Modulating plasticity in the motor cortex using repetitive transcranial magnetic stimulation to improve motor function in autism spectrum disorder

Protocol Identifying Number: 180/2023

Principal Investigator: Dr. Pushpal Desarkar

Version Number: 1.0

Version Date: 04-April -2024

Principal Investigator:	Dr. Pushpal Desarkar 1025 Queen Street West, Toronto, ON M6J 1H1 416-535-8501 ext. 32726
Co-Investigators:	Stephanie Ameis, MD, MSc, FRCPC Daniel M. Blumberger, MD, MSc, FRCPC Meng-Chuan Lai, MD, PhD Hsiang-Yuan Lin, MD Yona Lunsky, PhD Stewart Mostofsky, MD Tarek Rajji, MD, FRCPC Marcos Sanches, MSc Christoph Zrenner, MD
Funder:	Canadian Institutes of Health Research (CIHR)
Research Analyst :	Ashna Imran

camh
mental health is health

Table of Contents

STATEMENT OF COMPLIANCE

A statement confirming the clinical trial will be conducted in compliance with the protocol, ICH GCP, applicable regulatory bodies and institutional requirements must be included here.

For multi-site clinical trials: A statement of compliance should also be included for each site, with the site Principal I*nvestigator's (PI)* signature.

This clinical trial will be carried out in accordance with the following:

- International Conference on Harmonisation Good Clinical Practice (ICH GCP)
- Tri-Council Policy Statement 2018 (TCPS 2)
- ISO 14155:2020 for Medical Device Clinical Trials
- Personal Health Information Protection Act (PHIPA), 2004; Chapter 3 Schedule A (PHIPA) and applicable regulations
- Food and Drugs Act
  - Part C, Division 5 of the Food and Drug Regulations
  - Part 3, Medical Device Regulations
  - Part 4, Natural Health Products Regulations
- U.S. Federal Policy for the Protection of Human Subjects (Common Rule)
- U.S. FDA Regulations
- Institutional and REB policies and procedures

_____ _____31st October, 2023

_____

Signature of PI Date

or

Signature of site PI (multi-centre clinical trials)

LIST OF ABBREVIATIONS

AE	Adverse Event
ASD	Autism Spectrum Disorder
CRF	Case report form(s)
DSMB	Data Safety & Monitoring Board
GCP	Good Clinical Practice
iTBS	Intermittent Theta Burst Stimulation
ICF	Informed consent form
LTP	Long Term Potentiation
MEP	Motor Evoked Potentials
NT	Neurotypical
PHI	Personal Health Information
PHIPA	Personal Health Information Protection Act
PI	Principal Investigator
QI	Qualified Investigator
RMT	Resting Motor Threshold
SAE	Serious Adverse Event
SUSAR	Suspected unexpected serious adverse reaction
TBS	Theta Burst Stimulation
TCPS 2	Tri-Council Policy Statement
rTMS	Repetitive Transcranial Magnetic Stimulation

1.0 INTRODUCTION

1.1 Background

Autism Spectrum Disorder (ASD) is one of the most common neurodevelopmental disorders, affecting ~ 1 in 66 children and youth in Canada[5]. ASD is a persistent disabling condition accounting for 111 disability-adjusted life-years per 100,000 population[6]. Motor function difficulties involving fine and gross motor skills such as coordination, strength, balance and mobility in ASD are very common[7-13] and they emerge early[14-17] and persist into adulthood[13,18-21]. These motor function difficulties significantly contribute to core challenges in social and communication skills[19,22-25] and negatively impact quality of life[26-27] and daily living skills[28] throughout the lifespan[28]. Interventions improving daily functioning in autistic adults have been identified as one of the top research priorities[29] in the autism community. Thus, the motor system represents a key therapeutic target for biological interventions. However, despite the centrality of motor functions in daily functioning, there is paucity of research in autistic adults. At this point, there is no quality evidence supporting the clinical use of any motor skill intervention in ASD[30]. Although alterations of functional connectivity and white matter pathways involving motor networks, including primary motor cortex (M1), have been found to underlie motor function difficulties in ASD[31-42], the findings are inconsistent and the brain mechanisms informing biological interventions remain elusive. Converging evidence indicates that the neurobiology of ASD is characterized by atypical plasticity. In particular, an excessive plasticity (i.e. hyperplasticity) in the form of excessive long-term potentiation (LTP), operationally indexed by a significantly longer lasting facilitation of motor evoked potentials (MEPs), was observed in M1 of human participants with ASD, when compared to neurotypical (NT) controls, based on the theta-burst stimulation (TBS)[43-46]. We recently replicated the finding of M1 hyperplasticity in autistic adults[46]. Our finding of hyperplasticity (i.e. excessive LTP) in autistic adults is a direct human translation of the consistent finding of excessive LTP found in valproic acid animal models of ASD[47-48]. One key insight from animal models of ASD is that hyperplasticity adversely affects behavior [47,49]. Besides replicating the finding of M1 hyperplasticity, as a foundation for intervention, we also collected pilot data using a repetitive transcranial magnetic stimulation (rTMS) protocol designed to strengthen inhibitory mechanisms, which reduced hyperplasticity in autistic adults[46]. Here we propose a new line of translational inquiry: i) Testing whether M1 hyperplasticity underlies motor function difficulties in ASD, and ii) Using 'mechanism-driven' rTMS with autistic adults to examine whether resulting reduced M1 hyperplasticity is associated with clinical improvements in motor function. If successful, our project will identify a brain mechanism, i.e. hyperplasticity, underlying motor function difficulties in ASD and will also identify a 'mechanism-driven' neurostimulation treatment to reduce hyperplasticity and improve motor function difficulties in ASD.

1.1.1 The rationale for targeting motor function difficulties in autistic adults

Although motor function difficulties are seen in a range of neurodevelopmental disorders such as attention-deficit/hyperactivity disorder, such deficits in ASD are significantly more common[50], more severe[50], and are linked withthe core behavioral characteristics of ASD[19,22-25,51-52]. Two meta-analyses confirm consistent and robust motor function difficulties in individuals with ASD, involving a large effect size[12-13]. Such motor function difficulties involve a broad range of skills necessary to fine and gross motor coordination, arm movement, walking speed, and balance. These difficulties appear early[14-17] and even precede social-communication deficits in ASD[53]. They tend to be present across the lifetime[13], e.g., they persist into adolescence[54-58] and young adulthood[18-20], are present in older adults[21] and may even get worse with aging[59-60]. Further, motor function difficulties in ASD negatively affect quality of life[26-27], autonomy, independence, community participation[61] and daily adaptive living skills in autistic adults[28]. A recentsystematic review identified that interventions improving daily functioning in autistic adults have been identified as one of the top research priorities[29] in the autism community. Thus, given the centrality of motor function in daily functioning, motor systems represent an important therapeutic target for biological interventions to improve outcomes in autistic adults. To highlight the paucity of research in this area, at present there is no ongoing trial to improve motor function in autistic adults registered on Clinicatrials.gov. Stakeholder input : We have engaged with a panel of advisors comprising of autistic adults at the Centre for Addiction and Mental Health (CAMH) during grant development. The feedback has consistently been that this is one of the high priority areas of significant unmet need for autistic adults that has been much neglected. The group welcomed the effort to identify brain mechanism underlying motor function difficulties and felt improving motor skills could 'significantly boost their confidence, improve their appearance, increase community participation, and overall, improve their dailyliving skills'. The other feedback was to use 'motor function difficulties' instead of 'motor deficits' and add an exploratory objective to test any potential association between changes in the adaptive daily living skills and changes in the motor function following rTMS. The feedback on the rTMS course for this project (5-session) and duration of visits (described later) was positive.

1.1.2 What is known about the neurobiology of motor function difficulties in ASD?

Even though neurobiology of ASD is characterized by atypical plasticity, to our knowledge, no study yet looked into the relationship between atypical plasticity in M1 and motor function difficulties in ASD.Existing studies on the neurobiology of motor function in autism used neuroimaging research and the findings are inconsistent, i.e. the findings include both reduced[31-35] and increased[36-37] functional connectivity of motor networks. Further, reduced asymmetry of functional connectivity[38], altered organizationof the motor network[39] and increased white matter volume in M1[40], and

atypical functional connectivityof cerebellar network involving motor function were also reported[41-42]. Overall, these findings are inconsistent and the brain mechanisms informing new biological interventions remains elusive.

1.1.3 Our published data showing hyperplasticity (excessive LTP) in M1 in autistic adults

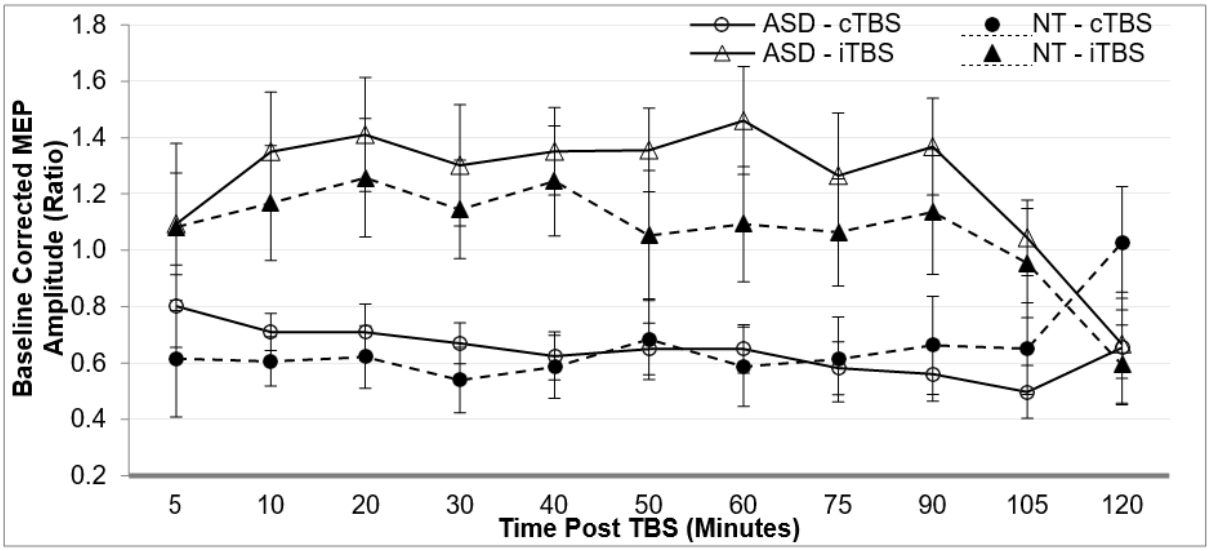

Figure 1: This figure depicts baseline-corrected MEP amplitude following iTBS and cTBS in the ASD and NT groups at 11time points up to 120 min post-TBS. LTP following iTBS (ASD: 106.93±30.37minutes; NT: 86.33±38.23minutes; p=0.023;partial η2=0.092, i.e. medium effect size) was clearly excessive in the ASD group, indicating hyperplasticity[46]. We also found excessive long-term depression (LTD) following continuous TBS or cTBS. Error bars indicate standard error of means.

Using a randomized, cross-over design, we (PI: Desarkar) assessed plasticity using TBS in the left M1 in 31 right-handed autistic adults and 30 handedness, sex, intelligence quotient (IQ), and age-matched controls[46]. During TBS, the TMS stimulation pattern involves the delivery of a biphasic burst of 3 pulsesat 50Hz at intervals of 200ms (i.e. 5 Hz) (total 600 pulses)[62]. In the iTBS paradigm, a 2-second train of TBS is repeated intermittently (hence intermittent TBS or iTBS), i.e. every 10-second for a total of 190 seconds[62]. We calculated LTP by measuring the duration of enhancement (after iTBS) of TMS-evoked MEPs (Figure 1) that reflect cortico-spinal excitability, which is indexed by the size ofthe peak-to-peak amplitude of MEPs evoked by single pulse TMS delivered at a rate of 0.1Hz and at the intensity of 120% of each participant's resting motor threshold (RMT).

1.1.4 Measurement of LTP

The duration of facilitation of MEP amplitude, is indexed by the time for the MEP amplitude to return to baseline values following iTBS. The selection of the time point at which MEP values were judged to have returned to baseline following iTBS was

based on published criteria[44,46]: a) the time point when the mean MEP value reaches 'within the 95% confidence interval of the baseline amplitude', and b) does not go 'outside that interval on subsequent time point measures'. MEP values for each participant was standardized by baseline correction. Standardized values represent a ratio of post-/average baseline MEPamplitude. Thus, for iTBS, values >1 represent facilitation. For the ASD and NT control groups, we usedone sample t test (against 1) to test if facilitation was significant[46]. After controlling for sex, age, IQ, andTBS order, we found that LTP was significantly increased in the ASD group, indicating hyperplasticity(Figure 1).

1.2 Study Intervention

1.2.1 The rationale for using rTMS to modulate hyperplasticity in M1 and improve motor function diffic ulties in ASD

1.2.1.1 Our pilot data show promising evidence that rTMS can reduce an excessive LTP in M1 in autistic adults [46]

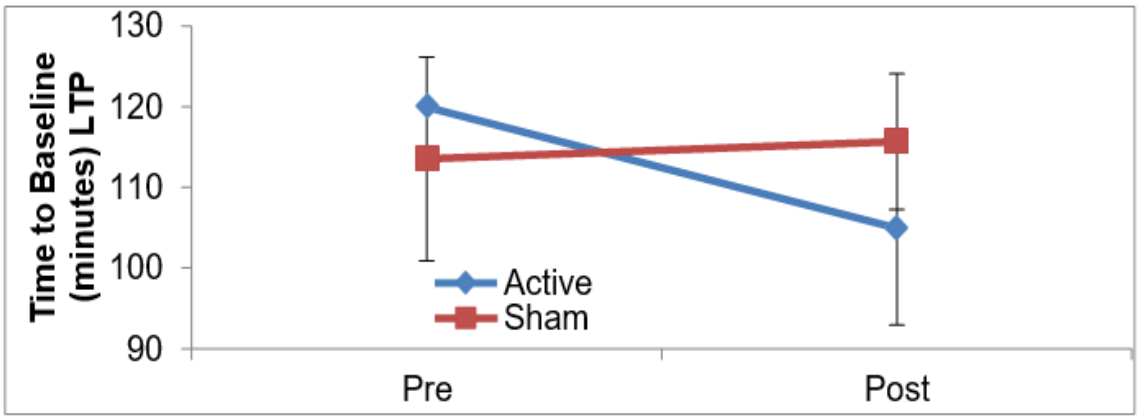

Figure 2: Our pilot data show the attenuation of LTP (assessed by iTBS) in the ASD group following active vs. sham rTMS.

We (PI: Desarkar) recently published pilot data[46] showing preliminary evidence that rTMS, which was previously shown to maximally potentiate brain inhibitory mechanisms in M1, reduced excessive LTP in M1 in autistic adults. In our study, 29 autistic adults were randomized (1:1) to receive a single session of active (n=14) or sham (n=15) (6,000 pulses at 20Hz) over left M1 and plasticity was reassessed on the next day following rTMS. The mean reduction of LTP ('meanpre – meanpost rTMS') assessed using iTBS in the active rTMS group was 15.00 minutes and -2.14 minutes in the sham group (Figure 2), indicating a large effect size (partial η^2=0.167) of active rTMS on LTP. The inhibitory effectof such rTMS was previously documented by our group[81-82]. Compared to 1Hz or 10Hz rTMS, 20Hz rTMS with an extended delivery of pulses had more pronounced 'inhibitory' effect[81], and such 'inhibitory' effect was maximal when 20 Hz rTMS was delivered for 6,000 total pulses[82]. In the altered excitation/inhibition model of ASD[77],

hyperplasticity in M1 is likely linked with the increased excitation/inhibition ratio and the reduction of hyperplasticity in the ASD group in our published work (PI Desarkar) by the rTMS could be due to facilitation of inhibition[46]. We had previously published the rationale behindsuch approach[83].

1.2.1.2 Why study primary motor cortex (M1)?

The human motor cortex is comprised of three areas: the primary motor cortex (M1), pre-motor area and supplementary motor area. Human motor control network involves communication between motor cortex and other areas including basal ganglia system, frontal lobe, cerebellum, sensory cortex, thalamus, and medulla. Within motor cortex, M1 receives inputs from pre-motor areas, supplementary motor areas and plays a critical role in encodingforce, direction, extent and speed of a movement[84-86]. The well-defined links between M1 and motor control provides strong biological validity for our approach, as does published data revealing M1 hyperplasticity in autistic adults and preliminary data suggesting that rTMS may rectify hyperplasticity in M1. Further, stimulation of M1 is an established method to improve motor function in neurological conditions such as Parkinson's disease[87] and stroke[88].

1.2.1.3 Why not other therapies?

Common motor skills intervention in ASD involved teaching and strengthening locomotor and various object control skills such as balance, throwing, running, etc. and training fine motor skills. Other studies used robot-assisted training and training using video games[30]. A recent review[30] of motor skills interventions for children and adolescents with ASD found preliminary suggestion of possible beneficial effects of these interventions; however, only two studies were randomized controlled trials (RCTs)[89-90]. While one did not report significant improvement after motor skills intervention[90], the other RCT did not compare thetwo active intervention programs[89]. Further, the training sessions in these intervention studies required more time commitment, i.e., up to 5 days a week for 6-12 weeks, than what is proposed in this project (i.e., 2 hours/day for 5 days)[30]. At this point, there is no quality evidence supporting the clinical use of any motor skill intervention in ASD.

1.2.1.4 How would hyperplasticity in the M1 affect motor function?

Hyperplasticity can be a compensatory mechanism[91] or part of physiological development[92]. The relationship between plasticity and behavior/cognition is 'inverted U'[49]. While deficient plasticity prevents brain to adequately adjust itself to changing conditions, one key insight from animal experiments is that hyperplasticity may compromise behavior[47,49]. A model of plasticity pathology continuum posits that, at the circuit level, persistent and excessive LTP could lead to excitotoxicity, which leads to neuronal loss and reduced synaptic density, compromising behavior[93]. In the context of M1 hyperplasticity, a meta-analysis of post-mortem studies did reveal reduced dendritic spines in M1 in ASD[94].

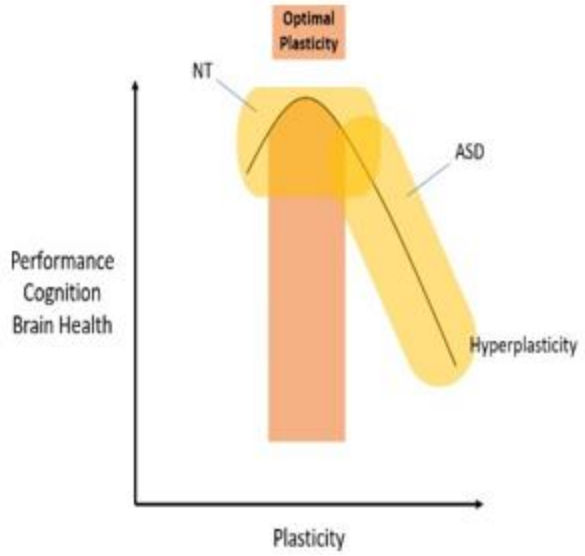

Figure 3: Illustrating the incomplete inverted U relationship in relation to hypothesis 1b.

1.3 Preclinical Data to Date

The role of atypical plasticity in the neurobiology of ASD is supported by a growing number of genes associated with ASD that are involved in synaptic plasticity[63-69]. Among animal models of ASD, while deficient plasticity was found in some[70-72], e.g. FMR1, SHANK3, etc, hyperplasticity was observed in valproic acid models[47-48]. By contrast, a more direct evidence of hyperplasticity was consistently observed in human M1 using TMS[43-46] with one exception[73]. Our finding replicating M1 hyperplasticity in autistic adults using TBS is consistent with what was observed in 3 studies (Cohen's d 1.21-2.47)[43-45]. The only TMS study[73] that found reduced LTP in M1 using paired associative stimulation, another way to assess plasticity, had a small sample (n=9) with a mixed population of children and adults, and the effects of sex and intellectual ability were not controlled for. The generation of LTP in the classical post-synaptic model is mediated via N-methyl-D-aspartate (NMDA) receptors[74]. Consistent with this model, it was shown that the LTP-like after-effects of patterned high-frequency stimulation such as TBS was NMDA-dependent[75]. At the synaptic level, post-synaptic NMDA[74] and both gamma aminobutyric acid (GABA) A and B receptors play a critical role in the LTP generation[76]. One explanation of observed hyperplasticity is the excitation/inhibition imbalance created by over-expression of NMDA[77-78] and/or reduced expression of $GABA_A$ or $GABA_B$ receptors observed in the ASD brain[78-79]. A systematic review of TMS studies found evidence of increased excitation/inhibition ratio in M1 in ASD[80].

1.4 Risks/Benefits

1.4.1 Risk

1.4.1.1 Assessments

Assessments may impose some risk due to possible emotional discomfort and possible fatigue.

1.4.1.2 TMS and TMS-EEG

The most commonly reported side effect of TMS is headache (~5%). Participants may also experience some discomfort under the coil due to contraction of muscles and stimulation of nerves on the scalp. These reactions are generally minor and lack serious sequelae. If the participant is discomforted by the headache, it is usually easily managed with standard analgesics. Earplugs may be used during each TMS session to prevent discomfort from the clicking noise generated by the stimulation. No hearing loss has been found in humans exposed to single or paired pulse TMS.

The occurrence of fainting from TMS has been reported but may not be very common. To minimize the risk of this, brain stimulation will be discontinued if participants feel significant dizziness or nausea through the study visits.

The TMS-electroencephalography (TMS-EEG) neurophysiology sessions will be conducted during 2nd (all participants), 8th and 9th (ASD participants only) visits. This TMS protocol does not deliver repetitive pulses that can cause sustained therapeutic neuromodulation. The pulses delivered are thought only to be sufficient to assess brain functioning and it is not physiologically plausible that the TMS protocols intended to assess GABAergic neurotransmission would have a sustained effect on mood or cognition. In numerous studies, single or paired-pulse TMS has been found to pose no significant health risk to properly screened normal volunteers.

Single and paired-pulse TMS is now in routine clinical diagnostic use in hundreds of neurophysiological laboratories worldwide. The FDA has concluded that stimulation at <1 Hz carries only a remote likelihood of seizure and is therefore classified as a non-significant risk device. All subjects will be screened for risk of seizure using the TMS adult safety screen[84].

Safety data: The rTMS protocol (6000 pulses, 20Hz, delivered at 90% of the resting motor threshold is within the safety parameters for rTMS[138]. In our pilot study[137], 29 autistic adults and 30 control participants received rTMS and no participant reported any adverse effects.

1.4.2 Benefit

There may not be any direct benefit to study participants. The study finding may advance our understanding of the brain mechanisms underlying motor function difficulties in ASD.

1) Brain-behavior relationship in ASD: if successful, our project will identify a brain mechanism, i.e. hyperplasticity, underlying motor function difficulties in ASD;

2) Novel neurostimulation intervention for ASD: if successful, our project will also identify a 'mechanism-driven' neurostimulation treatment to reduce hyperplasticity in the brain and improve motor function difficulties and thus, outcomes in ASD. The estimated lifetime cost of supporting an individual with ASD in Canada is between $1.2 million to $4.7 million[95]. Thus, increasing daily functioning and independence will have significant cost-benefit.

3) Informing future trials: This information will provide a foundation to test similar neurostimulation approach in the less able ASD population subgroups in the future.

2.0 CLINICAL TRIAL OBJECTIVES

2.1 Primary Objective

Objective 1: To examine the strength and nature of association between plasticity in M1 and motor function in autistic adults and neurotypical (NT) controls. Hypothesis 1a: Compared to NT controls, autistic adults will display greater plasticity in M1. Hypothesis 1b: There will be a non-linear incomplete 'inverted U' shaped association (plasticity on the x axis, motor performance on the y axis) between plasticity in M1 and motor function, however, the association will be different for the two groups, i.e. autistic adults will mainly be in the right slope of the inverted U reflecting hyperplasticity associated with impaired motor function, while NT controls will cluster around the center (Figure 3).

2.2 Secondary Objective

Objective 2: To examine the efficacy of bilateral rTMS delivered to M1 in reducing hyperplasticity in M1 and improving motor function in autistic adults via a randomized, double-blind, sham-controlled experiment. Hypothesis 2a: Autistic adults receiving active rTMS will have lower plasticity in M1 immediately, and 1 and 4 weeks after the course compared to autistic adults receiving sham rTMS. Hypothesis 2b: Autistic adults receiving active rTMS will have better motor function immediately, and 1 and 4 weeks after the course compared to autistic adults receiving sham rTMS.

2.3 Tertiary Objective

Objective 3: To examine if changes in the M1 plasticity correlate with changes in the motor function in autistic adults following active bilateral rTMS. Hypothesis 3: Changes in the M1 plasticity will correlate with changes in the motor function in autistic adults following active bilateral rTMS.

2.4 Exploratory Objective

Exploratory objective 1: To examine if changes in the motor function correlate with changes in theadaptive daily living skills in autistic adults following active bilateral rTMS.
Exploratory hypothesis 1: Changes in the motor function will positively correlate with changes in the adaptive daily living skills in autistic adults following active bilateral rTMS.

3.0 CLINICAL TRIAL DESIGN

3.1 Overall Design

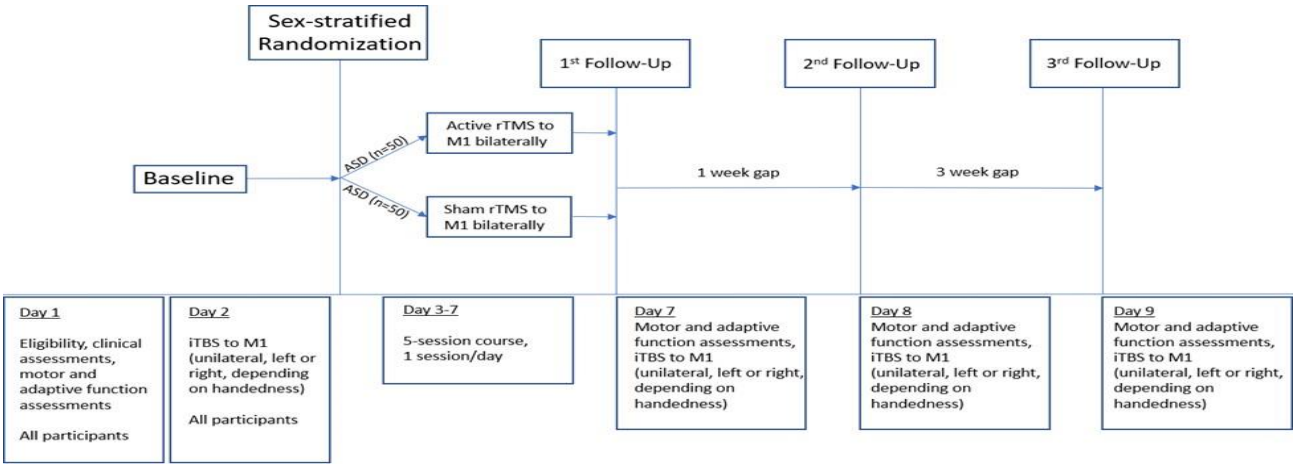

Figure 4: The outline of the project

We will recruit 100 autistic adults and significant motor function difficulties (based on standardized motor assessment – see below in section 3.2.1.) and 50 NT controls matched 2:1 based on age, sex and IQ. Day 1: all participants will complete clinical, adaptive and motor function assessments (~3hours). Day 2: Plasticityin the left or right M1 (depending on handedness, see below) will be assessed with iTBS in all participants (~2.5hours). Day 3-7: Following Day 2 procedures, 100 ASD participants will be randomized (sex-stratified, 1:1, double-blind) to receive active (n=50) or sham (n=50) rTMS delivered to M1 bilaterally, 1session/day for 5 days (total 5 sessions) (~1.5hours/day). On the last day of rTMS (i.e., Day 7) ASD participants will repeat motor and adaptive function assessments and iTBS will be used to assess plasticity. (~4.5-5hours). Assessment of motor and adaptive function and plasticity using iTBS will be repeated 1-week (Day 8) (3-3.5hours) and 4-week (Day 9) (3-3.5hours) after the final day of rTMS (i.e., Day 7). In order to avoid any potential influence of iTBS on motor function assessments, we will always carry out motor assessments before iTBS.

3.1.1 Project Timeline

We anticipate that a timeline of 5 years is necessary to complete all aspects of thisproject.

An initial 3 months start-up period is anticipated to hire and train the research staff. After this, we plan to recruit about 1-2 new ASD participants and 1 NT controls/month. This timeline will allow usto complete recruitment of all participants by month-53. Participants recruited in month-53 will completethe project in month-54, leaving us about 6 months to complete our analyses and report our results.

3.1.2 Clinical Assessments

All clinical and baseline motor assessments will be done on Day 1 (~3hours)(Figure 4). The Wechsler Abbreviated Scale of Intelligence, Second Edition[105] will be used to ensure participants have IQ>70. Edinburgh Handedness Inventory[106] will be used to assess handedness. The daily living skills domain of Adaptive Behavior Assessment System-3rd edition[107] (~15-20 minutes) will be used to assess adaptive daily living skills. All ASD participants will have a DSM-5[96] diagnosis, confirmed by ADOS-2[97]. This visit includes a screening for any contraindication to TMS[108].

3.1.3 Assessment of motor function in ASD and NT Controls

Bruininks-Oseretsky Test of Motor Proficiency, Second Edition (BOT-2)[98] will be used to assess motor function of ASD and NT control participants. The BOT-2 is a standardized comprehensive test battery assessing fine manual control, manual and body coordination, strength and agility domains and takes about 45-60 minutes to complete. The total motor composite score will be used as the primary measure of motor function. Rationale for usingBOT-2: BOT-2 is validated in the ASD population[98,109-110], has most-validated age-norms for adults[98, 110-111], can be repeated to monitor progress[98,109], and includes domains found to be impaired in ASD.

3.2 Primary Endpoints

3.2.1 Assessment of Plasticity in M 1 using Intermittent Theta -burst Stimulation iTBS

3.2.1.1 Primary measure of plasticity

Plasticity using iTBS will be assessed at the right M1 in left-handed, and left M1 in right- and mixed- handed participants. Rationale: Given the high variability of handedness in ASD[99], in order to be inclu sive, we will include participants with left, right or mixed handedness. The motor “hotspot” will be determined as the coil location over M1 that will consistently produce MEPs at the contralateral relaxed hand muscles at the lowest stimulator intensity. The coil will be positioned flat on the scalp over the motor hotspot of M1 (right or left) such that the main component of the induced electric field points ina postero-lateral to anteromedial direction, making a 45° angle with the midline[112]. iTBS will be administered using a MagPro stimulator (MagVenture Inc). The Resting Motor Threshold (RMT) is defined as the minimal TMS intensity that produces an MEP of >50

μV peak-to-peak amplitude in 5 of 10 trials in relaxed right first dorsal interosseous muscle[113-114]. iTBS pulses will be delivered at 80% of the RMT. The baseline measure of corticospinal excitability will be defined as the mean MEP amplitude across 150 single pulses. In order to track changes in corticospinal excitability following iTBS over time, we will follow current recommendation, i.e., blocks of 20 single pulses[114] will be delivered at 5, 10, 20, 30, 40, 50, 60, 75, 90, 105, and 120 min following iTBS. Mean amplitude of 20 MEPs will represent the measure of corticospinal excitability at each time point. The LTP will be calculated using published method[46] described earlier.

The duration of facilitation of MEP amplitude, is indexed by the time for the MEP amplitude to return to baseline values following iTBS. The selection of the time point at which MEP values were judged to have returned to baseline following iTBS was based on published criteria[44,46]: a) the time point when the mean MEP value reaches 'within the 95% confidence interval of the baseline amplitude', and b) does not go 'outside that interval on subsequent time point measures'. MEP values for each participant was standardized by baseline correction. Standardized values represent a ratio of post-/average baseline MEP amplitude. Thus, for iTBS, values >1 represent facilitation. For the ASD and NT control groups, we will use one sample t test (against 1) to test if facilitation was significant[46].

3.2.1.2 Secondary measure of plasticity using E EG

As a secondary assessment of plasticity in M1 using iTBS, we will also be recording EEG before, during iTBS, and also at all 11 time points i.e. at 5, 10, 20, 30, 40, 50, 60, 75, 90, 105, and 120 min following iTBS when single pulse TMS will be delivered (see above).

In this TMS-EEG method (i.e. use of EEG to index iTBS-induced plasticity), iTBS- induced LTP is defined by potentiation of cortical evoked activity (CEA), which is measured by EEG. Previously we reported the validity of CEA by measuring strong correlation between MEP and CEA measures[123]. CEA will be defined as the area under rectified curve for averaged EEG recordings in electrode over M1 between 50-275 millisecond post-stimulus. The 50 millisecond cutoff is chosen as it represents the earliest artefact free data that can be recorded post-stimulus. The 275 millisecond cutoff is chosen as TMS-induced CEA subsides almost to 0 by 275 millisecond post-TMS[123]. iTBS-induced LTP is indexed by maximum post-iTBS/pre-iTBS CEA ratio of the 11 time points i.e. at 5, 10, 20, 30, 40, 50, 60, 75, 90, 105, and 120 min following iTBS.
Prior to iTBS procedure, we will record the resting state EEG for 10 minutes (five minutes with eyes closed, and five minutes with eyes open).

EEG will be acquired through a 64-channel Synamps 2 EEG system. A 64 channel EEG cap will be used to record the cortical signals, and 4 electrodes will be placed around the eyes to correct for eye movement artefacts. EEG signals will be recorded using direct current mode at 20 kiloHz sampling rate, which was shown to avoid saturation of amplifiers and minimize TMS artefact[123]. All EEG processing and analysis will be done offline using EEGLAB toolbox of Matlab.

3.2.2 Primary endpoint of rTMS intervention

In this study, our second objective is to examine the efficacy of bilateral rTMS delivered to M1 in reducing hyperplasticity in M1 and improving motor function in autistic adults via a randomized, double-blind, sham-controlled experiment. We anticipate that autistic adults receiving active rTMS will have lower plasticity in M1 immediately, and 1 and 4 weeks after the course compared to autistic adults receiving sham rTMS. Further, we anticipate that autistic adults receiving active rTMS will have better motor function immediately, and 1 and 4 weeks after the course compared to autistic adults receiving sham rTMS.

The assessment of plasticity immediately, 1 and 4 week after rTMS will be assessed using the same methods to assess plasticity described above (using changes in MEPs, i.e. primary, and CEA ratio, i.e. secondary).

Bruininks-Oseretsky Test of Motor Proficiency, Second Edition (BOT-2)[98] will be used to assess motor function as described above. The total motor composite score will be used as the primary measure of motor function. Rationale for usingBOT-2: BOT-2 is validated in the ASD population[98,109-110], has most-validated age-norms for adults[98, 110-111], can be repeated to monitor progress[98,109], and includes domains found to be impaired in ASD.

3.3 Secondary Endpoints

There are no secondary endpoints for this study.

4.0 PARTICIPANT SELECTION AND WITHDRAWAL

4.1 Target Population

ASD participants and healthy NT controls will be recruited for this study.

4.2 Participant Recruitment and Screening

ASD participants will be recruited from i) CAMH Adult Neurodevelopmental Servicesthat sees ~ 320 autistic adults without ID (~250 new, about 35%, i.e. ~ 112 females assigned at birth)every year, ii) the youth ASD clinic in the CAMH that sees ~ 300 new youth with ASD/year; iii) community partners serving autistic adults such as Autism Ontario, Kerry's Place Autism, Redpath Centre,etc. Targeting a male: female (sex assigned at birth) ratio of 2:1, we aim for recruiting at least 34 femaleASD participants over 5 years and believe this is highly feasible. Considering the increased rate of gender diversity among autistic adults[4], we will collect gender identity information for all participants as a part of sociodemographic characterization. NT control participants will be recruited from CAMH healthy control registry, through advertising at universities, newspapers and online classified advertisements, and from the current and past projects of co-applicants Drs. Ameis, Lai and Lunsky. Initial contact will be made via email and/or telephone. Contact information will be provided on all advertising materials.

CLEARR will be used to recruit participants for this clinical trial. All new referrals will be reviewed by the CLEARR coordinator and CLEARR physician for eligibility to participate using minimal inclusion/exclusion criteria outlined. Once a patient is identified as potentially suitable for the clinical trial, the attending physician will be notified via outlook calendar invite or email that their patient may be eligible for the clinical trial. The attending physician will decide whether research is appropriate for the patient and if so, they will ask the patient for consent to be contacted regarding the clinical trial. If the patient provides verbal consent to be contacted to receive more information about the clinical trial, the physician will connect the patient with the CLEARR coordinator or research team who will further explain the clinical trial. No personal health information (PHI) will be given to the research team prior to obtaining the patient's consent.

The CAMH Research Registry will be used to recruit participants for this clinical trial. Upon REB approval to use the Research Registry as a recruitment strategy, authorized research personnel will search and contact potential research participants included within the member database of the Research Registry for study participation. This clinical trial will also be posted on the Research Registry website, as well as the public CAMH website. Once posted, interested participants can use the "Find a CAMH study" feature to explore clinical trials that they are interested in.

4.3 Equity, Diversity and Inclusion Considerations

Emerging evidence indicates sex differences in brain plasticity in ASD[1]. We will address the potential effect of sex by a) matching patient (i.e. ASD) and control group on sex, and b) recruiting a higher number of female ASD participants. A male to female (assigned at birth) ratio of 2.5:1 has been described in recent clinical samples[2], compared to an earlier 3:1[3]. In this study, we will recruit a higher number of female ASD participants targeting a male to female ratio of 2:1. Further, in order to balance sex across active and sham rTMS groups, we will conduct a sex-stratified randomization. We will include sex as fixed independent factors in our analyses plans so that we could study the sex moderation effects. We will describe sex-stratification of findings using means/estimates and 95% confidence intervals, recognizing this is exploratory given that the design is likely underpowered for this.

At this point, there is no known effect of gender identity on plasticity or motor function in ASD. Therefore, we will not control for gender identity in the analysis plan. However, considering the increased rate of gender diversity among autistic adults[4], we will collect gender identity information for all participants as a part of sociodemographic characterization and will conduct gender identity stratified subgroup analysis for descriptive purpose.

4.4 Eligibility Criteria

4.4.1 Inclusion Criteria

ASD or control participants must meet all of the inclusion criteria to eligible for this study:

1. Aged between 18 and 40 years old. 40 years is chosen as the cut-off because of the report of high rates of Parkinsonism in autistic adults>39years[60];
2. Have IQ>70;
3. Are able to read, write and communicate effectively in English;
4. Are able to provide informed consent. We will recruit only intellectually-able autistic adults. The intellectual ability will be determined using WAIS. The ability to provide consent will be determined using clinical assessment.
5. Have no prior history of seizure;
6. Must sign and date the informed consent form;
7. Stated willingness to comply with all study procedures;
8. Agreement to adhere to Lifestyle Considerations (section 4.5) throughout study duration.

All ASD participants:

1. Will have DSM-5[96] diagnosis of ASD without intellectual disability, confirmed by clinical assessment and the Autism Diagnostic Observation Schedule – 2 (ADOS-2)[97];
2. Will have significant motor function difficulties defined as a standard composite score <40 (i.e., >1 standard deviation below the mean) on either fine or gross motor composite scores of the Bruininks-Oseretsky Test of Motor Proficiency, Second Edition or BOT-2[98];
3. Are clinically stable as determined by clinical assessment, with no medication changes over the past 4 weeks. Given the high variability of handedness in ASD[99], we will include participants with left, right or mixed handedness.

4.4.2 Exclusion Criteria

ASD or control participants will be excluded if they experience/have:

1. current pregnancy;
2. current or past history of co-morbid medical condition that may require urgent medical intervention;
3. DSM-5 substance use disorder (other than tobacco) within the past 6 months; however, all participants will be asked to refrain from smoking or taking caffeine four hours prior to theiTBS session;
4. significant hearing or visual impairment interfering with the ability to read or hear instructions;
5. significantly debilitating medical or neurologic illness (e.g., encephalitis, aneurysms, tumors, central nervous system infections), or acute or unstable medical illnesses as determined by project physician (e.g., uncontrolled diabetes);
6. metal implants or a pace-maker;
7. prior rTMS treatment;
8. claustrophobia;

In addition ASD participants will be excluded if they report taking benzodiazepines or anticonvulsants currently.

NT controls will be excluded if they have:

1. presence of psychopathology other than specific phobia, as screened by Personality Assessment Inventory[100] and;
2. a known diagnosis of Pervasive Developmental Disorder or ASD among any biologicallyrelated family members

4.5 Lifestyle Considerations

During this study, participants are asked to:

- Refrain from consumption of alcohol, tobacco, marijuana or caffeine on the day of study visits.

4.6 Screen F ailures

Screen failures are defined as participants who consent to participate in the clinical trial but do not meet one or more eligibility criteria required for participation. We will record information including demography, screen failure details, eligibility criteria, and any serious adverse events (SAE). If there is a screen failure due to medication changes, we will invite back the participant after 4 weeks for their medication to stabilize.

4.7 Participant Withdrawal Criteria

4.7.1 When and How to Withdraw Participants

Participants are free to withdraw from participation in the clinical trial at any time.
An investigator may discontinue or withdraw a participant from the clinical trial for the following reasons:

1. Unable to tolerate the procedure
2. develop significant adverse events (e.g., seizure);
3. Participant missing or is unable to receive 2 consecutive scheduled rTMS treatment.
4. Withdraw consent

The reason for participant discontinuation or withdrawal from the study will be recorded within the participant's research record.

4.7.2 Follow -up for Withdrawn Participants

If a participant withdraws consent, they can also request the withdrawal of their subject to any research-specific restrictions. Once withdrawn from the clinical trial, no further research procedures or evaluations will be performed, or additional research-specific data collected on the participant. Reasonable effort will be made to obtain permission to document the reason for withdrawal.

4.7.3 Early Termination Visit

If a participant withdraws from the clinical trial, every effort should be made to perform an Early Termination Visit. In the termination visit, we will assess AEs.

4.7.4 Participants who are Lost to Follow -up

A participant will be considered lost to follow-up if they fail to return for the 2 scheduled visits after the completion of the 5-day rTMS course and is unable to be contacted by the research team.

The following actions will be taken if a participant fails to attend a required study visit:

- The research team will attempt to contact the participant and reschedule the missed visit within a week, counsel the participant on the importance of maintaining the assigned visit schedule, and reconfirm whether the participant wishes to and/or should continue in the clinical trial.
- Before a participant is deemed lost to follow-up, the research team will make every effort to regain contact with the participant (where possible, three telephone calls and/or, sending e-mails). These contact attempts should be documented in the participant's research record. .
- Should the participant continue to be unreachable, they will be considered to have withdrawn from the clinical trial with a primary reason of lost to follow-up.

5.0 STUDY INTERVENTION

5.1 Description

5.1.1 Repetitive Transcranial Magnetic Stimulation (rTMS) t rial

5.1.1.1 Trial Design

This project has an RCT (not as major component). We will use a randomized, double-blind, sham-controlled design comparing the efficacy of active vs. sham rTMS (6000 pulses at 20 Hz/session) delivered to M1 using bilaterally, 1 session/day for 5 days (5 sessions). The pulses will be delivered first to one side (right or left), which will be then followed by the pulses being delivered to the opposite side. The sequence will be randomized per participants.

5.1.1.2 Duration of rTMS effect – will the effect be transient?

While the effect of a single rTMS session on motor function may be transient, studies using rTMS for motor function in neurological condition such as Parkinson's disease showed gradual development of long-lasting treatment effects of multiple sessions of rTMS[115] that persisted when studied 4 weeks after the last session[87]. In depression, besides clinical improvement, rTMS led to even remission in a subset of patients that persisted when assessed 12 weeks after the last session[116]. In our study, outcome measures of motor function and plasticity (LTP), as well as adaptive function, will be

evaluated at baseline, immediately, and then 1 and 4 weeks after the last rTMS session. No medication changes will be allowed from 4 weeks prior to the trial until its conclusion, i.e. day 9. (Figure 4). Project investigators including biostatistician, raters and TMS technicians will remain blind to treatment allocation until after project data has been analyzed.

5.1.1.3 Controlling confounding effects of rTMS – the rationale behind choosing 'sham – stimulation'

The two methods to control for the confounding effects of active rTMS are (i) using a sham coil, which is by far the most widely used method, or (ii) using an 'off-target' active brain stimulation control[117]. Sham stimulation closely mimics placebo, however, some indirect stimulation aspects of active rTMS related to sensory, auditory and vibration are not always adequately matched using sham conditions. To minimize this, we will use active and sham adapters for active and sham stimulation and the coil will remain the same in both conditions blinding both patients and technicians. The sham adapter causes identical auditory and similar somatosensory (vibration) stimulation without cortical stimulation. Unlike sham stimulation, actively stimulating another brain region as a control site may address the specificity of the effect of active rTMS, however it comes with an additional risk associated with the added dose of active brain stimulation. A technical paper[117] on the experimental control for brain stimulation recommended using active stimulation control as the last resort. The ethical decision-making to choose an appropriate control in rTMS trials depends on two factors: the safety of the population studied and quality and reliability of data[117]. We considered stimulating 5cm in front of M1 as a control site. However, we preferred to choose sham control for this project because 1) stimulating another site actively will add to the risk of rTMS exposure in an already vulnerable ASD population who have a baseline higher risk of seizures compared to general population[118]; 2) the requirement to add another sham-control for the added active rTMS control site (i.e. 2 active and 2 sham stimulation in total) will add to the burden and complexity of the study; and 3) stimulating 5cm in front of M1 will likely have significant confounding effects on motor control[119]. We will use electrocardiography to monitor heart rate throughout the rTMS session to control for arousal related confounding effects of active rTMS.

5.2 Treatment Regimen

Rationale: Bilateral stimulation of M1 is chosen because a meta-analysis of rTMS clinical trials for the treatment of motor function difficulties in neurological conditions such as Parkin*son's* disease showed a clearly significant benefit of bilateral over unilateral M1 stimulation[87]. Further, a significantly greater and longer lasting effect of rTMS on motor *function was dependent on the use of 'high-frequency' pulses, >1 trial session, an*d the *total 'dose' of pulses delivered during the trial*[87], i.e. it was found that studies with total stimulation pulses around 18,000 to 20,000 pulses had the most clinical benefit[87]. Thus, the total number of pulses delivered in the current project will be 30,000/ASD participant over 5 days.

5.3 Method for Assigning Participants to Treatment Groups

We will conduct a sex-stratified randomization in which within each sex groups, i.e., male and female assigned at birth, ASD participants will be randomly (1:1) allocated to active or sham rTMS groups. Randomization will be completed using random permuted blocks of varying sizes with project personnel blinded to the block sizes.

5.4 Administration of Study Intervention

Active or sham rTMS will be delivered bilaterally to M1. The rTMS paradigm comprises of the delivery of 6,000 pulses (120 trains of 50 pulses with an inter-train interval of 30 seconds) of active or sham 20Hz rTMS[46]. The Magstim Horizon TMS Therapy System with EZ Coil with true and sham adapters (Magstim, Plymouth, MN) will be used for rTMS. Participants will remain seated in a comfortable chair in semi-reclined position and the coils will be machine-held. rTMS will be delivered at 90% of the RMT in both conditions[46].

5.5 Participant Compliance Monitoring

Not applicable.

5.6 Concomitant Therapy

Not applicable.

5.7 Packaging

Not applicable.

5.8 Blinding of Study Intervention

An independent assistant external to the project will manage the randomization of subjects. The clinician, investigators, participant and technician will all be blinded. To ensure blinding during treatment, either the active or sham adapter will be connected to the Magstim Horizon, the coil will remain the same. To ensure blinding of the technician and the participant an independent study assistant will connect the active or sham adapter for the Magstim Horizon. Both heads have identical external appearances, and stimulation of either coil generates identical auditory and somatosensory (vibration) stimuli. All raters obtaining outcome measures will also be blinded to treatment assignment.

5.9 Receiving, Storage, Dispensing and Return

5.9.1 Receipt of Study Intervention Supplies

Not applicable.

5.9.2 Storage

Not applicable.

5.9.3 Dispensing of Study Intervention

Not applicable.

5.9.4 Return or Destruction of Study Intervention

Not applicable.

6.0 RESEARCH PROCEDURES

6.1 Research Visits

Screening Visit (Visit 1) :
During the screening visit, the following steps will be done:

- Reviewing and signing the consent form
- Completing the demographic questionnaire
- Ensure that it is safe for participants to get Transcranial Magnetic Stimulation (TMS)
- Completing assessments:
    - Wechsler Abbreviated Scale of Intelligence – Second Edition (all participants)
    - Autism Diagnostic Observation Schedule – 2 (ASD participants only)
    - Bruininks-Oseretsky Test of Motor Proficiency Second Edition (BOT-2) – (ASD participants only)
    - Edinburgh Handedness Inventory (all participants)
    - Adaptive Behavior Assessment System-3rd (ASD participants)
    - Personality Assessment Inventory (control participants only)

The screening visit will take about 3 hours.

Study Visit 2

During the second visit, we will use iTBS to assess the plasticity in one side of the brain at the motor cortex. This visit will take about 2.5 hours. This will be done for all participants. We will then randomize the ASD participants into active vs sham rTMS groups for visits 3 to 7. There will be no more visits for control participants.

Study Visits 3 to 7

During visits we will administer active or sham rTMS bilaterally at the motor cortex to ASD participants only. These visits will be about 1.5hours each.

Study Visit 8

Exactly 1 week after visit 7, we will again repeat motor and adaptive function assessments. We will then again assess plasticity on one side of the brain using iTBS same as Day 2.

Study Vis it 9

Exactly 3 weeks after visit 8, we will again repeat motor and adaptive function assessments. We will then again assess plasticity on one side of the brain using iTBS same as Day 2.

6.2 Schedule of Events

Procedures	Screening and Baseline Visit 1, Day 1	Study Visit 2, Day 2	Study Visit 3, Day 3	Study Visit 4, Day 4	Study Visit 5, Day 5	Study Visit 6, Day 6	Study Visit 7, Day 7	Study Visit 8, Day 14	Study Visit 9, Day 35
Informed consent	X								
Demographics	X								
Medical history	X								
Edinburgh Handedness Inventory	X								
Wechsler Abbreviated Scale of Intelligence – Second Edition	X								
Autism Diagnostic Observation Schedule – 2 (ASD only)	X								
Bruininks-Oseretsky Test of Motor Proficiency Second Edition	X								
Adaptive Behavior Assessment System-3rd	X								
Personality Assessment Inventory (control only)	X								
Randomization		X							
iTBS		X						X	X
rTMS			X	X	X	X	X		
Adverse event review and evaluation									
Concomitant medication review									
Complete Case Report Forms (CRFs)	X	X	X	X	X	X	X	X	X

7.0 STATISTICAL PLAN

7.1 Sample Size Determination

Hypothesis 1a – In a sample of 31 ASD and 30 NT participants, we found LTP to be 20.6 points higher in the ASD group (Cohen's d = 0.54)[46]. Therefore, a sample of 80 ASD and 40 control participants will allow us to detect with 80% power differences of 17 points in LTP, equivalent to Cohen's d = 0.48. Hypothesis 1b – A sample of 80 ASD and 40 NT participants will provide 80% power to detect a regression coefficient equivalent to a standardized effect Cohen's f^2 = 0.08 and 0.16 respectively in the ASD and control group, which are small and medium effect sizes. Hypothesis 2a and 2b – In both cases, considering a mixed effect model with 3 time points and an overall test for the main effect of group (active rTMS, sham rTMS), 40 participants per group will provide 80% power to detect a standardized effect size Cohen's f = 0.26, i.e., a medium effect size, which is consistent with our recently published work[46]. Hypothesis 3 – A sample of 40 ASD participants expected to receive active bilateral rTMS will provide 80% power to detect a within subject medium effect size of 0.4. We will recruit 100 ASD and 50 NT participants, allowing for a 10-20% drop out rate.

7.2 Statistical Methods

Following intent-to-treat principles, all randomized participants and available data will be considered in the analyses. All tests will use significance level 0.05.

7.2.1 Sex and gender consideration

Given the normative sex differences in plasticity[1], we will address the potential effect of sex by matching ASD and NT controls (2:1) on sex and recruiting at least 34 female (assigned at birth) ASD participants, reflecting a 2:1 male:female ratio (compared to 2.5-3:1 male:female ratio described in clinical samples[2-3]) to allow for sex-focused explorations. Further, in order to balance sex across active and sham groups, we will conduct a sex-stratified randomization. We will include sex as fixed independent factors in our analyses so that we could study sex-moderation effects. We will describe sex-stratification of findings using means/estimates and 95% confidence intervals, recognizing this is exploratory given that the design is likely underpowered for this. At this point, there is no known effect of gender identity on plasticity or motor function in ASD, therefore, we will not control for gender identity. A gender identity stratified subgroup analysis will be completed for descriptive purpose.

7.2.2 Testing Hypothesis 1a

This hypothesis will be tested with an analysis of covariance model, where study group (ASD, NT) is the primary predictor of interest, and plasticity will be entered as the dependent variable, controlling for age, sex, hemisphere of iTBS administration, baseline MEP values, and IQ. To ensure robustness, model diagnostic will be checked through analysis of residuals. Potential confounding effect of medication use and attention-deficit/hyperactivity disorder comorbidity among ASD participants on plasticity will be investigated in a post-hoc sensitivity analysis.

7.2.3 Testing Hypothesis 1b

A Generalized Additive Model (GAM)[122] will be used with motor performance as dependent variable andan interaction between plasticity and study group (ASD, NT) as the effect of interest. GAM is flexible and works in a regression framework, without imposing an assumption of linearity, while still allowingtesting the association between the predictor and the outcome by use of basis function. A cubic spline smoother with up to 5 knots will be adjusted for plasticity, addressing the expected non-linearity of the association. The model will control for sex, age, and IQ.

7.2.4 Testing Hypothesis 2a and 2b

Mixed effect models with random intercepts will be used to compare the overall randomization group effect for plasticity and motor function (two separate models, one for each hypothesis). Initially, the main effect of group (active, sham rTMS), regardless of time (baseline, immediately, 1 and 4 weeks after rTMS) will be accessed. We will also add to the model the group-by-time interaction and access the evidence that the group difference depends on time.

7.2.5 Testing Hypothesis 3

Using mixed models, we will examine within subject association between motor function and plasticity by separating between and within subject effects. The same approach will be used to examine our exploratory hypothesis 1.

8.0 SAFETY AND ADVERSE EVENTS

8.1 Definitions

Adverse Event

An adverse event (AE) is any untoward medical occurrence in a research participant administered an investigational product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the investigational product.

Serious Adverse Event

A serious adverse event (SAE) is any AE that is:

- Fatal;
- Life-threatening;
- Requires or prolongs hospital stay;
- Results in persistent or significant disability or incapacity;
- A congenital anomaly or birth defect; or
- An important medical event (events that may not be life threatening but are of major clinical significance, such as a drug overdose or seizure that did not result in in-patient hospitalization).

Adverse Event Collection Period
The period during which adverse events must be collected is normally defined as the period from the initiation of any research procedures to the end of the study intervention follow-up. For this study, the study intervention follow-up is defined as up to 4 weeks after the last rTMS session.

Preexisting Condition
A preexisting condition is one that is present at the start of the clinical trial. A preexisting condition will be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period. Throughout the clinical trial, any new clinically significant findings/abnormalities that meet the definition of an adverse event will be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events will be followed by the PI until the events are resolved/stable; the participant is lost to follow-up; or the adverse event is otherwise explained.

At the last scheduled visit, the PI will instruct each participant to report any event(s) that the participant believes might reasonably be related to participation in this clinical trial.

8.2 Recording of Adverse Events

All adverse events occurring during the study period will be recorded. At each contact with the research participant, the research team will seek information on adverse events.

8.3 Reporting of Serious Adverse Events

8.3.1 Investigator Reporting: Notifying the Sponsor

Not applicable.

8.3.2 Investigator Reporting: Notifying the REB

The process for notification to the REB for applicable serious adverse events (SAEs) will be completed as per REB reporting requirements.

8.3.3 Sponsor Reporting of SUADRs: Notifying Health Canada

Not applicable – not a regulated trial.

8.3.4 Sponsor Reporting of SUADRs: Notifying Sites

Not applicable.

8.4 Reporting of Device Deficiencies

Not applicable.

8.5 Safety Management Plan

TBS is well-established and safe in the ASD populations[108,120]. Our protocol (6000 pulses, 20Hz, delivered at 90% of RMT) is also within the safety parameters for rTMS[108]. In our pilot study[46], 33 autistic adults and 30 NT control participants were recruited and 31 autistic adults (11 females assigned at birth) received TBS. One ASD participant experienced vasovagal attack and theother was excluded as motor threshold could not be safely determined. No control participant reported any adverse effects. Further, 2 participants dropped out because they were unable to commit further; therefore, 29 autistic adults completed rTMS trial phase. None of the 29 ASD participants reported any adverse effects of the rTMS. The overall study dropout (4/33) rate was 12.1%. In another completed RCT[121] for youth with ASD (n=40), our team used bilateral 20Hz rTMS (20 session, 5 days a week, for4-week) and had 95% retention rate. The recruitment goal was met successfully on time and the rate of adverse events was no different between the active and sham rTMS groups. These pilot data clearly show that TBS and bilateral rTMS proposed in this project are safe and can be feasibly implemented in autistic adults. Recruitment is also highly feasible because of the high number of autistic adults without intellectual disability attending our primary recruitment clinics. Because of these reasons, we are uniquely positioned to meet our recruitment goals on time and believe our approach is highly feasible.

8.6 Unblinding Procedures

Unblinding will occur for a safety reasons for an SAE that is unexpected and thought to be related to the intervention device.

8.7 Data and Safety Monitoring Board

A data and safety monitoring board is not required for this study.

9.0 CLINICAL TRIAL DISCONTINUATION AND CLOSURE

9.1 Clinical Trial Discontinuation

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause (i.e. closure based on PI decision, sponsor/funder decision, REB or other oversight bodies' decision; review of serious, unexpected and related AEs; noncompliance; futility). Notification, which includes the reason for study suspension or termination, will be provided by the suspending or terminating party to research participants, the PI, funding agency, CAMH, and regulatory authorities. If the clinical trial is prematurely terminated or suspended, the PI will promptly inform research participants, the REB, and the sponsor, and will provide the reason(s) for the termination or suspension. All communication with participants for this purpose will go through REB review and approval. Research participants will then be contacted, as applicable, and be informed of changes to the study visit schedule.

10.0 DATA HANDLING AND RECORD KEEPING

10.1 Source Documents & Case Report Forms

Please reference this study's Data Management Plan (DMP).

Data for this clinical trial will be managed using REDCap electronic case report forms. This system is maintained on central CAMH servers, with data backed up daily, and is supported by the Research Informatics department.

10.2 Protocol Deviations

No deviations from or changes to the protocol will be implemented without prior agreement from the sponsor as required, and approval from the REB, unless to eliminate an immediate hazard to a participant.

10.3 Record Retention

Study records and data will be kept for 10 years after the completion of study.

10.4 Clinical Trial Registration

This study will be registered on www.clinicaltrials.gov.

11.0 STUDY MONITORING, AUDITING, AND INSPECTING

11.1 Study Monitoring Plan

Independent monitoring is not required.

We are proposing a study that is investigator-initiated with a device and coil that has a Health Canada (HC) license. We will be using the Magstim Horizon TMS Therapy System for the delivery of active and sham rTMS. According to HC an ITA is not required for this trial.

Below is the license for the Magstim system:
License No.: 102253
Type: System
Device class: 3
Device first issue date 2019-06-20
License name: MAGSTIM HORIZON TMS THERAPY SYSTEM

11.2 Auditing and Inspecting

Not applicable.

12.0 ETHICAL CONSIDERATIONS

Clinical trial materials (e.g., protocol, ICF, recruitment materials, written information provided to participants, etc.) must be submitted to the research ethics board (REB) for review and approval in accordance with REB requirements. Approval must be obtained prior to initiating any clinical trial-specific tasks, and maintained throughout the course of the clinical trial in accordance with REB requirements. Any amendments will require review and approval by the REB before the changes are implemented in the clinical trial, unless to eliminate an immediate hazard to the participant. The REB must be notified of any unanticipated issue or event that may increase the level of risk to participants or that has other ethical implications that may affect participants' welfare.

12.1 Research Ethics Board (REB) Approval

Research Ethics Board (REB) approval will be obtained prior to beginning any research-specific procedures. Following initial ethics approval, ongoing ethical approval will be maintained and the clinical trial will undergo REB review at least annually, in accordance with regulatory and REB requirements. The clinical trial will be conducted in accordance with the REB-approved study documents and the determinations (including any limitations) of the REB, and in compliance with REB requirements.

Whenever new information becomes available that may be relevant to participant consent, a consent form and/or consent for addendum will be presented to the REB for review and approval prior to its use. Any revised written information will receive REB approval prior to use.

12.2 Informed Consent Process & Documentation

Informed consent is a process that is initiated prior to the individual agreeing to take part in the clinical trial and continues throughout their participation.

If consent is done in person:

Informed consent will be obtained from each participant prior to their participation in the study. Informed consent will be obtained by appropriately trained and qualified CAMH research personnel who do not have an existing clinical relationship with the participant. The PI will not obtain participant consent.

Each participant will be provided with a current copy of the REB approved ICF prior to the consent discussion. Research personnel will explain the clinical trial to the participant and answer any questions that may arise. This discussion will include an explanation of the clinical trial purpose, procedures, potential risks and benefits, confidentiality considerations and participant rights (e.g. participants will not be penalized or lose any benefits regardless of what they decide and they have the right to withdraw from the clinical trial at any time). Participants may take as much time as they need to make their decision, and may consult with others (e.g. family members, other health care providers, etc.) if they like. Following the consent discussion, and once the participant has decided to take part, the participant and the person conducting the consent discussion will personally sign and date the ICF. Each participant will be provided with a complete (fully signed) copy of the ICF. The original ICF(s) and the informed consent process will be documented in the source documents.

Written Paper Consent:

Following the consent discussion, the participant and the person conducting the consent discussion will each personally sign and date the ICF. This will occur by emailing the ICF to the participant, the participant signing the ICF, and the participant emailing or faxing the original, scan or photograph of the consent back to CAMH. The person conducting the consent discussion will also sign the ICF once received. No research procedures will begin until after the ICF signed by the participant is received by CAMH, and the ICF is signed by the person conducting the consent discussion (i.e. the ICF and documentation has been completed).
After informed consent has been obtained, a complete (fully signed) copy of the ICF will be provided to participants by email.

13.0 PRIVACY AND CONFIDENTIALITY

All study related documents and data will be held in strict confidence and stored at CAMH or on CAMH servers, and will follow CAMH policies and procedures to ensure participant privacy and confidentiality.

All research activities will be conducted in as private a setting as possible. The study monitor, other authorized representatives of the sponsor, representatives of the REB, regulatory agencies may inspect all documents and records required to be maintained by the PI, including but not limited to, medical records and pharmacy records for the participants in this clinical trial. The participant's contact information will be securely stored at CAMH for internal use during the clinical trial. At the end of the clinical trial, all records will continue to be kept in a secure location in accordance to applicable institutional and regulatory requirements.

14.0 CLINICAL TRIAL FINANCES

14.1 Funding Source

This study is funded through a grant from the Canadian Institute of Health Research.

14.2 Conflict of Interest

None of the investigators have any conflicts to share.

15.0 PUBLICATION POLICY/DATA SHARING

15.1 Future Secondary Use of Dat a

De-identified data from this project may be used for future research by internal and/or external project collaborators.

16.0 **REFERENCES**

1. Mottron L, Duret P, Mueller S, et al. (2015). Sex differences in brain plasticity: a new hypothesis for sex ratio bias in autism. Molecular Autism, 6, 33.

2. Posserud MB, Skretting Solberg B, Engeland A, et al (2021). Male to female ratios in autism spectrum disorders by age, intellectual disability and attention- deficit/hyperactivity disorder. Acta Psychiatrica Scandinavica, 144(6), 635-646.

3. Loomes R, Hull L, Mandy WPL (2017). What Is the Male-to-Female Ratio in Autism Spectrum Disorder? A Systematic Review and Meta-Analysis. Journal of AmericanAcademy of Child & Adolescent Psychiatry, 56(6), 466-474.

4. Warrier V, Greenberg DM, Weir E, et al. (2020). Elevated rates of autism, other neurodevelopmental and psychiatric diagnoses, and autistic traits in transgender and gender-diverse individuals. Nature Communications, 11, 3959.

5. Ofner M, Coles A, Decou ML, et al. (March 2018). Autism spectrum disorder amongchildren and youth in Canada 2018: A report of the National Autism Spectrum Disorder Surveil-lance System. Retrieved from https://www.canada.ca/en/public-health/services/publications/diseases-conditions/autism-spectrum-disorder-children-youth-canada-2018.html.

6. Murray CJ, Vos T, Lozano R, et al. (2012). Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet, 380(9859), 2197-223.

7. Page J, Boucher J (1998). Motor impairments in children with autistic disorder. ChildLanguage Teaching and Therapy, 14, 233–259.

8. Ming X, Brimacombe M, Wagner GC (2007). Prevalence of motor impairment in autismspectrum disorders. Brain and Development, 29, 565–570.

9. Green D, Charman T, Pickles A, et al (2009). Impairment in movement skills of children with autistic spectrum disorders. Developmental Medicine and Child Neurology, 51, 311–316.

10. Whyatt CP, Craig CM (2012). Motor skills in children aged 7–10 years, diagnosed with autism spectrum disorder. Journal of Autism and Developmental Disorders, 42, 1799– 1809.

11. Whyatt C, Craig C (2013). Sensory-motor problems in Autism. Frontiers in IntegrativeNeuroscience, 7, 1–12.

12. Fournier KA, Hass CJ, Naik SK, et al (2010). Motor coordination in autism spectrum disorders: a synthesis and meta-analysis. Journal of Autism and Developmental Disorders, 40(10), 1227-1240.

13. Coll SM, Foster NEV, Meilleur A, et al (2020). Sensorimotor skills in autism spectrum disorder: A meta-analysis. Research in Autism Spectrum Disorders, 76(10), 101570

14. Teitelbaum P, Teitelbaum O, Nye J, et al (1998). Movement analysis in infancy may be useful for early diagnosis of autism. Proceedings of the National Academy of Sciences of the United States of America, 95,13982–13987.

15. Bryson SE, Zwaigenbaum L, Brian J, et al (2007). A prospective case series of high-risk infants who developed autism. Journal of Autism and Developmental Disorders, 37, 12– 24.

16. Matson JL, Mahan S, Fodstad JC, et al (2010). Motor skill abilities in toddlers with autistic disorder, pervasive developmental disorder—not otherwise specified, and atypical development. Research in Autism Spectrum Disorders, 4:444–449.

17. Bhat AN, Galloway JC, Landa RJ (2012). Relation between early motor delay and later communication delay in infants at risk for autism. Infant Behavior and Development, 35, 838–846.

18. Glazebrook CM, Elliott D, Lyons J (2006). A kinematic analysis of how young adults with and without autism plan and control goal-directed movements. Motor Control,10(3), 244-264.

19. Cassidy S, Hannant P, Tavassoli T, et al. (2016). Dyspraxia and autistic traits in adults with and without autism spectrum conditions. Molecular Autism 7, 48.

20. Fukui T, Sano M, Tanaka A, et al. (2018). Older Adolescents

and Young Adults With Autism Spectrum Disorder Have Difficulty Chaining Motor Acts When Performing Prehension Movements Compared to Typically Developing Peers. Frontiers in Human Neuroscience 12.

21. Linke AC, Kinnear MK, Kohli JS, et al. (2020). Impaired motor skills and atypical functional connectivity of the sensorimotor system in 40- to 65-year-old adults with autism spectrum disorders. Neurobiology of Aging, 85, 104-112.

22. Freitag CM, Kleser C, Schneider M, et al. (2007). Quantitative assessment of neuromotorfunction in adolescents with high functioning autism and Asperger Syndrome. Journal of Autism and Developmental Disorders, 37(5), 948-959.

23. Bhat AN, Galloway JC, Landa RJ (2012). Relation between early motor delay and later communication delay in infants at risk for autism. Infant Behavior and Development, 35(4), 838-846

24. Wymbs NF, Nebel MB, Ewen JB, Mostofsky SH (2021). Altered Inferior Parietal Functional Connectivity is Correlated with Praxis and Social Skill Performance in Children with Autism Spectrum Disorder. Cerebral Cortex, 31(5), 2639-2652.

25. Ohara R, Kanejima Y, Kitamura M, et al. (2019). Association between Social Skills and Motor Skills in Individuals with Autism Spectrum Disorder: A Systematic Review.European Journal of Investigation in Health, Psychology and Education, 10, 276–296;

26. Baranek GT (1999). Autism during infancy: a retrospective video analysis of sensorymotor and social behaviors at 9-12 months of age. Journal of Autism and Developmental Disorders, 29(3), 213-224.

27. Hedgecock JB, Dannemiller LA, Shui AM, et al. (2018). Associations of Gross Motor Delay, Behavior, and Quality of Life in Young Children With Autism Spectrum Disorder. Physical Therapy, 98(4), 251-259.

28. Travers BG, Bigler ED, Duffield TC, et al. (2017). Longitudinal development of manual motor ability in autism spectrum disorder from childhood to mid-adulthood relates to adaptive daily living skills. Developmental Science, 20(4), 10.1111/desc.12401.

29. Roche L, Adams D, Clark M (2021). Research priorities of the autism community: A systematic review of key stakeholder perspectives. Autism, 25(2),336-348.

30. Ceccarelli SB, Ferrante C, Gazzola E, et al. (2020). Fundamental Motor Skills Intervention for Children with Autism Spectrum Disorder: A 10-Year Narrative Review. Children (Basel), 7(11), 250.

31. Anderson JS, Druzgal TJ, Froehlich A, et al. (2011). Decreased interhemispheric functional connectivity in autism. Cerebral Cortex, 21, 1134–1146.

32. Floris DL, Barber AD, Nebel MB, et al. (2016). Atypical lateralization of motor circuit functional connectivity in children with autism is associated with motor deficits. Molecular Autism 7, 35.

33. Lee JM, Kyeong S, Kim E, et al. (2016). Abnormalities of Inter- and Intra-Hemispheric Functional Connectivity in Autism Spectrum Disorders: A Study Using the Autism Brain Imaging Data Exchange Database. Frontiers in Neuroscience, 10, 191.

34. Nebel MB, Eloyan A, Nettles CA, et al. (2016). Intrinsic Visual-Motor Synchrony Correlates With Social Deficits in Autism. Biological Psychiatry, 79(8), 633-641.

35. Mostofsky SH, Powell SK, Simmonds DJ, et al. (2009). Decreased connectivity and cerebellar activity in autism during motor task performance. Brain, 132, 2413–2425.

36. Nebel MB, Eloyan A, Barber AD, Mostofsky SH (2014a). Precentral gyrus functional connectivity signatures of autism. Frontiers in Systemic Neuroscience, 8, 80.

37. Nebel MB, Joel SE, Muschelli J, et al. (2014b) Disruption of functional organization within the primary motor cortex in children with autism. Human Brain Mapping, 35(2), 567–580.

38. Carper RA, Solders S, Treiber JM, et al. (2015). Corticospinal tract anatomy and functional connectivity of primary motor cortex in autism. Journal of American Academy of Child and Adolescent Psychiatry, 54(10), 859–867.

39. Nunes AS, Peatfield N, Vakorin V, et al. (2018). Idiosyncratic organization of cortical networks in autism spectrum disorder.

NeuroImage, 190, 182-190.

40. Mostofsky SH, Burgess MP, Gidley Larson JC (2007). Increased motor cortex white matter volume predicts motor impairment in autism. Brain, 130(8), 2117-2122.

41. Arnold Anteraper S, Guell X, D'Mello A, et al. (2018). Disrupted Cerebrocerebellar Intrinsic Functional Connectivity in Young Adults with High-Functioning Autism Spectrum Disorder: A Data-Driven, Whole-Brain, High-Temporal Resolution Functional Magnetic Resonance Imaging Study. Brain Connectivity, 9(1), 48-59.

42. Khan AJ, Nair A, Keown CL, et al. (2015). Cerebro-cerebellar Resting-State Functional Connectivity in Children and Adolescents with Autism Spectrum Disorder. Biological Psychiatry, 78(9), 625–634.

43. Oberman L, Ifert-Miller F, Najib U, et al. (2010). Transcranial magnetic stimulation provides means to assess cortical plasticity and excitability in humans with fragile X syndrome and autism spectrum disorder. Frontiers in Synaptic Neuroscience, 2, 26.

44. Oberman L, Eldaief M, Fecteau S, et al. (2012). Abnormal modulation of corticospinal excitability in adults with Asperger disorder. European Journal of Neuroscience, 36, 2782–2788.

45. Oberman L, Ifert-Miller F, Najib U, et al. (2016). Abnormal Mechanisms of Plasticity and Metaplasticity in Autism Spectrum Disorders and Fragile X Syndrome. Journal of Child and Adolescent Psychopharmacology, 26 (7), 617-624.

46. Desarkar P, Rajji TK, Ameis SH, Blumberger DM, Lai MC, Lunsky Y, Daskalakis ZJ. (2022). Assessing and Stabilizing Atypical Plasticity in Autism Spectrum Disorder using rTMS: Results from a Proof-of-Principle Study. Clinical Neurophysiology, 141, 109-118.

47. Markram K & Markram H (2010). The intense world theory – a unifying theory of the neurobiology of autism. Frontiers in Human Neuroscience, 21(4), 224.

48. Silva GT, Le Bé JV, Riachi I, et al. (2009). Enhanced Long-Term Microcircuit Plasticity in the Valproic Acid Animal Model of Autism. Frontiers in Synaptic Neuroscience, 1, 1.

49. Pascual-Leone A, Freitas C, Oberman L, et al. (2011). Characterizing brain cortical plasticity and network dynamics across the age-span in health and disease with TMS- EEG and TMS-fMRI. Brain Topography, 24, 302–315.

50. Ament K, Mejia A, Buhlman R, et al (2015). Evidence for specificity of motor impairments in catching and balance in children with autism. Journal of Autism and Developmental Disorders, 45(3), 742-751.

51. Dziuk MA, Larson JC, Apostu A, et al. (2007). Dyspraxia in autism: Association with motor, social, and communicative deficits. Developmental Medicine and Child Neurology, 49(10), 734–739.

52. Dowell LR, Mahone EM, Mostofsky SH (2009). Associations of postural knowledge and basic motor skill with dyspraxia in autism: Implication for abnormalities in distributed connectivity and motor learning. Neuropsychology, 23(5), 563–567.

53. Leary MR & Hill DA (1996). Moving on: autism and movement disturbance. Mental Retardation, 34, 39–53.

54. Ghaziuddin M & Butler E. (1998). Clumsiness in autism and Asperger syndrome: a further report. Journal of Intellectual Disability Research, 42(1), 43–48.

55. Green D, Baird G, Barnett AL, et al. (2002). The severity and nature of motor impairment in Asperger's syndrome: a comparison with specific developmental disorder of motor function. Journal of Child Psychology and Psychiatry and Allied Disciplines, 43, 655– 668.

56. Jansiewicz EM, Goldberg MC, Newschaffer CJ, et al. (2006). Motor signs distinguish children with high functioning autism and Asperger's syndrome from controls. Journal ofAutism and Developmental Disorders, 36(5), 613–621.

57. McPhillips M, Finlay J, Bejerot S, et al. (2014). Motor Deficits in Children With Autism Spectrum Disorder: A Cross-Syndrome Study. Autism Research, 7(6), 664-676.

58. Miyahara M, Tsujii M, Hori M, et al. (1997). Brief report: motor incoordination in children with Asperger syndrome and learning disabilities Journal of Autism and Developmental Disorders, 27(5), 595–603.

59. Croen LA, Zerbo O, Qian Y, et al. (2015). The health status of adults on the autism spectrum. Autism, 19(7), 814–823.

60. Starkstein S, Gellar S, Parlier M, et al. (2015). High rates of parkinsonism in adults with autism. Journal of Neurodevelopmental Disorders 7, 29.

61. Lord C, Charman T, Havdahl A, et al. (2022). The Lancet Commission on the future of care and clinical research in autism. Lancet, 399(10321), 271-334.

62. Huang YZ, Edwards MJ, Rounis E, et al. (2005). Theta burst stimulation of the human motor cortex. Neuron, 45, 201–206.

63. Ebert DH & Greenberg ME (2013). Activity-dependent neuronal signalling and autism spectrum disorder. Nature 493, 327–337.

64. Neale B M, Yan K, Polak P, et al. (2012). Patterns and rates of exonic de novo mutations in autism spectrum disorders. Nature 485, 242–245.

65. O'Roak BJ, Vives L, Turner E, et al. (2012). Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. Nature 485, 246–250.

66. Krumm N, O'Roak B J, Shendure J, et al. (2014). A de novo convergence of autism genetics and molecular neuroscience. Trends in Neuroscience, 37, 95–105.

67. Samocha K E, Robinson EB, Sanders SJ, et al. (2014). A framework for the interpretationof de novo mutation in human disease. Nature Genetics, 46, 944–950.

68. Iossifov I, O'Roak BJ, Sanders SJ, et al. (2014). The contribution of de novo coding mutations to autism spectrum disorder. Nature, 515, 216–221.

69. De Rubeis S, He X, Goldberg AP, et al. (2014). Synaptic, transcriptional and chromatin genes disrupted in autism. Nature 515, 209–215.

70. Larson J, Jessen R E, Kim D, et al. (2005). Age-dependent and selective impairment of long-term potentiation in the anterior piriform cortex of mice lacking the fragile X mentalretardation protein. Journal of Neuroscience, 25, 9460–9469.

71. Uchino S, Wada H, Honda S, et al. (2006). Direct interaction of post-synaptic density- 95/Dlg/ZO-1 domain-containing synaptic molecule Shank3 with GluR1 α-amino-3- hydroxy-5-methyl-4-isoxazole propionic acid receptor. Journal of Neurochemistry, 97, 1203–1214.

72. Gilbert J, Man HY (2017). Fundamental Elements in Autism: From Neurogenesis and Neurite Growth to Synaptic Plasticity. Frontiers in Cellular Neuroscience, Nov 20;11, 359.

73. Jung NH, Janzarik WG, Delvendahl I, et al. (2013). Impaired induction of long-term potentia-tion-like plasticity in patients with high-functioning autism and Asperger syndrome. Developmental Medicine Child Neurology, 55(1), 83-89.

74. Citri A, Malenka RC (2008). Synaptic plasticity: multiple forms, functions, and mechanisms. Neuropsychopharmacology, 33(1), 18-41.

75. Huang YZ, Chen RS, Rothwell JC, et al. (2007). The after-effect of human theta burst stimulation is NMDA receptor dependent. Clinical Neurophysiology, 118(5), 1028-1032.

76. Jones EG (1993). GABAergic neurons and their role in cortical plasticity in primates. Cerebral Cortex, 3(5), 361-372.

77. Rubenstein JL & Merzenich MM (2003). Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain and Behavior, 2(5), 255–267.

78. Baroncelli L, Braschi C, Spolidoro M, et al. (2011). Brain plasticity and disease: a matter of inhibition. Neural Plasticity, 2011, 286073.

79. Fatemi SH, Folsom TD, Reutiman TJ, et al. (2009). Expression of GABA(B) receptors isaltered in brains of subjects with autism. Cerebellum, 8(1), 64–69.

80. Masuda F, Nakajima S, Miyazaki T, et al. (2019). Motor cortex excitability and inhibitory imbalance in autism spectrum disorder assessed with transcranial magnetic stimulation: a systematic review. Translational Psychiatry, 9(1), 110.

81. Daskalakis ZJ, Möller B, Christensen BK, et al. (2006). The effects of repetitive transcranial magnetic stimulation on cortical inhibition in healthy human subjects. Experimental Brain Research, 174, 403–412.

82. de Jesus DR, Favalli GPD, Hoppenbrouwers SS, et al. (2014). Determining optimal rTMS parameters through changes in cortical inhibition. Clinical Neurophysiology, 125(4), 755–762.

83. Desarkar P, Rajji TK, Ameis SH, et al. (2015). Assessing and Stabilizing Aberrant Neuroplasticity in Autism Spectrum Disorder: The Potential Role of Transcranial Magnetic Stimulation. Frontiers in Psychiatry, 6, 124.

84. Ohbayashi M (2021). The Roles of the Cortical Motor Areas in Sequential Movements. Frontiers in Behavioral Neuroscience, 15:640659.

85. Graziano MS, Taylor CS, Moore T (2002). Complex movements evoked by microstimulation of precentral cortex. Neuron, 34(5), 841-851.

86. Georgopoulos AP, Kalaska JF, Caminiti R, et al (1982). On the relations between the direction of two-dimensional arm movements and cell discharge in primate motor cortex. Journal of Neuroscience, 2(11), 1527-1537.

87. Yang C, Guo Z, Peng H, et al. (2018). Repetitive transcranial magnetic stimulation therapy for motor recovery in Parkinson's disease: A Meta-analysis. Brain and Behavior, 8(11), e01132.

88. Xiang H, Sun J, Tang X, et al. (2019). The effect and optimal parameters of repetitive transcranial magnetic stimulation on motor recovery in stroke patients: a systematic review and meta-analysis of randomized controlled trials. Clinical Rehabilitation, 33(5), 847-864.

89. Srinivasan SM, Kaur M, Park IK, et al. (2015). The Effects of Rhythm and Robotic Interventions on the Imitation/Praxis, Interpersonal Synchrony, and Motor Performance of Children with Autism Spectrum Disorder (ASD): A Pilot Randomized Controlled Trial. Autism Research and Treatment, 736516.

90. Felzer-Kim IT, Hauck JL. (2020). How Much Instructional Time Is Necessary? Mid-intervention Results of Fundamental Movement Skills Training within ABA Early Intervention Centers. Frontiers in Integrative Neuroscience, 14, 24.

91. Tan HY, Callicott JH, Weinberger DR (2007). Dysfunctional and compensatory prefrontal cortical systems, genes and the pathogenesis of schizophrenia. Cerebral Cortex, 17 S1, 171-181.

92. Wallace GL, Dankner N, Kenworthy L, et al. (2010). Age-related temporal and parietal cortical thinning in autism spectrum disorders. Brain, 133(12), 3745–3754.

93. McEachern JC, Shaw CA. (1999). The plasticity-pathology continuum: defining a rolefor the LTP phenomenon. Journal of Neuroscience Research, 58(1), 42-61.

94. Fetit R, Hillary RF, Price DJ, et al. (2021). The neuropathology of autism: A systematic review of post-mortem studies of autism and related disorders. Neuroscience and Biobehavioral Reviews, 129, 35-62.

95. Dudley C & Emery JCH (2014). The Value of Caregiver Time: Costs of Support and Care for Individuals Living with Autism Spectrum Disorder. SPP Research Paper , 7-1

96. American Psychiatric Association (2013) Diagnostic and Statistical Manual of Mental Disorders (Fifth Ed.) Arlington, VA: American Psychiatric Association.

97. Lord C, Rutter M, DiLavore P C, et al. (2012). Autism diagnostic observation schedule, second edition. Torrance, CA: Western Psychological Services.

98. Bruininks RH & Bruininks BD(2005). Bruininks–Oseretsky test of motor proficiency: Examiner's manual (2nd ed.).Windsor, UK: NFER-Nelson.

99. Markou P, Ahtam B, Papadatou-Pastou M. (2017). Elevated Levels of Atypical Handedness in Autism: Meta-Analyses. Neuropsychology Review, 27(3), 258-283.

100. Morey LC (1991). Personality Assessment Inventory – Professional Manual. Florida: Psychological Assessment Resources.

101. Lai MC, Kassee C, Besney R, et al. Prevalence of co-occurring mental health diagnosesin the autism population: a systematic review and meta-analysis. Lancet Psychiatry,6(10), 819–829.

102. Abdallah MW, Greaves-Lord K, Grove J, et al. (2011). Psychiatric comorbidities in autism spectrum disorders: Findings from a Danish Historic Birth Cohort. European Child and Adolescent Psychiatry, 20(11-12),599-601.

103. Leyfer OT, Folstein SE, Bacalman S, et al. (2006). Comorbid psychiatric disorders in children with autism: Interview development and rates of disorders. Journal of Autism Developmental Disorder, 36(7), 849-861.

104. Simonoff E, Pickles A, Charman T, et al.(2008). Psychiatric Disorders in Children With Autism Spectrum Disorders: Prevalence, Comorbidity, and Associated Factors in a Population-Derived Sample. Journal of American Academy of Child and Adolescent Psychiatry, 47(8), 921-929.

105. Wechsler D (2011). Wechsler Abbreviated Scale of Intelligence, Second Edition (WASI- II). San Antonio TX: NCS Pearson.

106. Oldfield RC (1971). Assessment and Analysis of Handedness – Edinburgh Inventory. Neuropsychologia, 9 (1), 97 -113.

107. Sparrow SS, Cicchetti DV (1985). Diagnostic uses of the Vineland adaptive behavior scales. Journal of Pediatric Psychology, 10(2), 215e25.

108. Rossi S, Antal A, Bestmann S, et al. (2021). Safety and recommendations for TMS use inhealthy subjects and patient populations, with updates on training, ethical and regulatory issues: Expert Guidelines. Clinical Neurophysiology, 132(1), 269-306.

109. Downs SJ, Boddy LM, McGrane B, et al. (2020). Motor competence assessments for children with intellectual disabilities and/or autism: a systematic review. BMJ Open Sportand Exercise Medicine, 6(1):e000902.

110. Deitz JC, Kartin D, Kopp K. (2007). Review of the Bruininks-Oseretsky Test of Motor Proficiency, Second Edition (BOT-2). Physical and Occupational Therapy in Pediatrics, 27(4), 87-102

111. Hands B, Licari M, Piek J (2015). A review of five tests to identify motor coordination difficulties in young adults. Research in Developmental Disabilities, 42, 40-51.

112. Dominici F, Popa T, Ginanneschi F, et al. (2005). Cortico-motoneuronal output to intrinsic hand muscles is differentially influenced by static changes in shoulder positions. Experimental Brain Research, 164, 500–504.

113. Kujirai T, Caramia MD, Rothwell JC, et al. (1993). Corticocortical inhibition in human motor cortex. Journal of Physiology, 471, 501–519.

114. Corp DT, Bereznicki HGK, Clark GM, et al. (2020). 'Big TMS Data Collaboration'. Large-scale analysis of interindividual variability in theta-burst stimulation data: Results from the 'Big TMS Data Collaboration'. Brain Stimulation, 13(5), 1476-1488.

115. Khedr, EM, Farweez HM, Islam H (2003). Therapeutic effect of repetitive transcranial magnetic stimulation on motor function in Parkinson's disease patients. European Journal of Neurology, 10(5), 567–572.

116. Blumberger DM, Vila-Rodriguez F, Thorpe KE et al. (2018). Effectiveness of theta burst versus high-frequency repetitive transcranial magnetic stimulation in patients with depression (THREE-D): a randomised non-inferiority trial. Lancet, 391(10131), 1683- 1692.

117. Davis NJ, Gold E, Pascual-Leone A, et al. (2013). Challenges of proper placebo control for non-invasive brain stimulation in clinical and experimental applications. European Journal of Neuroscience, 38(7), 2973-2977.

118. Liu X, Sun X, Sun C, et al. (2022). Prevalence of epilepsy in autism spectrum disorders: A systematic review and meta-analysis. Autism, 26(1), 33-50.

119. Takarae Y, Minshew NJ, Luna B, et al. (2007). Atypical involvement of frontostriatal systems during sensorimotor control in autism. Psychiatry Research, 156(2), 117-127.

120. Oberman LM, Rotenberg A, Pascual-Leone A. (2015). Use of Transcranial Magnetic Stimulation in Autism Spectrum Disorders. Journal of Autism and Developmental Disorder, 45, 524-536.

121. Ameis SH, Blumberger DM, Croarkin PE, Mabbott DJ, Lai MC, Desarkar P, Szatmari P, Daskalakis ZJ (2020). Treatment of Executive Function Deficits in autism spectrum disorder with repetitive transcranial magnetic stimulation: A double-blind, sham-controlled, pilot trial. Brain Stimulation,13(3),539-547.

122. Fahrmeir, Ludwig, Thomas Kneib, Stefan Lang, et al. (2013). Regression: Models, Methods and Applications. Springer Science

& Business Media.

123. Daskalakis ZJ, Farzan F, Barr MS, et al. (2008). Long-interval cortical inhibition from the dorsolateral prefrontal cortex: a TMS-EEG study. Neuropsychopharmacology, 33(12), 2860–2869.