Title: Eye Tracking as a Predictor of Methylphenidate Response in Autism with Co-morbid Attention Deficit Hyperactivity Disorder

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1.0. Background

Recent changes in the DSM-5 (APA, 2013) have removed Autism Spectrum Disorders (ASD) as an exclusionary criteria for Attention Deficit Hyperactivity Disorder (ADHD). This major revision has been strongly supported by several lines of genetic, clinical, and neuroimaging data that suggests ADHD co-occurs in individuals with ASD (Sprenger et al., 2013). It is estimated between 16 to 54% of individuals with ASD have symptoms of inattention, hyperactivity, and impulsivity and meet criteria for ADHD (Sinzig, Walter, & Doepfner, 2009; Sprenger et al., 2013). Individuals with a co-diagnosis of ADHD and ASD (ASD+ADHD) generally have a higher severity of pathophysiology which his reflected in higher rates of hospitalization, medication treatment, and behavioral therapy than ASD alone (J. A. Frazier et al., 2001). In addition, children with a diagnosis of ASD+ADHD have therapeutic implications including distinct behavioral therapies, pharmacotherapy, and neural correlates (Antshel et al., 2011; Geurts, Verte, Oosterlaan, Roeyers, & Sergeant, 2004; RUPP, 2005).

Recent estimates suggest one-third of children diagnosed with ASD+ADHD are treated with psychostimulants (T. W. Frazier et al., 2011). In the past, the routine use of psychostimulants were discouraged in ASD over concern that they might precipitate increased aggression, repetitive behaviors, or irritability (Campbell, 1975). More recently, in the largest randomized control trial (RCT) of methylphenidate (MPH) in children with ASD and symptoms of ADHD, approximately 49% of the 72 enrolled subjects had a favorable response (RUPP, 2005). Despite this reported benefit in some subjects, 18% of participants discontinued MPH primarily due to irritability or other adverse effects. The results of this trial are in contrast to the NIMH Multimodal Treatment Study of ADHD of otherwise typical youth with ADHD, in which nearly three-quarters of the subjects were successfully maintained on MPH monotherapy with only a 4% dropout rate (MTA, 1999).

Recently, Tye and colleagues (2014) attempted to further clarify electroencephalography (EEG) differences between children with ASD, ASD+ADHD, and ADHD using event related potentials (ERPs). They used ERPs to measure temporal characteristics of different stages of emotional processing (emotional face task) to identify early encoding deficits (N170) in ASD and later contextual processing deficits (N400) in ADHD. Of interest, the ASD+ADHD group demonstrated ERP features consistent with both ASD and ADHD. Furthermore, the magnitude of these changes correlated with clinical measures of symptom severity including Social Communication Questionnaire and the Connors' questionnaire. The implications of this innovative study demonstrate a unique electrophysiology signature of ASD and ADHD, including shared features in the ASD+ADHD group. As a stated limitation, the selection of subjects including mostly higher functioning ASD (average full-scale IQ = 115.7) and may not be easily generalizable to children with ASD+ADHD and more severe cognitive limitations.

Eye Tracking Rational: Eye tracking is a non-invasive neurophysiological measure that can clarify a wide range of cognitive processes and well suited to investigate the primary goal of this proposal in children with more impaired ASD. Eye tracking offers a window into a "hardwired" circuit into the brain in a patient population that may not easily tolerate more invasive diagnostic procedures. Hands-free eye tracking can be readily used with children with ASD who have a more severe behavioral phenotype including limited receptive language, hypersensitivity, and hyperactive/impulsive behaviors. The basic parameters measured by modern eye trackers have been well characterized in typical and atypical development (Karatekin, 2007). These parameters can be obtained in real-time and can be readily transformed into a spatial and temporal time-series which can model and make inferences about cognitive processes including memory, attention, socio-emotional processes, and motivation. Children with ADHD consistently have

been reported to make more premature saccades and more errors on anti-saccade tasks than TDC suggesting difficulties in cognitive inhibition (Klein, Raschke, & Brandenbusch, 2003; Munoz, Armstrong, Hampton, & Moore, 2003). Recently, Fried and colleagues studied a cohort of 22 subjects with ADHD, on and off their medication, and 22 healthy controls while performing the Test of Variables of Attention (TOVA) (Fried et al., 2014). Off medication, the ADHD group displayed a higher number of microsaccades (3-fold), eye blinks (8-fold), and a decrease in pupil size over time compared to controls. Of interest, after treatment with psychostimulants, the ADHD group largely normalized all three parameters and was similar to TDC. Eye tracking has been extensively studied in ASD, primarily to assess social cognition and previously reviewed (Boraston & Blakemore, 2007). Of importance, despite frequent use of eye tracking in ASD, no published study has applied current ADHD eye tracking paradigms and analysis such as described in Fried (2014) to investigate attentional features of ASD.

Secondary Measure: SICI is a TMS measure of the efficiency of inhibitory interneurons in the primary motor cortex (M1) (Kujirai et al., 1993). Over the last decade supported by extensive NIH funding (R01/MH095014) we identified SICI as a TMS biomarker of ADHD with the following properties: (1) M1 SICI is *reduced* in 8-12 year old children with ADHD compared to TDC (Figure 1), (2) reduced SICI correlates with symptom severity, (3) reduced SICI is a robust predictor of ADHD diagnosis (even adjusted for SES, IQ, reading scores, and gender) (Gilbert, Isaacs, Augusta, MacNeil, & Mostofsky, 2011). SICI is routinely and rapidly measured in our laboratory and is well tolerated by children. Of interest, one previous study has shown no difference between SICI between adults with ASD and matched TDC (Enticott et al., 2013). In sub-analysis, a difference in SICI emerged between ASD subjects with early language delay compared to TDC, further supporting the use of SICI as a symptom specific biomarker, rather than a global deficit in ASD. As a quantitative physiological marker of motor function that can be obtained quickly, SICI is an ideal candidate which to clarify fundamental mechanisms of cerebral function that underlie impaired behavioral control.

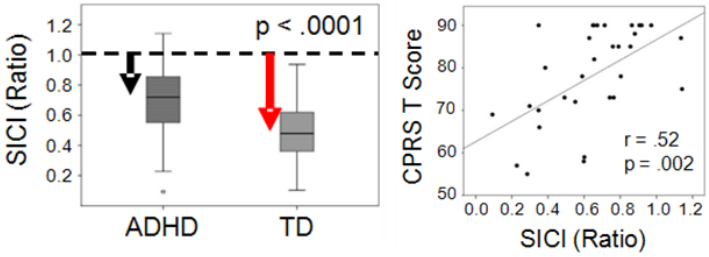


Figure 1: SICI ratios are significantly higher in ADHD children than in TD children (ADHD showing less motor cortex SICI, p<0.001). Higher parent-rated severity of ADHD symptoms (right) on the Conner's scale (CPRS T Score) correlates with larger ratios/less motor cortex SICI in children with ADHD (r=.52,p=0.002) (Gilbert et al. 2011)

Presently, it remains *unresolved* in the current literature if (1) co-occurrence of ASD+ADHD represents an additive co-morbidity or an endophenotype of ASD with a distinct etiology, symptoms, and prognosis and (2) a lack of a reliable clinical methodology for the diagnosis and management of ASD+ADHD (Matsuura et al., 2014), especially in regards to pharmacotherapy response. Children with ADHD either alone or in co-occurrence with ASD have difficulty with response selection and control, which is reflected in more variable and disinhibited behavior. Diagnosis and treatment of ADHD symptoms in the ASD could be greatly enhanced if the physiological substrates of treatment response variability were known.

1.1. Summary

The proposed project "Eye Tracking as a Predictor of Methylphenidate (MPH) Response in Autism Spectrum Disorders (ASD) with comorbid ADHD" will investigate the role of a non-invasive neurobehavioral biomarker in an underserved clinical population to clarify diagnosis and

guide treatment decisions. Specifically, we will modify an existing eye tracking paradigm that discriminates between ADHD and typical youth to use in an ASD cohort with ADHD (ASD+ADHD) and without ADHD comorbidity. A case-control design (Aim 1) comparing neurobehavioral measures will lead into a randomized placebo controlled trial of MPH in ASD+ADHD (Aim 2). We hypothesize that children with ASD+ADHD will demonstrate specific abnormalities in microsaccades, eye blink frequency, and pupil dilation on continuous performance testing that will predict MPH treatment response on standardized clinical outcomes for ADHD. As a secondary measure, we perform a brief electrophysiological measure, short interval cortical inhibition (SICI), as measured by transcranial magnetic stimulation (TMS). We have extensively investigated this measure as a robust predictor of ADHD diagnosis and symptom severity in otherwise typical youth with ADHD. We anticipate this personalized medicine-based approach to identify ADHD co-morbidity in ASD will produce a neurophysiological biomarker to enhance diagnostic reliability and match appropriate pharmacotherapy in a complex neurodevelopmental disease.

2.0. Rationale and Specific Aims

2.1. Rationale

The overall goal of this proposal to use neurophysiological measures to profile attentional and response control deficits for ADHD co-morbidity in ASD to clarify diagnosis and to predict treatment response. The central hypothesis posits that neurophysiological differences between ASD and ADHD can be reliably differentiated by eye tracking to enhance diagnosis and treatment. For Aim 1, our *primary outcome* is to validate critical eye tracking parameters to classify ASD and ASD+ADHD. As a *secondary outcome*, we will measure SICI, a well-established electrophysiological TMS parameter in ADHD, to identify differences in motor cortex inhibition. For Aim 2, we propose a single dose methylphenidate (methylphenidate) RCT exclusively in ASD+ADHD participants to identify neurophysiological correlates of treatment response and provide critical preliminary data for additional imaging or EEG studies to investigate mechanisms of action. To approach these goals we specifically propose:

2.2. Specific Aims and Hypotheses

Aim 1: To identify neurophysiological measures that discriminate between children with ASD and ASD+ADHD, which specifically correlate with symptom severity as measured by standardized clinical scales.

1.1 To use eye-tracking to identify critical parameters that highly correlate with ADHD comorbidity in ASD during continuous performance testing (CPT). Hypothesis: Children diagnosed with ASD+ADHD will have (1) an increased number of microsaccades and eye blinks and a decreased pupil size over time compared to children with ASD during a CPT and (2) severity of these markers will correlate with symptom burden.

1.2 To measure resting paired pulse TMS evoked cortical inhibition (SICI) and determine the relationship between SICI and ADHD symptoms in youth with ASD. Hypothesis: Baseline SICI will be (1) predictive of baseline symptom severity and (2) SICI will be decreased in ASD+ADHD compared to ASD.

Aim 2: To perform a pilot randomized control trial (RCT) of a single dose of MPH or placebo in children with ASD+ADHD to determine the neurophysiological correlates of treatment response as measured by computerized CPT testing.

1.1 To specific eye tracking parameters (microsaccades, eye blinks, and pupil size) to predict response of MPH on improved CPT scores on the Test of Attentional Performance for Children (KiTAP). Hypothesis: Discriminatory eye tracking parameters for ASD+ADHD will be highly correlated with response to MPH on the KiTAP and unrelated to placebo.

1.2 We will measure effects the change of SICI pre- and post-dosing to evaluate change associated with MPH and correlation with treatment. Hypothesis: MPH treatment will improve performance on CPT and increase SICI (decrease SICI ratio) compared to placebo.

2.3. Innovation

Our long-term goal is to elucidate the contribution of neurophysiological mechanisms in the neural pathology of ASD, specifically for rigorous classification and to predict treatment response to behavioral and pharmacological interventions. We believe the published work demonstrate innovation as it capitalizes on a novel approach to quantify deficits in neural circuits hypothesized to be involved in ADHD comorbidity in ASD and leverages the unique strength of our Autism research group and pediatric electrophysiology/TMS program at CCHMC. No published study has examined these particular eye tracking or TMS measures within a subgroup of ASD as well as including a clinical trial to assess treatment response. Successfully completing the aims of this project will establish a key quantitative measure to characterize a highly prevalent and distressing comorbidity in ASD. Though this proposal highlights the clinical consequences of this work, i.e. diagnostic and therapeutic biomarkers, a critical application of this technique would be to assist with specific enrichment of research cohorts of individuals with ASD. Such work is essential for the development of future targeted treatments developed to focus on subgroups defined by neurophysiological features and future genetic, neuroimaging, and phenotypical studies investigating the etiology of ASD.

3.0. Inclusion and Exclusion Criteria

3.1. Inclusion Criteria

1. DSM-V-TR diagnosis of Autism Spectrum Disorder not otherwise specified (NOS) based on a semi-structured review of DSM-V criteria and mental status examination as well as a a complete systematic patient interview utilizing the Autism Diagnostic Observation Schedule (ADOS)
2. Males and females ages 8-21 years.
3. Subjects must not be taking any psychotropic drugs affecting glutamate neurotransmission (riluzole, memantine, acamprosate, topiramate, amantadine, among others) which may interfere with TMS recording. If patient is on a home psychostimulants medication this will be held on the day of testing. Subjects may not be taking more than two psychotropic drugs. Dosing of all concomitant psychotropic drugs targeting core social and/or communication impairment must be stable for four weeks prior to randomization. Dosing of all concomitant psychotropic drugs targeting other features associated with ASD (insomnia, inattention, hyperactivity, anxiety, irritability among others) must be stable for two weeks (with the exception of four weeks for fluoxetine) prior to randomization.
4. Stable seizure disorder (no seizures in 6 months prior to enrollment; on same anticonvulsant dose > 60 days or)

5. Able to participate in neurophysiological testing including EEG and TMS portions of the experiment based on patient comfort and examiner judgement
6. Legal guardian has provided written informed consent and the subject has provided written informed assent. Expectation that a majority of subjects will be able to assent but the potential for the younger children and/or those that are cognitively impaired will not be able to assent.

3.2. Exclusion Criteria

1. Subjects exhibiting significant disruptive, aggressive, self-injurious, or sexually inappropriate behavior will not be eligible for enrollment
2. Presence of current DSM-V psychiatric disorders that may require alternative pharmacotherapy or different treatment unrelated to Autism Spectrum Disorder or Attention Deficit Hyperactivity Disorder.
3. Presence of any medical condition that would make treatment with MPH less safe. Subjects with significant cardiac, hepatic, or renal disease will be excluded due to concerns about pharmacokinetic alterations or adverse effects. Because of the unknown effects of MPH on the developing human fetus, females of childbearing potential will be given a urine pregnancy test and required to use a suitable form of birth control during the study. A positive pregnancy test result excludes the subject.
4. Presence of any other condition that would make the participants unable to comply with the requirements of the study for any reason.
5. Prohibited Concomitant Medications: Methylphenidate is primarily excreted by the kidneys and has few known pharmacokinetic drug interactions. The following medications are not allowed due to the potential for a pharmacodynamic interaction: monoamine oxidase inhibitors or atomoxetine.

4.0. Enrollment and Randomization

4.1. Enrollment

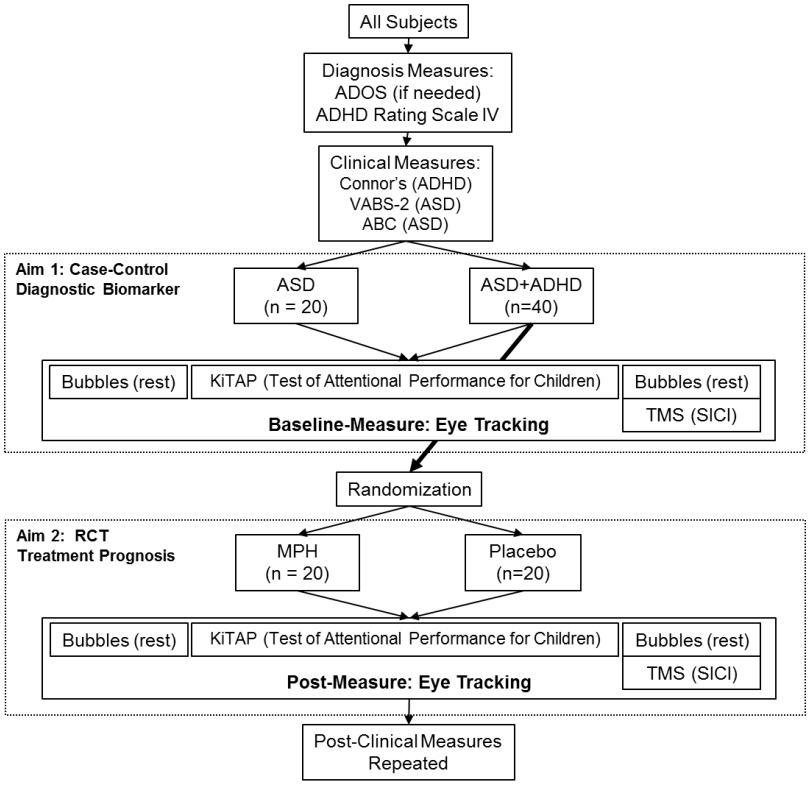
We plan on enrolling 25 children with ASD and 40 children with ASD+ADHD for Aim 1. For Aim 2, we will target a total of 40 children with ASD+ADHD (20 for MPH and 20 for placebo) which may include subjects who elect to remain in the study after completing Aim 1 tasks.

Figure 1 gives the experimental design and study flow. This is a single visit study. Total enrollment of participants will be 60 ages 8 to 21 years.

4.2. Randomization

Subjects with ASD complete the study following a single neurophysiological session. Subjects with ASD and ADHD will be randomized to either single dose MPH or placebo in 1:1 ratio after they have been determined to meet all eligibility criteria through screening. We will use block randomization (4 subjects a group) to assure age and gender matching based on recruitment. This computer-generated randomization list will be generated by the statistician through statistical software (e.g., Splus6) and passed to the investigational pharmacy for treatment assignment. The pharmacist keeps a log of what regimen is dispensed at each visit that can then be subsequently verified. Only the investigational pharmacist will have access to the randomization list.

Figure 1 Study Design and Flow. Single visit design



5.0. Study methods

5.1. Baseline characterization

We plan to recruit from an ASD clinical treatment population of several thousand patients. On average the Kelly O'Leary Center Autism Research Group enrolls four new subjects of all ages with ASD into research protocols per week. The majority of persons diagnosed at the center participate in research and all subjects receive a rigorous clinical diagnostic protocol following the specifications of the Autism Treatment Network (ATN). This comprises a large existing cohort of subjects available to immediately participate in studies, including the ASD and ASD+ADHD sample we propose in this study. **Autism Diagnosis:** If a subject has not received gold-standard diagnostic testing and is considered highly qualified candidate by the study physician, we will arrange for a research reliable psychologist to administer the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2)(Lord et al., 2012) to confirm autism diagnosis and the ADOS Calibrated Severity Score (Gotham, Risi, Pickles, & Lord, 2007)will be used to assess deficits in communication and reciprocal social interaction that occur in a clinical context. **ADHD diagnosis:** To critically group ASD subjects with and without ADHD co-morbidity, we will rely on a clinician's diagnosis based and described methods (Matsuura et al., 2014) with confirmation by ADOS-2 report and ADHD Rating Scale IV (DuPaul, Ervin, Hook, & McGoey, 1998). **Severity Scales:** In addition, a parent/guardian will be asked to complete the Social Responsiveness Scale (SRS)(Constantino & Gruber, 2005), the Vineland Adaptive Behavior Scales-2nd Edition (VABS-II)(Sparrow, Balla, & Cicchetti, 2005), Conners' Parent Rating Scale–Revised (CPRS; ADHD severity T score accounts for age and gender)(Conners, Sitarenios, Parker, & Epstein, 1998), and Social Communication Questionnaire (SCQ)(Rutter,

Bailey, & Lord, 2003) to assess adaptive behavior, problem behaviors and competencies, attention, health, and social behavior. Developmental or Cognitive Assessments including but not limited to the Leiter R, Differential Ability Scales, Stanford Binet 5th Edition, Bayley Developmental Scales, Wechsler Intelligence Scale for Children, Wechsler Adult Intelligence Scale, Kaufmann Brief Intelligence Test-2 may also be performed. Finally, the Physical and Neurological Exam for Soft Signs (PANESS) will be used to assess subtle soft neurological signs and motor skills in children that have been demonstrated to be aberrant in ASD and ADHD (Dziuk et al., 2007). Additional assessments include complete medical and psychiatric history, mental status examination, height, weight, head measurements, vitals, and TMS tolerance rating. If study requires access to participant's hospital or healthcare provider records or access to participant's data from a prior research study in which they have participated, a Data Use Agreement will be appropriately filled out and submitted.

5.2. Neurobehavioral measures

5.2.1. Computerized Attention Testing

KiTAP: Attentional functions will be assessed using the KiTAP (Fimm & Zimmermann, 2003). KiTAP is an easy to follow computer-assisted test battery for non-verbal attentional functioning across several key domains and has been validated in a DD population (Knox et al., 2012). Subtests will be performed in the following order: Alertness, Distractibility, Flexibility, Go/NoGo, Visual Scanning, Vigilance, Sustained Attention, and Divided attention. Following previous methods in a lower-functioning population (Knox et al., 2012), the shortest subtests will be administered first to give the widest number of children the greatest chance to complete an entire subtest.

5.2.2. Eye Tracking

A Tobii (Stockholm, Sweden) T300 Infrared Eye Tracker controlled with Tobii Studio software (Version 3.0) and integrated with a 17-inch thin film transistor monitor will be used to measure gaze fixation in response to presented visual stimuli. *Eye Movements* Participants will be monitored or asked to make specific eye/eyelid movements. A false belief behavioral task may be used in conjunction to monitor eye movements (Senju, Southgate, White, & Frith, 2009). This task involves the subject to look at a sequence of an actor and a puppet moving a ball between two boxes. The resulting ocular movements will be recorded using a Tobii Eye Tracker (Tobii Technology AB, Danderyd Sweden).

5.2.3. Single and paired-pulse TMS procedure

TMS will be performed using a Magstim 200® stimulator (Magstim Co., New York, NY, USA) connected through a Bistim® module to either a 90 mm circular coil or a double 70 mm figure 8 coil as described in CHMC 06-10-11; 05-05-19; 03-05-52; 03-05-53. The subject sits in a comfortable chair. The TMS devices are maintained in the A7-EEG suite or CCHMC Medical Office Building 3430 Burnett Ave 3-113 Neurophysiology Suite, which is under direct supervision of laboratory personnel during business hours and is locked with restricted key access during all other hours.

Single and Paired Pulse TMS: TMS will be performed as described in our prior CCHMC IRB approved protocol CHMC IRB 2008-0061. TMS involves non-invasive administration of magnetic pulses of a strength comparable to, at maximum, the magnetic field intensity of clinical MRI scanners. Motor cortex stimulation for upper limb: The round coil is placed with its center near the vertex in the optimal position and orientation for producing a motor evoked potential (MEP) in the bicep, tricep, abductor pollicis brevis (APB) muscle, first digital inter-osseous (FDI)

muscle, or abductor digiti minimi (ADM) muscle, as the clinical context requires. The figure 8 coil is similarly placed. TMS pulses over motor cortex generate a motor evoked potential whose amplitude reflects the number of neurons which depolarized. We record MEPs using a Micro 1401 processor (CED, Cambridge, UK) and process the EMG data using Signal® software (CED). The EMG is recorded with surface electrodes taped to the skin. This is comfortable for the patient as no needle is required to be inserted in the muscle. The signal is amplified, and filtered (100/1000 Hz) (Coulbourn Instruments, Allentown, PA) before being digitized at 2 kHz and stored for analysis using Signal® software and a Micro1401 interface (Cambridge Electronic Design, Cambridge, UK). Resting SICI: Once the MEP "hotspot" is identified and depolarization thresholds measured, resting SICI measures require 6 minutes. Briefly, resting SICI is a measure of the baseline efficiency of inhibitory interneurons in motor cortex (M1). Ten single, suprathreshold pulse TMS trials, muscle evoked response amplitudes are a measure of motor cortex responsiveness or excitability. In paired pulse TMS trials, the first conditioning subthreshold pulse briefly activates inhibitory interneurons, so that the subsequent suprathreshold pulse, on average, evokes a smaller response in muscle (a smaller motor evoked potential, or MEP).(Chen et al., 1998) SICI may be expressed as either a ratio of Paired (smaller) to Single (larger) pulses (so a small ratio means more inhibition), or as a percent (100*(1 minus the ratio)). So if the average paired pulse MEP amplitude is 0.4 mV and the single pulse is 1.0 mV, SICI may be equivalently expressed as a ratio of 0.4 or inhibition of 60%. Initially, single pulse stimulation will be performed over the left primary motor cortex to elicit MEP in the right FDI and to determine active and resting motor thresholds (AMT, RMT) using standard methods (Mills & Nithi, 1997). Then 40 trials of brief single or paired pulse stimulation at an interval of 6 seconds with one of 2 conditions in randomized order: 1) single (test) pulses and 2) SICI: paired pulses (condition/test) at an inhibitor 3-ms interval. The test pulse will be set above RMT intensity and the condition pulse set at approximately 60% RMT.

Additional information and neurophysiological TMS measures: Thresholds defined using conventional criteria as the lowest stimulator intensity that produces measurable responses in 3 of 6 trials. Intracortical inhibition (SICI) and facilitation (ICF) are measured with a paired-pulse paradigm using three conditions: single pulse, paired pulse at 3 msec interstimulus intervals, paired pulse at 10 msec interstimulus intervals. Subthreshold pulse precedes suprathreshold pulse for paired pulses. Peripheral conditioning pulses are administered 20-30 msec prior to motor cortex. Short ICF will be also performed with a paired-pulse paradigm (Chen & Garg, 2000; Hanajima et al., 2002; Ziemann et al., 1998). Cerebellar conditioning pulses are administered 5-10 msec prior to motor cortex. Twenty trials are performed for each ISI and for the test stimulus alone. The order of the intervals is varied randomly, and the interval between trials varies randomly by <10% around a mean of 6 seconds. Transcortical inhibition to the left dominant hemisphere/ right dominant hand is measured by stimulating over right motor cortex, while the child simultaneously contracts muscles in both hands. This produces an evoked potential in this ipsilateral and contralateral hand, followed by periods of EMG silence (the "silent period"). Latency and duration of this silent period are affected both by age and by ADHD diagnosis (Garvey et al., 2005). Latencies are measured by subtracting MEP onset time from TMS artifact time. Amplitudes are measured as peak to peak and area under the curve.

Bimanual Tasks In each experiment, the child has both hands relaxed on his lap, on a pillow supporting at the elbows. For the <u>single finger tapping motor task</u>, the child is instructed to repeatedly tap the task hand index finger while keeping the non-task hand in the same position, but relaxed. To ensure consistency in performance speed and coordination, the a computer instructions will be shown to indicate resting, tapping one finger, tapping all fingers sequentially, and sustaining a squeezing of the index finger to the thumb. TMS will be administered as above but during the task.

Response Inhibition Tasks In each experiment, the child keeps both hands relaxed, but on a cue from the computer has to push an X button with the right hand or O button with the left hand, on a game controller. On some trials, a "stop" auditory tone will occur. At time = 0ms, an image of either an X or O will be displayed on the screen. TMS will be administered as above but during the task. Slater-Hammel procedure, another response-inhibition task, may also be used to assess motor response time (Slater-Hammel, 1960). This task requires the subject to hold a button and release before a moving marker reaches a predefined time point (800 milliseconds after the start signal). If the marker stops in its trajectory, the participant is instructed to not let go of the button.

Reward Paradigm In each experiment, the participant watches while smiling or frowning faces appear on the computer screen. Three smiles will result in the participant receiving a monetary award ($0.25). TMS will be administered as above, but during the task.

Money Bags Paradigm In each experiment, the participant clicks the mouse control each time a quarter appears above the money bag on a computer screen. If they click accurately, the quarter drops into the money bag and ($0.25) is registered as gained by the participant. The difficulty and perceived degree of difficulty varies. TMS will be administered during the task.

Behavioral Tasks Various behavioral task(s) may be performed to assess the effects on motor control.

5.2.4. Electroencephalography (EEG)

EEG will be used to assess the electrophysiologic correlates of behavioral computerized testing, behavior, or motor function with TMS. EEG will be recorded with a whole dense array (dEEG) with 256 channel electrode cap (HydroCel Geodesic Sensor Net) continuous recording EEG system (Electrical Geodesics, Inc. (EGI), Eugene, OR, USA). The sensor net uses a mild, fragrance-free, saline-based solution to contact the scalp, requires approximately 10 minutes to position the net and does not require abrasion of the skin as the EGI amplifiers are design to tolerate normal skin impedances. This system of electrodes and amplifiers can be left in place during TMS stimulation. Emotiv® wireless EEG (Emotiv, San Francisco, CA USA) headset or Wearable Sensing (San Diego, CA) dry electrode system will also be used to capture scalp EEG data.

5.3. Drug dosing: MPH and placebo

MPH or placebo will be administered 60-120 minutes prior to the repeat neurophysiological test session. The timing of this dose is consistent based on the well-described response of psychostimulants. It will also allow us to monitor compliance since dosing will occur during the study visit. The dose of MPH will be 0.3 mg/kg, up to 20 mg; rounding to the nearest 2.5mg increment This dose of 0.3 mg/kg is also comparable to studies of stimulant use in children with autism that have explored doses ranging from 0.3 mg/kg to 0.6 mg/kg (Handen, Johnson, & Lubetsky, 2000). Placebo capsules will be identical in appearance to MPH capsules. All participants and study investigators will remain blind to drug assignment during the trial. In case of emergency, the investigational pharmacy will be able to break the blind.

5.4. Primary Outcome Measure

Eye tracking is a non-invasive neurophysiological measure that can clarify a wide range of cognitive processes and well suited to investigate the primary goal of this proposal in children

with more impaired ASD. The basic parameters measured by modern eye trackers have been well characterized in typical and atypical development (Karatekin, 2007). In this study, we will be replicating findings from Fried colleagues who studied a cohort of 22 subjects with ADHD, on and off their medication, and 22 health controls while performing the Test of Variables of Attention (TOVA) (Fried et al., 2014). Off medication, the ADHD group displayed a higher number of microsaccades (3-fold), eye blinks (8-fold), and a decrease in pupil size over time compared to controls.

5.5. Secondary Outcome Measures

A number of additional secondary outcome measures will be administered. SICI is a TMS measure of the efficiency of inhibitory interneurons in the primary motor cortex (M1) (Kujirai et al., 1993). Over the last decade supported by extensive NIH funding (R01/MH095014) we identified SICI as a TMS biomarker of ADHD with the following properties: (1) M1 SICI is *reduced* in 8-12 year old children with ADHD compared to TDC (Figure 1), (2) reduced SICI correlates with symptom severity, (3) reduced SICI is a robust predictor of ADHD diagnosis (even adjusted for SES, IQ, reading scores, and gender) (Gilbert et al., 2011). SICI is routinely and rapidly measured in our laboratory and is well tolerated by children. Of interest, one previous study has shown no difference between SICI between adults with ASD and matched TDC (Enticott et al., 2013). In sub-analysis, a difference in SICI emerged between ASD subjects with early language delay compared to TDC, further supporting the use of SICI as a symptom specific biomarker, rather than a global deficit in ASD. As a quantitative physiological marker of motor function that can be obtained quickly, SICI is an ideal candidate which to clarify fundamental mechanisms of cerebral function that underlie impaired behavioral control.

5.6. Assessment of adverse events (AEs)

Assessment of AEs will be collected at each visit by asking the subject and caregiver if the subject has experienced any new symptoms, visits to the doctor, or taken any new medication. In addition, a TMS 16-point review of systems may be used pre and post TMS to assess any adverse effects due to TMS. This scale was recently published and used frequently in our lab (Hong et al., 2015). The physician will keep a running log of AEs that will record the date of onset, date of resolution, severity, and relationship to study intervention (e.g., definite, probable, possible, remote, or none). A schedule of measures is shown in **Table 1**.

Table 1. Schedule of Measures

Study Time point (duration)	Initial Measures	Pre-MPH Testing	Post-MPH Testing (only for ASD/ADHD subjects randomized to clinical trial)
Consent/Assent	X		
Developmental, medical and psychiatric history; MSE and history of psychotropic medications	X		
Heart rate, Blood Pressure, height, weight, head circumference	X		
Concomitant Medications	X		
Urine Pregnancy Test (sexually active females of child bearing potential)	X		
Adverse Events (baseline, pre, and post testing)	X	X	X
Parent Interviews			
ADHD-Rating-Scale-IV	X		
Connor's Rating Scale	X		
VABS-2	X		
Aberrant Behavior Checklist	X		
Social Responsiveness Scale (SRS)	X		
Social Communication Questionnaire (SCQ)	X		
Physical and Neurological Exam for Soft Signs (PANESS)	X		
Primary Outcome Measure			
Eye Tracking		X	X
Secondary Outcome Measures			
SICI (TMS)		X	X
Resting State EEG		X	X
KiTAP Attentional Profile		X	X
ADOS Diagnostic Testing**			
Developmental/Cognitive Testing**			

** Completed only at baseline if testing no completed previously

6.0. Data Analysis and Management

6.1. Sample Size

Fried et al. (2014) reported a large effect size (d=1.0) between youth with ADHD and TDC in comparisons of microsaccades difference between groups, a primary outcome in our study. Based on this effect size, our proposed sample size of 20-25 per a group will provide 87% power to detect a significant difference in means of our primary outcome. The proposed project will provide crucial pilot data that will be used for sample size estimates for future studies. The proposed sample size will also be sufficient to identify barriers to enrollment and completion of the study.

6.2 Statistical Analysis

Eye tracking data: Raw eye tracking data will be acquired by Tobii Studio (Version 3.0; Switzerland, Sweden). Analysis will be performed based on previous methods (Fried et al., 2014). Briefly, eye blinks will be detected as periods of no tracking data. Microsaccade rate will be detected with a post-processing algorithm of raw eye tracking data adapted from previously described methods (Bonneh et al., 2010) and using a velocity-threshold filter (V-T): Eye tracking data will first be processed using a 120 Hz cut-off frequency. The algorithm will identify sequences of eye movements that represent an eye movement for at least 6 ms in a the same direction (with a 30 degree window) with a minimum velocity of 10 degrees/s, peek velocity greater than 18 degrees/s, and a saccade amplitude greater than 0.1 degrees. To account for false detection of microsaccades, eye movements 20 ms before and after each blink will be excluded. Pupil diameter data will be measured in millimeters using a conversion factor by a standard measure during each experimental setup. Intervention analysis: For statistical analysis in the RCT, 2-way repeated measures ANOVA will be used to compare eye tracking measures between MPH and placebo, pre and post intervention [GROUP*TIME]. TMS data: Motor physiology measures (SICI, thresholds) will be compared between ASD and ASD+ADHD groups using t tests. In the intervention group, motor physiology measures will be compared pre- and post- using 2 way repeated measures ANOVA. Computerized Neurocognitive Testing: KiTAP results of the five summary domain scores will be compared between groups using a multivariate analysis of variance (MANOVA) in SPSS and followed up with independent sample *t* tests. In addition, we will examine the effect sizes for the individual primary scores. Neurophysiological Data and Clinical Measures: A Pearson product moment correlation of age, eye tracking measures (eye blinks, microsaccade rate, pupil diameter), neurobehavioral measures (KiTAP), clinical measures (VABS, Connor's), and motor physiology will be explored within the entire sample and stratified by diagnosis.

6.3. Data Management

When a participant is enrolled in the study, the participant will be assigned a unique identification number that is used to identify all data associated with that person, including hard copy and computerized data. Data will be collected on hard-copy forms and then verified by data entry personnel. All of the hard copy research data is kept in locked file cabinets at the Kelly O'Leary Center for Autism. Only the PI and primary research staff will have access to these files, ensuring security of the hard copy records.

6.4 Reporting Results to Participants

Patients may receive a copy of testing results, psychological evaluations or a statement disclosing drug randomization after completion of study procedures if they elect to have that information disclosed.

7.0 Feasibility

The Autism Research Group and Kelly O'Leary Center at CCHMC has a large existing cohort of subjects available to immediately participate in studies, including the ASD and ASD+ADHD sample we propose in this study. We have a strong research team with a diverse set of expertise in clinical trial research, and clinical assessment and treatment. Drawing from an ASD clinical treatment population of several thousand patients, on average the Autism Research Group and Kelly O'Leary Center enrolls four new subjects of all ages with ASD into research protocols per week. The proposed project builds on that work and because it employs similar tasks, measures, and participants, it has a high potential for success.

8.0. Project timeline

We are anticipating requiring two years to complete this project and the associated RCT. We will enroll 1 subject with ASD or 1 subject with ASD+ADHD per a week over an 84 week recruitment period. We will spend the first 6 weeks of the grant period obtaining regulatory approval, preparing case report forms, and other study materials. Over the final 10 weeks of the grant period we will analyze the data, prepare an initial study abstract(s) for presentation at the AACAP Annual Meeting, and initiate primary manuscript preparation.

9.0. Special considerations

1. Radiation Safety: NA
2. Investigational Devices: The TMS apparatus is investigational, although it has recently been cleared for treatment of refractory depression. We consider 2 Tesla or less stimulation or brain, cerebellum, spinal cord, and nerves to be a non-significant risk use of the TMS device. Manufacturer's information for the Magstim device has previously been submitted to the IRB and is available on request.
3. IND (Investigational New Drug): NA
4. Emergency Use: NA
5. CCHMC Pharmacy: NA.
6. Discarded Tissues: NA
7. Tissue Banks: NA
8. Genetic Studies: NA
9. Institutional Biohazard Committee: NA
10. Imaging: NA

10.0. Facilities

The facilities for this proposal at CCHMC including the clinical/cognitive testing in the A8-neurology clinic area, E2-Psychiatry clinic area, MOB3430 research suites or the Schubert Research Clinic. Single pulse and rapid TMS will occur in the A7 in the EEG area in the TMS lab on A7, MOB3430 or the Schubert Research Clinic.

11.0. Human Subjects

11.1. Human Subjects Involvement and Characteristics

A total of 60 children between the ages of 8 years and 21 years meeting DSM-V and ADOS criteria for ASD (n=20) or ASD+ADHD (n=40) will participate in the study of eye tracking as a predictor of methylphenidate response. There are no restrictions on gender, ethnic or social background. We plan to include females, children, cognitively impaired, and members of minority groups and their subpopulations in this research. By gender, the projected composition is female (20%) and male (80%). The rationale for the anticipated inequality by gender is based on a higher frequency of ASD in males as detailed below.

11.2. Sources of Material

The neuropsychiatric history, medical history, and overall clinical assessment will be utilized in the research period. Specific research material includes the behavioral rating data, which will be obtained for research purposes only. There are no plans to make use of records or other data.

12.0. Risks and Benefits

12.1. Risks to the subject

The primary risks of this study include those associated with MPH drug administration and TMS. Minor risks are associated with EEG. Other risks are those associated with the potential for loss of confidentiality. Finally, there are minimal risks commonly associated with a medical office visit and collection of data.

12.1.1. MPH

MPH 0.3 mg/kg will be dosed 60-120 minutes prior to the post-drug neurophysiological session. The dose of 0.3 mg/kg (maximum dose 20 mg) was chosen to approximate the range of most participants in similar studies (Handen et al., 2000; Harfterkamp et al., 2013) and is within range of doses found to effectively treat ADHD in human studies (MTA, 1999). In addition, MPH is only being given as a single dose. Because of this, we do not expect to have any serious adverse effects. Higher doses are routinely used clinically with all dosing occurring at home. At higher doses, MPH can cause tachycardia, hypertension, headache, tremor, confusion, nervousness, irritability, psychosis, twitching, and visual disturbances. There is a remote risk of sudden death in patients with specific preexisting heart defects. Despite this reported benefit in some subjects, 18% of participants discontinued MPH primarily due to irritability or other adverse effects. The results of this trial are in contrast to the NIMH Multimodal Treatment Study of ADHD of otherwise typical youth with ADHD, in which nearly three-quarters of the subjects were successfully maintained on MPH monotherapy with only a 4% dropout rate (MTA, 1999). In order to ensure safety of subjects, additional criteria must be met for a subject to be randomized to drug treatment phase of the study. Subjects must have 1) no significant heart disease, 2) no history of a first degree relative with early, sudden cardiac death, 3) no hypertension, 4) no history of severe adverse response to MPH,

12.1.2. Single and Paired-pulse TMS

Single and paired pulse TMS has been used at CCHMC under Dr. Gilbert's direction since 2001 for research only. Potential discomforts are mild and transient. In a prior study of 40 healthy and ADHD children, Garvey et al asked children to rank TMS compared to other childhood activities. TMS was ranked preferable to 1) a "shot"; 2) going to the dentist; and 3) a long car ride (Garvey, Kaczynski, Becker, & Bartko, 2001). The following mild, transient effects were reported in our prior study of 35 children and adults: scalp discomfort (12%), hand weakness (9%), headache, neck pain, arm pain, and arm tingling (6%), hand pain, decreased hand dexterity, hearing

changes, and tiredness (3%). All of these had resolved by the following day. There were no physical findings after TMS supporting the subjective descriptions of loss of strength or dexterity. We have also recently published a series of > 100 youth who have underwent TMS in our laboratory without any serious adverse effects (Hong et al., 2015). A prior common concern about use of TMS was the risk of seizures(Wassermann, 1998). We follow recommended guidelines (Wassermann, 1998) and have seen no seizures in children or adults studied at our center. In addition, more recent studies even in children with epilepsy suggest that the risk of TMS, especially single or paired pulse TMS, inducing seizures is quite low.

12.1.3. Loss of Confidentiality

Loss of confidentiality is a potential risk in most clinical research. During the conduct of clinical research, personal information about the participant or family could become known to others against the participant's wishes. These episodes are not common, but occasionally occur due to the large number of different people that may interact or "run in to" the participants or family members during a course of a clinical trial with frequent visits. Acquiring the behavioral and treatment records and video, of the participants creates the potential risk of breech in confidentiality. In unusual cases, the mandatory reporting of certain events (e.g., child abuse or neglect) would be required by law.

12.1.4. Minimal Risks

Beyond those risks noted above, there are minimal risks associated with office visits and the collection of behavioral data. This would include the inconvenience of the frequency of study visits, potential embarrassment over discussing psychiatric symptoms, and feeling uncomfortable during procedures (e.g., physical exam, vital signs). There are no substantial risks involved with participating in social skills training. However, should a child become a danger to the child's self, others or objects, physical intervention may be deemed necessary. The risk of this latter event will be minimal since subjects must be free of disruptive, aggressive, self-injurious, or sexually inappropriate behavior in order to be eligible for enrollment.

12.2 Risks for ASD without ADHD

The risks for typically developing peers will be less since they will not be administered MPH and randomized to the clinical trial.

12.2. Adequacy of protection against risks

12.2.1. Recruitment and informed consent

Recruitment of subjects will be conducted by notifying individuals with ASD, their families, treating clinicians and agencies throughout Ohio, and those within our existing clinical services, residential facilities, schools and group homes for the developmentally disabled of the availability of this program of research. Approved recruitment materials will be shared when notifying individuals with ASD, their families, treating clinicians and agencies throughout Ohio and within our existing clinical services. The study may also be advertised in hospital/clinic and general public areas via print, email, electronic and social media using the recruitment materials. The available historical and clinical data will be reviewed with the referring clinician, and if it appears that the subject would satisfy entry criteria for the study, the study will be described to the subject and his/her legal guardian. Subjects and their legal guardians interested in participating in the study will have a face-to-face interview with the principal investigator and/or the Research Coordinator where the nature of the project, the risks, the benefits, and the alternatives to

participation in the project are discussed with the subject (when possible) and the subject's family. If following these discussions the subject and family continue to be interested in the project, and assent is obtained from the subject *(*when possible depending on cognitive ability) and formal written consent is obtained from the parent(s)/legal guardian(s), clinical responsibility for the care of the subject is then assumed by the principal investigator and the other members of the research team. All potential subjects and their legal guardians will be encouraged to ask questions about any aspect of the study that is unclear. All questions will be answered and uncertainties clarified before the consent is signed. All potential subjects and their legal guardians will be consented in their native language or a language that they find understandable. Written assent will only be obtained from those subjects deemed to have the cognitive ability to fully comprehend the document. This will be determined by a qualified member of the research team based on direct observation, clinical judgment and parent/guardian report. All subjects and their legal guardians will be provided with copies of the consent form for future reference. Appropriate clinical evaluation and treatment of the referring problem will be offered regardless of the subject's/legal guardian's decisions regarding participation in the study. All of the research recruitment data is kept in locked files at the TKOC, which ensures that no one other than the study investigators have access to health information. The other procedures to ensure confidentiality follow the regulations and policies of the Cincinnati Children's Medical Hospital Center.

If the legal guardian withdraws consent prior to completion of the study, the reason for withdrawal will be documented. This will not affect the subject's ability to get clinical treatment for their condition either at our Center or elsewhere. The principal investigator also has the right to withdraw subjects from the study in situations that increase risk to subjects (e.g., development of new medical or psychiatric illness that would make participation less safe) or jeopardizes integrity of data (e.g., subject refusing to follow protocol).

12.2.2. Protection against risk for MPH adverse effects

Effective screening (using medical history and physical exam) will be used to eliminate subjects with ASD+ADHD who will be randomized to the controlled trial and who are at greatest risk for adverse effect from MPH because of concurrent medical conditions. Subjects will only be accepted into the study if they are free of any significant medical illness as determined by a comprehensive history taking, medical review of systems and physical examination.

Methylphenidate is an U.S. FDA Pregnancy Category C drug. Because of the unknown effects of methylphenidate on the developing human fetus, females of childbearing potential will be given a urine pregnancy test and will be excluded from the study if this is positive. Since our sample will include some pre-pubertal children ages 9 to 21 years with an autism spectrum disorder, we do not expect that many subjects will be sexually active. However, both females and males who are sexually active will be required to use a suitable form of birth control during the study. If a positive pregnancy test is obtained, we will confirm with a serum HCG, inform the parents and transfer the care to the child's primary care physician. Since the some of the children in this study are under the age of 14 years, this will also require a mandatory report to Child Protective Services.

The subjects will be evaluated and cared for in an advanced well-staffed pediatric neuropsychiatric research environment. Thus, the direct observation by nursing staff, research psychiatrists, and research staff will allow for careful monitoring of potential adverse effects including drug side effects. If adverse reactions become excessive, the subject will be treated and removed from the study. Hospitalization is available for any subject whose symptoms become

difficult to manage or dangerous. Any subjects leaving the study will be evaluated post-study until AEs have stabilized. There will be repeat monitoring of behavior and vital signs that allow the treatment team to assess the status of the subject and alter or terminate the study if it is warranted. Any serious adverse event will be reported to our local IRB, the drug manufacturer, Human Research Protection Office (HRPO), and the FDA.

We have several mechanisms to reduce the risk of loss of confidentiality. All of the research data is kept in locked files at the TKOC, which ensures that no one other than the study investigators have access to health information. The database is locked and only accessible by those with server access and an additional password unique to the database. Each participant will be given a unique alphanumeric code and this will serve as the only connection between the locked files, the hard-copy forms, and the electronic database. The key to the code is only available to the study investigators. All forms used in the study contain only this alphanumeric code so no personal information is exposed to potential hazards. All visits are conducted in private offices, exam rooms and classrooms. Additionally, other procedures to ensure confidentiality will follow the regulations and policies set by Cincinnati Children's Medical Hospital Center, including those of the Health Insurance Portability and Accountability Act.

Finally, the minimal risks associated with office visits will be reduced even further. The guiding principle in all interactions is respect for the dignity of each participant and their family. The scheduling of study visits takes into account school and work schedules to minimize the disruption for the participant and family. All procedures (e.g., physical exam) will be explained verbally or by demonstration prior to their completion including prepared social stories for more technical aspects of testing. Frequent breaks are allowed during visits as needed in order to reduce fatigue. The use of praise, rewards, visually stimulating objects and gadgets (e.g., bubble machine, Koosh ball) are also encouraged while interacting with younger participants. In order to minimize any financial hardship, free parking will be provided.

Since persons with significant disruptive behavior will be excluded from participation, we do not expect subjects to exhibit any severe maladaptive behaviors. Disruptive behavior will initially be handled by redirection. Behavior that is attention seeking may be managed but the use of brief time-outs. Should behaviors occur that threaten to jeopardize the safety of subjects or staff, crisis intervention-trained staff may utilize de-escalation and self-protection as prescribed by the Crisis Prevention Institute.

All written information will be kept confidential and separate from the clinical chart. All data and information accumulated in person, through observation, video, photograph, survey, or via checklist, pertaining to the participant will only be discussed and shared with the participant and necessary personnel and agencies. Participant identity will be held in confidence in reports in which the project may be published.

13.0. Potential benefits of the proposed research

The potential benefits that a subject could have from entering this project include the following: subjects will receive an extensive psychiatric and medical evaluation that is provided free-of-charge. Forty children with ASD+ADHD will be assigned to either MPH or placebo and will receive a very carefully controlled drug treatment trial which may treat psychiatric symptoms. There will be no direct benefit from single/paired pulse TMS as used in this study as a diagnostic measure. A study completion letter will be provided to the patient's parent at the end of the study. The letter will include information from the psychiatric evaluation including a clinical diagnosis and any recommendations for follow-up.

13.1. Risk-Benefit Ratio

The subjects will be exposed to the risks of loss of confidentiality and the potential adverse effects of MPH. MPH has been shown to have excellent efficacy in the treatment of ADHD symptoms in ASD. The potential benefits outweigh the risks.

14.0. Compensation

There will be no charges to patients or to third party payers related to participation in this study. All subjects will be provided free parking for visits. Subjects with ASD who complete clinical measures and a single session of the neurophysiological assessment will be paid $35. Subjects with ASD+ADHD who continue to complete the controlled trial and repeat the neurophysiological assessments will be paid $70. Payment will be in the form of a reloadable debit card (ClinCard). We will provide the card and load money onto the card after each completed visit based on the schedule listed above. We will also administer a handout that will explain how to use it.

Because this research study involves payment for participation, we are required by federal Internal Revenue Service (IRS) rules to collect and use participants' social security or tax ID number (SSN) in order to track the amount of money that we pay. Unless we have been given specific permission for another use of participants' SSN related to this research, we will only use the participants' SSN to keep track of how much money we pay to them and their SSN will not be used as part of this research.

14.1. Funding

This project is directly supported by the American Association of Child and Adolescent Psychiatry (AACAP) Junior Investigator Award funded by the AACAP Research Initiative. Additional funding will be provided by the P.I. Business Plan funded by the Children's Hospital Research Foundation if needed. All of the listed investigators will be contributing time and support and a variety of projects will generate data to secure more extramural funding.

15.0. Data Plan

A rigorous and systematic approach to data management is critical for the quality of any study. The substantial effort and resources that will be devoted to collecting data in this project will be matched by an equally substantial commitment of effort and resources to edit, verify, correct, update, and assemble the resulting data files. Our data management system incorporates quality control at every juncture from data collection through analysis. We have found that this bottom-up approach to data quality is essential, since there is no single procedure that will verify and correct erroneous data. The PI and research assistant are responsible for data collection and accuracy of record keeping and the researchers will convey an attitude that the data management procedures be treated with unwavering gravity, therefore maintaining a high level of quality for this project.

When a participant is enrolled in the study, they will be assigned a unique identification number that is used to identify all data associated with that person, including hard copy, biological specimens, and computerized data. Unique identifiers will be linked to personal data such as names and addresses only by use of a restricted password, thus assuring confidentiality. Data will be collected on hard-copy forms and then verified by data entry personnel. All of the hard copy research data is kept in locked file cabinets at TKOC. Only the PI and primary research assistants will have access to these files, ensuring security of the hard copy records.

We have taken several steps to ensure the quality of data entry. Once data is obtained, the clinician will review the form to make sure that all required items are completed and to clarify any ambiguous notations before giving it to the research assistant responsible for data entry. Several quality control measures are built into our computerized data management. Data entry forms have been designed that correspond to the measures used in this study. These forms are configured so that out-of-range values cannot be entered; data entry prompts appear in the correct order, including skipping questions when appropriate; and entered values cannot be inadvertently overwritten. Immediately after data entry, a series of logic check programs are run automatically, indicating any entered values that appear incorrect and the reason why. Logic check reports will be reconciled with the hardcopy and, if necessary, the clinician. Changes will then be entered, and the logic check programs executed again in order to detect any new errors resulting from the changed values. The process will continue until the logic check programs detect no further errors.

The electronic database used to house these data until analyses are needed will be a web-based electronic data capture program created uniquely for this project. The database will be password protected and only certain users will be given access to the web-based program. This will protect the electronic data against any unauthorized persons from entering the dataset and jeopardizing the integrity of the data or engaging in some sort of malicious piracy. As this grant matures into the data analysis stage, data will be queried and exported to statistical software, in most cases SAS, for data analysis.

16.0. Protocol Deviations

All protocol deviations will be reported to the IRB and the HRPO. Unanticipated deviations deemed to involve subject safety, subject/study data reliability or validity, or any other deviation in the view of the investigator that cannot wait until time of continuing review for reporting will be reported to the IRB and the HRPO within (5) working days of the date the principal investigator or other research personnel first became aware of the deviation. All other deviations will be reported at the time of continuing review. Examples of these protocol deviations include those that 1) have no substantive effect on the risks to research subjects and 2) do not affect the value of the data collected (e.g., the deviation does not confound the scientific analysis).

17.0. SAFETY Monitoring:

A physician will monitor adverse effects at each visit. In addition, the physician will review vital signs and laboratory data, as they become available. All of these values are recorded and then reviewed by the PI. All adverse events that arise are recorded by a physician. This includes documenting the date of onset, duration, severity, seriousness, and relationship to study medication. Any severe or serious AEs will be reported as soon as possible to the PI. All significant adverse events as well as the progress of the study will be reviewed and discussed in detail at a biannual meeting of the Section of Child and Adolescent Psychiatry Data and Safety Monitoring Board (DSMB).

17.1. Data and Safety Monitoring Board (DSMB)

Data safety and monitoring will occur at several levels. First, we will systematically elicit and document adverse events on a monthly basis. This review includes the possibility of systematic worsening or medication side effects. The Principal Investigator will review reported AEs at regular research meeting. The results will be recorded under de-identified subject ID numbers. This information will be forwarded to Dr. Sergio Delgado. Dr. Delgado will serve as an independent external observer for adverse events. Dr. Delgado is a pediatric psychiatrist at

Cincinnati Children's Hospital Scores from these clinical rating scales will be routinely sent to Dr. Delgado for review every six months. Dr. Delgado will then report back to the investigator and forward the report to the IRB. Initially Dr. Delgado will evaluate safety data after the first 10 youth have been enrolled (5 diagnosed with ASD and 5 ASD+ADHD) or three months after study initiation, whichever comes first. This medical monitor will have the authority to stop the research project or take other steps in order to protect the safety and well-being of research volunteers until the IRB and HRPO can assess the written report.

17.2. Reporting Adverse Events

All serious or unexpected AEs that are possibly related to drug treatment will be reported to the Cincinnati Children's Hospital Medical Center Investigational Review Board within 3 working days. Examples of serious AEs include death, life-threatening event, inpatient hospitalization, persistent or significant disability/incapacity, congenital anomaly/birth defect, and events that require medical or surgical intervention to prevent death, disability, or hospitalization. An unexpected AE is one that is not described in the protocol. In addition, the HRPO, FDA, and drug manufacturer will be notified of any serious or unexpected AE that is possibly related to MPH. Any action resulting in temporary or permanent suspension of the study will be reported to our IRB, the HRPO, and the drug manufacturer.

17.3. Assuring Protocol Compliance and Data Accuracy

All research staff participating in this study have thoroughly reviewed the protocol prior to the initiation of the study. The principal investigator and primary research assistant are responsible for data collection and accuracy of record keeping. They review the study weekly in rounds to monitor recruitment, data collection, and protocol compliance. IRB approval notification, approved continuing review reports, and the final study report will be submitted to HRPO as soon as these documents become available.

18.0. Inclusion of Special Populations

18.1. Inclusion of Women and Minorities

Multiple studies have confirmed that autism is four to five times more common in males than females. Therefore, our projected study composition will reflect this epidemiological fact and propose that 80% of our participants will be male and 20% will be female. Enrollment at the Cincinnati recruitment site will reflect the 4:1 male:female ratio in autism. Recruitment estimates in Cincinnati are based upon the composition of the Greater Cincinnati metropolitan area including areas of Southwest Ohio, Northern Kentucky, and Southeast Indiana. The Greater Cincinnati area breakdown is as follows: White 54.3%, Black or African American 39.8%, Hispanic 2.8%, American Indian or Alaska Native 0.3%, Asian 1.8%, Native Hawaiian and Other Pacific Islander 0.1%.

18.2. Inclusion of Children

ASD is a disorder of childhood onset that are usually diagnosed by the age of three years. It has a severe impact on the individual's development from the time of onset onwards. Early intervention is critical to outcome. Most treatment studies include minors with hopes that early treatment of symptoms will result in better long-term outcome. Informed Consent and Assent: Informed Consent of at least one parent or legal guardian of each subject will be obtained voluntarily after study procedures, risks, and benefits to participation have been explained and all questions are answered. Oftentimes, only one parent is reasonably available for study visits due to work responsibilities, caring for other children, or living arrangements (i.e., one parent lives in

another city or state). When reasonably possible, informed consent will be obtained from both parents. This is consistent with 21 CFR 50. The parent or guardian will be encouraged to ask questions at any time and will be given adequate time to review the consent form. Based on parent report, and/or clinical judgment, if the subject is able to comprehend the assent document and the risks and benefits involved in the study, written, assent will be obtained after the child is given adequate time to review the document. Cognitive and communication deficits are common in this population; therefore, obtaining written assent will not always be possible.

The PI and his collaborators have extensive experience in working with children. The PI is a Triple Board trained, board-certified pediatrician and a child and adolescent psychiatrist and has spent the entirety of his career focused on studying drug treatments for children with autism and other pervasive developmental disorders.

18.3. Inclusion of Cognitively Impaired

Written informed assent will be obtained from those subjects who have the cognitive ability to fully comprehend the document. This will be primarily determined by parent/guardian report, as well as a qualified member of the research team, based on direct observation and clinical judgment. Given that some of the children with autism and pervasive developmental disorders may have additional cognitive or communication deficits, it is possible that a few younger children will not be able to give assent. Efforts will be made to obtain informed assent from each child who is at least 11 years old.

These efforts include the following steps:
The subject's parent or legal guardian will be asked whether the child can read, sign their name, or reliably provide a yes/no answer to a question.
Children who are able to read will be given the assent form to read and will likely be able to provide signed or verbal assent.
If the child is unable to read, but the parent thinks the child is able to understand the assent form if read to him/her and then reliably provide a yes/no answer as to their assent, the assent will be read to them.
If the parent is unsure as to the child's ability to understand the assent form, we will attempt to read the assent to the child/adolescent unless it becomes clear to the investigator and parent that the subject is not listening or understanding. 5. Verbal assent or nonverbal gestures (nodding head "yes" or shaking head "no") will be acceptable if the parent agrees that this was a reliable response to the question.
Assent will not be obtained in children where it would be unreasonable to do so because of the severity of their cognitive limitations.
Although verbal assent may be obtained with some children, it may not be possible to obtain written assent with those children, as they may not be able to write their name and/or initials. The process will be clearly documented by the person obtaining assent with this documentation being put in the note to file and reviewed by the PI.

Only the parent or legal guardian will be asked to give consent on the behalf of the child. The informed consent clearly states that if they agree to participate, they will allow their child to be a part of the research study. Parents/legal guardians are told this verbally as well. It is also explained verbally and in the consent that the study is completely voluntary and they can withdraw at any time and for any reason.

The parent/legal guardian will be asked to inform the child's primary care physician. In addition, the parent/legal guardian will be asked to provide authorization for release of information for

their child's physician, so that additional information can be conveyed, as necessary, throughout the duration of the study.

The research is not likely to interfere with ongoing therapy or regimens. The study physician routinely obtains information regarding concomitant medications, therapies, or regimens. Parents are encouraged to maintain their child's current, non-medical psychosocial treatments throughout the study. In addition, all medications are carefully reviewed for any potential interactions with the study drug. The study medication has associated possible adverse effects. However, there is a greater probability of direct benefit to the subject.

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