Large-Scale Brain Organization During Cognitive Control in ADHD

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Complete Title: Large-Scale Brain Organization During Cognitive Control in ADHD

Short Title: Large-Scale Brain Organization During Cognitive Control in ADHD

Drug or Device Name(s): Methylphenidate

Sponsor: University of North Carolina, Chapel Hill

Protocol Date: 05/15/2020

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I confirm that I have read this protocol and understand it.

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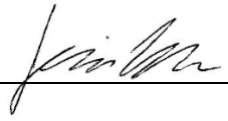
Principal Investigator Signature: 

Date: 5/15/20

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ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AAL	Automatic Anatomical Labeling
ADHD	Attention-deficit/hyperactivity disorder
ARI	Affective Reactivity Index
ASD	Autism spectrum disorder
BIS/BAS	Behavioral Inhibition System/Behavioral Approach System
BRIC	Biomedical Research Imaging Center
BRIEF	Behavior Rating Inventory of Executive Functioning
BSSS-C	Brief Sensation Seeking Scale for Children
CBCL	Child Behavior Checklist
CDW-H	Carolina Data Warehouse for Health
CON	Cingulo-opercular network
CRS-3	Conners' Rating Scale-3
CV	Coefficient of Variation
dB	Decibels
DAN	Dorsal Attention Network
DCC	Dynamic Conditional Correlation
DISC-IV	Diagnostic Interview Schedule for Children 4
DMN	Default Mode Network
DOSPERT	Domain-Specific Risk-Taking
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders 4
ERC	Emotion Regulation Checklist
FDA	Food and Drug Administration
fMRI	Functional MRI
FPN	Fronto-parietal Network
FSIQ	Full Scale Intelligence Quotient
GNG	Go/no-go
HIPAA	Health Insurance Portability and Accountability Act
IDS	Investigational Drug Services
MD	Doctor of Medicine
MRI	Magnetic resonance imaging
MPRAGE	Magnetization-Prepared Rapid Gradient Echo
PDS	Pubertal Development Scale
PRI	Perceptual Reasoning Index
REDCap	Research Electronic Data Capture
ROI	Region of Interest
RT	Reaction Time
SCQ	Social Communication Questionnaire
SES	Socioeconomic Status
SNAP-IV	Swanson, Nolan and Pelham Questionnaire 4
SRT	Serial Reaction Time
TD	Typically developing

TPN	Task-positive Network
UPPS-P	Urgency, Premeditation, Perseverance, Sensation Seeking and Positive Urgency
VAN	Ventral Attention Network
VCI	Verbal Comprehension Index
WIAT-III	Wechsler Individual Achievement Test - Third Edition
WISC-V	Wechsler Intelligence Scale for Children - Fifth Edition

PROTOCOL SYNOPSIS

Study Title	Large-Scale Brain Organization During Cognitive Control in ADHD
Funder	National Institute of Mental Health (NIMH)
Clinical Phase	Early Phase 1
Study Rationale	Attention deficit hyperactivity disorder (ADHD) is the most commonly diagnosed developmental disorder of childhood, affecting approximately 8% of children worldwide. ADHD is a great public health concern as its core symptoms, which include increased impulsivity/hyperactivity and difficulty in sustaining attention, increase the risk for poor academic achievement, substance abuse, and criminal behavior. Research into the neural basis of ADHD is crucial to improve early detection and treatment of the disorder. We propose to use innovative imaging methods to examine ADHD-associated dysfunction in brain network dynamics and to quantify how stimulant medication normalizes that dysfunction. The findings will lead to the development of biomarkers for early detection and more effective treatments of the disorder.
Study Objective(s)	Primary • To assess network topology during resting state and go/no-go (GNG) tasks using functional connectivity estimates • To assess the reconfiguration of network topology between the GNG tasks and the resting state • To assess how changes in functional connectivity and brain network topology relate to improvements in behavioral performance on the GNG task Secondary • To evaluate commission errors, omission errors, and response time variability assessed during the GNG tasks
Test Article(s)	Methylphenidate (FDA approved)
Study Design	This is a randomized, double blind, placebo-controlled study of the effects of methylphenidate on brain organization and behavior in children with ADHD. It will consist of multiple testing sessions. After a telephone screening that consists of basic screening and a diagnostic interview with a parent or guardian, electronic

	questionnaires will be sent to the parent or guardian and to a non-parent adult to confirm diagnosis and ask additional questions about the subject. Next, an initial in-person session will occur during which consent will be obtained. This session will include neuropsychological assessments, behavioral testing , and mock scan preparation to confirm eligibility. If eligible, the interventional phase will consist of one MRI scan on placebo and one MRI scan on methylphenidate, counterbalanced.
Subject Population **key criteria for Inclusion and Exclusion:**	Inclusion Criteria 1. Between 8-12 years old 2. Diagnosis of ADHD; ADHD group only can have comorbid Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) diagnoses of oppositional defiant disorder, conduct disorder, depressive disorders, or anxiety disorders 3. ADHD subjects must never have been treated with medication for their ADHD Exclusion Criteria 1. Wechsler Intelligence Scale for Children-Fifth Edition Full-Scale Intelligence Quotient (FSIQ) < 80 2. Wechsler Individual Achievement Test-Third Edition Word Reading < 85 3. Any neurologic or developmental disabilities 4. Any reading or learning disabilities 5. Visual impairment that cannot be corrected-to-normal 6. Color blindness 7. Documented hearing impairment greater than 25 decibels (dB) loss in either year 8. Have already gone through puberty (Tanner Stage II or higher) 9. Medical contraindication to MRI 10. Any psychoactive medication
Number Of Subjects	32 (anticipated)
Study Duration	Each subject's participation will last for approximately 10 hours across three visits: 6 hours during the behavioral visit and 2 hours for each of the two interventional visits. The entire study is expected to last 3.5 years.
Study Phases **Screening** **Study Treatment** **Follow-Up**	1. Screening: Screening for eligibility will occur via telephone interview. 2. Parent and Non-Parent Adult Online Surveys: Links to parent and non-parent adult questionnaires will be e-mailed to be completed securely online via REDCap. 3. Behavioral Session: One in-person study session involving consent, neuropsychological testing, self-report

	questionnaires, introduction to mock scanner and behavioral tasks, and behavioral testing; if parent questionnaires are incomplete, parent may complete them during this session. 4. Interventional Sessions: Two in-person sessions involving drug or placebo administration and an MRI scan. Each session will be approximately one week apart.
Efficacy Evaluations	Change in brain network topology and improved performance on a GNG task
Pharmacokinetic Evaluations	N/A
Safety Evaluations	The safety of the subjects will be ensured by trained research staff and the MD collaborator, with the supervision of the PI. All subjects will have undergone a physical examination by an MD collaborator to ensure they can safely take this single dose of medication, and will be adequately monitored for 24-36 hours following the administration of methylphenidate to ensure no adverse side effects. If the parents report that any side effect has not resolved at the time the survey is completed, the survey will instruct parents to page the MD collaborator. The MD collaborator or a trained designee will review every survey within 24 hours of its completion by the parents to ensure no ongoing side effects, and the MD collaborator will contact the parents if a side effect is reported as not yet resolved, even if the parent does not page the MD collaborator. There is a medium probability of the occurrence of a low-severity event that is completely reversible (e.g., trouble sleeping, loss of appetite, etc.), and very low probability of a more serious adverse event.
Statistical And Analytic Plan	To address primary objectives, we will calculate brain metrics of interest using standard functional connectivity and graph theory methodology. To address secondary objectives, we will calculate behavioral metrics of interest using data from the GNG tasks. Paired t-tests comparing methylphenidate session to placebo session will test for within-subject improvements in network organization and behavior. All tests will be conducted with a significance level of 0.05 correcting for multiple comparisons. We will report the magnitude of all statistical effects, as well as confidence intervals for all analyses conducted, as p-values greater than .05 cannot be interpreted as anything other than a lack of statistical significance
DATA AND SAFETY MONITORING PLAN	The PI and project coordinator will be responsible for data quality management and ongoing assessment of safety. All data collected for the study will be stored on secure, password-protected servers maintained by the Department of Psychology and Neuroscience or

	on secure, password-protected computers in the laboratory. Safety monitoring will be provided by the PI, the MD collaborator, and the project coordinator.

1 BACKGROUND AND RATIONALE

1.1 Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a debilitating disorder characterized by difficulty in sustaining attention and/or increased hyperactivity and impulsivity. ADHD is highly prevalent, affecting approximately 8% of children worldwide, and it dramatically increases the risk for poor academic achievement, substance abuse, and criminal behavior. Longitudinal studies suggest that current pharmacologic and behavioral approaches, while effective in the short term, do not substantially reduce long-term adverse social outcomes. There is therefore tremendous need to better understand the biological substrates of ADHD, which can lead to more targeted diagnosis and treatment for individuals with the disorder. Neuroimaging is currently being utilized to identify neural mechanisms contributing to ADHD. Research thus far has primarily focused on specific brain regions, yet structural and functional anomalies are consistently found distributed across the entire brain.

Recent advances in neuroimaging methods have allowed researchers to move beyond examining dysfunction limited to individual brain regions to explore dysfunctional communication between brain regions. The overall goal of this study is to apply these innovative methods to better understand the neural basis of ADHD, focusing on dysfunction in network organization and determining how that relates to deficits in behavior and symptoms in children with the disorder. It has been proposed that ADHD may be a disorder of brain connectivity. Despite evidence that abnormal development and function distributed across the entire brain may underlie ADHD, investigations thus far have focused on connectivity patterns limited to specific networks or pairs of regions. For example, much research has focused on connections between the default mode network (DMN) and task-positive networks (TPNs), such as the cognitive-control related fronto-parietal network (FPN), dorsal attention network (DAN), ventral attention network (VAN), and cingulo-opercular network (CON). The current study will take advantage of the recent application of graph theoretical tools from mathematics to functional neuroimaging data, which will allow us to expand these relatively localized examinations of dysfunctional network organization in ADHD to the entire brain. The major goals of this study characterize network organization and dynamics—how network organization *changes*, or reconfigures,

from an intrinsic state (i.e., during rest) when confronted with cognitive control demands, as well as in response to the administration of stimulant medication in children with ADHD.

This study will allow for a more specific characterization of dysfunction in network dynamics that relates to global organization in the brains of children with ADHD, and how this dysfunction relates to behavior. This knowledge may lead to improved success in early diagnosis and the development of treatments targeting the dysfunctional systems, reducing the burden of the disorder both for the patients themselves and in terms of healthcare costs.

1.2 Name and Description of Investigational Product or Intervention

This study entails a one-time dose of short-acting methylphenidate to assess its immediate/short-term effects on brain connectivity and on behavior. As the subjects enrolled in the medication challenge are medication-naïve, the administration of methylphenidate departs from the subject's routine clinical care.

1.3 Non-Clinical and Clinical Study Findings

Methylphenidate (Ritalin, Metadate, Concerta) is FDA-approved for treatment of ADHD in children and adolescents. It is used as part of routine clinical care for ADHD and is increasingly used in preschoolers. FDA-approved single dose range is between 5mg and 30mg for children aged 8-12 (daily range 10mg-60mg); subjects in this study will receive a dose within the FDA-approved single dose range. Minimal increases in blood pressure and heart rate, anorexia, insomnia, rash, and tics have been reported during oral methylphenidate administration. Long-term effects are not pertinent as this is a single dose study. Administration of methylphenidate vs. placebo will take place in the research laboratories of the Biomedical Research Imaging Center (BRIC) at the UNC School of Medicine, in a clinical environment and within a few minutes walk to the hospital's emergency room. Parents will be advised of possible side effects that, if they do occur, should be transient and mild. Mild side effects of methylphenidate are infrequent (occurring between 1-10% of the time), and serious adverse effects are extremely rare (< 1%). The MD collaborator and other members of the on-site study team will be available to monitor for any side effects after medication administration and the MD collaborator or designee will be available by cell phone for 24-36 hours after the study visit for any questions about adverse events related to study medication.

1.4 Relevant Literature and Data

ADHD is the most commonly diagnosed developmental disorder of childhood, and can cause widespread deficits and incur staggering healthcare costs [1]. Uncovering the neurological basis of ADHD is critical to improving early diagnosis and treatment. It is thought that dysfunctional connections between brain regions may underlie ADHD [2]. This study aims to identify dysfunctional functional connectivity in children with ADHD and how that underlies deficits in behavior and ADHD-related symptomatology.

While promising, research into dysfunctional connectivity patterns in ADHD has been limited by either: 1) focusing on the resting state (when a subject is lying in the scanner and otherwise not engaged in a task) instead of taking cognition-related network structure into account; or 2) probing specific networks or connections, despite evidence for diffuse alterations in brain structure and function across the entire brain [2]. Mathematical tools based on graph theory have recently emerged as a powerful method to quantify the complexities of global, local, and dynamic properties of large-scale brain

networks using functional connectivity measurements [3]. Such tools are crucial for studying the distributed disruption of functional connectivity observed in ADHD.

This study will apply graph theoretical tools to functional connectivity estimates across the entire brain while children are engaged in cognitive control processes that are core deficits of ADHD: attentional and response control [4]. We will extend research that has found that stimulants improve both behavior and functional connectivity across specific connections in children with ADHD [5–7], with a goal of identifying changes in network dynamics (i.e., organization and reconfiguration), behavior, and symptomatology that are associated with stimulant administration in medically-naive children with ADHD. By implementing graph theoretical analyses we will capture the complexity of network dynamics across the entire brain.

In sum, this research will characterize dysfunction in whole-brain network dynamics in ADHD and increase understanding of how stimulant medication can normalize this dysfunction. This will provide novel insights into the function of cognitive control networks in ADHD, establishing biomarkers to facilitate early detection and providing a foundation for research into both pharmacological and behavioral interventions targeting dysfunctional systems.

2 STUDY OBJECTIVE

The purpose of this study is to examine the changes in brain organization (intrinsic organization, task-related organization, and task-related reconfiguration) that result from stimulant administration in children with ADHD and how those changes relate to changes in behavior.

2.1 Primary Objectives

- To assess network topology during resting state and GNG tasks using functional connectivity estimates
- To assess the reconfiguration of network topology between the GNG tasks and the resting state
- To assess how changes in functional connectivity and brain network topology relate to improvements in behavioral performance on the GNG task

2.2 Secondary Objective

- To evaluate commission errors, omission errors, and response time variability assessed during the GNG tasks

3 INVESTIGATIONAL PLAN (brief overview)

3.1 Study Design

This is a randomized, double blind, placebo-controlled study of the effects of methylphenidate on brain organization and behavior in children with ADHD. The study phases include:

Screening: Screening for eligibility will occur via telephone interview.

Parent and Non-Parent Adult Online Surveys: Links to parent and non-parent adult questionnaires will be e-mailed to be completed securely online via REDCap.

Behavioral Session: One in-person study session involving consent, neuropsychological testing, self-report questionnaires, introduction to mock scanner and behavioral tasks, and behavioral testing; if parent questionnaires are incomplete, parent may complete them during this session. This session will last approximately 6 hours, including lunch and breaks.

Interventional Sessions: Two in-person sessions involving drug or placebo administration and an MRI scan. Each session will be approximately one week apart. Each session will last approximately 2 hours.

3.2 Allocation to Treatment Groups and Blinding (if applicable)

A study team member not directly involved in subject testing will assign methylphenidate/placebo order to each subject in a pseudo-random fashion, with the only constraint being that 50% of subjects receive placebo first, and 50% of subjects receive methylphenidate first, with gender being balanced (i.e., 50% of females will receive placebo first, and 50% of males will receive placebo first). The MD collaborator who is monitoring safety and possible adverse events while the subjects are on methylphenidate or placebo will be unblinded during the study and therefore able to identify which pill was taken if there are any significant adverse effects felt by the subject after taking the pill. The MD collaborator will not have direct contact with subjects during the experimental visits. Thus, no study team member who interacts with subjects or their families on the methylphenidate/placebo days will know whether a subject is on placebo or methylphenidate. After data collection for the medication challenge portion of the study is completed, the team member who made order assignments will reveal participation order (placebo/methylphenidate or methylphenidate /placebo) so appropriate analyses can be conducted.

3.3 Study Duration, Enrollment and Number of Subjects

This study is expected to be active for 3.5 years, and to enroll 32 subjects. Each subject's participation will last for approximately 10 hours across three visits: 6 hours during the behavioral visit and 2 hours for each of the two interventional visits.

3.4 Study Population

Children must be between the ages of 8 years, 0 months and 12 years, 11 months, 30 days. They will be excluded if they have any of the following, established via review of medical and developmental history: 1) diagnosis of Mental Retardation, Developmental Language Disorder, Reading Disability, Autism Spectrum Disorder, or a Pervasive Developmental Disorder; 2) visual impairment, including color blindness that will be ruled out using Ishihara plates because the Go/No-go tasks involve the use of colored stimuli; 3) neurologic disorder (e.g., epilepsy, cerebral palsy, traumatic brain injury, Tourette syndrome); 4) documented hearing impairment greater than 25 dB loss in either ear; 5) have already gone through puberty (as assessed via responses on the Pubertal Development Scale; for girls, this means that in addition to other physical developmental stages they have experienced menarche); and 6) medical contraindication to MRI (e.g., implanted electrical devices, dental braces). In addition, children will be excluded if there exists history of speech/language disorder or a Reading Disability

(RD), based on prior school assessment completed within one year of enrollment in the study, or if our own examination reveals a statistically significant discrepancy between a child's Full Scale IQ score as determined using the WISC-V and his/her Word Reading subtest score from the WIAT-III, or a standard score below 85 on the Word Reading Subtest, regardless of IQ score.

Research Criteria for ADHD: The diagnosis of ADHD will be based on DSM-IV criteria (ADHD/Inattentive Type, Hyperactive/Impulsive Type, and Combined Type, at the time of assessment) using a structured parent interview–the Diagnostic Interview Schedule for Children, version 4 (DISC-IV). The DISC-IV will be administered via telephone after an oral consent process by a highly trained research assistant under the supervision of a clinical psychologist, Dr. Margaret Sheridan. Dr. Sheridan was trained by the developers of the DISC-IV in administration and scoring. The DISC-IV is a computerized diagnostic interview designed to be used by non-psychologists (such as study staff) for the purposes of identifying children who appear to meet criteria for specific psychological disorders. It does not require specialized training aside from the training that will be provided by Dr. Sheridan. DSM-IV criteria requires that symptoms be present in at least two settings; therefore, two questionnaires/rating scales will be administered to each child's parent as well as a selected non-parent adult, who can be a teacher, coach, tutor, club leader, baby-sitter, or someone in another comparable role. The Conners Parent Rating Scale, Third Edition, will be administered to each child's parent; the Conners Teacher Rating Scale, Third Edition will be administered to each child's non-parent adult. The Parent and Teacher versions of the Swanson, Nolan and Pelham Questionnaire, Version 4 will be administered to the parent and the non-parent adult respectively. While the two non-parent adult versions are referred to as Teacher versions, they can be appropriately completed by any adult who knows the child well. Subjects must meet criteria for ADHD on at least one of the two parent questionnaires/rating scales and on at least one of the two non-parent adult questionnaires/rating scales. Following this, the child must meet criteria on the DISC-IV as described above. The results of the questionnaires/rating scales will also be considered as measures of ADHD symptom severity, available for use in statistical analyses examining effect of diagnosis on behavioral and imaging findings, as well as impact of intervention for the subjects enrolled in the medication challenge.

Subjects with ADHD will be still be included from this study if there are any additional psychiatric diagnoses (or sub-threshold diagnoses) including major depression, bipolar disorder, conduct disorder, adjustment disorder, obsessive-compulsive disorder and other anxiety disorders based on parent responses from the DISC-IV. However, subjects with autism spectrum disorder and/or reading disability are being excluded so that we can examine neurologic abnormalities associated specifically with ADHD.

Only medication-naïve children with ADHD are eligible for this study. Subjects will undergo a physical examination to ensure that they can safely take a single dose of a stimulant. The physical will involve a comprehensive examination of all major systems, with an emphasis on cardiac status. Additional exclusion criteria include: hypertension (either systolic or diastolic blood pressure above the 95th percentile on two separate measurements separated by at least 10 minutes), history of seizures or seizure disorder, significant arrhythmia or history of EKG abnormality, and liver disease. The physical exam will be conducted by the MD collaborator. If the subject is deemed eligible, the MD collaborator will write the prescription. The subject will be excluded from the study if the MD collaborator determines that the child cannot safely take a single dose of a stimulant. Further, subjects must be able to swallow capsules in order to be included in this portion of the study.

4 STUDY PROCEDURES (what will be done)

4.1 Screening/Baseline Visit procedures

Prior to initial entry, all children recruited will undergo an initial screening lasting 15 to 20 minutes with a parent to determine their general suitability for inclusion or exclusion, including information about MRI eligibility and the medication challenge (ADHD subjects only). Before the screening questions are administered to the parent, oral consent will be obtained. If it seems likely that the child meets criteria for inclusion in the study based on the parent's answers to the screening questions, following a second oral consent process a parent will be administered the Diagnostic Interview Schedule for Children, version 4 (DISC-IV) via phone by a trained study examiner. This will last approximately 30-90 minutes and may occur in the same phone call as the screening, or in a separate phone call.

Parents of eligible children who wish to participate in this study will be e-mailed a web link to questionnaires/rating scales to be completed securely online via REDCap, as will a selected non-parent adult. The e-mail to parents will also include a link to an informational video about MRI with instructions to show it to their child to ensure that both the parent and child are interested in the child having an MRI scan (https://www.youtube.com/watch?v=duQR23cR5Gs). It will be requested of the parent to complete the questionnaires in advance. The e-mail to the non-parent adult will indicate that, by completing the questionnaires and mailing them in, he/she provides implicit consent. It will be requested that the non-parent adult complete the questionnaires before the first in-person session. Parents, if they agree, will provide contact information for the non-parent adult so study personnel can follow up to ensure receipt of the questionnaires. If the parent does not complete the questionnaires in advance, the parent will have the opportunity to complete them during the initial in-person study visit. If, based on initial screening, the child's eligibility is unclear, the parent will receive a bonus for both completing his/her questionnaires and for ensuring that the non-parent adult completes his/hers.

The in-person behavioral day of testing will last approximately 6 hours (including approximately 4 hours of testing, lunch, and breaks). This session will determine the appropriateness of each subject's inclusion in the study and involves the following:

- Consent/Assent (~30 m)
- Physical Exam (~15 m; including assessment of comfort with pill swallowing)
- Neuropsychology Testing for eligibility (~60 m)
- Parent questionnaires collected and reviewed for completion (~30 m)
- Mock MRI Training (~60 m)
- Training on/Performance of Behavioral Tasks (~60 m)

The administration of behavioral tasks both inside of and outside of the MRI machine will be standardized; this is discussed in detail below. The administration of all interviews and cognitive testing will also be standardized.

4.2 Intervention/Treatment procedures (by visits)

The interventional visits are two additional testing sessions that include MRI scans. The two sessions will be approximately one week apart, and will last approximately 2 hours each. Subjects will come in for one session on placebo and one session on methylphenidate (0.3 mg/kg, in a pseudo-random, double-blind, crossover design). They will be administered in identical capsules to ensure that members of the study team and subjects are blind to the identity of the medication until after completion of the study. Medication/placebo will be administered with food at the start of the testing session. If subjects have trouble swallowing the pills, the researcher will provide an Oraflo cup, which is a cup with a spout specifically designed to make it easier to swallow a pill with water or juice. Oraflo cups have been demonstrated to make pill swallowing easier for individuals who typically have trouble swallowing them. The time of day for administration of all testing (including MRI scanning) will be standardized. Furthermore, the administration of behavioral tasks inside of the MRI machine will be standardized.

4.3 Follow- up procedures (by visits)

We will request that parent(s) fill out a survey 24-36 hours after intake of methylphenidate/placebo to ensure safe monitoring of potential (but unexpected) adverse side effects. The 24-36 hour range allows for monitoring to last at least 24 hours, but accepts evening responses if the parent(s) work and cannot fill the survey out during the work day of the day following methylphenidate/placebo administration. To increase convenience (and therefore compliance), we will provide parents with a link to an online survey via REDCap (Research Electronic Data Capture), a user-friendly and secure online resource for collecting, organizing, and storing survey data. Parent(s) will therefore be able to answer a series of questions probing their child's response to the methylphenidate/placebo to ensure that there are no adverse side effects. A member of the study team will send out a reminder to the parent at 24 hours after methylphenidate/placebo administration, and again at 36 hours if the survey has not yet been completed. If the survey is not completed the day after the testing session, the parent(s) will be called by a study team member the day following to ensure the safety of the subject. The MD collaborator will review survey responses within 24 hours of survey completion to ensure safety and will be available via pager during that 24-36 hour period if a subject or parent becomes concerned about the effects of the medication.

There are no plans to follow-up with subjects beyond monitoring potential adverse side effects of the medication through the day following drug administration in subjects enrolled in the medication challenge.

4.4 Subject Completion/ Withdrawal procedures

Compensation will be based on session instead of hourly so as to not rush the subjects or the experimenters. Therefore, child subjects who participate in the behavioral testing session but are found to not be eligible for the MRI session will receive a check for $40.00 four to six weeks after testing to help defer expenses related to participation in this study, such as travel to and from the institute and any meals purchased during the testing day. Subjects who participate in the intervention portion of the study will receive $60.00 per MRI session. Therefore, if they complete the entire study they will receive a check for $160.00 four to six weeks after completion of participation. For any

session that is terminated early, the subject will get compensated for the entirety of that session, but will not receive compensation for any sessions the subject did not begin. Since subjects completing all testing sessions is particularly important for this within-subject design, the intervention portion of the study in particular, subjects and their parent who complete the study in its entirety will receive a $50.00 completion bonus. Furthermore, if parents complete their questionnaires and ensure that the non-parent adult's questionnaire is submitted before the first in-person session, they will receive an additional bonus of $25. This is because it is necessary to evaluate questionnaires for complete diagnosis (and therefore assessment of eligibility). We may gather address information from subjects in order to send them a check for compensation after completion of the study.

Throughout the study, subjects will receive points that they can cash in for prizes based on their ability to remain still during the mock scanning and actual scanning sessions, as well as behavioral performance on a subset of tasks. Subjects will have the option of cashing in on points immediately after earning them (for "smaller" prizes worth fewer points), or saving points until they have earned more to cash them in for "larger" prizes worth more points. We will record when subjects choose to cash in on points as a measure of delay of gratification. They will receive extra points as a completion bonus if the entire study is completed. Prizes will be toys, games, books, and gift cards.

In addition, subjects will receive an additional monetary reward of up to $15 based on performance on the Reward GNG task. This reward will be disbursed in cash at the end of each study session the subject completes the Reward GNG task. The purpose of this is to increase the salience of the incentive. The cash will be stored in a locked safe, in a locked drawer/cabinet, in a locked office. Only approved study personnel will have access to the safe.

If parents drive, parking passes will be provided so they will not have to pay for parking. If subjects and their parents take public transportation, they will be additionally reimbursed for the cost of the public transportation.

Non-parent adults will be compensated for their time in filling out the online surveys about ADHD symptoms with a $10 gift card to amazon.com. After completion of the surveys, non-parent adults will be brought to a page where they can enter their e-mail address for gift card receipt.

5 STUDY EVALUATIONS AND MEASUREMENTS (how measurements will be made)

Neuropsychology Testing for Eligibility Measures:

- *The Wechsler Intelligence Scale for Children - Fifth Edition* (WISC–V) is comprised of ten core subtests which generate a Full Scale score (FSIQ), as well as five composite scores (indices): Verbal Comprehension Index (VCI), Processing Speed Index (PSI), Visual-Spatial Index (VSI), Fluid Reasoning Index (FRI), and Working Memory Index (WMI). The WISC-V will be administered via iPad to all subjects on the first day of testing to determine eligibility. To meet eligibility requirements, a subject's FSIQ must be > 80, unless FRI is > 80, in which case FSIQ must be > 65.
- *The Wechsler Individual Achievement Test - Third Edition* (WIAT-III) is a wide-range, individually administered achievement battery used as a measure of academic achievement. The Word

Reading subtest demonstrates the ability to recognize and read single words that increase in their level of difficulty and provides an assessment of basic reading skills. The Word Reading subtest from the Wechsler Individual Achievement Test (Version 3) will be administered via iPad to subjects on the first day of testing to screen for disability in basic reading skills. To meet eligibility requirements, a subject must have a standard score above 85 on the Word Reading Subtest, regardless of IQ score.

Self-Report, Non-Parent Adult, and Parent Questionnaires:

- *Conners Rating Scales 3 (CRS-3)* uses observer ratings to help assess ADHD-related symptomatology and evaluate problem behavior in children and adolescents. A parent and a selected non-parent adult will be asked to complete the Long Versions of the Parent and Teacher forms to provide a perspective of the child's behavior from those who interact with the child on a daily basis. Raw and scaled total scores will be used as dependent variables in combination with scores from the Swanson, Nolan and Pelham Questionnaire. Any adult who knows the child well can complete the Teacher form; it does not have to be completed by a teacher.
- *Swanson, Nolan and Pelham Questionnaire 4 (SNAP-IV),* ADHD-related questions only is a 20 item questionnaire which is used to assess ADHD-related symptomatology. A parent and selected non-parent adult will be asked to complete the Parent and Teacher Versions. Raw and scaled total scores will be used as dependent variables in combination with scores from the Conners Rating Scales. Any adult who knows the child well can complete the Teacher form; it does not have to be completed by a teacher.
- *Social Communication Questionnaire (SCQ*) is an instrument for screening for autism spectrum disorders (ASD) in individuals over the age of 4 with a mental age over 2 years. The SCQ contains 40 yes/no items, which can be completed in less than 10 minutes by a parent or other caregiver. The version of the SCQ that we are administering, the Current Form, focuses on behavior during the most recent month. The instrument yields a Total Score for comparison to defined cutoff points. Given the high comorbidity of ADHD and autism spectrum disorders, this questionnaire is being used to screen out subjects who may qualify for a diagnosis of ASD, and will not be used as a dependent variable.
- *Child Behavior Checklist (CBCL)* is a 113 item parent-report questionnaire on which the child is rated on various behavioral and emotional problems. It assesses internalizing (i.e., anxious, depressive, and overcontrolled) and externalizing (i.e., aggressive, hyperactive, noncompliant, and undercontrolled) behaviors. Sub-areas include social withdrawal, somatic complaints, anxiety and depression, destructive behavior, social problems, thought problems, attention problems, aggressive behavior, and delinquent behaviors. This questionnaire is being used to screen out subjects who have psychopathology that is grounds for exclusion, and will not be used as a dependent variable. Medical and Developmental History Form will be used to collect the child's medical and developmental history from his or her parent. To meet eligibility requirements, a subject must not have a history of Mental Retardation, Developmental Language Disorder, Reading Disability, or a Pervasive Developmental Disorder.
- *Pubertal Development Scale (PDS)* is a validated, brief questionnaire describing physical and sexual development. In order to minimize the potential age and pubertal effects on behavioral

and MRI measurement, subjects will only be included if they are pre-pubertal or in the early stages of puberty as assessed by the PDS. Menarche in girls is exclusion criteria.

- *Edinburgh Handedness Inventory* is a 12 item questionnaire which is used to assess dominance of a person's right or left hand in everyday activities. In order to minimize the potential effects of handedness on tasks assessing response control that involve manual motor skills, handedness will be used as a covariate in analyses.
- *MacArthur Scale of Subjective Social Status* measures both subjective and objective socioeconomic status (SES) of the adult(s) of the subjects families, to be filled out by the parent. As SES is related to education and cognitive and behavioral outcomes, this questionnaire will be used as a matching variable to ensure that enrolled children with ADHD and TD children do not have significantly different levels of SES.
- *Behavior Rating Inventory of Executive Functioning (BRIEF)* is an 86 item assessment designed to assess executive functioning in the home and school environments. The BRIEF is useful in evaluating children with a wide spectrum of developmental and acquired neurological conditions, including ADHD. A parent will be asked to complete the Parent Form of this questionnaire. Raw and scaled total scores will be used as dependent variables to assess executive functioning, which is known to be impaired in children with ADHD.
- *Urgency, Premeditation, Perseverance, Sensation Seeking and Positive Urgency Scale (UPPS-P)* is a self-report and parent-report 40 item, 4-point scale designed to assess various factors of impulsivity and risk-taking, behaviors that are elevated in ADHD. Total scores will be used as dependent variables.
- *Behavioral Inhibition System/Behavioral Approach System Scales (BIS/BAS)* is a 24 item self-report questionnaire that assesses sensitivity to punishment/avoidance (BIS) and sensitivity to reward/approach (BAS) on a 4-point scale. These systems are thought to be imbalanced in ADHD, and potentially altered via stimulant medication (i.e., methylphenidate). Scores on each of the subscales will be used as dependent variables to assess sensitivity to punishment and reward.
- *Domain-Specific Risk-Taking Children's Scale (DOSPERT)* risk-taking scale is a 40 item, 7-point self-report questionnaire that assesses how likely children are to engage in a variety of risky behaviors. Children with ADHD are more impulsive and risky than TD children on average, and the dopamine system, which is manipulated with methylphenidate, is related to risk-taking. Therefore, the DOSPERT, in combination with the Brief Sensation Seeking Scale for Children, will be used as a dependent variable to assess risk-taking behavior and attitudes.
- *Brief Sensation Seeking Scale for Children* (BSSS-C) is a 14 item, 5-point self-report questionnaire that assesses sensation seeking and risk-taking behavior. The BSSS-C, in combination with the DOSPERT, will be used as a dependent variable to assess risk-taking behavior.
- *Affective Reactivity Index* (ARI) is a brief rating scale that assesses symptoms and impairment associated with childhood irritability over the prior 6 months. The measure contains six symptom items that examine the child's threshold for an angry reaction as well as the frequency and duration of angry feelings/behaviors. Items are rated on a three-point scale: Not True, Somewhat True, or Certainly True. Both a parent-report and self-report version will be used. Self-report scores will be used as a dependent variable. Parent-report scores will be used as a dependent variable in combination with parent-report scores on the Emotion Regulation Checklist.

- *Emotion Regulation Checklist* (ERC) is a 24-item, parent-report measure regarding parents' perceptions of their child's typical methods of managing emotional experiences. Items are rated on a 4-point Likert scale (1=rarely/never to 4=almost always). Higher scores are reflective of poorer emotion regulation (i.e., greater dysregulation). The ERC has two subscales: Lability/Negativity (L/N) which assesses inflexibility, lability, and dysregulated negative affect (e.g.,"Exhibits wide mood swings") and Emotion Regulation, which measures appropriate emotional expression, empathy, and emotional self-awareness. Scores will be used as a dependent variable in combination with parent-report scores on the Affective Reactivity Index.

Behavioral Tasks:

Behavioral tasks assessing response control, learning, executive functioning, and reward processing/reward responsivity will be administered to subjects in the MRI machine or behaviorally, with training occurring in the mock scanner and behaviorally outside of the mock scanner on the first day of testing. Tasks will be based on the Go/No-go (GNG) paradigm and the Serial Reaction Time (SRT) paradigm.

The *Simple GNG task* consists of simple images, such as shapes, for Go trials (~75% of trials) and different simple images for No-go trials (~25% of trials), presented one at a time. Subjects will be instructed to press a button on a button box (MRI compatible if task is being completed inside the MRI machine) with their right index finger as quickly as possible in response to Go stimuli only. They will be instructed to withhold a button press when they see a No-go stimulus. The weighting of approximately 3:1 Go:No-go trials is to ensure that responding becomes a prepotent motor response, and withholding a response is therefore more difficult. Each stimulus will be presented for between 300-600 ms, with a delay of approximately 1500ms between stimuli. To allow for appropriate MRI data analysis, in the MRI version there will either be null events interspersed throughout the run, or the length of the inter-trial interval will be jittered (ranging from 500ms - 5000ms). Trial order will be pseudo-random. Each run will last approximately six minutes.

The *Reward GNG task* is identical to the Simple GNG task, except that subjects will be rewarded for responding quickly and for correctly withholding responses on a subset of trials (proportion of rewarded trials will be between 50%-100%). For versions in which less than 100% of stimuli are rewarded, in order to indicate which trials will be rewarded the structure of the trials will be slightly different: before the stimulus, subjects will see a cue for approximately 300-600ms, followed by a delay that may be jittered similarly to the delay subsequent to the stimulus. Total trial time, therefore, will be longer than that of the Simple GNG task. Cues will indicate whether the subsequent trial will be rewarded. For versions in which 100% of stimuli are rewarded, there will not be a cue. For all versions, feedback will be presented to the subject after each trial based on performance. If a subject successfully responds within 400-800ms to a rewarded Go trial (specific value will be chosen to ensure approximately 75% successful Go trials), or successfully inhibits his or her response to a rewarded No-go trial, the subject will receive a small reward of between 1-10 cents per trial. Non-rewarded trials will not result in a reward regardless of performance. At the end of each session, subjects will receive the money earned on the rewarded trials (maximum if all trials are rewarded across all sessions will be $15). The number of trials of Go and No-go stimuli will remain the same as for the Simple GNG task.

The Reward GNG task will always occur after the simple GNG tasks. Tasks without a reward component will be administered before tasks with a reward component to avoid the situation where a lack of reward is perceived as punishment (as opposed to neutral). While this approach is not ideally suited for statistical analysis of order effects with regard to the reward tasks, it provides a system by which potential confounds related to task interference will be avoided.

For all of the GNG tasks, the primary variables of interest relevant to response control will be coefficient of variation (CV) and the total number of inhibitory errors (commission errors). CV is calculated as the standard deviation of response time divided by the mean of response time, to control for baseline differences in response time. We will also examine response time variance using ex-Gaussian approaches, with the normal distribution standard deviation (sigma) and the exponential component (tau) calculated as additional variables of interest.

In addition to the GNG tasks, subjects may be asked to complete a single block consisting only of Go trials in order to get a baseline measure of Go response time and variability. This block would be administered as part of practice before any of the GNG tasks.

The *Alternating SRT task* consists of four stimuli displayed horizontally across the screen, each spatially mapped to one of four buttons on a button box or computer keyboard. During each trial, one of the stimuli changes to become the "target". Subjects will be instructed to press a button with the finger corresponding to the position of the target. The target will disappear immediately after the subject's button press, and there will be a delay of less than 1 second between targets. Unknown to subjects, there is a pattern underlying some of the targets that repeats throughout the experiment. This task will assess differences in responding to the patterned, as compared to the non-patterned (random), targets. Stimuli will be presented in blocks of 60 trials with breaks between each block. Each block will begin with non-patterned practice trials that end with feedback encouraging speed and accuracy. On 50% of the blocks, subjects will be told before the block begins that they will receive a reward at the end of the block. After the task is finished, explicit knowledge of the pattern will be tested using a combination of techniques including the following: (1) a questionnaire asking subjects about their knowledge of any pattern they observed, (2) a generation task in which subjects will generate a typical pattern they saw during the task, (3) a card sorting task in which subjects are given cards with various combinations of patterns they may have seen and are asked to sort them according to the frequency with which they were seen during the task. At the end of the task, the accumulated reward will be converted into points that can be used to cash in on prizes. This task is expected to take approximately 30 minutes (including breaks).

Eye tracking measurements will be measured during the alternating SRT task using a corneal reflection, SR Eyelink 1000 eye tracker. Eye tracking is safe and physically unobtrusive except for the chin rest that will be used to assist with head stillness, similar to what is required for the MRI scan. A camera positioned in front of the computer screen records eye movements as subjects complete the task. A visual image of the eye itself is not recorded. The only data that are recorded are data corresponding to eye movements. The data will not contain sensitive information and will be deidentified. To calibrate the eye tracker, subjects will be asked to follow an object on the screen with their eyes. A sticker will also be placed on their face so the eye tracker can judge the distance between the eye tracker and the head. Subjects will rest their chins on a padded chin rest to assist them with keeping

still. The computer screen will be approximately 40-70 cm in front of the subjects. They will use the keyboard or a stand alone keypad to respond to the stimuli. To control the brightness in the room, subjects will be in a room with windows darkened.

Subjects will also stare at a stationary, neutral picture for 2-5 minutes to count how often they blink. Eye blink rate has been shown to be associated with dopamine levels, which is implicated in learning processes, such as those probed via the alternating SRT task. Subjects will also stare at the task stimuli for up to 3 minutes to get a baseline measure of pupil size. Pupil size has been shown to be associated with norepinephrine levels, which are disrupted in ADHD.

Eye blink rate may also be collected during the MRI sessions. This will be achieved by a small MR-compatible video camera focused on one of the subject's eyes. The image is in black and white and low resolution. The rest of the subject's face will not be part of the recording. The camera is unobtrusive and will not impact subject comfort. The eye will be recorded for the duration of the scan, and eye blinks will be manually counted by between 1-3 trained and reliable raters to ensure accurate counts. The camera will be stored securely in a locked cabinet. Recordings will be removed from the camera within 1 week and uploaded to One Drive, which requires a UNC ONYEN and password to access.

For the SRT task, the primary variables of interest will be accuracy, mean response time, CV, pupil size, and eye blink rate. We will also examine response time mean and variance using ex-Gaussian approaches as described above, with the added ex-Gaussian parameter of interest, mu, which indicates the normal distribution mean.

MRI Scanning Methods:

Subject preparation for scanning: All subjects will participate in a mock scan training protocol during the behavioral visit that utilizes Applied Behavior Analysis techniques to train subjects to cooperate with MRI protocols. The mock scan protocol helps to improve comfort, decrease potential anxiety, and train subjects to lie still; it also gives subjects exposure to alternative versions of fMRI paradigms so that they learn to maintain vigilance to test stimuli and how to respond properly. These procedures make use of a simulation area with a life-size mock scanner.

MRI Scanning: All MRI scans will be acquired using a 3-Tesla Siemens MAGNETOM Prisma MRI machine at the Biomedical Research Imaging Center at UNC School of Medicine, where Dr. Cohen (the PI) is an affiliated faculty member. Before scanning, parents will fill out an MRI safety screening form to ensure that the child (and the parent, if the parent wants to accompany the child into the scanner room) is free of metal and can safely participate in an MRI scan. All subjects will undergo a high-resolution anatomical scan (MPRAGE) to be used for individual subject brain registration to a standard template. MPRAGE acquisition parameters will be similar to the following: TR=8ms, TE=3.76ms, Flip Angle=8°; Slice thickness=1mm; FOV= 256mm, Matrix size: 256x256. A localizer scan will be run with parameters similar to the following: TR=20.0ms, TE=5.00ms, Flip Angle=40°, Slice thickness=8.0mm, FOV=280mm, Matrix size=280x280, and a low resolution anatomical scan will be acquired with parameters similar to the following: TR=5850.0ms, TE=65.0ms, Flip Angle=120°, Slice thickness=3.0mm, FOV=230mm, Matrix size=230x230. Subjects will also undergo functional MRI scans with acquisition parameters similar to the following: gradient-echo EPI pulse sequence, TR=2s, TE=40ms, Flip Angle=90°, 31 axial slices

oriented for best whole-brain coverage, Slice thickness 3mm, voxel size 3.125x3.125x3mm with a .3mm interslice gap. Due to the development of new sequences that allow for better brain coverage, lower signal dropout and improved robustness to subject motion, parameters may be tweaked slightly at the recommendation of BRIC MRI technologists. This will influence data quality, but will not change the subjective experience or safety for the subjects. Functional runs will include task runs (as described above), and resting state runs (10 minutes; subjects will be instructed to lie still and stay awake while maintaining fixation on a crosshair in the middle of an otherwise blank screen). Diffusion weighted imaging (DWI) data will also be collected to measure white matter connectivity. DWI acquisition parameters will be similar to the following: posterior to anterior phase encoding direction: 83 directions, b=0 s/mm 2, TR = 3700 ms, TE = 69.0 ms, echo spacing = 0.56 ms, FOV = 240 mm2, slice thickness = 2.5 mm, slices: 37. Anterior to posterior phase encoding direction: 83 directions, b=2000 s/mm2, TR=3700ms, TE=69.0ms, echo spacing = 0.56 ms, FOV = 240mm2, slice thickness=2.5mm, slices: 37. Finally, arterial spin labelling will be carried out with parameters similar to the following: post-labelling delay: 1000000.00 us, labelling scheme: pseudo-continuous ASL, TR=4000ms, TE=16.0ms, FOV=220 mm2, slice thickness=4.0mm. Head movement will be minimized using a foam pillow and cushions. Subjects will view task stimuli presented with a computer-controlled LCD projector onto a back-projection screen placed in the back of the scanner bore through a mirror mounted on the head coil. An MR-compatible camera will be attached to the back of the mirror and focused on the subject's eye. This allows for on-line monitoring of subject compliance, as well as real-time feedback if a subject is falling asleep or moving too much. This data may be recorded to count eye blinks. When necessary, vision will be corrected to normal using MRI compatible plastic lenses and frames. For tasks that require button presses, responses will be recorded using MRI compatible button boxes. Psychophysiological data will also be collected for the purposes of measuring heart rate and respiration rate to control for the effects of medication on physiological processes that can affect neural processes that are unrelated to cognition. This will be measured either by the use of electrodes placed on the shoulders, neck, chest, and back, which measure cardiac impedance and electrocardiogram activity, or by the use of a heart rate monitor placed on the finger, and a respiration belt wrapped around the chest. If electrodes are used, parents will be present during their placement. Subject comfort will be monitored during the scan and subjects may be asked to rate their comfort after scanning concludes.

6 STATISTICAL CONSIDERATION

6.1 Statistical Methods

To address primary objectives, we will calculate brain metrics of interest using standard functional connectivity and graph theory methodology, as detailed below:

Functional Connectivity Analysis: FMRI data will be preprocessed using standard methods used in the literature, and implemented by Dr. Cohen in preliminary data analyses. Given recent evidence that subject motion can cause spurious correlations between two brain regions we will implement procedures developed to reduce the effects of motion on functional connectivity estimates. Individual scans with excessive motion (as defined by a spike >3mm in any direction or average displacement across the entire scan of >0.5mm) will be excluded from all analyses. The brain will be parcellated into

regions of interest (ROIs) using a publicly available brain atlas such as the Automated Anatomical Labeling (AAL) atlas. Voxel timeseries will be averaged within each ROI and these averages will be bandpass filtered (0.005-0.08 Hz) to remove physiological noise such as cardiac and respiratory artifact. Functional connectivity will be assessed in each subject by correlating average timeseries across ROIs, resulting in an NxN correlation matrix for each subject, with N being the number of ROIs.
Typically, these timeseries analyses are conducted across an entire run. However, it is possible to calculate meaningful connectivity estimates and, importantly for this proposal, graph theoretical metrics, from using dynamic conditional correlation (DCC) analysis, which produces a connectivity matrix for each data acquisition time point. Given that children with ADHD show increased RT variability and more frequent lapses of attention than TD children, we will not only look at connectivity across an entire timeseries, but will look at changes in connectivity across time as well. Therefore, we will conduct the same graph theoretical analyses (detailed below) with multiple functional connectivity correlation matrices per run to get a measure of the variability of graph metrics across time. Additionally, we will select time windows that include particularly slow RTs (thought to reflect lapses of attention) and compare them to time windows that have more stable RTs.

Graph Theory Analysis and Metrics: To implement a graph theoretical analysis, first the correlation matrices (for both rest and task data) will be fisher-transformed to standardize the values. Then weighted graphs will be constructed, where the strength of the correlation between any given pair of brain regions becomes the weight of the edge between those two nodes of the graph. Each whole-brain graph will be separated into modules (or individual networks) derived by optimizing Newman's modularity. Modularity is the ratio of the number of within- compared to between-module connections. It reflects the strength of a graph's modular organization, with higher modularity indicating lower integration across networks. The global similarity of two graphs, their mutual information, will be calculated across contexts (i.e., between rest and task within an individual). Lastly, the flexibility of individual nodes will be quantified. To measure flexibility, we will calculate the number of times a node changes network membership across time during a task run.

To address secondary objectives, we will calculate behavioral metrics of interest using data from the go/no-go tasks. We will calculate “Go”-related metrics and “No-go”-related metrics. Go-related metrics will include mean response times and variability of response times. We will calculate variability in two ways. First, coefficient of variation (CV), which is calculated as the standard deviation of response time divided by the mean of response time, to control for baseline differences in response time. Second, we will use ex-Gaussian approaches, with the normal distribution standard deviation (sigma) and the exponential component (tau) calculated as additional variables of interest. Go-related metrics will include the total number of inhibitory errors (commission errors), as well as the number of omitted responses (omission errors).

Statistical Analysis: Paired t-tests comparing methylphenidate session to placebo session will test for within-subject improvements in network dynamics, behavior, symptoms, executive functioning, and emotion regulation. All tests will be conducted with a significance level of 0.05 correcting for multiple comparisons. We will report the magnitude of all statistical effects, as well as confidence intervals for all analyses conducted, as p-values greater than .05 cannot be interpreted as anything other than a lack of statistical significance

6.2 Sample Size and Power

We selected our proposed sample size after conducting a power analysis. The effect size of preliminary resting state analyses was d=0.5; similar to that observed in analyses of a distinct sample in the lab of Dr. Cohen's previous mentor, Dr. Stewart Mostofsky, with the same number of subjects (d=0.7). Emphasizing the importance of studying network dysfunction during cognitive tasks, the effect size during task performance in Dr. Mostofsky's sample was significantly higher than during rest (d = 2). Importantly, in a study comparing the change from rest to task with 16 adolescents with ADHD and 16 TD adolescents, the effect size was 0.8. Therefore, assuming a moderate effect size of 0.7, we will have at least 80% power to detect significant effects with the proposed sample size of 32 subjects. As these are across-subject effect sizes, we anticipate greater power for the within-subject analyses of the medication challenge (N = 32).

7 STUDY INTERVENTION (drug, device or other intervention details)

Description. Methylphenidate (Ritalin, Metadate, Concerta) is FDA-approved for treatment of ADHD in children and adolescents. It is used as part of routine clinical care for ADHD and is increasingly used in preschoolers. FDA-approved single dose range is between 5mg and 30mg for children aged 8-12 (daily range 10mg-60mg); subjects in this study will receive a dose within the FDA-approved single dose range. Minimal increases in blood pressure and heart rate, anorexia, insomnia, rash, and tics have been reported during oral methylphenidate administration. Long-term effects are not pertinent as this is a single dose study.

Receipt/Storage. A study team member will pick up the medication at the Investigational Drug Services (IDS) pharmacy the day prior to the MRI session. Medication will be stored in a room temperature locker in the Biomedical Research Imaging Center (BRIC) at the UNC School of Medicine. Only study personnel will have the combination for this locker.

Packaging/Labeling. Medication will be packaged and labeled in accordance with IDS protocol.

Dosing. Each subject will receive a one-time dose of methylphenidate based on their weight: 0.3 mg/kg (range 5mg-30mg). This dose is equivalent to a conservative initial dose utilized when beginning treatment with a stimulant for children of this age group, and comparable to other doses used for research in medication-naïve children with ADHD**.** Each subject will also receive a matching placebo pill. We will use over-encapsulation to make the drug and placebo appear identical. We will completely cover the drug and the placebo pill with an identical covering so they look, feel, and taste the same. The UNC pharmacy will encapsulate both the drug and the placebo**.**

Drug Return/Destruction. If a subject does not show up for their appointment, we will return the drug to IDS and they will destroy it in accordance with their protocol.

Administration of methylphenidate vs. placebo will take place in the research laboratories of the BRIC, in a clinical environment and within a few minutes walk to the hospital's emergency room. Parents will be advised of possible side effects that, if they do occur, should be transient and mild. Mild side effects of methylphenidate are infrequent (occurring between 1-10% of the time), and serious adverse effects are extremely rare (< 1%). The MD collaborator and other members of the on-site study team will be available to monitor for any side effects after medication administration and the MD collaborator or

designee will be available by cell phone for 24-36 hours after the study visit for any questions about adverse events related to study medication.

8 STUDY INTERVENTION ADMINISTRATION (if applicable)

A study team member not directly involved in subject testing will assign methylphenidate/placebo order to each subject in a pseudo-random fashion, with the only constraint being that 50% of subjects receive placebo first, and 50% of subjects receive methylphenidate first, with gender being similarly balanced (i.e., 50% of females will receive placebo first, and 50% of males will receive placebo first). The MD collaborator who is monitoring safety and possible adverse events while the subjects are on methylphenidate or placebo will be unblinded during the study and therefore able to identify which pill was taken if there are any significant adverse effects felt by the subject after taking the pill. The MD collaborator will not have direct contact with subjects during the experimental visits. Thus, no study team member who interacts with subjects or their families on the methylphenidate/placebo days will know whether a subject is on placebo or methylphenidate. After data collection for the medication challenge portion of the study is completed, the team member who made order assignments will reveal participation order (placebo/methylphenidate or methylphenidate /placebo) so appropriate analyses can be conducted.

9 SAFETY MANAGEMENT

For subjects enrolled in the medication challenge, there is a small chance that adverse side effects of the medication may occur. This portion of the study entails a minor increase over minimal risk. In this portion of the study, subjects who have a confirmed diagnosis of ADHD will receive a single, low dose of methylphenidate (0.3 mg/kg), a drug and dose that is FDA-approved to safely treat ADHD in children of this age range (8-12). All subjects who will receive a single dose of methylphenidate will have undergone a physical examination to ensure they can safely take this single dose of medication, will have provided informed assent (and their parent informed consent), and will be adequately monitored for 24-36 hours following the administration of methylphenidate to ensure no adverse side effects. There is a medium probability of the occurrence of a low-severity event that is completely reversible (e.g., trouble sleeping, loss of appetite, etc), and very low probability of a more serious adverse event.

If a subject was excluded from the medication challenge on the basis of the physical examination, the parent will be told what finding (i.e., blood pressure, arrhythmia, etc) caused the child to be excluded and given the option to report that finding back to the child's pediatrician.

Parent(s) of subjects enrolled in the medication challenge will be required to fill out a survey 24-36 hours after intake of methylphenidate/placebo to ensure safe monitoring of potential (but unexpected) adverse side effects. The 24-36 hour of methylphenidate/placebo to ensure safe monitoring of potential (but unexpected) adverse side effects. The 24-36 hour range allows for monitoring to last at least 24 hours, but allows for evening responses if the parent(s) work and cannot fill the survey out during the work day of the day following methylphenidate/placebo administration. To increase convenience (and therefore compliance), we will provide parents with a link to an online survey via REDCap (Research Electronic Data Capture), a user-friendly and secure online resource for collecting, organizing, and storing survey data. Parent(s) will therefore be able to answer a series of

questions probing their child's response to the methylphenidate/placebo to ensure that there are no adverse side effects. A member of the study team will send out a reminder to the parent at 24 hours after methylphenidate/placebo administration, and again at 36 hours if the survey has not yet been completed. If the survey is not completed the day after the testing session, the parent(s) will be called by a study team member the day following to ensure the safety of the subject. The MD collaborator or a trained designee will review survey responses within 24 hours of survey completion to ensure safety and will be available via pager during that 24-36 hour period if a subject or parent becomes concerned about the effects of the medication. The REDCap survey will ask questions about common side effects, including: head aches, stomach aches, appetite, changes in mood (i.e., irritability, anxiety, increased emotionality, depression), difficulty falling asleep, tiredness/fatigue, heart racing/palpitations, chest pain, unusual sensory experiences (i.e., hearing or seeing things that aren't there), and nausea/vomiting. For each side effect, the survey will ask: 1) did your child experience any of the following; and 2) have these symptoms resolved? If any side effect is endorsed, the survey will ask the parent to report if the side effect was mild (easily tolerated, no intervention needed), moderate (severe enough to make your child pause his or her activities, but able to be managed at home), or severe (difficult to tolerate, stopped the child in his or her tracks). Parents will be told that if at any point their child experiences a severe side effect they should page the MD collaborator, who will be available. If the parents report that any side effect has not resolved at the time the survey is completed, the survey will instruct parents to page the MD collaborator. As stated above, the MD collaborator or a trained designee will review every survey within 24 hours of its completion by the parents to ensure no ongoing side effects, and the MD collaborator will contact the parents if a side effect is reported as not yet resolved, even if the parent does not page the MD collaborator.

Dr. Cohen (the PI) and the MD collaborator will make up the committee responsible for receiving and reviewing adverse events in aggregate across the entire study population. This is sufficient given that this is a Level 2 (minor increase over minimal risk) study that consists only of a single dose of methylphenidate, which is FDA-approved to safely treat ADHD in children of the age range in this study (8-12). For subjects without serious adverse events, Dr. Cohen and the MD collaborator will review data every two weeks. If a subject experiences a serious adverse event, the MD collaborator, who will be either a child psychiatrist or a child neurologist, will be contacted by the experimenter or parent immediately and will take appropriate action. Any adverse events that occur will be reported to the IRB according to UNC IRB policy by the PI. Adverse events that occur during the study or that are noted during the 24-36 hour follow-up will be reported to the FDA in accordance with 21 CFR 312, in addition to the IRB under HRPP policies.

10 DATA COLLECTION AND MANAGMENT

We will take steps to ensure that data are stored securely and appropriately over time to protect the privacy of all subjects as specified below.

The privacy of participating children and families will be protected, as all data will be used only for research purposes. Data will be de-identified by the research team and will be stored in password protected files, locked offices, locked cabinets, and coded with a master list that is similarly secured and kept separately from all data. Only identification numbers, never names, will be indicated on records obtained from the subject. Only personnel trained in procedures to maintain confidentiality

will handle study information and documentation. Findings may be made available to legitimate agents of the subjects (parents, schools, treating psychologists, physicians), but only with the express, written consent of the parent(s).

Data collected for the study will be stored on secure, ONYEN-protected servers maintained by UNC (MRI data, eye tracking data) or on secure, password-protected computers in the laboratory. To log on to the servers, a UNC ONYEN username and password are needed. All hard copies of the data will be de-identified and kept in a locked laboratory, in a locked filing cabinet. Data may be transmitted electronically among the research team via password-protected files.

All identifiers will be destroyed at the end of the study by (1) shredding all hard copies of data, and (2) purging all electronic databases. This will be done unless the parent has indicated on the parental permission form and the child has indicated on the assent form that they are willing to be contacted for participation in future research studies, in which case contact, demographic and eligibility information, as well as date of testing, will be saved in a secure REDCap database independent from any study-specific data. Only approved personnel will have access to the REDCap database.

11 RECRUITMENT STRATEGY

Subjects will be recruited through multiple methods.

- In person at local community events such as the UNC Science Expo. In person recruitment will be conducted by trained research personnel. Potential subjects will have the opportunity to take study flyers or brochures home or to provide their names and e-mail addresses or phone numbers to be contacted in the future for more information. If subjects provide contact information, it will be on a sheet of paper that is stored securely so no one other than the trained research personnel will be able to see their contact information, and no other identifying information will be stored with it. The paper will be destroyed as soon as the potential subject is contacted by study staff.
- Educational information sessions on ADHD will be conducted in person at local community locations such as the library. The information sessions will be conducted by trained research personnel. After these sessions, audience members will be welcome, but not required, to take study flyers or brochures home or to provide their names and e-mail addresses or phone numbers to be contacted in the future for more information. If subjects provide contact information, it will be on a sheet of paper that is stored securely so no one other than the trained research personnel will be able to see their contact information, and no other identifying information will be stored with it. The paper will be destroyed as soon as the potential subject is contacted by study staff.
- Email or listserv announcements through UNC and community listservs such as the Carolina Institute for Developmental Disabilities Child Development Research Registry, the UNC all-campus listserv, and parent group listservs. For all listservs, parents who are interested in participating in our study will be given contact information for a member of the study team and will voluntarily contact that individual if they are interested in participating. No study staff will directly contact a listserv member without that member voluntarily contacting study staff first. The Carolina Institute for Developmental Disabilities Child Development Research Registry is for parents in Wake, Durham, Chatham, Orange, and Alamance counties, who are invited to voluntarily enroll their babies and typically developing children in this registry. Parents who

enroll their children indicate that they are interested in hearing about research opportunities at UNC-CH, which they can then choose to participate in or not. A member of the research team will send an informative e-mail to parents of children who are in the age range of the initial testing session of our study (10-12). Based on the average rate of ADHD, we anticipate that approximately 9% of school-aged children who were typically developing at birth will have developed ADHD. Parents who are interested in participating in our study will be given the contact information of a member of the study team and will voluntarily contact that individual if they are interested in participating.

- Newspaper and website-based advertisements
- Flyers and brochures placed in lobbies and given to clients of local clinics, including the Carolina Institute for Developmental Disabilities, the Duke ADHD Clinic, local chapters of Children and Adults with Attention-Deficit/Hyperactivity Disorder, local pediatrician and mental health offices, and public spaces such as public libraries, public schools, and after-school programs. Each of these community resources will be contacted via phone, e-mail, mail or in person and asked if they are willing to distribute study flyers. These community-based resources are expected to provide a representative sample of children with ADHD with respect to ethnicity, intelligence, socioeconomic status, sex, and ADHD subtype. Flyers and brochures may be in paper format, posted on websites, or sent electronically.
- We will get records from the Carolina Data Warehouse through i2b2 at UNC. A HIPAA waiver is requested to obtain access to patient records to determine study eligibility. We will send an email or a letter to identified patients' parents. If they choose to opt out, we will cease all contact. If they do not choose to opt out, we will contact them for initial screening
- We will use Join the Conquest to post our study. Parents who are interested in participating in our study will be given study information and contact information for a member of the study team and will voluntarily contact that individual if they are interested in participating. No study staff will directly contact a listserv member without that member voluntarily contacting study staff first.
- We will advertise on the Chapel Hill Transit buses for recruitment.
- We will use Facebook sponsored ads targeted to parents in the triangle area

Subject privacy during recruitment for subjects recruited through CDW-H will be maintained by keeping any identifying information in a secure REDCap database. For subjects recruited via other methods, we have no access to any private information. If parents are interested in having their children participate, they will contact a member of the research team directly. We will conduct an initial telephone screening to determine eligibility before bringing families in to participate. Private information will be discussed during that telephone screening, but if a subject is deemed ineligible, or if a subject is deemed eligible but later chooses not to participate, all information from the telephone screening will be destroyed immediately.

12 CONSENT PROCESS

For the study, after initial telephone contact parents will undergo an oral consent process then, if they consent, a brief telephone screening. If it seems likely that their child meets eligibility criteria for the

study based on the telephone screening, parents will next undergo a second oral consent and, if they consent, a diagnostic interview will be conducted over the telephone. The interview will further probe eligibility by determining diagnostic status of the child. In some cases, the oral consent/screening and the oral consent/diagnostic interview will take place during the same phone call. In other cases they will take place during two separate phone calls. If parents provide oral consent and their child is eligible, the first behavioral testing session will be scheduled where in-person written consent and assent will be obtained. For the behavioral pilot study, upon initial telephone contact parents will complete an oral consent and, if they agree, telephone screening for eligibility. If their child is eligible, the behavioral testing session will be scheduled where in-person written consent and assent will be obtained.

Both parents (consent) and children (assent) will be together when consent/assent is obtained, and as much time as needed will be provided for subjects to make a decision regarding whether to participate. It will be emphasized that participation is voluntary, that reluctance to participate will not compromise availability of care or the eligibility to participate in other studies, and that participation may be discontinued with no penalty at any time. Parents will be encouraged to read the consent form carefully and ask any questions they may have. Children will be read the child assent form, and will be asked multiple times in different ways whether they want to participate to ensure that they have made up their mind and clearly and consistently indicate that they do or do not want to participate.

In order to screen for eligibility, which requires first a brief screening conducted verbally, then a diagnostic interview in addition to further screening questions, we will ask those screening questions and, if the parent orally consents, conduct a diagnostic interview over the telephone. This is to allow for the appropriate screening and diagnostic information to be gathered before the first on-site visit. If, after screening, a subject is deemed ineligible or a parent is not comfortable with his or her child participating, then all information collected during the telephone screening will be immediately destroyed. Further, if a parent provides oral consent but during the first on-site visit the parent or child chooses not to participate, or the child is deemed ineligible, all information collected during the telephone screening will be immediately destroyed. This information will only be maintained (in a confidential manner as described in this application) if the child participates in this study. Therefore, this phase of the study involves minimal risk and only includes a questionnaire, thus written consent for the telephone screening can be waived. Other phases of the study will still require written consent

13 PLANS FOR PUBLICATION

We plan to submit the results from this study to peer-reviewed psychology and neuroscience journals.

14 REFERENCES

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