

Predicting Treatment Response to Memantine in Autism Spectrum Disorder using MR
Spectroscopy

Protocol 1

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Abstract

Memantine, an N-methyl-D-aspartate receptor antagonist, has been explored as a possible therapeutic agent that reduces the excitatory (glutamate) – inhibitory (gamma amino-butyric acid, GABA) imbalance in autism pathology and improves social and communication deficits. While some studies have shown positive results, a large clinical trial failed to show benefit possibly because different subsets of autism responded differently to the treatment. We propose a pilot, exploratory, clinical follow-on study using proton magnetic resonance spectroscopy ($^1\text{H-MRS}$) to determine whether baseline glutamate/GABA levels in certain regions of the brain may help predict treatment response to Memantine in autistic subjects. At study onset, subjects will be assessed on the behavioral scales such as the Aberrant Behavior Checklist and Clinical Global Impressions scale, followed by MRS imaging. Memantine treatment will be started post imaging. Assessment measures will be repeated at week 12 during treatment. Glutamate and GABA levels in brain regions will be correlated to improvements on assessment measures. We expect higher glutamate and/or lower GABA levels in the anterior cingulate cortex at baseline in responders to memantine. If our hypotheses are confirmed, we will have established a relevant neural biomarker to predict treatment response to memantine with important implications for clinical care including improving accuracy of individual prognosis and individualizing treatments.

Objectives

Specific Aim 1: We propose a pilot, exploratory, clinical follow-on study using proton magnetic resonance spectroscopy (^1H -MRS) to examine whether the following measures can be used to predict treatment response to memantine in ASD:

- (a) Glx and/or GABA and the Glx/GABA ratio in the anterior cingulate cortex,
- (b) Glx levels in the dorsolateral prefrontal cortex and the cerebellum
- (c) Markers of neuronal integrity, N-acetyl aspartate (NAA), myo-inositol, choline and creatine/phosphocreatine levels in the anterior cingulate, dorsolateral prefrontal cortex and cerebellum

Specific Aim 2: To examine whether resting state functional connectivity between the dorsolateral prefrontal cortex, anterior cingulate and the cerebellum, holds predictive value for treatment response to memantine in ASD.

Background and Rationale

Autism Spectrum Disorder (ASD) is a behaviorally defined complex neurodevelopmental disorder characterized by early childhood onset of marked deficiencies in social and communication skills and presence of restrictive, repetitive behaviors. Its phenotypic heterogeneity makes studying ASD and its treatment a challenging task. Currently, only two FDA approved drugs, risperidone and aripiprazole, are available to treat ASD¹ that manage irritability associated with the disorder. None have shown conclusive benefit for core features of social and communication deficits.

Research indicates that a disrupted balance between excitation (glutamate) and inhibition (gamma amino-butyrac acid, GABA) is likely to be one of the underlying mechanisms of ASD. This

hypothesis is supported by a number of magnetic resonance spectroscopy studies that report an excess of glutamatergic excitation and/or a reduction in GABAergic inhibition observed in different brain regions including the limbic system and the anterior cingulate cortex.²⁻⁹ Similar findings have also been reported in post mortem studies of the ASD brain¹⁰⁻¹². Thus, it is expected that drugs targeting this imbalance could be beneficial. For example, the GABA_B agonist, arbaclofen, was tested in ASD for this reason and showed promising results but a large trial failed to show improvement on the primary endpoints of lethargy and social withdrawal.¹³

Memantine, a moderate affinity N-methyl-D-aspartate (NMDA) receptor antagonist, is known to improve communication in patients with Alzheimer's disease and is an FDA approved treatment for that disease¹⁴. Examination of the efficacy of memantine in treating ASD has also been driven by the excitatory (glutamate) - inhibitory (gamma amino-butyric acid, GABA) imbalance hypothesis of ASD pathology. Memantine binds to the glutamatergic NMDA receptors and attenuates glutamatergic excitation.¹⁵ This reduction in excessive glutamatergic activation is thought to decrease the "noise" in the neural network system and facilitate learning and memory¹⁶. A few small studies have shown effectiveness for memantine in the treatment of social and communication aspects of ASD spectrum disorder (ASD) as well^{1,15-18}. However, one large clinical trial using memantine in ASD reported a failure to respond¹⁸. These discrepancies in results could possibly be due to the heterogeneity in the excitatory-inhibitory imbalance between ASD patients thereby causing a variation in response¹. In such a scenario, having a biomarker to predict an individual's treatment response would be invaluable.

MR Spectroscopy is a non-invasive tool used to examine the biochemical profile of brain tissue associated with different psychiatric and neurological conditions including ASD¹⁹⁻²¹.

Specifically, ¹H-MRS studies in ASD demonstrated the excitatory-inhibitory imbalance in various cortical and subcortical regions of the brain ^{2,3,5-8}, a finding also reported in post mortem brain tissue studies ¹⁰⁻¹². The use of this technique to determine if Glutamate/GABA concentrations in certain regions of the brain can predict response to treatment with memantine would be an innovative breakthrough in providing more effective individualized treatment for patients since very little research has been done to explore this possibility.

A similar approach has been adopted in a recent ongoing study that uses proton MR spectroscopy to map changes in glutamate and GABA following use of riluzole in ASD²². Riluzole is a drug commonly used in amyotrophic lateral sclerosis (ALS) and is a glutamate antagonist that works by blocking presynaptic glutamate release and noncompetitive inhibition of NMDA receptors.²³ However, riluzole is not clinically used in the treatment of ASD. To our knowledge, only one study so far (apart from the ongoing study mentioned) has examined the effects of riluzole in ASD, but as an adjunct to risperidone²⁴. The primary outcomes of the Ghaleiha et al. study were irritability and repetitive motor behaviors and not core social and communication deficits as in the present proposal. The purpose of riluzole in this MR spectroscopy study is a proof of principle for its effects on spectroscopy based on its glutamate antagonistic effects. By contrast, memantine, which will be studied in the present proposal, has some documented evidence from small studies of being beneficial in treating social and communication deficits associated with ASD in clinical settings, despite the failure of the larger trial.^{16-18,25}

Our lab has previously utilized MR spectroscopy techniques to map these different compounds in a group of 14 subjects with ASD and identify glutamate and GABA alterations in the

cerebellum, discovering a relationship to cerebrocerebellar connectivity and behavioral outcomes in individuals with ASD²⁶.

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Specific Aim 2: To examine whether resting state functional connectivity between the dorsolateral prefrontal cortex, anterior cingulate and the cerebellum, holds predictive value for treatment response to memantine in ASD.

The anterior cingulate cortex, dorsolateral prefrontal cortex and the cerebellum have been chosen as ROIs due to known atypical glutamate and GABAergic profiles in these regions associated with ASD^{2,5,7,9,25-32}. The approach of limiting to selected ROIs has been adopted in order to ensure patient comfort during the imaging process by reducing the time spent in the scanner. Since altered levels for markers of neuronal integrity, such as NAA, choline, creatine/phosphocreatine and myoinositol have also been reported in ASD,^{3,5,6,9,29,33-36} we will be mapping these biochemicals as well in order to explore their effect on treatment response. Resting state functional connectivity will also be analyzed for possible association with treatment outcome prediction since a previous study from our lab has shown a link between alterations in resting state

cerebrocerebellar connectivity, excitatory-inhibitory ratio in the cerebellum and behavioral outcome in ASD using MR spectroscopy²⁶.

Study Design

We propose to enroll 25 subjects with ASD for this pilot, exploratory study who are interested in starting memantine off-label as a clinical follow on. Glutamate concentration variations with MRS have been successfully reported with this sample size by Page et al., 2006³⁷. Subjects will be recruited from PI's clinic at the Thompson Center for Autism and Neurodevelopmental Disorders at the University of Missouri. The Thompson Center (TC) is one of the leading sites for recruitment within the Autism Treatment Network with more than 375 participants recruited since 2008. Our group and other TC researchers have successfully recruited imaging patients from this resource for multiple federally funded research projects. We have preliminary behavioral data on 15 ASD patients currently on memantine treatment showing mixed responses.

Subjects will be approached to participate in the study during their clinic visit with a healthcare professional. For potential subjects in clinics of healthcare professionals who are not co-investigators in the study, a recruitment flyer will be provided with contact information for questions and queries and instructions to let their healthcare provider know if they are interested in participating in the study. The recruitment flyer will be provided to healthcare professionals at the Thompson Center who will distribute it to potential subjects upon request. Following indication of interest, the researcher from the study team will go over the consent (/assent, as applicable) form with the subject in detail. Subjects will be informed that if they agree to participate in the study, they will be asked to hold off on starting the drug, memantine, till the

imaging session of the study is completed. Subjects will also be informed that they can choose not to be a part of study and start taking memantine immediately if they so wish. The subject/guardian will be able to ask questions throughout the process and the researcher will pause multiple times throughout the consent process to ensure understanding. The study physician, who is authorized by the IRB to obtain consent, will obtain signatures from the subjects and their parents/caregivers (as applicable). Following informed consent, a researcher from the study team will go over the MRI screening form with the subject and ensure compliance with MRI safety requirements. If the MRI screening requirements are not met, the subject will be taken off the study and the study physician will discuss appropriate alternatives with the patient.

Once informed consent is obtained, trained researchers will obtain information regarding autism diagnosis, medication information, IQ and medical history from the subject's medical records. For subjects who do not have an Autism Diagnostic Interview - Revised (ADI-R) in their medical record, the study physician will administer the ADI-R over the phone and document the information. After the required information is obtained and the subject is found to meet the inclusion and exclusion criteria for the study, the subject will attend 3 sessions (with an optional 4th session for mock scanning, per subject request).

Session 1 will involve collection of basic demographic information and behavioral assessments using the Aberrant Behavior Checklist (ABC), Social Responsiveness Scale (SRS), Clinical Global Impressions – Severity (CGI-S) Scale, General Social Outcomes Measure (GSOM) and the Test of Language Competence (TLC). Photographs and videos of the subjects will be recorded during the GSOM test for scoring by an independent rater. A Wechsler Abbreviated Scale of Intelligence (WASI) test will be administered to subjects who do not have IQ information on file.

Female subjects of childbearing age will undertake a urine pregnancy test. If pregnancy is indicated, the subject will no longer be eligible to participate in the study and will be withdrawn from the study. This session will last no longer than 2 hours.

Session 2 will be either the optional mock scanning session (if requested for by the subject) or the MR imaging session. For the mock scanning session, the scanning environment of the real scanner will be mimicked. For the MR imaging session (session 2 for subjects who do not require a mock scanning session and session 3 for subjects who do), the researcher will go over the MRI screening form with the participant in detail to ensure compliance. The imaging session will last no longer than 90 minutes during which the subject will be asked to lie still in an MRI scanner. For subjects with potential behavioral concerns that are manageable by support staff or family, support staff or family will be present during the imaging session to ensure safety. Subjects with potential behavioral concerns that cannot be managed by support staff or family will not be imaged and will no longer be eligible to be a part of the study. The study physician will discuss appropriate alternatives with the subjects.

Post imaging, subjects will be started on memantine. Memantine is marketed in three forms: Namenda (pill), Namenda XR (capsule) and the liquid formulation of Namenda. Depending on subject preference and insurance approval, the subjects will be started on one of these three forms of memantine. Namenda (pill) will be started at doses of 5 mg/day and titrated up to 20 mg/day based on patient tolerance and response. Namenda XR (capsule) will be started at doses of 7 mg/day and titrated up to 28 mg/day based on patient tolerance and response. The liquid formulation of Namenda will be started at a doses of 5 mg/day and titrated up to 20 mg/day based on patient tolerance and response.

After week 12 of treatment with memantine, the subjects will have a follow-up behavioral assessment session (Session 3 for subjects who do not require a mock scanning session and Session 4 for those who do) using Clinical Global Impressions - Improvement scale, Social Responsiveness Scale, Aberrant Behavior Checklist, the General Social Outcomes Measure (GSOM) and the Test of Language Competence (TLC). The GSOM test will involve recording of photographs and videos for scoring by an independent rater. This session will last no longer than 2 hours.

Inclusion and Exclusion Criteria:

Potential participants will be asked to take part in this study because he/she:

- 1) has autism spectrum disorder
- 2) is starting memantine off label for managing their autism symptoms
- 3) is deemed safe to enter the MR environment using the attached screening form, and
- 4) is capable of lying still for approximately 1.5 hour.

Subjects would be excluded if:

- 1) they have certain types of metallic implants, risk of exposure to metallic foreign bodies, pacemakers, magnetically sensitive implants that cannot be removed or are not securely attached,
- 2) pregnancy, or
- 3) claustrophobia.

All of these issues will be addressed with the MR screening form. Additional exclusion criteria include:

- 4) memantine intolerance
- 5) known hypersensitivity to memantine hydrochloride or

6) inability to lie still for approximately 90 minutes.

7) subjects with potential behavioral concerns that cannot be managed by support staff or family.

Behavioral Assessments:

The Clinical Global Impressions – Severity (CGI-S) scale is a clinician rated scale to assess severity of symptoms and is used fairly commonly in ASD research.^{13,17,18}

The Social Responsiveness Scale (SRS) is a well validated 65 item questionnaire that specifically evaluates social deficits associated with ASD^{38,39}. Each question on the SRS is rated on a scale of 0-3 based on behavior in the past 6 months and is designed to provide information on the degree of social deficits present. The SRS is particularly well suited to this project since we intend to monitor improvements in social and communication deficits following the use of memantine. A number of other studies in ASD have used SRS to track behavioral outcomes following pharmacological intervention^{1,3,8,9,13,26,40–42}.

The General Social Outcomes Measure (GSOM) is a relatively new assessment tool that also assesses social deficits in ASD⁴³. The GSOM is experimenter rated and evaluates subjects on five core domains of conversational reciprocity, facial expression recognition, social problem solving, affect demonstration and emotional perception. Photographs will be taken during the mimicking of facial expressions test of the GSOM. These photographs will be used for scoring by an independent rater, not present at the time of testing. Photographs will be captured and stored electronically on a firewall and password protected network drive accessible only by authorized study personnel in Dr. Beversdorf's lab. All photographs will be used just for the study and will be deleted after responses are scored. The higher the score on the GSOM scale, the better level of

social responsiveness in the subject. The SRS and the GSOM, used in combination, would provide a more detailed picture of the subject's social and communication skills by including both the subject's or caretaker's and the trained researcher's observations. The GSOM has successfully been used in our lab previously for the evaluation of treatment response to propranolol.⁴⁴

The Aberrant Behavior Checklist (ABC) was developed specifically with the intention of assessing effectiveness of therapeutic interventions.⁴⁵ The ABC assesses subjects on five subscales of irritability, lethargy/social withdrawal, stereotypic behavior, hyperactivity or noncompliance and inappropriate speech and has been used widely in ASD research to assess effectiveness of treatment.^{13,17,18}

The WASI test involves defining and relating certain words and solving puzzles to obtain a measure of intelligence.

The Test of Language Competence (TLC) is a test that assesses delays in language skills and the use of higher level language skills.

If not available from medical records, ASD subject diagnosis will be confirmed by the parent completing an Autism Diagnostic Inventory-Revised (ADI-R) with Dr. Beversdorf. If not available from medical records the Social Responsiveness Scale and Aberrant Behavior Checklist (ABC) will be given to the parent/guardian in person/over the phone/or through mail/email to assess the subject's social skills and behaviors.

¹H-MRS acquisition and analysis:

Following behavioral assessment, at the second session, subjects will undergo an MRI assessment, including structural MRI and ¹H-MRS. This assessment will be performed on a

research dedicated, Siemens 3-Tesla TIM Trio MRI scanner located in the Brain Imaging Center at the MU. Participants will be asked not to consume any forms of caffeine or alcohol 8 hours before the scanning to eliminate possible effects from caffeine/alcohol on GABA metabolites⁴⁶. Subjects will be given detailed instructions on how to successfully and safely complete these scans and will be monitored by study staff at all times.

High-resolution T1-weighted structural images are acquired using the three-dimensional multiplanar rapidly acquired gradient echo (MP-RAGE) pulse sequence: repetition time (TR), 2500ms; echo time (TE), 438ms; flip angle, 8°, 256x256 voxel matrix; field of view (FOV), 256 mm; 176 axial slices; thickness, 1 mm. This is collected to quantify the brain tissue composition with spectroscopic voxel and exclude any pathology.

For specific aim 1, based on anatomical landmarks, we prescribe a single voxel spectroscopy (SVS, $2 \times 2 \times 2 \text{cm}^3$) in our region of interest, the anterior cingulate cortex. The size of the voxel and number of averages acquired will be optimized for better signal-to-noise ratio while keeping ensuring scanning time does not get prolonged. GABA and a complex consisted of glutamate and its precursor glutamine (labeled as Glx) are collected using MESHcher-Garwood-edited Point RESolved Spectroscopy (MEGA-PRESS, TE=68ms, TR=2000ms). To avoid lipid artifact from the skull, we prescribe outer voxel suppression saturation bands around the SVS. We perform automated, followed by manual, shimming to achieve an optimal full-width at half maximum of <20Hz of the water signal from the entire excitation volume⁵⁴. We will also acquire internal reference water signal by using non-water suppressed MRS imaging to calculate absolute concentrations of our neurochemicals of interest. A recent study from our laboratory has

successfully used these techniques to map these different compounds in a group of 14 subjects with ASD and identify glutamate and GABA alterations in the cerebellum in relation to cerebrocerebellar connectivity and behavioral outcomes in a subset of individuals with ASD²⁶.

We have also recently succeeded to pilot a new sequence, TE-averaged semi-LASER, for glutamate measurement. Representative spectra for GABA and Glx (A) and Glu (B) acquired from a SVS located in primary motor cortex and

prefrontal areas respectively in a healthy control are shown in Fig. 1. High spectral quality (high signal-to-noise ratio, lower Cramér-Rao bounds from LCModel, see below) was consistently obtained in a

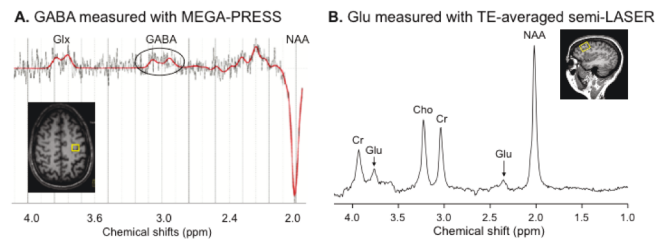


Fig. 1 **A.** ¹H-MRS spectrum using MEGA-PRESS. The LCModel output consisted in the acquired ¹H-MRS spectrum (black line) and estimated spectrum (red). **B.** ¹H-MRS spectrum using TE-averaged semi-LASER. Glx-glutamate-glutamine, NAA-Nacetylaspartate, Cr-creatine, Cho-choline; ppm, parts per million.

sample of 5 controls. Peaks from GABA, Glx, and glutamate are clearly discernible. Dr. Cirstea will supervise these scans. Our previous studies found that subjects with ASD are compliant with the ~60 minutes acquisition time. ¹H-MRS data analysis was detailed previously²⁹. Briefly, we use LCModel to quantify absolute GABA, Glx, and glutamate concentrations and other metabolites mentioned. LCModel-concentrations are corrected for partial brain tissue volume within voxel using a software written in Matlab software (2013a Mathworks, Natick, MA) and segmented structural images (SPM8, London, UK). For specific aim 1(b) and (c), spectroscopy voxels will be placed over the dorsolateral prefrontal cortex and cerebellum and concentrations of glutamate/glutamine, N-acetyl aspartate (NAA), myo-inositol, choline and creatine/phosphocreatine levels will be extracted. Resting state functional connectivity data will also be acquired during the imaging session for specific aim 2.

Following the imaging session, subjects will be started on memantine, starting at 5 mg/day doses to be titrated up 20 mg/day based on response and tolerability, for a period of 12 weeks as part of clinical care.

Follow-up assessments:

Subjects will be assessed after week 12 of memantine administration using the same clinical testing battery used prior to drug administration. We will also use an additional scale to determine improvement in symptoms: Clinical Global Impression – Improvement (CGI-I) scale. The CGI-I scale assesses the patient’s improvement on a scale of 1-7 where 1 represents very much improved and 7 represents very much worse when compared to baseline (before initiation of memantine). The reference for baseline severity will be derived from the CGI-S score obtained before initiation of memantine. Subjects who score 1-3 on the CGI-I will be considered responders while those who score 4-7 will be considered non-responders, a technique commonly used to categorize responders vs. non-responders in treatment response monitoring studies ^{40,47,48}. Subjects will be assessed for improvements on the SRS, GSOM, TLC and each of the 5 subscales of the ABC described earlier. Additionally, research staff will review ASD subject medical records to gain a better understanding of clinical benefit from drug administration. This will include physician clinical notes and any standardized assessments of clinical/behavioral improvement.

Information produced by this study will be stored in a locked cabinet or secure server in the PI’s offices in Galena Hall. Each subject will be assigned an anonymous study specific code. The code key will be kept on a separate secure server. Information contained in records will not be given to anyone not associated with the study without written consent, except as required by law.

Analyses:

Using data from the MR spectroscopy study performed at baseline, the means (SD) for each of the different compounds – GABA (in the anterior cingulate cortex only), Glx, NAA, choline, myo-inositol, creatine and phosphocreatine - measured from the different ROIs mentioned above will be analyzed for differences in responders vs. non-responders to memantine treatment. The ratio of Glx/GABA from the anterior cingulate will also be included in all analyses to explore effects of the excitatory-inhibitory ratio of the region on treatment response. Means (SD) of the different compounds and Glx/GABA ratio will also be correlated to CGI-I and changes in SRS scores and GSOM and ABC measures for each ROI to explore additional specific differences using Spearman's correlation coefficients. A significance level of 0.05 will be used. Since demographic factors such as age and gender are known to influence the results of MR spectroscopy studies⁴⁹, demographic factors will be included in the analysis to account for the associated changes in metabolite concentrations.

For resting state functional connectivity, we will extract time series from the regions of interest and correlation analyses will be carried out. The strength of functional connectivity between the regions will then be correlated to changes on scores in the battery of clinical tests to determine whether resting state functional connectivity might have a role in treatment response to memantine.

Hypothesis and Expected Results: We hypothesize that the best responders will have higher concentrations of glutamate/glutamine and reduced levels of GABA in the anterior cingulate cortex at baseline. Consistent with previous studies, we also expect to see increased glutamate/glutamine levels in the dorsolateral prefrontal cortex and cerebellum^{38,39} and increased

resting state functional connectivity in cortical networks⁵¹ that will be related to treatment response. Data from this pilot, exploratory study will be used to derive power for a larger, follow up study.

Potential Problems & Alternative Strategies: Neurochemicals depend on the brain tissue sampled in the spectroscopic voxels. We will use brain tissue-corrected concentrations. The phases of menstrual cycle for female subjects and smoking status will be included in our model due to their known effects on GABA concentrations^{46,52,53}. If recruitment falls behind, we will expand our recruitment to other institutions in Missouri, with whom the Thompson Center already has relationships for research purposes.

Timeline: Subjects will be recruited through the Thompson Center for Autism and Neurodevelopmental Disorders and would involve patients who are interested in starting memantine off-label as a clinical follow-on. Subjects will be informed about the study while in clinic by the study physician and the researcher will go over the consent(/assent as applicable) with the subject (and guardian, as applicable) in detail. The study physician will obtain signatures on the consent and/or assent as applicable. Following informed consent to participate in the study, behavioral assessments and imaging sessions will be scheduled at the earliest date possible (within 30 days of each other). Subject recruitment will extend over a period of 18-20 months. Data collection will commence as soon as subjects are enrolled into the study. Since the follow-up assessments extend to 12 weeks post the start of the drug, data collection will continue into the latter half of the second year of the project as well. Each subject will be in the study for

approximately 20 weeks. Imaging and behavioral assessment data will be analyzed starting in the beginning of the first year and will continue till the end of the project timeline.

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