Motor and Neurophysiological Changes after Ischemic Conditioning in Individuals with Stroke

Cover Page

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Table of Contents

LIST OF ABBREVIATIONS

10MWT	10-meter walk test
AHS	Applied Health Sciences
AMT	Active motor threshold
BDNF	Brain derived neurotrophic factor
BP	Blood Pressure
CCTS	Center for Clinical and Translational Science (CCTS)
CME	Corticomotor excitability
EMG	Electromyography
FDA	Food and Drug Administration
FMLE	Fugl Meyer Lower Extremity Scale
HIPAA	Health Insurance Portability and Accountability Act
HR	Heart rate
HRR	Heart rate reserve
IC	Ischemic Conditioning
iSP	Ipsilateral silent period
M1	Lower limb primary motor cortex
MEP	Motor-evoked potential
MMSE	Mini-Mental State Examination
MRC	Medical Research Council
MRI	Magnetic resonance imaging
MVC	Maximum voluntary contraction
MVIC	Maximum voluntary isometric contraction

MVT	Maximum voluntary torque
NIH	National Institutes of Health
PAR-Q	Physical Activity Readiness Questionnaire
PI	Principal Investigator
RMT	Resting motor threshold
RPE	Rating of perceived exertion
SICI	Short intracortical inhibition
TA	Tibialis anterior
TCI	Transcallosal inhibition
tDCS	Transcranial direct current stimulation
TMS	Transcranial magnetic stimulation
UIC	University of Illinois at Chicago

Motor and Neurophysiological Changes after Ischemic Conditioning in Individuals with Stroke

1.0 Study Summary

Functional ambulation remains a top priority for many stroke survivors living with chronic gait impairments. Current rehabilitation techniques to restore ambulatory capacity in stroke have shown promising, yet varying, results, and there is a critical need to maximize current benefits by designing optimal interventions. Neuromodulation is increasingly investigated as a promising strategy to promote neuroplasticity and accelerate motor recovery in stroke; however, no motor priming modality has emerged as the most effective method to prime the central nervous system for rehabilitation. Aerobic exercise is an effective neuromodulatory approach and advantageous for gait recovery in stroke, but some patients, especially those with moderate or severe impairments, lack the functional capacity to perform aerobic exercise. Thus, there is a need for alternative modalities. Ischemic conditioning (IC) is a procedure when the limb is exposed to brief, repeated bouts of reduced blood flow immediately followed by restored blood flow. Ischemic conditioning is a possible neuromodulatory technique that has shown to increase muscle hypertrophy, improve aerobic capacity, and increase walking speeds in both healthy and clinical populations. However, research investigating ischemic conditioning in stroke rehabilitation is limited. In the current study, our aim is to quantify the effectiveness of ischemic conditioning as a neuromodulatory modality to promote gait rehabilitation in individuals with stroke. Outcome measures will include gait variables, lower limb strength, lower limb muscle volume, ankle motor performance, and neurophysiological measures of corticomotor excitability (CME), intracortical inhibition, and transcallosal inhibition measured from the tibialis anterior (TA) muscles using transcranial magnetic stimulation (TMS).

2.0 Objectives*

Aim 1. To quantify changes in CME and TCI of the paretic TA following a single session of IC in comparison to sham IC in individuals with stroke.
Hypothesis 1. Individuals with stroke will display greater increases in CME of the paretic TA and greater increases in TCI from the lesioned to the non-lesioned hemisphere after IC in comparison to sham IC.

Aim 2. To quantify changes in lower limb strength and ankle motor control following a single session of IC in comparison to sham IC in individuals with stroke.
Hypothesis 2. Individuals with stroke will show greater improvements in lower limb strength and ankle motor control following IC in comparison to sham IC.

3.0 Background*

Stroke is the leading cause of serious long-term disability in the United States resulting in mild to severe chronic gait impairments, such as hemiparesis, reduced gait speeds and endurance, and instability during gait (Jørgensen et al. 1995; Olney et al., 1996; Mayo et al. 1999). The primary focus of gait rehabilitation has been the development of interventions to restore ambulatory capacity which has shown promising results, but there is a critical need to maximize benefits of current gait rehabilitation. The concept of neuromodulation has emerged as an adjuvant tool to enhance the effects of stroke rehabilitation by modulating neural

activity to restore cortical balance and enhance behavioral outcomes (Stoykov & Madhavan, 2015). Neuromodulatory modalities can be categorized as movement-based (continuous passive or active movements), stimulation-based (non-invasive brain and spinal stimulation), motor imagery and action observation, sensory-based (electrical stimulation, vibration), and pharmacological-based (Stoykov & Madhavan, 2015). To date, no neuromodulatory modality has emerged as the most effective method to prime the central nervous system for rehabilitation. Due to the limited number of studies in stroke, the effects and cortical mechanisms of neuromodulatory modalities particularly targeting the lower limb primary motor cortex (M1) are still not completely understood.

Aerobic exercise is considered a movement-based modality that may upregulate corticomotor pathways and enhance the effects of subsequent motor training (Stinear et al., 2014; Stoykov et al., 2017). In healthy populations, previous studies demonstrated that a single session of aerobic exercise increased corticospinal excitability (Hendy et al., 2022), enhanced performance on a perceptual learning task and simple motor task (Perini et al., 2016), improved implicit motor learning (Mang et al., 2014), enhanced motor memory retrieval (Mang et al., 2016), enhanced motor skill consolidation (Stavrinos et al., 2017), and facilitated responses to neuromodulation (McDonnell et al., 2013; Mang et al., 2014; Singh et al., 2014). Previous research in stroke demonstrated that a single session of aerobic exercise increased responses to subsequent therapies by increasing lesioned hemisphere motor cortical excitability (Boyne et al., 2019; Li., et al 2019) and circulating levels of brain derived neurotrophic factor (BDNF) (Boyne et al., 2019) - a neural substrate underlying motor learning. Aerobic exercise has led to positive outcomes in the studies previously mentioned, however, other studies have reported mixed results. In one instance, aerobic exercise increased walking speeds but suppressed corticomotor excitability (Madhavan et al., 2020). Another study found aerobic exercise had no influence on motor learning (Snow et al 2016). One explanation for the inconsistency could be exercise intensity. Stroke literature suggests exercising at higher intensities for optimal outcomes (Boyne et al., 2019; Luo et al., 2019; Munari et al., 2018), however, many stroke patients, especially those with severe impairments, lack the mechanistic properties required to perform high-intensity exercise. Furthermore, individuals with stroke lack the aerobic capacity necessary to perform most aerobic exercise protocols. Mixed results, inconsistencies in current aerobic exercise protocols, and the lack of neuromodulatory procedures for those with severe impairments demand investigation into alternative methods for individuals with stroke.

Ischemic conditioning (IC) could be a potential modality to improve function post-stroke. In IC, the limb is exposed to brief, repeated bouts of ischemia (reduced blood flow) immediately followed by reperfusion. Priming procedures increase the brain's responsiveness to subsequent training protocols (Stoykov & Madhavan, 2015), and previous studies have revealed the neuromodulatory effects of IC. In rats, aerobic exercise and IC share mechanisms to induce neuroprotection and stimulate neuroplasticity by increasing levels of neurotrophic factors like BDNF and proteins responsible for building new connections within the central nervous system to support motor learning (Wang et al., 2020). Additionally, prior research suggests short (0-12

hours) and long (24-72) windows of action following IC (Koch et al., 2014; Durand et al., 2019), and it has been implied by several studies that the delayed, or long-term, action may be more robust, especially as applied to neural plasticity (Perez-Pinzon, 2004; Dirnagl et al., 2009; Koch et al., 2014). In humans, IC has shown to improve muscle performance in healthy populations (Paradis-Deschênes et al., 2016). Local and remote IC performed in healthy individuals improved motor learning (Cherry-Allen et al., 2015), increased fatigue resistance (Barbosa et al., 2015), enhanced running performance (Bailey et al., 2012), and increased maximal power output (De Groot et al., 2010). Given the positive results in healthy populations, IC could potentially exert even larger effects in clinical populations with diminished motor output like stroke. In fact, an acute exposure to IC increased paretic muscle strength and muscle activation in individuals with stroke (Hyngstrom et al., 2018). Also in chronic stroke, prolonged IC exposure improved walking speed, cognitive function, and reduced paretic muscle fatigue (Durand et al., 2019; Feng et al., 2019). Increased fatigue resistance at submaximal contraction levels following IC may be due to increased neural activation which may be particularly beneficial following cortical reorganization after stroke (Hyngstrom et al., 2018).

Thus, **the objective of this work is to establish IC as an acute neuromodulation modality by measuring changes in corticomotor outcomes and subsequent motor performance in individuals with stroke after a single session of IC and sham-IC.** This study is unique as it seeks to address the need for alternative priming modalities to promote motor function in individuals with stroke, particularly individuals with severe impairments. This study aims to develop a rehabilitation technology which can enhance motor outcomes for any severity of stroke by supplying an alternative method to traditional motor priming procedures.

4.0 Study Endpoints*

Primary

Corticomotor excitability (CME): Motor evoked potentials (MEPs) recorded from the TA muscles of the paretic limbs for individuals with stroke using single-pulse TMS targeting the lower limb primary motor cortex.

Transcallosal inhibition (TCI): TCI from the stimulated hemisphere to the non-stimulated hemisphere will be quantified as a measure of the ipsilateral silent period (iSP). During a 50% maximum voluntary contraction, single-pulse TMS will be delivered to the lower limb primary motor cortex ipsilateral to the active lower limb. The iSP in the active lower limb will be defined as the time after the MEP when electromyography (EMG) activity is below 25% of the background EMG.

Muscle Strength: Ankle dorsiflexion and plantarflexion strength will be measured using a simple hand-held strength testing device (dynamometer) and using the Medical Research Council (MRC) Scale for muscle strength.

Ankle Motor Control: With a custom-built ankle tracking device, we will measure reaction time using a choice reaction time task. While seated, subjects will perform ankle dorsiflexion or plantarflexion using the paretic foot as quickly as possible in response to a rising or falling visual bar signal displayed on a screen. Bar signals will show in a random order and interval. EMG activity will be recorded during the task. Participants will perform a total of 30 trials with a 60-second rest after every five trials. Reaction time will be defined as the duration from the signal presentation to the onset of the EMG response.

Secondary

Pain: Subjective measures of pain will be reported during IC using a Numerical Rating Scale (NRS) from 0 (no pain) to 10 (worst pain). If cuff inflation becomes too painful for the participant, the session will be terminated.

5.0 Study Intervention/Investigational Agent

Ischemic Conditioning

A rapid inflation cuff (Hokanson SC12 thigh cuff), similar to those used to measure blood pressure, will be placed on the proximal thigh of the paretic leg while subjects are seated in a chair. Prior to IC procedures, the cuff will be inflated to determine each subjects' limb occlusion pressure (leg receiving IC) and will last <1-minute. For all Aims in the proposed study, the cuff will be inflated to 225mmHg for real IC and to 25mmHg for sham IC. The exact same set up used for real IC will be used for sham IC (same cuffs, same device, same body positions, etc.). The only difference between real and sham IC will be the amount of pressure in the cuff (225mmHg vs. 25mmHg). For all Aims in the proposed study, the cuff will be inflated for 5 minutes followed by 5 minutes of no inflation (0mmHg). Each cycle lasts 10 minutes (5 minutes of inflation followed by 5 minutes of no inflation). Subjects will complete 5 cycles of real and sham IC for a total of 50 minutes. These exact methods have been used previously in stroke patients without any adverse events or negative feedback from participants (Hyngstrom et al., 2018; Durand et al., 2019; Hyngstrom et al., 2020). A more detailed description of IC procedures is provided under Section 11.0 'Procedures Involved'.

The equipment used for IC will be stored in a locked room in Dr. Madhavan's laboratory. Only key research personnel will have key access to the storage room.

For Aim 2, to distinguish the acute effects of both neuromodulatory modalities (sham IC and real IC) we will record measures of ankle strength and ankle motor control after one session of sham IC and real IC. Force output will be used to measure ankle (dorsiflexion and plantarflexion) strength. Ankle motor control (ankle dorsiflexion and plantarflexion reaction time) will be measured with a custom-built ankle-tracking device.

6.0 Study Timelines*

In Aims 1 and 2, subjects will make three visits to the lab. The first lab visit will be for screening and clinical measures and will last approximately 2-3 hours. If

deemed eligible, subjects will make two more visits to the lab: 1 sham IC session and 1 real IC session. Each priming session will last 3-4 hours (including 50 minutes of sham IC and real IC).

We anticipate enrolling all study subjects within 3-5 years. Primary analyses will be completed one year following the completion of subject recruitment.

7.0 Inclusion and Exclusion Criteria*

During recruitment procedures, when potential subjects contact the Brain Plasticity Lab, key research personnel will explain the study and answer all questions. Before admission to the study, subjects will undergo a brief screening pertaining to the inclusion and exclusion criteria listed below to assess their qualification to participate in the study. If the individual is interested, they will be screened over the phone using the attached screening documents (Supplemental_Phone Screen Stroke). If the individual is determined to be eligible over the phone using the eligibility criteria listed below, they will be scheduled for a visit to the laboratory for an in-person screening (Supplemental_Screening Stroke). All subjects will be screened in person in the Brain Plasticity Lab regardless of the initial mode of recruitment (i.e., initial phone screens will be screened again in person). Screen failures will be excluded from the study. To ensure that subjects continue to meet the eligibility criteria of this study, at the start of each session, subjects will be asked to notify the investigators of any change in their medical or physical condition or change in their prescription medications. All screening and patient consent will be done by key research personnel at the University of Illinois at Chicago (UIC).

To determine whether potential subjects are to be excluded from the current study based on the presence of thrombosis, including deep vein thrombosis (DVT), we have included two additional risk assessment questionnaires, the Wells clinical prediction guide and the Caprini risk assessment model for venous thromboembolism. The Wells clinical prediction guide quantifies the probability of deep venous thrombosis (DVT). The Wells clinical prediction guide incorporates risk factors, clinical signs, and the presence or absence of alternative diagnoses to enable physicians to reliably stratify patients into high-, moderate-, and low-risk categories (McLendon et al., 2017). The Caprini Risk Assessment Model is an ordinal scoring tool used to quantify and categorize a patient's risk for venous thromboembolism (VTE—an aggregate disease that includes both pulmonary embolism [PE] and deep vein thrombosis [DVT]). Similar to the Wells prediction guide, the Caprini Risk score stratifies patients into risk categories: very low risk, low risk, moderate risk, and three separate high-risk categories. Any potential subject that scores higher than moderate risk, will be excluded from the study.

In addition to pre-study assessments, to continuously monitor risks for DVT, we have incorporated safety measures during the ischemic conditioning procedure including safety checks for skin integrity (bruising or blistering on the skin), measures of blood pressure, oxygen saturation, and pain.

Additionally, we will assess potential subjects' risk for vascular disease using the ankle-brachial index (ABI) test. The ankle-brachial index (ABI) is a non-invasive tool for the assessment of vascular status (McClary & Massey, 2022). It consists of the ratio between the systolic blood pressure of the lower extremity, specifically the ankle, and the upper extremity (McClary & Massey, 2022). The ankle-brachial index test compares the blood pressure measured at the ankle with the blood pressure measured at the arm to determine if patient's have narrowed arteries or significant reductions in blood flow to the arms and legs. This ratio compares the resistance of the blood vessels, with one of the primary factors being the diameter of the vessels (McClary & Massey, 2022). To calculate the ankle brachial index, the blood pressure in an artery in the leg is divided by the blood pressure in an artery in the arms. The final product is a ratio representative of subject's potential risk for vascular disease. A low ankle brachial index number can indicate narrowing or blockage of the arteries in the legs. The opposite is true if blood pressure from the upper limb is divided by blood pressure from the lower limb (i.e., to determine narrowing in the arms). If the lower limb to upper limb ratio is less than 0.9, it may mean that a person has vascular disease in the blood vessels in his or her legs (McClary & Massey, 2022). If potential subjects' ratios are less than 0.9, they will be excluded from the current study.

Prior to enrollment of stroke subjects, we will also request access to medical records (Supplemental_Authorization for Release of Medical Information Cover Letter) to verify subject's history of medical conditions prior to enrollment in the study.

Eligibility Criteria:

Inclusion Criteria:

- Age >21 years old
- Single, monohemispheric stroke > 6 months since onset
- Residual hemiparetic gait deficits (e.g., abnormal gait pattern)

Exclusion Criteria:

- Lesions affecting the brainstem or cerebellum
- Other neurological disorders that may interfere with motor function
- Unhealed decubiti, persistent infections that may interfere with ability to perform test procedures
- Significant cognitive or communication impairment (Mini-Mental State Examination (MMSE<21)), which could impede the understanding of the purpose of procedures of the study
- Botulinum toxin (Botox) treatments to the lower limb within the past 6 months
- Pregnant women
- Contraindications to TMS or IC (Listed below)

TMS General Exclusion Criteria*:*

- Previous adverse reaction to TMS

- Skull abnormalities or fractures
- Concussion within the last 6 months
- Unexplained, recurring headaches
- Implanted cardiac pacemaker
- Metal implants in the head or face
- History of seizures or epilepsy
- Use of medications that could alter cortical excitability or increase risk of seizure (e.g., antidepressants, antipsychotics, anxiolytics, anticonvulsants)
- Current pregnancy

IC General Exclusion Criteria:

- History of thrombosis (i.e., blood clots) including venous thrombosis or deep vein thrombosis (DVT).
- Blood clots in the leg, or any condition in which compression of the thigh or transient ischemia is contraindicated (i.e., open wounds in the leg, bruising, nerve damage, etc.)
- Peripheral arterial grafts in the lower extremity
- History of uncontrolled hypertension
- History of peripheral vascular disease or hematological disease

8.0 Vulnerable Populations*

Pregnant women and children below the age of 18 will be excluded from the research. TMS is contraindicated in women who are pregnant as the effect of TMS on the unborn fetus is unknown. Women of childbearing age will be asked their pregnancy status during phone screening procedures and answers will be recorded in the appropriate phone screen documents (Supplemental_Phone Screen Healthy; Supplemental_Phone Screen Stroke; Supplemental_Phone Screen 2week IC Stroke). When women of childbearing age visit the lab, they will be given a pregnancy test to confirm they are not pregnant prior to enrollment. Results of the pregnancy test will be recorded in the appropriate screening documents (Supplemental_Screening Healthy; Supplemental_Screening Stroke; Supplemental_Screening 2week IC Stroke). Children are excluded from this study because of concerns regarding maturation of the nervous system that could introduce confounding variables into the TMS related measures in the study.

9.0 Number of Subjects

50 individuals with stroke will be required to attend up to one screening session and two training sessions (sham IC and real IC). All subjects will complete both conditions (sham IC and real IC) prior to corticomotor (CME and TCI) and behavioral (lower limb strength and ankle motor control) testing, and the order will be randomized, counterbalanced, and separated by at least one week.

As this study intends to include individuals with stroke of all severities, we expect most screened subjects to be enrolled. Those with contradictions to TMS or IC

during screening will not be included. In total we expect to screen 75 individuals with stroke and anticipate enrolling 50 individuals with stroke.

10.0 Recruitment Methods

Individuals with stroke regardless of gender, racial or ethnic status will be recruited using a flyer (Supplemental_Flyer Stroke) and email announcement (Supplemental_Email Announcement Stroke), which will be posted at UIC, the Chicago metropolitan area, and via the web (UIC Announcements, UIC-AHS labs webpage, UIC Massmail). In addition, we will make in-person vocal announcements regarding our research in classrooms on the UIC campus for additional recruitment. Flyers (Supplemental_Flyer Stroke) and email announcements (Supplemental_Email Announcement Stroke) will also be shared with stroke support groups and outpatient clinics for additional recruitment of individuals with stroke. Flyers (Supplemental_Flyer Stroke) and email announcements (Supplemental_Email Announcement Stroke), will provide contact information for interested individuals to directly contact a member of the research team. When potential subjects contact the Brain Plasticity Lab, key research personnel will explain the study and answer all questions. If the individual is interested, they will be screened over the phone using the attached screening documents (Supplemental_Phone Screen Stroke). If the individual is determined to be eligible over the phone, they will be scheduled for a visit to the laboratory for an in-person screening (Supplemental_Screening Stroke). All subjects will be screened in person in the Brain Plasticity Lab regardless of the initial mode of recruitment (i.e., initial phone screens will be screened again in person). Screen failures (phone or in-person) will be excluded from the study, and data from screen failures will be securely disposed.

Furthermore, individuals with stroke will also be recruited by physician referral from the Department of Neurology at UIC. Physicians will inform eligible patients about the study during their routine visits to the clinic by providing them with a copy of the flyer (Supplemental_Flyer Stroke). It will be made clear that the study is solely being conducted for research purposes and will in no way provide treatment for their symptoms. If the patient is interested in knowing more about the study, the patient will initiate contact with key research personnel. Screening and informed consent will be conducted by key research personnel only and not by the referring physician (to avoid possible coercion by the treating neurologist who may be the patient's primary care physician).

To aid recruitment, we will also use the UI Health EPIC electronic health record system. Specifically, we will use EPIC to identify potential participants who have had a stroke and have been treated at UI Health. Individuals who have had a stroke and meet other study inclusion criteria will be contacted first with one email (Supplemental_Email Script). After 7 days, if the research team has not received a response to the email, we may contact the individual with one phone call (Supplemental_Phone Script). No additional attempts will be made to contact potential subjects if no response is received to

the email or phone call. If a response is received from the individual indicating interest in the study, they will undergo screening procedures.

Subjects will receive $20 per screening session and $20 per training session up to $60 for three sessions. Compensation will be provided in cash immediately at the end of each visit. If subjects discontinue, we will arrange to send payment in the mail or arrange for subjects to pick up payments.

11.0 Procedures Involved*

Subjects will be screened for study eligibility prior to participation in all Aims.
All key research personnel will be provided the adequate training and equipment needed to carry out the study. Training will be repeated as necessary. Data collection and analyses will be performed by authorized personnel only. Subjects will be identified using a unique alpha-numeric code. Only the authorized study personnel will have access to the name associated with this code. This code and name will be in a secure, password protected database accessible only to study personnel.

The principal investigator (PI) will be primarily responsible for monitoring the safety of subjects, and the safety and confidentiality of data. This will be done by following the best practices to ensure that we strictly adhere to the inclusion and exclusion criteria, and protections against risks outlines. The safety of each subject will be assessed on an individual basis throughout his or her involvement in the study. Any decline in function or subject responses that indicates a potential adverse effect will be reviewed individually. In the case of a subject being excluded from research participation based on TMS, or IC safety questionnaires, the PI will maintain confidentiality of these data.

A member of the study team will be available to each subject throughout testing and will be monitoring participant safety. Subjects will be instructed to immediately inform study personnel if any adverse effects of treatment are experienced. We will ask subjects to provide us with a phone number of a caregiver (or friend or family member) to contact in case of emergency. Any adverse events or unanticipated problems will be recorded and communicated to all study personnel as soon as possible. Subjects will be removed from the study if the study procedures threaten the subjects' safety or study procedures are unbearable. Any adverse events will be noted within the subject file.

Any unexpected experience, adverse event or outcome related to the research will be closely monitored by the research personnel and reported to Dr. Madhavan immediately. Dr. Madhavan will take necessary action according to the nature of unforeseen problem which may include reporting of adverse events to the IRB and funding agency (if funded), suspension of research procedures in currently enrolled subjects; modification of research procedures for new subjects; changes of the informed consent document/process to ensure subject safety and privacy, modification of inclusion or exclusion criteria to mitigate the newly identified risks; implementation of additional procedures for monitoring subjects; and/or suspension of enrollment of new subjects.

Motor and Neurophysiological Changes after Ischemic Conditioning in Individuals with Stroke

General Overview:

Aims 1 and 2 are a repeated measures cross-over study design with each subject undergoing two sessions of IC (real and sham).

Aims 1 and 2:

If potential subjects are deemed eligible for the study through phone screening, subjects will visit the lab for on-site screening (see details below). Once qualified, clinical measurements including motor impairment, ankle range of motion, cognition, quality of life, disability, and walking analyses (walking endurance and spatiotemporal characteristics) will be assessed. All of the tests to be used are commonly used in the clinic to measure motor and cognitive function as well as quality of life in individuals with stroke. A research investigator will be in attendance and will ensure safety at all times during the session.

After screening procedures, each subject will come to the lab on two separate occasions separated by at least one week (2 weeks total). Subjects will experience one session of sham IC and one session of real IC (randomized order). Baseline measures of TMS followed by lower limb strength and ankle motor control will be recorded to begin both sessions (sham IC and real IC). The duration of sham IC and real IC will be 50 minutes in total (cycles of 5-min of occlusion followed by 5-min of reperfusion). TMS, strength, and ankle motor control will be recorded again immediately after sham IC and real IC procedures as well as 30-minutes post both procedures. To maintain subject safety and study validity, subjects with severe impairments who cannot perform the 10MWT safely, will not perform tests of gait speed (10MWT) but will experience all other study endpoints (corticomotor excitability, transcallosal inhibition, muscle strength, ankle motor control). If subjects with severe impairments are able to perform 10MWT safely using an assistive device (i.e., cane or walker), then gait speed will be collected using the 10MWT. Subjects with mild or moderate impairment levels will experience all study endpoints including gait speed.

Motor and Neurophysiological Changes after Ischemic Conditioning in Individuals with Stroke

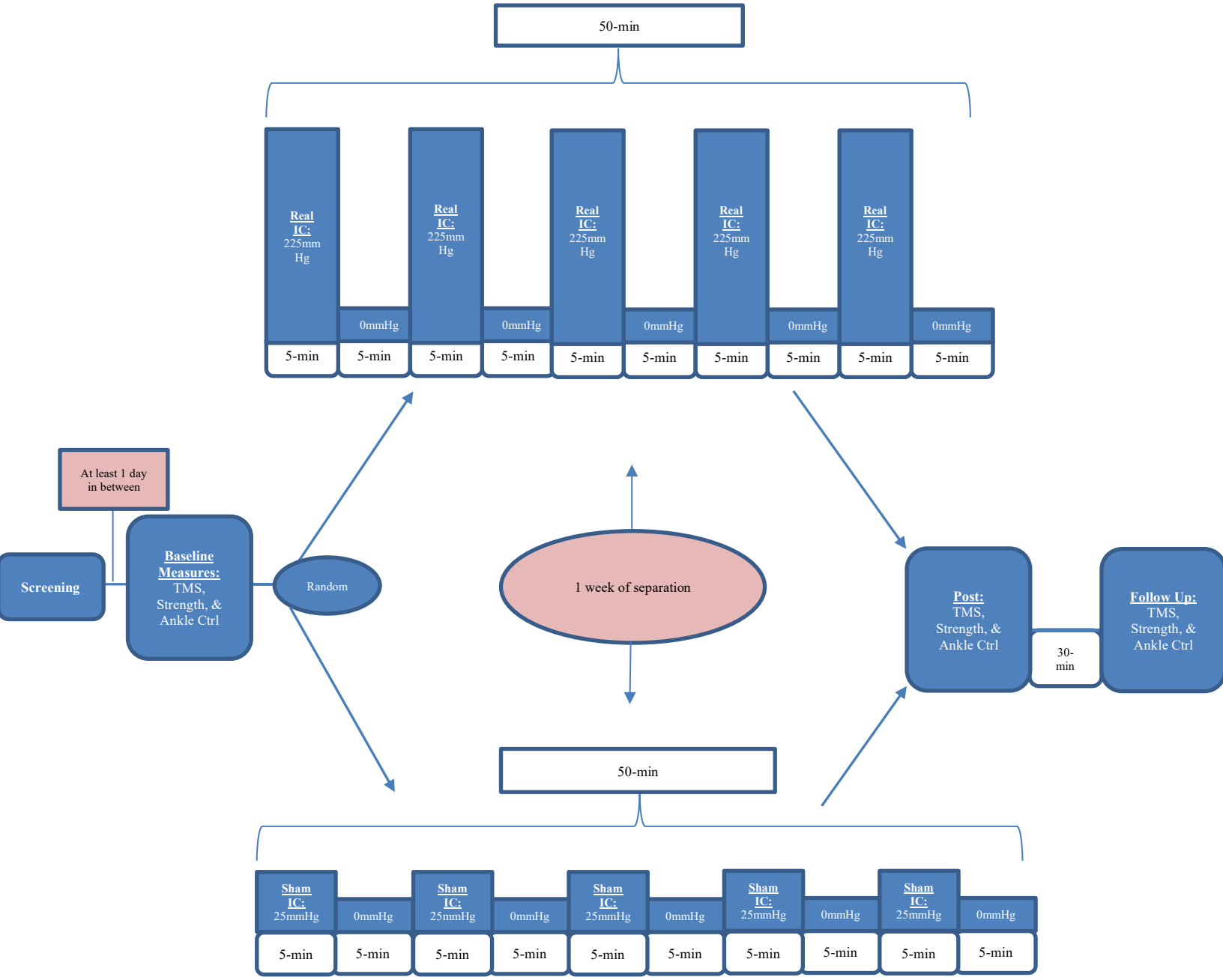

Figure 1: Aims 1 & 2 Study Design. Stroke subjects will be randomized to receive either sham IC or real ICseparated by at least one week (2 weeks total). For IC sessions, with subjects in a seated position, the cuff will be placed over subjects' paretic thigh and inflated to either 225mmHg (real IC) or 25mmHg (sham IC) for 5 minutes followed by 5 minutes of no inflation (0mmHg). This will be repeated five times for a total of 50 minutes. Measures of TMS, lower limb strength, and ankle motor control will be recorded prior to, immediately after, and 30-minutes after both procedures (sham IC and real IC).

Motor and Neurophysiological Changes after Ischemic Conditioning in Individuals with Stroke

Screening Procedures:

Aims 1 and 2:

Subjects will be screened to determine if they qualify for the study using the listed inclusion and exclusion criteria. We will interview subjects and use their medical records to obtain stroke-related, demographic, and descriptive information. For subjects seen at UI Health, stroke-related and descriptive information will be obtained through the UI Health EPIC electronic health record system. For subjects not seen at UI Health, we will complete a separate authorization form (Supplemental_Authorization for Release of Medical Information Cover Letter) requesting their non-UI doctor to give the information to Dr. Sangeetha Madhavan. We will also have subjects complete the 'Supplemental_Screening Stroke' document which includes the TMS and IC safety questionnaire to ensure safety with all study procedures. Once subjects are determined to be eligible to participate, we will obtain informed consent from subjects and perform clinical assessments of movement impairment, ankle range of motion, cognitive function, quality of life, disability, and walking analyses (walking endurance and spatiotemporal characteristics). See below for detailed explanations. During the screening process, we will collect contact information for each subject, including: name, address and zip code, phone numbers, and email addresses (Supplemental_Screening Stroke).

Ankle Range of Motion: Ankle range of motion will be tested by a trained evaluator using a manual range of motion testing tool (goniometer). We will test ankle dorsiflexion and plantarflexion range in the supine lying position (active and passive).

Cognitive Function: Cognitive function will be assessed with the Mini-Mental State Examination (MMSE), a quantitative assessment of cognitive impairment.

Motor Impairment: Lower extremity impairment will be measured using the Fugl Meyer Lower Extremity Scale (FMLE) - a series of tests of movement, reflex activity, coordination/ speed, sensation, and range of motion.

Walking Endurance: Walking endurance will be measured using the 6-Minute Walk test. Subjects will walk as far as possible within 6 minutes.

Walking spatiotemporal characteristics: During the 6-Minute Walk test, spatiotemporal parameters of walking will be assessed using APDM wearable sensors. Outcome measures of walking capacity will include cadence, stride length, individual step lengths, step times, and the absolute and relative times of stance and swing phases. Measures of interlimb symmetry, defined as ratios of paretic to nonparetic values of the spatial and temporal variables, will be calculated.

Quality of life: Quality of life will be measured with the EuroQol-5D (EQ-5D), a questionnaire with questions designed to assess aspects of quality of life.

Disability: Global disability will be measured with the modified Rankin Scale (mRS), a simple 0-6 rating scale. Disability will also be measured using the Barthel Index which assesses a person's ability to perform activities of daily living by assigning a numerical score based on individual performance in tasks such as feeding, bathing, grooming, dressing, using the toilet, transferring from a bed to a chair, walking, and climbing stairs. Scores typically range from 0 to 100, with lower scores indicating greater disability.

Transcranial Magnetic Stimulation (TMS) Procedure:

TMS is a safe, non-invasive, and painless method of brain stimulation that has been widely used to study the physiology of the muscle representations in the motor cortex in healthy and neurologically disordered individuals (Anand & Hotson, 2002). Very short duration (<1 ms) magnetic pulses are applied via an insulated wire coil placed on the intact scalp overlaying the motor cortical area projecting to a target muscle. Each pulse induces a MEP in the target muscle that can be readily monitored by recording EMG with electrodes taped to the muscle of interest.

Prior to the start of TMS testing, while seated, maximum voluntary isometric contractions (MVIC) for each target muscle will be obtained with the ankle in neutral position restricted by a tightened metal clamp attached to a wooden board. EMG electrodes will be applied over the muscle bellies of each target muscle. Standard skin preparation techniques (light abrasion and cleansing with alcohol) will be completed prior to application of the electrodes. A ground electrode will be applied over the patella or other bony prominence. The ground electrode will be placed over a bony prominence at the level of the 7th cervical vertebra (C7) or below. This ground electrode will require small amounts of ultrasound transmission gel (Park Laboratories Inc, Fairfield, NJ, USA) which may be uncomfortable for some subjects, but the area will be cleaned thoroughly with paper towels and alcohol wipes upon session completion. EMG recordings will be amplified (Bagnoli, Delsys, Boston, MA), band-pass filtered (10-1000 Hz), and sampled at 2000 Hz. EMG activity will be collected from the all the muscles bilaterally. Three five-second contractions will be conducted with real-time visual feedback.

Magnetic stimuli will be delivered via a double cone coil (diameter 110mm) with a posterior-anterior orientation connected to a Magstim 200 unit (Magstim Company, Boston MA). A double cone coil is typically used to deliver focal magnetic pulses to a number of scalp sites over the cortical area representing a muscle of interest. First, the TMS coil will be systematically moved over the targeted M1 to identify the location of the "hotspot", defined as the location with the largest, consistent contralateral MEP, while maintaining a tonic contraction corresponding to 10% MVIC of the target muscle. For stroke subjects, the hotspot

location of the lesioned M1 will be mirrored from the location determined for the non-lesioned M1. Resting motor threshold (RMT) for the muscle of interest will be defined as the stimulator output intensity that can elicit a MEP with peak-to-peak amplitude of at least 0.075 mV in four out of eight trials. It will be determined by increasing stimulus intensity in steps of 1% stimulator output. Active motor thresholds (AMT) will also be determined with the same protocol and defined by the lowest stimulus intensity required to elicit a MEP with a peak-to-peak amplitude of at least 0.1 mV in four out of eight trials with the subject contracting the muscle of interest to about 10% MVIC.

CME of the paretic TA muscle representations will be measured with single pulse TMS (Magstim, Dyfed, Wales, UK). To assess corticospinal excitability of the M1, the double cone coil will be placed over the M1 cortical area (near the vertex of the head), and the subject will then receive 15 stimuli at intensities corresponding to 130% of rest and active motor threshold at each designated timepoint (See Figures 1, 2, and 3). Changes in descending CME will be quantified from TMS as changes in mean peak-to-peak MEP amplitude. These measures will assist the investigators to assess the contribution of the motor cortex to lower limb muscles.

Transcallosal inhibition (TCI) will also be measured with single-pulse TMS to assess the changes in interhemispheric inhibition from the lesioned to non-lesioned hemisphere at designated timepoints (See Figure 1): before/ after real IC and sham IC. TCI from the stimulated hemisphere to the non-stimulated hemisphere will be quantified as a measure of the ipsilateral silent period (Davidson et al., 2016). During TCI, the participant will be instructed to maintain ~ 50% maximum voluntary contraction while a suprathreshold pulse (130% of active motor threshold) is delivered to the hemisphere ipsilateral to the active muscle. This would enable the measurement of the ipsilateral silent period (iSP) which serves as a measure of TCI. The iSP in the contralateral TA is defined as the time after the MEP when EMG is below 25% of the background EMG. We will perform 5 trials to obtain iSP with an interval of ~60 seconds between trials to avoid fatigue.

Ischemic Conditioning Procedure:

A rapid inflation cuff (Hokanson SC12 thigh cuff) similar to those used to measure blood pressure, will be placed on the proximal thigh of the paretic leg while subjects are seated in a chair.
Prior to IC procedures, the cuff will be inflated to determine each subjects' limb occlusion pressure (leg receiving IC) which will last <1-minute. For all Aims in the proposed study, the cuff will be inflated to 225mmHg for real IC, and 25mmHg for sham IC. The exact same set up used for real IC will be used for sham IC (same cuffs, same device, same body positions, etc.). The only difference between real and sham IC will be the amount of pressure in the cuff (225mmHg vs. 25mmHg). For all Aims in the proposed study, the cuff will be inflated for 5 minutes followed by 5 minutes of no inflation (0mmHg). Each cycle lasts 10 minutes (5 minutes of inflation followed by 5

minutes of no inflation). Subjects will complete 5 cycles of real and sham IC for a total of 50 minutes. These exact methods have been used previously with stroke participants without any adverse events or negative feedback from participants (Hyngstrom et al., 2018; Durand et al., 2019; Hyngstrom et al., 2020). During IC, we will visibly monitor signs of skin integrity by loosening the cuff after the first inflation cycle to examine for any bruising or blistering on the skin. For all Aims in the proposed study, ratings of fatigue (0-10 scale) and pain (0-10 scale) will be monitored throughout the protocol and the pressure will be reduced or stopped completely if intolerable. Likewise for all Aims, signs of nerve compression will be monitored throughout IC procedures via verbal feedback regarding tingling or numbness. If these symptoms persist during periods of reperfusion (no cuff inflation), the activity will be stopped immediately. Blood pressure (BP), heart rate (HR), and oxygen saturation levels will be monitored throughout, and the activity will be stopped immediately if necessary. Participants will be contacted the day after their first IC treatment to follow-up regarding any current or lingering symptoms from the procedure.

The equipment used for IC will be stored in a locked room in Dr. Madhavan's laboratory. Only key research personnel will have key access to the storage room.

Summary of Procedures

Aims 1 and 2:

- Visit # 1 (2-3 hours) - Subjects will undergo:
  - Screening using the appropriate documentation (Supplemental_Screening Stroke) which will collect information related to demographics, study eligibility criteria, medical conditions (Medical Screening Questionnaire), and IC and TMS safety.
  - Clinical assessments of movement impairment (FMLE), ankle range of motion, cognitive function (MMSE), quality of life (EQ-5D), disability (modified Rankin scale, Barthel Index), and walking analyses (walking endurance and spatiotemporal characteristics).
- Visits # 2 and 3 (3-4 hours) - Subjects will undergo:
  - (Pre/ Baseline) TMS measures of CME and TCI
  - (Pre/ Baseline) assessments of lower limb strength and ankle motor control
  - 50 minutes of real IC or sham IC
  - (Post) TMS measures of CME and TCI
  - (Post) assessments of lower limb strength and ankle motor control
  - (30-minute Follow Up) TMS measures of CME and TCI
  - (30-minute Follow Up) assessments of lower limb strength and ankle motor control

For Studies that Collect Existing or Prospective Data:

Motor and Neurophysiological Changes after Ischemic Conditioning in Individuals with Stroke

We will be collecting prospective data from our participants, at the time points outlined above. Hard copies of any data will be stored in a locked office in a locked file cabinet in the UIC College of Applied Health Sciences (AHS), and all digital data will be stored in a password-protected computer on the AHS server. Data entered in any spreadsheet, either digital or print, will be presented de-identified via subject's unique alphanumeric code, save for the original screening forms and consent documents, which will contain participants names and contact information. Only authorized study personnel will have access to this data. All hard copies of data will be properly disposed of 10 years after the completion of the study.

12.0 Data and Specimen Banking*

NA

13.0 Sharing of Results with Subjects*

If requested, results of the experiments will be shared with subjects. However, raw data will not be shared with subjects. Study information from this study may be reviewed by representatives of the study group at University of Illinois at Chicago, the Institutional Review Board (IRB) at the University of Illinois at Chicago, and perhaps, the U.S. Food and Drug Administration (FDA), or the National Institutes of Health (NIH). Study records will be kept confidential to the extent provided by law. Names of individual subjects or any other identifying data will not be used in any report or publication of this study.

14.0 Withdrawal of Subjects*

Subjects will be reassured of their right to withdraw from the study at any time. The voluntary nature of participation will be reiterated, and subjects will be informed that their decision about participation will not affect their relationship with the UIC or any of its related entities. Subjects will be removed from the study if the study procedures threaten the subject's safety or study procedures are unbearable. Subjects will be compensated for completed sessions. All previously collected data for a subject will be de-identified and used for the final analysis following their withdrawal from the study. All personal identifiable data will be destroyed 10 years after study completion. Results published will not include names of subjects.

Any unexpected experience, adverse events or outcomes related to the research will be closely monitored by the research personnel and reported to Dr. Madhavan immediately. Dr. Madhavan will take necessary action according to the nature of the unforeseen problem which may include suspension of research procedures in currently enrolled subjects; modification of research procedures for new subjects; changes of the informed consent document/process to ensure subject safety and privacy, modification of inclusion or exclusion criteria to mitigate the newly identified risks; implementation of additional procedures for monitoring subjects; and/or suspension of enrollment of new subjects.

15.0 Risks to Subjects*

The measurements made in this study present a minimal risk. The following specific risks exist for this study:

Risks associated with TMS:

1) Seizures: rare cases have reported the development of seizures during or immediately after magnetic brain stimulation. Individuals who have a history of seizures or have been diagnosed with epilepsy will be excluded from this research study. Single and paired-pulse TMS that we use in this study have been deemed to carry little risk beyond occasionally causing local discomfort. Our stimulation procedures follow published safety guidelines. Seizure activation is extremely unlikely with the single/ paired-pulse low numbers of stimulation proposed in the current investigation.
2) Discomfort: a small number of people find TMS uncomfortable, particularly at high intensities of stimulation. If individuals report feelings of discomfort, stimulation intensity will be reduced or, if not feasible, testing will be terminated.
3) Muscle twitching: subjects may feel twitches in the muscles of the arm, leg or face during the magnetic stimulation.
4) Noise: a loud click during magnetic stimulation may be heard. Subjects will be provided with foam earplugs that can effectively prevent this discomfort.
5) Pregnant women: the risks of TMS on pregnant women are unknown. If there is any doubt, TMS testing will not be initiated until the nonpregnancy is confirmed. Pregnant women will be excluded from TMS.
6) Skin irritation: there is a risk of mild skin irritation at the location where the electrode sensors have been placed, but this usually consists of minor redness that will go away quickly after they are removed.

Risks associated with clinical assessments:

1) Muscle soreness or fatigue: during or following clinical assessments, subjects may experience muscle soreness 24-72 hours after testing or general fatigue. We will always check with the subject before proceeding with testing or training. Clinical assessments or exercise will be terminated if muscle soreness or fatigue becomes intolerable.
2) Loss of balance, falls, and/or orthopedic injuries: subjects may lose their balance while getting up from sitting or walking during study procedures. Subjects also have a risk of falling and/or orthopedic injuries during clinical assessments, although the risks are no more than those experienced in everyday life. All clinical assessments will be conducted under the supervision and instruction of study personnel.

Risks associated with ischemic conditioning:

1) Discomfort: subjects may experience discomfort due to the pressure of the blood pressure cuff.
2) Bruising and skin irritation: there is a risk of bruising and skin irritation around the area where the IC cuff is placed. To monitor signs of skin integrity, the cuff will be loosened after the first inflation cycle to examine for any bruising or blistering on the skin.
3) Tingling: subjects may experience tingling of the limb and feet and a rare but potential risk of nerve damage. Signs of nerve compression will be monitored throughout IC via verbal feedback regarding tingling or numbness. If these symptoms persist during periods of reperfusion, the activity will be stopped immediately. Subjects will be contacted the day after their first IC treatment to follow-up regarding any current or lingering symptoms from the procedure.

Other Risks:

1) Worsening of pre-existing medical condition: if a subject notes a worsening of a pre-existing medical condition because of IC or TMS, they will be instructed to notify study personnel immediately. Although the exact safety plan may be specific to the subject's underlying medical condition, information regarding an emergency contact number and contact details for the nearest clinic/hospital will be provided. Study procedures will be terminated immediately.
2) Loss of privacy or confidentiality: medical information and other study related data that is collected may be unintentionally accessed by non-study personnel. Subject data will be coded and stored in locked filing cabinets (paper format) or on password-protected computers (excel format) which are located in a locked office (AHSB Rm 810) to minimize this risk. Data access will be limited to key research personnel.
3) Uncomfortable emotions: subjects may experience mild anxiety or embarrassment in answering questions of personal nature regarding their health or well-being. However, all information collected through study questionnaires will be kept confidential and all personal identifiers will be removed. In addition, only the study's key personnel will have access to this information.

16.0 Potential Benefits to Subjects*

There are no direct benefits to individuals participating in this research project.

17.0 Data Management* and Confidentiality

Data and Statistical Analysis:

Strength will be represented as raw force output values and comparisons will be made between the paretic and nonparetic legs for stroke subjects. Ankle motor control will be measured as reaction time using the paretic ankle which will be defined as the duration between visual signal presentation and the onset of the EMG response in the TA muscle.

Changes in descending CME will be quantified from TMS as changes in mean peak-to-peak MEP amplitude obtained from the TA muscle in the paretic lower limb. TCI from the stimulated hemisphere to the non-stimulated hemisphere will be quantified as a measure of the ipsilateral silent period (Davidson et al., 2016).

Based on previous studies, a statistical power analysis was conducted for sample size estimation using G*Power software. We used change in peak MEP amplitude after TMS as the primary outcome measure. For a meaningful change in peak MEP amplitude, the estimated sample size is 35 which provides at least 80% power with $\alpha = 0.05$. Based on our previous experiences with attrition rates, we will recruit 45 subjects to achieve the desired power.

Normality of outcome distributions will be confirmed using Shapiro–Wilk tests for all dependent variables. Baseline comparisons of neurophysiological and behavioral outcomes between the two experimental conditions (real IC and sham IC) will be evaluated using change scores and paired samples t-tests. To assess the main effects and interactions of condition (IC vs. sham-IC) and time (pre, post, post-30), two-way repeated-measures ANOVAs will be conducted separately for each outcome variable (CME, TCI, strength, and motor control). When significant main effects or interaction terms are observed, follow-up pairwise t-tests will be performed. Statistical analyses will be conducted using RStudio (version 4.0.2) and statistical significance will be set at a *p*-value of 0.05. Effect sizes will be calculated for ANOVAs using eta squared (η^2) for ANOVA models and interpreted according to Cohen's benchmarks: small ($\eta^2 = 0.01$), medium ($\eta^2 = 0.06$), and large ($\eta^2 = 0.14$) (Cohen, 1988).

Data Management and Security:

The research will be conducted in compliance with state and federal laws, including the Health Insurance Portability and Accountability Act (HIPAA) which require researchers to protect an individual's health information. Data will be stored and analyzed in the Brain Plasticity Lab (Rooms 810, 834, 447), 1919 W. Taylor St., Chicago, IL 60612. To minimize the risk of the loss of privacy and confidentiality, subject data will be coded and stored on a secure HIPAA compliant application (Box for Healthcare – UIC Health Data Box folder). Data will be processed and stored under the assigned participant code. The key code linking the subject's participant number to his/her identity will be stored on a secure HIPAA compliant application (Box for Healthcare – UIC Health Data Box folder) separate from the data and destroyed ten years after study completion. All study personnel will have access to the key code and raw data. Study information from this study may be reviewed by representatives of the study group at University of Illinois at Chicago, the Institutional Review Board (IRB) at the University of Illinois at Chicago, and perhaps, the U.S. Food and Drug Administration (FDA), or the National Institutes of Health. Study records will be kept confidential to the extent provided by law. Names of individual subjects or any other identifying data will not be used in any report or publication of this study.

The principal investigator will be primarily responsible for monitoring the confidentiality of data. Authorized study personnel will have access to the raw data. The raw data will not be made available to any other individuals.

Quality Control Procedures:

We will employ several quality control procedures to maximize the validity and reliability of protocol delivery and outcome assessments namely written protocols for the research personnel, and training research personnel. Dr. Madhavan will be actively involved in supervising and monitoring the study daily. She will meet with all study personnel at least once a week to ensure that there is strict adherence to study protocol and outcome assessments. All key research personnel will be provided the adequate training and equipment needed to carry out the study. Training will be repeated as necessary. Dr. Madhavan will check and regularly review any adverse events or deviations from protocol. Study personnel will monitor subject compliance to the protocol and losses to follow up.

18.0 Provisions to Monitor the Data to Ensure the Safety of Subjects*

NA. The measurements made in this study present a minimal risk.

19.0 Provisions to Protect the Privacy Interests of Subjects

The PI will be primarily responsible for monitoring the safety of subjects. This will be done by following the best practices to ensure that we strictly adhere to the inclusion and exclusion criteria, and protections against risks outlines. The safety of each subject will be assessed on an individual basis throughout his or her involvement in the study. Any decline in function or participant responses that indicates a potential adverse effect will be reviewed individually.

Screening and testing of subjects will be conducted in private rooms. Subject's privacy will be protected at all times by ensuring that only key research personnel are present during sessions. An alphanumeric code will be used to identify each subject. Authorized research personnel will maintain a master sheet which has the subject's name linked to his/her code (initials followed by a number). This sheet will be maintained on the lab computer in a password protected file that only the research team directly working on the project has access to. Other data will be maintained on the lab computers which only authorized personnel have access to. Security of these computers are maintained by the College of Applied Heath Science IT personnel. All personal identifiable data will be destroyed 10 years after study completion. Results published will not include names of subjects.

A member of the study personnel will be available to each participant throughout testing and will be monitoring subject safety. Subjects will be instructed to immediately inform study personnel if any adverse effects of treatment are experienced. We will ask subjects to provide us with a phone number of a caregiver (or friend or family member) to contact

in case of emergency. Any adverse events or unanticipated problems will be recorded and communicated to all study personnel as soon as possible. Subjects will be removed from the study if the study procedures threaten the subject's safety or study procedures are unbearable. Any adverse events will be noted within the subject file.

Subjects will be reassured of their right to withdraw from the study at any time. The voluntary nature of participation will be reiterated, and subjects will be informed that their decision about participation will not affect their relationship with the UIC or any of its related entities.

Subjects will continuously be reminded of the rare/ minimal risks associated with study procedures and reminded that all information they supply is voluntary.

20.0 Compensation for Research-Related Injury

A member of the research team will be available to the subject throughout testing and will be monitoring subject safety throughout. We will ask subjects to provide us with a phone number of a person to contact in case of emergency. In the event of injury related to this research, treatment will be available. However, the subject or his/her third-party payer, if any, will be responsible for payment of this treatment. In the unlikely event that the subject requires emergency medical care during a study visit, 911 will be called immediately. There is no compensation and/or payment for such medical treatment for such injury except as may be required by law.

21.0 Economic Burden to Subjects

Participants will be required to provide their own means of travel and will be responsible for any travel-related costs which may be partially reimbursed.

22.0 Consent Process

Subjects will be provided with a written consent form and may be assisted with understanding by the research therapist, the investigators, attending clinicians, or family members. Voluntary, informed consent will be obtained from all subjects by key research personnel prior to participation in any study procedures. As part of the informed consent process, the key research personnel will discuss the written informed consent document approved by the IRB at UIC. All study information, including purpose, procedures, risks, and benefits will be reviewed. Subjects will be given sufficient time to understand the form and will have the opportunity to ask any questions during and after the consent process. Subjects will be reassured of their right to withdraw from the study at any time. The voluntary nature of participation will be reiterated, and potential subjects will be informed that their decision about participation will not affect their relationship with the UIC or any of its related entities. Only individuals with the capacity to consent will be included in the study. The research personnel will ask specific questions about the study

to assess whether the participant understands the involved procedures. If they choose to participate in the study, subjects will provide informed written consent in the presence of the investigators. Subjects will be provided with a copy of the informed consent, so they can reference any information or contact details.

Written consent will be obtained in person at Brain Plasticity Lab (Rooms 810, 834, 447), 1919 W. Taylor St., Chicago, IL 60612. Study personnel will explain the study, and subjects will review the informed consent document. All questions regarding the study will be answered by the study personnel obtaining informed consent. Informed consent will be obtained in English using lay language. The subject will receive a copy of the signed informed consent document, and the investigator will keep the original copy in the research records. The informed consent document will be accessible by all study personnel.

23.0 Process to Document Consent in Writing

We will be documenting consent in writing with HRP-091-SOP-Written Documentation of Consent.

24.0 Setting

All subject recruitment and research procedures will be performed at the UIC Brain Plasticity Lab located at 1919 W. Taylor St., Rooms 447/ 810/ 834 (Applied Health Sciences Building) Chicago, IL 60612.

25.0 Resources Available

The Brain Plasticity Lab has excellent access to subject recruitment due to its location in the Illinois Medical District of Chicago, near the John H Stroger Hospital of Cook County, Rush University Medical Center, University of Illinois Medical Center, and the Jesse Brown VA Medical Center. Subjects for this study will be recruited from the various outpatient clinics in the greater Chicagoland area, including the University of Illinois at Chicago, Shirley Ryan Ability Lab, Rush University Medical Center, Schwab Rehabilitation Hospital, and the University of Chicago. Through Dr. Madhavan's well-established working relationships with neurologists at UIC, we have access to a stroke registry maintained by the Department of Neurology at UIC. This registry gives us access to an additional ~1000 individuals with stroke who have given their consent to share their contact information with research personnel. The database is extremely useful for recruitment since it includes detailed medical information, such as lesion location and impairment level. Dr. Madhavan has also cultivated collaborative relationships with other hospitals in the area resulting in participant recruitment from these sites as well. In the past, previously mentioned recruitment resources have resulted in a complete R01-funded clinical trial (with strict recruitment criteria) with over 80 participants. Based on the resources above, we do not anticipate any problems in recruiting the 100 individuals required for the

proposed study. We anticipate enrolling all study subjects within 3-5 years, and primary analyses will be completed one year following the completion of subject recruitment.

Resources are available for consequences of human research. Measures of HR, BP, oxygen saturation, pain, and RPE will be taken during sessions to mitigate potential consequences of human research and monitor any serious adverse events. Additionally, during IC procedures, the rapid inflation cuff will be loosened to inspect any damage to the skin and subjects will be asked to give feedback regarding painful sensations. All study procedures will be stopped if the study safety criteria are violated. Water, Gatorade, snacks, and first aid kits are available in the event of non-serious medical events. The Brain Plasticity Lab contains plenty of seating and a physical therapy bed in case subjects need to rest in certain positions at any point during the study procedures. In the event a subject needs immediate medical attention, they will be taken to the University of Illinois Hospital (1740 W Taylor St, Chicago, IL 60612) located 0.4 miles from the Brain Plasticity Lab.

The Brain Plasticity Lab has a long history with priming procedures including stimulation-based priming (i.e., tDCS) and movement-based priming via ankle movements and aerobic exercise on either a treadmill or recumbent stepper. We have successfully completed numerous priming procedures without any serious adverse events. We will employ several quality control procedures to maximize the validity and reliability of protocol delivery and outcome assessments namely written protocols for the research personnel, and training research personnel. Dr. Madhavan will be actively involved with supervising and monitoring the study on a day-to-day basis. She will meet with all study personnel at least once a week to ensure that there is strict adherence to study protocol and outcome assessments. Dr. Madhavan will also hold bi-monthly journal club meetings to ensure that all key personnel are abreast of the scientific literature in the area and the importance of the current research and thereby motivate staff to ensure their best performance during training and assessment. All key research personnel will be provided the adequate training needed to carry out the study. Training will be repeated as necessary. Dr. Madhavan will check and regularly review any adverse events or deviations from protocol. Study personnel will monitor subject compliance to the protocol. In addition to quality control procedures, research personnel will have access to the UIC Center for Clinical and Translational Science (CCTS) which provides strategic services related to research design and analysis, research dissemination and implementation, biostatistics, community engagement, clinical research, regulatory and bioethics, recruitment and retention, and funding advice and support. Additionally, CCTS also helps young investigators navigate complex research processes and manage logistics.

26.0 Multi-Site Research*

NA

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Motor and Neurophysiological Changes after Ischemic Conditioning in Individuals with Stroke