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ABBREVIATIONS AND DEFINITIONS OF TERMS

ABC-C	**Aberrant Behavior Checklist-Community**
ADHD-IV	**Attention Deficit Hyperactivity Disorder Rating Scale-IV Home Version**
ADOS	**Autism Diagnostic Observation Schedule**
ADI-R	**Autism Diagnostic Interview-Reviewed Adolescent/Adult Sensory Profile (ages 11 and up)**
AE	**Adverse Event**
ASD	**Autism Spectrum Disorder**
BID	**Twice Daily**
BRIEF	**Behavior Rating Inventory of Executive Functioning [parent report]**
BUN	**Blood urea nitrogen**
CASI-Anx	**Child and Adolescent Symptom Inventory – 4 ASD Anxiety Scale**
CBCL	**Child Behavior Checklist**
CELF-IV	**Clinical Evaluation of Language Fundamentals – 4th Ed.**
CGI-S	**Clinical Global Impression-Improvement**
CHI-I	**Clinical Global Impression-Severity**
CYBOCS-PPD	**Children's Yale-Brown Obsessive Compulsive Scale-Pervasive Developmental Disorders**
KADI	**Krug Asperger's Disorder Index**
Leiter-R Brief IQ	**Leiter International Performance Scale-Revised Brief Intelligence Quotient Screener**
M50	**The ~50ms component of the auditory**

	evoked neuromagnetic field
M100	**The ~100ms component of the auditory evoked neuromagnetic field**
MEG	**Magnetoencephalography**
MMF	**Mismatch field**
MRI	**Magnetic Resonance Imaging**
MRS	**Magnetic Resonance Spectroscopy**
ODT	**Optimal Dose Tolerability**
PK	**Pharmacokinetics**
Placebo	**Placebo Condition**
PPVT-IV	**Peabody Picture Vocabulary Test-Fourth Edition**
RBANS	**Repeatable Battery for the Assessment of Neuropsychological Status**
RBS	**Repetitive Behavior Scale**
RT	**Reaction time**
RTP	**Rapid Temporal Processing**
SB5	**Stanford-Binet Intelligence Scale–5th Edition**
SCQ	**Social Communication Questionnaire-Current and Lifetime versions) [parent completed measure]**
SMURF	**The Safety Monitoring Uniform Report Form**
SRS-II	**Social Responsive Scale, 2nd edition) [parent completed measure]**
TID	**Three times a day**
VABS-II	**Vineland Adaptive Behavior Scales [parent/caregiver rating form]**
VAS-Target Behavior	**Parent Defined Target Symptoms Scale-Visual Analog Scale**
WASI-II	**Wechsler Abbreviated Scale of Intelligence – 2nd Edition**

ABSTRACT

Context: (Background)
Magnetoencephalographic (MEG) studies in autism spectrum disorders (ASD) have pointed to neural oscillation abnormalities.

Objectives: (primary and important secondary objectives)
A candidate therapeutic for ASD has been developed – STX209, a GABA-B agonist. STX209 targets synaptic activity that has clear electrophysiological correlates. This proposal assesses the responsiveness of neural oscillatory measures to acute administration of STX209.

Primary Objectives:
1. Determine whether STX209 produces a dose-dependent significant change in MEG target parameters compared to baseline as well as compared to placebo treatment.

2. Determine whether STX209 leads to a dose-dependent significant change in magnetic resonance spectroscopy (MRS)-obtained auditory cortex GABA levels compared with placebo treatment.

Study Design:
A 'randomized' acute dose-response design

Setting/Participants:
All imaging is performed at the Children's Hospital of Philadelphia. Participants will be forty male adolescent individuals (14- to 17.75-years-old) with an ASD diagnosis.

Study Interventions and Measures:
Each participant will receive (in increasing order) doses of 15mg and 30mg of STX209, with a single placebo treatment randomly administered (i.e., placebo exam could be first, second, or third exam). For the MEG primary target parameters are bilateral auditory cortex resting state gamma power and auditory evoked gamma power and synchrony as well as auditory evoked 100ms "M100" response latency. For MRS target parameters are bilateral auditory cortex GABA.

PROTOCOL SYNOPSIS

Study Title	MEG/MRS Dose Response Study of STX209 in ASD
Funder	Simons Foundation
Clinical Phase	Exploratory study for MEG
Study Rationale	Recent evidence from magnetoencephalographic (MEG) studies in ASD have pointed to abnormalities (specifically, delays) in auditory evoked neuromagnetic responses (e.g. M100 – see Roberts et al., 2010, and mismatch field, MMF – see Roberts et al., 2011) as well as abnormalities in the oscillatory behavior of auditory cortex, especially in the gamma band (30-50Hz), at rest and in response to simple auditory stimuli (see Gandal et al., 2010 and Cornew et al., 2012; Edgar et al., 2013). The local circuitry underlying such evoked activity and oscillations, and synaptic transmission, in general, requires an appropriate balance of excitation and inhibition, mediated by glutamate and GABA, respectively. One model of the neural oscillatory deficits in ASD suggests that impaired regulatory control by inhibitory interneurons onto pyramidal cells underlies abnormal auditory latency and oscillatory electrophysiological measures. As such, electrophysiological deficits are interpreted in terms of local circuitry abnormalities, with inferences at the molecular level of imbalances in the activity of glutamate and GABA. A candidate therapeutic for ASD has been developed – STX209, a GABA-B agonist. Since this pharmaceutical targets synaptic activity that has clear electrophysiological correlates, one goal of this proposal is to assess the responsiveness (sensitivity to change) of MEG measures to acute administration of STX209 at various doses in adolescents on the autism spectrum. The study also aims to establish the nature of the putative relationship between such electrophysiologic markers and GABA and glutamate levels using MEGAPRESS spectrally-edited magnetic resonance spectroscopy (MRS).
Study Objective(s)	1. To determine whether STX209 produces a significant change in MEG target parameters compared to baseline as well as compared to placebo treatment. Secondly, to determine if the magnitude of such change is influenced by STX209 dose: 0 mg (placebo) versus 15mg versus 30 mg. 2. To determine whether STX209 leads to a significant change in MRS-obtained auditory cortex GABA levels compared with placebo treatment. Secondly, to determine whether the magnitude of the GABA change is influenced by STX209 dose level. 3. To determine if dose-dependent changes in MEG target parameters are correlated to dose-dependent changes in MRS target parameters in auditory cortex.
Test Article(s) *(If Applicable)*	STX209 (Arbaclofen), a GABA-B agonist and a matching placebo. The active compound is made available to this project by the Clinical Research Associates, LLC (CRA).

Study Design	A randomized acute dose-response design will be employed with a total study duration of 3 weeks. At each visit, baseline MEG will be obtained followed by acute single-dose drug/placebo administration, followed 60 minutes later by repeat MEG. Each participant will receive a single dose of placebo in random order and a single dose of STX209 from smallest to largest (15mg and 30mg). MRI and MRS will be performed immediately following MEG to provide an anatomic basis for source localization as well as to assess acute effects of STX209 administration on MRS estimates of GABA and glutamate.
Number Of Subjects	This is a single site study. 40 male subjects with ASD will be recruited in order to get 38 evaluable subjects.
Study Duration	Subjects will be examined at three separate sessions, at each session receiving in random order a single-dose of placebo, and from smallest to largest a single-dose of STX209 (15mg and 30mg). Each subject's participation will last approximately 4 weeks although participation could be extended up to six or eight weeks if subjects are unable to come four consecutive weeks in a row. The entire study is expected to last one year.
Study Phases	
Screening	Screening for eligibility, obtaining consent, and cognitive and clinical symptom assessments
MEG Assessments	Over a period of 3 visits (at least 1 week apart), baseline MEG exam and then MEG/MRS assessment of STX209 at 15mg and 30mg doses or a single placebo.
Efficacy Evaluations	The measures used to assess efficacy of STX209 will be as follows: 1. Change from baseline to STX209 administration in left and right superior temporal gyrus auditory M50, M100 and MMF latencies. 2. Change from baseline to STX209 administration in left and right superior temporal gyrus auditory gamma total power and phase-locking measures (examined pre- and post-stimulus).
Safety Evaluations	All safety evaluations will be done by the study physician Dr. Amanda Bennett, a Developmental-Behavioral Pediatrician at the Children's Hospital of Philadelphia. The Safety Monitoring Uniform Report Form (SMURF) will be used to assess STX209 specific side effects. In addition, safety and tolerability of STX209 will be determined by Adverse Events (AE), Vital Signs (heart rate and blood pressure), and a blood draw for BUN and creatinine labs. Dr. Bennett or one of her attendings will be on call for any problems that might arise in between study visits. Families will be advised to call 911 in case of an emergency when they are not at our Center.
Statistical And Analytic Plan	Primary statistical analyses assess baseline versus post acute-dose (placebo, STX209 15 and 30 mg) changes for (1) M100 auditory latency, and (2) post-stimulus auditory gamma-band activity. For each dependent measure, ANOVAs with dose as a repeated measure will examine baseline versus post acute-dose changes (analyses showing no significant differences across time in baseline M100 and gamma measures will allow

	use of a single averaged baseline measure). Contrasts of *a priori* interest assess (1) a baseline versus post-acute dose change in the dependent measures averaging across STX209 15 and 30 mg, as well as (2) a change in dependent measures from baseline versus STX209 15 mg versus STX209 30 mg, with this test demonstrating an effect of dose. Given the possibility of between-subject variability in the degree of baseline auditory neural pathology, analyses will also examine response to treatment in those participants showing the greatest baseline auditory neural circuit abnormality (median split on M100 latency or gamma power), with a larger effect of treatment expected in those participants showing greater baseline auditory neural impairment. Secondary and exploratory analyses examine baseline to post acute-dose response changes in MRS auditory GABA concentration as well as associations between MEG functional measures and MRS GABA concentration.
DATA AND SAFETY MONITORING PLAN	The investigators will perform the study in accordance with this protocol, will obtain consent and assent (unless a waiver is granted), and will report unexpected problems in accordance with CHOP and federal requirements. Collection, recording, and reporting of data will be conducted to ensure the privacy, health, and welfare of research subjects during and after the study. Any episode of noncompliance will be documented in the CRF. Upon entry to the study, all subjects will be given a study identifier code. This code will be used in all data acquisition (MEG and MRS). During the study, the association between subject name and identifier will be kept in a electronic database with restricted encrypted access only (i.e., CHOP SAN drive). MEG and MRS data will be archived to permanent storage on the secure CHOP SAN drive. Hardcopy consent forms will be stored in a locked file cabinet located in Dr. Roberts' office. The Office of Research Compliance and Regulatory Affairs (ORCRA) at CHOP will conduct routine data safety monitoring of this sponsor-investigator trial. A data safety monitoring plan will be developed before the first subject is enrolled and will be maintained in the regulatory file for the study. Data monitoring will include informed consent form review, regulatory documentation review, source data verification and case report form data validation, sponsor documentation review and drug accountability. The frequency of monitoring may be modified as needed based on previous monitoring outcomes and/or rate of enrollment.

TABLE 1: SCHEDULE OF STUDY PROCEDURES

Study Phase	**Phone-Screen**	**Screening Visit**	**MEG Assessment**		
Visit Number			**Visit 1***	**Visit 2***	**Visit 3***
Study Days	**pre**	**0**	**7**	**14**	**21**
Phone screen / Eligibility / Verbal Informed Consent	X				
Pre-visit Intake Questionnaire	X				
Written Informed Consent/Assent		X			
Phone Parent Informed Consent	X				
Review Inclusion/Exclusion Criteria		X			
Demographics/Medical History		X			
Intervention History Form					
Prior/Concomitant Medications		X			
The Safety Monitoring Uniform Report Form (SMURF)		X	X	X	X
Vital Signs		X	X	X	X
Physical Examination		X			X
Blood Draw**		X			
Autism Diagnostic Observation Schedule (ADOS-II)		X			
Autism Diagnostic Interview-Revised (ADI-R)		X			
Child Behavior Checklist (CBCL)		X			
Clinical Evaluation of Language Fundamentals (CELF –IV)		X			
DSM-IV Checklist of Autism Symptoms		X			
Repetitive Behaviour Scale (RBS)		X			

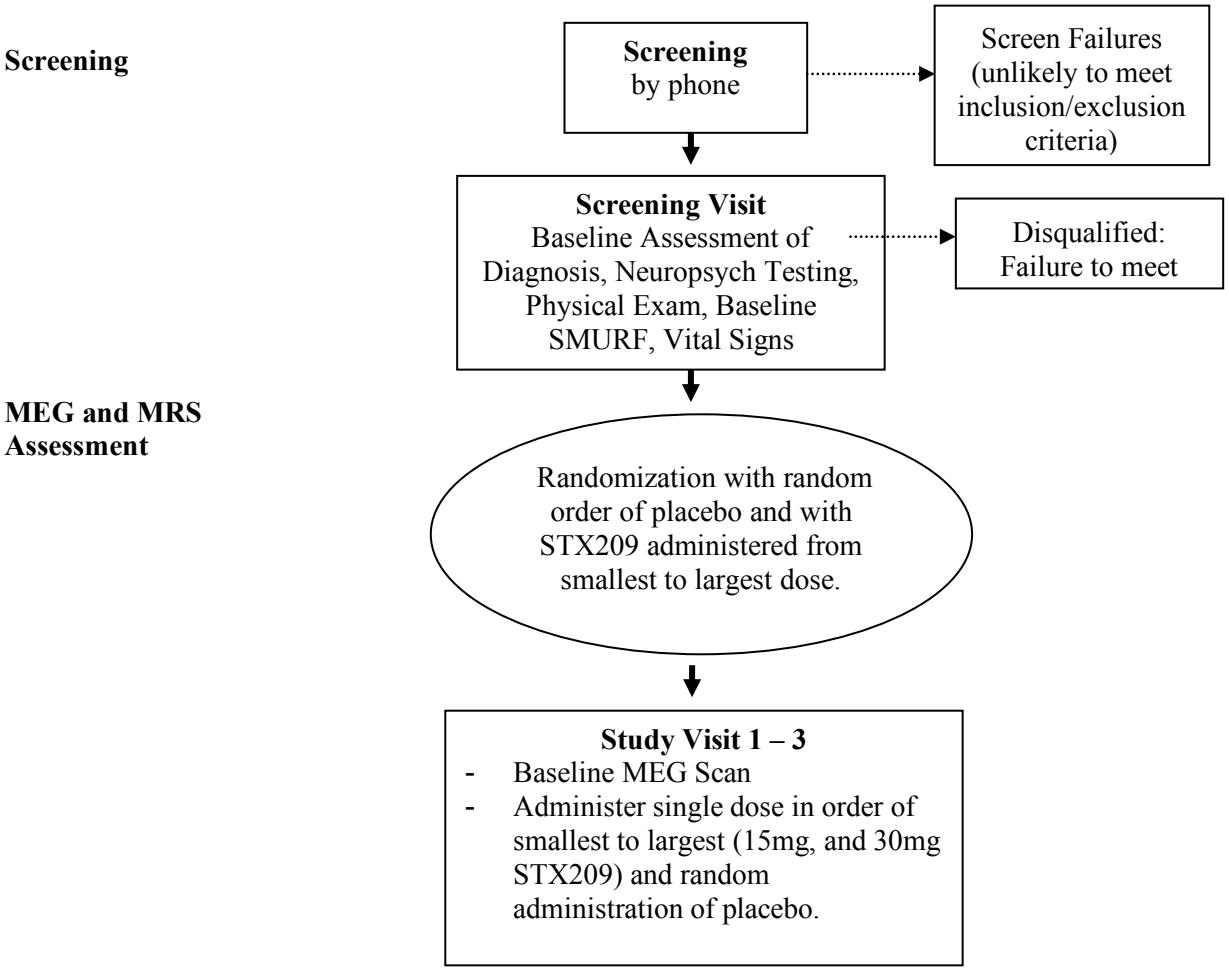
Social Communication Questionnaire (SCQ)		X			
Social Responsive Scale (SRS-II)		X			
Vineland Adaptive Behaviour Scales (2nd edition)		X			
Wechsler Abbreviated Scale of Intelligence (WASI-II)		X			
Randomization		X			
MEG Scan (pre/post drug administration)			X	X	X
MRI Scan			X	X	X
Dispense Study Drug			X	X	X
Adverse Event/Serious Adverse Event Assessment			X	X	X

NOTE: At each of the three visits, subjects will receive (in increasing order) doses of 15mg and 30mg of STX209 and a randomly administered single placebo exam. Each subject's participation will last approximately 3-4 weeks, although participation could be extended up to six or eight weeks if subjects are unable to come four consecutive weeks in a row. At each visit, baseline MEG will be obtained followed by acute single-dose drug/placebo administration, followed 60 minutes later by repeat MEG

*MEG Assessment visits will be scheduled no less than four days or more than 14 days apart.

** In cases where subject is unable to do a blood draw on the day of the screening visit due to anxiety/fear/discomfort if no medical risk factors are identified during medical history review, we will either accept BUN and Creatinine results from an outside source within 3 months of first imaging visit or attempt to do the blood draw on the day of the imaging visit

FIGURE 1: STUDY DIAGRAM



1 BACKGROUND INFORMATION AND RATIONALE

1.1 Introduction

The proposed study will use non-invasive imaging methods to examine the effect of STX209 on oscillatory brain activity. All study procedures will take place at the Children's Hospital of Philadelphia.

Glutamate is the most prevalent excitatory neurotransmitter. Gamma-aminobutyric acid (GABA) is the most prevalent inhibitory neurotransmitter. Disruptions in glutamate or GABA-ergic signaling have been associated with neurodevelopmental disorders including autism and epilepsy (Rubenstein and Merzenich [1]; Fatemi, Folsom et al. [2]). An increase in the excitatory:inhibitory (E/I) ratio is proposed to underlie brain dysfunction in individuals with ASD (Rubenstein and Merzenich [1]). GABA type B (GABA-B) receptors are crucial for maintaining the EI balance and pervasive defects in GAB-B receptor expression and activity have been associated with autism and are postulated to contribute to co-morbid seizure activity and cognitive impairment (Fatemi, Folsom et al. [2]).

STX209 (arbaclofen) is a selective GABA-B receptor agonist. STX209 augments GABA-ergic activity, inhibits presynaptic release of glutamate, inhibits postsynaptic transmission, and modulates intracellular signaling (Isaacson and Hille [3]; Scanziani, Capogna et al. [4]). STX209 is the active isomer of racemic baclofen, an approved GABA-B agonist. Baclofen has demonstrated efficacy in treating hyperactivity and audiogenic seizure phenotypes in the fragile X knockout mouse (Pacey, Heximer et al. [5]). Through elevation of GABA-ergic inhibitory activity, STX209 may act to alleviate ASD symptoms associated with social anxiety and emotional hyperarousal.

The accumulated scientific evidence in fragile X syndrome (FXS) mice suggest that the symptoms of FXS, which overlap significantly with autism, reflect activated metabotropic glutamate receptor mGluR1 and mGluR5 signaling resulting in excessive protein synthesis and exaggerated long-term depression (a form of synaptic plasticity). Glutamate signaling via mGluR5 has thus been identified as a valid target for the treatment of FXS (Bear [6]) and therefore may be relevant in the treatment of ASD. The efficacy for STX209 in the FXS mouse is comparable in magnitude to that produced by mGluR5 antagonists, both in reducing susceptibility to audiogeneic seizure and in reducing marble burying, an anxiety-driven stereotypical behavior (Seaside Investigator's Brochure).

The preclinical data on the effects of STX209, together with the understanding of its pharmacologic mechanism through GABA-ergic activity and modulation of mGluR signaling support evaluation of STX209 as a viable treatment in patients with ASD.

1.2 Name and Description of Investigational Product or Intervention

STX209 (arbaclofen) is a selective GABA-B receptor agonist and augments GABA-ergic activity, inhibits presynaptic release of glutamate, inhibits postsynaptic transmission, and modulates intracellular signaling (Isaacson and Hille [3]; Scanziani, Capogna et al. [4]). STX209 is the active isomer of racemic baclofen, an approved GABA-B agonist.

Baclofen has demonstrated efficacy in treating hyperactivity and audiogenic seizure phenotypes in the fragile X knockout mouse (Pacey, Heximer et al. [5]). FXS mouse models have been found to exhibit deficient GABA-mediated inhibitory neurotransmission particularly notable in the amygdala (Olmos-Serrano, Paluszkiewicz et al. [7]), a brain region associated with affective behaviors involving emotional understanding and social interaction. Through elevation of GABA-ergic

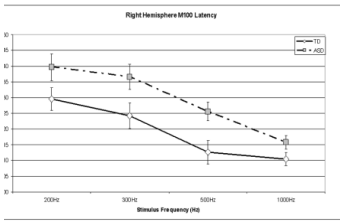
inhibitory activity, STX209 may act to alleviate ASD symptoms associated with social anxiety and emotional hyperarousal.

A candidate therapeutic has been developed – namely, STX209, a GABA-B agonist (made available to this project by the Clinical Research Associates, LLC (CRA)). Since this pharmaceutical targets synaptic activity that has clear electrophysiological correlates, one goal of this proposal is to assess the responsiveness (sensitivity to change) of MEG measures to acute administration of STX209 at various doses in adolescents on the autism spectrum. The study also aims to establish the nature of the putative relationship between such electrophysiologic markers and GABA and glutamate levels using MEGAPRESS spectrally-edited magnetic resonance spectroscopy (MRS), which we have previously used to demonstrate a relationship between GABA concentration and gamma oscillatory activity (Gaetz et al. [8]).

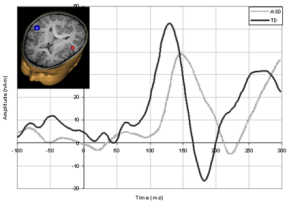
1.3 Findings from Non-Clinical and Clinical Studies

As detailed below, recent MEG studies point to delays in auditory processing in ASD revealed in the latencies of auditory responses. As detailed below, published as well as very recent preliminary data from our group also indicate that ASD is characterized by abnormal patterns of resting or background gamma-band activity as well as reduced stimulus evoked gamma-band responses with simple auditory tasks. In other studies from our laboratory, background gamma power predicted language skills and stimulus-evoked gamma responses predicted social cognition, supporting their clinical relevance. Finally, as shown below, recent MRS findings from our laboratory showed reduced GABA levels in auditory cortex of children with ASD. Although gamma and GABA measures have not yet been obtained in the same ASD subjects, these gamma and GABA findings suggest a relationship between the two (NOTE: we have previously shown a relationship between GABA levels and gamma band oscillatory activity in healthy adults (Gaetz et al. [8]).

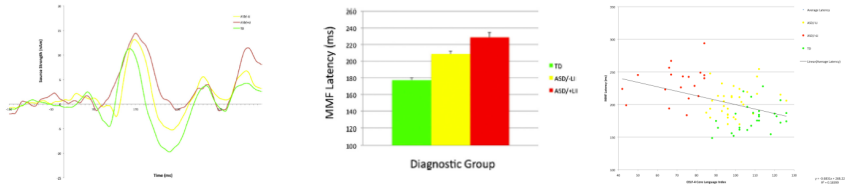
The following sections provide brief descriptions of our published studies examining auditory latency abnormalities in ASD.



Delayed M100 Latency – a Biomarker for ASD [9]: Delayed STG M100 responses to simple auditory stimuli are seen in children with ASD vs. TD (Fig left). Of ~10ms magnitude, this is apparent for stimuli in the range 200Hz-1kHz (Fig right). This prolongation persists after regressing out variance associated with age and language ability, suggesting it is a hallmark of ASD *per se*.



Mismatch Field Latency - a Biomarker for Language Impairment in ASD [10]: Mismatch fields are detected due to change of stimulus feature (e.g. vowel type). As shown in the figure, although similar in amplitude, the MMF response is profoundly (~50ms) delayed in children with ASD + language impairment (left). Although there is a main effect of diagnostic group, the bar chart (center) and scatter plot (right) illustrate stratification of MMF latency across the autism spectrum, with the measure correlating with a domain of symptom severity, namely language impairment (CELF-4 Core Language Index).

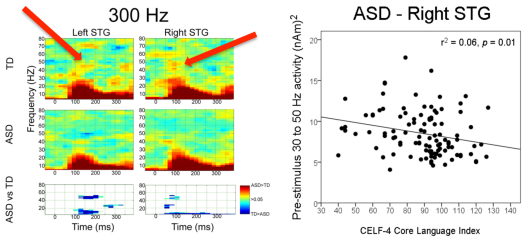


Specificity of M100 and MMF findings: comparison to specific language impairment, SLI[11]: To examine the specificity of the above M100 and MMF findings, we recruited age-matched children with SLI with no ASD symptoms. Whereas M100 latency results (upper) in SLI were not distinguish-able from TD (augmenting the claim for M100 prolongation as *specific* for ASD), MMF latencies were delayed an amount similar to children with ASD with concomitant language impairment (lower), suggesting the *sensitivity* of MMF latency to mechanisms underlying the phenotype of impaired language.

Considering translational findings, work from our preclinical collaborators identified a significant relationship between social interaction behaviors and deficits in baseline and auditory-evoked gamma synchrony in mouse models with altered excitatory/inhibitory balance. Further, modulation of electrophysiological response latencies was achieved through administration of an NMDA receptor blocker. Importantly, in these studies it was found that gamma abnormalities and social deficits were reversed with acute administration of the GABA-B agonist baclofen, but not GABA-A modulating compounds.

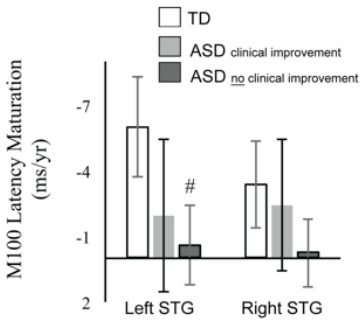
In terms of oscillatory activity. Our most recent findings show pre- and post-stimulus oscillatory abnormalities in ASD.

Gamma power and phase locking is reduced in ASD[12, 13]: Inter-trial coherence (ITC) (left, top) and familywise-corrected p-value plots comparing TD and ASD for 300Hz tones (left, bottom) show when greater ITC gamma activity was observed in TD than ASD (arrows). In ASD, lower CELF-4 Core Language Index scores were also associated with increased right STG pre-stimulus gamma activity (30-50Hz; right). Increased pre-stimulus activity in ASD likely demonstrates a fundamental signal-to-noise deficit, with elevations in oscillatory activity suggesting an inability to maintain an appropriate 'neural tone'.



Neural markers are prognostic of functional outcome

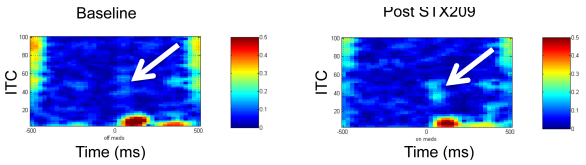
In a recent study examining the effect of cognitive training on auditory neural activity in schizophrenia, Popov et al. (2015) observed a normalization of auditory neural activity in approximately 50% of the individuals with schizophrenia who received 4 weeks of cognitive training. **These data suggest significant between subject differences in modulating brain rhythms, with some individuals showing a clear response to treatment and others showing a smaller or perhaps no response to treatment. Such between participant differences are likely due to the heterogeneity intrinsic to neurodevelopmental disorders, with not all individuals with a given neurodevelopmental disorder showing evidence of a specific brain pathology (e.g., auditory cortex neural circuit pathology).**



Recent findings from our own laboratory support the above. In a study from our laboratory (Port et al., 2016), MEG auditory measures were obtained in 9 typically developing (TD) children and 27 children with ASD aged 6- to 11-years-old and then two to five years later. A striking pattern was observed in a subgroup of children with ASD who no longer met diagnostic criteria for ASD at the second MEG exam (i.e., showed marked clinical improvement). In particular, as shown to the left, a faster rate of STG M100 latency maturation was observed in TD (white bars) versus ASD (black bars), with maturation rates in the children with ASD who showed significant clinical improvement in-between (gray bars). **These data again suggest subgroups within the ASD populations and thus likely between ASD subject differences in brain activity, in the above example observed as between subject differences with respect to 'normalization' of neural circuit activity across time. This along with the finding from Popov et al. (2015) thus indicates between subject differences in ability to change the auditory latency and gamma neural measures in response to treatment (for further details related to the above, see section 5.7).**

Finally, regarding STX209, preliminary data is presented.

Effects of a GABA-B agonist, STX209: Building upon the above time-frequency study and of direct relevance to the proposed study, the figure shows auditory cortex phase-locking (inter-trial coherence, ITC) to pure-tone auditory stimuli (onset 0ms) from an adolescent with ASD participating in a Phase II STX209 clinical trial at CHOP. Compared to the baseline period (left), after 12wks of treatment with STX209 (right) greater post-stimulus gamma-band ITC was seen, suggesting a normalization of STG neural circuits via STX209 treatment (arrows). Although only an individual result, preliminary data provide tantalizing evidence that STX209 has a resolvable neurophysiological effect – normalizing neural circuit activity. Of note, in this same individual **decreases in the latency of the M100 response pre- to post-treatment was also observed.**



1.3.1 Research Studies

1.3.1.1 Clinical Studies

Seaside Therapeutics completed a randomized, double-blind, placebo-controlled Phase 2 crossover study that explored the efficacy, safety, and tolerability of STX209 (capsule) for the treatment of irritability in subjects with FXS (Study 22001). In this crossover study, each treatment period was 4 weeks in duration. The study employed a flexible-dose schedule to define the optimal tolerated dose (OTD) for each subject. The OTD was defined as either the dose that achieved a behavior rating of very much improved on the CGI-I or as the maximum tolerated dose. Dosing decisions were based on the average response since the last dose change compared to baseline behavior. The starting dose of 1 mg twice daily (BID) could be increased every three days to 2 mg BID, 3 mg BID, 5 mg BID, and then 10 mg BID.

The dose for subjects who were at least 12 years of age could have been increased to 10 mg three times daily (TID). Thereafter, subjects continued on the OTD for the remainder of the 4-week treatment period. After completion of assessments at the 4-week time point, subjects were tapered

from study drug during a titrated withdrawal period lasting up to two weeks, followed by a 7- to 30-day washout period, and then entered the second treatment period, where they received the other study drug according to the same titration regimen. After completing the second 4-week treatment period, subjects completed assessments and began a titrated withdrawal period lasting up to two weeks.

Efficacy endpoints included the ABC-C, CGI-S, CGI-I, Global Assessment (Treatment and Improvement), VABS-II, CASI-Anx, VAS-Target Behavior, RBS-R, SRS, ADHD-IV, ADI-R, SB5, PPVT-IV, and RBANS-List learning/Story memory. Safety evaluations included AEs, laboratory assessments, vital signs, ECG, and physical examination.

The population for this study consisted of 63 subjects, including 55 males and eight females aged 6 to 39 years. Twenty-four subjects were between 6 to 11 years of age, 22 subjects were between 12 to 17 years of age, and 17 subjects were 18 to 39 years of age. Fifty-six (89%) subjects completed the study. One subject each was withdrawn for withdrawal of consent, protocol deviation, and travel difficulties; one subject was considered lost to follow-up. Three subjects discontinued as a result of AEs (two on placebo and one on STX209).

Although this study did not show statistically significant effects of STX209 on irritability symptoms, subjects showed evidence of global behavioral improvement. Subjects' most troublesome behaviors as judged by the parents/caregivers/legally authorized representatives (LARs) were rated on a Visual Analog Scale (VAS) as more improved when the subjects were on STX209 compared to placebo ($p<0.05$). In the Per Protocol (PP) study population, more clinicians and parent/caregivers rated the subjects' behavior as preferable on STX209 versus placebo by a 2:1 ratio. A post-hoc analysis showed that subjects with higher baseline scores on the Lethargy/Social Withdrawal scale of the ABC-C showed significantly greater improvement on that scale when treated with STX209, as well as on the Vineland Adaptive Behavior Scales-II (VABS-II) Socialization domain ($p<0.050$ for both comparisons). These subjects also showed significantly greater improvement on multiple measures of global improvement, including the CGI-I, CGI-S, and blinded treatment preference ratings by the clinicians and by parent/caregivers ($p\leq0.010$ for all comparisons) (22001 Clinical Study Report). No significant safety or tolerability concerns were apparent in Study 22001. Overall, 58 (92.1%) of 63 subjects reported AEs; the percentage was the same (45 subjects, 71.4%) during treatment with STX209 and placebo. AEs with incidence >5% and greater than twice the incidence in placebo included headache, sedation (both 8% on STX209 versus 2% on placebo), fatigue, and vomiting (both 6% on STX209 versus 2% on placebo).

The proportion of subjects with AEs deemed to be at least possibly related to study drug was higher with STX209 (53.9%) than with placebo (46.0%). The most commonly reported treatment-related AEs in STX209-treated subjects were sedation (7.9%), irritability (6.3%), increased appetite (6.3%), headache (6.3%), aggression (4.8%), anxiety (4.8%), and fatigue (4.8%). The majority of AEs were mild or moderate in severity, and most resolved spontaneously. Five subjects reported severe AEs (two subjects with increased irritability and one each with aggression, diarrhea, and dental caries requiring extraction). One subject who was tapering from STX209 treatment experienced an SAE of worsened irritability
that resulted in hospitalization and study withdrawal. This subject had a prior history of similar events requiring multiple hospitalizations. The Investigator assessed the event as severe in intensity and possibly related to study drug taper. Two subjects discontinued while on placebo, both for worsened irritability during tapering of study medication. No deaths or other significant AEs were reported.

The following data were reported from Seaside Therapeutics Study 22003, a Phase 2 open-label, flexible-dose evaluation of the safety and tolerability of STX209 for treatment of irritability in subjects with autism spectrum disorders. This open-label study employed a flexible-dose schedule to define the optimal tolerability dose (OTD) for each subject. The OTD was defined as either the dose that achieved a behavior rating of very much improved on the CGI-I or as the maximum tolerated dose. Dosing decisions were based on the average response since the last dose change compared to baseline behavior. The starting dose of 1 mg BID could be increased every three days to 2 mg BID, 3 mg BID, 5 mg BID and then 10 mg BID. The dose for subjects who were at least 12 years of age could have been increased to 10 mg TID. After the 8-week assessment, subjects were tapered from study drug during a 2-week titrated withdrawal period.

Efficacy endpoints included the ABC-C, CGI-S, CGI-I, CYBOCS-PDD, VABS-II, CASI-Anx, VAS-Target Behavior, SRS, ADHD-IV, and Leiter-R Brief IQ. Safety evaluations included AEs, laboratory assessments, vital signs, ECG, and physical examination.

The population for this study consisted of 32 subjects, including 29 males and three females, age 6 to 17 years. Twelve subjects were 6 to 11 years of age, and 20 subjects were 12 to 17 years of age. Two subjects discontinued due to AEs (increased impulsivity, aggression, and oppositionality in one subject; and increased agitation in a second subject). In addition, five other subjects did not complete the study. One subject withdrew consent, one subject was withdrawn at the Investigator's discretion, one subject started treatment with baclofen, one subject had a planned tonsillectomy, and one subject was considered lost to follow-up.

In the Intent-to-Treat population (ITT), there was significant improvement on the primary endpoint, the Irritability Subscale of the ABC-C, from baseline to Week 8 ($p<0.001$). Subjects also showed significant improvements on the ABC-Social Withdrawal score ($p= 0.001$), the Clinician's Global Impression – Improvement (CGI-I) ($p=0.023$), the Clinician's Global Impression – Severity (CGI-S), the ABC-Total score, the Attention Deficit Hyperactivity Disorder – IV (ADHD-IV) Rating Scale (all $p< 0.001$), and on other specific measures of social and communicative behaviors. STX209 showed broad beneficial effects on core symptoms of ASD in this open-label study (22003 Clinical Study Report).

No significant safety or tolerability concerns were evident from Study 22003. Of the 32 subjects enrolled in Study 22003, 28 (87.5%) reported treatment-emergent AEs. The most common adverse events (AEs) were agitation (7; 22%), irritability (7; 22%), fatigue (5; 16%), psychomotor hyperactivity (5: 16%), diarrhea (4; 13%) and insomnia (4; 13%). The majority of AEs were mild and resolved spontaneously or with dose adjustment. One serious AE (SAE), worsening of aggression, was reported during down-titration of STX209. Two subjects withdrew due to worsening of baseline symptoms, one during initial up-titration of drug and one during the treatment period. No deaths or other significant AEs were reported.

A recent Phase 2 study in subjects with ASD (209AS208) observed no significant safety or tolerability effects in subjects receiving up to 15mg dose TID of STX209 (arbaclofen), up to 45mg total. This was a randomized, double-blind, placebo controlled study of the efficacy, safety, and tolerability of STX209 (arbaclofen) administered for the treatment of social withdrawal in subjects (5 to 21 years old) with autism spectrum disorders. The study employed a flexible-dose schedule to determine the OTD for each subject. The OTD was defined as either the dose that achieved a behavior rating of very much improved on the CGI-I or as the maximum tolerated dose. Dosing decisions were based on the average response since the last dose change as compared to baseline behavior. Dosing regimens were stratified by age. For subjects in the younger age group, the starting dose was 5 mg QD, and could be increased every seven days to 5 mg BID, 10 mg BID, and then 10

mg TID. For subjects in the older age group, the starting dose was 5 mg BID, and could be increased every seven days to 10 mg BID, 10 mg TID, and then 15 mg TID.

Additionally, there are two human studies examining brain function after a single dose of 50 mg of baclofen (McDonnell et al. 2006, 2007). In McDonnell et al. (2006) transcranial magnetic stimulation was used to assess the motor cortex threshold and then motor cortex evoked potential amplitudes were examined before and after baclofen administration. The primary measures were ERP assessments of inhibitory synaptic transmission. Examining nine human subjects 21 to 41 years old, they observed a significant increase in long interval intracortical inhibition and a significant increase in short interval intracortical inhibition 90minutes following 50 mg of baclofen. After receiving 50 mg of baclofen, it was noted that all subjects reported mild adverse effects after the administration of baclofen, most commonly tiredness and light-headedness. It was also noted that these effects did not interfere with the ability of subjects to comply with all requirements of the study.

Examining seven adult subjects administered 50 mg baclofen, McDonnell et al. (2007) used transcranial magnetic stimulation to obtain measures of cortical and corticospinal excitability, they showed that the GABA-B receptor agonist baclofen decreases paired associative stimulation induced LTP-like plasticity in human motor cortex. This suggests that increased GABA-B mediated inhibitory postsynaptic potentials drive this effect, and that baclofen may have a negative impact on LTP-dependent behavioral processes such as motor learning. In this study Mcdonnell et al. noted that all subjects tolerated drugs and experimental procedures well except for slight tiredness in the baclofen and/or placebo conditions in some subjects, which did not interfere with the subjects' ability to comply fully with the demands of all experimental procedures.

With regard to the proposed study, the McDonnell et al findings are of particular interest, showing changes in cortical neural function 90 minutes after an acute single dose. They also show that the 50 mg dose of baclofen (including both enantiomers) was generally well tolerated. In the present study, the maximum does is 30mg (arbaclofen only). As such, we anticipate fewer side-effect than the already minimal side effects reported in McDonnell et al.. In addition, as Arbaclofen is associated with fewer side effects than baclofen, at the 30 ms dose we would again hypothesize few side effects.

1.3.1.2 Pharmacokinetics Results from Clinical Studies

Although the Pharmacokinetics (PK) of racemic baclofen has been studied in humans, minimal information has been available on the individual enantiomers, R-baclofen, and S-baclofen. In 1990, Mutschler et al. evaluated the PK of the baclofen enantiomers in humans. Two healthy volunteers received 10 mg of racemic baclofen IV. AUC and CL were essentially the same for both enantiomers. An additional seven healthy volunteers received a 20 mg oral dose of racemic baclofen and the enantiomers had elimination half-lives of 5.3 and 5.1 hours, respectively. A slightly higher fraction of R-baclofen was eliminated in the urine during the first four hours (39% versus 33% for S-baclofen). In summary, review of the literature suggests that R-baclofen and S-baclofen have comparable PK properties.

Of the 63 subjects in Study 22001 (55 males and eight females), plasma concentration data and dosing history were available from 48 subjects. All of these subjects were included in the pharmacokinetics analysis. Results from pharmacokinetics analysis shows that both the weight-normalized model and the allometric model fit the data well. Inter-individual variability was slightly smaller for the allometric model (49% vs. 52% for the weight-normalized model). Covariate graphics for the weight-normalized model showed a small residual relationship between CL/F and weight. In summary, PK parameters obtained in healthy adults (after adjustment for body size and allowing a

different relative bioavailability between the two studies) were applied to subjects in the present study, the fit was generally unbiased. However, in the weight-normalized model, there was a small residual relationship between post hoc values and weight. This relationship was not present in the allometric model. Inter-individual variability in CL/F, as assessed by variance, was slightly smaller for the allometric model.

Another study (209NV103) looked at the effects of the oral disintegrating tablet formulation of STX209. This study was conducted to characterize the PK of STX209 of the orally disintegrating tablet (ODT) formulation following administration of single oral doses of STX209 and racemic baclofen to healthy volunteers under fasting conditions. The potential for *in vivo* chiral inversion from STX209 to S-baclofen was also evaluated. Eight healthy, adult male volunteers aged 24 to 40 years received single doses of STX209 in a crossover design following a minimum 5-day washout period. Six subjects completed the study; one subject voluntarily withdrew prior to receiving period two treatment, and the other did not return to the clinic for Study Period 2 and was considered lost to follow-up.

As was observed in the PK study using STX209 capsules, the plasma concentration profiles were similar following oral administration of single doses of 5 mg STX209 ODT and 10 mg racemic baclofen tablets (which contain approximately 5 mg STX209) to healthy volunteers under fasting conditions. Noncompartmental analysis yielded similar geometric mean estimates for STX209 Cmax, Tmax, and AUC, and the point estimate for t½ was similar for the STX209 and racemic baclofen formulations (5.85 versus 5.04 hours, respectively). In addition, it should also be noted that the Tmax following administration of STX209 was approximately 60 minutes (Investigator's Brochure, Table 16).

Among the samples collected following STX209 administration, none of the plasma samples and only one urine sample collected yielded detectable concentrations of S-baclofen. Except for the single aberrant sample, these results support the previous claim that there is no *in vivo* stereoinversion between the two isomers. A single AE of headache (12.5%) was reported for one subject dosed with racemic baclofen. Concluding that the oral disintegrating tablet formulation of STX209 does demonstrates a pharmacokinetic (PK) profile comparable to that of racemic baclofen in healthy volunteers.

1.3.1.3 Non-Clinical Studies

Wuis studied the PK of racemic baclofen and the R- and S-enantiomers in dogs after IV infusions of 2 mg/kg of the racemate and 1 mg/kg of each enantiomer. Blood samples were collected at selected times for 25 hours after drug administration, and urine was collected for a period of 50 hours. Taking into account the small number of animals (n=3), the PK parameters of the racemate and individual enantiomers were comparable.

Blood-brain barrier transport profiles of racemic baclofen, STX209, and S-baclofen were assessed in rats using unit impulse response methodology. Although plasma and cerebral spinal fluid (CSF) elimination kinetics did not differ between STX209 and S-baclofen, there was a suggestion of stereo-selective transport of baclofen across the blood-brain barrier with STX209 transported more efficiently.

In a subsequent study, quantitative electroencephalography (EEG) parameters in conjunction with plasma concentration measurements and PK-pharmacodynamic (PD) modeling techniques were used to characterize both the PD and plasma-effect site equilibration kinetics of baclofen. This study again found similar PK for STX209 and S-baclofen. The rate constant for transport of STX209 into the CSF was 2-fold higher than for S-baclofen, but there was no difference in rate constants out of the

CSF, and there was no PK interaction between the 2 isomers with respect to transport into the CSF. The net result was that times for the CSF to reach 50% equilibrium were equivalent for racemic baclofen, STX209, and S-baclofen. PD parameters for the R-isomer were not different from the parameters of the racemate, indicating that PD interactions between the Rand S-isomers do not occur. Furthermore, the equilibration rate constants at the effect site were comparable after administration of the racemate or individual enantiomers.

The plasma and brain pharmacokinetics of STX209 was also evaluated in the mouse. In one study (STX-REP-0011), plasma and brain concentrations of STX209 were determined in C57BL/6 mice dosed at three doses of STX209 (1, 3 and 6 mg/kg) administered as intraperitoneal injections twice daily for 9 days. Dose-related increases in plasma and brain exposure were observed, with a rapid tmax in both tissues (0.25-0.5 hr) and short terminal elimination half-lives (< 2 hr). Low brain-to plasma concentration ratios were also observed (≤ 0.11 up to 2 hr; 0.16-0.28 at 8 hr).

STX209 brain and plasma levels were compared to those produced following administration of racemic baclofen in C57BL/6 mice. Animals were administered STX209 (1, 3 or 6 mg/kg) or racemic baclofen (2, 6 or 12 mg/kg) via intraperitoneal injection and brain/plasma levels of each enantiomer were measured at intervals up to 240 minutes. Blood plasma levels of R-baclofen were higher in mice administered 2x racemic baclofen compared to those receiving STX209. Conversely, R-baclofen levels in brain were higher in mice treated with STX209 compared to those receiving 2x racemic baclofen. Consistent with this, brain-to-plasma ratios of R-baclofen were higher in animals receiving 1 and 3 mg/kg STX209 than in animals receiving 2 and 6 mg/kg racemic baclofen.

In summary, these results indicate that the PK and PD properties after administration of the single isomer, STX209, are similar to those observed when STX209 is administered with S-baclofen as a racemic mixture.

1.4 Selection of Drugs and Dosages

This is a study examining the acute effect of STX209 on brain activity at two different doses: 15mg and 30mg. Subjects will receive STX209 via oral disintegrating tablets, administered in individual 15mg tablets. Over a period of three visits, participants will receive a single randomly administered dose of placebo and in order from smallest to largest a single-dose of STX209 (15mg, and 30mg). All visits subjects will receive two tablets in the following order, with placebo randomly administered:

Dosage	Tablets Administered
15mg	One 15mg tablet of STX209 and one placebo
30mg	two 15mg tablets STX209 and zero placebo
Placebo	Zero STX209 tablets and two placebo

Across the three visits, all subjects will receive all doses, with the subject blind to dose.

1.5 Compliance Statement

This study will be conducted in full accordance with all applicable Children's Hospital of Philadelphia Research Policies and Procedures and all applicable Federal and state laws including IND 21 CFR 312 and regulations. All episodes of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain consent (Appendix 1) and assent, and will report unanticipated problems involving risks to subjects or others in accordance with The Children's Hospital of Philadelphia IRB Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

2 STUDY OBJECTIVES

A candidate therapeutic for ASD has been developed – STX209, a GABA-B agonist (made available to this project by the Clinical Research Associates, LLC (CRA)). Since this pharmaceutical targets synaptic activity that have clear electrophysiological correlates, one goal of this proposal is to assess the responsiveness (sensitivity to change) of MEG measures to acute administration of STX209 at various doses in adolescents on the autism spectrum. The study also aims to establish the nature of the putative relationship between such electrophysiologic markers and GABA and glutamate levels using MEGAPRESS spectrally-edited magnetic resonance spectroscopy (MRS), which we have previously used to demonstrate a relationship between GABA concentration and gamma oscillatory activity (Gaetz et al. [8]).

2.1 Primary Objective (or Aim)

1. To determine whether STX209 produces a significant change in MEG target parameters compared to baseline as well as compared to placebo treatment. Secondly, to determine if the magnitude of such change is influenced by STX209 dose: 0mg (placebo) versus 15mg versus 30mg.

2. To determine whether STX209 leads to a significant change in MRS-obtained auditory cortex GABA levels compared with placebo treatment. Secondly, to determine whether the magnitude of the GABA change is influenced by STX209 dose level.

3. To determine if dose-dependent changes in MEG target parameters are correlated to dose-dependent changes in MRS target parameters in auditory cortex.

3 INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

3.1.1 Screening Phase

Screening - Contact Prior to Visits to CHOP

Determination of eligibility will be conducted over the phone after the parent has provided verbal informed consent. This phone screen determines eligibility, and only subjects who meet study inclusion and exclusion criteria will be enrolled in this study.

Pre-Visit Intake Questionnaire
The Pre-Intake Questionnaire is completed with a parent over the phone once a subject is deemed eligible for this study and wishes to participate. The parent is given the option of completing the short questionnaire immediately after the phone screen or scheduling a separate time to complete it

over the phone with one of the study staff. The purpose of the questionnaire is to provide the study staff with additional necessary information for scheduling, as well as information that will help the staff best prepare for the subject's visits. The hope is that both visits are positive experiences and that we can maximize compliancy. Therefore this questionnaire should be completed via phone prior to the subject's first study visit.

Once potential subjects have been identified, via a phone interview the study will be described. If potential subjects are interested, then they will be schedule for Visit 1. At this first visit the study will again be described and if the subject is still interested they consent for signed.

3.1.2 Screening Visit:

The screening visit for the study will be conducted at the Children's Hospital of Philadelphia and will entail the following procedures:

- Written informed consent provided by parent and child assent if appropriate
- Neuropsychological evaluation, including clinical and behavioral testing (*only if applicable*)
- Physical Examination (*include abdominal palpation to assess for liver enlargement, assessing for decreased tone and postural problems, increased drooling and poor orofacial tone, problems holding head up, and difficulty with coordination of motor actions),* Height and Weight, and Medical/Surgical History Review
- Vital Signs: Temperature, Blood Pressure, Heart Rate, and Respiratory Rate
- Blood Draw (BUN and Creatinine): *Arbaclofen (STX209) is elimanated in the urine, BUN and Creatinine are done to confirm that subject has normal urinary function.*

Neuropsychological Evaluation: Clinical and Behavioral Assessments

On the screening visit, clinical and behavioral testing will be performed to confirm diagnosis of autism spectrum disorder and to evaluate language function. Intelligence testing will be performed to obtain an IQ estimate. The following instruments will be used:

- ADOS-2 (Autism Diagnostic Observation Schedule- 2nd edition)- *A clinical tool that looks at a child's language, social skills and behavior. This updated version is attached to the eIRB application section 12.02 (2.0).*
- ADI-R (Autism Diagnostic Interview-Revised) [parent interview]- *A parent interview that is used to look at autism symptoms. The interview asks about a child's language, social skills and behavior.*
- CBCL (Child Behavior Checklist) [parent report]- *This is a parent completed rating scale that asks about behaviors like attention problems, aggression, anxiety, and depression.*
- CELF –IV (Clinical Evaluation of Language Fundamentals- 4th edition)- *This tool evaluates a child's language understanding and expression. This updated version is attached to the eIRB application section 12.02 (2.0).*
- Intervention History Form- *This form is based on one created for the NIH funded autism research network (CPEA: Collaborative Programs of Excellence for Autism). Our revised version is attached in the eIRB application section 12.02 (2.0). It was created by an expert panel of autism treatment researchers from throughout the country, so as to capture*

information about past treatments. This information can be an important moderating influence on the developmental trajectory of each child.

- SCQ (Social Communication Questionnaire-Lifetime version) [parent completed measure]- *This is a parent completed rating scale that asks about a child's social and communication skills both over the span of the child's lifetime.*
- SRS-II (Social Responsive Scale, 2nd edition) [parent completed measure]- *This is a parent completed rating scale that asks about a child's social development*
- Vineland Adaptive Behavior Scales (2nd edition) [Parent/Caregiver Rating Form]- *This is a parent completed measure used to support the diagnoses of intellectual and developmental disabilities.*
- WASI-II (Wechsler Abbreviated Scale of Intelligence (2nd edition)) - *This is a standardized tool that measures a child's problem-solving and reasoning skills*.

Tests will not be administered if results from recent testing (less than 12 months prior to screening visit) are available (data from tests will only be used if subjects who previously participated in one of our approved research studies, agreed to share their data on future studies). Depending on the availability of previous test results it is anticipated that behavioral and cognitive test duration will be between 1 and 4 hours. The ADI-R parent interview will be administered as needed when further information about ASD symptoms is required (e.g., when children with no prior ASD diagnosis have screened positive on the other autism diagnostic measures). The parent will be told during the child's screening visit if we would like to do the ADI-R interview. The interview will then be conducted during the child's first imaging study visit.

Testing will be performed in the Division of Child Development, Children's Seashore House, Dept. of Radiology, Roberts Center for Pediatric Research 2716 South St 5th floor at Specialty Care at 3550 Market Street .

In the case that a standing ASD diagnosis is called into question during the neuropsychological evaluation, the neuropsychological report will document that the child's prior ASD diagnosis received in the community cannot be confirmed. The psychologist who did the child's testing will also contact the subject's parent via phone to discuss the results. The diagnostic information is obtained for research purposes only, and is not sufficient for a clinical diagnosis, therefore the psychologist will provide appropriate referral information if the parent wishes to pursue further clinical evaluation. These results will not affect any educational, medical, or special services that the child might currently be receiving. It will be up to the participant or family to accept, use or reveal this information. Results from this testing will not appear in the subject's medical record.

In the case that there are any additional concerns following a child's neuropsychological evaluation, the psychologist will document the concerns in the written report as well as speak to the parent via phone. Again, appropriate referral information will be provided if the parent wishes to pursue further evaluation, since the diagnostic information obtained is not sufficient for a clinical diagnosis. Results from this testing will not appear in a subject's medical record.

3.1.3 MEG Assessments: Visits 1-3

On the next three visits (scheduled to occur within a range of 3-12months of the clinical/behavioral testing) MEG, MRI, and MRS measures will be collected at each study visit in the Dept. of Radiology. MEG testing will last approximately 3 hours (pre- and post STX209 scanning, with 1 hour period between baseline and post-STX209 administration) and MRI will take approximately an additional 1 hour (after the post-MEG exam) For MRI, no sedation or contrast will be used.

Specifically, we will accept behavioral testing within a 6-12 month timeframe and will accept Blood Draw results and Physical Examination review within 3 months of the first imaging visit. For cases where there has been a 3 month or more lag between screening visit and imaging visit, the Blood Draw and Physical Exam will be repeated to ensure safety and adequate monitoring (as noted above, in cases where subject is unable to do a blood draw on the day of the screening visit due to anxiety/fear/discomfort if no medical risk factors are identified during medical history review, we will either accept BUN and Creatinine results from an outside source within 3 months of first imaging visit or attempt to do the blood draw on the day of the imaging visit.).

Upon arrival, MEG data will be collected to obtain baseline measures. At the conclusion of the baseline MEG scan, participants will be randomly administered a single dose of placebo and in order of increasing order a single-dose of STX209 (15mg and 30mg). Based on advice from Seaside Therapeutics, given the time course of plasma STX209 levels, the MEG examination will be repeated 60 minutes after drug administration. Following the MEG exam, subjects will obtain a 3T MRI to obtain an anatomic 3D image (MP-RAGE) and bilateral STG GABA and glutamate measures (MEGAPRESS MRS). Subjects will return for their imaging study visits no less than four days or more than 14 days to repeat the MEG and MRI and MRS exams, each time with a different drug/placebo dose.

3.2 Blinding

Across three visits, participants will be randomly assigned a single dose of placebo, and in order from smallest to largest a single-dose of STX209 (15mg, and 30mg). Participants will be blind to dosage order. Clinical Research Associates, LLC (CRA) will provide the needed STX209 tablets and identical placebo tablets. For each subject, the pharmacist at CHOP will create three 2-pill combinations. Order of the STX209/placebo conditions will differ for each subject. Upon entry into the study, the study pharmacist will assign each subject to one of the below dosage orders, and at each visit providing the research coordinator with the needed pills for that particular visit.

Option 1	Option 2	Option 3
Placebo	15mg	15mg
15mg	Placebo	30mg
30mg	30mg	Placebo

Subjects will receive study drug regimen in increasing dosage from smallest to largest and a randomly administered single-dose of placebo.

3.3 Study Duration, Enrollment and Number of Sites

3.3.1 Duration of Study Participation

40 participants will take part in a 4-week placebo controlled, randomized, acute dose response trial. Participation will be extended up to six or eight weeks if subjects are unable to come in 4 consecutive weeks in a row. Subjects will first come in for their screening visit followed by 3 MEG/MRI/MRS assessment visits (total of 4 visits).

3.3.2 Total Number of Study Sites/Total Number of Subjects Projected

The study will be conducted at one investigative site in the United States – at the Children's Hospital of Philadelphia. The clinical and neuropsychological testing will occur at the Roberts Pediatric Center at the Children's Hospital of Philadelphia (2716 South St.) or at Main Hospital or at Clinical and Translational Research Center at 3550 Market Street and the medical evaluation on screening

visit and Visit 3 will occur either at the Clinical and Translational Research Center at 3550 Market Street, or at the main hospital at 3401 Civic Center Boulevard. Dr. Amanda Bennett, M.D., a Developmental-Behavioral Pediatrician at the Children's Hospital of Philadelphia will be the primary physician on this project.

We will enroll 40 subjects in order to get 38 evaluable subjects.

3.3.3 Inclusion Criteria

1) Males aged 14 to 17.75 years.

2) Confirmation of diagnosis of ASD within the past 12 month, according to the DSM-IV criteria, including Autism, Pervasive Developmental Disorder – Not Otherwise Specified (PDD-NOS), and Asperger's Syndrome but excluding Childhood Disintegrative Disorder and Rett Symdrome.

3) Current pharmacological treatment regimen has been stable for at least 4 weeks prior to Screening.

4) If the subject is already receiving stable non-pharmacologic educational, behavioral, and/or dietary interventions, participation in these programs must have been continuous during the 2 months prior to Screening and subjects or their parent/caregiver may not electively initiate new or modify ongoing interventions for the duration of the study. Typical school vacations are not considered modifications of stable programming.

5) Prior to the conduct of any study-specific procedures, the subject must provide verbal assent to participate in the study (if developmentally appropriate), and the parent/caregiver must provide written informed consent. If the caregiver attending the clinic visits is not the parent, written consent must be obtained from the parent for the caregiver's participation in the study.

3.3.4 Exclusion Criteria

1) No known neurological impairment (e.g., head trauma with loss of consciousness for more than 10 minutes, stroke, seizure disorder).

2) Claustrophobia

3) Metallic implanted prosthetic or stimulation device (including pacemaker)

4) Excessive metallic dental work (including braces, non-removable retainers)

5) Subjects who are currently receiving treatment with racemic baclofen, vigabatrin, tiagabine, or riluzole.

6) Subjects who have taken another investigational drug within the last 30 days.

7) Subjects who are not able to take oral medications.

8) Subjects who have a history of hypersensitivity to racemic baclofen.

9) Any concomitant medication deemed necessary for the welfare of the subject during the study may be given at the discretion of the Investigator. No more than 2 psychoactive medications (including antiepileptics) may be used with the exception of medications being combined to treat ADHD symptoms and sleep problems. Psychoactive medications must be FDA-approved for use for the disease or symptom being treated; off-label use is not permitted. If the subject is taking fewer than 2 psychoactive medications, and during the study it is deemed the subject needs an additional psychoactive medication, the Medical Monitor must be consulted before the subject can continue in the study. Antipsychotic medications are not permitted.

   Medications with anxiolytic properties (serotonin re-uptake inhibitors, tricyclic anti-depressants, venlafaxine, buspirone, and propranolol) are not permitted. Melatonin, diphenhydramine, and other medications administered regularly for insomnia are permitted. The use of vigabatrin, tiagabine, riluzole, or racemic baclofen is prohibited

10) Parents/guardians or subjects who, in the opinion of the Investigator, may be non-compliant with study schedules or procedures.

4 STUDY PROCEDURES

4.1 Screening: Phone

Participants who respond to our recruitment procedures will be called by a Research Assistant. These trained staff members will describe the intent of the study, what is involved for the child and parent, and discuss basic elements of the consent (**Study Description Phone Script and Informed Consent for Phone Screening**). The parent/guardian will be invited to complete a brief, preliminary screening interview in order to determine eligibility for the study and will be consented via the telephone for an eligibility screening. If parents/guardians choose not to continue or decline to consent to screening, they will be thanked for their time and asked if they would like to receive any information about research participation or clinical services. If so, general information about ongoing research and clinical services at CHOP will be provided (as described in the phone script).

Once the parent/legal guardian verbally consents to the screening call, trained staff will complete the "**Screen for Eligibility Criteria**". This interview takes approximately 10 minutes to complete. *Upon completion, eligibility criteria will be reviewed immediately*. If the child is likely to meet eligibility criteria, the parent is invited to continue. A "**Pre-Visit Intake Questionnaire**" will be conducted over the phone with the parent of subjects who are both eligible for the study and want to participate. This short questionnaire needs to be completed prior to the subject coming in for his/her first study visit.

If the parent has endorsed any exclusionary criteria, they will politely be informed that they are not eligible for the current study and provided with the opportunity to seek referrals for other research projects or to be contacted by a clinician to receive appropriate clinical referrals.

4.2 Screening Visit

On the screening visit participants will be given Informed Consent and the following assessments and procedures will be administered to confirm eligibility and safety. Measures bearing on exclusion and inclusion criteria will be done first, and if determined eligible to participate, participants will complete the other measures.

- Neuropsychological evaluation, including both clinical and behavioral testing (*only if applicable*)
- Physical Examination (*include abdominal palpation to assess for liver enlargement, assessing for decreased tone and postural problems, increased drooling and poor orofacial tone, problems holding head up, and difficulty with coordination of motor actions), Height and Weight, and Medical/Surgical History Review*
- Vital Signs: *Temperature, Blood Pressure, Heart Rate, and Respiratory Rate*
- Blood Draw (*BUN and Creatinine)* (To minimize harm to the subject, in cases where subject is unable to do a blood draw on the day of the screening visit due to anxiety/fear/discomfort, if no medical risk factors are identified during medical history review, we will either accept BUN and Creatinine results from an outside source within 3 months of the first imaging visit or attempt to do the blood draw on the day of the imaging visit.)

4.3 MEG and MRI/MRS Assessment Phase: Visit 1-3

On the next three visits (scheduled to occur within 3 months of the physical examination and within 12 months of clinical/behavioral testing) MEG, MRI and MRS measures will be collected at each study visit. Upon arrival, vital signs will be collected. Then pre-drug MEG data will be collected to obtain baseline measures. At the conclusion of the baseline MEG scan, participants will be randomly administered a single-dose of Placebo and single-dose of STX209 in order of smallest to largest dose (15mg, 30mg) (see Section 3.2). Based on advice from Clinical Research Associates, LLC (CRA), given the time course of plasma STX209 levels, the MEG examination will be repeated 60 minutes after drug administration. Following the MEG exam, subjects will obtain a 3T MRI to obtain an anatomic 3D image (MP-RAGE) and bilateral STG GABA and glutamate measures (MEGAPRESS MRS). At the end of the scanning visit AE will be assed via the SMURF form. Subjects will return for their imaging visit no less than four days or more than 14 days to repeat the MEG, MRI and MRS exams, each time with a different drug/placebo dose.

4.4 Unscheduled Visits

The investigators will schedule visits based on participant's availability. Every effort will be made to accommodate the participants' scheduling needs. Participants will be given the contact information of Dr. Amanda Bennett for the drug portion of the study. They will be given instructions on how to contact Dr. Bennett should any issues arise.

4.5 Concomitant Medication

Any concomitant medication deemed necessary for the welfare of the subject during the study may be given at the discretion of the medical monitor and/or Investigator. No more than 2 psychoactive medications (including antiepileptics) may be used with the exception of medications being combined to treat ADHD symptoms and sleep problems. Psychoactive medications must be FDA-approved for use for the disease or symptom being treated; off-label use is not permitted. If the subject is taking fewer than 2 psychoactive medications, and during the study it is deemed the subject needs an additional psychoactive medication, the Medical Monitor must be consulted before the subject can continue in the study. **Antipsychotic medications are not permitted.**

Medications with anxiolytic properties (serotonin re-uptake inhibitors, tricyclic anti-depressants, venlafaxine, buspirone, and propranolol) are not permitted. Additionally, regular use of

benzodiazepines is not permitted. Melatonin, diphenhydramine, and other medications administered regularly for insomnia are permitted.

The use of vigabatrin, tiagabine, riluzole, or racemic baclofen is prohibited.

Examples of psychoactive medications that are allowed for FDA-approved indications are provided in Appendix A. All concomitant medication information, including indication, dose, route and dates of use, must be documented in full in the source notes and case report form (CRF), and should include any changes that have occurred during the study along with the reason for the medication or dose change. Any changes in the doses of concomitant medications should be reported to the Medical Monitor.

4.6 Rescue Medication Administration

There are no antidotes for STX209.

4.7 Subject Completion/Withdrawal

Criteria for withdrawal of subjects and plans for provision of care after withdrawal. Subjects may withdraw from the study at any time without prejudice to their care. They may also be discontinued from the study at the discretion of the Investigator for lack of adherence to study treatment or visit schedules. The Investigator may also withdraw subjects who violate the study plan, or to protect the subject for reasons of safety or for administrative reasons. It will be documented in the CRF whether or not each subject completes the clinical study. If the Investigator becomes aware of any serious, related adverse events after the subject completes or withdraws from the study, they will be recorded in the source documents.

At study completion (Visit 3) a final physical examination will be performed, AE assessed, and subjects debriefed.

4.7.1 Early Termination Study Visit

When a subject withdraws from the study, the reason(s) for withdrawal shall be recorded by the Investigator. Given that this is a study examining the acute effects of STX209 on brain activity, if a subject withdraws, they will be contacted by the study coordinator to elicit AE information.

5 STUDY EVALUATIONS AND MEASUREMENTS

5.1.1 Screening and Monitoring Evaluations and Measurements

Upon entry into this study, we will obtain informed consent/assent, review inclusion /exclusion criteria, clinical symptom ratings and intelligence testing will additionally be performed.

5.1.2 Clinical and Neuropsychological Assessment

Intelligence testing will additionally be performed to ensure no mental retardation. The following instruments will be used:

1) ADOS-2 (Autism Diagnostic Observation Schedule- 2nd edition) - *(ADOS-G; Lord et al. [14]; Lord et al. [15]). The ADOS-G is a semi-structured standardized assessment using developmentally appropriate social and toy-based interactions in a 30-45 minute evaluation session. The ADOS-G consists of four different modules, each directed at a particular level of language ability. The ADOS-G has been carefully psychometrically validated across a wide*

range of ages and severity levels in autism, and it is widely considered the single best gold standard diagnostic instrument (Lord et al. [16]).

2) ADI-R (Autism Diagnostic Interview-Revised) [parent interview] - *(ADI-R; Lord, Rutter, & LeCouteur [16]). The ADI-R will not be administered to control participants or to parents of children with ASD who have already completed an ADI-R with the Center for Autism Research. This interview typically takes 1- 2 hours and primary caregivers will be given the opportunity to complete this measure during the child testing. The ADI-R is appropriate for children 3 and older, and is a structured, standardized parent interview developed to assess the presence and severity of symptoms of autism in early childhood across all three main symptom areas involved in autism: social relatedness, communication, and repetitive and/or restrictive behaviors. The ADI-R has been carefully psychometrically validated across a wide range of ages and severity levels in autism. Considered a gold standard in assessment in autism, this instrument yields an algorithm score and cutoffs for a diagnosis of autism. An algorithm has been established that differentiates autism from other developmental disorders at high levels of sensitivity and specificity (over .90 for both) for subjects with mental ages of 18 months and older.*

3) CBCL (Child Behavior Checklist) [parent report] - *This is a parent completed rating scale that asks about behaviors like attention problems, aggression, anxiety, and depression.*

4) CELF –IV (Clinical Evaluation of Language Fundamentals- 4th edition) - *This tool evaluates a child's language understanding and expression. This updated version is attached to the eIRB application section 12.02 (2.0).*

5) Intervention History Form - *This form is based on one created for the NIH funded autism research network (CPEA: Collaborative Programs of Excellence for Autism). Our revised version is attached in the eIRB application section 12.02 (2.0). It was created by an expert panel of autism treatment researchers from throughout the country, so as to capture information about past treatments. This information can be an important moderating influence on the developmental trajectory of each child.*

6) SCQ (Social Communication Questionnaire-Lifetime version) [parent completed measure] - *This is a parent completed rating scale that asks about a child's social and communication skills both over the span of the child's lifetime.*

7) SRS-II (Social Responsive Scale, 2nd edition) [parent completed measure] - *This is a parent completed rating scale that asks about a child's social development*

8) Vineland Adaptive Behavior Scales (2nd edition) [Parent/Caregiver Rating Form] - *This is a parent completed measure used to support the diagnoses of intellectual and developmental disabilities.*

9) WASI-II (Wechsler Abbreviated Scale of Intelligence – 2nd Edition) - *This is a standardized tool that measures a child's problem-solving and reasoning skills*.

Tests will not be administered if results from recent testing (less than 12 months prior to screening visit) are available. Data from tests will only be used if subjects who previously participated in one of our research studies, agreed to share their data on future studies. The ADI-R will be administered only when further classification of subjects is needed

All tests will be promptly reviewed by one of the psychologists on the study team. Any significant clinical concerns on the Child Behavior Checklist or other measures will be verbally reported to the parent-- either in person during the study visit or later via telephone, depending on when the concern

is identified. When indicated, parents will be provided with mental health services referrals or will be advised to take their child to a hospital emergency room in the event of an acute care concern.

5.1.3 Physical Examination

Dr. Bennett will perform the physical examination (screening visit and visit 3) at the Clinical and Translational Research Center located at 3550 Market Street or at Main Hospital. However, for the imaging visits (visit 1-visit 3) the SMURF will be performed at the Department of Radiology to assess for adverse events. This can be performed by either Dr. Bennett or a trained research staff under the guidance and supervision of Dr. Bennett.

1) Physical Examination: *A Physical examination will be performed at Screening and at Visit 4. Physical examination findings will be classified using the standard categories listed on the CRF. A brief physical examination will be performed at screening visit and visits 1 through 3 and will include abdominal palpation to assess for liver enlargement, assessing for decreased tone and postural problems, increased drooling and poor orofacial tone, problems holding head up, and difficulty with coordination of motor actions. Any physical examination finding that is judged by the Investigator as a clinically significant change (worsening) from screening visit will be considered an AE, assessed and recorded on the CRF.*

2) Height and Weight: *Height and weight measurements must be performed without shoes on, and with only a single layer of light clothing. Height and weight measurements should be performed using consistent units (US).*

3) Medical/Surgical History Review: *The study doctor will review Medical/Surgical History*

4) Safety Monitoring Uniform Report Form (SMURF): The SMURF *contains a general inquiry, several questions about daily activities (e.g., sleep, appetite, energy level, bowel and bladder function). The general inquiry includes an open ended question about any problems or complaints, as well as questions regarding the need for other medications and doctor or health care encounters since the last study visit. The next section includes 20 to 25 specific queries. These items are rated as not present, mild, moderate or severe. The ratings mild, moderate, or severe are defined as follows: mild=present, but no intervention required; moderate=present, may be bothersome or may require intervention; severe=present, bothersome and requires intervention. The last section includes specific questions about daily activities. All new adverse events (mild, moderate, or severe) will be documented. The status of previously reported adverse events will be monitored as well.*

5.1.4 Vital signs

Depending on availability, vital signs will be measured either by the Imaging techs or Dr. Bennett at the Department of Radiology. Standard procedures will be followed.

1) Temperature: *Temperature will be measured orally using a digital thermometer.*

2) Blood Pressure: *Blood pressure will be measured using a Dyna-Map machine. A manual sphygmomanometer will be available for use as needed.*

3) Heart Rate: *Heart rate is measured using a Dyna-Map machine*.

4) Respiratory Rate: *Respiratory rate will be measured manually.*

5.1.5 Laboratory Evaluations

- Blood Draw: *blood draw testing will include the following: BUN and Creatinine*

*To minimize harm to the subject, in cases where subject is unable to do a blood draw on the day of the screening visit due to anxiety/fear/discomfort if no medical risk factors are identified during medical history review, we will either accept BUN and Creatinine results from an outside source within 3 months of first imaging visit or attempt to do the blood draw on the day of the imaging visit.)

5.1.6 MEG, MRI and MRS Assessment

1) **MEG:** During the MEG examination the child will sit or lie on a padded chair / bed with their head positioned inside a scanning helmet. The chair / bed and helmet are housed inside a special room (magnetically shielded room), free from external electromagnetic interference. Inside the helmet are highly sensitive magnetic field detectors which can passively detect brain activity within the head. There is no radiation involved. The helmet may gently touch the child's head, but there is no need for placement of electrodes directly on the scalp. The standard acquisition procedure employed in our studies is to place three fiducial markers (at left and right pre-auricular points and nasion) and instruct subjects to sit very still with their head inside the MEG helmet. In addition to MEG, electrodes at the outer canthi and supra- and suborbitally may be used to monitor EOG. Likewise, an electrode each may be attached to both the right and the left collar bone in order to monitor heartbeat. After attachment of the three localizing head coils and the EOG and cardiac electrodes, the location of points across the surface of the subjects head will be digitized using a Polhemus digitizer for later topographic and source-localization analyses and to facilitate co-registration with structural MRI. The child will be able to see clearly as the helmet covers only the upper and back portions of the head. Earphones will be placed in the child's ears to deliver auditory stimuli at a comfortable listening level (40dB above sensation level). A screen will be placed approximately 12 inches in front of the child's face and will display video images. A variety of auditory stimuli will be delivered. When auditory stimuli are being delivered, the child may watch a DVD movie or cartoons on the video screen. The experimental session is broken down into a series of short (5-10minute) data acquisition periods (see below).

   After each task, the child will be able, if they wish, to take a break from scanning. During the entire scanning session, the child will be monitored via closed-circuit video and communication will be possible through an intercom system.

   Three MEG recordings will be obtained (at baseline and 60 minutes following acute drug/placebo administration), with each MEG recording session lasting ~30 minutes, and consisting of the following paradigms:
   1) Resting MEG/EEG: 2 minutes eyes open and 5 minutes eyes closed (7 minutes)
   2) Binaurally presented 300 and 500Hz 300ms duration sinusoidal tone (45dB SL), using the task reported in Roberts et al. (2010). (2 minutes)
   3) Auditory 40 Hz steady-state task, presenting 200 trials of 40 Hz amplitude modulated (AM) sinusoidal tones (500 ms long, 45 dB SL) with a jittered 1.5 sec inter-stimulus interval. (7 minutes)
   4) Oddball auditory task with interleaved /a/ and /u/ vowel stimuli as in Roberts et al. (2011). (12 minutes)

2) **MRI/MRS** Magnetic resonance imaging will then be performed using a 3T Siemens Trio MR scanner in the Department of Radiology at Children's Hospital of Philadelphia. This state of the art equipment is equipped with multi-channel RF capabilities (allowing parallel imaging

(Pruessmann et al. [17]). A mock scanner (Nordic Neurolabs) is available for subject conditioning and paradigm practicing. Sequences will include:

1) localizer scouts (3-plane)
2) inversion-recovery prepped 3D gradient recalled echo imaging sequences for anatomic visualization (high resolution 256x256x160 matrix, isotropic 1mm resolution)
3) MEGAPRESS single voxel edited magnetic resonance spectroscopy sequence and analysis, as provided by Siemens Medical Solutions

All of the above MRI sequences are FDA-approved with the exception of the MEGAPRESS. The MEGAPRESS sequence was recently developed and measures brain chemistry (for example GABA).The MEGAPRESS sequence will take approximately 20 minutes and will obtained for all participants.

The MEGAPRESS MR pulse sequence is not specifically approved by the FDA. It was obtained from Siemens Medical Solutions as a Works-in-progress (WIP #529). The conditions of use of this WIP is that it is not to be used for clinical interpretation and that it should be used under IRB-approved protocols. Technically, it is a very simple modification of the conventional approved MR spectroscopy pulse sequence, in which a mathematical subtraction allows resolution of the previously obscured GABA resonance (by cancelling the overlapping Creatine resonance). It is extremely widely used in research studies worldwide. The conditions of use of a WIP is that it is not to be used for clinical interpretation and that it should be used under IRB approved protocols.

5.2 Efficacy Evaluations

The measures used to assess efficacy of STX209 are:

1. Change from baseline to STX209 administration in left and right superior temporal gyrus auditory M50, M100 and MMF latencies. To measure M50 (40-90ms) and M100 (90-180ms) STG latency (and amplitude), pre-stimulus baseline activity will be subtracted, and right and left M50 and M100 superior temporal gyrus latency and amplitude calculated from the largest amplitude point in the scoring windows using in-house MatLab software. These extended latency ranges allow for M50 and M100 latencies observed in younger children and for low frequency (e.g. 300Hz) stimuli.

2. Change from baseline to STX209 administration in left and right superior temporal gyrus auditory gamma total power and phase-locking measures (examined pre- and post-stimulus).Calculation of single-trial phase/magnitude for right and left STG sources will use complex demodulation procedures[18] implemented in BESA 5.3™, using frequencies between 4 and 80Hz, in steps of 2Hz. Continuous data will be analyzed relative to the tone onset every 25ms, using +/-39.4ms and +/- 2.83Hz (FWHM parameters) of contiguous data at each step. For evoked activity, background activity at each frequency (average power -400 to -100ms) will be subtracted. In addition to evoked power, for each time-frequency bin, a measure of phase-locking referred to as inter-trial coherence (ITC) will be computed, according to $ITC = abs\left\{\frac{1}{N}\sum_{k=1}^{N} e^{i\emptyset(k)}\right\}$, where N is the number of trials, and $\emptyset(k)$ is the phase of the signal in the k^{th} trial. ITC is a normalized measure with ITC=1 reflecting no phase variability and ITC=0 reflecting maximal phase variability across trials. The above time-frequency measures will be obtained for the pure-tone and steady-state tasks.

5.2.1 Diagnostic Tests, Scales, Measures, etc.

The following methods will be used to determine whether initial diagnostic criteria and inclusion/exclusion criteria are met, as well as to assess efficacy of intervention. They are listed with their timing of administration. The list provides an overview/summary of all measures given to child participants and their primary caregivers, including screening measures.

Screening - Contact Prior to Visits to CHOP	**Approximate Time Demand**
Parent/Legal Guardian	
Study description phone script	10 min
Collected by Phone:	
Verbal Consent for Phone Screening	5 min
Screen for Eligibility Criteria	10 min
Pre-Intake Questionnaire (Demographics)	20 min
Subtotal:	75 min

Screening Visit:
Medical, Diagnostic, and Neuropsychological Assessments

Procedures administered to Parent/Legal Guardian (at the same time as child testing)	
Intervention History Form	15 min
Medical History Form	30 min
Child Behavior Checklist (CBCL) *(if applicable)*	20 min
Social Communication Questionnaire (SCQ) *(if applicable)*	5 min
Social Responsive Scale-II (SRS-II) *(if applicable)*	10 min
Vineland Adaptive Behavior Scale *(if applicable)*	20 min
Autism Diagnostic Observational Scale (ADOS-2) *(if applicable)*	60 min
Subtotal:	2 hours and 40 min

Procedures individually administered to children:	
Physical Examination	15 min
Blood Draw	10 min
Vital Signs	10 min
Clinical Evaluation of Language Fundamentals (CELF –IV) *(if applicable)*	60 min
Wechsler Abbreviated Scale of Intelligence (WASI-II) *(if applicable)*	60 min
Baseline SMURF	20 min
MRI Mock Scan *(optional)*	30 min
Subtotal:	3 hours and 25 min

* Diagnostic and neuropsychological testing will not occur if testing scores are available (less than 12 months prior to screening visit) for re-recruited participants (data from tests will only be used if subjects who previously participated in one of our research studies, agreed to share their data on future studies). Only the medical assessments (e.g., vital signs, physical exam, medical history, intervention history form, and blood draw) will be completed for re-recruited participants at screening visit. Therefore, the screening visit will last approximately 80 minutes for re-recruited participants.

MEG Assessment 1: Visit 1

Procedures individually administered to participants at Visit 1	
Autism Diagnostic Interview – Revised (ADI-R) (*if applicable*)	180min
Vital Signs	10 min

Pre Drug MEG Scan	45 min
MRI Scan	30 min
Administration of STX209/Placebo	< 10 min
MRI Scan continued	30 min
Break	30-60 min
Post Drug MEG Scan	45 min
Adverse Event Assessment (SMURF)	20 min
Subtotal:	4 hours and 50 min

MEG Assessment 2: Visit2

Procedures individually administered to participants at Visit 2	
Vital Signs	10 min
Pre Drug MEG Scan	45 min
MRI Scan	30 min
Administration of STX209/Placebo	< 10 min
MRI Scan continued	30 min
Break	30-60 min
Post Drug MEG Scan	45 min
Adverse Event Assessment (SMURF)	20 min
Subtotal:	4 hours and 10 min

MEG Assessment 3 + Close Out: Visit3

Procedures individually administered to participants at Visit 3	
Vital Signs	10 min
Pre Drug MEG Scan	45 min
MRI Scan	30 min
Administration of STX209/Placebo	< 10 min
MRI Scan continued	30 min
Break	30-60 min
Post Drug MEG Scan	45 min
Physical Examination	15 min
Adverse Event Assessment (SMURF)	20 min
Subtotal:	4 hours and 40 min

5.3 Safety Evaluation

There were no significant safety or tolerability concerns from previous studies with STX209. AEs reported that were higher in frequency during the STX209 period of the double-blind study, or with incidence >15% in the open-label study included: headache, sedation, fatigue, increased agitation, increased irritability, vomiting, and psychomotor hyperactivity. Other AEs with incidence >5% of subjects included: diarrhea, insomnia, aggression, disturbance in attention, nasopharyngitis, abdominal pain, cough, decreased appetite, pharyngitis streptococcal, self-injurious behavior, asymptomatic decreased heart rate, and upper respiratory tract infection.

Based upon information on the action of STX209 (published reports and information from Seaside Therapeutics), several procedures will be followed to ensure subject safety. First, since STX209 is excreted renally, subjects will be excluded if laboratory tests indicate abnormal renal function (i.e., for their age, abnormal BUN and Creatinine levels) at the Screening Visit (To minimize harm to the subject, in cases where subject is unable to do a blood draw on the day of the screening visit due to anxiety/fear/discomfort, if no medical risk factors are identified during medical history review, we

will either accept BUN and Creatinine results from an outside source within 3 months of the first imaging visit or attempt to do the blood draw on the day of the imaging visit.). Second, since hypotension is a potential risk with STX209, blood pressure will be documented at each visit. Individuals with a significant change in blood pressure (determined by the medical monitor or PI) following administration of STX209 will be discontinued from the study visit if it is a Minor Adverse Event or will be discontinued from the entire study if it is a Severe Adverse Event.

Subject safety will be monitored by adverse events, blood draw, vital signs, and physical examination findings and concomitant medication usage.

STATISTICAL CONSIDERATIONS

5.4 Primary Endpoint

The primary endpoints are (1) change from baseline to STX209 administration in left and right superior temporal gyrus auditory M50, M100 and MMF latencies, and (2) change from baseline to STX209 administration in left and right superior temporal gyrus auditory gamma total power and phase-locking measures (examined pre- and post-stimulus).

5.5 Secondary Endpoints

Secondary endpoints will include the following:

- The change from baseline to STX209 administration in left and right superior temporal gyrus GABA levels.
- Safety and tolerability of STX209 based on Adverse Events. Measurements and evaluations

5.6 Statistical Methods

Primary statistical analyses assess baseline versus post acute-dose (placebo, STX209 15 and 30 mg) changes for (1) M100 auditory latency, and (2) post-stimulus auditory gamma-band activity. For each dependent measure, ANOVAs with dose as a repeated measure will examine baseline versus post acute-dose changes (analyses showing no significant differences across time in baseline M100 and gamma measures will allow use of a single averaged baseline measure). Contrasts of *a priori* interest assess (1) a baseline versus post-acute dose change in the dependent measures averaging across STX209 15 and 30 mg, as well as (2) a change in dependent measures from baseline versus STX209 15 mg versus STX209 30 mg, with this test demonstrating an effect of dose. Secondary and exploratory analyses examine baseline to post acute-dose response changes in MRS auditory GABA concentration as well as associations between MEG functional measures and MRS GABA concentration.

5.6.1 Baseline Data

Baseline and demographic characteristics will be summarized by standard descriptive summaries (e.g. means and standard deviations for continuous variables such as age).

5.6.2 Efficacy Analysis

Our principal hypotheses relate to baseline to STX administration changes in the latency of the auditory M50, M100 and MMF responses, and gamma-band total power and inter-trial coherence (examined pre- and post-stimulus). To this end, for each dependent measure, ANOVAs with dose as a repeated measure will examine baseline versus post acute-dose changes (analyses showing no significant differences across time in baseline M50, M100, MMF and gamma measures will allow use of a single averaged baseline measure). Contrasts of *a priori* interest assess (1) a baseline versus post-acute dose change in the dependent measures averaging across STX209 15 and 30 mg, as well as (2) a change in dependent measures from baseline versus STX209 15 mg versus STX209 30 mg, with this test demonstrating an effect of dose. For the secondary endpoints, associations between MRS GABA/Cr ratios and the MEG dependent measures will be assessed using Pearson correlation analyses.

5.6.3 Pharmacokinetic Analysis

N/A

5.6.4 Safety Analysis

All subjects entered into the study at screening visit will be included in the safety analysis. The frequencies of AEs by type, body system, severity and relationship to study drug will be summarized. SAEs (if any) will be described in detail.

5.7 Sample Size and Power

Forty individuals with ASD (aged 14 to 18 years) will be recruited. In each individual, baseline and post acute-dose imaging data will be obtained at three doses: (1) placebo, (2) STX209 15 mg, or (3) STX209 30 mg. Imaging measures at these three doses will be obtained at least 1 week apart, with the order of placebo and STX209 pseudo-randomized with the constraint that the STX209 15mg dose will be administered before the STX209 30 mg dose (FDA requirement). Assuming lost data or incomplete data sets from 4 individuals, the following power calculations were performed assuming a final sample of 36. As detailed in Section 1.3, given significant between subject variability in auditory cortex neural circuit pathology and thus potential between subject differences with regard to normalization of brain activity in response to STX209, the below statistical analyses conservatively assume a response to STX209 from approximately 50% of the participants. As detailed below, although the study is sufficiently powered to assess a baseline versus a 'general' post acute-dose change (i.e., averaging 15mg and 30mg), there is insufficient data to determine if the study is powered to observe a difference between STX209 15mg and STX209 30mg acute dose brain responses. As such, present data will provide preliminary data to guide future studies investigating STX209 dose differences.

Regarding the primary aims, assuming baseline to post acute-dose changes in M100 latency of approximately 2.5 ms (5 ms = one-half the ASD and control group differences reported in Roberts et al. (2010) and in the range observed in the single Arbaclofen study patient (2-8ms), but half of 5 ms given acute responses in only 50% of the participants), and assuming a standard deviation of similar size (which may be conservative given initial cross-dose averaging), and assuming a response in only 50% of the population, given a sample of 36 and $p = 0.05$, the power to detect a main effect on M100 latency is equal to 0.80. Assuming baseline to post acute-dose changes in post-stimulus gamma-band activity approximately half that reported in Edgar et al. (2015), the power to detect a main effect on gamma activity is approximately 0.70.

Given between subject variability in baseline auditory latency and gamma measures, and given a hypothesis that those participants showing the most pronounced M100 and gamma neural circuit pathology will show the largest response to STX209, the above analyses will be repeated with Group as a factor, with grouping obtained via a median split of M100 latency or gamma activity to identify those participants with more versus less auditory cortex neural circuit pathology. A Group X baseline to post acute dose interaction is expected, with significantly greater change observed in the more impaired (N = ~18) versus less impaired group (N = ~18). Given an expected greater effect in the participants showing greater impairment at baseline (e.g., a 5+ ms delay in the group showing greater auditory neural abnormalities), the study is powered at approximately 0.80 to observe an effect of this size.

Thus, the present study is sufficiently powered to assess the primary study aims.

5.8 Interim Analysis

N/A

6 STUDY MEDICATION (STUDY DEVICE OR OTHER STUDY INTERVENTION)

6.1 Description

STX209 (arbaclofen) is a selective GABA-B receptor agonist. STX209 augments GABA-ergic activity, inhibits presynaptic release of glutamate, inhibits postsynaptic transmission, and modulates intracellular signaling (Isaacson and Hille [3]; Scanziani, Capogna et al. [4]). STX209 is the active isomer of racemic baclofen, an approved GABA-B agonist. Baclofen has demonstrated efficacy in treating hyperactivity and audiogenic seizure phenotypes in the fragile X knockout mouse (Pacey, Heximer et al. [5]). Through elevation of GABA-ergic inhibitory activity, STX209 may act to alleviate ASD symptoms associated with social anxiety and emotional hyperarousal.

6.1.1 Packaging

Tablets are packed and labeled in blister strips of 7 tablets with expiry dates listed on the labels.

6.1.2 Labeling

Tablets are packed and labeled in blister strips of 7 tablets with expiry dates listed on the labels.

6.1.3 Dosing

This is a study examining the acute effect of STX209 on brain activity at two different doses: 15mg and 30 mg. Subjects will receive STX209 via oral disintegrating tablets, administered in individual 15 mg tablets. Over a period of three visits, participants will randomly receive a single-dose of Placebo and in order of smallest to largest a single-dose of STX209 (15mg, and 30mg).

Dosage	Tablets Administered
15mg	One 15mg tablets STX209 and one placebo
30mg	Two 15mg tablets STX209 and no placebo
Placebo	Zero STX209 tablets and two placebo

Across the three visits, all subjects will receive all doses, with the subject and the study investigators blind to dose until the close of the study.

6.1.4 Treatment Compliance and Adherence

Participants are asked to undergo imaging with *three* different dosages (15mg, 30mg, and placebo). Imaging is obtained weekly. If a subject misses an appointment they will be asked to come the following week. The dosage order for that subject will be maintained. If a subject does not complete the entire study the imaging data that was obtained from that subject will be included in the analyses.

6.1.5 Drug Accountability

Adequate records of study drug receipt and disposition will be maintained by the CHOP Pharmacy Records of receipts, investigational drug orders, dispensing records, and disposition forms will be examined during the course of the study. The purpose of these records is to ensure regulatory authorities and the Sponsor that the investigational new drug will not be distributed to any person who is not a study subject under the terms and conditions set forth in this protocol. The study medication is to be prescribed by the Investigator or designee and may not be used for any purpose

other than that described in this protocol. At study completion, all drug supplies including partially used and empty containers must be returned to Sponsor or designee.

7 SAFETY MANAGEMENT

Clinical adverse events (AEs) will be monitored throughout the study.

Subject safety will be monitored by adverse events, blood draw, vital signs, and physical examination findings and concomitant medication usage.

Dr. Bennett or the developmental behavioral pediatrician on call, will be on call during all MEG exams. Specifically, Dr. Bennett will review pre-MEG vital signs (either in person or over the phone) prior to medication administration. Dr. Bennett will administer the SMURF and evaluate the vital sign pre-and post-MEG in person for the initial and final study visits. For the visits in between SMURF and vital signs pre- and post-MEG will be done by an approved study staff and Dr. Bennett will be on call to evaluate the results.

All tests will be promptly reviewed by one of the psychologists on the study team. Any clinical significant findings will be verbally reported to the parent-- either in person during the study visit or later via telephone, depending on when the concern is identified. Parents will be provided with mental health services referrals or will be advised to take their child to a hospital emergency room in the event of an acute care concern.

7.1 Adverse Event Reporting

The risks to potential participants are expected to be minimal and precautions will be taken to minimize all risks. All unanticipated problems related to research events will be reported to the IRB. A log of all unexpected adverse events will be kept at the study coordinators office for tracking these events and the potential impact on the participants and study.

7.2 Definition of an Adverse Event

An adverse event is any untoward medical occurrence in a subject who has received an intervention (drug, biologic, or other intervention). The occurrence does not necessarily have to have a causal relationship with the treatment. An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

All AEs (including serious AEs) will be noted in the study records and on the case report form with a full description including the nature, date and time of onset, determination of non-serious versus serious, intensity (mild, moderate, severe), duration, causality, and outcome of the event.

7.3 Definition of a Serious Adverse Event (SAE)

An SAE is any adverse drug experience occurring at any dose that results in any of the following outcomes:

- death,
- a life-threatening event (at risk of death at the time of the event),
- requires inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant disability/incapacity, or
- a congenital anomaly/birth defect in the offspring of a subject.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug event when, based upon appropriate medical judgment,

they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

A distinction should be drawn between serious and severe AEs. A severe AE is a major event of its type. A severe AE does not necessarily need to be considered serious. For example, nausea which persists for several hours may be considered severe nausea, but would not be an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke, but would be an SAE.

7.3.1 Relationship of SAE to study drug or other intervention

The relationship of each SAE to the study intervention should be characterized using one of the following terms in accordance with CHOP IRB Guidelines: definitely, probably, possibly, unlikely or unrelated.

7.4 IRB/IEC Notification of SAEs and Other Unanticipated Problems

The Investigator will promptly notify the IRB of all on-site unanticipated, serious Adverse Events that are related to the research activity. Other unanticipated problems related to the research involving risk to subjects or others will also be reported promptly. Written reports will be filed using the eIRB system and in accordance with the timeline below. External SAEs that are both unexpected and related to the study intervention will be reported promptly after the investigator receives the report.

Type of Unanticipated Problem	Initial Notification (Phone, Email, Fax)	Written Report
Internal (on-site) SAEs Death or Life Threatening	24 hours	Within 2 calendar days
Internal (on-site) SAEs All other SAEs	7 days	Within 7 business days
Unanticipated Problems Related to Research	7 days	Within 7 business days
All other AEs	N/A	Brief Summary of important AEs may be reported at time of continuing review

7.4.1 Follow-up report

If an SAE has not resolved at the time of the initial report and new information arises that changes the investigator's assessment of the event, a follow-up report including all relevant new or reassessed information (e.g., concomitant medication, medical history) should be submitted to the IRB. The investigator is responsible for ensuring that all SAE are followed until either resolved or stable.

7.5 Investigator Reporting of a Serious Adverse Event to Sponsor

Reporting must be consistent with regulatory, sponsor or GCRC requirements (if applicable)

8 STUDY ADMINISTRATION

8.1 Treatment Assignment Methods

8.1.1 Randomization

Across three visits, participants will be randomly assigned a single dose of placebo and in order of smallest to largest a single dose of STX209 (15mg, 30mg). Upon entry into the study, the study pharmacist will assign each subject to one of the below dosage orders, and at each visit providing the research coordinator with the needed pills for that particular visit.

Imaging Assessment 1 Visit 1	Imaging Assessment 2 Visit 2	Imaging Assessment 3 Visit 3
Placebo	15mg	15mg
15mg	Placebo	30mg
30mg	30mg	Placebo

8.1.2 Blinding

Participants will be blind to dosage order. Clinical Research Associates, LLC (CRA) will provide the needed STX209 tablets and identical placebo tablets. For each subject, the pharmacist at CHOP will create three 2-pill combinations.

8.1.3 Unblinding

In case an emergency arises or a situation where unblinding becomes necessary. The pharmacy will unblind the medical monitor and the PI.

8.2 Data Collection and Management

An electronic database (REDCap) will be used to store all data relating to this study. All electronic data will be stored on the CHOP SAN. We will design case report forms (CRF) using the database itself and in a manner to enable both easy completion of forms and easy data entry. Additionally, the CRF form also acts as a document to record direct source data (i.e. vitals). The database enables modifications of data collection forms and data entry using computer screens that are identical to the data collection forms. Subjects will be assigned a unique identification number and the database will be password protected to insure confidentiality and security. Database system (REDCap) will be established to allow query of information relating to relevant aspects of the datasets. Through queries to the database, we will be able to create summary reports as needed, and prepare data sets for statistical analyses. The CHOP SAN drive is backed up daily to insure data safety.

8.3 Confidentiality

All data and records generated during will be kept confidential in accordance with Institutional policies and HIPAA on subject privacy and that the Investigator and other site personnel will not use such data and records for any purpose other than conducting the study (see exception below).

In order to keep protected health information (PHI) from disclosure, all data will be coded and identified only by a code label assigned to the participant upon entry into the study. All information will be stored on a CHOP SAN password protected server. Hardcopy documents will be stored in a locked filing cabinet in a locked office of one of the approved study staff. When a need for further

medical evaluation or treatment is identified during the research, participants will be appropriately referred and upon obtaining consent, study information made available to the health-care provider.

The information collected as a part of this study will be retained for at least 5 years or until the study is completed, whichever is longer. At that time, the research information will either be destroyed or all the information that identifies the subject will be removed from the study results.

8.4 Regulatory and Ethical Considerations

8.4.1 Data and Safety Monitoring Plan

The Office of Research Compliance and Regulatory Affairs (ORCRA) will begin monitoring activities for this study within three months of the first subject's enrollment into the study. Thereafter, ORCRA will monitor this study annually unless circumstances necessitate more frequent monitoring, e.g., an increase in risk to the subjects, less than ideal study conduct, higher than expected accrual rates. Monitoring activities will be guided by ICH E6 section 5.18.
Monitoring of subject data will be initiated at 100% verification of the data recorded. This may be amended, and a tapered approach to monitoring may be employed, if conduct and documentation of the study reaches a level of reliability that would permit valid conclusions based upon a sampling of data.

Monitoring reports will be communicated to the sponsor, the principal investigator, and the IND/IDE Support Program within 30 days after the monitoring visit has concluded.

Any serious or persistent noncompliant activity or violation that is observed during the course of monitoring will be reported to the sponsor and/or the principal investigator, and to the ORCRA Director. The ORCRA Director will evaluate the report of noncompliant activities and discuss with the sponsor and/or principal investigator any necessary reporting requirements and corrective and preventative actions plans.

8.4.2 Risk Assessment

As with any study of a novel therapeutic, there are at least minor risks associated for participants receiving the study drug. Adverse effects associated with STX209 in human trials have included sedation, agitation, irritability, psychomotor hyperactivity, headache, fatigue, diarrhea, and vomiting. However, rates of adverse effects and rates of withdrawal due to adverse effects were lower than for racemic baclofen, which is already in clinical use. Additional adverse effects reported with use of racemic baclofen, and rarely (< 1%) occurring in previous studies of STX209, included neuromuscular weakness, worsening of seizures in a known seizure disorder, and mild hallucinations. In addition, a time commitment of approximately 12.5 hours is expected in total for the four visits, which may be inconvenient. Some of the questions the children will be asked during the WASI testing may be hard for them (e.g., challenging problem solving, block design), which may cause some nervousness. The examiner will make every effort to reduce frustration and provide breaks when needed. In the second session, children may find lying on the MEG bed slightly uncomfortable and/or feel mildly "closed-in" (or claustrophobic) -with his/her head inside the MEG machine. We will make every effort to reduce any discomfort including pillows, blankets and the availability of video movies (during times when visual stimuli are not presented). There are no known medical risks associated with magnetic resonance imaging (MRI). However, children may experience mild discomfort or feelings of claustrophobia. Each child will be provided with a squeeze ball alarm as well as voice intercom to indicate that they would like to stop the MRI scan. Ear plugs will be provided to reduce the noise of the MRI scan. A video movie will be presented during MRI to make the experience more comfortable

8.4.3 Potential Benefits of Trial Participation

This information is likely to help us understand more about the children with an autism spectrum disorder. Participants will not benefit directly from the study. Science and society in general will benefit from our improved understanding of the risks associated with autism spectrum disorder. This information may lead to possible intervention and prevention strategies for developmental problems. These benefits are believed to outweigh the minimal risk to individual participants. Subjects may benefit from the opportunity to contribute to the improvement of scientific understanding of Autism Spectrum Disorders.

8.4.4 Risk-Benefit Assessment

Since risks associated with the procedures are negligible the risk/benefit ratio is favorable.

8.5 Recruitment Strategy

Recruitment will first focus on families of children between the ages 14-17.75 years who have a pre-existing ASD diagnosis, have participated in a previous MEG study (#5385) in the Radiology Department, and meet this study's inclusion and exclusion criteria. Only families who have agreed to be contacted about future studies and to have their data used for other research studies can be contacted. The study coordinator will contact these families via phone, mailing or email. If families who agreed to participate in future research are contacted via email, the initial email will only include the IRB-approved recruitment flyers. If the family is interested, the coordinator will proceed with screening, which will include phone screen, eligibility, verbal consent and pre-intake questionnaire. The coordinator will then schedule these subjects for their study visit.

Additional recruitment sources for participants with autism spectrum disorders (ASD) will be CHOP's Regional Autism Center. An additional recruitment source will be AutismMatch via the Center for Autism Research at CHOP. Potential participants will be identified by CAR staff via the autismMatch database. Subjects who agreed to be contacted about future research will also potentially be recruited from the Center for Autism Research studies including: #7275: Autism, Genes, Brain, and Behavior; 11-00824: Oxytocin and Learning in Autism. These names will be given to the research coordinator of this study, who will then send out recruitment letters to these families. Additionally, Dr. Amanda Bennett (study physician) and/or Dr. Nathan Blum (division chief) from the Division of Developmental Behavioral Pediatrics will send out a recruitment letter (attached to eIRB) to the parents or guardians of eligible children in the desired age range from those seen at the Division of Developmental Behavioral Pediatrics.

We have attached our flyers for review. These flyers will be posted and/or distributed in public spaces at CHOP as well as in cafes, libraries, schools, hospitals and other public places; we will obtain permission from these location prior to posting/distributing our flyers. We will also post these flyers on Internet sites, such as Craig's List, in local newspapers and magazines.

In addition, we will also send out emails/eBLASTs or provide information for research study listings on autism-related listservs and websites, such as the Autism Society of America (ASA) local Philadelphia chapter listserv, the Interactive Autism Network (IAN) database, the Center for Autism Research (CAR) at CHOP and/or post our approved study flyer on the CAR website. In these cases, we will send an email to the listserv/website administrator and ask them to email or post the IRB-approved study flyer and/or IRB-approved email text (these documents are separately attached to the eIRB application and labeled appropriately). Contacts from the IAN database will receive the recruitment letter (attached to the eIRB application) via email and interested individuals will have the option to either select "interested" or "not interested." For individuals who select "interested" they will be prompted to complete a REDCap screening form (attached to the eIRB application -

https://redcap.chop.edu/surveys/?s=TAR9EPWFED) online to give us permission to contact them in regards to the study.

Some of the organizations utilize social media; in these cases, we will provide short headline text (see document in eIRB) that then links to the IRB-approved flyer or email text. Additionally, Autism related agencies/organizations such as Autism Speaks and the Simons Foundation occasionally do news features or interviews with Dr. Roberts (PI) on his research studies. In these cases, we will provide the approved email/eBLAST text with pertinent recruitment information to be included at the end of the features. The eBLAST text (attached to the eIRB application) will be included in the body of emails for the listed listservs for the groups mentioned above, in addition to the eBLAST text a link to the study flyer will also be provided in the email.

We will also post the MEG Lab's "My MEG Scan" video (https://www.youtube.com/watch?v=zhXTX7IiS3U) with study information on CHOP's Research Institute YouTube channel, blog and/or CHOP Research Institute related CHOP only social media outlets. Currently, the social media websites/outlets are not maintained by the MEG Lab (i.e. CHOP Research Institute, ASA, IAN or CAR) and will be monitored by the groups controlling/managing these pages. Interested families are encouraged to contact us to begin the process of enrolling in the study.

We will also utilize CHOP's Recruitment Enhancement Core (REC). The Recruitment Enhancement Core (REC) provides assistance with recruitment plan development and may assist in identifying and contacting potential participants using the CRU, the CHOP Recruitment Registry and internal communication resources. REC will contact potential subjects directly, on behalf of the investigators, and that the investigators will not have access to their names or contact information until interested individuals contact them directly. Families will be contacted through the REC by either postal mail or email and the correspondence will contain opt out language for future communications. Recruitment documents for REC will be generated in collaboration with the REC Director, Chris Gantz, and then submitted for IRB approval prior to distribution.

We will utilize Simons Foundation's autism research database SPARK (Simons Foundation Powering Autism Research for Knowledge) to recruit participants (https://sparkforautism.org/). SPARK is a collaborative online initiative that aims to recruit individuals from North America with a professional diagnosis of autism, and their family members, into an online research cohort. Based on our recruitment requests, SPARK will contact each potentially eligible family to obtain permission to send their contact information to our lab. Once granted permission, we will be granted access to contact information on participants through a secure web portal.

8.6 Informed Consent and HIPAA Authorization

The adult consent for the study is attached. Study staff will send home a copy of the informed consent to all participants before they come in for their study visit. Subjects are encouraged to contact the study coordinator with any questions or concerns about the study if they arise. The study coordinator or one of the study investigators will obtain consent when the subject arrives for their screening visit and answer any questions they may have. Informed consent will be obtained prior to any data collection.

8.6.1 Waiver of Documentation of Consent

A waiver of documentation of consent for the screening portion only (consisting of the phone screen) is necessary since study staff will be communicating with interested subjects over the phone in order

to conduct a short phone screen, during which PHI will be collected in order to determine eligibility. This waiver will not adversely affect the rights and welfare of the subjects because written consent will be obtained when the subject comes in for the study visit.

8.6.2 Alteration of HIPAA Authorization

Alteration of HIPAA authorization is necessary to obtain verbal HIPAA authorization for the phone screen. The phone screener, and thus the screening portion for this study, cannot practically be conducted without this alteration of HIPAA since PHI will be collected without written consent over the phone. An appropriate plan is in place to protect identifying information from disclosure as well as a plan to destroy identifiers.

8.7 Informed Consent/Assent and HIPAA Authorization

In-Person Consent

Study staff will go over all study procedures with potential participants both over the phone and when they come in for their study visit. Consent will take place a in a private room at one of the following CHOP locations: 3550 Market Street, Roberts Pediatric Research Center or Main Hospital (depends on location of Screening Visit and Family and/or Clinician Preference of Location). Parents are encouraged to ask the study coordinator questions/voice any concerns at any time. The study staff will make sure all questions are satisfactorily answered before the parent grants consent at the beginning of the study visit. Dr. Bennett is immediately available at the time of the consent discussion, if desired or needed by the family Consent from both parents and assent from the child participant (if applicable) will be obtained before any data is collected.

Phone Consent:

We require both parents to sign and complete a hardcopy of the consent form. It is in our best practice to have at least one parent complete consent in person for child's participation. However, due to unforseen and uncontrollable ciricumstances there can be incidences where both parents are unble to attend the child's first study visit and in these cases where both parents are involved in the consent processvia phone the study physician will lead the consent conversation. A hardcopy of the consent form is sent to us via mail before the child's first study visit. In both cases the following procedures will take place during an informed consent process via phone:

- Study Staff will document reasons why parent(s)/legal guardian(s) are unable to come in person to consent for their child to participate
- Consent forms will be made available to the parent(s)/legal guardian(s) prior to phone consent process
- During the phone consent process study staff will ensure it is communicated that the parent(s)/legal guardian(s) can talk to the PI or study physician at any time to discuss any issues they are concerned about. When both parents are involved in the consent conversation over the phone, a physician will lead the consent conversation.
- Study staff will share PI and study physician contact information with the parent(s)/legal guardian(s)
- In cases where the parent(s)/legal guardian(s) communicate they would like to speak to the PI and/or study physician the study coordinator will assist in scheduling a time for both parties to talk. In all cases the study physician will always be available and connected to the parents via the consent conference.

- The study staff will make a plan to get original copies of signed consent forms (i.e. bring during screening visit, mail before screening visit etc.)
- The study staff will draft a detail note to file in real time outlining the ICF phone procedures that occurred.

8.8 Payment to Subjects/Families

There will be no cost to the families for participating in our study. Subjects will be compensated at a total rate of $20 per hour for their participation Payment of $20 per hour will be given for participation in the study. Payment of $20 per hour will be calculated from the start of the testing visit to the end of the visit appointment. Full payment will be given to the participant's parent in the form of a bankcard (18+). Some of this hourly payment is to reimburse them for costs including parking and transportation. The remaining dollars are for their time and inconvenience for taking part in the study. Subjects' parents will additionally receive a parking pass to validate parking after each visit. Additionally, participants who successfully complete all 4 study visits will be given an additional $100 as a completion bonus, which will be added to the assigned bankcard.

If participation is discontinued voluntarily or by the request of the PI, the subject will receive part of the payment according to the duration of their participation.

Each subject will receive a small gift regardless of whether they complete all study procedures or not, as a token of appreciation.

9 PUBLICATION

If the results of this study are presented at scientific meetings or published in professional journals, the report will not contain any information that could be used to identify participants or family members.

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APPENDIX A

ALLOWED CONCOMITANT PSYCHOACTIVE MEDICATIONS

The following drugs are examples of concomitant medications that are allowed during the study. Drugs prescribed for any condition besides the FDA-approved indication(s) are prohibited. This list is not exhaustive and other drugs that meet all requirements in Section 7.4 may be permitted. All questions on psychoactive medications should be directed to the Medical Monitor.

Drug Name FDA-Approved Indication(s)

atomoxetine Attention–Deficit/Hyperactivity Disorder
clonidine Attention–Deficit/Hyperactivity Disorder, Hypertension
dexmethylphenidate Attention–Deficit/Hyperactivity Disorder
dextroamphetamine Attention–Deficit/Hyperactivity Disorder, Narcolepsy
guanfacine Attention–Deficit/Hyperactivity Disorder
lacosamide Seizures
lamotrigine Epilepsy, Bipolar Disorder
levetiracetam Seizures
methylphenidate Attention–Deficit/Hyperactivity Disorder, Narcolepsy
oxcarbazepine Seizures
pregabalin Seizures, Fibromyalgia, Neuropathic Pain, Neuralgia
topiramate Seizures, Migraine prophylaxis
valproic acid Seizures, Mania associated with Bipolar Disorder, or Migraine prophylaxis
vyvanse Attention–Deficit/Hyperactivity Disorder
zonisamide Seizures