networks architecture of top-down control. Trends Cogn. Sci. 2008; 12: 99–105.

Dosenbach NUF, Koller JM, Earl EA, Miranda-Dominguez O, Klein RL, Van AN, et al. Real-time motion analytics during brain MRI improve data quality and reduce costs. NeuroImage 2017; 161: 80–93.

Dosenbach NUF, Nardos B, Cohen AL, Fair DA, Power JD, Church JA, et al. Prediction of Individual Brain Maturity Using fMRI. Science 2010; 329: 1358–1361.

Du W, Calhoun VD, Li H, Ma S, Eichele T, Kiehl KA, et al. High Classification Accuracy for Schizophrenia with Rest and Task fMRI Data [Internet]. Front. Hum. Neurosci. 2012; 6[cited 2017 Jul 21] Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3366580/

Emerson RW, Adams C, Nishino T, Hazlett HC, Wolff JJ, Zwaigenbaum L, et al. Functional neuroimaging of high-risk 6-month-old infants predicts a diagnosis of autism at 24 months of age. Sci. Transl. Med. 2017; 9: eaag2882.

Erus G, Battapady H, Satterthwaite TD, Hakonarson H, Gur RE, Davatzikos C, et al. Imaging Patterns of Brain Development and their Relationship to Cognition. Cereb. Cortex 2015; 25: 1676–1684.

Fair DA, Cohen AL, Power JD, Dosenbach NUF, Church JA, Miezin FM, et al. Functional Brain Networks Develop from a “Local to Distributed” Organization. PLOS Comput. Biol. 2009; 5: e1000381.

Fair DA, Dosenbach NUF, Church JA, Cohen AL, Brahmbhatt S, Miezin FM, et al. Development of distinct control networks through segregation and integration. Proc. Natl. Acad. Sci. 2007; 104: 13507–13512.

Fair DA, Nigg JT, Iyer S, Bathula D, Mills KL, Dosenbach NUF, et al. Distinct neural signatures detected for ADHD subtypes after controlling for micro-movements in resting state functional connectivity MRI data [Internet]. Front. Syst. Neurosci. 2013; 6[cited 2017 Jul 11] Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3563110/

Fan Y, Liu Y, Wu H, Hao Y, Liu H, Liu Z, et al. Discriminant analysis of functional connectivity patterns on Grassmann manifold. NeuroImage 2011; 56: 2058–2067.

Fox MD, Greicius M. Clinical Applications of Resting State Functional Connectivity [Internet]. Front. Syst. Neurosci. 2010; 4[cited 2017 Jul 21] Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2893721/

Fox MD, Raichle ME. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat. Rev. Neurosci. 2007; 8: 700–711.

Fox MD, Snyder AZ, Vincent JL, Corbetta M, Essen DCV, Raichle ME. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. Proc. Natl. Acad. Sci. U. S. A. 2005; 102: 9673–9678.

Fox MD, Zhang D, Snyder AZ, Raichle ME. The Global Signal and Observed Anticorrelated Resting State Brain Networks. J. Neurophysiol. 2009; 101: 3270–3283.

Franke K, Luders E, May A, Wilke M, Gaser C. Brain maturation: Predicting individual BrainAGE in children and adolescents using structural MRI. NeuroImage 2012; 63: 1305–1312.

Giedd JN, Rapoport JL. Structural MRI of Pediatric Brain Development: What Have We Learned and Where Are We Going? Neuron 2010; 67: 728–734.

Gogtay N, Giedd JN, Lusk L, Hayashi KM, Greenstein D, Vaituzis AC, et al. Dynamic mapping of human cortical development during childhood through early adulthood. Proc. Natl. Acad. Sci. U. S. A. 2004; 101: 8174–8179.

Gordon EM, Laumann TO, Adeyemo B, Gilmore AW, Nelson SM, Dosenbach NUF, et al. Individual-specific features of brain systems identified with resting state functional correlations. NeuroImage 2017a; 146: 918–939.

Gordon EM, Laumann TO, Glimore AW, Newbold DJ, Greene DJ, Berg JJ, et al. Precision functional mapping of individual human brains. Neuron 2017b; (in press)

Greene DJ, Black KJ, Schlaggar BL. Considerations for MRI study design and implementation in pediatric and clinical populations. Dev. Cogn. Neurosci. 2016a; 18: 101–112.

Greene DJ, Church JA, Dosenbach NUF, Nielsen AN, Adeyemo B, Nardos B, et al. Multivariate pattern classification of pediatric Tourette syndrome using functional connectivity MRI. Dev. Sci. 2016b; 19: 581–598.

Greene DJ, Koller JM, Hampton JM, Wesevich V, Van AN, Nguyen AL, et al. Behavioral interventions for reducing head motion during MRI scans in children. NeuroImage 2018; 171: 234–245.

Greene DJ, Laumann TO, Dubis JW, Ihnen SK, Neta M, Power JD, et al. Developmental Changes in the Organization of Functional Connections between the Basal Ganglia and Cerebral Cortex. J. Neurosci. 2014; 34: 5842–5854.

Guyon I, Elisseeff A. An Introduction to Variable and Feature Selection. J. Mach. Learn. Res. 2003; 3: 1157–1182.

Hazlett HC, Gu H, Munsell BC, Kim SH, Styner M, Wolff JJ, et al. Early brain development in infants at high risk for autism spectrum disorder. Nature 2017; 542: 348–351.

Jimura K, Poldrack RA. Analyses of regional-average activation and multivoxel pattern information tell complementary stories. Neuropsychologia 2012; 50: 544–552.

Khundrakpam BS, Tohka J, Evans AC. Prediction of brain maturity based on cortical thickness at different spatial resolutions. NeuroImage 2015; 111: 350–359.

Koch W, Teipel S, Mueller S, Benninghoff J, Wagner M, Bokde ALW, et al. Diagnostic power of default mode network resting state fMRI in the detection of Alzheimer's disease. Neurobiol. Aging 2012; 33: 466–478.

Laumann TO, Gordon EM, Adeyemo B, Snyder AZ, Joo SJ, Chen M-Y, et al. Functional System and Areal Organization of a Highly Sampled Individual Human Brain. Neuron 2015; 87: 657–670.

Liang SF, Hsieh TH, Chen PT, Wu ML, Kung CC, Lin CY, et al. Differentiation between resting-state fMRI data from ADHD and normal subjects: Based on functional connectivity and machine learning. In: 2012 International conference on Fuzzy Theory and Its Applications (iFUZZY2012). 2012. p. 294–298.

Ling J, Merideth F, Caprihan A, Pena A, Teshiba T, Mayer AR. Head injury or head motion? Assessment and quantification of motion artifacts in diffusion tensor imaging studies. Hum. Brain Mapp. 2012; 33: 50–62.

Marek S, Hwang K, Foran W, Hallquist MN, Luna B. The Contribution of Network Organization and Integration to the Development of Cognitive Control. PLOS Biol. 2015; 13: e1002328.

Meier TB, Desphande AS, Vergun S, Nair VA, Song J, Biswal BB, et al. Support vector machine classification and characterization of age-related reorganization of functional brain networks. NeuroImage 2012; 60: 601–613.

Mueller S, Wang D, Fox MD, Yeo BTT, Sepulcre J, Sabuncu MR, et al. Individual Variability in Functional Connectivity Architecture of the Human Brain. Neuron 2013; 77: 586–595.

Nielsen JA, Zielinski BA, Fletcher PT, Alexander AL, Lange N, Bigler ED, et al. Multisite functional connectivity MRI classification of autism: ABIDE results [Internet]. Front. Hum. Neurosci. 2013; 7[cited 2017 Jul 11] Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3782703/

Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. NeuroImage 2012; 59: 2142–2154.

Power JD, Cohen AL, Nelson SM, Wig GS, Barnes KA, Church JA, et al. Functional Network Organization of the Human Brain. Neuron 2011; 72: 665–678.

Power JD, Mitra A, Laumann TO, Snyder AZ, Schlaggar BL, Petersen SE. Methods to detect, characterize, and remove motion artifact in resting state fMRI. NeuroImage 2014; 84: 320–341.

Pruett JR, Kandala S, Hoertel S, Snyder AZ, Elison JT, Nishino T, et al. Accurate age classification of 6 and 12 month-old infants based on resting-state functional connectivity magnetic resonance imaging data. Dev. Cogn. Neurosci. 2015; 12: 123–133.

Reuter M, Tisdall MD, Qureshi A, Buckner RL, van der Kouwe AJW, Fischl B. Head motion during MRI acquisition reduces gray matter volume and thickness estimates. NeuroImage 2015; 107: 107–115.

Rudolph MD, Miranda-Domínguez O, Cohen AO, Breiner K, Steinberg L, Bonnie RJ, et al. At risk of being risky: The relationship between “brain age” under emotional states and risk preference. Dev. Cogn. Neurosci. 2017; 24: 93–106.

Saad ZS, Gotts SJ, Murphy K, Chen G, Jo HJ, Martin A, et al. Trouble at Rest: How Correlation Patterns and Group Differences Become Distorted After Global Signal Regression. Brain Connect. 2012; 2: 25–32.

Santarnecchi E, Galli G, Polizzotto NR, Rossi A, Rossi S. Efficiency of weak brain connections support general cognitive functioning. Hum. Brain Mapp. 2014; 35: 4566–4582.

Satterthwaite TD, Elliott MA, Gerraty RT, Ruparel K, Loughead J, Calkins ME, et al. An improved framework for confound regression and filtering for control of motion artifact in the preprocessing of resting-state functional connectivity data. NeuroImage 2013a; 64: 240–256.

Satterthwaite TD, Wolf DH, Ruparel K, Erus G, Elliott MA, Eickhoff SB, et al. Heterogeneous impact of motion on fundamental patterns of developmental changes in functional connectivity during youth. NeuroImage 2013b; 83: 45–57.

Shulman GL, Pope DLW, Astafiev SV, McAvoy MP, Snyder AZ, Corbetta M. Right Hemisphere Dominance during Spatial Selective Attention and Target Detection Occurs Outside the Dorsal Frontoparietal Network. J. Neurosci. 2010; 30: 3640–3651.

Siegel JS, Mitra A, Laumann TO, Seitzman BA, Raichle M, Corbetta M, et al. Data Quality Influences Observed Links Between Functional Connectivity and Behavior. Cereb. Cortex 2016: 1–11.

Solé RV, Ferrer-Cancho R, Montoya JM, Valverde S. Selection, tinkering, and emergence in complex networks. Complexity 2002; 8: 20–33.

Sundermann B, Herr D, Schwindt W, Pfleiderer B. Multivariate Classification of Blood Oxygen Level–Dependent fMRI Data with Diagnostic Intention: A Clinical Perspective. Am. J. Neuroradiol. 2014; 35: 848–855.

Supekar K, Musen M, Menon V. Development of Large-Scale Functional Brain Networks in Children [Internet]. PLoS Biol. 2009; 7Available from: /pmcc/articles/PMC2705656/?report=abstract

Supekar K, Uddin LQ, Prater K, Amin H, Greicius MD, Menon V. Development of functional and structural connectivity within the default mode network in young children. NeuroImage 2010; 52: 290–301.

Uddin LQ, Supekar K, Lynch CJ, Khouzam A, Phillips J, Feinstein C, et al. Salience Network–Based Classification and Prediction of Symptom Severity in Children With Autism. JAMA Psychiatry 2013; 70: 869–879.

Van Dijk KRA, Sabuncu MR, Buckner RL. The influence of head motion on intrinsic functional connectivity MRI. NeuroImage 2012; 59: 431–438.

Vergun S, Deshpande AS, Meier TB, Song J, Tudorascu DL, Nair VA, et al. Characterizing Functional Connectivity Differences in Aging Adults using Machine Learning on Resting State fMRI Data [Internet]. Front. Comput. Neurosci. 2013; 7[cited 2017 Jul 21] Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3635030/

Wang D, Buckner RL, Fox MD, Holt DJ, Holmes AJ, Stoecklein S, et al. Parcellating cortical functional networks in individuals. Nat. Neurosci. 2015; 18: 1853–1860.

Wee C-Y, Yap P-T, Zhang D, Denny K, Browndyke JN, Potter GG, et al. Identification of MCI individuals using structural and functional connectivity networks. NeuroImage 2012; 59: 2045–2056.

Yendiki A, Koldewyn K, Kakunoori S, Kanwisher N, Fischl B. Spurious group differences due to head motion in a diffusion MRI study. NeuroImage 2014; 88: 79–90.

Yeo BTT, Krienen FM, Sepulcre J, Sabuncu MR, Lashkari D, Hollinshead M, et al. The organization of the human cerebral cortex estimated by intrinsic functional connectivity. J. Neurophysiol. 2011; 106: 1125–1165.

Yu L, Liu H. Efficient Feature Selection via Analysis of Relevance and Redundancy. J. Mach. Learn. Res. 2004; 5: 1205–1224.

Chapter 3: Atypical functional connectivity in Tourette syndrome differs between children and adults

This chapter has been reviewed as a journal article and revisions have been requested. The citation is:

Nielsen, Ashley N., Caterina Gratton, Jessica A. Church, Nico UF Dosenbach, Kevin J. Black, Steven E. Petersen, Bradley L. Schlaggar, and Deanna J. Greene. "Atypical Functional Connectivity in Tourette Syndrome Differs Between Children and Adults."

3.1 Abstract

Tourette syndrome (TS) is a neuropsychiatric disorder characterized by motor and vocal tics that typically change over development. Whether and how brain function in TS also differs across development has been largely understudied. Here, we used functional connectivity MRI to examine whole brain functional networks in children and adults with TS. Multivariate classification methods were used to find patterns among functional connections that distinguish TS from controls separately for children and adults (total N = 202). We tested whether the patterns of connections that classify diagnosis in one age group (e.g., children) could classify diagnosis in another age group (e.g., adults). We also tested whether the developmental trajectory of these connections were altered in TS. Patterns of functional connections that distinguished TS from controls were generalizable to an age-matched independent test set, but not to other age groups. While diagnostic classification was successful in children and adults separately, the connections that best distinguished TS from controls were age-specific. When contextualized with typical development, some functional connections exhibited accelerated maturation in childhood TS, while others exhibited delayed maturation in adulthood TS. Our results demonstrate that brain networks are differentially altered in children and adults with TS, and that the

developmental trajectory of affected connections is disrupted. These findings further our understanding of neurodevelopmental trajectories in TS and carry implications for future applications aimed at predicting the clinical course of TS in individuals over development.

3.2 Introduction

Tourette syndrome (TS) is a developmental neuropsychiatric disorder characterized by motor and vocal tics (Leckman *et al.*, 2014) that affects 1-3% of children (Khalifa and Knorring, n.d.; Scahill *et al.*, 2009; Cubo *et al.*, 2011). Tics are brief, unwanted, repetitive movements or noises that can be intrusive in daily life. On average, tic onset occurs at age 5-7 years, with tic severity peaking during late childhood/early adolescence (10-12 years). Tics usually continue into adulthood (Goetz *et al.*, 1992; Pappert *et al.*, 2003a), but with marked improvement or even remission after adolescence (Erenberg *et al.*, 1987; Leckman *et al.*, 1998; Peterson *et al.*, 2001a; Bloch *et al.*, 2006; Hassan and Cavanna, 2012). However, symptom progression varies substantially across individuals, with a sizeable subgroup of patients (~60%) experiencing moderate to severe tics that persist into adulthood (Leckman *et al.*, 1998; Pappert *et al.*, 2003b). Understanding how the brain changes over the course of development in TS may provide insight into its clinical manifestation across development and aid prediction of the disorder's trajectory in individuals.

Most neuroimaging studies of TS treat it as a singular disorder, unchanging across development, by grouping together patients from a wide age range (Tobe *et al.*, n.d.; Amat *et al.*, 2006; Sowell *et al.*, 2008; Fahim *et al.*, 2010; Miller *et al.*, 2010) or focusing on a single age cohort (Roessner *et al.*, n.d.; Bloch *et al.*, 2005; Baym *et al.*, 2008; Mazzone

et al., 2010; Debes *et al.*, 2011), often by necessity. However, there is evidence that differences in brain structure and function in TS vary by age (Peterson *et al.*, 2001b; Raz *et al.*, 2009; Pépés *et al.*, 2016). Comparing the brain differences observed in children and adults with TS is necessary to reveal effects that are present in both age groups (i.e., "age-invariant" TS effects) as well as effects that differ between age groups (i.e., "age-specific" TS effects). Critically, a more complete understanding of the differences observed in children or adults with TS also requires taking into account typical maturational changes in the brain. Given a context of typical development, one can determine whether brain differences reflect atypically shifted development (e.g., accelerated or delayed maturation) or an anomalous difference not observed in typical development, potentially providing clues into etiology. While several TS neuroimaging studies have interpreted their findings in the context of brain maturity (Muellner *et al.*, n.d.; Peterson *et al.*, 2001c; Raz *et al.*, 2009; Worbe *et al.*, 2012; Pépés *et al.*, 2016), few have included typical developmental comparisons to contextualize the differences observed in TS (Marsh *et al.*, 2007; Church *et al.*, 2009b; Debes *et al.*, 2015).

The potential presence of both maturity- and disorder-related differences in the brain in TS is made more complex by considering where these differences are localized. While many studies of TS have primarily identified differences within a select few brain regions or networks, the findings together suggest that TS involves many cortical and subcortical brain regions (for reviews, see (Greene *et al.*, 2013, 2015)). Thus, capturing the developmental trajectory of brain function in TS might be facilitated by a multivariate approach that combines information from many brain regions and identifies complex patterns in the data that distinguish individuals by diagnosis and/or age. Multivariate

machine learning techniques have been applied to neuroimaging data in an attempt to identify patterns of diagnosis-related differences in neuropsychiatric disorders (Arbabshirani *et al.*, 2017) and age-related differences in typical development (Brown *et al.*, 2012; Franke *et al.*, 2012; Erus *et al.*, 2015; Khundrakpam *et al.*, 2015). Notably, these methods require validation in an independent group of subjects to ensure that the identified differences do not represent idiosyncratic or spurious group differences (Varoquaux *et al.*, 2017), which is often not possible in small sample studies.

Here, we used a whole-brain, multivariate approach to investigate if and how brain networks in TS differ from controls in children and adults. Functional connectivity MRI, which measures the temporal correlations between spontaneous fluctuations in the blood oxygen level-dependent signals across the brain (Biswal *et al.*, 1995), was used to examine functional brain networks in separate cohorts of children and adults with TS. We previously demonstrated that multivariate approaches applied to functional connectivity can distinguish children with TS from controls (Greene *et al.*, 2016b) and typically developing children from adults (Nielsen *et al.*, n.d.; Dosenbach *et al.*, 2010). In the present work, we use a similar approach, first validating that multivariate patterns of functional connections that distinguish TS and controls can generalize to an independent sample. Then, we test whether the patterns of functional connections that differ in TS in one age group (e.g., children) can also distinguish individuals with TS in the other age group (e.g., adults). Finally, we test whether the functional connections that differ in TS (in either children or adults) exhibit altered developmental trajectories by placing these differences in the context of typical development.

3.3 Material and Methods

3.3.1 Participants

A total of 172 individuals with TS, ages 7.3-35.0 years, were recruited from the Washington University School of Medicine Movement Disorders Center and the Tourette Association of America Missouri chapter. After quality control assessments of the neuroimaging data (see below), 101 children, adolescents, and adults with TS were included (Table 3-1). A group of 101 control participants was selected from an extant database (*n*=487, ages 6.0–35.0 years, 206 males; recruited from the Washington University campus and surrounding community) and matched to the TS group on age, sex, IQ, handedness, and in-scanner movement (Table 3-1). Conditions commonly comorbid with TS (e.g., ADHD, OCD, anxiety) and medication use were not considered exclusionary for the TS group (Greene *et al.*, 2016a) (Table 3-S1) but were for the control group. All participants completed assessments of IQ, and TS participants completed additional assessments of symptom severity for TS, ADHD, and OCD (Supplement 1.1). Adult participants and a parent or guardian for all child participants gave informed consent and all children assented to participation.

Table 3-1. Participant characteristics.

	TS group	Control group
N	101	101
Male/Female	62/39	61/40
Age (Years)	17.5 (7.6); 7.6-35.0	17.5 (7.5); 7.4-34.2
Handedness (R/L)	95/6	95/6
IQ	113 (13.3); 83-139	115 (13.8); 83-145
Residual in-scanner movement (mean FD)	0.11 (0.015); 0.063-0.14	0.11 (0.013); 0.067-0.13
Amount of data ("good" frames)	287.5 (100.8); 121-573	262.8 (102.1); 122-668
YGTSS Total Tic Score	17.4 (8.2); 0-37	N/A
ADHD Rating Scale	11.2 (10.2); 0-44	N/A
CY-BOCS Score	5.5 (6.2); 0-24	N/A
Number on medications	52	0
Number with comorbidities	67	0

Where applicable values are displayed as Average (Standard Deviation); Range
FD = Frame-wise Displacement (in millimeters) (Power *et al.*, 2012a)
YGTSS = Yale Global Tic Severity Score (Total Tic Score) (Leckman *et al.*, 1989)
CY-BOCS Score = Children's Yale-Brown Obsessive-Compulsive Scale (Scahill *et al.*, 1997)

3.3.2 Functional Connectivity Network Construction

Resting-state fMRI data were collected as participants viewed a centrally presented white crosshair on a black background. Participants were instructed to relax, look at the plus sign, and hold as still as possible. The duration and number of resting-state scans varied across participants (Supplement 1.2). Imaging data were collected using a 3T Siemens Trio Scanner with a 12-channel Head Matrix Coil. Images were pre-processed to reduce artifacts (Shulman *et al.*, 2010). Additional pre-processing steps were applied to the resting-state data to reduce spurious correlated variance unlikely related to neuronal

activity. Stringent frame censoring (frame-wise displacement>0.2 mm) and nuisance regression (motion estimates, global signal, and individual ventricular and white matter signals) were used to reduce spurious individual or group differences in functional connectivity related to head movement in the scanner (Power *et al.*, 2012b, 2014; Ciric *et al.*, 2017). Participants with at least 5 minutes of low-motion data were included. See Supplement 1.2-1.4 for details.

For each participant, resting-state time-courses were extracted from a set of 300 regions of interest (ROIs) (Figure 3-1) covering much of the cortex (Power *et al.*, 2011), subcortex, and cerebellum (available at https://greenelab.wustl.edu/data_software). Functional connectivity was measured as the correlation (Fisher z-transformed) between the resting-state time-courses for each pair of ROIs.

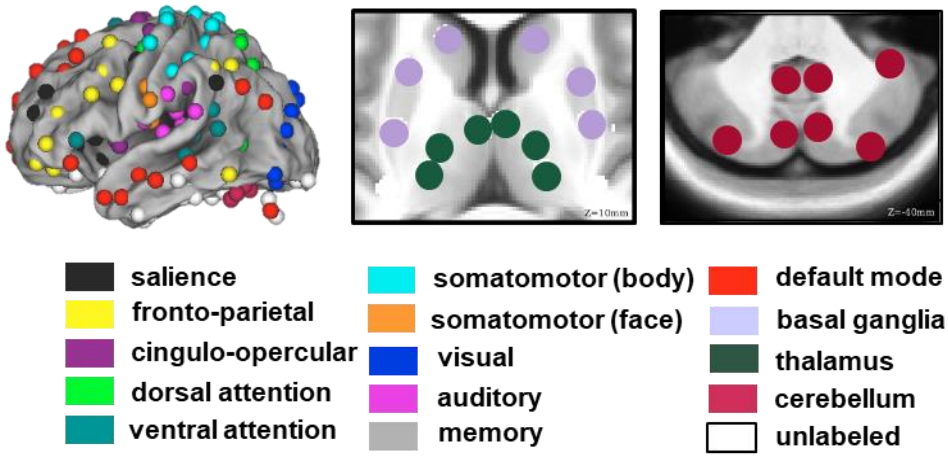

Figure 3-1. R*egions of interest.* Cortical regions were previously defined from a combination of task fMRI activation and resting-state fMRI studies (Power et al. 2011). Subcortical and cerebellar regions were defined from a combination of resting-state functional connectivity and review of the anatomical literature (Greene et al., 2014; Seitzman et al. (under review)). Cortical regions have been previously characterized as organizing into distinct functional networks (denoted by color).

3.3.3 Support Vector Machine Learning

Support vector machine (SVM) learning was implemented (Nielsen *et al.*, n.d.; Dosenbach *et al.*, 2010; Greene *et al.*, 2016b) to distinguish individuals with TS from controls based on patterns of functional connections (Supplement 1.5*)*. SVM classification is a powerful tool for finding differences across many features in a multivariate dataset (here, functional connections) that, in aggregate, best discriminate groups (here, TS vs. controls). Patterns of features that best distinguish individuals by group in a training set are weighted in the resulting classifier and can be subsequently applied to classify new test individuals. All 44,850 functional connections among the 300 ROIs were included as features.

Using SVM, three separate diagnostic classifiers were built to distinguish individuals with TS from controls using functional connectivity from three different training sets (Table 3-2). Leave-one-out cross validation (LOOCV) was used to assess classification accuracy within the training sets. The classifiers were then tested using independent test samples to answer several questions. A “YOUTH” diagnostic classifier was used to validate that patterns of functional connectivity that classify TS diagnosis are generalizable to an age-matched independent test set (see below). “CHILD” and “ADULT” diagnostic classifiers were used to test whether patterns of functional connectivity that classify TS diagnosis are age-specific or age-invariant (see below).

SVM classification can also be extended to find patterns among features that predict a continuous variable (here, age) with support vector regression (SVR). Using SVR, developmental models were built to predict age using functional connectivity from

the controls (Table 3-2), and assessed with LOOCV, to generate a context of typical development.

Table 3-2. Overview of participants included in the training and testing sets for each diagnostic classifier and developmental model.

SVM

Diagnostic Classifier			N	Ages
YOUTH	***Train***	***Youth sample***	**39 TS / 39 Controls**	**8.0 – 16.6 years**
	Test	*Independent youth sample*	23 TS / 23 Controls	7.4 – 16.5 years
CHILD	***Train***	***Children***	**39 TS / 39 Controls**	**7.4 – 13.1 years**
	Test	*Adolescents*	23 TS / 23 Controls	13.1 – 16.6 years
		Adults	39 TS / 39 Controls	18.1 – 35 years
ADULT	***Train***	***Adults***	**39 TS / 39 Controls**	**18.1 – 35 years**
	Test	*Children*	39 TS / 39 Controls	7.4 – 13.1 years
		Adolescents	23 TS / 23 Controls	13.1 – 16.6 years

SVR

Developmental Model			N	Ages
Typical Development	***Train***	***Control sample***	**101**	**7.4 – 34.2 years**
	Test	*TS sample*	101	7.6 – 35 years

48 out of 78 children in the YOUTH training set were used in the CHILD training set
84 out of 124 children and adolescents (TS and controls) were used in Greene et al. 2016

3.3.4 Validating diagnostic classification in an age-matched independent test set

We previously demonstrated that SVM can be used to classify children and adolescents with TS vs. controls based on patterns of functional connectivity (Greene *et al.*, 2016b). Here, we first wanted to ensure that the identified differences in functional connectivity

characterize the disorder rather than idiosyncratic or spurious group differences within a specific sample. Because the TS sample contained many young individuals within an age range similar to that in Greene *et al*. 2016, we built a "YOUTH" diagnostic classifier trained to discriminate 39 children and adolescents with TS from 39 matched controls (8.0-16.6 years; Table 3-2) using SVM. The remaining 46 individuals were kept separate as an age-matched, independent youth sample (7.4-16.5 years; Table 3-2). We tested whether the YOUTH diagnostic classifier could accurately classify TS and controls in the independent youth sample.

3.3.5 Testing for age-invariant or age-specific differences in functional connectivity in TS

We tested whether the patterns of functional connections that distinguished TS and controls were common or distinct between children and adults. Separate SVMs were used to build a CHILD diagnostic classifier trained to separate 39 children with TS from 39 matched controls (7.0-12.9 years; Table 3-2) and an ADULT diagnostic classifier trained to separate 39 adults with TS from 39 matched controls (18.0-35.0 years; Table 3-2). The remaining 23 adolescents with TS and 23 matched controls were kept as a separate adolescent test set (13.1-16.6 years; Table 3-2) to test whether the patterns of functional connections that classify diagnosis in children or adults can also classify diagnosis in adolescents.

We tested if the patterns of functional connections that distinguish TS from controls in one age group (child or adult) could generalize to accurately classify individuals in another age group. We evaluated whether the performance of a diagnostic classifier significantly differed across age groups using a binomial significance test (Supplement

1.6). As sex, comorbidities, and current medications were not matched across age groups (Table 3-S2), we also tested if the generalizability of the CHILD or ADULT diagnostic classifiers (or lack thereof) was driven by these characteristics (Supplement 2.1).

If the patterns of functional connections used to distinguish individuals with TS from controls in one age group are “age-specific,” the classifier should not generalize well to the other age group (i.e., the CHILD diagnostic classifier will not accurately distinguish adults with TS from adult controls, and vice versa). If these patterns are “age-invariant,” the classifier should generalize well to the other age group. We also directly tested for age-invariant differences using an ALL-AGES diagnostic classifier (Supplement 2.2).

We extracted the top 1000 (out of 44,850) most strongly weighted functional connections in each of the CHILD and ADULT diagnostic classifiers and examined the percentage overlap of those functional connections. Few overlapping connections would suggest age-specific differences between TS and controls, while many overlapping connections would suggest age-invariant differences (Supplement 2.3).

3.3.6 Testing for anomalous or atypically shifted development of functional connectivity in TS

As previously reported, many functional connections vary systematically according to age in typical development (Nielsen *et al.*, n.d.). The functional connections that differ by diagnosis (TS vs. controls) may also vary according to age in typical development. To test this, we used SVR to build a developmental model using the top 1000 most strongly weighted functional connections from either the CHILD or ADULT diagnostic classifier, and tested if those features could also distinguish individuals by age in the control sample (7.4-34.2 years; Table 3-2). The developmental models built using the CHILD TS or

ADULT TS features were also compared with developmental models built using randomly selected sets of functional connections to evaluate the utility of these specific features against a null model (Supplement 2.4).

We tested whether the developmental models built to predict age in the controls could also accurately predict age in the TS sample (7.6-35.0 years; Table 3-2). To benchmark the generalizability of age prediction to the TS sample, we also tested whether additional developmental models built to predict age in controls could accurately predict age in TS using 1) all 44,850 functional connections or 2) the top 1000 connections that differed most between control children and control adults (Supplement 2.5).

Determining if the most strongly weighted functional connections used for diagnostic classification can also predict age places the TS vs. control differences in the context of typical development, allowing interpretations pertaining to brain maturity. If the patterns of functional connections that distinguish TS from controls reflect an anomalous divergence unrelated to development, 1) the CHILD TS or ADULT TS features will not successfully predict age in controls or 2) those functional connections will predict age equivalently in both the control and TS samples. By contrast, if the patterns of functional connectivity that distinguish TS from controls reflect an atypically shifted developmental trajectory, the CHILD TS or ADULT TS features will predict age well in controls but inaccurately in TS. Predicted ages in TS that are older than in age-matched controls would indicate accelerated maturation of brain networks, while predicted ages in TS that are younger than in age-matched controls would indicate delayed/incomplete maturation of brain networks. Alternatively, if predicted ages in TS fluctuate near the mean age, the maturational changes present in typical development may be absent in TS.

3.4 Results

3.4.1 Classification of TS vs. controls based on functional connectivity generalizes to an age-matched independent test set.

Using SVM, we successfully classified individuals as TS or controls based on patterns of functional connectivity. The YOUTH diagnostic classifier, which included children and adolescents (8.0-16.6 years; Table 3-2), was 64% accurate when estimated with LOOCV, significantly above chance (p=0.01). Importantly, this diagnostic classifier successfully generalized to an independent youth sample of age-matched children and adolescents with 67% accuracy (Figure 3-2A). By demonstrating generalizability in an age-matched independent test set, we can better interpret the generalizability of the CHILD and ADULT diagnostic classifiers to different age groups; poor generalizability can likely be attributed to age-related differences in how brain networks are altered in TS rather than idiosyncratic group differences related to data quality or overfitting.

3.4.2 Patterns of functional connections can classify TS diagnosis in children and in adults, but do not generalize across age groups.

The CHILD diagnostic classifier (7.4-13.1 years; Table 3-2) was 71% accurate (LOOCV, p<0.001). The ADULT diagnostic classifier (18.1-35.0 years; Table 3-2) was 72% accurate (LOOCV, p<0.001). However, neither classifier accurately classified TS diagnosis in the other age groups (Figure 3-2 B-C). Specifically, the CHILD diagnostic classifier did not distinguish TS from controls in adolescents (accuracy: 48%, p=0.48) or adults (accuracy: 49%, p=0.43). Similarly, the ADULT diagnostic classifier did not distinguish TS from controls in adolescents (accuracy: 48%, p=0.49), though it was slightly better in children (accuracy: 57%, p=0.11). Classification of the other age groups was significantly less accurate than classification in the training sample (see Figure 3-2).

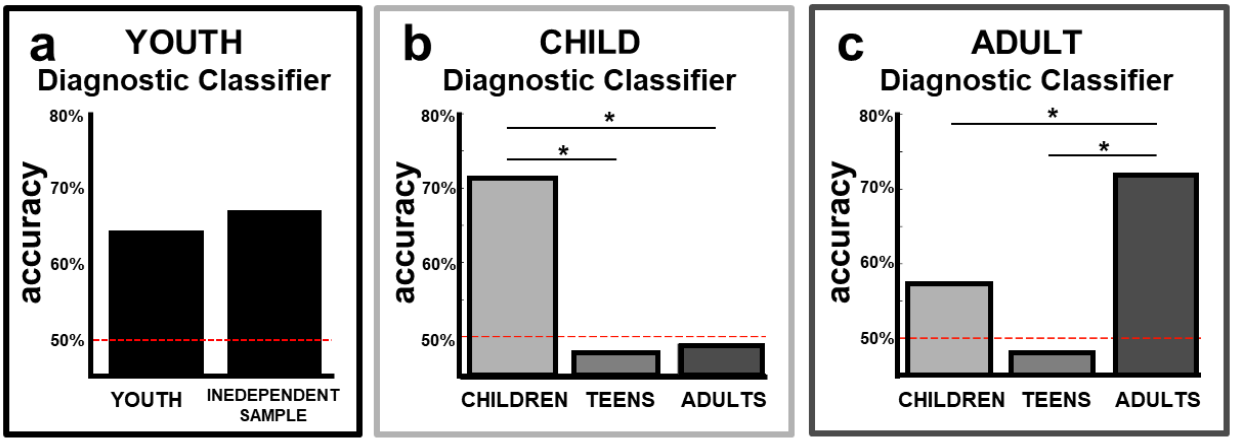

Figure 3-2. *Functional connections that best distinguished TS from controls were age-specific.* **a.)** Performance of the YOUTH diagnostic classifier was significantly better than chance in the independent sample (p = 0.01). **b.)** Performance of the CHILD diagnostic classifier was not significantly better than chance in adolescents (accuracy: 48%, sensitivity: 91%, specificity: 4%, p = 0.48) or adults (accuracy: 49%, sensitivity: 97%, specificity: 0%, p = 0.43) and was significantly less accurate in classifying adolescents and adults than children (adolescents: p < 0.001; adults: p < 0.001). **c.)** Performance of the ADULT diagnostic classifier was not significantly better than chance in adolescents (accuracy: 48%, sensitivity: 17%, specificity: 78%, p = 0.49) or children (accuracy: 57%, sensitivity: 31%, specificity: 85%, p = 0.11) and was significantly less accurate in classifying children and adolescents than adults (adolescents: p < 0.001; children: p = 0.012).

Given the successful generalizability of the YOUTH diagnostic classifier (described above), poor generalizability is likely not solely related to data quality or overfitting. Moreover, poor generalizability was not driven by sex, comorbid disorders, or medication status (Supplement 2.1, Table 3-S3). These results suggest that the CHILD and ADULT diagnostic classifiers relied on age-specific differences in functional connectivity to best discriminate TS from controls. We also found evidence for age-invariant differences in functional connectivity in TS (Supplement 2.2). However, those age-invariant patterns were not the primary features used to distinguish TS and controls when considering children and adults separately.

3.4.3 Top functional connections that distinguish TS and controls were distinct in children and adults.

Regions associated with the top weighted functional connections from the CHILD and ADULT diagnostic classifiers are displayed in Figure 3-3, and show that these functional connections were within and between many different functional networks (Supplement 2.3, Figure 3-S3). Only 33 (3%) of the top 1000 functional connections overlapped between the CHILD and ADULT diagnostic classifiers (Figure 3-3C), indicating different patterns of region involvement (Figure 3-3 A-B) and providing further evidence that the functional connections involved in TS differ in children and adults.

3.4.4 Functional connections that differ in TS reflect atypically shifted development.

Using SVR, the top weighted functional connections from the CHILD diagnostic classifier and the ADULT diagnostic classifier were each able to predict age well in the controls (CHILD: r=0.62, R^2=0.39, p<0.001; ADULT: r=0.74, R^2=0.55, p<0.001; Figure 3-4, *red)* when evaluated against a null model (Supplement 2.4, Figure 3-S4). By contrast, these developmental models did not predict age well in TS. Specifically, the developmental model built to predict age in controls using the CHILD TS features did not significantly predict age in TS, r=0.11, R^2=0.012, p=0.27 (Figure 3-4A, *blue*) such that the children with TS were inaccurately predicted as older than age-matched controls. Note that these predicted ages were shifted above the age expected if predicted spuriously (Supplement 2.6, Figure 3-S5), suggesting accelerated maturation of these functional connections in childhood TS. The developmental model built to predict age in controls using the ADULT TS features also did not significantly predict age in TS, r=0.11, R^2=0.013, p=0.27 (Figure 3-4B, *blue*) such that the adults with TS were inaccurately predicted as younger than age-matched controls. These predicted ages were shifted below the mean age (Supplement

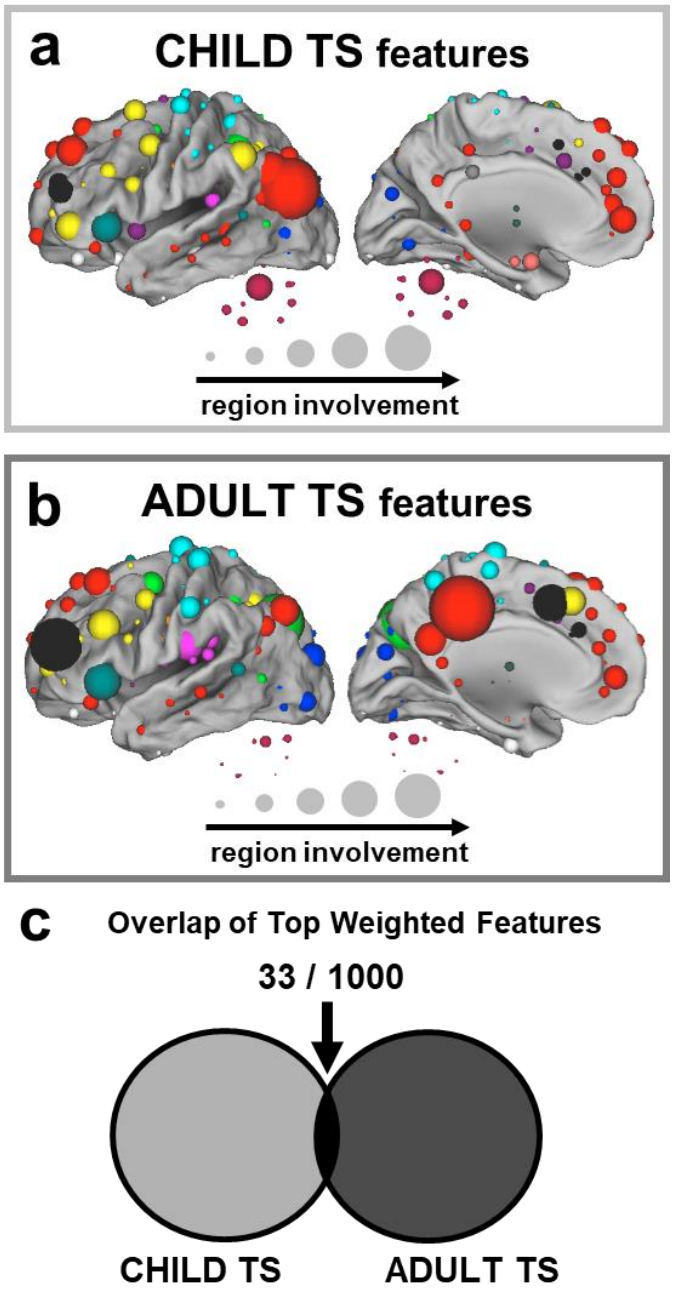

Figure 3-3. *Functional connections that best distinguished TS from controls differed between children and adults.* **a.)** Regions are shown from the top weighted 1000 functional connections used to distinguish TS from controls in the CHILD diagnostic classifier. The size of each sphere represents region involvement (i.e., number of functional connections in the feature set involving a region). Region colors indicate the network to which that region belongs, labeled in Figure 3-1. **b.)** Regions are shown from the top weighted 1000 functional connections used to distinguish TS from controls in the ADULT diagnostic classifier. The size of each sphere represents region involvement and the color represents network affiliation. **c.)** The overlap of the top weighted functional connections from the CHILD and ADULT diagnostic classifiers was only 33 out of 1000.

2.6, Figure 3-S5), suggesting delayed maturation of these functional connections in adulthood TS.

Not all development of functional connectivity was disrupted in TS. We found that additional developmental models could accurately predict age in the TS sample using 1) whole-brain functional connectivity (r=0.71, R^2=0.50, p<0.001) and 2) the functional connections that differ most between control children and adults (r=0.62, R^2=0.38, p<0.001; Supplement 2.5). Thus, only the top functional connections used to distinguish TS from controls in each age group demonstrated altered developmental trajectories in TS.

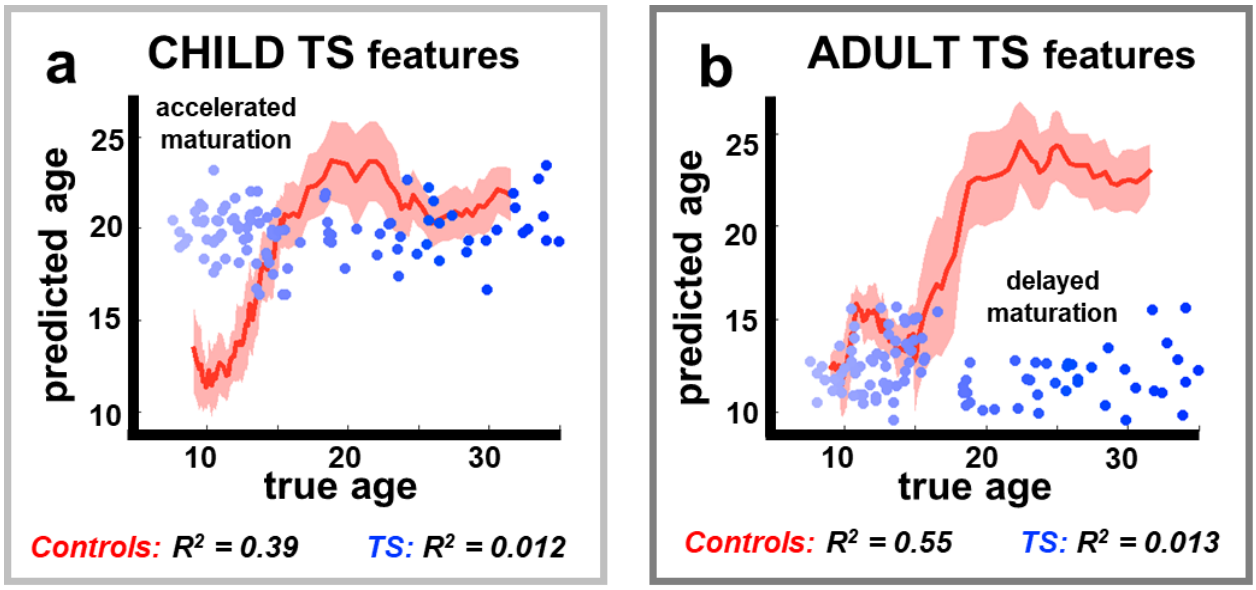

Figure 3-4. *Functional connections that best distinguished TS from controls reflect atypically shifted development.* **a.)** The developmental model built using CHILD TS features was able to predict age well in the control sample (*red*) but not in the TS sample (*blue*). Predicted ages of children with TS were older than the predicted ages of age-matched controls indicating accelerated maturation of the CHILD TS features. **b.)** The developmental model built using ADULT TS features was able to predict age well in the control sample (*red*) but not in the TS sample (*blue*). Predicted ages of adults with TS were younger than the predicted ages of age-matched controls indicating delayed or incomplete maturation of the ADULT TS features.

3.5 Discussion

In the present work, we applied multivariate machine learning methods to resting-state functional connectivity MRI data to understand how functional brain organization is altered in TS over development. We found that the patterns of functional connections that best distinguished TS from controls were generalizable to an age-matched independent sample, but not to other age groups. Rather, the functional connections involved in TS differed between children and adults, suggesting they are age-specific. In addition, we found that these functional connections reflected atypical development in TS. Specifically, those functional connections that differed the most in childhood TS exhibited accelerated maturation (i.e., resembled brain networks of older subjects), while those that differed the most in adulthood TS exhibited delayed maturation (i.e., resembled brain networks of younger subjects). By directly examining TS across a wide age range (7-35 years), comparing children to adults, and contextualizing these results with typical development, our findings provide evidence that the neural underpinnings of TS differ in childhood and adulthood, and involve changes to the typical brain maturation timeline.

It has been argued that childhood and adulthood TS are fundamentally different, given the common clinical trajectory in which many patients experience significant improvement or remission in adulthood (Eichele and Plessen, 2013). Our results extend this argument to the brain's functional connections. Past studies have also identified age-specific effects in TS, yet primarily within single brain regions. For example, some cortical regions (dorsal prefrontal, orbitofrontal, parieto-occipital cortex) exhibit distinct, even sometimes opposing, volumetric differences in children and adults with TS (Peterson *et al.*, 2001c). Previous research has also shown that motor excitability is selectively altered

in children with TS (Pépés *et al.*, 2016) and atypical development of fronto-striatal self-regulatory signals only emerges in adulthood TS (Raz *et al.*, 2009). These findings in combination with ours suggest that treatments may need to be tailored differently for children and adults with TS.

We also characterized functional connectivity in TS in the context of typical development. In childhood TS, we found differences indicative of accelerated development. It has been proposed that living with chronic tics accelerates the maturation of control systems in children with TS as a result of the need to regularly suppress tics (Plessen *et al.*, 2009; Eichele and Plessen, 2013). In line with this idea, previous studies have reported enhanced cognitive control as well as putatively adaptive changes in brain function and structure in children with TS (Jackson *et al.*, 2011, 2015; Jung *et al.*, 2013). In heathy children, cognitive training yields modifications of the intrinsic connectivity among brain networks (Astle *et al.*, 2015). Thus, it is possible that the development of compensatory tic-suppression mechanisms is reflected in the patterns of functional connectivity that best distinguish children with and without TS. It is possible that these alterations support the improvement of tic symptoms during adolescence and early adulthood experienced by many patients (Spessot *et al.*, 2004).

In adulthood TS, we found differences in functional connectivity indicative of delayed maturation. Thus, adults that experience persistent tics may have maladaptive brain function that either developed with prolonged symptoms or led to the prolonged symptoms. As mentioned above, some argue that childhood TS and adulthood TS are fundamentally different, given the commonly held belief that most patients with TS experience substantial symptom improvement or remission into adulthood (Leckman *et*

al., 1998). Therefore, by studying a sample of adults with current tics, we may have captured the subsample who do not experience remission. By contrast, any sample of children with TS will include a mixture of individuals whose tic symptoms will go on to improve and those whose tics will persist. However, there is evidence that remission is likely much rarer than previously estimated (10%, rather than 40%; (Pappert *et al.*, 2003b)), and in our sample, many of the adults with TS reported improvement from childhood even if they did not report remission. Longitudinal data and studies of adults with remitted tics are necessary to determine whether immature brain function in adulthood TS is a cause or consequence of prolonged symptom burden. There have been previous reports of immature brain structure and function in TS (Church *et al.*, 2009b, a; Worbe *et al.*, 2012, 2015). However, methodological concerns related to head motion artifact in MRI data have called some of these conclusions into question (Power *et al.*, 2012b; Van Dijk *et al.*, 2012; Satterthwaite *et al.*, 2013; Reuter *et al.*, 2015; Alexander-Bloch *et al.*, 2016). In the present study, we implemented strict processing methods that have been shown to best mitigate the artifactual effects of motion (Power *et al.*, 2012b; Ciric *et al.*, 2017). Evidence for altered maturation of functional connectivity in TS remains even when potential artifactual confounds have been addressed.

Notably, not all maturation of functional connectivity was altered. When the complete set of functional connections across the brain were included in the developmental model, age was predicted well in both TS and controls. Further, those functional connections that varied with age the most in controls could also predict age well in TS. Thus, only specific patterns of functional connections – those that best discriminated TS and controls within each age group – exhibited shifted developmental

trajectories in TS, while much of the typical maturation of functional connectivity was preserved. This finding may correspond to the clinical observation that although TS can involve diminished academic achievement and quality of life, most individuals with TS lead relatively normal lives (Evans *et al.*, 2016; Pérez-Vigil *et al.*, 2018).

It is important to note that our TS sample was heterogeneous with respect to comorbid neuropsychiatric disorders and medication status, representative of the TS population (Freeman *et al.*, 2000; Greene *et al.*, 2016a). As brain network function can be affected by medications (Mueller *et al.*, n.d.) and other neuropsychiatric conditions (Fair *et al.*, 2013), the diagnostic classifiers here might have included medication-induced or comorbidity-related differences in brain function between the TS and control groups. Additionally, our child and adult samples differed with respect to sex; the children included more boys than girls, while the adults were more balanced. This difference reflects epidemiological data, as the sex imbalance (4:1 male:female) reported in childhood TS is attenuated in adulthood TS (Lichter and Finnegan, 2015). Nevertheless, examination of the misclassified individuals demonstrated that poor generalizability across age groups was not driven by these factors. Future studies with larger samples will be useful for parsing the influence of medications, comorbidities, and sex on brain function in TS.

The success of multivariate machine learning classification applied to functional brain networks holds promise for clinical application of these methods. Given the heterogeneity in the developmental course of TS symptoms, there is a great need to predict future clinical outcome for individuals. Being able to predict whether a given child with tics will go on to improve or not would have high clinical utility, providing important information to families, guiding treatment plans, and affording the opportunity for early

intervention. Our findings suggest that functional connectivity contains signals that can be used for these types of predictions, and that the best predictions will likely rely upon modeling these effects in a rich typical developmental context.

3.6 Acknowledgments

We thank Rebecca Coalson, Rebecca Lepore, Kelly McVey, Jonathan Koller, Annie Nguyen, Catherine Hoyt, Lindsey McIntyre, and Emily Bihun for assistance with data collection, the children and adults who participated in this study and their families, and Deanna Barch for her input on this manuscript. This project was supported by: Tourette Association of America fellowships (DJG, JAC), Tourette Association of America Neuroimaging Consortium pilot grant (KJB, BLS), Tourette Association of America research grant (DJG), NARSAD Young Investigator Award (DJG), NIH K01MH104592 (DJG), NIH R21MH091512 (BLS), NIH R01HD057076 (BLS), NIH R01NS046424 (SEP), NIH R21 NS091635 (BLS, KJB), NIH R01MH104030 (KJB, BLS), K12HD076224 (NUFD, Scholar of the Child Health Research Center at Washington University), NIH K23NS088590 (NUFD), NIH F32NS065649 (JAC), NIH F32NS092290 (CG), NIH F32NS656492, NIH K23DC006638, P50 MH071616, P60 DK020579-31 American Hearing Research Foundation, and The Simons Foundation Autism Research Initiative ("Brain circuitry in Simplex Autism," SEP). Research reported in the publication was supported by the Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health under Award Number U54 HD087011 to the Intellectual and Developmental Disabilities Research Center at Washington University. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

3.7 Supplemental Material

1. Supplemental Methods

1.1 Participants

A total of 101 children and adults with Tourette syndrome (TS) and 101 healthy control children and adults were included in the present study. All participants were native English speakers. All participants underwent a 2-scale brief assessment of IQ (WASI). For TS participants, the experimenter completed the following measures of "past week" symptom severity: Yale Global Tic Severity Score (Total Tic Score) (Leckman *et al.*, 1989), Children's Yale-Brown Obsessive Compulsive Scale (Scahill *et al.*, 1997), and ADHD Rating Scale (Conners *et al.*, 1998). All participants self- or parent-reported any history of neuropsychiatric diagnoses and current medications (Table 3-S1). For the control participants, any history of neuropsychiatric or neurological diagnoses prohibited participation in the study.

Table 3-S1. Comorbid diagnoses and current medications in participants with TS.

	Children with TS *N* = 39 (7.4 – 13.1 years)	**Adolescents with TS** *N* = 23 (13.1-16.6 years)	**Adults with TS** *N* = 39 (18.0-35 years)
Comorbid Diagnosis			
ADHD/ADD	18	11	11
OCD	9	8	13
Anxiety Disorders	5	4	9
Depression	1	2	9
ODD	0	2	0
Migraines	1	0	6
Medications			

Centrally acting adrenergic agents	13	9	3
Stimulants	10	3	7
Anti-depressants	2	5	6
Anti-anxiety	1	0	2
Antipsychotics	0	1	1

1.2 Imaging Acquisition

Data were acquired on a Siemens 3T Trio scanner (Erlanger, Germany) with a Siemens 12-channel Head Matrix Coil. Each child was fitted with a thermoplastic mask fastened to the head coil to help stabilize head position. T1-weighted sagittal MP-RAGE structural images in the same anatomical plane as the BOLD images were obtained to improve alignment to an atlas (1 sequence acquisition for each of the 101 control participants (child, adolescent, and adult) and for 88 of the TS participants (child, adolescent, adult): slice time echo = 3.06 ms, TR = 2.4 s, inversion time = 1 s, flip angle = 8°, 176 slices, 1 × 1 × 1 mm voxels; 2 sequence acquisitions for each of the 13 remaining child and adolescent TS participants: slice time echo = 2.34 ms, TR = 2.2 s, inversion time = 1 s, flip angle = 7°, 160 slices, 1 × 1 × 1 mm voxels). Functional images were acquired using a BOLD contrast-sensitive echo-planar sequence (TE = 27 ms, flip angle = 90°, in-plane resolution 4x4 mm; volume TR = 2.5 s). Whole-brain coverage was obtained with 32 contiguous interleaved 4 mm axial slices. Steady-state magnetization was assumed after 4 volumes. For most participants, 2-4 resting state scans lasting 5-5.5 min each were acquired, but the duration of each scan ranged from 3.2 minutes to 30 minutes. In the TS group, 388 ± 61.5 (range 264-528) total functional volumes were acquired, and in the control group, 372 ± 130 (range 260-724) total functional volumes were acquired.

1.3 Imaging preprocessing

Functional images from each participant were preprocessed to reduce artifacts (Shulman et al. 2010). These steps included: (i) temporal sinc interpolation of all slices to the temporal midpoint of the first slice, accounting for differences in the acquisition time of each individual slice, (ii) correction for head movement within and across runs, and (iii) intensity normalization of the functional data was computed for each individual via the MP-RAGE T1-weighted scans. Each run was then resampled in atlas space on an isotropic 3 mm grid combining movement correction and atlas transformation in a single interpolation. The target atlas was created from thirteen 7-9 year old children and twelve 21-30 year old adults using validated methods (Black et al. 2004). The atlas was constructed to conform to the Talairach atlas space.

1.4 Functional Connectivity Preprocessing

Several additional pre-processing steps were applied to reduce spurious variance unlikely to reflect neuronal activity (Fox et al. 2009). These functional connectivity pre-processing steps included: (i) demeaning and detrending each run, (ii) multiple regression of nuisance variables, (iii) frame censoring (discussed below) and interpolation of data within each run, (iv) temporal band-pass filtering ($0.009\ Hz < f < 0.08\ Hz$), and (v) spatial smoothing (6 mm full width at half maximum). Nuisance variables included motion regressors (e.g. original motion estimates, motion derivatives, and Volterra expansion of motion estimates), an average of the signal across the whole brain (global signal), individualized ventricular and white matter signals, and the derivatives of these signals.

We applied a procedure determined and validated to best reduce artifacts related to head motion (Power et al. 2014; Ciric et al. 2017). Specifically, frame-by-frame head

displacement (FD) was calculated from preprocessing realignment estimates, and frames with FD > 0.2 mm were removed. An FD threshold of 0.2 mm was chosen because it best reduced the distance-dependence related to individual differences in head motion (mean FD) in this developmental dataset, as assessed using procedures from Power et al. (2012) and Ciric et al. (2017). Data were considered usable only in contiguous sets of at least 3 frames with FD < 0.2 and a minimum of 30 frames within a functional run. Motion-contaminated frames were censored from the continuous, processed resting-state time series before computing resting-state correlations. Notably, the global signal was included as a nuisance regressor (mentioned above) in order to further reduce global, motion-related spikes in BOLD data (Power et al. 2014; Ciric et al. 2017) and reduce patterns of spurious functional connectivity that might be utilized for prediction with machine learning (Nielsen et al. 2018).

1.5 Parameters for Support Vector Machine Learning

The parameters used for support vector machine (SVM) learning were the same as those used in Dosenbach et al. 2010 and Greene et al., 2016. We used the Spider Machine Learning Toolbox implemented in Matlab for SVM training and testing. In SVMs, each of the samples (here, participants) is treated as a point in multidimensional space defined by as many dimensions as features (here, 44,850 functional connections). In training an SVM classifier, a penalty is incurred for misclassified data in the training set (points on the wrong side of the multivariate decision boundary). The parameter C describes the margin used in training. For a larger C, a larger penalty is assigned to misclassification

errors. All SVM classifications described in this work used soft-margin SVMs with the default setting of C = 1. Leave-one-out cross validation (LOOCV) was used to assess how well a classifier can distinguish individuals from different groups. In turn, each individual was removed from the training set, a diagnostic classifier was built to distinguish TS from controls in the remaining participants, and the left out subject was classified with the resulting diagnostic classifier.

We empirically tested whether a diagnostic classifier performed significantly above chance. We randomly sorted individuals with and without TS into two classes and trained a classifier distinguish the two arbitrary classes. LOOCV was used to determine the diagnostic classification accuracy of each classifier (expected accuracy is near 50%). We repeated this randomization process 100 times. By comparing the observed classification accuracy in the CHILD or ADULT diagnostic classifiers to the classification accuracy of the diagnostic classifiers trained with arbitrary classes, we can determine whether the CHILD or ADULT diagnostic classifier can discriminate TS from controls above chance.

The parameters used for support vector regression (SVR) were the same as those used in Dosenbach et al. 2010 and Nielsen et al. 2018. SVR retains some of the main features of binary SVM classification. In SVR, a penalty is incurred for data that is too far from the regression line in multivariate space. Epsilon-insensitive SVR defines a tube of width epsilon around the regression line in multivariate space. Any data points (i.e., subjects) within this tube carry a loss of zeros, meaning there is no penalty. In SVR, the C parameter controls the trade-off between how strongly subjects beyond the epsilon insensitive tube are penalized and the flatness of the regression line (larger C allows the regression line to be less flat). All SVR predictions described here used epsilon-

insensitive SVRs with the Spider Machine Learning Toolbox default setting of C = Infinity and epsilon = 0.00001.

1.6 Binomial Significance Test

To determine whether the performance of a diagnostic classifier significantly differed from an expected performance, we used a binomial significance test. We determined the probability density function for an observed classification accuracy *x*, given *n* independent test subjects and *p*, the expected accuracy of a diagnostic classifier, as follows.

$$y = f(x|n,p) = \binom{n}{x} p^x (1-p)^{(n-x)} I_{(0,1,\ldots,n)}(x)$$

The result, *y*, is the probability of observing *x* in *n* independent trials, where the probability of correctly classifying TS in any given subject is *p*.

This approach was used to assess whether:

A. CHILD diagnostic classifier performed significantly differently in adolescents and adults than in children
B. ADULT diagnostic classifier performed significantly differently in children and adolescents than in adults
C. CHILD and ADULT diagnostic classifiers performed significantly differently than the ALL-AGES diagnostic classifier (see Supplement 2.2 *ALL-AGES Diagnostic Classifier*)
D. Misclassification of individuals according to sex, comorbid disorders, or current medications was significantly different than expected given the composition of the test set (see Supplement 2.1 *Misclassification of potentially confounding characteristics*).

2. Supplemental Analyses

2.1 Misclassification of potentially confounding characteristics

As shown in Figure 3-2, the CHILD and ADULT diagnostic classifiers did not generalize well to other age groups. Notably, these classifiers systematically misclassified individuals from other age groups, as evidenced by the imbalanced sensitivity and specificity. The CHILD diagnostic classifier misclassified control adolescents and adults as TS, while the ADULT diagnostic classifier misclassified children and adolescents with TS as controls. The CHILD diagnostic classifier misclassified 24 out of 46 adolescents (2 TS / 22 control) and 40 out of 78 adults (1 TS / 39 control). The ADULT diagnostic classifier misclassified 24 out of 46 adolescents (19 TS / 5 control) and 33 out of 78 adults (27 TS / 6 control). While the total TS and control samples were matched on age, sex, IQ, handedness, and in-scanner movement, not all of these characteristics were matched across age groups (Table 3-S2). Sex ratio, frequency of comorbid disorders, and the number of individuals currently taking medications varied across the groups of children, adolescents, and adults.

Table 3-S2. TS participant characteristics per age group.

	Age range	Sex Ratio	Comorbidities	Medications	YGTSS
Children	7.4 – 13.1 years	61 M: 17 F	23	19	17.5 (8.1)
Adolescents	13.1 – 16.6 years	30 M: 16 F	16	14	18.2 (8.2)
Adults	18.1 – 35 years	32 M: 46 F	28	18	16.8 (8.4)

To test whether these characteristics affected classification, we first used a binomial significance test to determine if the composition of misclassified individuals from the CHILD or ADULT diagnostic classifiers significantly differed from the overall

composition of the test set. A summary of these results is shown in Table 3-S3. Overall, misclassified individuals were representative of the test set containing largely the same sex ratio and percentage of individuals with comorbidities or current medications.

Table 3-S3. Comparing the composition of misclassified individuals from the CHILD and ADULT Diagnostic Classifiers to that of the original test set.

CHILD Diagnostic Classifier	Test Set	Number misclassified	Composition of Test Set	Composition of Misclassifications	Difference uncorrected p-value
Sex % males	*Adolescents*	24	65%	63%	0.16
	Adults	40	41%	40%	0.13
Comorbidities % with comorbidities	*TS Adolescents*	2	70%	50%	0.42
	TS Adults	1	72%	100%	0.72
Medications % on medications	*TS Adolescents*	2	61%	50%	0.48
	TS Adults	1	46%	0%	0.54
ADULT Diagnostic Classifier	**Test Set**	**Number misclassified**	**Composition of Test Set**	**Composition of Misclassifications**	**Difference uncorrected p-value**
Sex % males	*Children*	33	78%	64%	0.021*
	Adolescents	24	65%	63%	0.16
Comorbidities % with comorbidities	*TS Children*	27	59%	59%	0.15
	TS Adolescents	19	70%	63%	0.16
Medications % on medications	*TS Children*	27	49%	41%	0.15
	TS Adolescents	19	61%	53%	0.14

As the sex ratio of the misclassified children using the ADULT diagnostic classifier differed from the sex ratio of the entire set of children ($p = 0.021$, *uncorrected*), we wanted to ensure that poor generalizability was not due to sex differences between age groups. Thus, we built sex-matched CHILD and ADULT diagnostic classifiers and tested how well these diagnostic classifiers generalized to other age groups. The sex-matched training

sets were smaller (n=66 rather than n=78) and sampled from a broader age range (Table 3-S4) than the training sets used in the main text.

Table 3-S4. Training sets for sex-matched CHILD and ADULT diagnostic classifiers

	N	Ages	Sex
Sex-matched CHILD Diagnostic Classifier	33 TS / 33 Controls	**7.4 – 16.6 years** TS: 11.2 (2.6) 7.6 – 16.6 Controls: 11.7 (2.4) 7.4 – 16.3	33 males 33 females
Sex-matched ADULT Diagnostic Classifier	33 TS / 33 Controls	**18.1 – 34.1 years** TS: 23.4 (4.3) 18.4 – 34.1 Controls: 23.8 (3.4) 18.1 – 30.8	33 males 33 females

The sex-matched CHILD diagnostic classifier (7.4-16.6 years; Table 3-S5) was 64% accurate with LOOCV, akin to the YOUTH diagnostic classifier (also 64%). The sex-matched ADULT diagnostic classifier (18-31 years; Table 3-S5) was 88% accurate with LOOCV. All diagnostic classifiers were accurate significantly above chance, which is 50% (sex-matched CHILD: $p = 0.01$; sex-matched ADULT: $p < 0.001$). However, these sex-matched classifiers still did could not accurately distinguish TS from controls as well in the other age group. Specifically, the sex-matched CHILD diagnostic classifier was 56% accurate for classifying diagnosis in the sex-matched adults, and the sex-matched ADULT diagnostic classifier was 59% accurate for classifying diagnosis in the sex-matched children. These classifiers performed significantly worse in the other age groups (see Figure 3-S1). Thus, the poor generalizability observed in Figure 3-2 is likely due to age-related differences rather than sex differences.

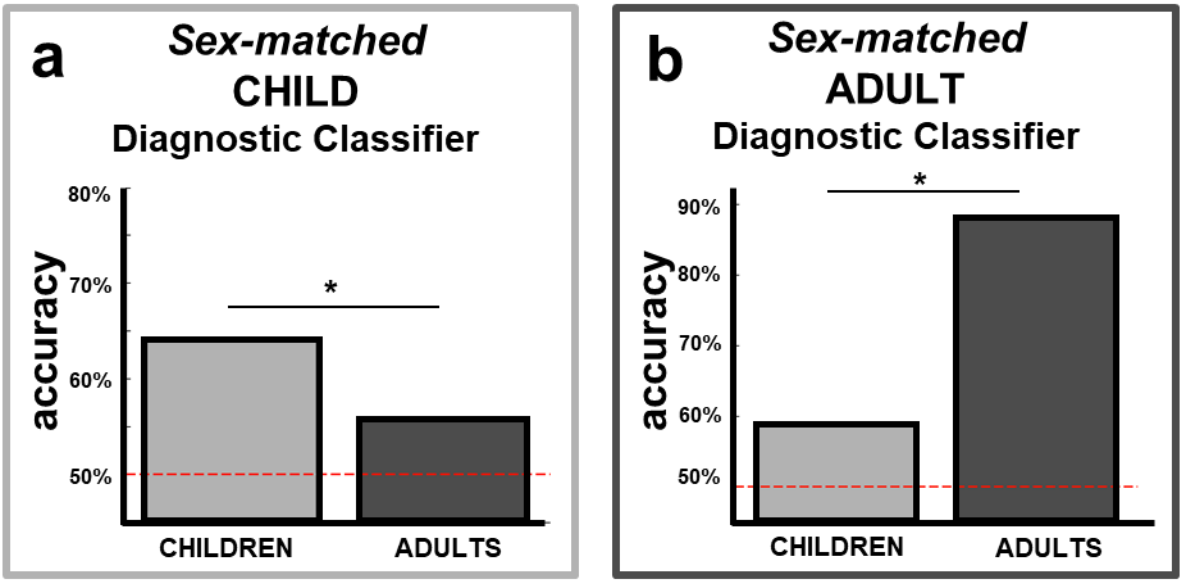

Figure 3-S1. *Functional connections that best distinguish TS from controls were age-specific, even when age groups were matched on sex* **a.)** Performance of the sex-matched CHILD diagnostic classifier to classify adults was significantly less accurate than performance in children (p = 0.01). **b.)** Performance of the sex-matched ADULT diagnostic classifier to classify children was significantly less accurate than performance in adults (p < 0.001).

2.2 ALL-AGES Diagnostic Classifier

To specifically target age-invariant differences in functional connectivity, we used SVM to build an ALL-AGES diagnostic classifier trained to distinguish TS from controls across the age range of our subjects (ages 7.4-34.2 years; Table 3-S5). If some of these age-invariant differences are utilized by the CHILD or ADULT diagnostic classifiers, the CHILD and ADULT classifiers will generalize across age groups at least as well as the ALL-AGES diagnostic classifier. We used a binomial significance test to determine whether the performance of the CHILD or ADULT diagnostic classifiers significantly differed from the ALL-AGES diagnostic classifier (Supplemental Methods, *Binomial Significance Test*).

Table 3-S5. Training and testing sets used for the ALL-AGES diagnostic classifier

SVM **Diagnostic Classifier**		**N**	**Ages**
ALL-AGES	***Train***	***All age sample***	**39 TS / 39 Controls**	**7.4 – 34.2 years**
	Test	*Children*	24 TS / 24 Controls	8.0 – 13.1 years
		Adolescents	12 TS / 12 Controls	13.5 – 16.6 years
		Adults	26 TS / 26 Controls	18.1 – 35 years

The ALL-AGES diagnostic classifier, which included children, adolescents, and adults (7-31 years; Table 3-S5) was 60% accurate, marginally significant (p = 0.05). As the CHILD and ADULT diagnostic classifiers significantly outperformed the ALL-AGES diagnostic classifier (p = 0.015), the patterns of functional connections that best distinguished TS from controls in children and adults separately included age-specific differences.

We extracted the top 1000 (out of 44,850) functional connections most strongly weighted in the ALL-AGES diagnostic classifier. Regions involved in these functional connections were distributed among many processing, control, and default mode networks (Figure 3-S2).

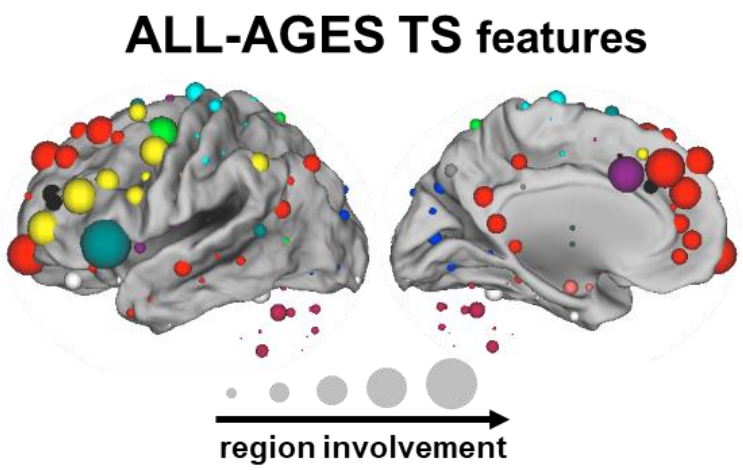

Figure 3-S2. *Functional connections that best distinguished TS from controls across children, adolescents, and adults.* Regions are shown from the top 1000 weighted functional connections used to distinguish TS from controls in the ALL-AGES diagnostic classifier. The size of each sphere represents region involvement (i.e., number of functional connections from the feature set involving a particular region). Region colors indicate the network to which that region belongs.

2.3 CHILD TS and ADULT TS Features

The top 1000 (out of 44,850) functional connections most strongly weighted in the CHILD or ADULT diagnostic classifiers were extracted. The top weighted functional connections are displayed in Figure 3-S3 and show that these functional connections were within and between many different functional networks. Only 33 (3%) of the top 1000 functional connections overlapped between the CHILD and ADULT diagnostic classifiers. Top functional connections appear to be organized loosely by "blocks" of functional connections, either within a single network or between a pair of networks. Some blocks appear more heavily weighted in the ADULT diagnostic classifier (e.g., THAL-VIS or SM_{body}-VIS) and other blocks appear to have different portions more heavily weighted by the CHILD and ADULT diagnostic classifiers (e.g., DMN). The different patterns of

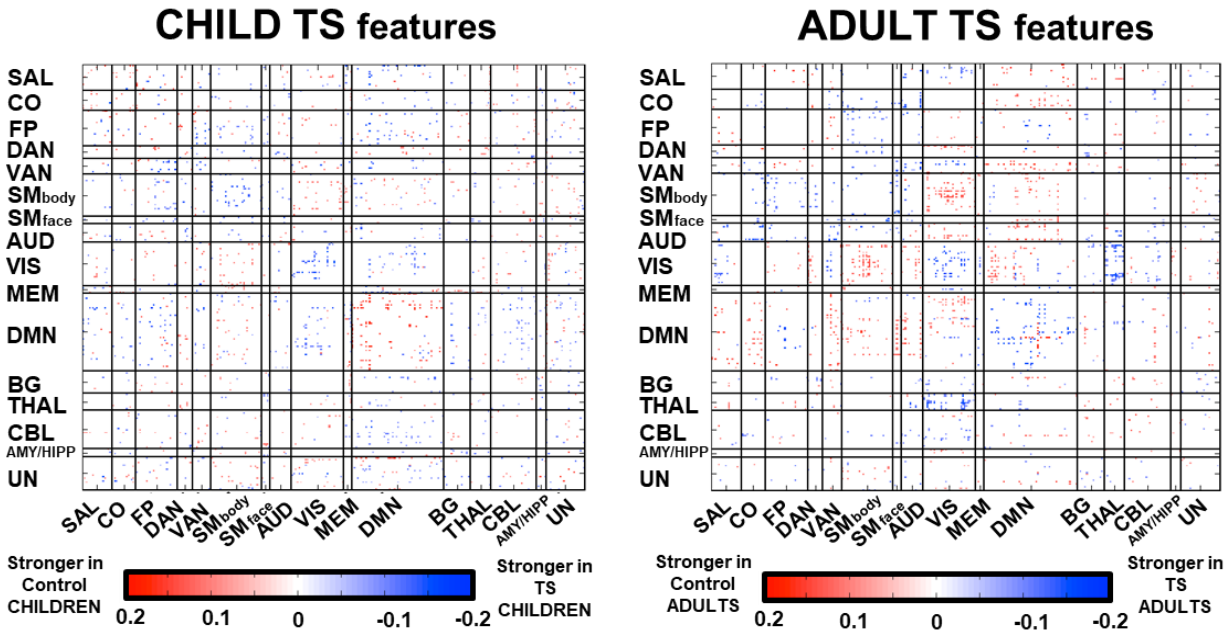

Figure 3-S3. *Functional connections selected as CHILD TS and ADULT TS features.* Functional connections are shown from the top 1000 weighted functional connections used to distinguish TS from controls in the CHILD diagnostic classifier (*left*) and in the ADULT diagnostic classifier (*right*). Functional connections are shown between regions that are sorted by functional network and then from left to right. The average difference between TS and controls is depicted for each connection in both children (*left*) and adults (*right*).

functional connections involved in the CHILD and ADULT diagnostic classifiers provide further evidence that TS differs between children and adults.

2.4 Age Prediction with Random Features

In Nielsen et al. 2018, we found that many sets of functional connections, even randomly selected, can be used to predict the age of typically developing individuals, using SVR. In fact, in some cases, randomly selected features outperformed features selected based on *a priori* hypotheses. To evaluate whether the CHILD TS and ADULT TS features (i.e., those features most heavily weighted in each of these diagnostic classifiers) carry sufficient information related to age, we wanted to ensure that a matched number of

randomly selected features did not outperform the disorder-related features. One hundred sets of 1000 randomly selected functional connections were used to build developmental models to predict age in the controls using SVR. Leave-one-out cross-validation was used to assess performance of each developmental model. Figure 3-S4 shows the amount of age-related variance explained by the predicted ages in controls from each set of randomly selected features, and how this compares to the CHILD TS and ADULT TS features. Randomly selected features did not outperform the CHILD TS and ADULT TS features and, thus, these disorder-related features carry sufficient information related to age.

2.5 Intact Development in TS

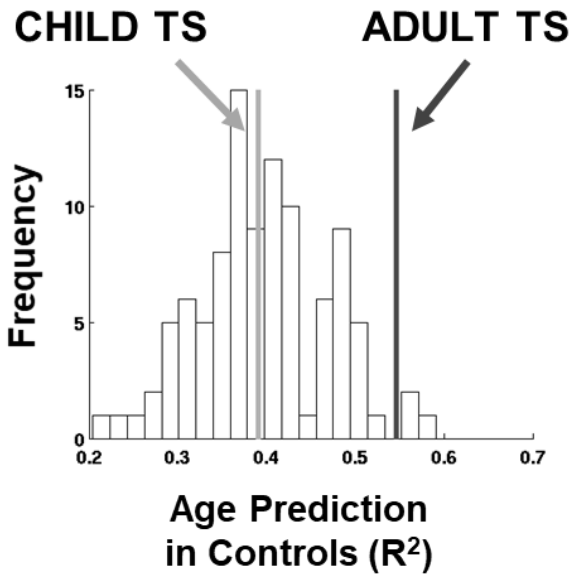

Figure 3-S4. *CHILD TS and ADULT TS features have sufficient information to predict age in controls*. Variance explained by age in 100 developmental models built from 1000 randomly selected functional connections using SVR is displayed. The performance of the developmental models built from the CHILD TS and ADULT TS features is on par with those built from randomly selection connections.

To further explore typical development of functional connectivity in TS, we used SVR trained on other feature sets in the controls to predict age in the TS participants. First, we included all possible features (all functional connections among the 300 regions across the whole brain), and as in Nielsen et al. 2018, we found that whole-brain functional connectivity contained age-related patterns that could be used to predict age well in controls (r = 0.73, R^2 = 0.54, p < 0.001). These age-related patterns were maintained in TS, as the model also predicted age well in the TS group (r = 0.71, R^2 = 0.50, p < 0.001). Second, we included the features that change the most in typical development by selecting the top 1000 features from a developmental classifier trained on 39 control children and 39 control adults (Table 3-2, controls used to train the CHILD and ADULT diagnostic classifiers) using SVM. These functional connections predicted age well in controls (r = 0.74, R^2 = 0.56, p < 0.001) and in TS (r = 0.62, R^2 = 0.38, p < 0.001), indicating that the age-related patterns in these developmentally relevant functional connections appear to be largely intact in TS.

Finally, we used SVR to predict age from the functional connections that differ most in the ALL-AGES diagnostic classifier, i.e., the top-weighted 1000 functional connections from the ALL-AGES diagnostic classifier. We found that these functional connections predicted age well in controls (r = 0.57, R^2 = 0.32, p < 0.001) and in TS (r = 0.57, R^2 = 0.32, p < 0.001), indicating that the developmental differences in the connections exhibiting age-invariant TS effects were largely intact in TS.

2.6 False Age Prediction

Figure 3-4 in the main text of Chapter 3 indicates that the predicted ages of TS individuals differ from the predicted ages of controls. This offset between the actual age and predicted age of TS individuals might arise if (1) TS individuals lack the age-related patterns of functional connectivity differences identified by the developmental model or (2) TS individuals have functional connectivity that exhibits accelerated or delayed maturation. To sort out these possibilities, we used SVR trained on the CHILD TS or ADULT TS features to build developmental models (n=100) to predict age in the controls using false, permuted age labels. Then, we tested how these false developmental models predicted age in the TS participants. As expected, false developmental models did not predict the age of TS individuals well (CHILD TS: average R^2 = 0.021; ADULT TS: average R^2 = 0.044). Rather, the predicted ages fell near the mean age of the training set, indicating a failure of the model (Figure 3-S5, *grey*). Further, these predicted ages differed from the predicted ages of TS individuals when the real (not false) ages of the controls were used to the build the model (see main text), indicating that the functional connections that differ in TS reflect shifted development.

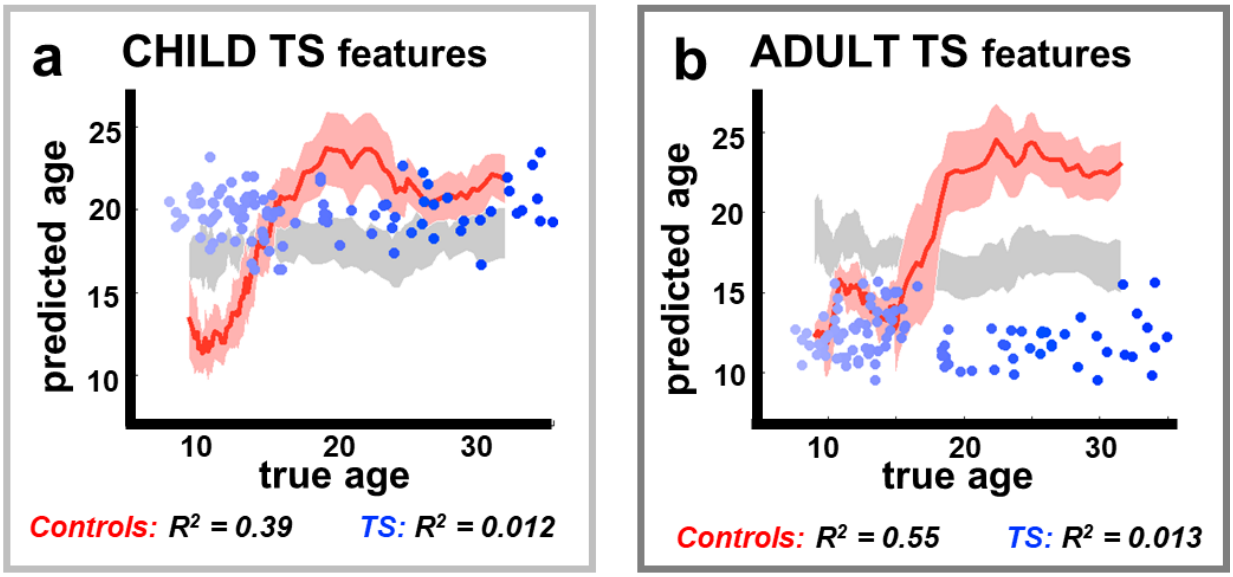

Figure 3-S5. *Predicted ages with CHILD TS and ADULT TS features reflect shifted rather than absent development.* **a.)** The developmental model built using the CHILD TS features was able to predict age well in the control sample (*red*) but not in the TS sample (*blue*). The false developmental models using CHILD TS features did not predict age well in the TS sample (*grey*). Predicted ages of children with TS were older than the predicted ages of age-matched controls and older than their predicted ages form the false developmental models, indicating acceleration maturation in the CHILD TS features. **b.)** The developmental model built using the ADULT TS features was able to predict age well in the control sample (*red*) but not in the TS sample (*blue*). The false developmental models using the ADULT TS features did not predict age well in the TS sample (*grey*). Predicted ages of adults with TS were younger than the predicted ages of age-matched controls and younger than their predicted ages from the false developmental models, indicating delayed or incomplete maturation in the ADULT TS features.

3.8 References

Alexander-Bloch A, Clasen L, Stockman M, Ronan L, Lalonde F, Giedd J, et al. Subtle in-scanner motion biases automated measurement of brain anatomy from in vivo MRI. Human Brain Mapping 2016; 37: 2385–2397.

Amat JA, Bronen RA, Saluja S, Sato N, Zhu H, Gorman DA, et al. Increased Number of Subcortical Hyperintensities on MRI in Children and Adolescents With Tourette's Syndrome, Obsessive-Compulsive Disorder, and Attention Deficit Hyperactivity Disorder. AJP 2006; 163: 1106–1108.

Arbabshirani MR, Plis S, Sui J, Calhoun VD. Single subject prediction of brain disorders in neuroimaging: Promises and pitfalls. NeuroImage 2017; 145: 137–165.

Astle DE, Barnes JJ, Baker K, Colclough GL, Woolrich MW. Cognitive Training Enhances Intrinsic Brain Connectivity in Childhood. J. Neurosci. 2015; 35: 6277–6283.

Baym CL, Corbett BA, Wright SB, Bunge SA. Neural correlates of tic severity and cognitive control in children with Tourette syndrome. Brain 2008; 131: 165–179.

Biswal B, Zerrin Yetkin F, Haughton VM, Hyde JS. Functional connectivity in the motor cortex of resting human brain using echo-planar mri. Magn. Reson. Med. 1995; 34: 537–541.

Bloch MH, Leckman JF, Zhu H, Peterson BS. Caudate volumes in childhood predict symptom severity in adults with Tourette syndrome. Neurology 2005; 65: 1253.

Bloch MH, Peterson BS, Scahill L, Otka J, Katsovich L, Zhang H, et al. Adulthood outcome of tic and obsessive-compulsive symptom severity in children with Tourette syndrome. Arch Pediatr Adolesc Med 2006; 160: 65–69.

Brown TT, Kuperman JM, Chung Y, Erhart M, McCabe C, Hagler Jr. DJ, et al. Neuroanatomical Assessment of Biological Maturity. Current Biology 2012; 22: 1693–1698.

Church JA, Fair DA, Dosenbach NUF, Cohen AL, Miezin FM, Petersen SE, et al. Control networks in paediatric Tourette syndrome show immature and anomalous patterns of functional connectivity. Brain 2009a; 132: 225–238.

Church JA, Wenger KK, Dosenbach NUF, Miezin FM, Petersen SE, Schlaggar BL. Task control signals in pediatric Tourette syndrome show evidence of immature and anomalous functional activity [Internet]. Front. Hum. Neurosci. 2009b; 3[cited 2018 Jun 19] Available from: https://www.frontiersin.org/articles/10.3389/neuro.09.038.2009/full

Ciric R, Wolf DH, Power JD, Roalf DR, Baum GL, Ruparel K, et al. Benchmarking of participant-level confound regression strategies for the control of motion artifact in studies of functional connectivity. NeuroImage 2017; 154: 174–187.

Conners CK, Sitarenios G, Parker JDA, Epstein JN. The Revised Conners' Parent Rating Scale (CPRS-R): Factor Structure, Reliability, and Criterion Validity. J Abnorm Child Psychol 1998; 26: 257–268.

Cubo E, Galán JMTG y, Villaverde VA, Velasco SS, Benito VD, Macarrón JV, et al. Prevalence of Tics in Schoolchildren in Central Spain: A Population-Based Study. Pediatric Neurology 2011; 45: 100–108.

Debes N, Jeppesen S, Raghava JM, Groth C, Rostrup E, Skov L. Longitudinal Magnetic Resonance Imaging (MRI) Analysis of the Developmental Changes of Tourette Syndrome Reveal Reduced Diffusion in the Cortico-Striato-Thalamo-Cortical Pathways. J Child Neurol 2015; 30: 1315–1326.

Debes NMMM, Hansen A, Skov L, Larsson H. A Functional Magnetic Resonance Imaging Study of a Large Clinical Cohort of Children With Tourette Syndrome , A Functional Magnetic Resonance Imaging Study of a Large Clinical Cohort of Children With Tourette Syndrome. J Child Neurol 2011; 26: 560–569.

Dosenbach NUF, Nardos B, Cohen AL, Fair DA, Power JD, Church JA, et al. Prediction of Individual Brain Maturity Using fMRI. Science 2010; 329: 1358–1361.

Eichele H, Plessen KJ. Neural Plasticity in Functional and Anatomical MRI Studies of Children with Tourette Syndrome [Internet]. Behavioural Neurology 2013[cited 2018 Jun 29] Available from: https://www.hindawi.com/journals/bn/2013/376590/abs/

Erenberg G, Cruse RP, Rothner AD. The natural history of Tourette syndrome: a follow-up study. Ann. Neurol. 1987; 22: 383–385.

Erus G, Battapady H, Satterthwaite TD, Hakonarson H, Gur RE, Davatzikos C, et al. Imaging Patterns of Brain Development and their Relationship to Cognition. Cereb Cortex 2015; 25: 1676–1684.

Evans J, Seri S, Cavanna AE. The effects of Gilles de la Tourette syndrome and other chronic tic disorders on quality of life across the lifespan: a systematic review. Eur Child Adolesc Psychiatry 2016; 25: 939–948.

Fahim C, Yoon U, Das S, Lyttelton O, Chen J, Arnaoutelis R, et al. Somatosensory–motor bodily representation cortical thinning in Tourette: Effects of tic severity, age and gender. Cortex 2010; 46: 750–760.

Fair D, Nigg JT, Iyer S, Bathula D, Mills KL, Dosenbach NU, et al. Distinct neural signatures detected for ADHD subtypes after controlling for micro-movements in resting state functional connectivity MRI data [Internet]. Front. Syst. Neurosci. 2013; 6[cited 2018 Jun 29] Available from: https://www.frontiersin.org/articles/10.3389/fnsys.2012.00080/full

Franke K, Luders E, May A, Wilke M, Gaser C. Brain maturation: Predicting individual BrainAGE in children and adolescents using structural MRI. NeuroImage 2012; 63: 1305–1312.

Freeman RD, Fast DK, Burd L, Kerbeshian J, Robertson MM, Sandor P. An international perspective on Tourette syndrome: selected findings from 3500 individuals in 22 countries. Developmental Medicine and Child Neurology 2000; 42: 436–447.

Goetz CG, Tanner CM, Stebbins GT, Leipzig G, Carr WC. Adult tics in Gilles de la Tourette's syndrome. Neurology 1992; 42: 784.

Greene DJ, Black KJ, Schlaggar BL. Neurobiology and functional anatomy of tic disorders. Oxford University Press: Oxford; 2013.

Greene DJ, Black KJ, Schlaggar BL. Considerations for MRI study design and implementation in pediatric and clinical populations. Developmental Cognitive Neuroscience 2016a; 18: 101–112.

Greene DJ, Church JA, Dosenbach NUF, Nielsen AN, Adeyemo B, Nardos B, et al. Multivariate pattern classification of pediatric Tourette syndrome using functional connectivity MRI. Dev Sci 2016b; 19: 581–598.

Greene DJ, Schlaggar BL, Black KJ. Neuroimaging in Tourette Syndrome: Research Highlights from 2014 to 2015. Curr Dev Disord Rep 2015; 2: 300–308.

Hassan N, Cavanna AE. The prognosis of Tourette syndrome: implications for clinical practice. Funct. Neurol. 2012; 27: 23–27.

Jackson GM, Draper A, Dyke K, Pépés SE, Jackson SR. Inhibition, Disinhibition, and the Control of Action in Tourette Syndrome. Trends in Cognitive Sciences 2015; 19: 655–665.

Jackson SR, Parkinson A, Jung J, Ryan SE, Morgan PS, Hollis C, et al. Compensatory Neural Reorganization in Tourette Syndrome. Current Biology 2011; 21: 580–585.

Jung J, Jackson SR, Parkinson A, Jackson GM. Cognitive control over motor output in Tourette syndrome. Neuroscience & Biobehavioral Reviews 2013; 37: 1016–1025.

Khalifa N, Knorring A-L von. Prevalence of tic disorders and Tourette syndrome in a Swedish school population. Developmental Medicine & Child Neurology; 45: 315–319.

Khundrakpam BS, Tohka J, Evans AC. Prediction of brain maturity based on cortical thickness at different spatial resolutions. NeuroImage 2015; 111: 350–359.

Leckman JF, King RA, Bloch MH. Clinical features of Tourette syndrome and tic disorders. Journal of Obsessive-Compulsive and Related Disorders 2014; 3: 372–379.

Leckman JF, Riddle MA, Hardin MT, Ort SI, Swartz KL, Stevenson J, et al. The Yale Global Tic Severity Scale: Initial Testing of a Clinician-Rated Scale of Tic Severity. Journal of the American Academy of Child & Adolescent Psychiatry 1989; 28: 566–573.

Leckman JF, Zhang H, Vitale A, Lahnin F, Lynch K, Bondi C, et al. Course of Tic Severity in Tourette Syndrome: The First Two Decades. Pediatrics 1998; 102: 14–19.

Lichter DG, Finnegan SG. Influence of gender on Tourette syndrome beyond adolescence. European Psychiatry 2015; 30: 334–340.

Marsh R, Zhu H, Wang Z, Skudlarski P, Peterson BS. A Developmental fMRI Study of Self-Regulatory Control in Tourette's Syndrome. AJP 2007; 164: 955–966.

Mazzone L, Yu S, Blair C, Gunter BC, Wang Z, Marsh R, et al. An fMRI Study of Frontostriatal Circuits During the Inhibition of Eye Blinking in Persons With Tourette Syndrome. AJP 2010; 167: 341–349.

Miller AM, Bansal R, Hao X, Sanchez-Pena JP, Sobel LJ, Liu J, et al. Enlargement of Thalamic Nuclei in Tourette Syndrome. Arch Gen Psychiatry 2010; 67: 955–964.

Mueller S, Costa A, Keeser D, Pogarell O, Berman A, Coates U, et al. The effects of methylphenidate on whole brain intrinsic functional connectivity. Human Brain Mapping; 35: 5379–5388.

Muellner J, Delmaire C, Valabrégue R, Schüpbach M, Mangin J-F, Vidailhet M, et al. Altered structure of cortical sulci in gilles de la Tourette syndrome: Further support for abnormal brain development. Movement Disorders; 30: 655–661.

Nielsen AN, Greene DJ, Gratton C, Dosenbach NUF, Petersen SE, Schlaggar BL. Evaluating the Prediction of Brain Maturity From Functional Connectivity After Motion Artifact Denoising [Internet]. Cereb Cortex[cited 2018 Jun 19] Available from: https://academic.oup.com/cercor/advance-article/doi/10.1093/cercor/bhy117/5025421

Pappert EJ, Goetz CG, Louis ED, Blasucci L, Leurgans S. Objective assessments of longitudinal outcome in Gilles de la Tourette's syndrome. Neurology 2003a; 61: 936–940.

Pappert EJ, Goetz CG, Louis ED, Blasucci L, Leurgans S. Objective assessments of longitudinal outcome in Gilles de la Tourette's syndrome. Neurology 2003b; 61: 936–940.

Pépés SE, Draper A, Jackson GM, Jackson SR. Effects of age on motor excitability measures from children and adolescents with Tourette syndrome. Developmental Cognitive Neuroscience 2016; 19: 78–86.

Pérez-Vigil A, Fernández de la Cruz L, Brander G, Isomura K, Jangmo A, Kuja-Halkola R, et al. Association of Tourette Syndrome and Chronic Tic Disorders With Objective Indicators of Educational Attainment: A Population-Based Sibling Comparison Study. JAMA Neurol 2018

Peterson BS, Pine DS, Cohen P, Brook JS. Prospective, longitudinal study of tic, obsessive-compulsive, and attention-deficit/hyperactivity disorders in an epidemiological sample. J Am Acad Child Adolesc Psychiatry 2001a; 40: 685–695.

Peterson BS, Staib L, Scahill L, Zhang H, Anderson C, Leckman JF, et al. Regional Brain and Ventricular Volumes in Tourette Syndrome. Arch Gen Psychiatry 2001b; 58: 427–440.

Peterson BS, Staib L, Scahill L, Zhang H, Anderson C, Leckman JF, et al. Regional Brain and Ventricular Volumes in Tourette Syndrome. Arch Gen Psychiatry 2001c; 58: 427–440.

Plessen KJ, Bansal R, Peterson BS. Imaging evidence for anatomical disturbances and neuroplastic compensation in persons with Tourette syndrome. J Psychosom Res 2009; 67: 559–573.

Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. NeuroImage 2012a; 59: 2142–2154.

Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. NeuroImage 2012b; 59: 2142–2154.

Power JD, Cohen AL, Nelson SM, Wig GS, Barnes KA, Church JA, et al. Functional Network Organization of the Human Brain. Neuron 2011; 72: 665–678.

Power JD, Mitra A, Laumann TO, Snyder AZ, Schlaggar BL, Petersen SE. Methods to detect, characterize, and remove motion artifact in resting state fMRI. NeuroImage 2014; 84: 320–341.

Raz A, Zhu H, Yu S, Bansal R, Wang Z, Alexander GM, et al. Neural Substrates of Self-Regulatory Control in Children and Adults with Tourette Syndrome , Neural Substrates of Self-Regulatory Control in Children and Adults with Tourette Syndrome. Can J Psychiatry 2009; 54: 579–588.

Reuter M, Tisdall MD, Qureshi A, Buckner RL, van der Kouwe AJW, Fischl B. Head motion during MRI acquisition reduces gray matter volume and thickness estimates. NeuroImage 2015; 107: 107–115.

Roessner V, Overlack S, Schmidt-Samoa C, Baudewig J, Dechent P, Rothenberger A, et al. Increased putamen and callosal motor subregion in treatment-naïve boys with Tourette syndrome indicates changes in the bihemispheric motor network. Journal of Child Psychology and Psychiatry; 52: 306–314.

Satterthwaite TD, Elliott MA, Gerraty RT, Ruparel K, Loughead J, Calkins ME, et al. An improved framework for confound regression and filtering for control of motion artifact in

the preprocessing of resting-state functional connectivity data. NeuroImage 2013; 64: 240–256.

Scahill L, Bitsko RH, Visser SN, Blumberg SJ. Prevalence of diagnosed tourette syndrome in persons aged 6-17 years - United States, 2007. Morbidity and Mortality Weekly Report 2009; 58: 581–585.

Scahill L, Riddle MA, McSWIGGIN-HARDIN M, Ort SI, King RA, Goodman WK, et al. Children's Yale-Brown Obsessive Compulsive Scale: Reliability and Validity. Journal of the American Academy of Child & Adolescent Psychiatry 1997; 36: 844–852.

Shulman GL, Pope DLW, Astafiev SV, McAvoy MP, Snyder AZ, Corbetta M. Right Hemisphere Dominance during Spatial Selective Attention and Target Detection Occurs Outside the Dorsal Frontoparietal Network. J. Neurosci. 2010; 30: 3640–3651.

Sowell ER, Kan E, Yoshii J, Thompson PM, Bansal R, Xu D, et al. Thinning of sensorimotor cortices in children with Tourette syndrome. Nature Neuroscience 2008; 11: 637–639.

Spessot AL, Plessen KJ, Peterson BS. Neuroimaging of developmental psychopathologies: the importance of self-regulatory and neuroplastic processes in adolescence. Ann. N. Y. Acad. Sci. 2004; 1021: 86–104.

Tobe RH, Bansal R, Xu D, Hao X, Liu J, Sanchez J, et al. Cerebellar morphology in Tourette syndrome and obsessive-compulsive disorder. Annals of Neurology; 67: 479–487.

Van Dijk KRA, Sabuncu MR, Buckner RL. The influence of head motion on intrinsic functional connectivity MRI. NeuroImage 2012; 59: 431–438.

Varoquaux G, Raamana PR, Engemann DA, Hoyos-Idrobo A, Schwartz Y, Thirion B. Assessing and tuning brain decoders: Cross-validation, caveats, and guidelines. NeuroImage 2017; 145: 166–179.

Worbe Y, Malherbe C, Hartmann A, Pélégrini-Issac M, Messé A, Vidailhet M, et al. Functional immaturity of cortico-basal ganglia networks in Gilles de la Tourette syndrome. Brain 2012; 135: 1937–1946.

Worbe Y, Marrakchi-Kacem L, Lecomte S, Valabregue R, Poupon F, Guevara P, et al. Altered structural connectivity of cortico-striato-pallido-thalamic networks in Gilles de la Tourette syndrome. Brain 2015; 138: 472–482.

Chapter 4: Evidence for divergent and attenuated development of functional networks in Tourette syndrome

4.1 Abstract

Tourette syndrome (TS) is a neurodevelopmental disorder characterized by motor and vocal tics. TS is complex, with symptoms that involve sensory, motor, and top-down control processes and that fluctuate over the course of development. While multiple investigators have studied atypical brain structure and function associated with TS, the neural substrates supporting the complex range and timecourse of symptoms is largely understudied. Here, we used functional connectivity MRI to examine functional networks across the whole-brain in children and adults with TS. To determine whether the influence of age and TS on functional networks is network-dependent, we separately considered the sets of connections within each functional network and those between each pair of functional networks. We tested whether age, TS, or an interaction between these factors was present among these connections. We found that the development of most functional networks was intact in TS (i.e., developmental differences in TS were similar to those in typically developing children and adults). While there was some suggestive evidence for consistent functional network differences in childhood and adulthood TS, most functional networks that were significantly affected by TS differed between children and adults. Several within-network and cross-network connections exhibited either divergent or attenuated development in TS. Connections involving the somatomotor, cingulo-opercular, auditory, dorsal attention, and default mode networks diverged from typical development in TS, demonstrating enhanced functional connectivity in adulthood TS. In

contrast, development of connections involving the basal ganglia, thalamus, cerebellum, auditory, visual, reward, and ventral attention networks was attenuated in TS. By placing these findings in a context of previous evidence, we developed a novel model of the development of atypical brain function, connections, and cognitive processes associated with TS. We contend that adulthood TS may be characterized by divergent development of systems implicated in suppressing, producing, and attending to tics; connectivity was greater than what is observed in typically developing individuals. In contrast, systems implicated in the initiation and production of tics demonstrated attenuated development in TS, predominantly exhibiting immaturity in adulthood TS. Jointly, our results inform a model of how several cortical and subcortical functional networks involved in the initiation, production, and/or suppression of tics interact and differ across development in TS.

3.2 Introduction

Tourette syndrome (TS) is a developmental neuropsychiatric disorder that affects 1-3% of children (Khalifa and Knorring, n.d.; Scahill *et al.*, 2009; Cubo *et al.*, 2011) and is characterized by motor and vocal tics (Leckman *et al.*, 2014). Tics are brief, unwanted, repetitive movements or noises. Tics are often accompanied by a preceding perceived sensation of discomfort called a premonitory urge (Leckman *et al.*, 1993); urges to tic can be suppressed, but only temporarily (Himle *et al.*, 2007). Thus, TS is a complex disorder which affects multiple sensory, motor, and top-down control processes (Mink, 2001). Additionally, tic symptoms are not static and often fluctuate over the course of development. Tic onset typically occurs at age 5-7 years, with tic severity peaking during late childhood/early adolescence (10-12 years), and with marked improvement or even remission after adolescence and into adulthood (Erenberg *et al.*, 1987; Leckman *et al.*,

1998; Peterson *et al.*, 2001a; Bloch *et al.*, 2006; Hassan and Cavanna, 2012); However, symptom progression varies substantially across individuals, with a sizeable subgroup of patients (~60%) experiencing moderate to severe tics that persist into adulthood (Leckman *et al.*, 1998; Pappert *et al.*, 2003b). Taking into account the complexity of the nature and course of symptoms when studying the neural abnormalities in TS may provide better targets for diagnosis, treatments, and prognosis.

Many cortical and subcortical systems likely support the initiation, production, and suppression of tics and other symptoms associated with TS. The most prominent theory in TS is that disruption of cortico-striato-thalamo-cortical loops leads to the production of tics; activity in the striatum propagates through these loops and leads to the disinhibition of unwanted motor plans and the production of tics (Mink, 2001). Several lines of research support this hypothesis as 1) disrupting activity in the basal ganglia produces tic-like movements (Alexander and DeLong, 1985; McCairn *et al.*, 2009), 2) the basal ganglia, thalamus, motor cortex, and cerebellum are co-activated at the time of tic action in patients with TS (Bohlhalter *et al.*, 2006; Wang *et al.*, 2011; Neuner *et al.*, 2014), and 3) reduced caudate volume and thinning of sensorimotor cortex have been reported in children and adults with TS (Peterson *et al.*, 1993; Bloch *et al.*, 2005, but see Greene *et al.*, 2017). Concurrently, motor control and the suppression of unwanted movements are also atypical in TS and thought to be supported by a group of regions including frontal cortex. Regions in frontal cortex (and others) are active during the time preceding tics (premonitory urge) (Bohlhalter *et al.*, 2006; Wang *et al.*, 2011; Neuner *et al.*, 2014) and during instructed eye blink suppression (Mazzone *et al.*, 2010) in patients with TS. Control signals in frontal and other associated regions during non-motor tasks are also atypical

in TS (Church *et al.*, 2009) and thinning of frontal cortex has been observed in children with TS (Sowell *et al.*, 2008). Less is known about the neurobiology supporting the initiation of tics; the frequency of tics can be modulated by many environmental factors (e.g., stress, fatigue, diverted attention; for review see Conelea and Woods, 2008) which might suggest that the attention and sensory systems responsible for processing and orienting to external triggers might play an important role in the initiation of tics.

Most neuroimaging studies of TS treat it as a singular disorder, unchanging across development, by grouping together a wide range of patients or focusing on a single age cohort. However, as symptoms vary by age, there is evidence that differences in brain structure and function in TS also vary by age (Peterson *et al.*, 2001b; Raz *et al.*, 2009; Pépés *et al.*, 2016). Considering whether brain differences in TS differ between childhood and adulthood enables one to determine whether an observed difference is necessary for the manifestation of tics over age. Further, given a context of typical development, one can determine whether brain differences observed in those with TS reflect atypically shifted development (e.g., accelerated or delayed maturation), potentially providing clues into etiology. Comparing the neural substrates supporting the initiation, production, and suppression of tics and other symptoms between children and adults with TS may reveal how maturation and/or experience with tics affect symptom course.

In combination, prior work suggests that many regions across the cortex and subcortex contribute to the manifestation of TS symptoms and any of these abnormalities may change over the course of development. Thus, studying the development of the network organization of the whole brain in TS is critical for a more complete understanding of neurobiology of tics and the broader symptoms of TS. Functional brain networks can

be examined using resting-state functional connectivity MRI: as fMRI signals from a pair of functionally related regions are often highly correlated even at rest (Biswal *et al.*, 1995), measurements of "functional connectivity" have been used to identify collections of functionally related regions, or functional networks (Power *et al.*, 2011; Yeo *et al.*, 2011). The measured strength of functional connectivity is thought to reflect a history of co-activation across the lifespan (Lewis *et al.*, 2009) thus tracking the atypical coordination (or lack of coordination) of different functional networks in TS. Thus, functional networks implicated in the initiation (e.g., sensory and attention), production (e.g., subcortical and somatomotor), and suppression (e.g., control and default-mode) of tics can be rapidly and simultaneously assessed using resting-state functional connectivity.

Placing any differences in TS within a context of functional networks also facilitates more specific and powerful interpretations of the cognitive manifestations observed in individuals with TS. For example, functional or structural differences observed in frontal cortex can be difficult to interpret as multiple functional networks reside in frontal cortex (e.g. fronto-parietal, cingulo-opercular, default mode, ventral attention, salience). Delineating the specific functional networks that are affected in TS would facilitate interpretations that leverage the extensive work elucidating the functional properties of these networks in healthy controls. For example, if differences observed in frontal cortex in TS are associated with the regions belonging to the cingulo-opercular network, then these differences might suggest atypical executive control signals related to task-set maintenance or the detection of errors (Neta *et al.*, 2014) in TS. Whereas if differences in frontal cortex in TS are associated with the neighboring ventral attention network, then these differences might suggest atypical stimulus-oriented attention (Corbetta and

Shulman, 2002). It is important to be cautious when making these interpretations though, as many functions are carried out by these networks (Poldrack, 2006).

Previously, we demonstrated that patterns of resting-state functional connectivity across the whole brain contain information that can distinguish individuals with TS from controls (Greene *et al.*, 2016; *Chapter 3*) and predict an individual's maturity (Dosenbach *et al.*, 2010; *Chapter 2; Chapter 3*). However, the specific functional networks that are altered in TS and how these connections are influenced by age and diagnosis in TS remains unknown. Here, we used a whole-brain network-level approach to investigate the development of functional networks in TS in relation to the typical developmental pattern observed in healthy controls. To determine whether the influence of age and/or TS on functional networks is network-dependent, we separately considered sets of functional connections within each network and those between each pair of networks across the brain. We first examined functional connectivity differences due to age, TS, and their interaction in children and adults with and without TS. We identified within-network and cross-network functional connections that differed between children and adults separately in TS and controls and then compared these differences in order to describe the intact, attenuated, and divergent development of functional networks in TS.

4.3 Materials and Methods

4.3.1 Participants

Individuals with TS (n=172, ages 7.3-35.0 years) were recruited from the Washington University School of Medicine Movement Disorders Center and the Tourette Association of America Missouri chapter. After quality control assessments of the neuroimaging data

(see below) and to provide consistency with Chapter 3, 78 individuals, comprising 39 children (ages 7.6-13.1 years) and 39 adults (ages 18.4-35.0 years), with TS were included (Table 4-1). A group of 39 child and 39 adult control participants was selected from an extant database (n=487, ages 6.0–35.0 years, 206 males; recruited from the Washington University campus and surrounding community) and matched to the TS group on age, sex, IQ, handedness, and in-scanner movement (Table 4-1). Conditions commonly comorbid with TS (e.g., ADHD, OCD, anxiety) and medication use were not considered exclusionary for the TS participants (Greene *et al.*, 2016a) (Table 4-S1) but were for the control participants.

All participants completed assessments of IQ, and TS participants completed additional assessments of symptom severity for TS, ADHD, and OCD (Supplement A). All aspects of the study were completed with approval from the Washington University School of Medicine Institutional Review Board. Adult participants gave informed consent. For children, a parent or guardian gave informed consent and all children gave verbal assent.

Table 4-1. Participant Characteristics

Children	TS group	Control group
N	39	39
Male/Female	31/8	30/9
Age (Years)	10.9 (1.6); 7.6-13.1	10.7 (1.5); 7.4-12.9
Handedness (R/L)	36/3	37/2
IQ	110 (12.3); 87-135	115 (12.1); 90-139
Residual in-scanner movement (mean FD)	0.11 (0.011); 0.092-0.14	0.11 (0.013); 0.067-0.13
Amount of data (“good” frames)	241.2 (69.4); 121-363	242.6 (88.2); 139-555

YGTSS Total Tic Score	17.5 (5.5); 0-37	N/A
ADHD Rating Scale	11.0 (8.2); 0-34	N/A
CY-BOCS Score	4.8 (5.5); 0-19	N/A
Number on medications	19	0
Number with comorbidities	23	0
Adults	**TS group**	**Control group**
N	39	39
Male/Female	16/23	16/23
Age (Years)	25.9 (5.3); 18.4-35.0	25.9 (4.6); 18.1-34.2
Handedness (R/L)	36/3	36/3
IQ	119 (12.7); 83-139	119 (14.8); 83-145
Residual in-scanner movement (mean FD)	0.11 (0.017); 0.063-0.13	0.10 (0.012); 0.077-0.13
Amount of data ("good" frames)	349.7 (103.9); 153-573	311.3 (115.9); 170-668
YGTSS Total Tic Score	16.7 (8.3); 0-32	N/A
ADHD Rating Scale	11.9 (12.3); 0-44	N/A
CY-BOCS Score	6.8 (6.6); 0-24	N/A
Number on medications	18	0
Number with comorbidities	28	0

Where applicable values are displayed as Average (Standard Deviation); Range
FD = Frame-wise Displacement (in millimeters) (Power *et al.*, 2012a)
YGTSS = Yale Global Tic Severity Score (Total Tic Score) (Leckman *et al.*, 1989)
CY-BOCS Score = Children's Yale-Brown Obsessive-Compulsive Scale (Scahill *et al.*, 1997)

4.3.2 Image Acquisition and Processing

Resting-state fMRI data were collected as participants viewed a centrally presented white crosshair on a black background. Participants were instructed to relax, look at the plus sign, and hold as still as possible. The duration and number of resting-state scans varied across participants (Supplement B). Imaging data were collected using a 3T Siemens Trio

Scanner with a 12-channel Head Matrix Coil. Images were pre-processed to reduce artifacts (Shulman *et al.*, 2010). Additional pre-processing steps were applied to the resting-state data to reduce spurious correlated variance unlikely related to neuronal activity. Stringent frame censoring (frame-wise displacement>0.2 mm) and nuisance regression (motion estimates, global signal, and individual ventricular and white matter signals) were used to reduce spurious individual or group differences in functional connectivity related to head movement in the scanner (Power *et al.*, 2012b, 2014; Ciric *et al.*, 2017). Participants with at least 5 minutes of low-motion data were included.

4.3.3 Regions, Networks, and Blocks

For each participant, resting-state time-courses were extracted from a set of 300 regions of interest (ROIs) covering much of the cortex (Power *et al.*, 2011), subcortex, and cerebellum (Figure 4-1; available at https://greenelab.wustl.edu/data_software). Functional connectivity was measured as the correlation (Fisher z-transformed) between the resting-state time-courses for each pair of ROIs. Whole-brain functional connectivity among all 300 ROIs was determined for each of the four groups (control children, control adults, TS children, TS adults).

Whole-brain functional connectivity has been shown to have modular organization such that different collections of ROIs assemble into separate functional networks thought to support different types of functions. These include processing networks (i.e., visual (VIS), auditory (AUD), and somatomotor (SM_{body}, SM_{face}) that interface with external world, control networks (i.e., fronto-parietal (FP), cingulo-opercular (CO, salience (SAL), dorsal attention (DAN), and ventral attention (VAN)) that direct cognitive resources, and

other association networks (i.e., default mode (DMN), parietal memory (PM), middle temporal lobe (MTL), and reward (RW)) that support internal associations. ROIs were also present within the basal ganglia (BG), thalamus (THAL), and cerebellum (CBL), which often link with multiple cortical networks (Choi *et al.*, 2012; Greene *et al.*, 2014; Marek *et al.*, 2018). Due to non-uniform associations with cortex, as a first step, we treated these ROIs as three separate sets of ROIS, distinct from cortex.

To precisely describe developmental differences in functional networks across the brain, we separately considered “blocks” of connections *within* each functional network and those *between* pairs of functional networks. Within-network blocks represent the set of connections among a group of ROIs belonging to a single functional network (e.g., among DMN regions). Cross-network blocks represent connections between two groups of ROIs from separate functional networks (e.g. between DMN regions and VIS regions). Figure 4-1 provides an overview of our investigation of the influence of age and diagnosis on functional networks.

We used two approaches to reduce the dimensionality of each block for statistical group comparisons. First, we averaged the strength of functional connectivity from an entire block. While this dimensionality reduction approach is straightforward, averaging across the connections in a block can obfuscate true effects of age, TS, or the interaction. Averaging inherently assumes that (1) the strength and sign of the connections in a block are uniform and that (2) the developmental or diagnostic differences present between groups are uniform and in the same direction. Differences in functional connectivity that are non-uniform (e.g., half connections increase and half connections decrease) are not

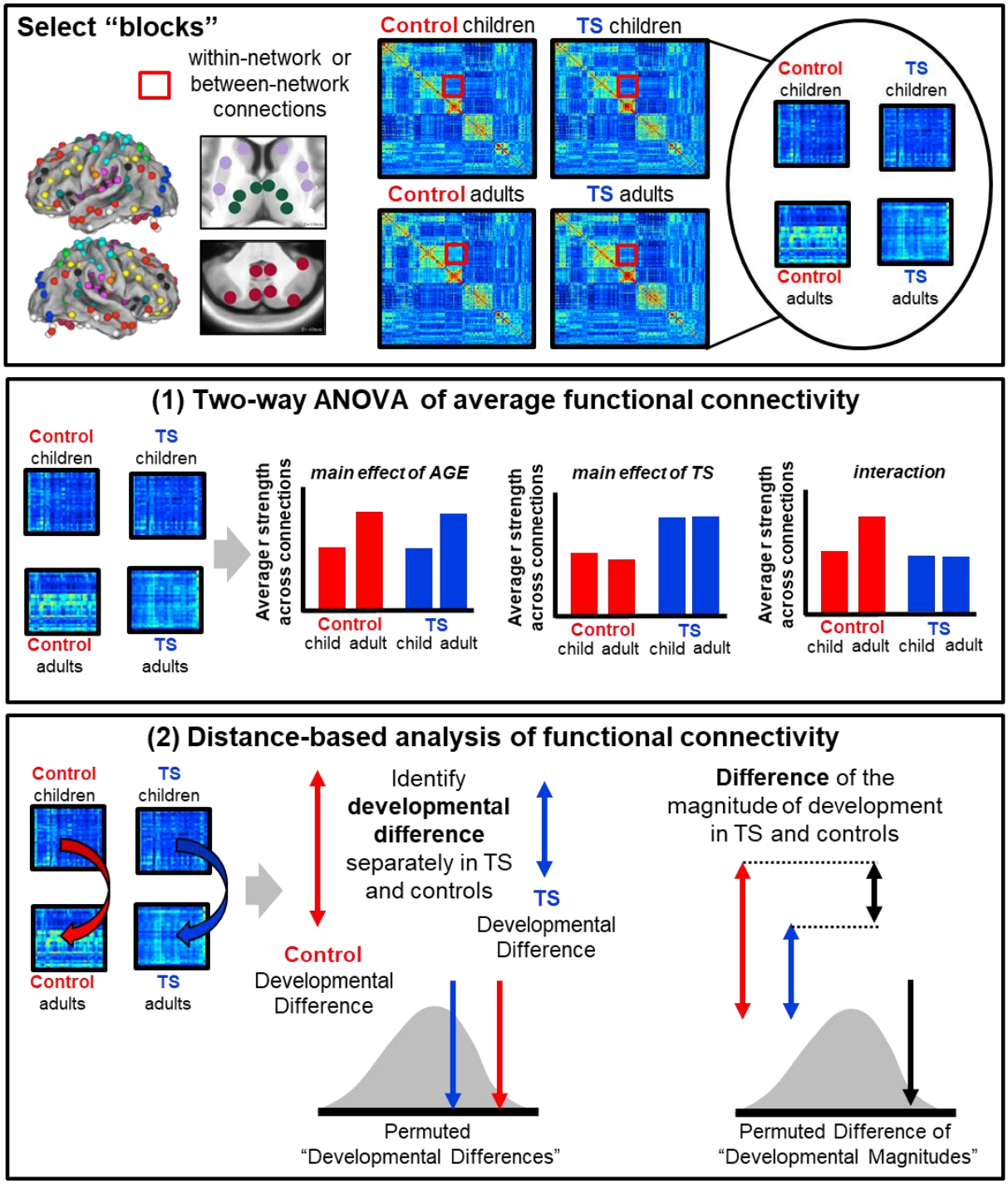

Figure 4-1. *Overview of approaches to investigate the development of functional networks in Tourette syndrome.*

easily detected by differences in average connectivity. Thus, to complement this approach we used a second distance-based approach that computes the difference between groups separately for each connection in a block before being combined (Supplement E). Distance-based approaches are sensitive to non-uniform differences in functional connectivity across a block. However, distance-based approaches alone cannot distinguish (1) the direction of group differences (e.g., children > adults or children < adults) or (2) the relative position of group differences (e.g, overall delay in TS). A combination of distance- and average-based approaches helps well detect and describe atypical development in TS.

4.3.4 Average-based analysis of functional connectivity across blocks

Two-way ANOVA of average functional connectivity across blocks

As a first step towards describing the atypical development of functional networks in TS, we performed a balanced two-way ANOVA to determine the effect of age, TS diagnosis, and the interaction between these factors on the average functional connectivity of each block from children and adults with and without TS. P-values were corrected for multiple comparisons across the total number of blocks tested (n = 136) using the FDR approach (requiring that P(FDR) < 0.05).

4.3.5 Distance-based analysis of functional connectivity across blocks

Distance-based identification of developmental differences in TS and controls

Next, the difference in functional connectivity between children and adults was determined for each block using Euclidean distance (see Formula S1), which maintains

the relationship of specific region pairs and allows detection of complex developmental differences that might be non-uniform. We calculated developmental differences across all blocks in both the control and TS groups.

Permutation testing was used to determine whether the magnitude of the developmental differences in the control group or the TS group are greater than expected by chance. Children and adults in the control group and TS group were separately permuted (N = 1000 times) and randomly assigned into a “child” or “adult” group. Developmental differences were then calculated as described above. For each block, the true developmental difference in the control group or the TS group was contrasted with the distribution of permuted “developmental differences” to generate a P-statistic. P-values were corrected for multiple comparisons across the total number of tested blocks (n = 136) using the FDR approach (requiring that P(FDR) < 0.05).

Distance-based comparison of developmental differences in TS and controls

Next, we compared the magnitude of developmental differences in TS to the developmental differences identified in controls. Comparisons were limited to the blocks with significant developmental differences in either TS or controls. For these blocks, we calculated the difference between the magnitude (as defined by Euclidean distance) of developmental differences in TS and the that of the developmental differences in controls.

Permutation testing was used to determine whether the difference in magnitude of developmental differences in the control and TS group are greater than expected by chance. Individuals in the control group and TS group were simultaneously, randomly permuted across age and diagnosis (N = 1000 times) and assigned into the “control child”,

“control adult”, “TS child”, or “TS adult” group. Developmental differences were then calculated and compared as described above. For each tested block, the true difference in the magnitude of developmental differences in TS and controls was contrasted with the distribution of permuted differences to generate a P-statistic. P-values were corrected for multiple comparisons across the total number of tested blocks, using the FDR approach (requiring that P(FDR) < 0.05).

We also conducted further analyses to test whether the observed effects on development in TS could be attributed to confounded characteristics of individuals with TS (e.g., co-morbid ADHD, tic severity, medications) (Supplement F).

4.3.6 Grouping Significantly Altered Development in TS

To aid visualization and interpretation, we grouped blocks with similarly altered developmental differences in TS using average functional connectivity. For example, suppose two tested blocks exhibited an attenuated magnitude of developmental differences in TS. Distance-based approaches alone cannot distinguish (1) the direction of the typical developmental differences (e.g., children > adults or children < adults) or (2) the direction of the atypical developmental differences in TS (e.g, accelerated in childhood TS, immature in adulthood TS, etc.). Using a data-driven modularity-based approach, we grouped blocks with similarly altered functional connectivity. For each tested block with significantly altered development in TS, we calculated the average functional connectivity across these connections for each subject. Then, we identified paired blocks in which the variability in average functional connectivity across individuals was similar (i.e., correlation between blocks > 0.3). Optimized modularity was then used to group linked

sets of similarly altered blocks (Supplement G). Further, post-hoc t-tests were used to determine whether the atypical development observed across blocks stems from TS-related differences in the children and/or the adults with TS.

4.4 Results

We found that many within-network and cross-network blocks were impacted by age and TS diagnosis to different extents (Figure 4-2; two-way ANOVA). Numerous within-network (on-diagonal in Figure 4-2) and cross-network (off-diagonal in Figure 4-2) blocks exhibited a main effect of age in both TS and controls (Figure 4-2A & D). Interestingly, select blocks, largely between control networks and the somatomotor networks, exhibited a main effect of TS in both children and adults (Figure 4-2B); however, these effects did not survive the correction for multiple comparisons (Figure 4-2E). Further, an interaction between age and diagnosis (Figure 4-2C) was seen in several blocks including within the default-mode network, between the dorsal attention and somatomotor networks, and between the basal ganglia and the visual network; some of these effects were significant after multiple comparisons correction (Figure 4-2F).

When we compared the functional connectivity among 300 regions spanning the whole brain between children and adults (i.e., developmental differences) with a distance-based approach, we also found many significant within-network and cross-network developmental differences in both the control group (Figure 4-3A) and the TS group (Figure 4-3B). Several within-network blocks (on-diagonal in Figure 4-3) differed between children and adults in only the control group (e.g. basal ganglia), only the TS group (e.g., cingulo-opercular), or in both (e.g., somatomotor (body)). In addition, numerous cross-

network blocks (off-diagonal in Figure 4-3) exhibited significant developmental differences in the control group (e.g. THAL-VIS), TS group (e.g., CO-DAN), or both (e.g., BG-SMbody). Critically, many of the developmental differences were observed in both groups; specifically, 4/10 within network blocks and 38/69 cross-network blocks shared significant developmental differences in TS and controls.

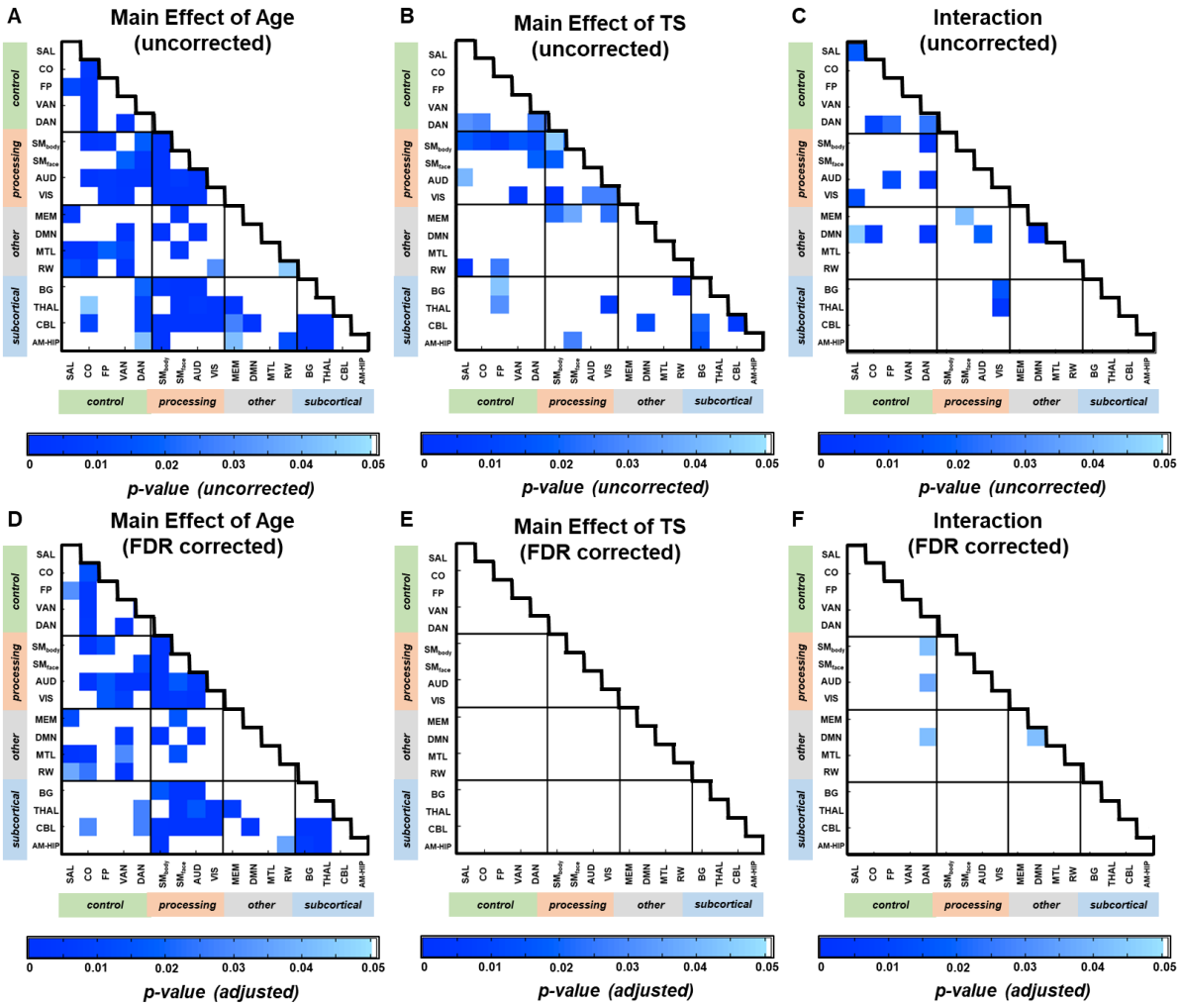

Figure 4-2. *ANOVA of the average functional connectivity from each block.* Within-network and cross-network blocks in which the average functional connectivity exhibits a main effect of age (A), diagnosis of TS (B), or an interaction between the two factors (C) before correcting for multiple comparisons. D-F depict the blocks with effects that survive after multiple comparisons correction.

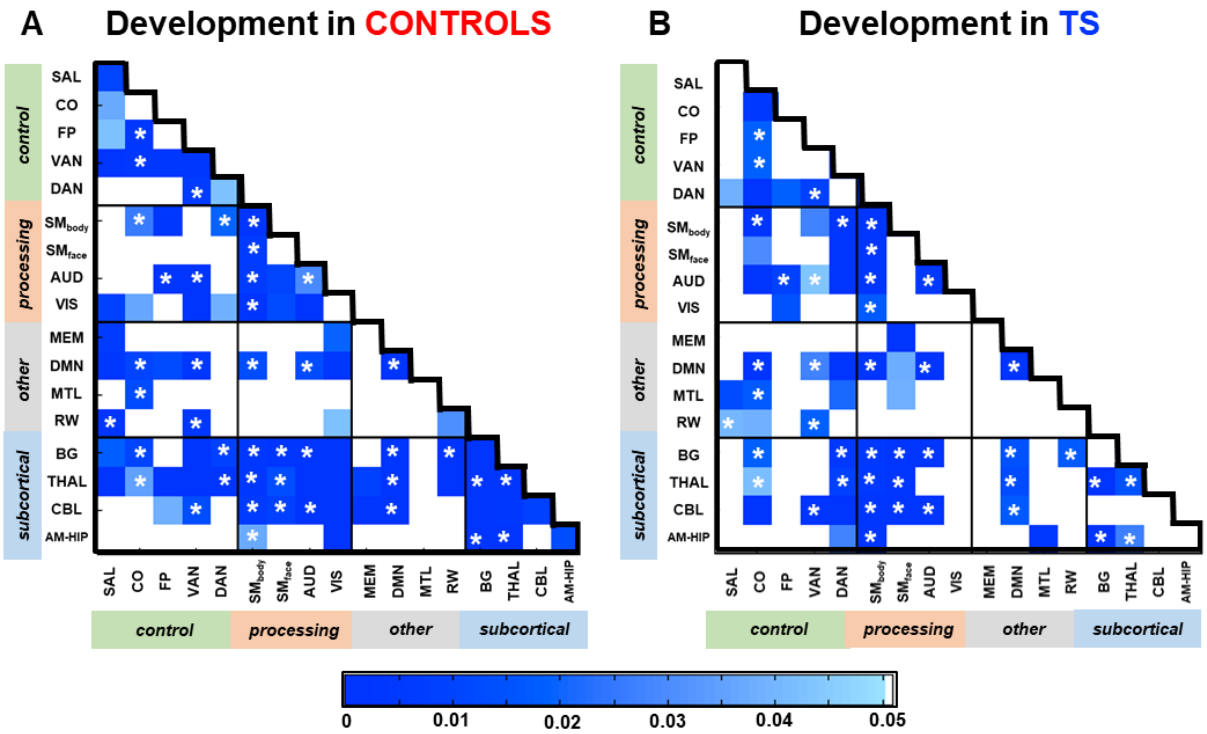

Figure 4-3. *Developmental differences for each block in TS and controls.* Within-network and cross-network blocks with significant developmental differences in the control group (A) and the TS group (B). The (*) indicates a developmental difference in both the control and TS groups.

We next compared the development of functional networks in the TS and control groups and found that the magnitude of developmental differences within specific blocks was altered in TS (Figure 4-S1). For several blocks (Figure 4-4A) and the block of connections within the cingulo-opercular network, the developmental differences observed in TS were greater than those observed in controls. Many (7/9) of these blocks with "divergent" development in TS did not exhibit significant developmental differences in the control group. In other cross-network blocks (Figure 4-4B) and the blocks of connections within the ventral attention network, basal ganglia, and amygdala/hippocampus, the developmental differences observed in TS were *smaller* compared to those observed in controls. Many (15/17) of these blocks with "attenuated"

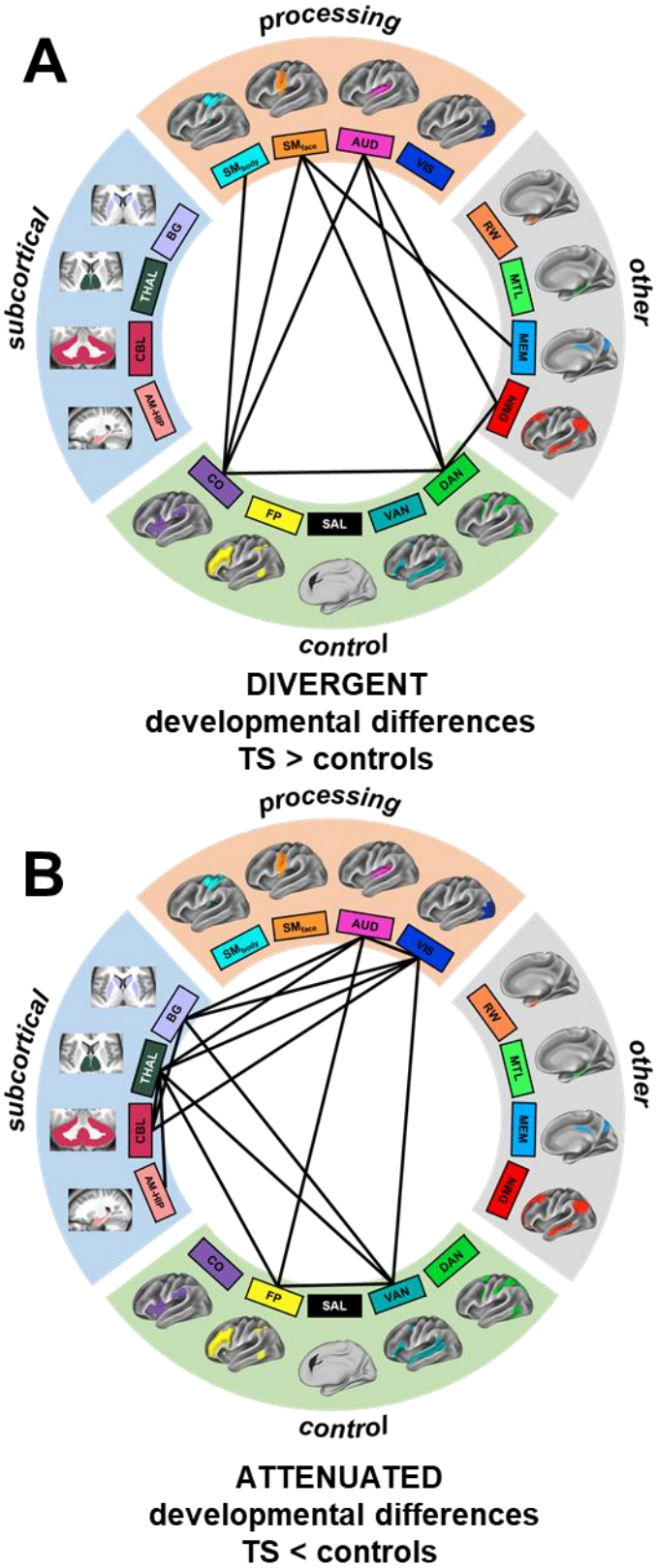

Figure 4-4. *Functional network interactions exhibiting altered developmental differences in TS.* Cross-network blocks with developmental differences that are (A) significantly greater in TS than in controls or (B) significantly greater in controls than in TS.

development in TS actually did not exhibit significant developmental differences in the TS group.

Next, we grouped blocks with average functional connectivity that was similarly altered across development in TS. Four types of similarly atypical development were identified (modularity of this grouping = 0.59) that were organized largely by type of alteration in TS (divergent vs. attenuated development) and by the direction of the developmental differences (children>adults or adults>children). The remaining blocks with idiosyncratically altered development \ are described separately (Supplement H).

One group involved connections among the cinguloopercular, dorsal attention, somatomotor (body), somatomotor (face), and auditory networks (Figure 4-5A). These blocks exhibited divergent increases in functional connectivity across development in TS. Many of these blocks did not exhibit significant developmental differences in the controls (except CO-SM_{body}). The connections among these control (cingulo-opercular and dorsal attention) and processing systems were stronger in TS adults than in TS children (Figure 4-5B-C) and the average strength of many of these blocks was significantly greater in TS adults than in control adults (Table 4-2).

A second group involved connections between the default mode network and the dorsal attention and auditory networks (Figure 4-5D). These blocks exhibited divergent decreases in functional connectivity across development in TS. These blocks did not exhibit significant developmental differences in the controls, but were more strongly negative in the TS adults compared to the TS children (Figure 4-5E). These connections were significantly more negative in adulthood TS than in controls (Table 4-2).

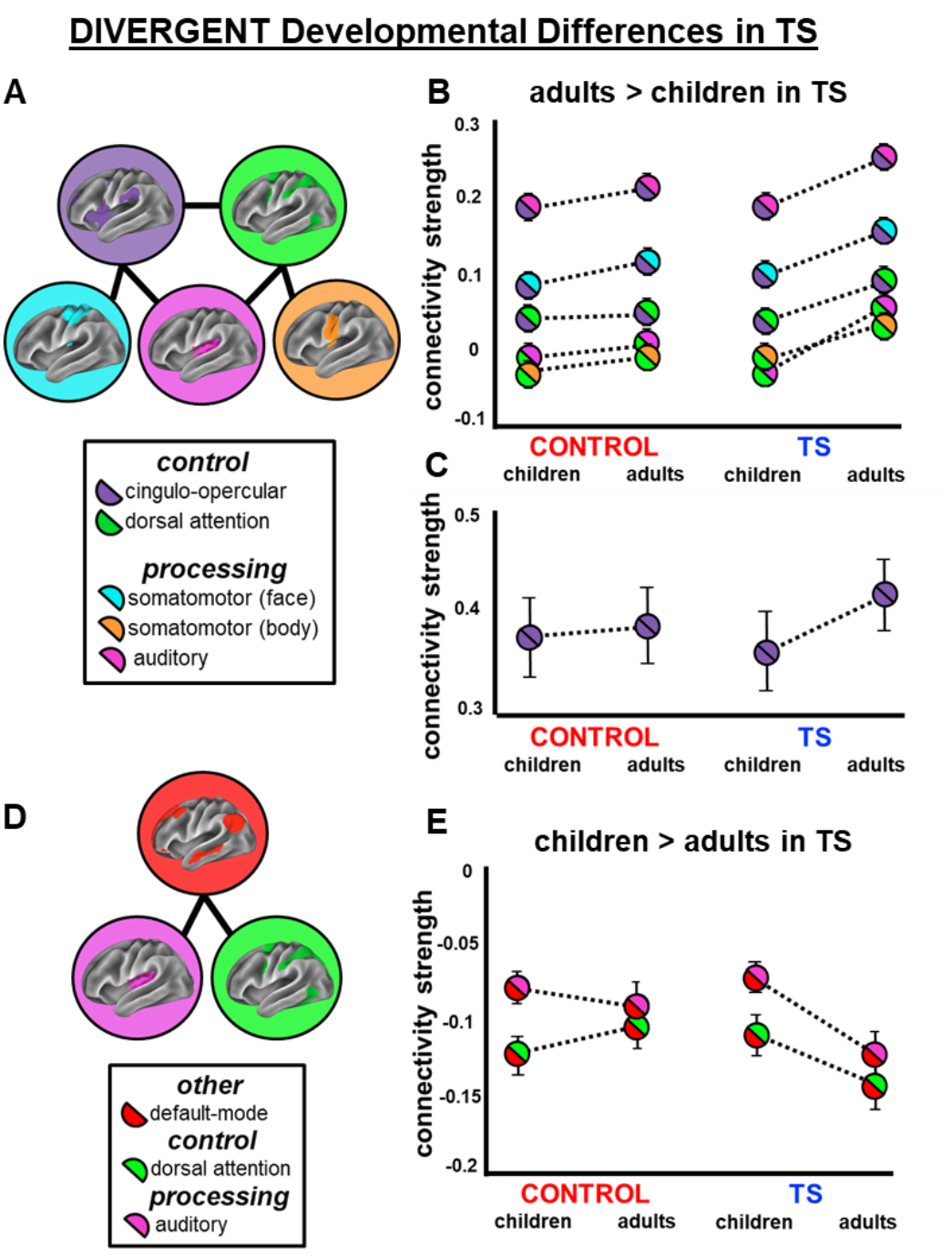

Figure 4-5. *Functional network interactions exhibiting divergent developmental differences in TS.* (A) Cluster of network interactions with similarly altered development in TS. Average functional connectivity of these cross-network (B) and within-network (C) blocks from (A) in the control children, control adults, TS children, and TS adults. (D) Cluster of network interactions with similarly altered development in TS. Average functional connectivity of these cross-network blocks (E) from (D) in the control children, control adults, TS children, and TS adults. Error bars are the stand error of the mean.

Table 4-2. Comparison of TS vs. controls in children and adults

	Child TS vs. Controls FDR corrected p-values	**Adult TS vs. Controls** FDR corrected p-values
Cluster #1		
CO – CO	0.4256	0.0571
CO - DAN	0.7371	0.0034*
CO - SM_{body}	0.3342	0.0161*
DAN – SM_{face}	0.4002	0.0166*
CO – AUD	0.9232	0.0273*
DAN – AUD	0.1137	0.0006*
Cluster #2		
DMN – DAN	0.2196	0.0007*
DMN – AUD	0.5840	0.0053*
Cluster #3		
VAN – VIS	0.2273	0.0043*
AUD – VIS	0.7994	0.0083*
Cluster #4		
BG – AUD	0.9138	0.0319*
BG – VIS	0.1323	0.0597
THAL – AUD	0.8639	0.2421
THAL – VIS	0.8471	0.0005*
THAL – RW	0.3963	0.9021
VIS – CBL	0.8324	0.1451
VAN – BG	0.1287	0.2565
VAN – THAL	0.1250	0.4687
Remaining		
THAL – CBL	0.7607	0.1133
THAL – AM/HIP	0.9007	0.5477
AM/HIP-AM/HIP	0.7190	0.0628*
SMface – MEM	0.9708	0.0041*
VAN – VAN	0.2035	0.3706
SAL – DMN	0.4746	0.0396*
BG – BG	0.6652	0.0872
FP – THAL	0.2244	0.0680

The third group involved connections between the visual network, the ventral attention, and the auditory network (Figure 4-6A). These blocks exhibited attenuated increases in functional connectivity across development in TS. All of these blocks exhibited significant developmental differences in controls, but not in TS. Both blocks

were more strongly positive in adults compared to children in typical development, but not in TS (Figure 4-6B). The strength of connections between the visual network and the ventral attention and auditory networks was significantly weaker in the TS adults than in the control adults (Table 4-2).

The fourth group involved connections between regions of the basal ganglia, thalamus, and cerebellum and several processing (auditory, visual), control (ventral attention), and other association (reward) networks (Figure 4-6C). These blocks exhibited attenuated decreases in functional connectivity across development in TS. Specifically, there were significant developmental differences in the controls, with stronger functional connectivity in the control children than in the control adults, yet the magnitude of these developmental differences was attenuated in TS. For the connections between the subcortical and processing functional networks (Figure 4-6D), the attenuation was driven by adulthood TS. The strength of the connections in these blocks was significantly less negative in TS adults than in control adults (Table 4-2). For the connections between the subcortical and ventral attention network (Figure 4-6E), the attenuation was driven by initial negative connectivity in childhood TS. While not significant (Table 4-2, VAN-BG: $p = 0.13$, VAN-THAL: $p = 0.12$), the average strength of the connections in these blocks was more negative in TS children than in the control children.

Lastly, post-hoc analyses suggested that neither comorbid ADHD, tic severity, or the use of medications contributed to the observed altered development in TS (Supplemental Table 4-S2). Most differences between individuals with TS and controls were present even when individuals with ADHD, high tic severity, and current medications were removed.

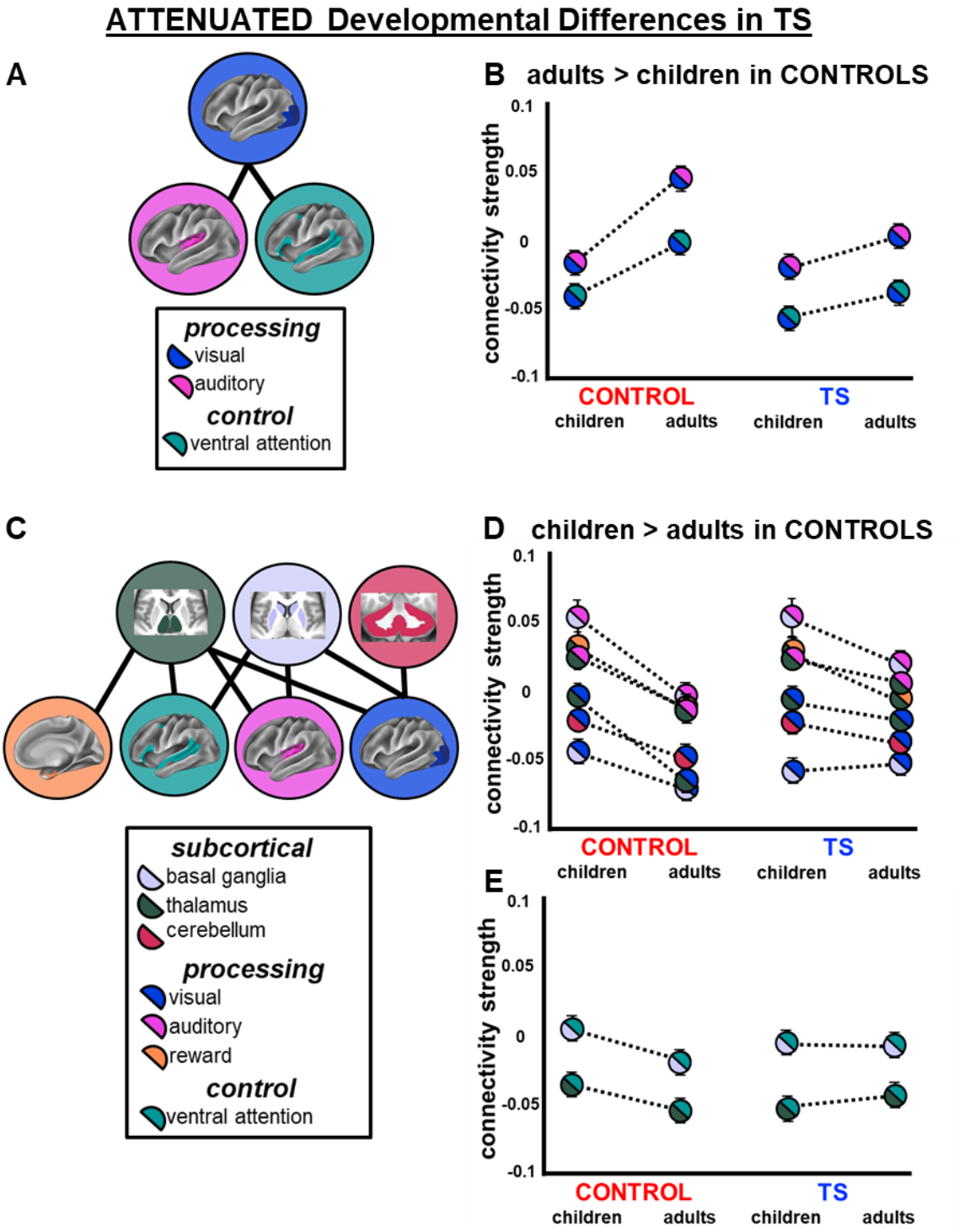

Figure 4-6. *Functional network interactions exhibiting attenuated developmental differences in TS.* (A) Cluster of network interactions with similarly altered development in TS. Average functional connectivity of these cross-network (B) blocks from (A) in the control children, control adults, TS children, and TS adults. (C) Cluster of network interactions with similarly altered development in TS. Average functional connectivity of these cross-network blocks (D & E) from (C) in the control children, control adults, TS children, and TS adults. Error bars are the stand error of the mean.

4.5 Discussion

In the present work, we applied a whole-brain network-level approach to functional connectivity MRI data in order to interrogate differences in functional brain organization in TS in two age groups: late childhood and early adulthood. We found that the organization of most functional networks in the TS groups was similar to that seen in typical development. While there was some suggestive evidence for consistent functional network differences across both age groups in TS, most functional networks that were significantly affected in TS differed between children and adults. Several within-network and cross-network blocks exhibited either divergent or attenuated development in TS. Development of connections involving the somatomotor, cingulo-opercular, auditory, dorsal attention, and default mode networks diverged from typical development, with stronger functional connectivity in adulthood TS than in typically developing individuals and children with TS. Alternatively, typical developmental differences observed in connections involving the basal ganglia, thalamus, cerebellum, auditory, visual, reward, and ventral attention networks were all reduced in TS. By combining the present investigation of functional relationships across the brain in TS with prior studies of brain function in TS and extant knowledge about the role of different functional systems in healthy controls we propose a novel model of the development of atypical brain function, connectivity, and concomitant cognitive resources associated with TS.

A whole-brain approach that places atypical brain function in TS in a context of functional networks can provide a novel and more comprehensive understanding of the neurobiology underlying TS. Figure 4-7 depicts regions that have been previously found to be activated in patients with TS during the period preceding tics (premonitory urge),

during tic action, and during instructed eye-blink suppression (Bohlhalter *et al.*, 2006; Mazzone *et al.*, 2010) and their overlap with functional networks defined in healthy control adults (Power *et al.*, 2011). In the discussion below, we outline how the functional networks that exhibit atypical development in TS might be associated with the complexity and course of tic symptoms.

In alignment with prominent theories of the production of tics, we found that the development of connections involving somatomotor networks and the basal ganglia and thalamus was altered in TS. Mink (2001) proposed that there is aberrant activity in cortico-striato-thalamo-cortical loops that leads to the disinhibition of unwanted motor plans and the production of tics. Consistent with this model, microstimulation and bicuculline injections of the basal ganglia, specifically the putamen, in rhesus monkeys yields tic-like movements movements (Alexander and DeLong, 1985; McCairn et al., 2009). Regions in sensorimotor cortex, the basal ganglia, thalamus, and cerebellum are consistently activated at the time of tic action (Bohlhalter et al., 2006; Wang et al., 2011; Neuner et al., 2014, Figure 4-7B) and transcranial magnetic stimulation in humans reveals hyperexcitability in motor cortex in TS (Ziemann et al., 1997). Additionally, smaller caudate and putamen volumes, as well as thinning in sensorimotor cortex, have been linked to more severe tics (Peterson et al., 1993; Bloch et al., 2005). Interestingly, our

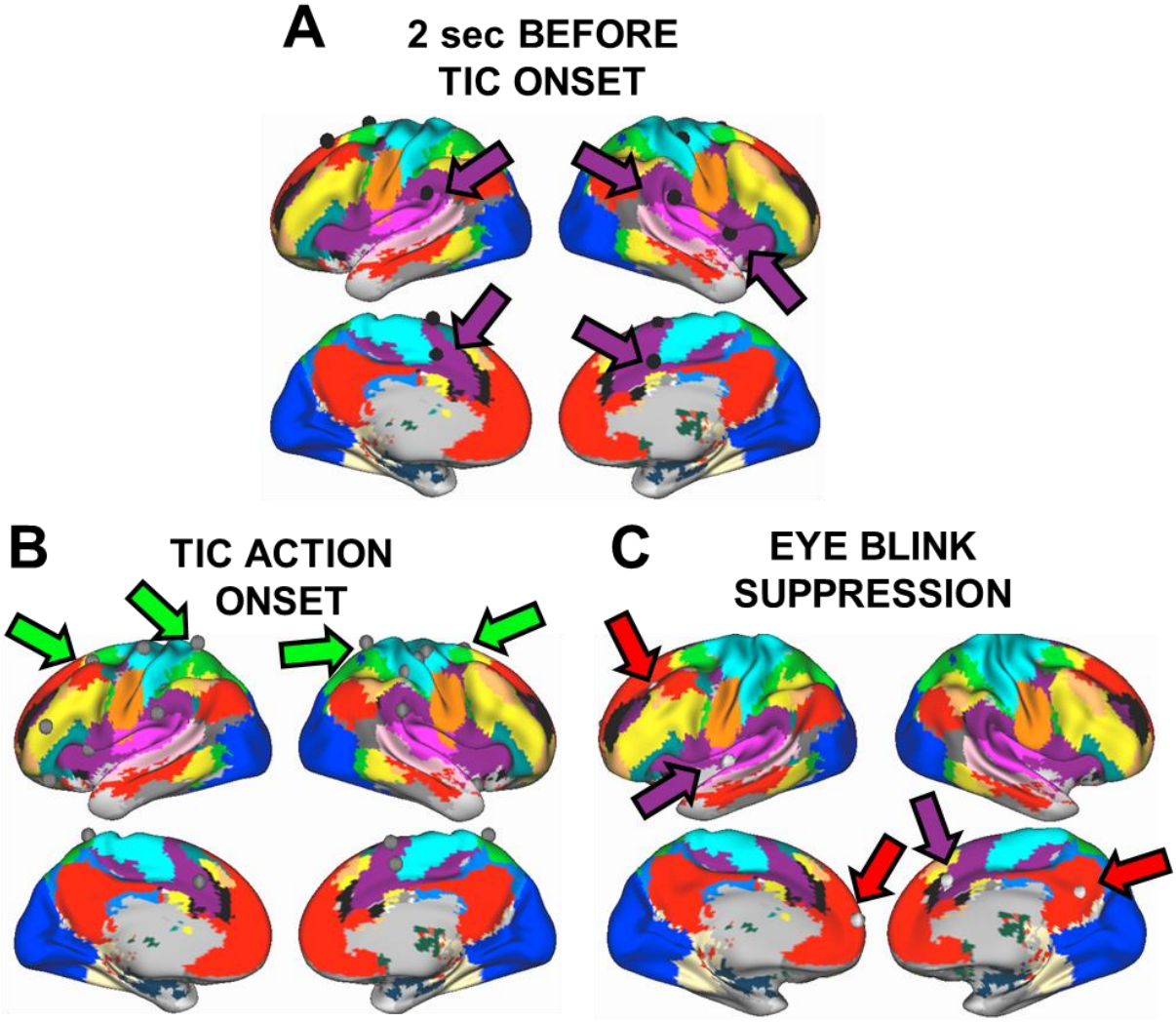

Figure 4-7. *Overlap of atypical brain function in TS and the functional network organization of the brain*. The functional networks depicted were defined in a set of 120 healthy control adults (Power et al. 2011). Spheres represent the peak activity related to the premonitory urge (A) and tic action (B) in adults with TS from Bohlhalter et al. 2006 and TS-specific activity during eye blink suppression (C) in children and adults with TS from Mazzone et al. 2010.

results suggest that these cortical and subcortical regions shown to be involved in the production of tics are altered in different ways across development in TS. Development of select blocks of connections involving the somatomotor networks diverged from typical development producing stronger associations with cortical control networks in adulthood

TS. In contrast, development of connections with the basal ganglia, thalamus, and cerebellum was attenuated in comparison to the development in healthy controls producing immature associations in adulthood TS. This distinction suggests that the development trajectories of the cortical and subcortical components of cortico-striato-thalamo-cortical circuitry differ and that the developmental course of tic symptoms is supported by a combination of developmental mechanisms.

We also found that the development of connections involving the cingulo-opercular network diverged such that these connections were stronger in adulthood TS which suggests that the neural substrates implicated in motor control and tic suppression differ in childhood and adulthood TS. In healthy controls, the cingulo-opercular network has been shown to be important for executive control, producing sustained signals that maintain goal-directed behaviors, detecting errors, conflict, and ambiguity (Dosenbach *et al.*, 2007; Neta *et al.*, 2014). In TS, regions in the cinguloopercular network are consistently activated during the time preceding a tic (Bohlhalter *et al.*, 2006, Figure 4-7). This time window is thought to reflect the premonitory urge, in which individuals with TS often describe a sensation of discomfort that can be relieved by performance of the tic. Activation of the cingulo-opercular network during the premonitory urge might reflect a time-limited attempt to suppress the urge to tic or the detection of an errant motor plan. Specifically, we found stronger functional connectivity between the cingulo-opercular network and somatomotor network in adulthood TS, which may indicate more common co-activation of these functional networks. Premonitory urges are more commonly reported in adulthood TS than in childhood TS (Leckman *et al.*, 1993) which may account for this developmental difference. Alternatively, the stronger link between the cingulo-

opercular network and sensorimotor network may be indicative of extended experience perceiving and suppressing the premonitory urges associated with tics. This enhanced functional connectivity that is present in adulthood, but not childhood, TS might be a maladaptive, neutral, or compensatory consequence of living with tics.

As the development of connections involving the dorsal attention network also diverged in TS, the developmental course of tic symptoms in TS might involve changes to directed attention. The dorsal attention network is important for directing attentional resources (Corbetta and Shulman, 2002). Individuals with parietal lesions in the dorsal attention network have difficulties attending to external (Heilman and Valenstein, 1979) and internal (Bisiach and Luzzatti, 1978) stimuli in specific spatial locations. Interestingly, regions within the dorsal attention appear to be activated at the time of tic action (Bohlhalter et al. 2006, Figure 4-7). Activation of the dorsal attention network during tics might suggest that the production of tics engages attentional resources. In line with this explanation, engaging attention elsewhere often reduces the production of tics (O'Connor *et al.*, 2003; Eapen *et al.*, 2004; Conelea and Woods, 2008b). Stronger correlations between the dorsal attention network and the somatomotor and cingulo-opercular networks indicate that tics and premonitory urges may more strongly engage attention in adulthood TS.

Further, connections between the dorsal attention and default mode network were also altered in adulthood TS, indicating that coordination of networks associated with directed attention and internal processing differs between childhood and adulthood TS. Regions in the default mode are atypically activated in both children and adults with TS when instructed to suppress eye blinks (Mazzone et al. 2010, Figure 4-7). As the default

mode network appears important for internal processing (e.g., thinking about oneself (Kelley *et al.*, 2002), retrieving autobiographical memories (Kim *et al.*, 2010), monitoring social aspects of self (Schilbach *et al.*, 2008)), a more negative relationship with the dorsal attention network might suggest that how attentional resources are directed towards internal stimuli differs over the course of development in TS.

In contrast to the functional networks linked to tic suppression and directed attention, the development of connections involving the visual, auditory, and ventral attention networks was attenuated in TS in comparison to development in healthy controls. Typically, visual and auditory networks process sensory inputs while the ventral attention network is involved with reorienting attention in response to external stimuli (Corbetta and Shulman, 2002; Fox *et al.*, 2006). In TS, tics can often be associated with environmental triggers (e.g., school vs. home setting). Further, tic frequency can increase under stress, different emotional contexts, or different social situations and decrease when attention is allocated elsewhere (Conelea and Woods, 2008a). Therefore, the functional networks responsible for processing and orienting to these external triggers might play an important role in the initiation of tics. We found that, typically, the connections among the visual, auditory, and ventral attention networks become stronger across development, but, in TS, this development is attenuated. Weaker relationships between these functional networks in adulthood TS might be an indicator of immature and imbalanced sensory processing and externally driven attention that facilitates the initiation of tics. In contrast, connections between the visual, auditory, and ventral attention networks and the basal ganglia, thalamus, and cerebellum typically decrease over the course of development, but this development was attenuated in TS. Connections

between the sensory networks and the subcortex and cerebellum remained atypically stronger in adulthood TS, perhaps indicating immature segregation of these inputs to cortico-striatal-thalamo-cortical loops.

Considered together, our results inform a model of how several cortical and subcortical functional networks involved in the initiation, production, and/or suppression of tics interact and differ across development in TS (Figure 4-8). We found that functional networks related to the premonitory urge (CO), directing attentional resources (DAN), internal processing (DMN), and the production of tics (SM) exhibited divergent development in adulthood TS. Adulthood TS can be characterized by strongly linked systems responsible for suppressing, producing, and attending to tics beyond what is observed in typical development. In contrast, the development of functional networks related to processing environmental triggers (AUD/VIS), orienting to external stimuli (VAN), and the production of tics (BG/THAL) was attenuated. Typical development of the systems facilitating the initiation and production of tics is disrupted in TS.

A likely important, but missing, component in this model is the differences in functional networks that are consistent across childhood and adulthood TS. In our sample, we were unable to identify significant TS-related differences in functional connectivity present in both children and adults with either an average- or distance-based approach. While not significant after correcting for multiple comparisons, intriguingly, connections involving the somatomotor network were most consistently affected in children and adults with TS (Figure 4-2B). Since TS-related differences were smaller in childhood TS, it is possible that our sample of children with TS is heterogeneous

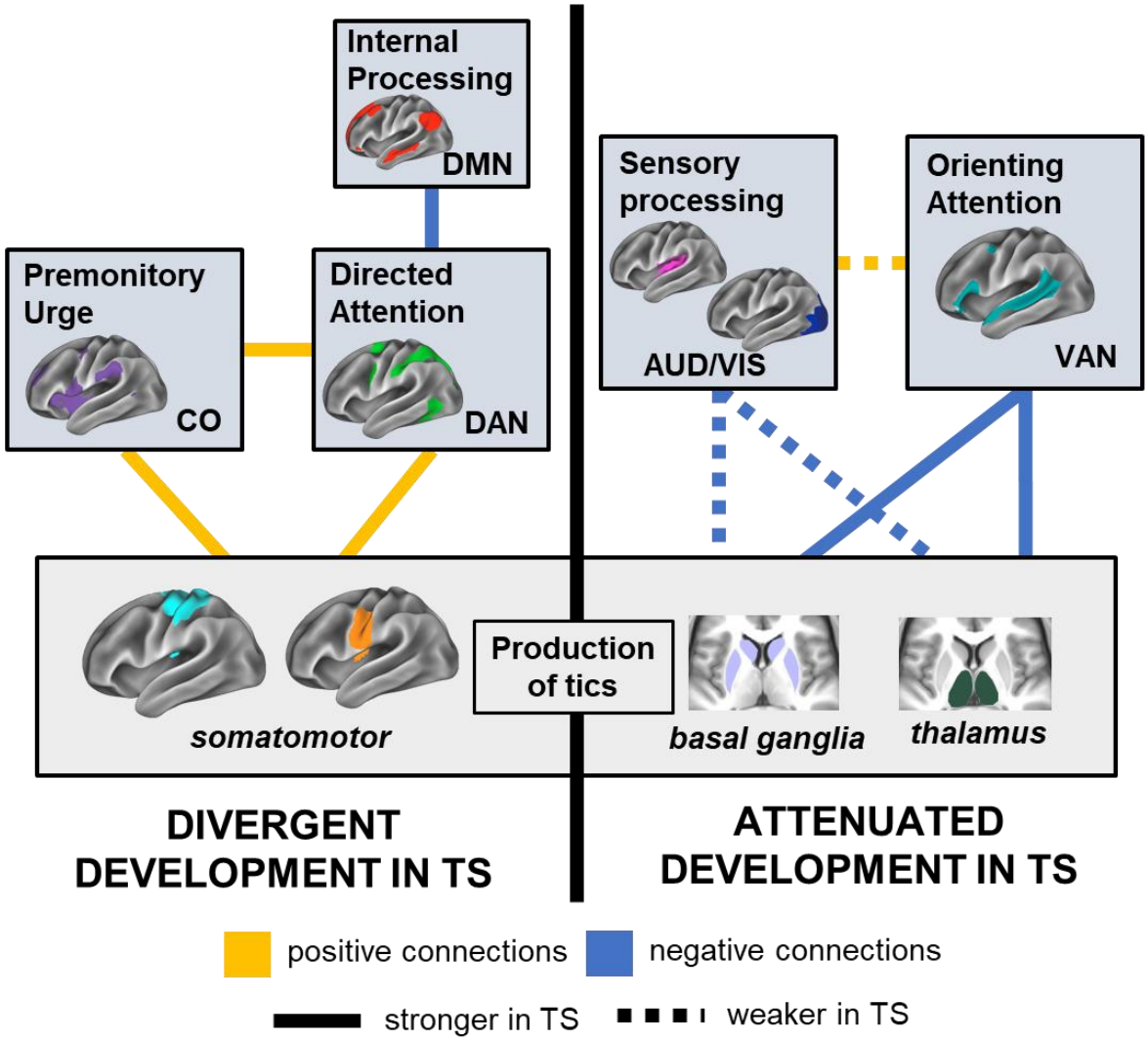

Figure 4-8. *A developmental model of functional networks associated with TS.*

preventing the identification of consistent TS-related differences in functional networks. Alternatively, TS-related differences in functional networks may not be represented at the network-level and may require patterns across many functional networks.

In addition, the present investigation and the proposed model may oversimplify the functional organization of the basal ganglia, thalamus, and cerebellum. Cortico-striato-thalamo-cortical loops between different pieces of cortex and subcortex appear devoted

to different functions (e.g, motor, control) (Haber, 2003). These associations can be illuminated with resting-state functional connectivity such that different pieces of the subcortex and the cerebellum exhibit stronger functional connectivity with specific functional networks (Choi *et al.*, 2012; Greene *et al.*, 2014; Marek *et al.*, 2018). While this work identified connections between the subcortex and specific cortical functional networks (e.g., visual, auditory, ventral attention) with atypical development in TS, it is possible that connections between specific pieces of the subcortex and cortical functional networks develop differently in TS. Investigation of the functional organization of the basal ganglia, thalamus, and cerebellum at a finer scale may provide a more complete model of the neural substrates underlying the developmental course of TS.

While the present work has illuminated specific blocks of functional networks affected in TS and how these networks differ between children and adults, the source of these developmental differences is still undetermined. Some argue that childhood TS and adulthood TS are fundamentally different, given the commonly held belief that most patients with TS experience substantial symptom improvement or remission into adulthood (Eichele and Plessen, 2013). Therefore, by studying a sample of adults with current tics, we may have captured the subsample who do not experience remission. By contrast, any sample of children with TS will include a mixture of individuals whose tic symptoms will go on to improve and those whose tics will persist. However, there is evidence that remission is likely much rarer than previously estimated (10%, rather than 40%; %; Pappert *et al.*, 2003b), and in our sample, many of the adults with TS reported improvement from childhood even if they did not report remission. Longitudinal data and

studies of adults with remitted tics are necessary to determine to what extent the altered brain function in adulthood TS is a cause or consequence of prolonged symptom burden.

Further, while this work identified specific blocks that were altered in TS, the source of these disorder-related differences remains difficult to disentangle. As an example, we identified atypically stronger functional connectivity between the cingulo-opercular network and the somatomotor network. These strengthened connections might be a change in the brain that facilitates tics (e.g., atypically coordinated inhibitory control of motor function). Alternatively, the strengthened connections between the cingulo-opercular and motor networks might be a consequence of having tics; experience with tics might produce maladaptive, neutral, or compensatory changes in the brain. Finally, the enhanced connections between the cingulo-opercular and somatomotor networks might reflect state differences between groups; if the TS group was unconsciously suppressing tics while in the scanner, these amplified connections might be attributable to this behavior rather than the underlying neurophysiology in TS. Further investigation of the networks in TS with longitudinal developmental designs, links to experience with tics, and comparisons to studies of tic suppression is needed to shed light on the potential contribution of these various sources of disorder-related differences in functional connectivity.

It is important to note that our TS sample was highly representative of the TS population in that it was heterogeneous with respect to comorbid neuropsychiatric disorders, tic severity, and medication status (Freeman *et al.*, 2000; Greene *et al.*, 2016a). As brain network function can be affected by medications (69) and other neuropsychiatric conditions (Mueller *et al.*, n.d.), differences in functional connectivity observed in

childhood and/or adulthood TS might have included medication-induced or comorbidity-related differences in brain function. Additionally, our child and adult samples differed with respect to sex; the children included more boys than girls, while the adults were more balanced. This difference reflects epidemiological data, as the sex imbalance (4:1 male:female) reported in childhood TS is attenuated in adulthood TS (Lichter and Finnegan, 2015). Nevertheless, the developmental differences in functional connectivity observed in the TS and control groups might have included sex-related differences in brain function. Future studies with larger samples will be useful for directly parsing the influence of medications, comorbidities, and sex on brain function in TS.

4.6 Acknowledgments

We thank Rebecca Coalson, Rebecca Lepore, Kelly McVey, Jonathan Koller, Annie Nguyen, Catherine Hoyt, Lindsey McIntyre, and Emily Bihun for assistance with data collection, the children and adults who participated in this study and their families, and Deanna Barch for her input on this manuscript. This project was supported by: Tourette Association of America fellowships (DJG, JAC), Tourette Association of America Neuroimaging Consortium pilot grant (KJB, BLS), Tourette Association of America research grant (DJG), NARSAD Young Investigator Award (DJG), NIH K01MH104592 (DJG), NIH R21MH091512 (BLS), NIH R01HD057076 (BLS), NIH R01NS046424 (SEP), NIH R21 NS091635 (BLS, KJB), NIH R01MH104030 (KJB, BLS), K12HD076224 (NUFD, Scholar of the Child Health Research Center at Washington University), NIH K23NS088590 (NUFD), NIH F32NS065649 (JAC), NIH F32NS092290 (CG), NIH F32NS656492, NIH K23DC006638, P50 MH071616, P60 DK020579-31 American Hearing Research Foundation, and The Simons Foundation Autism Research Initiative

("Brain circuitry in Simplex Autism," SEP). Research reported in the publication was supported by the Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health under Award Number U54 HD087011 to the Intellectual and Developmental Disabilities Research Center at Washington University. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

4.7 Supplemental Material

A. Participants

A total of 78 children and adults with Tourette syndrome (TS) and 78 healthy control children and adults were included in the present study. All participants were native English speakers. All participants underwent a 2-scale brief assessment of IQ (WASI). For TS participants, the experimenter completed the following measures of "past week" symptom severity: Yale Global Tic Severity Score (Total Tic Score) (Leckman *et al.*, 1989), Children's Yale-Brown Obsessive Compulsive Scale (Scahill *et al.*, 1997), and ADHD Rating Scale (Conners *et al.*, 1998). All participants self- or parent-reported any history of neuropsychiatric diagnoses and current medications (Table 4-S1). For the control participants, any history of neuropsychiatric or neurological diagnoses prohibited participation in the study.

Table 4-S1. Comorbid diagnoses and current medications in participants with TS.

	Children with TS *N* = 39; (7.4 – 13.1 years)	**Adults with TS** *N* = 39; (18.0-35 years)
Comorbid Diagnosis		
ADHD/ADD	18	11

OCD	9	13
Anxiety Disorders	5	9
Depression	1	9
ODD	0	0
Migraines	1	6
Medications		
Centrally acting adrenergic agents	13	3
Stimulants	10	7
Anti-depressants	2	6
Anti-anxiety	1	2
Antipsychotics	0	1

B. Imaging Acquisition

Data were acquired on a Siemens 3T Trio scanner (Erlanger, Germany) with a Siemens 12-channel Head Matrix Coil. Each child was fitted with a thermoplastic mask fastened to the head coil to help stabilize head position. T1-weighted sagittal MP-RAGE structural images in the same anatomical plane as the BOLD images were obtained to improve alignment to an atlas (1 sequence acquisition for each of the 101 control participants (child, adolescent, and adult) and for 88 of the TS participants (child, adolescent, adult): slice time echo = 3.06 ms, TR = 2.4 s, inversion time = 1 s, flip angle = 8°, 176 slices, 1 × 1 × 1 mm voxels; 2 sequence acquisitions for each of the 13 remaining child and adolescent TS participants: slice time echo = 2.34 ms, TR = 2.2 s, inversion time = 1 s, flip angle = 7°, 160 slices, 1 × 1 × 1 mm voxels). Functional images were acquired using a BOLD contrast-sensitive echo-planar sequence (TE = 27 ms, flip angle = 90°, in-plane resolution 4x4 mm; volume TR = 2.5 s). Whole-brain coverage was obtained with 32

contiguous interleaved 4 mm axial slices. Steady-state magnetization was assumed after 4 volumes. For most participants, 2-4 resting state scans lasting 5-5.5 min each were acquired, but the duration of each scan ranged from 3.2 minutes to 30 minutes. In the TS group, 388 ± 61.5 (range 264-528) total functional volumes were acquired, and in the control group, 372 ± 130 (range 260-724) total functional volumes were acquired.

C. Imaging preprocessing

Functional images from each participant were preprocessed to reduce artifacts (Shulman et al. 2010). These steps included: (i) temporal sinc interpolation of all slices to the temporal midpoint of the first slice, accounting for differences in the acquisition time of each individual slice, (ii) correction for head movement within and across runs, and (iii) intensity normalization of the functional data was computed for each individual via the MP-RAGE T1-weighted scans. Each run was then resampled in atlas space on an isotropic 3 mm grid combining movement correction and atlas transformation in a single interpolation. The target atlas was created from thirteen 7-9 year old children and twelve 21-30 year old adults using validated methods (Black et al. 2004). The atlas was constructed to conform to the Talairach atlas space.

D. Functional Connectivity Preprocessing

Several additional pre-processing steps were applied to reduce spurious variance unlikely to reflect neuronal activity (Fox et al. 2009). These functional connectivity pre-processing steps included: (i) demeaning and detrending each run, (ii) multiple regression of nuisance variables, (iii) frame censoring (discussed below) and interpolation of data within each run, (iv) temporal band-pass filtering ($0.009\ Hz < f < 0.08\ Hz$), and (v) spatial

smoothing (6 mm full width at half maximum). Nuisance variables included motion regressors (e.g. original motion estimates, motion derivatives, and Volterra expansion of motion estimates), an average of the signal across the whole brain (global signal), individualized ventricular and white matter signals, and the derivatives of these signals.

We applied a procedure determined and validated to best reduce artifacts related to head motion (Power et al. 2014; Ciric et al. 2017). Specifically, frame-by-frame head displacement (FD) was calculated from preprocessing realignment estimates, and frames with FD > 0.2 mm were removed. An FD threshold of 0.2 mm was chosen because it best reduced the distance-dependence related to individual differences in head motion (mean FD) in this developmental dataset, as assessed using procedures from Power et al. (2012) and Ciric et al. (2017). Data were considered usable only in contiguous sets of at least 3 frames with FD < 0.2 and a minimum of 30 frames within a functional run. Motion-contaminated frames were censored from the continuous, processed resting-state time series before computing resting-state correlations. Notably, the global signal was included as a nuisance regressor (mentioned above) in order to further reduce global, motion-related spikes in BOLD data (Power et al. 2014; Ciric et al. 2017) and reduce patterns of spurious functional connectivity that might be utilized for prediction with machine learning (Nielsen et al. 2018).

E. Euclidean Distance Formula

Euclidean distance was used measure the difference in functional connectivity between children and adults and between TS and controls. This measure reduces the dimensionality of a set of connections in order to determine whether function connectivity

differed between groups. For a set of functional connections the difference between two groups, children and adults, was calculated using Formula S1.

Formula S1. Euclidean Distance Formula

$$\Delta FC = \sqrt{(FC_{child,1} - FC_{adult,1})^2 + (FC_{child,2} - FC_{adult,2})^2 \ldots + (FC_{child,n} - FC_{adult,n})^2}$$

FC: functional connection
N: number of connections
Child: average functional connectivity across child group
Adult: average functional connectivity across adult group

F. Effects of co-morbid ADHD, tic severity, medications on functional connectivity in TS

TS is a complex condition. Many of the patients with TS had comorbid diagnoses of the other neuropsychiatric disorders and/or were taking medications (see Table 4-S1). In addition, tic severity varied within the children and adults with TS. It is possible that the differences in functional connectivity observed in TS are indicative of these factors. We investigated whether these factors contributed to the results observed in the main text.

Patients with TS in our sample were most commonly also diagnosed with ADHD. We examined whether TS-related differences in functional connectivity were present in individuals with and without an additional diagnosis of ADHD in children and adults separately. Next, we split our TS sample into two groups with low tic severity and high tic severity according to YGTSS. We then examined whether TS-related differences in functional connectivity were present in individuals with less severe and more severe tic symptoms in children and adults separately. Finally, we examined whether TS-related differences in functional connectivity were present in individuals that were and were not using medications. Comparisons were limited to blocks with significantly altered development in TS and are reported in Table 4-S2.

Table 4-S2. TS-related differences in functional connectivity before and after removing individuals with ADHD, high tic severity, and current medications.

Children with TS vs. Controls	Total sample	w/ ADHD	w/o ADHD	High tic severity	Low tic severity	w/ current medications	w/o current medications
CO-CO	0.426	0.455	0.549	0.845	0.274	0.707	0.308
CO-DAN	0.737	0.385	0.991	0.431	0.879	0.434	0.820
CO-SMbody	0.334	0.335	0.315	0.410	0.253	0.068	0.915
DAN-SMface	0.400	0.054	0.169	0.131	0.075	0.016	0.309
CO-AUD	0.923	0.958	0.485	0.838	0.597	0.569	0.873
DAN-AUD	0.114	0.820	0.487	0.765	0.338	0.786	0.776
SMface-PM	0.971	0.970	0.981	0.553	0.511	0.734	0.781
DAN-DMN	0.220	0.132	0.149	0.314	**0.047***	0.177	0.109
AUD-DMN	0.584	0.847	0.850	0.854	0.539	0.593	0.862
VAN-VAN	0.204	0.594	0.130	0.564	0.109	0.265	0.346
VAN-VIS	0.227	0.474	0.293	0.319	0.443	0.649	0.188
AUD-VIS	0.799	0.629	0.462	0.858	0.605	0.993	0.807
SAL-DMN	0.475	0.278	0.439	0.448	0.251	0.247	0.492
VAN-BG	0.129	0.283	0.085	0.045	0.489	0.065	0.342
AUD-BG	0.914	0.059	0.647	0.112	0.563	0.102	0.616
VIS-BG	0.132	0.297	0.438	0.735	0.176	0.996	0.106
BG-BG	0.665	0.450	0.217	0.906	0.469	0.499	0.240
FP-THAL	0.224	0.207	**0.032***	0.220	**0.026***	0.039	0.176
VAN-THAL	0.125	**0.027***	0.445	**0.034***	0.381	0.152	0.108
AUD-THAL	0.864	**0.001***	0.821	**0.032***	0.592	0.099	0.273
VIS-THAL	0.847	0.736	0.304	0.812	0.231	0.832	0.077
RW-THAL	0.396	0.923	0.383	0.248	0.931	0.748	0.193
VIS-CBL	0.832	0.357	0.277	0.122	0.727	0.141	0.559
THAL-CBL	0.761	0.656	0.778	0.666	0.765	0.895	0.567
THAL-AMY-HIP	0.901	**0.001***	0.147	**0.008***	0.060	**0.039***	**0.013***
AMY/HIP-AMY/HIP	0.719	0.488	0.206	0.902	0.611	0.644	0.315

Adults with TS vs. Controls	Total sample	w/ ADHD	w/o ADHD	High tic severity	Low tic severity	w/ current medications	w/o current medications
CO-CO	0.057	0.276	0.063	0.068	0.208	**0.020***	0.466
CO-DAN	**0.003***	**0.021***	**0.001***	**0.000***	**0.026***	**0.002***	**0.005***
CO-SMbody	**0.016***	**0.044***	**0.015***	**0.008***	0.070	**0.007***	0.081
DAN-SMface	**0.017***	0.050	**0.009***	**0.006***	0.057	**0.026***	**0.015***
CO-AUD	**0.027***	0.083	**0.027***	0.189	**0.005***	0.051	**0.038***
DAN-AUD	**0.001***	0.067	**0.000***	**0.002***	**0.002***	**0.001***	**0.006***
SMface-PM	**0.004***	**0.017***	0.058	0.157	**0.007***	**0.031***	0.077
DAN-DMN	**0.001***	**0.023***	**0.004***	**0.000***	0.097	**0.000***	0.083
AUD-DMN	**0.005***	**0.018***	**0.006***	**0.023***	**0.003***	**0.014***	**0.007***
VAN-VAN	0.371	**0.023***	0.786	0.563	0.380	0.493	0.439
VAN-VIS	**0.004***	**0.045***	**0.024***	**0.003***	0.139	**0.008***	0.067
AUD-VIS	**0.008***	0.170	**0.040***	**0.001***	0.523	**0.030***	0.154
SAL-DMN	**0.040***	0.728	**0.047***	0.419	0.095	0.062	0.481
VAN-BG	0.257	0.079	0.878	0.370	0.537	0.072	0.997
AUD-BG	**0.032***	**0.009***	0.620	0.078	0.431	**0.019***	0.569
VIS-BG	0.060	0.375	**0.000***	**0.003***	**0.028***	**0.033***	**0.002***
BG-BG	0.087	0.151	0.167	**0.027***	0.423	**0.003***	0.918
FP-THAL	0.068	**0.042***	**0.044***	**0.009***	0.153	0.214	**0.009***

VAN-THAL	0.469	0.069	0.714	0.394	0.744	0.382	0.926
AUD-THAL	0.242	**0.028***	0.510	0.361	0.523	0.179	0.449
VIS-THAL	**0.001***	**0.033***	**0.000***	**0.000***	**0.001***	**0.010***	**0.000***
RW-THAL	0.902	0.748	0.848	0.770	0.782	0.355	0.345
VIS-CBL	0.145	0.303	0.170	**0.031***	0.759	0.068	0.459
THAL-CBL	0.113	0.146	0.274	0.187	0.369	**0.012***	0.973
THAL-AMY-HIP	0.548	0.244	**0.036***	0.190	**0.023***	0.132	0.060
AMY/HIP-AMY/HIP	0.063	**0.032***	0.180	0.176	0.080	**0.004***	0.647

G. Optimized Modularity to group blocks with similarly altered development in TS

Modularity optimization was used to group blocks with similarly altered development of functional connectivity in TS. First, we averaged the functional connectivity across each set of functional connections for each individual. Then we calculated how correlated this individual variability in average functional connectivity was between pairs of different blocks. We tested several thresholds (r = 0.1 – 0.5, increments of 0.05) to define "similarly altered" pairs of blocks. We calculated the modularity, a graph theoretical measure, of each network of similarly altered connections (Newman, 2006). Figure 4-S1 depicts the modularity across these thresholds. We chose r = 0.3 in order to maximize modularity and retain more blocks.

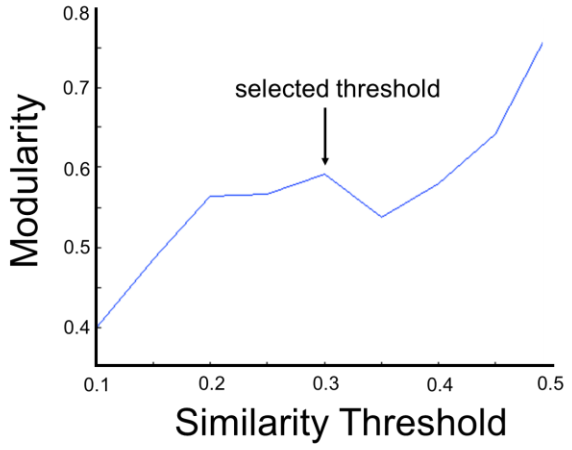

Figure 4-S1. *Modularity of the similarity between blocks across all individuals at different thresholds.*

H. Divergent and attenuated developmental differences in TS

In the main text, we found that within-network and cross-network blocks with divergent and attenuated developmental differences in TS. Figure 4-S2 depicts the FDR adjusted p-values that describe these effects.

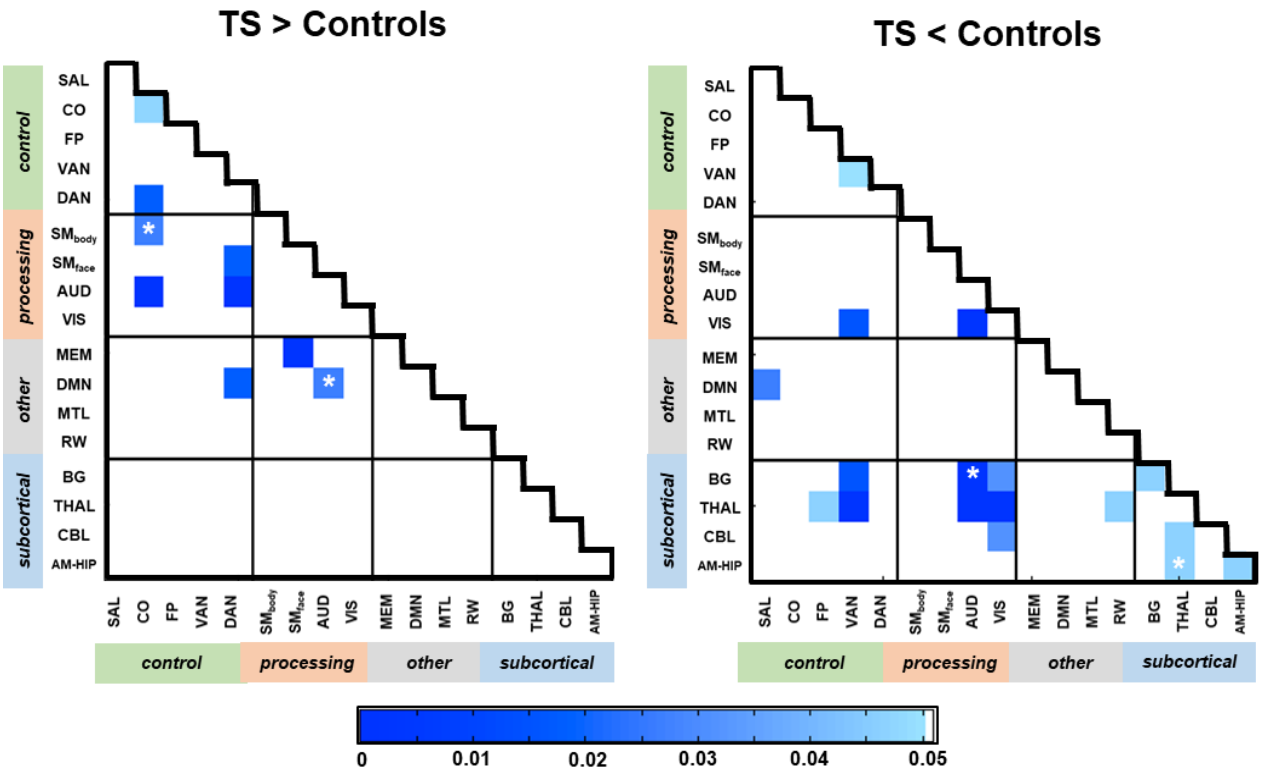

Figure 4-S2. *Blocks of within-network and cross-network functional connections in which the magnitude of developmental differences significantly differs in the control and TS groups.* The left panel depicts blocks with divergent developmental differences that are greater in TS than in controls. The right panel depicts blocks attenuated developmental differences that are smaller in TS than in controls. The (*) indicates a significant developmental difference in both the control and TS groups. P-values are FDR adjusted.

I. Remaining blocks with altered development in TS

Some blocks with significantly altered development in TS could not be grouped with other blocks. The average functional connectivity of these blocks across the children and adults with and without TS are shown in Figure 4-S3.

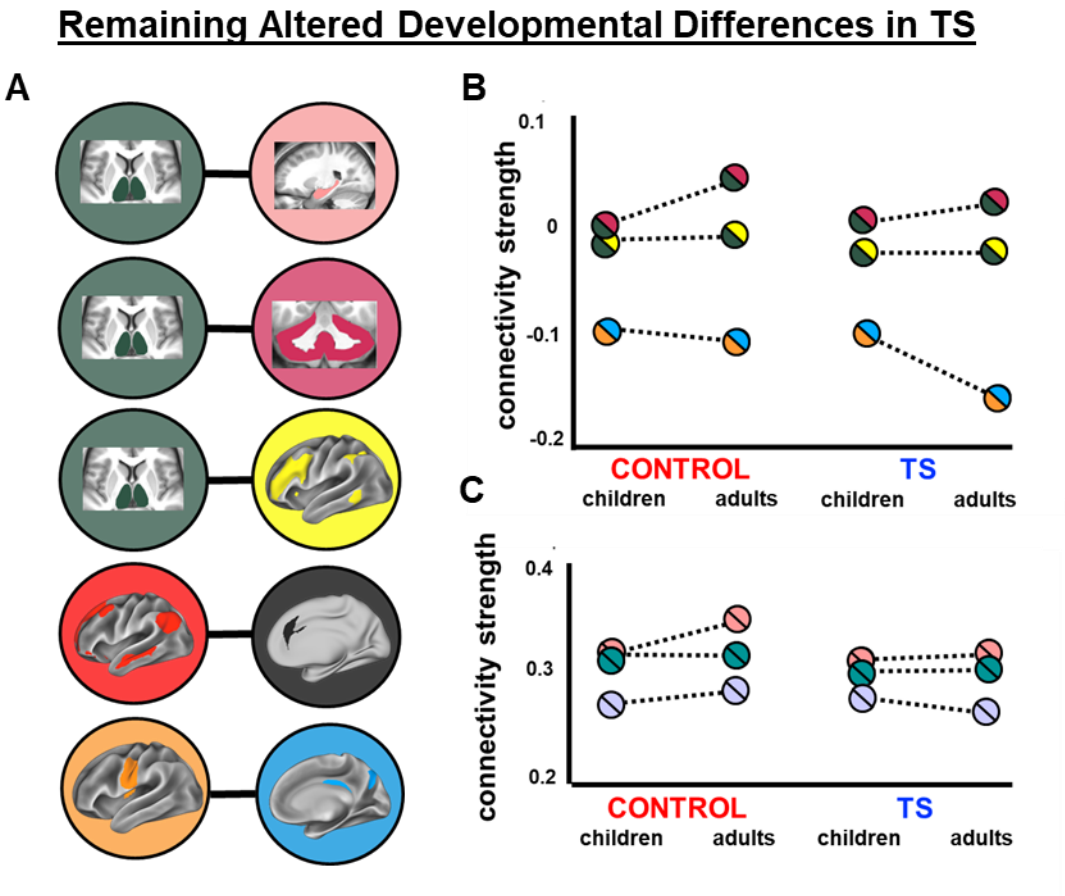

Figure 4-S3. *Blocks with altered developmental differences in TS that were not similar to other atypical development in TS.*

4.8 References

Alexander GE, DeLong MR. Microstimulation of the primate neostriatum. II. Somatotopic organization of striatal microexcitable zones and their relation to neuronal response properties. J. Neurophysiol. 1985; 53: 1417–1430.

Bisiach E, Luzzatti C. Unilateral neglect of representational space. Cortex 1978; 14: 129–133.

Biswal B, Yetkin FZ, Haughton VM, Hyde JS. Functional connectivity in the motor cortex of resting human brain using echo-planar mri. Magn. Reson. Med. 1995; 34: 537–541.

Bloch MH, Leckman JF, Zhu H, Peterson BS. Caudate volumes in childhood predict symptom severity in adults with Tourette syndrome. Neurology 2005; 65: 1253–1258.

Bloch MH, Peterson BS, Scahill L, Otka J, Katsovich L, Zhang H, et al. Adulthood outcome of tic and obsessive-compulsive symptom severity in children with Tourette syndrome. Arch. Pediatr. Adolesc. Med. 2006; 160: 65–69.

Bohlhalter S, Goldfine A, Matteson S, Garraux G, Hanakawa T, Kansaku K, et al. Neural correlates of tic generation in Tourette syndrome: an event-related functional MRI study. Brain 2006; 129: 2029–2037.

Choi EY, Yeo BTT, Buckner RL. The organization of the human striatum estimated by intrinsic functional connectivity. J. Neurophysiol. 2012; 108: 2242–2263.

Church JA, Fair DA, Dosenbach NUF, Cohen AL, Miezin FM, Petersen SE, et al. Control networks in paediatric Tourette syndrome show immature and anomalous patterns of functional connectivity. Brain 2009; 132: 225–238.

Ciric R, Wolf DH, Power JD, Roalf DR, Baum GL, Ruparel K, et al. Benchmarking of participant-level confound regression strategies for the control of motion artifact in studies of functional connectivity. NeuroImage 2017; 154: 174–187.

Conelea CA, Woods DW. The influence of contextual factors on tic expression in Tourette's syndrome: A review. J. Psychosom. Res. 2008a; 65: 487–496.

Conelea CA, Woods DW. Examining the impact of distraction on tic suppression in children and adolescents with Tourette syndrome. Behav. Res. Ther. 2008b; 46: 1193–1200.

Conners CK, Sitarenios G, Parker JDA, Epstein JN. The Revised Conners' Parent Rating Scale (CPRS-R): Factor Structure, Reliability, and Criterion Validity. J. Abnorm. Child Psychol. 1998; 26: 257–268.

Corbetta M, Shulman GL. Control of goal-directed and stimulus-driven attention in the brain. Nat. Rev. Neurosci. 2002; 3: 201–215.

Cubo E, Galán JMTG y, Villaverde VA, Velasco SS, Benito VD, Macarrón JV, et al. Prevalence of Tics in Schoolchildren in Central Spain: A Population-Based Study. Pediatr. Neurol. 2011; 45: 100–108.

Dosenbach NUF, Fair DA, Miezin FM, Cohen AL, Wenger KK, Dosenbach RAT, et al. Distinct brain networks for adaptive and stable task control in humans. Proc. Natl. Acad. Sci. 2007; 104: 11073–11078.

Dosenbach NUF, Nardos B, Cohen AL, Fair DA, Power JD, Church JA, et al. Prediction of Individual Brain Maturity Using fMRI. Science 2010; 329: 1358–1361.

Eapen V, Fox-Hiley P, Banerjee S, Robertson M. Clinical features and associated psychopathology in a Tourette syndrome cohort. Acta Neurol. Scand. 2004; 109: 255–260.

Eichele H, Plessen KJ. Neural Plasticity in Functional and Anatomical MRI Studies of Children with Tourette Syndrome [Internet]. Behav. Neurol. 2013[cited 2018 Jun 29] Available from: https://www.hindawi.com/journals/bn/2013/376590/abs/

Erenberg G, Cruse RP, Rothner AD. The natural history of Tourette syndrome: a follow-up study. Ann. Neurol. 1987; 22: 383–385.

Fox MD, Corbetta M, Snyder AZ, Vincent JL, Raichle ME. Spontaneous neuronal activity distinguishes human dorsal and ventral attention systems. Proc. Natl. Acad. Sci. 2006; 103: 10046–10051.

Freeman RD, Fast DK, Burd L, Kerbeshian J, Robertson MM, Sandor P. An international perspective on Tourette syndrome: selected findings from 3500 individuals in 22 countries. Dev. Med. Child Neurol. 2000; 42: 436–447.

Greene DJ, Black KJ, Schlaggar BL. Considerations for MRI study design and implementation in pediatric and clinical populations. Dev. Cogn. Neurosci. 2016a; 18: 101–112.

Greene DJ, Church JA, Dosenbach NUF, Nielsen AN, Adeyemo B, Nardos B, et al. Multivariate pattern classification of pediatric Tourette syndrome using functional connectivity MRI. Dev. Sci. 2016b; 19: 581–598.

Greene DJ, Laumann TO, Dubis JW, Ihnen SK, Neta M, Power JD, et al. Developmental Changes in the Organization of Functional Connections between the Basal Ganglia and Cerebral Cortex. J. Neurosci. 2014; 34: 5842–5854.

Greene DJ, Williams Iii AC, Koller JM, Schlaggar BL, Black KJ. Brain structure in pediatric Tourette syndrome. Mol. Psychiatry 2017; 22: 972–980.

Haber SN. The primate basal ganglia: parallel and integrative networks. J. Chem. Neuroanat. 2003; 26: 317–330.

Hassan N, Cavanna AE. The prognosis of Tourette syndrome: implications for clinical practice. Funct. Neurol. 2012; 27: 23–27.

Heilman KM, Valenstein E. Mechanisms underlying hemispatial neglect. Ann. Neurol. Off. J. Am. Neurol. Assoc. Child Neurol. Soc. 1979; 5: 166–170.

Himle MB, Woods DW, Conelea CA, Bauer CC, Rice KA. Investigating the effects of tic suppression on premonitory urge ratings in children and adolescents with Tourette's syndrome. Behav. Res. Ther. 2007; 45: 2964–2976.

Kelley WM, Macrae CN, Wyland CL, Caglar S, Inati S, Heatherton TF. Finding the self? An event-related fMRI study. J. Cogn. Neurosci. 2002; 14: 785–794.

Khalifa N, Knorring A-L von. Prevalence of tic disorders and Tourette syndrome in a Swedish school population. Dev. Med. Child Neurol.; 45: 315–319.

Kim H, Daselaar SM, Cabeza R. Overlapping brain activity between episodic memory encoding and retrieval: Roles of the task-positive and task-negative networks. NeuroImage 2010; 49: 1045–1054.

Leckman JF, King RA, Bloch MH. Clinical features of Tourette syndrome and tic disorders. J. Obsessive-Compuls. Relat. Disord. 2014; 3: 372–379.

Leckman JF, Riddle MA, Hardin MT, Ort SI, Swartz KL, Stevenson J, et al. The Yale Global Tic Severity Scale: Initial Testing of a Clinician-Rated Scale of Tic Severity. J. Am. Acad. Child Adolesc. Psychiatry 1989; 28: 566–573.

Leckman JF, Walker DE, Cohen DJ. Premonitory urges in Tourette's syndrome. Am. J. Psychiatry 1993; 150: 98.

Leckman JF, Zhang H, Vitale A, Lahnin F, Lynch K, Bondi C, et al. Course of Tic Severity in Tourette Syndrome: The First Two Decades. Pediatrics 1998; 102: 14–19.

Lewis CM, Baldassarre A, Committeri G, Romani GL, Corbetta M. Learning sculpts the spontaneous activity of the resting human brain. Proc. Natl. Acad. Sci. 2009: pnas.0902455106.

Lichter DG, Finnegan SG. Influence of gender on Tourette syndrome beyond adolescence. Eur. Psychiatry 2015; 30: 334–340.

Marek S, Siegel JS, Gordon EM, Raut RV, Gratton C, Newbold DJ, et al. Spatial and Temporal Organization of the Individual Human Cerebellum. Neuron 2018; 100: 977-993.e7.

Mazzone L, Yu S, Blair C, Gunter BC, Wang Z, Marsh R, et al. An fMRI Study of Frontostriatal Circuits During the Inhibition of Eye Blinking in Persons With Tourette Syndrome. Am. J. Psychiatry 2010; 167: 341–349.

McCairn KW, Bronfeld M, Belelovsky K, Bar-Gad I. The neurophysiological correlates of motor tics following focal striatal disinhibition. Brain 2009; 132: 2125–2138.

Mink JW. Basal ganglia dysfunction in Tourette's syndrome: a new hypothesis. Pediatr. Neurol. 2001; 25: 190–198.

Mueller S, Costa A, Keeser D, Pogarell O, Berman A, Coates U, et al. The effects of methylphenidate on whole brain intrinsic functional connectivity. Hum. Brain Mapp.; 35: 5379–5388.

Neta M, Schlaggar BL, Petersen SE. Separable responses to error, ambiguity, and reaction time in cingulo-opercular task control regions. NeuroImage 2014; 99: 59–68.

Neuner I, Werner CJ, Arrubla J, Stöcker T, Ehlen C, Wegener HP, et al. Imaging the where and when of tic generation and resting state networks in adult Tourette patients [Internet]. Front. Hum. Neurosci. 2014; 8[cited 2019 Jan 19] Available from: https://www.frontiersin.org/articles/10.3389/fnhum.2014.00362/full

Newman MEJ. Modularity and community structure in networks. Proc. Natl. Acad. Sci. 2006; 103: 8577–8582.

Nielsen AN, Greene DJ, Gratton C, Dosenbach NUF, Petersen SE, Schlaggar BL. Evaluating the Prediction of Brain Maturity From Functional Connectivity After Motion Artifact Denoising [Internet]. Cereb. Cortex[cited 2018 Jun 28] Available from: https://academic.oup.com/cercor/advance-article/doi/10.1093/cercor/bhy117/5025421

O'Connor K, Brisebois H, Brault M, Robillard S, Loiselle J. Behavioral activity associated with onset in chronic tic and habit disorder. Behav. Res. Ther. 2003; 41: 241–249.

Pappert EJ, Goetz CG, Louis ED, Blasucci L, Leurgans S. Objective assessments of longitudinal outcome in Gilles de la Tourette's syndrome. Neurology 2003; 61: 936–940.

Pépés SE, Draper A, Jackson GM, Jackson SR. Effects of age on motor excitability measures from children and adolescents with Tourette syndrome. Dev. Cogn. Neurosci. 2016; 19: 78–86.

Peterson B, Riddle MA, Cohen DJ, Katz LD, Smith JC, Hardin MT, et al. Reduced basal ganglia volumes in Tourette's syndrome using three-dimensional reconstruction techniques from magnetic resonance images. Neurology 1993; 43: 941.

Peterson BS, Pine DS, Cohen P, Brook JS. Prospective, longitudinal study of tic, obsessive-compulsive, and attention-deficit/hyperactivity disorders in an epidemiological sample. J. Am. Acad. Child Adolesc. Psychiatry 2001a; 40: 685–695.

Peterson BS, Staib L, Scahill L, Zhang H, Anderson C, Leckman JF, et al. Regional Brain and Ventricular Volumes in Tourette Syndrome. Arch. Gen. Psychiatry 2001b; 58: 427–440.

Poldrack RA. Can cognitive processes be inferred from neuroimaging data? Trends Cogn. Sci. 2006; 10: 59–63.

Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. NeuroImage 2012a; 59: 2142–2154.

Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. NeuroImage 2012b; 59: 2142–2154.

Power JD, Cohen AL, Nelson SM, Wig GS, Barnes KA, Church JA, et al. Functional Network Organization of the Human Brain. Neuron 2011; 72: 665–678.

Power JD, Mitra A, Laumann TO, Snyder AZ, Schlaggar BL, Petersen SE. Methods to detect, characterize, and remove motion artifact in resting state fMRI. NeuroImage 2014; 84: 320–341.

Raz A, Zhu H, Yu S, Bansal R, Wang Z, Alexander GM, et al. Neural Substrates of Self-Regulatory Control in Children and Adults with Tourette Syndrome
, Neural Substrates of Self-Regulatory Control in Children and Adults with Tourette Syndrome. Can. J. Psychiatry 2009; 54: 579–588.

Scahill L, Bitsko RH, Visser SN, Blumberg SJ. Prevalence of diagnosed tourette syndrome in persons aged 6-17 years - United States, 2007. Morb. Mortal. Wkly. Rep. 2009; 58: 581–585.

Scahill L, Riddle MA, McSWIGGIN-HARDIN M, Ort SI, King RA, Goodman WK, et al. Children's Yale-Brown Obsessive Compulsive Scale: Reliability and Validity. J. Am. Acad. Child Adolesc. Psychiatry 1997; 36: 844–852.

Schilbach L, Eickhoff SB, Rotarska-Jagiela A, Fink GR, Vogeley K. Minds at rest? Social cognition as the default mode of cognizing and its putative relationship to the "default system" of the brain. Conscious. Cogn. 2008; 17: 457–467.

Shulman GL, Pope DLW, Astafiev SV, McAvoy MP, Snyder AZ, Corbetta M. Right Hemisphere Dominance during Spatial Selective Attention and Target Detection Occurs Outside the Dorsal Frontoparietal Network. J. Neurosci. 2010; 30: 3640–3651.

Sowell ER, Kan E, Yoshii J, Thompson PM, Bansal R, Xu D, et al. Thinning of sensorimotor cortices in children with Tourette syndrome. Nat. Neurosci. 2008; 11: 637–639.

Wang Z, Maia TV, Marsh R, Colibazzi T, Gerber A, Peterson BS. The Neural Circuits That Generate Tics in Tourette's Syndrome [Internet]. Am. J. Psychiatry 2011[cited 2019 Jan 19] Available from: https://ajp.psychiatryonline.org/doi/abs/10.1176/appi.ajp.2011.09111692

Yeo BTT, Krienen FM, Sepulcre J, Sabuncu MR, Roffman JL, Smoller JW, et al. The organization of the human cerebral cortex estimated by intrinsic functional connectivity. J. Neurophysiol. 2011; 106: 1125–1165.

Ziemann U, Paulus W, Rothenberger A. Decreased motor inhibition in Tourette's disorder: evidence from transcranial magnetic stimulation. Am. J. Psychiatry 1997; 154: 1277.

Chapter 5: Discussion

5.1 Summary of Results

In this thesis, resting-state functional connectivity was used to study the development of neural systems in typically developing individuals and in individuals diagnosed with Tourette syndrome.

In Chapter 2, I presented our work using multivariate machine learning to identify developmental patterns in functional networks across the brain in healthy controls. After motion de-noising, these developmental patterns were found to be independent of patterns related to head motion. Reducing motion-related artifact also revealed that age prediction did not rely upon characteristics of functional connections previously hypothesized to mediate development (e.g., connection distance). Instead, successful age prediction relied upon sampling functional connections across multiple neural systems with strong, reliable functional connectivity within an individual.

Chapter 3 applied the approaches presented in Chapter 2 to the study of atypical development in TS. This work tested whether the patterns of functional connections across the whole brain that classify diagnosis in one age group (e.g., children) could classify diagnosis in another age group (e.g., adults). We also tested whether the typical developmental trajectory of these connections was altered in TS. While diagnostic classification was successful in children and adults separately, the connections that best distinguished TS from controls were age-specific. When contextualized with typical

development, some functional connections exhibited accelerated maturation in childhood TS, while others exhibited delayed maturation in adulthood TS.

In Chapter 4, I focused on identifying the specific functional networks and connections with atypical developmental trajectories in TS. We found that, as in Chapter 3, the development of most functional networks was intact in TS (i.e., similar to the developmental differences in typically developing children and adults). While there was some suggestive evidence for consistent functional network differences in childhood and adulthood TS ("age-invariant" effects), most functional networks that were significantly affected in TS differed between children and adults. Several within-network and cross-network functional connections exhibited either divergent or attenuated development in TS. We found that the divergent development observed in adulthood TS could be characterized by strongly linked neural systems that might be responsible for suppressing, producing, and attending to tics beyond what is observed in typical development. In contrast, the typical development of the systems potentially facilitating the initiation and production of tics was attenuated in TS. Considered together, our results inform a model of how several cortical and subcortical functional networks likely involved in the initiation, production, and/or suppression of tics interact and differ across development in TS.

In the following section, I will elaborate on some of the themes of this work with a focus on potential future directions.

5.2 Comments on using resting-state functional connectivity to study the development of functional systems.

Our results suggest that resting-state fMRI can be used to study the development of the system-level organization of the brain in school-age children and adults at both the group- and individual-level. After adequately correcting for submillimeter subject-motion, patterns of functional connectivity no longer carry information that can be used to predict individual differences in motion—even with "data greedy" machine learning algorithms. Remaining developmental differences in functional connectivity can be identified using both machine learning and group-level studies. Removing artifactual differences in functional connectivity illuminated some of the principles that do (and do not) organize the development of functionals systems. While the "local-to-distributed" hypothesis (i.e., short-range connections are weakened, and long-range connections are strengthen over development) for the development of functional networks (Fair *et al.*, 2009) was not supported by this thesis, we found that functional connections with strong positive or strong negative resting-state correlations, not weak correlations, carry developmental information about individuals. These results are promising as they suggest that there are developmental differences in neural systems measured with functional connectivity which might be associated with the ongoing changes in cognitive and behavioral capabilities occurring through adolescence and into adulthood.

The extent of developmental modifications to whole-brain functional connectivity was relatively small (i.e., correlation differences < 0.2) and fairly wide-spread, affecting many functional systems. We found that while the functional connections associated with each system were useful for age prediction, no single system could predict age as well as when connections were selected from many systems. As each functional system

contained information useful for age prediction, it appears that no functional system, including systems thought to mature early in development (Gogtay *et al.*, 2004), is entirely adult-like in children. The continuing development of most functional systems and their functional connections makes linking specific changes in functional systems to the emergence and improvement of different behaviors in this age range (e.g., reading, inhibitory control, decision-making) more difficult. There are several approaches (described below) which might facilitate a better understanding of the development of neural systems and their relation to developmental changes in behavior.

First, more sophisticated modeling of the developmental trajectories of functional systems might be crucial to teasing apart different developmental mechanisms that accompany developmental changes in behavior. In this thesis, I applied multivariate approaches in order to capture the complex, inter-dependence of developmental changes across functional connections. However, these approaches assume that these developmental differences are linear and may not capture more complex developmental trajectories (e.g., parabolic, growth curve). By more finely ascertaining the timing of developmental differences in functional connectivity, one may be able to better link the developmental trajectory of a particular system (or set of systems) to the emergence or maturation of a particular ability or behavior.

Second, attempts to describe the development of neural systems would be improved by more precise developmental data. The functional organization of the brain as measured with resting-state functional connectivity can differ between individuals (Gordon *et al.*, 2017). It is possible that the regions that describe a functional system across a group (e.g., Power *et al.*, 2011) do not accurately describe that functional system

within each individual. In an extreme hypothetical case, if the “ground truth” were that only a single functional system develops from childhood to adulthood, but the location of that functional system varies widely across individuals, it might be expected that randomly selected functional connections will predict age better than the group-level description of that functional system. This scenario is fairly unlikely, as the functional organization of the brain appears highly conserved across healthy control adults when highly sampled (Gordon *et al.*, 2017; Gratton *et al.*, 2018), but systematic individual differences may contribute to the muddiness of developmental differences in functional connectivity. Similarly, as these data were cross-sectional and chronological age was used as a proxy for maturity, individual differences in developmental trajectories might have weakened the observed developmental differences. A better description of the development of systems will likely require highly-sampled individuals to well-describe the functional organization of the brain and longitudinal study designs to capture true developmental trajectories.

Finally, since the differences in neural systems measured with functional connectivity between school-age children and adults were subtle, larger developmental differences in functional systems might be observed earlier on in development. Several have used fMRI to measure functional connectivity in sleeping infants and toddlers (Fransson *et al.*, 2011; Smyser *et al.*, 2011). As in school-age children, there is some debate as to whether and to what extent the functional networks observed in infancy differ from those observed in adulthood (Cusack *et al.*, 2018). Infant and toddler imaging data also contain head motion-related artifact (Cusack *et al.*, 2017) which might produce motion-contaminated developmental differences. But even properly de-noised functional connectivity may include additional differences related to the state of being asleep or

awake (Larson-Prior *et al.*, 2009; Tagliazucchi and Laufs, 2014). Aside from these methodological issues, imaging the infant brain may be easier to link to behavior as changes in behavior are more striking (e.g., crawling to walking) and have been well characterized by developmental psychologists (e.g., language acquisition (Kuhl, 2004), perceptual development (Slater and Kirby, 1998)) .

5.3 Comments on using multivariate machine learning to study developmental patterns in functional connectivity.

Many researchers apply multivariate machine learning to resting-state functional connectivity with the intent to make accurate predictions about individuals and to interrogate the neurobiological mechanisms underlying a predicted characteristic. This thesis has shown that resting-state functional connectivity provides a robust neurobiological measurement of an individual, sufficient to make predictions about that individual's chronological age and diagnostic status with relatively high accuracy even, notably, after correcting for systematic differences in functional connectivity related to subject head motion. The success of multivariate machine learning classification applied to functional brain networks in TS holds promise for clinical application of these methods. Given the heterogeneity in the developmental course of TS symptoms, there is a great need to predict future clinical outcome for individuals. Being able to predict whether a given child with tics will go on to improve or not would have high clinical utility, providing important information to families, guiding treatment plans, and affording the opportunity for early intervention. These findings suggest that functional connectivity contains signals that can be used for these types of predictions, and that the best predictions will likely rely upon modeling these effects in a rich typical developmental context.

However, this thesis has also shown that the potential of using machine learning to interrogate the specific features facilitating prediction in the hopes of understanding the neural mechanisms underlying typical or atypical brain development is somewhat limited. For example, identifying a unique set of functional connections that carry information useful for age prediction with functional connectivity is difficult due to the intercorrelated and distributed nature of developmental differences in resting-state functional connectivity. When evaluated against an appropriate null model, we found that most sets of selected features, while useful for prediction, were not exclusively meaningful nor indicative of a unique solution to age prediction from functional connectivity. In TS, we did find that only a select set of functional connections exhibited atypical developmental trajectories. However, those identified connections in Chapter 3 were not particularly useful for illuminating the etiology of TS, especially in comparison to the results from the group-level studies presented in Chapter 4. Machine learning identified seemingly random functional connections within and between many functional systems whereas group-level studies identified sets of connections with similarly altered functional connectivity from a limited number of functional systems. Machine learning algorithms are built to optimize the utility rather than the relevance of features for prediction (Guyon and Elisseeff, 2003) and may not be appropriate for hypothesis testing in all situations.

However, the field of machine learning is growing rapidly and more sophisticated algorithms are being developed. The support vector machine learning algorithm used in this thesis were fairly simple; this algorithm was chosen to improve our chances of being able to interpret the features used for prediction. Other newer algorithms such as deep learning, decision trees, and multilayer perceptrons might be able to improve overall

prediction accuracy with functional connectivity. Nonetheless, it is important to make sure each algorithm is applied to appropriate questions, internally validated, and then externally validated in order to ensure generalizability and avoid overfitting. Further, any identified features should be compared against an appropriate null model before interpreting the significance of a set of features.

In this thesis, it became apparent that multivariate machine learning models are built to make predictions, and can only test hypotheses about neurobiological mechanisms indirectly. Both approaches that make individual-level predictions and those that test group-level differences are important to our understanding of typical and atypical development. Multivariate prediction complemented by alternative approaches directed at more mechanistic questions (e.g., group-level studies, highly sampled individuals, within-subject longitudinal studies) will likely yield the best mechanistic understanding of typically and atypically developing individuals.

5.4 Comments on the atypical development of functional networks in Tourette syndrome

Our results suggest that studying the development of the neurobiology underlying TS can shed light on important features of the disorder and provide important context for illuminating the magnitude, extent, and nature of the abnormalities observed in TS. We found that the functional connectivity that best characterized TS differed between children and adults. Regardless of the reason for this difference (see below), these results suggest that diagnosis and treatment of TS may need to be tailored differently for children and adults. Further, studying the disorder-related differences in functional systems in the context of typical development is crucial for understanding the disorder's overall impact

on functional brain organization. In comparison to developmental differences in functional connectivity, disorder related differences were also small (correlation differences < 0.2) but not as widespread. Only patterns among select functional connections exhibited atypical development in TS, while most sets of connections could be used to predict age well in TS and controls. Our results suggest that the main effect of TS is smaller in comparison to the main effect of chronological age on the brain and that TS-related differences in the brain are best revealed by examining the statistical interaction of age and diagnosis. Further, by considering the typical development of the connections with atypical functional connectivity in TS, we were able to better understand the nature of these differences. As an example, we found that connections between the cingulo-opercular system and the somatomotor system were stronger in adulthood TS than in controls; this enhanced connectivity was only observed in adulthood TS and represents a divergent developmental difference in TS. In contrast, the connections between the basal ganglia and visual system were less negative in adulthood TS than in control adults; however, these connections typically become more negative from childhood to adulthood and thus, appear immature in adulthood TS. Using this approach, the work in this thesis was able to demonstrate that the developmental trajectories of different functional systems were altered in distinct ways.

Further, this thesis demonstrated that using a whole-brain approach and placing atypical brain function in TS in a context of functional networks provides a novel and more comprehensive understanding of the neurobiology underlying TS. By examining both cortical and subcortical functional connectivity, we identified regions affected in TS beyond the previously reported basal ganglia and frontal cortex. Examining how the

statistical functional relationships between the basal ganglia/frontal cortex and the rest of the brain differ in TS provides a more comprehensive picture of the circuitry that is disrupted (e.g., basal ganglia to visual system). We were also able to more precisely describe the functional systems affected in the frontal cortex (e.g., cingulo-opercular and ventral attention) and elsewhere. Additionally, by leveraging the extant research illuminating the properties of different functional systems in healthy controls, we were able to bridge neuroimaging research in TS with theories of the functional network organization of the brain. We showed that regions activated in the time preceding tics associated with a premonitory urge (Leckman *et al.*, 1993) largely include the cingulo-opercular network. Additionally, we found that regions activated at the time of tic action (Bohlhalter *et al.*, 2006) include the dorsal attention network, a functional system important for the direction of attentional resources (Corbetta and Shulman, 2002). Regions activated by children and adults with TS when instructed to suppress eye blinks include the default-mode network (Mazzone *et al.*, 2010). By combining our study of the functional relationships between regions with previous studies of brain function in TS and the extant knowledge about the role of functional systems in healthy controls, this thesis provides a novel model of the atypical brain function, connections, cognitive processes associated with TS.

Studying the functional organization of the brain in TS with functional connectivity faces many of the same issues discussed above in studying typical development. As in typical development, more precise data from individuals and longitudinal data would greatly benefit the study of the atypical development of functional systems. In TS, there are also additional benefits to these approaches. Symptoms in TS are highly heterogenous; TS is commonly associated with other comorbid diagnoses such as ADHD

and OCD (Freeman *et al.*, 2000). Even tics, the characteristic symptom of the disorder, manifest differently in different individuals (Leckman *et al.*, 1989). Understanding the neurobiology in TS might require an individualized approach to localize regions associated with an individual's specific symptoms (tics or otherwise) and determine how these correspond to that individual's functional brain organization. Similarly, the developmental course of symptoms varies widely in TS (Leckman *et al.*, 1998; Pappert *et al.*, 2003); it is possible that the patients with TS reported in this thesis were imaged at different points in the course of their symptoms (e.g., rise, peak, remission) even if measures of tic severity were similar. Longitudinal assessment of the functional networks underlying TS would facilitate a better understanding of how typical developmental processes and the developmental course of symptoms interact.

In addition, the present investigation and model proposed to describe atypical development in TS may oversimplify the functional organization of the basal ganglia, thalamus, and cerebellum. Cortico-striato-thalamo-cortical loops between different pieces of cortex and subcortex appear devoted to different functions (e.g, motor, control) (Haber, 2003). These associations can be illuminated with resting-state functional connectivity such that different pieces of the subcortex and the cerebellum exhibit stronger functional connectivity with specific functional networks (Choi *et al.*, 2012; Greene *et al.*, 2014; Marek *et al.*, 2018). While this thesis identified connections between the subcortex and specific cortical functional networks (e.g., visual, auditory, ventral attention) with atypical development in TS, it is possible that connections between specific pieces of the subcortex and cortical functional networks develop differently in TS. Investigation of the functional organization of the basal ganglia, thalamus, and cerebellum at a finer scale

may provide a more complete model of the neural substrates underlying the developmental course of TS.

While this thesis identified patterns of functional connectivity and specific sets of functional connections that were altered in TS, the source of these disorder-related differences remains difficult to disentangle. As an example, we identified atypically stronger functional connectivity in the set of connections between the cingulo-opercular system and the somatomotor system. (1) These strengthened connections might be a change in the brain that facilitates tics (e.g., atypically coordinated inhibitory control of motor function). (2) Alternatively, the strengthened connections between the cingulo-opercular and motor systems might be a consequence of having tics; experience with tics might produce maladaptive, neutral, or compensatory changes in the brain. (3) Finally, the enhanced connections between the cingulo-opercular and somatomotor systems might reflect state differences between groups; if the TS group was suppressing tics while in the scanner, these amplified connections might be attributable to this behavior rather than the underlying neurophysiology in TS.

Additional experiments and approaches could shed light on the potential contribution of these various sources of disorder-related differences in functional networks in TS. First, as mentioned above, studying the differences in TS over the course of development can prove useful; not everyone with tics (e.g., children with TS) have enhanced connectivity between the cingulo-opercular and motor systems so these connections must not be necessary for the production of tics. Second, linking functional connectivity to measurements of experience with tics such as tic severity, time since tic onset, or ability to suppress tics might determine whether the observed enhanced

connectivity is a consequence of maladaptive, neutral, or compensatory experience with tics, respectively. Finally, if differences in functional connectivity in TS are confounded by state-related differences between the TS and control group, controlling what participants are doing in the scanner by instructing both the TS and control groups to suppress eye blinks (as in Mazzone *et al.*, 2010) should mitigate these differences.

Interpreting the atypical developmental differences in TS reported in this thesis is also difficult due to the limitations of the study design. As mentioned above, we found that the functional connectivity that characterizes TS differs between children and adults. It is unclear whether this difference is a result of developmental change or cohort effects. Some argue that childhood TS and adulthood TS are fundamentally different, given the commonly held belief that most patients with TS experience substantial symptom improvement or remission into adulthood (Leckman *et al.*, 1998). Therefore, by studying a sample of adults with current tics, we have likely captured the subsample who do not experience significant remission. By contrast, any sample of children with TS will include a mixture of individuals whose tic symptoms will go on to improve and those whose tics will persist. Thus, it would seem that the observed developmental differences in atypical functional connectivity may in actuality be cohort effects describing the differences between persistent TS in adults and a mix of persistent and transient TS in children. However, there is evidence that complete remission is likely much rarer than previously estimated (10%, rather than 40%; (Pappert *et al.*, 2003)), and in our sample, many of the adults with TS reported improvement from childhood even if they did not report remission. Longitudinal studies and studies of individuals with remitted tics will be crucial to determine the origin of age-specific, atypical functional connectivity in TS.

5.5 Closing Comments

When I arrived at WashU, I wanted to study functional networks and how their organization contribute to complex, human behavior—I wasn't particularly interested in development. But shortly after my rotation in the lab, I became hooked. Development provides, like lesions, a unique window into how brain functioning and human thinking are affected if all of the necessary circuits are not properly in place. In my thesis work, I wanted to understand the mechanisms by which these functional networks change and coordinate over the course of development to support cognition. Even though my results were not as simple and satisfying as I had hoped, I learned a lot in my first project studying typical development—the power and limitations of machine learning, the widespread nature of developmental differences in functional networks, and the importance of reigning in open-ended questions. Subsequently through a series of tangential delays and detours, I serendipitously stumbled into studying development in Tourette syndrome (TS). Initially, I was solely interested in applying and assessing the diagnostic capability of the machine learning approaches I had previously developed. However, as these projects evolved, more and more interesting developmental questions emerged: Why do the functional networks that distinguish TS from controls differ between children and adults? How does development differ in TS? Which functional networks? What can this tell us about the nature and course of tics and other symptoms in TS? By broadening my thesis to include the atypical development in TS, I succeeded in examining the ways in which functional networks change and coordinate over the course of development to support cognition; I was able to dissect developmental trajectories of different functional networks that might support different aspects of tics and other symptoms in TS. Overall,

I am proud of what of this thesis has accomplished. While not necessarily ground-breaking for the neuroscience field as a whole, this series of projects taught me a lot about the scientific process and renewed my conviction that I want to continue in academia. The lessons that I have learned from studying the typical and atypical development of the brain's functional network architecture will surely aide me as I transition into a post-doc position at Northwestern University in the adjacent field of infant neuroimaging.

5.6 References

Bohlhalter S, Goldfine A, Matteson S, Garraux G, Hanakawa T, Kansaku K, et al. Neural correlates of tic generation in Tourette syndrome: an event-related functional MRI study. Brain 2006; 129: 2029–2037.

Choi EY, Yeo BTT, Buckner RL. The organization of the human striatum estimated by intrinsic functional connectivity. J. Neurophysiol. 2012; 108: 2242–2263.

Corbetta M, Shulman GL. Control of goal-directed and stimulus-driven attention in the brain. Nat. Rev. Neurosci. 2002; 3: 201–215.

Cusack R, Linke A, Zubiaurre-Elorza L, Duffy H, Herzmann C, Stojanoski B, et al. Differences in the spatial and temporal patterns of head motion during MRI of adults and infants. bioRxiv 2017: 114447.

Cusack R, McCuaig O, Linke AC. Methodological challenges in the comparison of infant fMRI across age groups. Dev. Cogn. Neurosci. 2018; 33: 194–205.

Fair DA, Cohen AL, Power JD, Dosenbach NUF, Church JA, Miezin FM, et al. Functional Brain Networks Develop from a "Local to Distributed" Organization. PLOS Comput. Biol. 2009; 5: e1000381.

Fransson P, Åden U, Blennow M, Lagercrantz H. The Functional Architecture of the Infant Brain as Revealed by Resting-State fMRI. Cereb. Cortex 2011; 21: 145–154.

Freeman RD, Fast DK, Burd L, Kerbeshian J, Robertson MM, Sandor P. An international perspective on Tourette syndrome: selected findings from 3500 individuals in 22 countries. Dev. Med. Child Neurol. 2000; 42: 436–447.

Gogtay N, Giedd JN, Lusk L, Hayashi KM, Greenstein D, Vaituzis AC, et al. Dynamic mapping of human cortical development during childhood through early adulthood. Proc. Natl. Acad. Sci. 2004; 101: 8174–8179.

Gordon EM, Laumann TO, Gilmore AW, Newbold DJ, Greene DJ, Berg JJ, et al. Precision Functional Mapping of Individual Human Brains. Neuron 2017; 95: 791-807.e7.

Gratton C, Laumann TO, Nielsen AN, Greene DJ, Gordon EM, Gilmore AW, et al. Functional Brain Networks Are Dominated by Stable Group and Individual Factors, Not Cognitive or Daily Variation. Neuron 2018; 98: 439-452.e5.

Greene DJ, Laumann TO, Dubis JW, Ihnen SK, Neta M, Power JD, et al. Developmental Changes in the Organization of Functional Connections between the Basal Ganglia and Cerebral Cortex. J. Neurosci. 2014; 34: 5842–5854.

Guyon I, Elisseeff A. An Introduction to Variable and Feature Selection. J. Mach. Learn. Res. 2003; 3: 1157–1182.

Haber SN. The primate basal ganglia: parallel and integrative networks. J. Chem. Neuroanat. 2003; 26: 317–330.

Kuhl PK. Early language acquisition: cracking the speech code. Nat. Rev. Neurosci. 2004; 5: 831–843.

Larson-Prior LJ, Zempel JM, Nolan TS, Prior FW, Snyder AZ, Raichle ME. Cortical network functional connectivity in the descent to sleep. Proc. Natl. Acad. Sci. 2009; 106: 4489–4494.

Leckman JF, Riddle MA, Hardin MT, Ort SI, Swartz KL, Stevenson J, et al. The Yale Global Tic Severity Scale: Initial Testing of a Clinician-Rated Scale of Tic Severity. J. Am. Acad. Child Adolesc. Psychiatry 1989; 28: 566–573.

Leckman JF, Walker DE, Cohen DJ. Premonitory urges in Tourette's syndrome. Am. J. Psychiatry 1993; 150: 98.

Leckman JF, Zhang H, Vitale A, Lahnin F, Lynch K, Bondi C, et al. Course of Tic Severity in Tourette Syndrome: The First Two Decades. Pediatrics 1998; 102: 14–19.

Marek S, Siegel JS, Gordon EM, Raut RV, Gratton C, Newbold DJ, et al. Spatial and Temporal Organization of the Individual Human Cerebellum. Neuron 2018; 100: 977-993.e7.

Mazzone L, Yu S, Blair C, Gunter BC, Wang Z, Marsh R, et al. An fMRI Study of Frontostriatal Circuits During the Inhibition of Eye Blinking in Persons With Tourette Syndrome. Am. J. Psychiatry 2010; 167: 341–349.

Pappert EJ, Goetz CG, Louis ED, Blasucci L, Leurgans S. Objective assessments of longitudinal outcome in Gilles de la Tourette's syndrome. Neurology 2003; 61: 936–940.

Power JD, Cohen AL, Nelson SM, Wig GS, Barnes KA, Church JA, et al. Functional Network Organization of the Human Brain. Neuron 2011; 72: 665–678.

Slater A, Kirby R. Innate and learned perceptual abilities in the newborn infant. Exp. Brain Res. 1998; 123: 90–94.

Smyser CD, Snyder AZ, Neil JJ. Functional connectivity MRI in infants: Exploration of the functional organization of the developing brain. NeuroImage 2011; 56: 1437–1452.

Tagliazucchi E, Laufs H. Decoding Wakefulness Levels from Typical fMRI Resting-State Data Reveals Reliable Drifts between Wakefulness and Sleep. Neuron 2014; 82: 695–708.