

**RESTING STATE FUNCTIONAL CONNECTIVITY MRI PREDICTS FUTURE REACTION TIME
VARIABILITY IN CHILDREN WITH AND WITHOUT ADHD**

By

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ABSTRACT

Background – ADHD is one of the most prevalent neurodevelopmental disorders in our society. One of the hallmark features of ADHD is increased reaction time variability (RTV), which is particularly significant among children, as RTV is implicated in many goal-directed behaviors and later academic achievement. Recent brain imaging studies have revealed that those with ADHD have atypical functional brain signatures compared with those without ADHD, suggesting a neurobiological basis for ADHD that can be measured using brain imaging. **Methods** – This is a historical cohort study of 32 children (7-14y) with ADHD and 40 children without ADHD. We administered a test of reaction time variability at study entry and then at least at one other time-point in the study. We use support vector machine-based multivariate pattern analysis to determine whether connection features derived from resting state functional connectivity MRI (rs-fcMRI) were able to predict longitudinal changes in a test of RTV. **Hypothesis** – We hypothesized that baseline rs-fcMRI measurements would predict RTV in the sample as a whole (i.e. ADHD and typically developing children as a single group). Furthermore, we expected that predictions of change in reaction time variability would become more robust when considering typically developing children and children with ADHD separately, as there are likely to be predictive systems that are distinct in these two groups, as well. **Results** – Connectivity between consensus features positively predicted changes in RTV when children with ADHD and TDC were evaluated as a single group (Adjusted $R^2 = 0.10$, $p = 0.0027$), and predictive functional networks include the cinguloopercular and ventral attention networks. When children with ADHD were considered alone, connectivity between consensus features improves in its ability to positively predict changes in RTV (Adjusted $R^2 = 0.3183$, $p < 0.0001$), and predictive

functional networks include the cinguloopercular, default, visual, and ventral attention networks. When considering TDC alone, predictive functional networks include the cinguloopercular, default, and dorsal attention networks, and consensus features also positively predict changes in RTV (Adjusted $R^2 = 0.2813$, $p < 0.0001$). Finally, group comparison between predictive networks in ADHD vs. TDC reveals a degree of overlap (particularly between the cinguloopercular and default networks), but also highlights specific subregions that are distinct within these networks, including the anterior insula/frontal operculum (within the cinguloopercular network), the inferior parietal subregion (within the frontoparietal network), the lateral parietal and inferior temporal subregions (within the default network), and the ventral vs. dorsal attention networks. **Conclusion** – We apply multivariate statistics along with non-invasive brain imaging to use baseline functional connectivity measurements to predict longitudinal change in RTV in children with and without ADHD. The functional connections that predict RTV in these two groups overlap in some regions, but are significantly distinct in other regions. While there are functional similarities in the brains of children with ADHD and TDC, there are also innate differences in the functional connectivity of these two populations, and these differences play a significant role in predicting longitudinal changes in RTV across these two groups. Ultimately, this work has the potential to characterize an approach aimed at identifying children at high-risk of deficits in future cognitive performance and thus associated life outcomes such as academic achievement.

INTRODUCTION

ADHD is reported to affect 9.0% of children ages 13 to 18 years in the US, with an average age of identification of 7 years (NIMH, ADHD). It is a neurodevelopmental disorder of childhood that often carries into adulthood, costing our society between \$36 billion to \$52.4 billion annually (Pelham et al., 2007). The main symptoms of inattention, hyperactivity, and impulsivity among children with ADHD lead to lifelong functional impairments that affect many life outcomes, including academic achievement. Children with ADHD are known to have academic underachievement compared to typically developing controls (TDC), and these differences persist across the lifespan from preschool readiness to university-level performance (Daley & Birchwood, 2010). Thus, ADHD is a significant public health issue with important consequences for the individual and society. However, one of the problems hindering our assessment of ADHD is that we lack objective, biologically informative measures that can aid our understanding of its etiology and to be used as stable prognosticators.

Neuroimaging offers a valuable tool to study the human brain non-invasively and may serve as an important tool to characterize etiology and may have prognostic value. Resting-state functional connectivity MRI (rs-fcMRI) in particular allows us to examine spontaneous activity between anatomically distinct but functionally linked regions of the brain. Rs-fcMRI rests on the principle that the spontaneous, low frequency (<0.1 Hz) blood oxygen level-dependent (BOLD) signal reflects neuronal activity between functionally connected regions of the brain (Biswal et al., 1995). In this way, changes in oxygen-rich blood flow serve as an indirect marker for

neuronal activation patterns. As described in detail by van den Heuvel and Hulshoff Pol (2010), several group-level rs-fcMRI studies have shown the construction of functionally linked brain networks during rest (Beckmann et al., 2005; Damoiseaux et al., 2006; Fox and Raichle, 2007; Fox et al., 2005). Although these studies all used various methods with different groups of subjects, they consistently demonstrated the emergence of the same or very similar functional brain networks during rest. These findings suggests external validity in resting-state functional networks. As a result, rs-fcMRI can be used to better elucidate group-level functional differences in the human brain. Indeed, rs-fcMRI has proven to be a valuable tool to better understand the neurobiology of ADHD.

In the quest to understand the biology of ADHD and the markers that reflect its clinical presentation, investigators often use measurements of executive functioning, reward, and impulse control. Unlike ADHD symptomatology, validated cognitive tasks offer objective data that can be measured with precision and accuracy. For example, a growing body of literature hypothesizes that one of the hallmark features of ADHD is increased reaction time variability (RTV). As described by Castellanos and Tannock (2007), reaction time variability serves as a surrogate marker of “the moment-by-moment process of task performance, in which individuals with ADHD have problems”. RTV is a particularly descriptive trait to examine, as it highlights fluctuations in behavior of a given child over a period of seconds or milliseconds, rather than hours, thus reflecting within-subject inconsistencies in behavior and task performance over a relatively short time span (Castellanos et al., 2005). These inconsistencies in behavior are an important finding in ADHD, in part because in order to maintain attention

toward efficient, goal-directed behaviors, one must be able to rapidly adapt thinking and behavior to both dynamic internal states and external conditions (McLoughlin et al., 2014). In this way, increased RTV suggests a deficit in cognitive flexibility. However, it is not entirely clear that RTV represents this ability alone. As described by Kofler et al. (2013), RTV has also been suggested to be secondary to difficulties in regulating the physiological system, particularly in relation to energy metabolism, such that individuals with ADHD are postulated to have astrocytes that produce insufficient lactate and thus problems with attention are said to be due to disruptions in the supply of energy (Russell et al., 2006). Moreover, other investigators have suggested that RTV is secondary to problems with maintaining arousal, such that individuals with ADHD have defective noradrenergic activation centrally, and this theory is supported by the improvement in arousal and attention exhibited with stimulant interventions, which are thought to affect dopaminergic and noradrenergic pathways (Biederman & Spencer, 1999).

Regardless of the underlying mechanism, the outcome in children with ADHD remains the same: under conditions that require fast and accurate responses, children with ADHD are generally slower, less accurate, and have more variable reaction times compared to typically developing children their age (Karalunas et al., 2012). Moreover, increased RTV has been reported as a mediating variable in the relationship between ADHD and functional impairments such as diminished academic achievement, particularly in relation to language skills and mathematics (Sjowall & Thorell, 2014). Therefore, it becomes evident that RTV is implicated in important outcomes such as educational achievement, which in itself is linked to other significant life consequences.

A meta-analysis of 319 studies examining RTV in children, adolescents, and adults with ADHD found that regardless of symptom presentation, the task used to assess RTV, and physiologic and/or cognitive state, increased RTV remains a reliable finding (Kofler et al., 2013). When comparing individuals with ADHD to typically developing controls, the effect size (converted to Hedges g) in this study ranges from 0.57 (corrected for sampling error in conjunction with publication bias) to 0.89 (corrected for measurement unreliability), with 95% confidence intervals ranging from 0.51-0.62 and 0.83-0.95, respectively. Thus, it becomes evident that statistical significance aside, the magnitude of the difference in RTV detected by this study when comparing individuals with ADHD to TDC is relatively strong. This is important, because given an N of 319 studies, one would expect to detect a relatively strong effect size (as opposed to a more nominal quantity) if there is indeed a true and meaningful underlying difference in RTV between ADHD and TDC. As described in the meta-analysis by Kofler et al. (2013), those with ADHD are noted to be “consistently inconsistent” (Rapport et al., 2001), and this trait is evident in performance on a variety of neuropsychological tests (Kofler et al., 2008; Klein et al., 2006), including tasks that assess reaction time related to working motor control (Klein et al., 2006).

Because increased RTV is a hypothesized feature of ADHD, the regions of the brain that are implicated in it could also further our understanding of ADHD. Given its pervasive nature, RTV has been suggested as a possible endophenotype for ADHD (Castellanos et al., 2005; Castellanos et al., 2009), where endophenotypes embody quantifiable characteristics that are

at least partially heritable and indicate an individual's predisposition to demonstrate a given condition or disease (Castellanos & Tannock, 2002). By studying a potential endophenotype of ADHD in conjunction with rs-fcMRI, we hope to further our understanding of ADHD and one of the potential markers of its etiology. Kofler et al. (2013) describe the considerable overlap between the regions of the brain that are often associated with ADHD and those that correspond to RTV. These regions include the anterior cingulate, dorsolateral prefrontal, and orbitofrontal cortices (Bellgrove et al., 2004; MacDonald et al., 2006).

Furthermore, Karalunas et al. (2012) highlight the complex interplay that is uncovered by RTV, including "stimulus encoding, rate of information processing, motor preparation and output, speed-accuracy trade-off effects, and response bias". Moreover, Karalunas et al. (2014) underscore the lack of consensus regarding the specific neural correlates of RTV. For example, they discuss the default mode network as a neural network that is thought to be very relevant to RTV given the default mode network's implications in attention. They describe results of a study that showed that increased RTV in children with ADHD is associated with attenuated deactivation of the default mode network (Fassbender et al., 2009). They suggest that this is in contrast, however, to another study that found a non-significant association between RTV in ADHD and levels of activation in the medial prefrontal cortex and precuneus, both of which are regions that also help comprise the default network (Christakou et al., 2013). Karalunas et al. (2014) also outline the results of two other fMRI studies that found an association between increased RTV in children with ADHD and decreased activation of fronto-parietal brain regions (Cao et al., 2008), as well as the right anterior cingulate gyrus (Konrad et al., 2006).

Taken together, these results point to the ambiguity of the neural correlates underlying RTV. As such, further clarification of the resting-state neural underpinnings of RTV, particularly in children with ADHD, would be useful. Perhaps a more robust and clinically useful approach to the question would be to determine whether brain signatures in children with and without ADHD are able to predict future improvement in reaction time variability. This particular question has not been previously elucidated. It is important because a stronger understanding of those features of ADHD that can aid in prediction of an outcome such as RTV would allow researchers to perhaps identify a critical period during which interventions could eventually change important outcomes relevant to children with ADHD.

The present study aims to examine a longitudinal sample of children with and without ADHD to determine whether SVM based multivariate pattern analysis, in combination with functional connectivity MRI, can predict changes in RTV at a later time-point. By examining an objective neuropsychological measure in conjunction with functional connectivity, we sought to further elucidate the neural correlates underlying RTV, as well as suggest a model to predict future RTV. In so doing, we hoped to create a stepping stone towards the identification of a potential critical period during which effective interventions could decrease the risk of long-term deficits in RTV. We hypothesized that baseline rs-fcMRI measurements would predict RTV in the sample as a whole (i.e. ADHD and typically developing children as a single group). Furthermore, we expected that predictions of change in reaction time variability would become more robust

when considering typically developing children and children with ADHD separately, as there are likely to be predictive systems that are distinct in these two groups as well.

MATERIAL & METHODS

Subject recruitment and diagnostic procedures below followed previously published procedures by Karalunas et al., 2014.

Subject Recruitment & Informed Consent: Study participants were ages 7 to 14 years old and were recruited between 2007-2013 using a community-based recruitment strategy via advertisements in mailing lists for parents of school-age children in public schools in Portland, OR. The Institutional Review Board at Oregon Health & Science University provided ethical approval of this study. Children provided written assent for the study, and their parent/legal guardian provided written informed consent.

Diagnostic Procedure: A multi-step diagnostic procedure was used to screen eligible volunteers and then classify them into one of two groups: ADHD vs. typically developing controls (TDC).

Those who did not meet our criteria for one of these two groups were excluded.

Parents completed standardized ratings of child behavior using the Conners' Rating Scale-Revised; the ADHD Rating Scale, the Child Behavior Checklist, and the Strengths and Difficulties Questionnaires (SDQ). Teachers completed a Conners-III, SDQ, and ADHD Rating Scale. Parents were also interviewed by a Master's-level clinician using the Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS) to rule out co-morbid psychiatric disorders. Children also completed a single short form of the Wechsler Intelligence Scale for Children (WISC-IV) under the supervision of a PhD level licensed psychologist, and these tests were used to estimate Full Scale IQ as described by Sattler (2004).

The above measures, along with written observation notes from both a short clinical interview of the child and from the tester who worked with the child, were compiled. These were presented to a clinical diagnostic team consisting of a board-certified child psychiatrist and a licensed clinical psychologist. They independently reviewed the cases and made an assignment; their agreement rate was satisfactory and disagreements were resolved by consensus. Children who did not meet criteria for ADHD and did not meet exclusion criteria were assigned to the control group (TDC). Some of the TDC subjects were siblings of the children diagnosed with ADHD. After all inclusion and exclusion criteria were applied, 32 children were assigned to the ADHD group, 40 children were assigned to the TDC group, and 7 sibling pairs were included (discussed below).

Exclusion Criteria: Subjects were excluded if they had: any major medical condition, neurological impairment, history of seizure, head injury with loss of consciousness, substance use disorder; prior diagnosis of mental retardation, Autism Spectrum Disorder, psychosis, or mania; estimated IQ < 70, use of long-acting psychotropic medications, or had a major depressive episode at study entry.

Demographics of ADHD group and TDC: Among those with ADHD, the mean age was 10.1 years. 11 children with ADHD were female. 78% of the ADHD group was White, non-Hispanic and 22% were non-White or Hispanic.

Among the TDC group, the mean age was 9.4 years, and 19 of these children were female. 80% of the TDC group were White, non-Hispanic and 20% were non-White or Hispanic.

Data Collection Overview: For each participant included in the study, two types of data were collected longitudinally: 1) rs-fcMRI and 2) administration of a dual-task measure called the Stop-Go Task, from which reaction time variability for go-trials was extracted, at each of at least two different time-points (baseline and one year later), each separated by at least 1 year. The task is described below. In order to be included in the study, a subject's first rs-fcMRI scan must have been completed within 6 months of first RTV measurement.

Predictive Variables (Features) – resting state functional connectivity MRI measurements

MRI acquisition:

3 five minute rs-fcMRI scans were acquired in a single session at baseline. All MRI acquisition steps closely follow the previously published work of Karalunas, Fair et al., 2014, Scanning was completed at the OHSU Advanced Imaging Research Center using a 3.0 Tesla Siemens Magnetom Tim Trio scanner with a twelve-channel head-coil. A high-resolution sagittal T1-weighted MPRAGE sequence was collected (T_e = 3.58 ms, TR = 2300 ms, 256x256 matrix, resolution = 1 mm, total scan duration = 9 min 14 sec). T2-weighted echo planar imaging (TR = 2000 ms, TW = 30 ms, flip angle = 90°, FOV = 240 mm, 36 slices encompassing the entire brain, slice thickness = 3.8 mm, in-plane resolution = 3.8 x 3.8 mm) in the oblique plane (i.e. parallel to the ACPC) was used to collect BOLD images under continuous rest while children kept their eyes

open, fixated on a crossbar. After approximately 10 seconds (5 frames), steady-state magnetization was presumed.

fMRI processing:

The following also closely follows the previously published work of Karalunas, Fair, et al., 2014.

In order to reduce artifacts, all functional images were processed using removal of a central spike due to MR signal offset; correction for interspersed image acquisition without gaps, which causes intensity differences in even vs. odd slices; head motion correction within and across runs; and use of a whole brain mode value of 1000 for within-run normalization of intensity.

The MPRAGE scan was used to configure atlas transformation of all functional data for all children. Movement correction and atlas transformation were then combined in one interpolation to resample each run in atlas space using an isotropic 3 mm grid. The atlas-transformed volumetric time-series was subsequently used for all remaining procedures.

rs-fcMRI data reduction:

Pre-processing steps included temporal bandpass filtering, spatial smoothing, regression of rigid body head motion correction, whole brain regression, and regression of white matter and ventricular signals, as described by Dosenbach et al., 2010. Motion correction is a critical issue and was handled using recently proposed procedures, directed at framewise displacement (FD), such that volumes greater than 0.3 FD were removed (Power et al., 2011; Fair et al., 2012).

The following procedures closely follow the previously published work of Dosenbach et al., 2010.

Generation of ROIs & correlation matrices subsequently used as features in prediction:

For each scan, the BOLD time-series was obtained for each of 99 predefined cortical and subcortical regions of interest (ROIs) as previously described by Power et al. (2011) and Gordon et al. (2014). Next, within each scan the BOLD time-series for each ROI was correlated (Pearson's r) with the BOLD time-series for all other ROIs from that scan, creating a square correlation matrix for each subject. This produced a 99x99 correlation matrix for each subject. R-values subsequently underwent Fisher's z-transformation in order to utilize normalized correlation values, i.e. functional connections, which served as the features used in all analyses that followed.

Outcome Measure: Change in Reaction Time Variability

The Stop-Go Task was administered by a Master's level clinician at each of at least two time-points in which subjects were assessed, as discussed above under Data Collection Overview. It is described in detail by Nigg (1999), Only the Go trials were used here. In the Go-task, children see either an "X" or an "O" (each is equally probable) on the screen and are instructed to press the corresponding key as quickly as possible. This type of simple reaction time task with only two options has many advantages for measuring RTV (Karalunas et al., 2014). This reaction time is measured in milliseconds by the computer clock. In the second half of the task, on 25% of trials, a tone sounds and the child is instructed to withhold his/her response, thus measuring

inhibitory control (in ms). These trials were ignored and their potential effect on RTV on the Go trials is discussed later.

The standard deviation (SD) of each subject's reaction time (i.e. Go trials) was calculated across all trials. Change in SD between at least two time-points (i.e. difference between a subject's first and last SD of reaction time) was calculated as a standard change score, and subsequently served as the outcome used in prediction.

Data analysis of functional brain networks: graph theory

Subsequent methods relied on graph theory for analysis of functional networks that would predict change in RTV. As described in detail by van den Heuvel and Hulshoff Pol (2010), brain networks can be described as graphs composed of nodes that are connected by functionally linked edges. After defining the collection of brain regions that will be represented as nodes, the functional connections, which reflect communication between these nodes, are defined. Within resting-state fMRI studies, the level of co-activation between brain regions is used as a measure of connectivity, defined by the level of correlation between the resting-state fMRI time-series. Using a graph approach, the level of functional connectivity (i.e. correlation) between each possible pair of nodes in the network is computed (i.e. between all possible regions or voxel pairs), resulting in a connectivity matrix. Finally, the existence of a connection between two points can be defined as whether their level of functional connectivity exceeds a certain predefined threshold (van den Heuvel & Hulshoff Pol, 2010). In this study, a predefined threshold was not used. Rather, all connections between regions of interest were examined to

see if patterns emerged that were predictive of change in RTV. This results in modeling the brain as a functional network with connections between regions that are functionally linked.

Support vector based multivariate pattern analysis (MVPA): predicting outcomes using rs-fcMRI

The present study used support vector machine (SVM) and support vector regression (SVR) based MVPA (as previously described by Dosenbach et al., 2010 and Fair et al., 2013) to predict future RTV at the level of the individual. Briefly, SVM based MVPA is an algorithm-based method that uses statistical learning theory to classify individual children. While there are certainly other multivariate pattern analysis methods, this particular machine learning approach was chosen based on the experience of this study's investigators, in addition to existing SVR approaches that have previously been used in other domains, e.g. in the prediction of age using resting-state data alone (Dosenbach et al., 2010), as well as the use of functional connectivity to distinguish between combined vs. inattentive subtypes of ADHD (Fair et al., 2013). Moreover, because functional connectivity MRI data is multi-dimensional, SVM is a useful approach given its ability to manage these dimensions, while generally maintaining resilience to overfitting (Dosenbach et al., 2010). Non-linear functions known as kernels are used to map input vectors onto a higher dimensional feature space. A hyperplane is subsequently designed in the feature space to maximize the margin between two classes of data and thus optimize distinguishing between the two sets.

Details regarding our SVR approach can be seen in Fair et al. (2013). In brief, for all SVR classifications we used epsilon-insensitive SVRs. Parameters were set with $C=\text{Infinity}$, $\text{epsilon}=0.00001$ with $\sigma = 2$ (as in (Dosenbach, et al., 2010)). We use Spider (<http://people.kyb.tuebingen.mpg.de/spider/main.html>), an object orientated environment for machine learning in Matlab (MATLAB 7.1.0, The Mathworks, Natick, MA), for generating the models (Fair et al., 2013).

Predictions of RTV for children with and without ADHD were based on the top 25 functional connections that were consistently predictive in each fold (or round) of leave-one-out-cross-validation (LOOCV). Here, the scan for each subject served as the test sample once, while the remaining scans for all other subjects served as the training sample, creating as many folds of LOOCV as there were subjects. This iterative method of training the sample developed a decision function in MATLAB that was later used to make a prediction about a given test subject. In this way, functional connectivity measurements informed the prediction of change in RTV.

We first used baseline functional connectivity measurements to predict changes in RTV for the sample as a whole (i.e. ADHD and TDC taken together as a single group, $n = 72$) and applied the same parcellation strategy to study the same 99 ROIs in each brain. After extraction of the BOLD time-series for each ROI for each child, next, within each scan, we examined the correlation (Pearson's r) between the BOLD time-series for each ROI with the BOLD time-series for all other ROIs from that scan. These correlation values represented functional connections

in the brain. Correlation values were subsequently placed in a 99x99 matrix for each of the 72 children, where each cell represented the strength of the functional connections between each of the 99 ROIs for that child. Next, each child was removed, one at a time, leaving 71 matrices, where each matrix represented the functional correlation matrix for each of the remaining children.

Change in RTV for each of the remaining 71 children was placed in a vector. Then the aforementioned correlation matrix and RTV vector were used to generate a new matrix, where each cell represented the correlation between functional connectivity and RTV for each of the remaining children. This new matrix was subsequently used to extract the top 25 features based on the strength of their r values, as is done under univariate feature selection. We then used MATLAB to generate a model that uses these top 25 features to predict change in RTV and then tested the model for its ability to predict RTV for the child that was removed. Next, we re-inserted the child that had been removed into the subject pool, removed the next child, and then repeated this process until we had removed each child, generated a model based on all of the remaining children, and put each child back.

Thus, there were 72 rounds (folds) in this process, which generated 72 models and 72 matrices that contained the top 25 features used with each of these models. Those features that consistently came out across all rounds of this process were termed consensus features. Consensus features were subsequently placed in a new matrix, and the r -values across each row were summed (i.e. node strength), as each sum contained the functional connections that

comprised each ROI. This information was then used to map the functional connections back onto the brain using CARET (Version 5.65).

Next, we divided the sample into ADHD only and TDC only and repeated the same methods above for each group. Given $n = 32$ for the ADHD group, 32 models were generated and 32 matrices that contained the top 25 features used with each of these models was considered. Similarly, for the TDC group ($n = 40$), 40 models and 40 matrices that contained the top 25 features for each of these models was derived.

Finally, we performed t-tests between the functional connections that were thought to be predictive in ADHD vs. those that were predictive in TDC, in order to highlight functional connections found to be significantly distinct ($p < 0.05$) between the two groups. In this case, summed t-scores were used, as these scores contained the functional connections between each ROI, in order to map these functional connections back onto the brain as ROIs (analogous to the procedure above using r values).

RESULTS

Baseline functional connectivity measurements have the potential to predict longitudinal changes in reaction time variability (Figure 1a). Predictive functional networks include the cinguloopercular and ventral attention networks. In particular, connectivity between consensus features in the sample as a whole (i.e. ADHD and controls combined), positively predicted changes in RTV in the current sample, such that connectivity between consensus features explained approximately 10% of the variance in change in RTV ($p = 0.0027$; Figure 1b).

However, as demonstrated in Figure 2b, this association is larger in the ADHD group than the control group, at least qualitatively. In the ADHD group, connectivity between consensus features positively predicted changes in RTV more strongly (Adjusted $R^2 = 0.3183$, $p < 0.0001$). When considering only children with ADHD, predictive functional networks include the default and visual networks in addition to the cinguloopercular and ventral attention networks (Figure 2a). Of note, this is a qualitative difference, as the ADHD-only model was not tested against the ADHD+TDC model to denote a comparative difference (which we test more directly below).

Moreover, predictions of RTV also appear to improve in strength qualitatively when considering only TDC (vs. ADHD+TDC taken together as a single group), and predictive functional networks include the cinguloopercular, default, and dorsal attention networks (Figure 3a). Connectivity between consensus features also appears to improve in its ability to explain the variance in predicted changes in RTV on a qualitative level, explaining approximately 28.13% of the variance of changes in RTV ($P < 0.0001$; Figure 3b).

Thus, there is overlap in the brain networks that predict these changes in TDC compared to children with ADHD, particularly between the cinguloopercular and default networks. However, on a qualitative level, there are also certain predictive subregions within these networks that appear to be significantly distinct in predicting changes in RTV in children with ADHD vs. TDC (Figure 4). These subregions include the anterior insula/frontal operculum (within the cinguloopercular network), the inferior parietal subregion (within the frontoparietal network), the lateral parietal and inferior temporal subregions (within the default network), and the ventral vs. dorsal attention networks.

DISCUSSION

In this preliminary study, we used baseline functional connectivity measurements in conjunction with multivariate statistics to predict longitudinal changes in RTV in children with and without ADHD. While there are functional similarities in the neural correlates thought to be predictive of RTV in children with ADHD compared to TDC (e.g. overlap in the cinguloopercular and default networks), there are also innate differences in the functional connectivity of these two populations. These differences lie mostly in specific subregions that are distinct within shared networks, including the anterior insula/frontal operculum (within the cinguloopercular network), the inferior parietal subregion (within the frontoparietal network), the lateral parietal and inferior temporal subregions (within the default network), and the ventral vs. dorsal attention networks. Moreover, these intrinsic differences appear to play a significant role in predicting longitudinal changes in RTV across these two groups, as evidenced by the fact that consensus features become more robust in their predictive ability, at least qualitatively, when the sample (ADHD+TDC) is separated into ADHD alone and TDC alone.

As described in Kofler et al. (2013), there are various etiological models that account for varying RTV as an outcome of ADHD. For example, Attentional Lapse Models describe RTV as an index of breaks in attention (Leth-Steensen et al., 2000). Some of the subregions within brain networks that are thought to be distinct in ADHD vs. TDC in our study, particularly the anterior insula/frontal operculum within the cinguloopercular network, are also suggestive of this, as these are regions of the brain implicated in maintaining alertness and attention (Sadaghiani & D'Esposito, 2014). Additionally, the lateral parietal and inferior temporal subregions within the

default mode network, which are also thought to be distinct in ADHD vs. TDC in our study, might also support models that consider RTV to represent lapses in attention or deficits in maintaining arousal, as the default mode network is most active when the mind wanders and is not engaged in a specific task (Karalunas et al., 2014). In fact, as described by Kofler et al. (2013) and proposed by Sonuga-Barke & Castellanos (2007), it is these regular oscillations in resting-state networks that lead to frequent lapses of attention in ADHD under the Default Mode Network Model of ADHD.

Of note, while the demographics of our subjects are grossly similar, we did not control for potential confounders, e.g. sex, age, race, SES, etc., in order to conserve power given our relatively modest sample size. Therefore it is possible that these confounders contribute to the magnitude of findings though it is unlikely that they explain the entirety of this magnitude. Moreover, learning changes functional connectivity and the resulting spontaneous activity of the human brain (Lewis et al., 2009). As such, rs-fcMRI serves as a reflection of the summed experiences of a given individual (Lewis et al., 2009), and it is plausible that these experiences are in part shaped by these potential confounders. What we have shown are regions of the brain that are predictive of this behavioral change, and these precursors may have had an effect on these brain regions or the functional connections between them. Thus, these brain regions serve as gross predictors and thus downstream, potential biomarkers, with the effects of these potential confounders inherent in them.

Additionally, 7 out of our 72 subjects were sibling pairs, creating a mixed sibling, non-independent design. There was no matching between cases and controls whom were unrelated, as the primary aim of this study was to use the brain to predict changes in RTV within participants in childhood, regardless of age, gender, and diagnosis. This study was not examining how the brain changes with RTV, which would necessitate matching. However, matching does become relevant in the secondary aim of determining how the models behave when considering ADHD-only and TDC-only. While the groups were generally matched based on age and race, gender does appear slightly skewed, and secondary analyses should control for gender as well.

Furthermore, RTV is a measure composed of several different parameters. For example, Karalunas et al. (2014) note that several different cognitive processes, including stimulus encoding, information processing (the speed of which is also affected by variables such as motivation, level of arousal, etc.), and motor preparation are involved in RTV, and separating RTV into these components is often completed using various statistical models. One such model is the diffusion model, which focuses on several parameters including drift rate. Drift rate is described as the rate at which information is collected in relation to the criteria an individual has set to elicit a response. In the diffusion model, slower drift rates denote information processing that is slower and less efficient. Children with ADHD are known to have slower drift rates, and this is said to, at least in part, explain increased RTV in children with ADHD (Karalunas et al., 2014). Therefore, while our results suggest a model to predict future RTV, our results are

not specific as to what is being predicted, and future studies may elicit better specific parameters of RTV to be used in prediction.

Moreover, individuals with ADHD are known to exhibit decreased post-error slowing compared to TDC, such that after making an error, controls have a tendency to slow subsequent reaction times as an adaptive cognitive mechanism in order to prevent further errors (Balogh & Czobor, 2014). The differential post-error slowing that is typical of children with ADHD compared to TDC was not controlled for in this study. As a result, it is possible that this difference in post-error slowing between the two groups could also confound estimated differences in RTV between these groups. Subsequent analyses should determine whether within a particular dataset, this differential post-error slowing confounds results.

While we recognize that this work has limited cross-replication given its small power, this work is still promising, at least as a stepping stone, in improving our assessment of cognitive development as it relates to RTV, both in children with and without ADHD. It is important to emphasize that this is a preliminary study illustrating proof of concept, and as such, we recognize that a larger sample size is necessary in order to test these conclusions, with a true training set and a true test set, so that LOOCV does not become necessary. Moreover, because we used LOOCV, when examining the sample as a whole, for example, we derived 72 models. Yet, in order for this to be truly clinically useful, we would need 1 model. Ultimately, however, given these limitations, this work is important from a more global perspective - if we can predict a given child's RTV trajectory, then we have the capacity to ultimately stratify children

according to risk, and to eventually create timely, targeted interventions to prevent long-term impairments in RTV. Thus, this work has the potential to characterize an approach aimed at identifying children at high-risk of deficits in future cognitive performance and thus associated life outcomes such as academic achievement.

SUMMARY & CONCLUSIONS

Overall we find that baseline rs-fcMRI has the potential to predict longitudinal changes in RTV in children with ADHD, as well as TDC. The functional connections that predict RTV in these two groups overlap in some regions, but are significantly distinct in other regions. These intrinsic differences in the functional connectivity of these two populations appear to play a significant role in predicting longitudinal reaction time variability across these two groups. Future work will be needed to confirm these findings, but the current results provide a needed and important stepping stone to using non-invasive imaging as a prognosticator of future cognitive functions in ADHD.

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APPENDIX

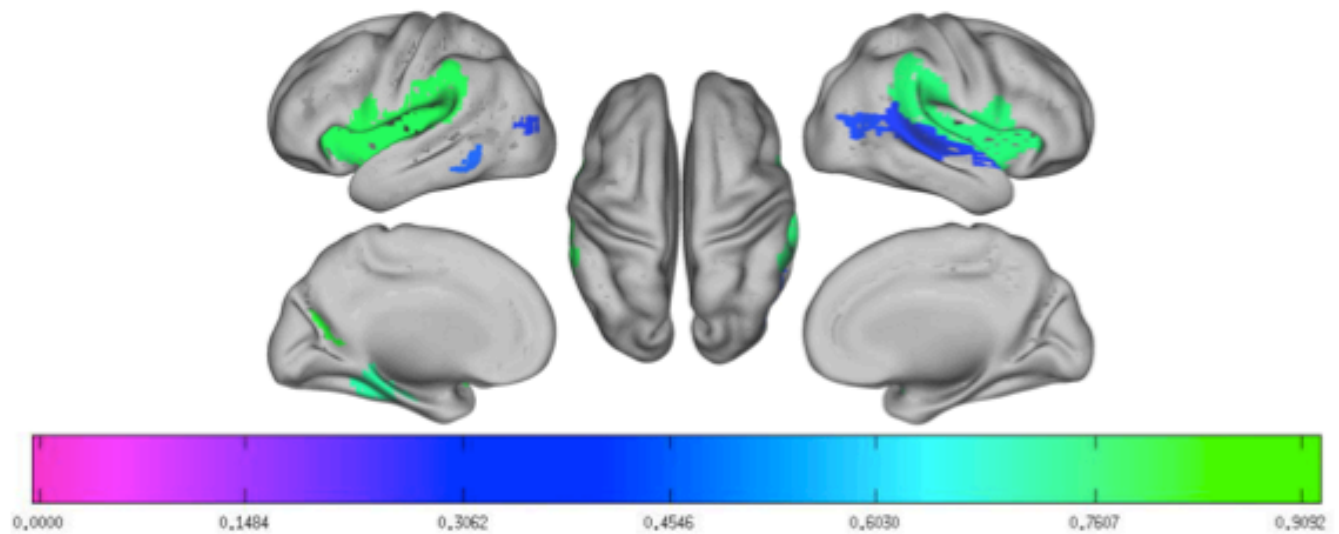


Figure 1a

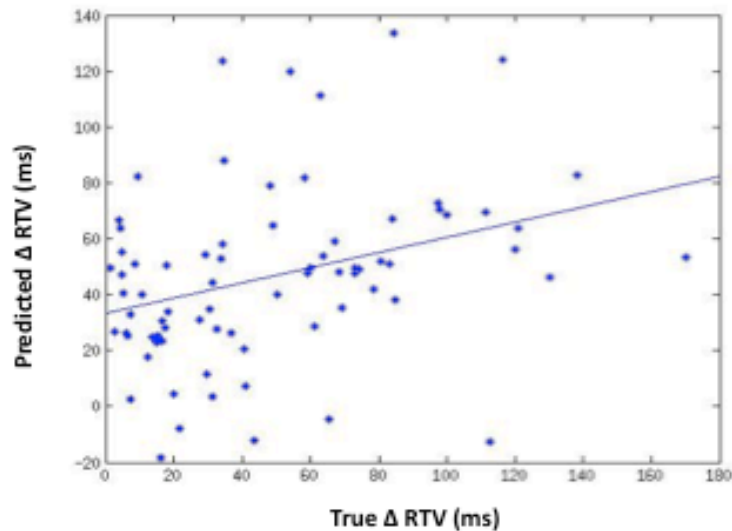


Figure 1b

Figure 1 a) Regions represent functional connections found to be consensus features in prediction. Each ROI served as a node in determining patterns of regions that were predictive. To determine the connection strength in each node, the strength assigned to an individual functional connection (i.e. between the two ROIs that make up that functional connection) was summed. Therefore, nodes with the greatest predictive connectivity are assigned a greater strength and are represented in green. Predictive functional networks include the cinguloopercular and ventral attention networks.

b) The regression line on the scatterplot reflects predicted vs. true change in reaction time variability (in milliseconds), where each subject is represented by an asterisk. Connectivity between consensus features positively predicts changes in reaction time variability in the current sample (Adjusted $R^2 = 0.10$, $p = 0.0027$).

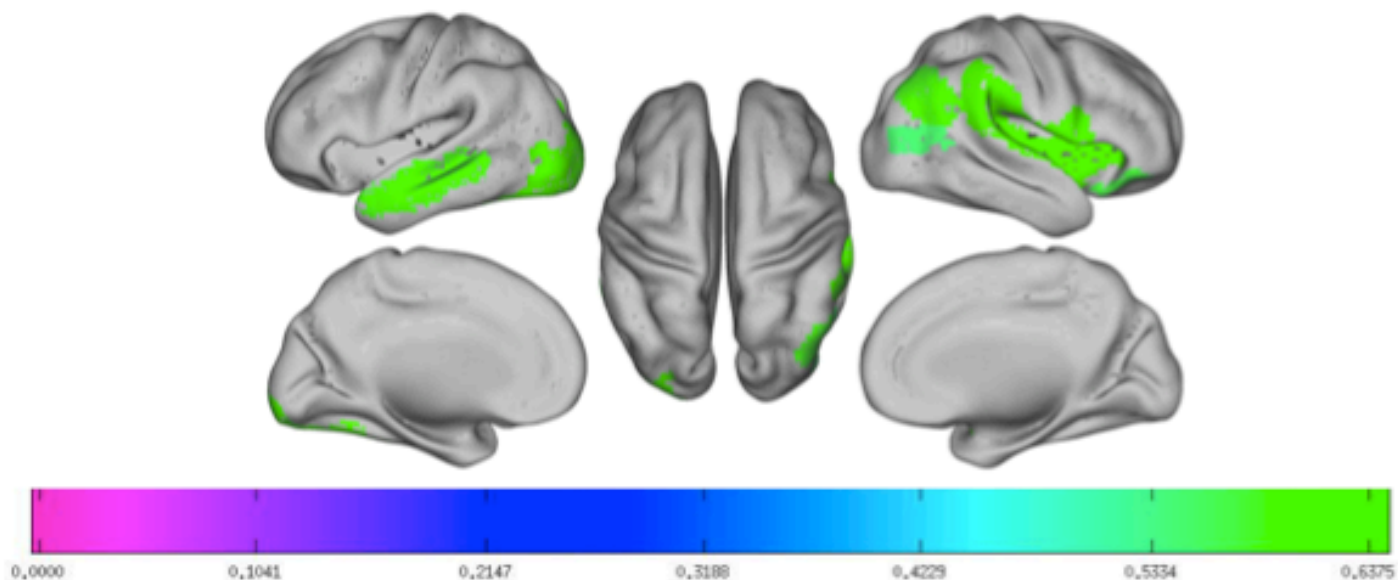


Figure 2a

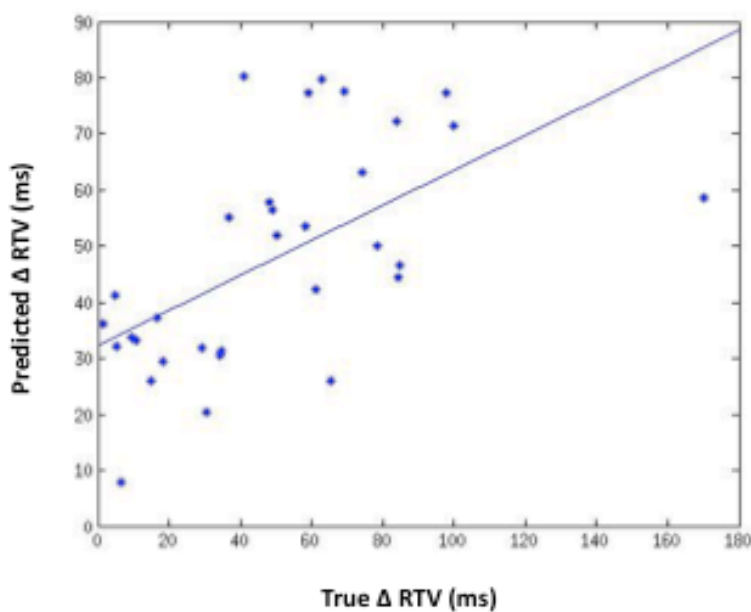


Figure 2b

Figure 2 a) When considering only children with ADHD, predictive functional networks improve in strength and include the cinguloopercular, default, visual, and ventral attention networks. **b)** Connectivity between consensus features also improves in its ability to positively predict changes in reaction time variability when the sample only includes children with ADHD (Adjusted $R^2 = 0.3183$, $p < 0.0001$).

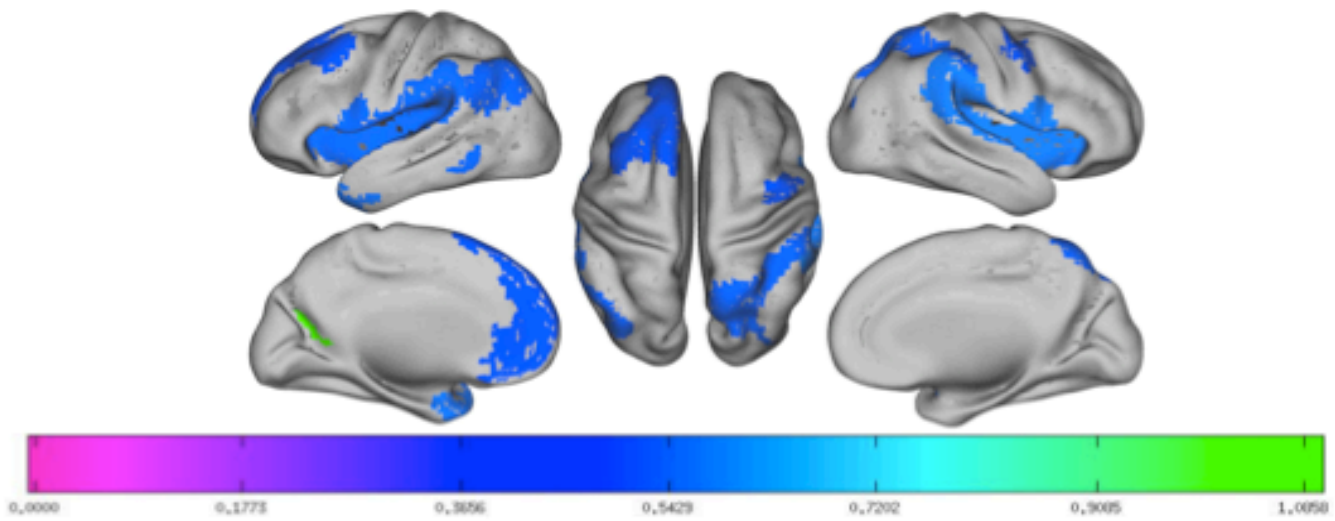


Figure 3a

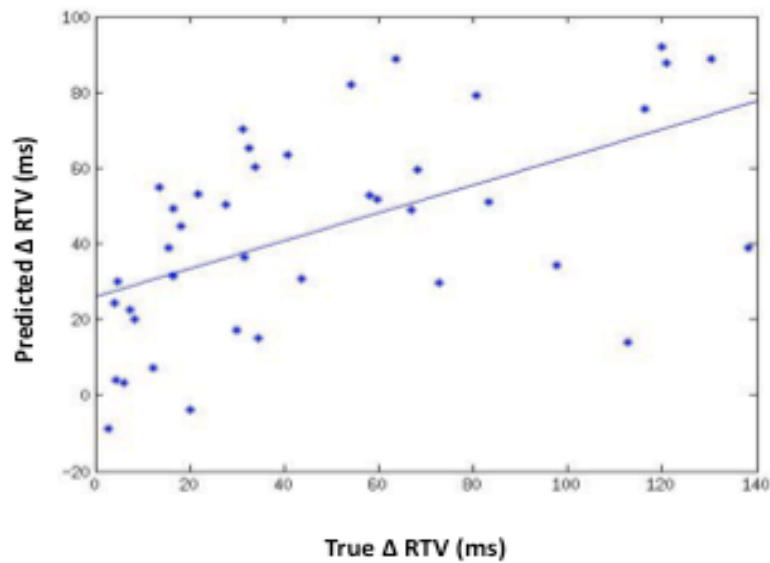


Figure 3b

Figure 3 a) When considering only TDC, predictive functional networks improve in strength (relative to ADHD & TDC taken together as a single group) and include the cinguloopercular, default, and dorsal attention networks, while certain predictive subregions are distinct in TDC. **b)** Connectivity between consensus features improves in its ability to positively predict changes in reaction time variability when the sample only includes TDC (Adjusted $R^2 = 0.2813$, $p < 0.0001$).

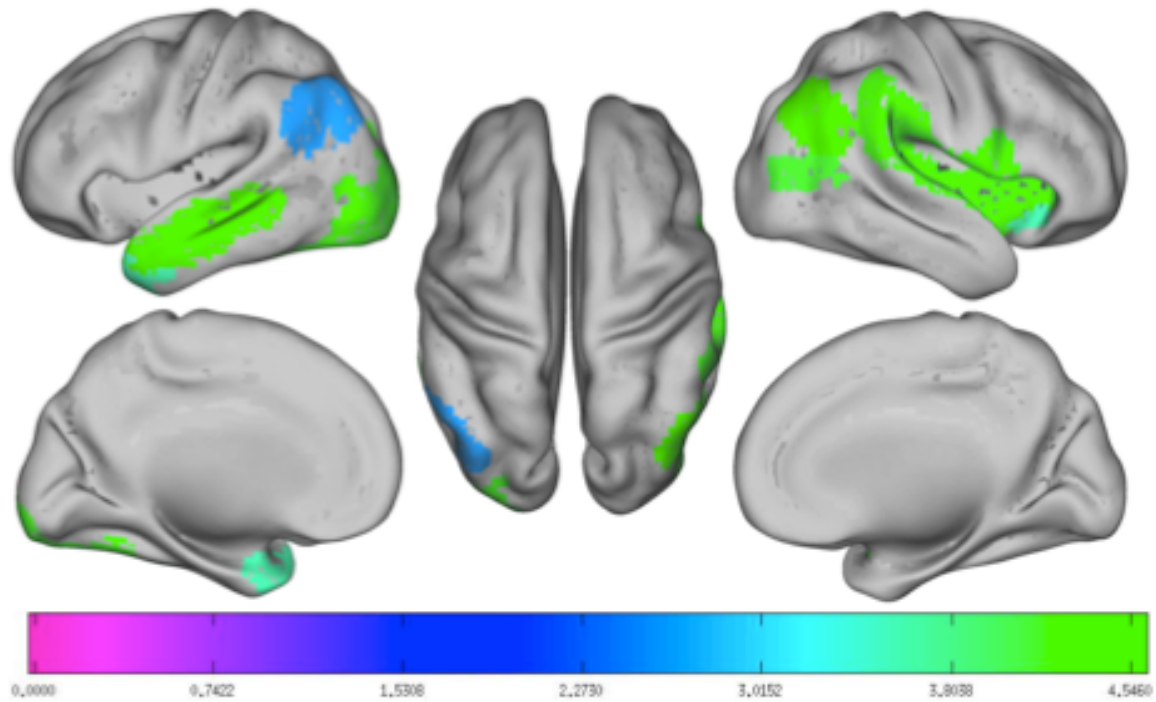


Figure 4

Figure 4) Group comparison between predictive networks in ADHD vs. TDC reveals a degree of overlap (particularly between the cinguloopercular and default networks), while uncovering specific distinct subregions within these networks. These subregions include the anterior insula/frontal operculum (within the cinguloopercular network), the inferior parietal subregion (within the frontoparietal network), the lateral parietal and inferior temporal subregions (within the default network), and the ventral vs. dorsal attention networks.