Behavioral and Neural Response to Memantine in Youth with ASD
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Study Protocol**:** Behavioral and Neural Response to Memantine in Youth with Autism Spectrum Disorder

Principal Investigator: Gagan Joshi, MD
Massachusetts General Hospital
Clinical and Research Program in Pediatric Psychopharmacology

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I.   BACKGROUND AND SIGNIFICANCE

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by difficulties with socialization & reciprocal communication, along with restricted, repetitive behavior[1]. An increasingly higher prevalence of ASD is documented in each successive epidemiological survey and is now estimated to affect more than 1% of youth[2].

Social Deficits in Autism: Deficits in social interaction are the central feature of autism and often result in significant impairment in cognitively capable individuals with ASD. In light of the growing recognition of ASD in intellectually capable individuals of all ages, there is an acute need for effective treatment for social deficits. Although there are drugs proven to be effective in treating target symptoms of hyperactivity, irritability, & repetitive behaviors in ASD, to date, no medications have consistently been shown to reliably improve social impairments in ASD[3-8]. Earlier studies of fenfluramine, secretin, & naltrexone have largely been disappointing[9-12]. Emerging evidence on the safety & efficacy of glutamatergic agents for the treatment of social deficits in ASD is encouraging.

Glutamatergic Dysregulation in Autism: Glutamate (Glu) is the primary excitatory amino acid neurotransmitter in the brain. Glu, through its activity at N-methyl-D-aspartate (NMDA) receptors, is crucial for neurodevelopmental processes, including neuronal plasticity & higher cognitive functioning[13]. Over-activation of Glu is associated with excitotoxicity & apoptosis. Dysregulation in glutamatergic activity has been hypothesized to contribute to the pathophysiology of ASD[14-15]. Evidence for increased Glu activity in autism comes from serological, postmortem brain, and preliminary genetic studies[16-24]. ^{1}HMRS in autism provides *in vivo* evidence of abnormal glutamatergic brain activity in autism by quantifying Glu levels in combination with glutamine (Gln) & gamma-aminobutyric acid (GABA; Glu+Gln+GABA=Glx). Although no Glx abnormality was identified in the temporal lobes or anterior cingulate cortex (ACC) by 1.5 T ^{1}HMRS in preschool-age children with ASD lower levels of Glx were observed in grey matter by 3T ^{1}HMRS in older school-age children with ASD[25-27]. On the contrary, in adults with ASD, Glx levels are found to be higher in the amygdalo-hippocampal region (on 1.5 T ^{1}HMRS) and decreased in the right ACC (on 3T ^{1}HMRS)[28-29]. More recently, Harada & colleagues[30] specifically examined Glu metabolites at 3T ^{1}HMRS in children with ASD and noted no abnormality in Glu levels in the frontal lobe & lenticular nuclei. Taken together, previous ^{1}HMRS studies in autism suggest Glx dysregulation in various regions, including regions implicated in autism (i.e., medial temporal lobe [MTL] & ACC). Our ^{1}HMRS study in adolescent males with high-functioning ASD (HF-ASD) suggest significantly increased Glu in the ACC with no change in the bilateral MTL regions[31].

Role of Glutamate Modulators in the Treatment of Autism: Glutamatergic agents lamotrigine, amantadine, & D-cycloserine have been studied as potential treatments for symptoms of ASD. Lamotrigine attenuates Glu release by inhibiting voltage-sensitive sodium channels in the presynaptic neuronal membranes[32]. In a randomized-controlled trial (RCT) of lamotrigine in 28 children with ASD, although there was marked improvement in autism behaviors, an equally robust response to placebo (PBO) resulted in the lack of a statistically significant separation between the two groups[33]. Amantadine, an antagonist at NMDA receptors, has also been studied in ASD. In a RCT of amantadine in 39 youth with ASD, amantadine was well tolerated and although

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amantadine was not superior to PBO in treating hyperactivity & irritability per parent report, it was associated with significant improvement in inappropriate speech & illness severity on clinician-rated measures of response[34]. D-cycloserine is a partial agonist at NMDA receptors[35]. In a single-blind PBO lead-in trial D-cycloserine was well tolerated & was associated with significant improvement in social withdrawal[36]. Thus, the empirical evidence for the efficacy of these glutamatergic agents for the treatment of social deficits in autism is modest at best. Memantine is a glutamatergic agent with a unique mechanism of action & the preliminary findings on safety & effectiveness are encouraging[37-38].

Memantine: Memantine hydrochloride is a moderate-affinity, non-competitive, NMDA receptor antagonist. Memantine treatment in adults with Alzheimer's disease improves cognition, as well as functional & behavioral symptoms[39-40]. Available data from limited retrospective & prospective treatment studies of memantine in individuals with ASD report an acceptable tolerability profile with improvement in a range of behavioral impairments including attention, hyperactivity, language, eye contact, social interaction & withdrawal, & repetitive behaviors[37-38].

Brain regions implicated in Autism: Several studies aiming to identify the etiology of autism have indicated involvement of limbic system structures including the amygdala, hippocampus, & the ACC[41-43]. These limbic structures also show a high affinity for NMDA receptor binding. ACC: is functionally associated with information processing and response to emotional cues and is, therefore, a region of interest in autism. The ACC also has close anatomic connections to the amygdala & participates in emotional regulation. Lesions of ACC are known to cause blunted affect, disinhibition, disabling repetitive behaviors, & impaired social judgment including the inability to interpret social cues[44]. Converging evidence from various investigative modalities suggests abnormalities in the ACC region in individuals with ASD including histopathological changes of increased cell packing density & decreased cell size, smaller in volume, decrease in regional cerebral blood flow, & metabolically less active[34-35, 38, 39-40] with abnormal functional activity (theory of mind task related & resting state)[45-52]. MTL: structures, the hippocampus & amygdala, play crucial roles in associative memory & social cognition, respectively, & lesions in the MTL are implicated in social impairments intrinsic to ASD[53-55]. Converging evidence of abnormalities in the MTL in ASD comes from various histopathological & imaging studies revealing decreased neuronal size and increased cell density and cytoarchitectural minicolumnar pathology, bilateral decreased volume & hypo-perfusion, and abnormal activation especially of amygdala on face recognition[41,45-46,48, 55-65].

II.   SPECIFIC AIMS

Primary Aims

Aim 1) To examine the clinical efficacy & tolerability of memantine for the treatment of social impairment in youth with ASD. We will study the short- & long-term clinical effects of memantine in 40 children and adolescents with ASD by conducting a 12-week randomized-controlled trial (RCT).

Aim 2) To examine the effect of memantine therapy on neural function in youth with ASD. We will assess neural response to memantine therapy by measuring spectroscopic

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& resting state functional connectivity (RsFc) changes with memantine treatment. We will also assess association of neural & clinical response to memantine therapy.

Aim 3) To characterize neural functional deficits and/or abnormalities in youth with ASD by comparing ASD subjects to healthy control subjects undergoing the same spectroscopic & RsFc imaging with two scans 12 weeks apart.

III.   LENGTH OF STUDY

This study may take up to 20 weeks from enrollment (allowing up to eight weeks to schedule and complete the initial screening process and baseline scan). Once subjects have completed the screening process and baseline characterization, subjects eligible for scanning will complete a baseline scan (for ASD subjects, this scan will be pre-treatment). ASD subjects will then begin the 12-week randomized-controlled trial. All subjects eligible for scanning, including healthy controls, will receive a follow-up scan approximately 12 weeks after the baseline scan. For ASD subjects, this scan will take place between the 10th and 12th week of treatment. All subjects will be taking the study medication at the time of the second brain scan. ASD subjects ineligible for scanning may participate in the 12-week randomized-control phase of the trial only.

IV.   SUBJECT SELECTION CRITERIA

A. Inclusion Criteria (all participants)
1. Male & female subjects ages 8-18 years (inclusive).

Participants with ASD
3. DSM-5 ASD diagnostic criteria as established by clinical diagnostic interview
4. At least moderate severity of social impairment as measured by a total raw score of ≥85 on the parent/guardian-completed Social Responsiveness Scale-Second Edition (SRS-2)[71] and a score of ≥4 on the clinician-administered **Clinical Global Impression-Severity scale (CGI-S)**.

Healthy Control Participants
3. Age-, sex-, & IQ-matched with ASD participants.
4. No Axis I diagnoses as established by the **Kiddie Schedule for Affective Disorders and Schizophrenia—Epidemiological Version (K-SADS-E)**[70] & confirmed by clinical diagnostic interview.
5. No significant traits of ASD as screened by **SRS-2** (raw score <60).

Exclusion Criteria (all participants)
1. IQ ≤70 based on the Wechsler Abbreviated Scale of Intelligence-II (WASI-II) Vocabulary and Matrix Reasoning subtests
2. Impaired communicative speech
3. Subjects currently treated with the following medications (known to impact glutamate levels):
    a. Lamotrigine
    b. Amantadine

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      c. N-acetylcysteine
      d. D-cycloserine

4. Subjects treated with a psychotropic medication not listed above on a dose that has not been stable for at least 4 weeks prior to study baseline.
5. Co-administration of drugs that compete with memantine for renal elimination using the same renal cationic system, including hydrochlorothiazide, triamterene, metformin, cimetidine, ranitidine, quinidine, and nicotine
6. Initiation of a new psychosocial intervention within 30 days prior to randomization.
7. Subjects who are pregnant and/or nursing.
8. Subjects with a history of non-febrile seizures without a clear and resolved etiology.
9. Subjects with a history of or a current liver or kidney disease.
10. Clinically unstable psychiatric conditions or judged to be at serious suicidal risk.
11. Subjects who meet on the K-SADS-E for alcohol or drug dependence or abuse. If the subject has a recent history of substance abuse, there will be a two-week washout period before initiating the trial as an added precaution. There are no known safety issues relating to memantine and recent history of substance abuse.
12. Serious, stable or unstable systemic illness including hepatic, renal, gastroenterological, respiratory, cardiovascular (including ischemic heart disease), endocrinologic, neurologic, immunologic, or hematologic disease.
13. Subjects with severe hepatic impairment (LFTs > 3 times ULN).
14. Subjects with genitourinary conditions that raise urine pH (e.g., renal tubular acidosis, severe infection of the urinary tract).
15. Known hypersensitivity to memantine.
16. Severe allergies or multiple adverse drug reactions.
17. A non-responder or history of intolerance to memantine, after treatment at adequate doses as determined by the clinician.
18. Investigator and his/her immediate family defined as the investigator's spouse, parent, child, grandparent, or grandchild.

Contraindications to MR scanning—braces, metal in the body, participant refusal to scan (e.g., due to severe anxiety or claustrophobia), etc.—are exclusionary for the scanning component of the trial. Subjects are allowed to have a security object (ei. ball or blanket) that is MR-safe and screened, in the scanner to help with possible anxiety they might experience due to the scanning. Healthy control subjects with contraindications to MR scanning will not be eligible to participate in the trial. However, in the event that an ASD subject with MR scanning contraindications otherwise meets all inclusionary criteria and does not meet any additional exclusionary criteria, that subject will be eligible to participate solely in the 12-week randomized-control phase of the study.

V.   SOURCE OF SUBJECTS

We propose to enroll up to 60 subjects with ASD and 30 healthy controls for a total of 90 subjects. Up to 60 children and adolescents with ASD who exhibit marked social impairment will be enrolled in order to randomize 40 eligible subjects. In addition, up to

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30 children and adolescents with no history of major psychiatric disorders, including ASD, will enroll in order for 20 eligible age-, sex-, & IQ-matched subjects to participate as neuroimaging healthy controls. Subjects will be recruited from the referral pool of existing and new patients at three different MGH sites – the Bressler Program for ASD, the Lurie Center for Autism, & the child psychiatry outpatient clinic – and from the general public by flyers and internet advertisements. Individuals who respond to local advertising will be screened for eligibility first by the study coordinator over the phone using an IRB-approved script prior to enrollment.

Subjects may also be recruited from our department's general pediatric screening research protocol, "A General Screening Protocol for Child and Adolescent Research Studies in the Pediatric Psychopharmacology Program" (2014-P-001103). After participating in the general screening protocol, subjects may be identified as potentially eligible for this study. In cases of potential eligibility, subjects and their parent/guardian will be informed of the study by their evaluating clinician. If they express interest in the study, the subject and their parent/guardian will be referred to this study's coordinator to schedule the first study visit, at which time a study clinician will review this study's informed consent and assent documents with the family. The screening protocol was designed so that some of its study procedures overlap with this study's screening procedures and that of other department studies. If a subject is referred from 2014-P-001103 and within the past 8 weeks, and there have been no changes in potential subject's medication or behavioral therapy, completed some of this study's required screening procedures under 2014-P-001103, we will use data previously collected so as to not burden the subject and/or their parent/guardian with redundant assessments.

In addition to the aforementioned sources, subjects for the healthy control arm of the study will be recruited from the pool of research subjects at the Gabrieli Lab at MIT who have consented to being contacted for future research participation opportunities. Individuals will be phoned and screened for eligibility by the study coordinator using the IRB-approved script.

School representatives in the Boston and Belmont areas (e.g., principals or heads of the parent teacher organization/association) will also be approached via telephone or email and asked if study advertising materials can be brought to their school or placed in their school communications (e.g., newsletter). Only upon hearing back from the school representative will any recruitment materials be provided.

VI.   SUBJECT ENROLLMENT

Informed consent/assent will be obtained prior to the performance of any protocol procedures and prior to administration of study drug for ASD subjects. The informed consent and assent documents will be used to explain, in simple terms, the risks and benefits of study participation to the subject and their parent/guardian. If a subject is of majority status, an investigator will obtain informed consent on the "Signature of Subject" signature line. The investigator will explain that "you and your child" and "your child" should be interpreted as applicable to the adult subject. Since this study involves

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significant involvement of the parent, an investigator will also attain informed consent from a parent, who will sign the same consent form on the "Signature of Consent" signature line. The nature of the study will be fully explained to the subject and his/her parent/guardian by a board-certified physician who is either the principal investigator or a co-investigator. The subject and his/her parent/guardian will be encouraged to ask questions pertaining to their participation in the study and the subject and his/her parent/guardian may take as much time as they feel necessary to consider their participation in the study, as well as to consult with family members or their physician. Participation in this study is voluntary and the subject and/or his/her caretaker may withdraw the subject from the study at any time. The IRB-approved informed consent/assent documents will be signed and dated by the subject's parent/guardian, the subject, and the physician obtaining consent.

VII.   STUDY PROCEDURES

This study includes two components: a 12-week randomized-controlled trial of memantine for the treatment of Autism Spectrum Disorder, and two ^{1}HMRS scanning sessions pre- and post-treatment.

After providing written informed consent and assent, all subjects will complete a clinical diagnostic interview with a study clinician to assess eligibility. All subjects will be administered a detailed assessment battery including a brief demographic interview, a pre-MR checklist to rule out contraindications to scanning, an indirect structured diagnostic interview (K-SADS-E), the SRS-2, and the following assessments to assess cognitive capabilities: WASI-II, the Wechsler Intelligence Scale for Children (WISC-IV; 8-16 year olds) and the Wechsler Adult Intelligence Scale (WAIS-IV; 17 and 18 year olds only) to assess cognitive capabilities.

If both parents of the subject are available, we will ask each parent to independently complete an SRS-2 form. Both forms will be scored, and the form with the higher score will be used to determine subject eligibility in order to reduce the possibility of under-recognition of ASD symptoms.

All participants will be required to give a urine sample to test for certain types of drugs. This includes prescription drugs, illegal drugs (street drugs), and controlled substances (substances that may be habit forming) that may affect behavior and that may be regulated by law. Results of the drug screen will be conveyed to the participant by the study clinician and if the results are positive for drug(s) there will be further discussion with the participant to determine if they are appropriate to participate in the trial.

In addition, female ASD and control subjects of childbearing potential will have a urine pregnancy test. If a participant has a positive urine pregnancy test, she will not be able to take part in the study. The study doctor will inform the participant of any positive test results. The decision whether to inform the subject's parent/guardian of these results will be made by the study doctor based on the participant's age and maturity level.

ASD subjects will also be assessed the Autism Diagnostic Observation Schedule (ADOS)[68]. This assessment is recommended, but due to limited ADOS rater availability, it will only be performed if time and schedule of participant and ADOS rater permit. If

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determined clinically necessary by study physician, the Clinical Evaluation of Language Fundamentals-Fourth Edition (CELF-4). ASD subjects will complete physical assessment measures (complete physical examination, tanner staging, vitals, height, ECG, and blood screening tests). If a subject refuses a blood draw during the screening visit due to extreme fear and anxiety, they will not be asked to provide a blood sample at the completion visit. For a complete schedule of assessments, refer to the Table I (page 18). The screening process may take place over multiple days, as necessary.

We anticipate that subjects may enter this study following completion of/withdrawal from other ASD protocols in our office, and that there may be procedural overlap. So as to not burden subjects/parents/guardians with redundant time commitments, we will use the following diagnostic data previously collected. If a subject has completed an evaluation with one of the study clinicians and/or the structured diagnostic assessment in the three years prior to entrance into this study, he/she will not be asked to repeat any overlapping diagnostic procedures. We will use the study diagnostic data that had been previously collected so as to not burden the subject with redundant time commitments. However, the study clinician will review the interval time period to assess for clinically significant medical or psychiatric history, to ensure that the subject meets appropriate study entrance criteria.

If a subject has been assessed with the WASI-II or WISC-IV/WAIS-IV in the 12 months preceding their entrance into this study, subjects will not be asked to repeat these procedures. We will use the data previously collected so as not to burden the subject with redundant time commitments.

All data will be collected and entered into StudyTRAX, an electronic data capture system that streamlines data collection and ensures data integrity. StudyTRAX software allows researchers to design and implement study surveys electronically for collecting, storing, retrieving, and manipulating data.

Parents/guardians and/or research staff will enter survey responses into electronic assessment forms using computers at the research site. The responses will then be transmitted securely via an encrypted connection and stored in a secured database. Electronic data capture eliminates the need for subsequent data entry by staff, thus minimizing human error. However, in the event that StudyTRAX is unavailable or malfunctioning, study staff will print all study instruments and study data will be collected in paper form.

12-Week RCT (ASD Subjects only):

Participating children and adolescents with ASD who meet the eligibility criteria will be randomly assigned to either memantine or placebo for the course of the 12-week RCT. Subjects will be assessed weekly during the titration phase (Weeks 1-4) & during the maintenance phase at midpoint (Week 6), Week 9, & at completion (Week 12/early termination). The same caretaker (a parent/guardian most familiar with the subject's day-to-day behavior) will participate in the assessment of behavioral symptoms at all visits of the treatment phases.

Randomization: The MGH Clinical Trials Pharmacy will prepare the blinded

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memantine and placebo capsules for the study. ASD subjects will be randomized to either active memantine or placebo in a 1:1 ratio after they have been determined to meet all eligibility criteria. Randomization lists stratified by gender and racial/ethnic minority status (minority vs. Caucasian) will be generated by the statistician & passed to the investigational pharmacy for assignment.

Washout Period: ASD subjects who are currently being treated with prohibited psychotropic medications as listed in the exclusion criteria must discontinue the use of their medication to be eligible for participation in this study. Medication washout is recommended by our clinicians to participants, their parent/guardian, and current providers – this is done based on a case-by-case assessment, considering the duration on drug, the dose, and the adverse effects associated with the treatment and effects of stopping that medication/treatment. Our office does not take over care for the patient, but remains available during this time period. The washout schedule –which will span two weeks or more -- will be discussed with the participant, their parent/guardian, and current providers. Individuals taking a medication that is effectively and safely treating their symptoms will not be taken off of such medication for the purpose of enrolling in this study.

Trial Phase (Weeks 0-12): Participants will be prescribed study medication for the period of 12 weeks. Study visits will have a visit window of +/- 3 days to facilitate scheduling. Although every effort will be made to encourage subjects to keep regularly scheduled appointments, in the event that a subject is unable to come into the office within a reasonable timeframe of a scheduled visit, and the treating research clinician feels that subject safety will not be jeopardized by doing so, the clinician can conduct the visit with the subject and parent/guardian over the phone. This will ensure that each subject will be continuously monitored by the clinician throughout the course of the study despite unforeseen scheduling circumstances. In the case of a phone visit, the subject will be dispensed two weeks of study drug. The following study visits cannot be conducted over the phone: Screening Visits (Week 99), Baseline (Week 0), Week 6, and Week 12. Additionally, phone visits may not occur for two consecutive visits. At each visit, safety & efficacy will be assessed by administering measures of efficacy (CGI & DSM Global Assessment of Functioning Scale [GAF]), tolerability (assessing treatment-emergent AEs), & safety (vital signs [blood pressure, pulse, weight]). Vital signs will not be collected if the visit is conducted over the phone. At baseline & endpoint (completion/drop-visit), the following assessment measures will be administered: MGH Social Emotional Competence Scale-Clinician Rated and –Informant Rated (MGH-SEC -S-C and –I), ABC, Children's Yale-Brown Obsessive Compulsive Scale modified for PDD (CY-BOCS-PDD), ADHD-Symptom Checklist (ADHD-SCL), Children's Depression Rating Scale-Revised (CDRS-R), Child & Adolescent Symptom Inventory-5-Anxiety (CASI-Anx), BRIEF-Parent, Social Adjustment Inventory for Children and Adolescents (SAICA), and Diagnostic Analysis of Nonverbal Accuracy (DANVA 2). If a subject is 18 years old, the subject will be administered the adult version of the DANVA2. The SRS-2, BRIEF-Parent, CY-BOCS-PDD, MGH-ASD-RS-C and MGH-ASD-RS-I, will also be administered at midpoint. At the endpoint visit of the trial, participants will be also reevaluated on the physical assessment measures, the SRS-2, and the CBCL.

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Dose Titration Phase (Weeks 1-4): Study medication will be initiated at 2.5 mg/day, will be raised to 5 mg/day on day 4, and will be gradually up-titrated by 5 mg/week to a maximum dose of 20 mg/day. Titration of the study medication will be guided by the (following) forced titration schedule with an option for slower titration or holding at lower dose per clinician judgment. Memantine will be administered in twice daily divided dosages with a total daily dose of ≥2.5 mg/day.

Study Medication Dosing: Study medication (memantine/placebo) will be titrated to the maximum daily dose during the first 4 weeks of the trial (dose titration phase). Subjects will follow the following forced titration schedule until Week 4 when subjects will be maintained on maximum achieved dose until the end of the trial (dose maintenance phase; Weeks 5-12). During the dose maintenance phase, there will be a one-time option to decrease the dose of the study medication.

Memantine Forced Titration Schedule

Visit	Day	Maximum Total Dose Prescribed (mg/day)
0	1	2.5
	5	5
1	8	10
2	15	15
3	22	20
4	28	Maintained on maximum achieved dose

Primary Outcome Measure of Efficacy: Clinician-rated CGI-Improvement (CGI-I) subscale and the parent-rated SRS-2. Treatment responders will be defined as those who demonstrated improvement of ≥25% on the SRS-2 total raw score *and* a score of 2 or 1 on the CGI-I subscale ("much" or "very much improved").

Secondary Outcome Measures of Efficacy: As with other psychotropic treatment outcomes in this population[72], response to memantine may not be uniform for all domains of ASD. The response differential will be examined by assessing change in the severity of the subscales of the SRS-2 representing core domains of ASD, change on the ABC-SW subscale, and change on the MGH-ASD-RS (clinician- and informant-rated). The CY-BOCS-PDD[73-74] will be administered to assess change with treatment in the ritualistic behaviors associated with ASD. Change with treatment in the level of adaptive functioning will be assessed by the CBCL. Change in the level of global functioning will be assessed by the clinician-rated GAF[76]. Change with treatment in social adjustment will be assessed by the parent/guardian-rated SAICA. Considering that features of ADHD, anxiety, & depression are frequently associated with ASD[4] & there is evidence of memantine's effectiveness in treating ADHD in adults, these symptoms will be assessed by administering the clinician-rated ADHD-SCL, CDRS-R[79], & CASI-Anx[80].

Safety & Tolerability Outcome Measures: The safety & tolerability of memantine treatment will be monitored by administering a complete physical examination, urine tests & ECG at baseline & endpoint of the trial, and by recording AEs and obtaining vital signs at each in-office study visit.

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Concomitant Medications/Treatments: As part of the initial psychiatric evaluation, a detailed history of past and present treatments (pharmacological and non-pharmacological) will be obtained. At each study visit, subjects will be assessed for the use of concomitant medication. Subjects will be allowed to take melatonin (up to 3 mg) or Benadryl (up to 50 mg) at bedtime as needed (pm) for insomnia. Non-pharmacological treatments such as supportive individual, family, or group therapy will be permitted provided they were in place for a substantial period of time (> 1 month) prior to the study participation and remain unchanged during the course of the trial. No new non-pharmacological treatments are to be initiated during the course of the trial.

Drop Criteria: A subject may be withdrawn from the study at any time if any of the following conditions are met:

- Worsening of ASD, ADHD, anxiety, depression, mania, OCD or psychosis, as reflected by respective Clinical Global Improvement score of 6 (Much worse) or 7 (Very Much Worse) for 2 visits in a row.
- Subjects who experience intolerable adverse effects, and/or clinically significant laboratory values inconsistent with continuation in the study as determined by PI.
- Unstable psychiatric condition that clinically requires 1) treatment with prohibited concomitant psychotropic medications or 2) subjects requiring inpatient psychiatric admission.
- Emergent suicidality
- Active substance abuse
- Pregnancy
- Allergic drug reaction
- Non-compliance (less than 70% compliance for 2 visits or longer based on parent report)
- Failure to return medication for 2 consecutive visits
- Failure to keep study appointments for more than 2 consecutive visits without justification
- Clinical judgment of the investigator
- Withdrawal of consent

If study participation is discontinued due to safety reasons, participants will receive three follow-up visits, giving adequate time for appropriate psychiatric referrals to treaters in their community. If emergent suicidality were to occur during the course of the study, the supervising clinician will then directly assess the level of risk and take the appropriate action (including contacting treatment providers, working on a safety plan, arranging for emergency evaluation via an ER, calling 911, etc.) The clinician will document the actions taken, and it will be noted in the participant's file. Subjects who discontinue due to non-compliance with the protocol will receive a referral to ASD treaters in the area.

^{1}HMRS Scanning (ASD and Healthy Control Subjects):

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All subjects eligible for scanning will complete two scanning sessions at the McLean Imaging Center over the course of the study. For subjects with ASD, the initial scan will take place after evaluation and screening and prior to randomization. ASD participants will have a second scanning session at McLean between the 10th and 12th week of treatment.

Upon signing consent/assent at MGH, healthy control subjects will participate in screening procedures to assess eligibility. Those who meet eligibility criteria and complete screening procedures will participate in two scanning sessions at McLean Imaging Center. The first scanning session will take place after screening procedures are completed at MGH, and the second scanning session will take place approximately 10-12 weeks after the first.

During the first scanning visit, subjects will undergo a urine drug screen to determine recent drug use, and all women of childbearing potential will be asked to undergo a urine pregnancy screen. A breath sample will be obtained to screen for alcohol. These procedures will be repeated when subjects return to complete for their second scanning visit. Additionally, parents/guardians of all eligible subjects will complete the Child Behavior Checklist (CBCL) during the first scanning visit at McLean Imaging Center in order to assess the child's maladaptive emotional and behavioral problems at the time of the scan.

At the first scanning visit, the MR protocol will consist of anatomical, diffusion weighted, resting state fMRI multiband, and spectral data acquisitions. The visit will consist of two separate scans: DTI, anatomical, and multiband resting state fMRI scans (35 minutes) will be performed on the 3 Tesla scanner (Siemens Trio) with a full TIM upgrade in place using a 32-channel phased-array head coil. MRS scans (60 minutes) will be acquired on a 4 Tesla Varian Unity/Inova whole body MR scanner (Varian NMR Instruments, Palo Alto, CA) equipped with proton TEM volumetric head coil (MR Instruments, Minneapolis, MN). At the second scanning visit, the MRS protocol will be repeated on the 4T scanner, and only the multiband resting state protocol will be repeated on the 3T scanner. Anatomical MR images will be used for ruling out clinically significant central nervous system disorders. It will be comprised of an axial high-resolution T1-weighted MRI obtained with a magnetization-prepared rapid acquisition of gradient echo sequence (TR/TE=2100/2.74 ms, acquisition time=5 minutes), a double echo T2-weighted conventional spin echo MRI (TR/TE=4000/33 ms, acquisition time=2 minutes), and a fluid-attenuated inversion recovery MR imaging sequence (TR/TE=9820/95 ms, acquisition time=3 minutes). No contrast agent will be injected. A board-certified neuroradiologist will review the anatomical MRIs. Individuals with structural brain abnormalities on MRI will be referred for a neurological evaluation and will not be included in the study.

Diffusion weighted images will be obtained with gradients applied in 72 directions (TE=92 ms, TR=6400 ms, matrix= 128x128, FOV=224x224 mm2, 42 continuous slices of 3.5 mm thickness, b value = 1000 s/mm2, acquisition time = 9 minutes).

Resting-state fMRI multiband EPI data will be obtained with the following parameters: TR/TE = 1500/35 ms, flip angle 66°, matrix = 106×106 on a 212 mm×212 mm FOV, multiband factor = 6, 72 2.0 mm slices, 520 measurements with acquisition time of 13 min 28 seconds. The total acquisition time on the 3 Tesla scanner will be about 35 minutes.

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Proton spectra will be acquired at 4T using a two-dimensional J-resolved (2D-JPRESS) ^{1}HMRS protocol. A 2D-JPRESS sequence was chosen to improve reliability for spectral fitting of the metabolites, allow analysis of Glu alone versus ratios or combined measures (e.g., Glx), and confirm that measured metabolic differences truly arise from differences in metabolic levels and not from T2 relaxation time differences. An 8 cc (2 x 2 x 2 cm) single voxel will be placed in the ACC along the midline such that the inferior edge of the voxel is parallel to the descending surface of the corpus callosum, as determined by longitudinal relaxation time (T1) images used also for tissue segmentation. Then, 3.375 cc (1.5 x 1.5 x 1.5 cm) a single voxel will be placed bilaterally the right MTL staying clear of the tip of the temporal bone to avoid shimming problems. Data will be collected in 12 TE-stepped spectra with the echo-time ranging from 30 to 250 ms in 20 ms increments, with TR = 2s, averages = 16 (for ACC) & 32 (for MTL), scan duration= 7 minutes (for ACC) & 13 minutes (for MTL). Spectral analysis will be conducted in a fully automated fashion using the commercially available LC Model package (version 6.2-1F). The total time of the MRS data acquisition will be up to 60 minutes, including a three plane set of fast localizers to ensure optimal patient positioning (12 seconds), T1-weighted sagittal and axial images for segmentation and voxel positioning (6 minutes), and local shimming/pulse optimization (15 minutes).

ASSESSMENTS (see Table I)

Autism Diagnostic Observation Schedule (ADOS)[67]
- The ADOS is a semi-structured assessment of communication, social interaction, and play (or imaginative use of materials) for individuals suspected of having autism or other pervasive developmental disorders.
- Consists of four modules, each of which is appropriate for children and adults of differing developmental and language levels, ranging from nonverbal to verbally fluent.

Kiddie Schedule for Affective Disorders and Schizophrenia-Epidemiologic Version (K-SADS-E)[70]
- This is a widely used, semi structured, diagnostic interview with established psychometric properties. It can be effectively administered by clinicians and/or trained non-clinician interviewers in 45 to 90 minutes, although more complex cases may require additional time.
- For all subjects, psychiatric data will be collected from the subject's parent/guardian.

Clinical Evaluation of Language Fundamentals-Fourth Edition (CELF-4)[81]
- The CELF-4 is an individually administered test for determining if an individual (5-21 years old) has a language disorder or delay.
- Assesses four aspects of language (morphology and syntax, semantics, pragmatics, and phonological awareness) and can be administered in 30-60 minutes.

Neuropsychological Battery

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- Wechsler Abbreviated Scale of Intelligence—Second Edition (WASI-II) Vocabulary and Matrix Subtests: to calculate verbal, performance, and full-scale IQ (Wechsler, 2011).
- The following subtests from the Wechsler Adult Intelligence Scale (WAIS-IV) for 17 and 18 year olds or the Wechsler Intelligence Scale for Children (WISC-IV) for 11-16 year olds: Digit Span, Arithmetic, and Letter/Number Sequencing to assess working memory and Digit/Symbol Coding and Symbol Search to assess processing speed (Wechsler, 2003; Wechsler 2008).

Demographic Interview
- A brief demographic interview will be conducted with the subject's parent to estimate socioeconomic status, as well as collect information about any educational accommodations and past head injuries and/or trauma.

Diagnostic Analysis of Nonverbal Accuracy Scale (DANVA 2)[84-85]
Subjects will be administered 2 tasks of social competence that test the subject's ability to recognize feelings expressed through faces and paralanguage by children: 1) Child (or Adult) Faces 2) Child (or Adult) Paralanguage. Each computer-administered subtest includes 24 photographs or 24 audio clips of child models (12 female, 12 male per subtest) displaying equal numbers of high- and low-intensity expressions of happiness, sadness, anger, and fear. Both subtests have been standardized and have acceptable internal consistency and reliability[82-83]. This test can be administered for testing social competence in children as young as 3 years of age.

Rating Scales

Parent Rated:
- Social Responsiveness Scale-Second Edition (SRS-2)[71] a 65-item rating scale completed by the parent used to measure the severity of autism spectrum symptoms as they occur in natural settings.
- Behavior Rating Inventory of Executive Function (BRIEF-Parent)[86] a 78-item rating scale to assess level of executive function deficits.
- Aberrant Behavior Checklist[69] a 58-item scale completed by the parent and reviewed by a clinician to establish the frequency of problematic or abnormal behaviors
- Child Behavior Checklist (CBCL)[88] is a parent-report questionnaire that evaluates maladaptive behavioral and emotional problems, both internalizing and externalizing, in children ages 6-18.
- Social Adjustment Inventory for Children and Adolescents (SAICA)[89] is a semi-structured interview administered to the child or parent/guardian that assesses social functioning in children 6-17 years old. Content areas include activities, peer relations, family relations, and academic performance (John et al., 1987).
- MGH- Social Emotional Competence Scale-Informant Rated (MGH-SECS-I): This is a 37-item scale that asks informants to rate subjects on their social competence and abilities on a Likert scale from 0 to 6.

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- <u>Pre-MR Checklist</u>: This checklist reviews basic medical history that may be pertinent to MR scanning and potential MR contraindications including metallic implants or claustrophobia.

Clinician Rated:

<u>Clinical Global Impression Scale (CGI)</u>[87]

- The CGI is a measure of illness severity, improvement, and efficacy of treatment (National Institute of Mental Health, 1985). The score for severity ranges from 1 (normal, not at all ill) to 7 (among the most extremely ill subjects). Improvement ranges from 1 (very much improved) to 7 (very much worse). And lastly, the effectiveness index measures to what extent the subject is experiencing therapeutic effects as well as the level of adverse events they are experiencing. The CGI scale will be used for the assessment of global functioning. Additionally, the following disorder-specific CGIs will also be administered: ASD, ADHD, OCD, psychosis, mania, depression, and anxiety.

<u>Depression</u>

- The Children's Depression Rating Scale (CDRS)[79] is a widely used observational rating measure of depression severity in children and adolescents.
- CGI-Depression.

<u>Anxiety</u>

- Children and Adolescent Symptom Inventory-5 Anxiety Scale (CASI-Anx)[80]
- CGI-Anxiety.

<u>ADHD</u>

- ADHD Symptom Checklist assesses each of the individual symptoms of ADHD based on the DSM (0-3 on a scale of severity)
- CGI-ADHD.

<u>Psychosis</u>

- CGI-Psychosis.

<u>Obsessive-Compulsive Disorder (OCD)</u>

- The Children's Yale Brown Obsessive Compulsive Scale for PDD (CY-BOCS-PDD)[74] will be used to assess obsessive and compulsive symptoms. This is a clinician-rated 10-item scale (total range from 0 to 40), with subtotals for obsessions (items 1-5) and compulsions (items 6-10).
- CGI-OCD.

<u>Autism Spectrum Disorder (ASD)</u>

- CGI- ASD

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- CGI- ASD– Social Interaction.
- CGI- ASD– Mannerisms.
- CGI- ASD– Social Communication.
- MGH ASD Symptom Checklist (MGH-ASD-SCL): The spectrum of ASD symptoms will be screened by using clinician administered MGH-ASD-SCL. This screening instrument adopted items from DSM-5 diagnostic criteria for ASD and assesses for the individual core domains and associated features of ASD.
- Social Emotional Competence Scale–Clinician Rated (MGH-SEC-S-C): This is a 37-item scale that assesses social competence and abilities in relation to ASD on a Likert scale from 0 to 8.

Level of Functioning
- DSM Global Assessment of Functioning Scale (GAF)[76]: a composite rating of an individual's overall level of functioning (1= worst to 100 = best).

Safety
- Adverse Experiences: to record any adverse health events experienced during the study, along with duration, severity, cause, treatment, and outcome.
- Concomitant Medications: to record additional medications taken during the study.

VI. BIOSTATISTICAL ANALYSIS

Clinical Data Analysis
Because this is a RCT following subjects over a short period of time, missing data are not expected to impact our analyses such that standard statistical tests will be employed. Changes in the primary outcome measures of efficacy (CGI-I & SRS-2) within & between study groups over time will be tested with longitudinal generalized estimating equation (GEE) regression models (RM) estimated using STATA 12.0 within the framework of the general linear model (GLM). For binary outcomes, logistic RMs will be fit with the binomial family & the logit link. For count data, Poisson RMs will be fit with the Poission family & the log link, for normally distributed data, linear RMs will be fit with the Gaussian distribution & identity link. Each model will predict outcome scores from treatment group (memantine vs. PBO), study visit (ordinal predictor), & the group by visit interaction, which is our test of efficacy. Secondary outcome measures (ABC-SW, GAF, ADHD-SCL, CY-BOCS-PDD, CDRS-R, CASI-Anx, & SAICA) will be examined using models similar to the primary outcome measures. Changes in the tolerability outcome measurements will be tested using Chi-squared tests. All analyses will be intention to treat (ITT).

Neuroimaging Data Analysis
After the 2D-JPRESS dataset is resolved into a series of one-dimensional spectra where each spectrum is modeled and fitted with GAMMA simulated J-resolved basis sets, Glu levels will be derived from the total integral across the J-series. Glu transverse relaxation time (T2) will be obtained from the raw peak vs. TE decay curve fitted with

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Levenberg-Marquardt algorithm using an exponential decay function (for Glu T2) convoluted with a polynomial function (for Glu J-coupling) that will be obtained from the GAMMA simulation run with previously measured spectral parameters. Data quality will be assessed by LCModel calculated Full Width Half Maximum (FWHM), Signal to Noise Ratio (SNR) and Cramer-Rao Lower Bounds (CRLB).

The following brain measures will be examined between groups and within-subject across time (e.g., pre- versus post-treatment and correlations between the measures of brain activity and clinical features):

1) ^{1}HMRS measures of brain Glu in:
    a) ACC
    b) MTL
2) measures of resting state functional connectivity of:
    a) dorsal & ventral ACC with prefrontal cortex including VMPFC;
    b) bilateral amygdala with insula & VMPFC;
    c) bilateral hippocampus with posterior cingulate cortex.

Pretest-posttest brain activity differences will be analyzed using linear regression models, where treatment group is the primary predictor and pretest measures of brain activity are covariates. A general linear regression model will evaluate the difference between the treatment groups in posttest brain measures while controlling for each subject's pretest measures of brain activity. This approach is equivalent to analyses of covariance (ANCOVA). We will also test if changes in Glu in memantine and placebo (PBO) responders differ from changes in Glu over time in healthy controls (HCs). This requires a three-group comparison: memantine responders vs. PBO responders vs. HCs. We will use a GLM to compare Glu changes from baseline to endpoint. If the direction and magnitude of change among the three groups are the same, there will be no group by time interaction. If the interaction is significant and responders show greater changes compared to HCs, it will indicate that the changes observed in responders are greater than expected from normal changes in Glu activity. We will do follow-up tests to separately test the interactions for each pair of the three groups analyzed.

We also predict normalization of brain activity measures with memantine treatment. "Normalization" will be defined as having achieved a resting state functional connectivity level that is no more extreme than the 95th percentile of the control distribution. Normalization ratings between the memantine and PBO groups will be compared using a logistic RMs. A significant effect would mean an increased likelihood of normalization in memantine-treated subjects.

To test our hypothesis of a significant association between neural and clinical response and between glutamate and functional connectivity response, we will use endpoint clinical efficacy scores as the dependent variable and change in brain activity measures from baseline to endpoint as the independent variables. For our exploratory hypothesis examining neural biomarkers of response to memantine therapy, we will use a GLM with endpoint clinical efficacy scores as the dependent variable, baseline brain activity measures as the independent variables, and baseline clinical scores as a covariate.

Resting state data will be analyzed using a seed driven approach with custom software (Conn)[88]. Data will be slice time corrected, realigned, coregistered, normalized, and spatially smoothed as necessary. Physiological & other spurious sources of noise

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will be estimated using the CompCor method, and removed together with movement-related covariates. The residual BOLD time-series will be band-pass filtered over a low-frequency window of interest (0.009Hz<f<0.08Hz). Correlation maps will be produced by extracting the residual BOLD time course from bilateral anatomically defined amygdala (WFU_Pickatlas) seed regions, and computing Pearson's correlation coefficients between that time course and the time course of all other voxels. Correlation coefficients will be converted to normally distributed scores using Fisher's transform to allow for second-level General Linear Model analyses. Between group differences will be calculated for each ROI.

Power Considerations

Spectroscopic: For our preliminary data from 7 ASD and 7 HC subjects, our significant finding (paired t-test, p =0.01) of 31% higher Glu levels in the ACC of ASD subjects compared to HCs was determined to have a large effect size of d=1.20 with an associated power= 0.55. Left MTL data produced an effect size d=1.03 with power = 0.43. Right MTL data produced an effect size d=1.15 with power =0.51. Assuming we observe the same sampling variability in the proposed study, we have greater than 90% power with a large effect size of d=1.10 to detect a 15% decrease in Glu in the ASD group with treatment.

Functional: In our preliminary studies (ASD: N=17; HC: N=16) of resting state functional connectivity with the bilateral amygdalae seeds, we found significant hyperconnectivity of the ASD group compared to the HC group (e.g., with the insula, p<0.0001 FWE cluster level corrected), and based on the preliminary data, power will be at 95% to detect between-group differences between the proposed ASD (N=40) and HC groups (N=20).

VII. RISKS AND DISCOMFORTS

Risks of Taking Memantine:

The most frequently observed adverse reactions with memantine are dizziness, confusion, headache, constipation, hypertension, cough, pain, hallucinations, somnolence, vomiting, dyspnea, and fatigue. Serious adverse reactions include Stevens-Johnson syndrome and seizures. All participants will be closely monitored for serious adverse reactions and drug-drug interactions with their ongoing concomitant medications. All serious unexpected adverse experiences of a research subject will be reported to the Partners Human Research Committee.

Problems and side effects not listed above and not known at this time could occur. Subjects will be told of any changes in the way the study will be done and any newly discovered risks to which they may be exposed.

Risks of Blood Draws:

When blood is drawn, some discomfort may be associated with it at the time of the blood draw. Bruising and/or bleeding at the needle site may occur. Occasionally a person feels faint. Rarely, an infection may develop. If an infection does occur, it can be treated. A topical anesthetic cream (Topicaine, EMLA, or ELA-MAX) will be applied to numb the skin where blood will be drawn if subjects prefer.

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Risks of ^{1}HMRS:

^{1}HMRS is not associated with any known adverse effects except to people with metal or magnetic implants (such as metal clips from surgery or a cardiac pacemaker). Therefore, if a subject has such metal objects in her/his body, s/he will be excluded from participating in the scanning component of the study.

There are no known risks of scanning for fetuses. However, the safety of scans for pregnant women and nursing mothers has not been established. Therefore, subjects must have a negative pregnancy test prior to each scan and nursing mothers cannot participate. If a participant has a positive pregnancy test, the study doctor will inform the subject and she will not be able to take part in the study. The decision whether to inform the parent of these results will be made by the physician based on the participant's age and maturity level and the requirements of the law, unless the participant agrees to parental notification. Birth control will be required for subjects who are sexually active. There may be some risk of emotional distress in the event of a positive pregnancy test.

Risks of Assessments:

Some of the questions asked in this study may make subjects feel uncomfortable, and some of the neuropsychological testing may be boring or frustrating. While we hope subjects and their parents will answer all questions, they may skip any questions they do not wish to answer.

Adverse events and unanticipated problems will be reported to the PHRC according to current guidelines. We will follow and adhere to all guidelines as defined and outlined on the Partners Human Research Committee web site:

(http://healthcare.partners.org/phsirb/adverse_events.htm).

VIII. POTENTIAL BENEFITS

There may be no direct benefit to subjects participating in this study. Potential benefits to the participants include education about ASD, a trial of medication that could be continued after the study, and the opportunity to contribute to medical science and thus help others with the disorder.

All subjects, including healthy controls, may receive up to $150 for completing both scanning visits ($75 will be paid per completed scanning visit).

IX. REFERENCES

1. Association AP, DSM-5 Sourcebook. Vol. 1. 2013, Washington, DC: American Psychiatric Association.
2. Autism and Developmental Disabilities Monitoring Network Surveillance Year 2008 Principal Investigators; Centers for Disease Control and Prevention. Prevalence of autism spectrum disorders--Autism and Developmental Disabilities Monitoring Network, 14 sites, United States, 2008. MMWR Surveill Summ. 2012;61(3):1-19. PMID: 22456193.
3. Research Units on Pediatric Psychopharmacology Autism Network: Risperidone in children with autism and serious behavioral problems. N Engl J Med. 2002;347: 314-321. PMID: 12151468.

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4. Research Units on Pediatric Psychopharmacology Autism Network. Randomized, controlled, crossover trial of methylphenidate in pervasive developmental disorders with hyperactivity. Arch Gen Psychiatry. 2005 Nov;62(11):1266-74. PMID: 16275814.

5. McDougle C, Holmes J, Carlson D, Pelton G, Cohen D, and Price L. A double-blind placebo-controlled study of risperidone in adults with autistic disorder and other pervasive development disorders. Arch Gen Psychiatry. 1998;55: 633-641. PMID: 9672054.

6. McDougle C, Naylor S, Cohen D, Volkmar F, Heninger G, and Price L. A Double-blind, Placebo-Controlled Study of Fluvoxamine in Adults with Autistic Disorder. Arch Gen Psychiatry. 1996;53: 1001-1008. PMID: 8911223.

7. Hollander E, Anagnostou E, Chaplin W, Esposito K, Haznedar MM, Licalzi E, Wasserman S, Soorya L, and Buchsbaum M. Striatal volume on magnetic resonance imaging and repetitive behaviors in autism. Biol Psychiatry. 2005;58: 226-32. PMID: 15939406.

8. Hollander E, Soorya L, Chaplin W, Anagnostou E, Taylor BP, Ferretti CJ, Wasserman S, Swanson E, and Settipani C. A double-blind placebo-controlled trial of fluoxetine for repetitive behaviors and global severity in adult autism spectrum disorders. Am J Psychiatry. 2012;169: 292-9. PMID: 22193531.

9. Campbell M, Adams P, Small AM, Curren EL, Overall JE, Anderson LT, Lynch N, Perry R. Efficacy and safety of fenfluramine in autistic children. J Am Acad Child Adolesc Psychiatry. 1988;27(4):434-9. PMID: 3053609.

10. Williams K, Wray JA, and Wheeler DM. Intravenous secretin for autism spectrum disorders (ASD). Cochrane Database Syst Rev. 2012;4: CD003495. PMID: 16034901.

11. Campbell M, Anderson L, Small A, Adams P, and Gonzalez N. Naltrexone in autistic children: Behavioral symptoms and attentional learning. J Am Acad Child Adolesc Psychiatry. 1993;32: 1283-1291. PMID: 8282676.

12. Willemsen-Swinkels S, Buitelaar JK, and van Engeland H. The effects of chronic naltrexone treatment in young autistic children: A double-blind placebo-controlled crossover study. Biol Psychiatry. 1996;39: 1023-1031. PMID: 8780837.

13. Cotman CW and Anderson AJ. A potential role for apoptosis in neurodegeneration and Alzheimer's disease. Mol Neurobiol. 1995;10: 19-45. PMID:7598831.

14. Carlsson ML. Hypothesis: is infantile autism a hypoglutamatergic disorder? Relevance of glutamate - serotonin interactions for pharmacotherapy. J Neural Transm. 1998;105: 525-35. PMID: 9720980.

15. McDougle CJ. Current and emerging therapeutics of autistic disorder and related pervasive developmental disorders. In: Davis KL, Charney D, Coyle JT, et al, eds. Psychopharmacology: The Fifth Generation of Progress. Philadelphia, PA: Lippincott Williams & Wilkins. 2002.

16. Shimmura C, Suda S, Tsuchiya KJ, Hashimoto K, Ohno K, Matsuzaki H, Iwata K, Matsumoto K, Wakuda T, Kameno Y, Suzuki K, Tsujii M, Nakamura K, Takei N, and Mori N. Alteration of plasma glutamate and glutamine levels in children with high-functioning autism. PLoS One. 2011;6: e25340. PMID: 3187770.

17. Lappalainen R and Riikonen RS. High levels of cerebrospinal fluid glutamate in Rett syndrome. Pediatr Neurol. 1996;15: 213-6. PMID: 8916158.

18. Blue ME, Naidu S, and Johnston MV. Altered development of glutamate and GABA receptors in the basal ganglia of girls with Rett syndrome. Exp Neurol. 1999;156: 345-52. PMID: 10328941.

19. Fatemi SH, Halt AR, Stary JM, Kanodia R, Schulz SC, and Realmuto GR. Glutamic acid decarboxylase 65 and 67 kDa proteins are reduced in autistic parietal and cerebellar cortices. Biol Psychiatry. 2002;52: 805-10. PMID: 12372652.

20. Jamain S, Betancur C, Quach H, Philippe A, Fellous M, Giros B, Gillberg C, Leboyer M, and Bourgeron T. Linkage and association of the glutamate receptor 6 gene with autism. Mol Psychiatry. 2002;7: 302-10. PMID: 2547854.

21. Shuang M, Liu J, Jia MX, Yang JZ, Wu SP, Gong XH, Ling YS, Ruan Y, Yang XL, and Zhang D. Family-based association study between autism and glutamate receptor 6 gene

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in Chinese Han trios. Am J Med Genet B Neuropsychiatr Genet. 2004;131: 48-50. PMID: 15389769.

22. Serajee FJ, Zhong H, Nabi R, and Huq AH. The metabotropic glutamate receptor 8 gene at 7q31: partial duplication and possible association with autism. J Med Genet. 2003;40: e42. PMID: 12676915.

23. Ramoz N, Reichert JG, Smith CJ, Silverman JM, Bespalova IN, Davis KL, and Buxbaum JD. Linkage and association of the mitochondrial aspartate/glutamate carrier SLC25A12 gene with autism. Am J Psychiatry. 2004;161: 662-9. PMID:15056512.

24. Purcell S and Sham P. Variance components models for gene-environment interaction in quantitative trait locus linkage analysis. Twin Research. 2002;5: 572-6. PMID: 12573188.

25. Friedman SD, Shaw DW, Artru AA, Richards TL, Gardner J, Dawson G, Posse S, and Dager SR. Regional brain chemical alterations in young children with autism spectrum disorder. Neurology. 2003;60: 100-7. PMID: 12525726.

26. Friedman SD, Shaw DW, Artru AA, Dawson G, Petropoulos H, and Dager SR. Gray and white matter brain chemistry in young children with autism. Arch Gen Psychiatry. 2006;63: 786-94. PMID: 16818868.

27. DeVito TJ, Drost DJ, Neufeld RW, Rajakumar N, Pavlosky W, Williamson P, and Nicolson R. Evidence for cortical dysfunction in autism: a proton magnetic resonance spectroscopic imaging study. Biol Psychiatry. 2007;61: 465-73. PMID: 17276747.

28. Page LA, Daly E, Schmitz N, Simmons A, Toal F, Deeley Q, Ambery F, McAlonan GM, Murphy KC, and Murphy DG. In vivo [1]H-magnetic resonance spectroscopy study of amygdala-hippocampal and parietal regions in autism. Am J Psychiatry. 2006;163: 2189-92. PMID: 17151175.

29. Bernardi S, Anagnostou E, Shen J, Kolevzon A, Buxbaum JD, Hollander E, Hof PR, Fan J. In vivo [1]H-magnetic resonance spectroscopy study of the attentional networks in autism. Brain Res. 2011;22: 198-205. PMID: 21185269.

30. Harada M, Taki MM, Nose A, Kubo H, Mori K, Nishitani H, and Matsuda T. Non-invasive evaluation of the GABAergic/glutamatergic system in autistic patients observed by MEGA-editing proton MR spectroscopy using a clinical 3 tesla instrument. J Autism Dev Disord. 2011;41: 447-54. PMID: 20652388.

31. Joshi G, Biederman J, Wozniak J, Goldin RL, Crowley D, Furtak S, Lukas SE, Gönenc A. Magnetic resonance spectroscopy study of the glutamatergic system in adolescent males with high-functioning autistic disorder: A pilot study at 4T. Eur Arch Psychiatry Clin Neurosci. 2012 Sep 18. [Epub ahead of print]. PMID: 22986449

32. Coulter DA. Antiepileptic drug cellular mechanisms of action: where does lamotrigine fit in? J Child Neurol. 1997;12 Suppl 1: 2-9. Review. PMID: 9429123.

33. Belsito KM, Law PA, Kirk KS, Landa RJ, and Zimmerman AW. Lamotrigine therapy for autistic disorder: a randomized, double-blind, placebo-controlled trial. J Autism Dev Disord. 2001;31: 175-81. PMID: 11450816.

34. King BH, Wright DM, Handen BL, Sikich L, Zimmerman AW, McMahon W, Cantwell E, Davanzo PA, Dourish CT, Dykens EM, Hooper SR, Jaselskis CA, Leventhal BL, Levitt J, Lord C, Lubetsky MJ, Myers SM, Ozonoff S, Shah BG, Snape M, Shernoff EW, Williamson K, and Cook EH, Jr. Double-blind, placebo-controlled study of amantadine hydrochloride in the treatment of children with autistic disorder. J Am Acad Child Adolesc Psychiatry. 2001;40: 658-65. PMID: 11392343.

35. D'Souza DC, Charney D, and Krystal J. Glycine Site Agonists of the NMDA Receptor: A Review. CNS Drug Rev. 1995;1: 227-260.

36. Posey DJ, Kem DL, Swiezy NB, Sweeten TL, Wiegand RE, and McDougle CJ. A pilot study of d-cycloserine in subjects with autistic disorder. Am J Psychiatry. 2004;161:2115-2117. PMID: 15514414.

37. Owley T, Salt J, Guter S, Grieve A, Walton L, Ayuyao N, Leventhal BL, and Cook EH. A prospective, open-Label trial of memantine in the treatment of cognitive, behavioral, and memory dysfunction in pervasive developmental disorders. J Child Adolesc Psychopharmacol. 2006;16: 517-24. PMID: 17069541.

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38. Chez M, Hing P, Chin K, Memon S, and Kirschner S. Memantine experience in children and adolescents with autism spectrum disorders. Ann Neurol. 2004;56.
39. Reisberg B, Doody R, Stöffler A, Schmitt F, Ferris S, and Möbius HJ. Memantine in moderate-to-severe Alzheimer's disease. N Engl J Med. 2003;348:1333–41. PMID: 12672860.
40. Tariot PN, Farlow MR, Grossberg GT, Graham SM, McDonald S, and Gergel I. Memantine treatment in patients with moderate to severe Alzheimer disease already receiving donepezil: A randomized con-trolled trial. J Am Med Assn. 2004;291:317–24. PMID: 14734594.
41. Baron-Cohen S, Ring HA, Wheelwright S, Bullmore ET, Brammer MJ, Simmons A, Williams SC. Social intelligence in the normal and autistic brain: an fMRI study. Eur J Neurosci. 1999;11(6):1891-8. PMID: 10336657.
42. Bauman ML and Kemper TL. Neuroanatomic observations of the brain in autism: a review and future directions. Int J Dev Neurosci. 2005;23: 183-7. PMID: 15749244.
43. Sokol DK, Dunn DW, Edwards-Brown M, and Feinberg J. Hydrogen proton magnetic resonance spectroscopy in autism: preliminary evidence of elevated choline/creatine ratio. J Child Neurol. 2002;17: 245-9. PMID: 12088077.
44. Devinsky O, Morrell MJ, and Vogt BA. Contributions of anterior cingulate cortex to behaviour. Brain. 1995;118 (Pt 1): 279-306. PMID: 7895011.
45. Bauman M and Kemper TL. Histoanatomic observations of the brain in early infantile autism. Neurology. 1985;35: 866-74. PMID: 4000488.
46. Kemper TL and Bauman M. Neuropathology of infantile autism. J Neuropathol Exp Neurol. 1998;57: 645-52. PMID: 9690668.
47. Simms ML, Kemper TL, Timbie CM, Bauman ML, and Blatt GJ. The anterior cingulate cortex in autism: heterogeneity of qualitative and quantitative cytoarchitectonic features suggests possible subgroups. Acta Neuropathol. 2009;118: 673-84. PMID: 19590881.
48. Ohnishi T, Matsuda H, Hashimoto T, Kunihiro T, Nishikawa M, Uema T, and Sasaki M. Abnormal regional cerebral blood flow in childhood autism. Brain. 2000;123 (Pt 9): 1838-44. PMID: 10960047.
49. Haznedar MM, Buchsbaum MS, Metyger M, Solimando A, Spiegel-Cohen J, and Hollander E. Anterior cingulate gyrus volume and glucose metabolism in autistic disorder. Am J Psychiatry. 1997;154:8. PMID: 9247387.
50. Di Martino A, Ross K, Uddin LQ, Sklar AB, Castellanos FX, and Milham MP. Functional brain correlates of social and nonsocial processes in autism spectrum disorders: an activation likelihood estimation meta-analysis. Biol Psychiatry. 2009;65: 63-74. PMID: 18996505.
51. Silk TJ, Rinehart N, Bradshaw JL, Tonge B, Egan G, O'Boyle M W, and Cunnington R. Visuospatial processing and the function of prefrontal-parietal networks in autism spectrum disorders: a functional MRI study. Am J Psychiatry. 2006;163: 1440-3. PMID: 16877661.
52. Di Martino A, Scheres A, Margulies DS, Kelly AM, Uddin LQ, Shehzad Z, Biswal B, Walters JR, Castellanos FX, and Milham MP. Functional connectivity of human striatum: a resting state fMRI study. Cereb Cortex. 2008;18: 2735-47. PMID: 18400794.
53. Baron-Cohen S, Ring HA, Bullmore ET, Wheelwright S, Ashwin C, and Williams SC. The amygdala theory of autism. Neurosci Biobehav Rev. 2000;24: 355-64. PMID: 10781695.
54. Stone VE, Baron-Cohen S, Calder A, Keane J, and Young A. Acquired theory of mind impairments in individuals with bilateral amygdala lesions. Neuropsychologia. 2003;41: 209-20. PMID: 12459219.
55. Sweeten TL, Posey DJ, Shekhar A, and McDougle CJ. The amygdala and related structures in the pathophysiology of autism. Pharmacol Biochem Behav. 2002;71: 449-55. PMID: 11830179.
56. Raymond GV, Bauman ML, Kemper TL. Hippocampus in autism: a Golgi analysis. Acta Neuropathol. 1996;91(1):117-9. PMID: 8773156.
57. Casanova MF, Buxhoeveden DP, Switala AE, and Roy E. Minicolumnar pathology in autism. Neurology. 2002;58: 428-32. PMID: 11839843.

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58. Bachevalier J and Loveland K, Early medial temporal dysfunction and autism, in Neurodevelopmental Mechanisms in Psychopathology, Cicchetti D and Walker EP, Editors. 2003, Cambridge University Press: Cambridge, England. 215-238.

59. Herbert MR, Ziegler DA, Deutsch CK, O'Brien LM, Lange N, Bakardjiev A, Hodgson J, Adrien KT, Steele S, Makris N, Kennedy D, Harris GJ, and Caviness VS, Jr. Dissociations of cerebral cortex, subcortical and cerebral white matter volumes in autistic boys. Brain. 2003;126: 1182-92. PMID: 12690057.

60. Aylward EH, Minshew NJ, Goldstein G, Honeycutt NA, Augustine AM, Yates KO, Barta PE, and Pearlson GD. MRI volumes of amygdala and hippocampus in non-mentally retarded autistic adolescents and adults. Neurology. 1999;53: 2145-50. PMID: 10599796.

61. Zilbovicius M, Boddaert N, Belin P, Poline JB, Remy P, Mangin JF, Thivard L, Barthelemy C, and Samson Y. Temporal lobe dysfunction in childhood autism: A PET study. Am J Psychiatry. 2000;157:1988-93. PMID: 11097965.

62. Boddaert N and Zilbovicius M. Functional neuroimaging and childhood autism. Pediatr Radiol. 2002;32: 1-7. PMID: 11819054.

63. Critchley HD, Daly EM, Bullmore ET, Williams SC, Van Amelsvoort T, Robertson DM, Rowe A, Phillips M, McAlonan G, Howlin P, and Murphy DG. The functional neuroanatomy of social behaviour: changes in cerebral blood flow when people with autistic disorder process facial expressions. Brain. 2000;123 (Pt 11):2203-12. PMID: 11050021.

64. Schultz RT, Gauthier I, Klin A, Fulbright RK, Anderson AW, Volkmar F, Skudlarski P, Lacadie C, Cohen DJ, and Gore JC. Abnormal ventral temporal cortical activity during face discrimination among individuals with autism and Asperger syndrome. Arch Gen Psychiatry. 2000;57: 331-40. PMID: 10768694.

65. Pierce K, Muller RA, Ambrose J, Allen G, and Courchesne E. Face processing occurs outside the fusiform 'face area' in autism: evidence from functional MRI. Brain. 2001;124: 2059-73. PMID: 11571222.

66. Petersen A, Crockett L, Richards M, and Boxer A. A self-report measure of pubertal status: Reliability, validity, and initial norms. J Youth and Adolesc. 1988;17: 117-133.

67. Lord C, Rutter M, and Le Couteur A. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. J Autism Dev Disord. 1994;24: 659-85. PMID: 7814313.

68. Lord C, Rutter M, Goode S, Heemsbergen J, Jordan H, Mawhood L, and Schopler E. Autism diagnostic observation schedule: a standardized observation of communicative and social behavior. J Autism Dev Disord. 1989;19: 185-212. PMID: 11055457.

69. Aman MG, Singh NN, Stewart AW, and Field CJ. The aberrant behavior checklist: a behavior rating scale for the assessment of treatment effects. Am J Ment Def. 1985;89: 485-491. PMID: 3993694.

70. Orvaschel H. Schedule for Affective Disorder and Schizophrenia for School-Age Children Epidemiologic Version. 5th Edition ed. 1994, Ft. Lauderdale: Nova Southeastern University, Center for Psychological Studies.

71. Constantino JN & Gruber CP. The Social Responsiveness Scale-Second Edition. 2012, Los Angeles: Western Psychological Services.

72. McDougle CJ, Scahill L, Aman MG, McCracken JT, Tierney E, Davies M, Arnold LE, Posey DJ, Martin A, Ghuman JK, Shah B, Chuang SZ, Swiezy NB, Gonzalez NM, Hollway J, Koenig K, McGough JJ, Ritz L, and Vitiello B. Risperidone for the core symptom domains of autism: results from the study by the autism network of the research units on pediatric psychopharmacology. Am J Psychiatry. 2005;162: 1142-8. PMID: 15930063.

73. Goodman WK, Rasmussen SA, Price LH, Mazure C, Heninger GR, and Charney DS, Yale-Brown obsessive compulsive scale (Y-BOCS). 1986 (Rev 89), Yale University.

74. Scahill L, McDougle CJ, Williams SK, Dimitropoulos A, Aman MG, McCracken JT, Tierney E, Arnold LE, Cronin P, Grados M, Ghuman J, Koenig K, Lam KS, McGough J, Posey DJ, Ritz L, Swiezy NB, and Vitiello B. Children's Yale-Brown Obsessive Compulsive Scale modified for pervasive developmental disorders. J Am Acad Child Adolesc Psychiatry. 2006;45: 1114-23. PMID: 16926619.

Behavioral and Neural Response to Memantine in Youth with ASD
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Last Modified: 03/14/2018

75. Sparrow S, Balla D, and Cicchette D, Vineland Adaptive Behavior Scales. 1984, Circle Pines, MN: American Guidance Service Publishing.

76. Endicott J, Spitzer RL, Fleiss JL, and Cohen J. The global assessment scale. A procedure for measuring overall severity of psychiatric disturbance. Arch Gen Psychiatry. 1976;33(6):766-71. PMID: 938196.

77. Achenbach T. (2001). Manual for the Child Behavior Checklist/6-18. Burlington, VT: University of Vermont Department of Psychiatry.

78. John K, Gammon GD, Prusoff BA, Warner V. (1987). The Social Adjustment Inventory for Children and Adolescents (SAICA): Testing of a new semi structured interview. *J Am Acad Child Adolesc Psychiatry*. 26(6):898-911. PMID: 3429410.

79. Poznanski E, Freeman L, and Mokros H. Children's depression rating scale-revised. Psychopharmacology Bulletin. 1985;21: 979-989.

80. Gadow KD and Sprafkin J. Adolescent symptom inventory-4 norms manual. 2005, Stony Brook, NY: Checkmate Plus.

81. Semel E, Wiig EH, & Secord WA. Clinical Evaluation of Language Fundamentals-Fourth Edition (CELF-4). 2008. Upper Saddle River, NJ: Pearson Education, Inc.

82. Roid GH & Barram RA. Essentials of Stanford-Binet Intelligence Scales (SB5) Assessment. 2004. Hoboken, NJ: John Wiley & Sons, Inc.

83. CANTABeclipse Test Administration Guide, 2004, Cambridge: Cambridge Cognition Limited. 1-164.

84. Nowicki, S., & Carton, J. (1993). The measurement of emotional intensity from facial expressions. Journal of Social Psychology, 133, 749–750.

85. Nowicki, S., & Duke, M. (1989). A measure of nonverbal social processing ability in children between the ages of 6 and 10. Paper presented at the American Psychological Society, Alexandria, VA.

86. Gioia GA, Isquith PK, Guy SC, Kenworthy L. Behavior Rating Inventory of Executive Function (BRIEF). 2000, Lutz, FL: Psychological Assessment Resources, Inc.

87. National Institute of Mental Health: CGI (Clinical Global Impression) Scale—NIMH. Psychopharmacol Bull 21:839–844, 1985.

88. Whitfield-Gabrieli S & Nieto-Castanon A. Conn: A functional connectivity toolbox for correlated and anticorrelated brain networks. Brain Connect. 2012;2:125-41. doi: 10.1089/brain.2012.0073.

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Table I. Study Schema

Week	Screening	Pre-BL	BL	1	2	3	4	6	9	12
Consent	XX									
Procedures										
Blood Draw	X									X
ECG	X									X
Physical Exam (including PPDS and waist circumference)	X									X
Tanner Staging	X									
Height	X		X							X
Vital Signs (weight, BP, pulse) ***	XX		X	X	X	X	X	X	X	X
Urine Drug Screen	XX							X		X
Urine Pregnancy (females only)	XX							X		X
Characterization Assessments										
ADOS	X									
K-SADS-E	XX									
Clinical Interview	XX									
Demographic Interview	XX									
Neuropsychological Battery	XX									
CELF-4*	X**									
DANVA 2			X							X
Clinician Rated Scales										
CGIs**	X		X	X	X	X	X	X	X	X
GAF			X	X	X	X	X	X	X	X
CY-BOCS-PDD			X					X		X
MGH-ASD-SCL	X									
MGH-ASD-RS-C			X					X		X
CDRS-R			X					X		X
ADHD-SCL			X					X		X
CASI-Anx			X					X		X
Adverse Events		XX	X	X	X	X	X	X	X	XX
Concomitant Medications		XX	X	X	X	X	X	X	X	XX
Patient/Parent-Rated Scales										
CBCL		X								X
SRS-2	XX							X		X
ABC			X					X		X
BRIEF Parent			X					X		X
MGH-ASD-RS-I			X					X		X
SAICA			X							X
Pre-MR Checklist		XX								
¹HMRS Scan	XX									XX***

X: ASD only tasks; XX: ASD and HC tasks; BL: Baseline
*If clinically necessary
**CGIs: General, ASD, ASD-SC, ASD-SI, ASD-M, ADHD, Anxiety, OCD, MDD, Mania, Psychosis.
***Will not be collected at phone visits
****Can be conducted anytime between Week 10 and Week 12