COVER SHEET

NCT03194464

Neural Mechanisms Mediating Interlimb Transfer Following Stroke

Study Protocol
and
Statistical Analysis Plan

14 June 2019

Research Design and Methods

For Aim 1 all participants will be studied in a single session. For Aim 2 participants will be studied over eight sessions distributed across four weeks. Transcranial Magnetic Stimulation (TMS) will be used to target motor responses in both paretic (P) and non-paretic (NP) first dorsal interosseus muscles (FDI) while stimulating the contralateral hemisphere primary motor cortex (M1). The primary neurophysiological outcome is short intracortical inhibition (SICI); the primary behavioral outcome is the Box and Blocks test (BBT)[1].

1. Participants: Justification and Recruitment

We will enroll a total of 24 persons post-stroke from the surrounding community. Through the PI's appointment at the VA Brain Rehabilitation Research Center (BRRC), we have access to the BRRC participant registry, which currently holds records for over 2500 individuals who have undergone an extensive screening process in which they are evaluated by a neurologist, physical therapist and speech pathologist (1-2 additional participants are typically added weekly). All are confirmed to have had a stroke by MRI or CT scan and have consented to be contacted for possible inclusion in research studies. Currently the database lists over 500 persons meeting our inclusion criteria. Given the large pool of appropriate candidates, the ongoing screening of new participants, and the interest expressed by persons post-stroke to participate in studies, we expect no difficulties in meeting our recruitment goals. Recruitment priority will be given to veterans. Approximately 25% of the individuals in the BRRC database are veterans.

2. Inclusion and Exclusion Criteria

Following screening and study enrollment, all participants will provide informed consent and be evaluated by a Physical Therapist. Individuals meeting the following inclusion criteria will be eligible for participation: 1) clinical presentation of a single hemispheric stroke (confirmed by CT or MRI) with resulting hemiparesis; of at least 6 months duration; 2) demonstrated ability to move the UE in the horizontal plane, 3) able to form and release a power grip; 4) freedom from significant UE joint pain, passive range of motion limitations, and marked sensory deficits as evidenced by absent proprioception at the elbow or shoulder joints[2]; 5) UE Fugl-Meyer score of ≥22/66 points; 6) absence of severe perceptual or cognitive deficits as determined by using the MoCA[3]; 7) absence of severe osteoarthritis or prior pathological fracture; and 8) absence of significant cardiovascular impairments contraindicative to exertion. **Exclusion criteria** include: 1) severe perceptual or cognitive deficits as identified using the MoCA[3]; 2) severe osteoarthritis or prior pathological fracture; 3) significant cardiovascular impairments contraindicative to exertion; 4) use of medications that may lower seizure threshold; 5) history of epilepsy, brain tumor, learning disorder, mental retardation, drug or alcohol abuse, dementia, major head trauma, or major psychiatric illness; 6) evidence of epileptiform activity on electroencephalography obtained prior to screening; 7) history or radiographic evidence of arteriovenous malformation, intracortical hemorrhage, subarachnoid hemorrhage, or bilateral cerebrovascular disease; 8) history of implanted pacemaker or medication pump, metal plate in skull, or metal objects in the eye or skull; 9) pregnancy; 10) inability to understand 3-step directions; 11) impaired corrected vision that would alter ability to perform force matching tasks with visual feedback.

3.Clinical Assessments

Participants will be assessed using a battery of clinical measures including assessments of impairment (UE Fugl-Meyer Motor Assessment[4], modified Ashworth Scale[5]). The Montreal cognitive assessment (MoCA) will be administered to screen for cognitive dysfunction [3, 6]. The Late Life Function and Disability Indicator[7, 8] will be obtained at study baseline to determine the self-reported level of disability.

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4. Study Variables, Data Acquisition and Data Analysis

4.a. Motor Evoked Responses (Cortical Excitability)

Participants will be positioned in sitting with the arm supported. Single-pulse TMS will be delivered (Magstim 200[2], Carmarthenshire, UK) over the primary motor cortex (M1) using an 8-shaped coil (MagStim, Carmarthenshire, UK) to elicit motor evoked potentials (MEPs) from the first dorsal interosseus (FDI). MEPs will be recorded from pre-amplified EMG electrodes (MA-411, Motion Lab Systems, Baton Rouge, LA). Resting motor threshold (rMT) will be defined experimentally as the minimum stimulator intensity (SI) required to elicit MEPs ≥50μV peak-peak in at least 3/5 consecutive stimulations[9]. Next, participants will produce submaximal isometric power grip corresponding with 10% MVC using visual feedback. Active motor threshold (aMT) will be defined experimentally as the minimum SI required to elicit MEPs ≥100μV peak-peak in at least 5/10 consecutive stimulations. Twelve trials of single-pulse TMS will be delivered at 1.5x rMT.

Analog signals will be collected (2 kHz sampling rate) using a Power 1401 data acquisition interface and controlled with Signal Software (Version 6.0, Cambridge Electronic Design, Cambridge, UK). MEPs will be analyzed offline using custom-written Matlab scripts (The Mathworks, Natik, MA). EMG data will be demeaned, bandpass filtered (10Hz – 1kHz), rectified, signal averaged (minimum 5 responses), and the area of the average rectified response (MEP_{area}) calculated.

4.b. Short Intracortical Inhibition (SICI)

Paired-pulse (conditioning-test) TMS will be delivered using two Magstim 200^2 stimulators coupled with a Bi-Stim unit (Magstim, Carmarthenshire, UK). Paired Conditioning-Test pulses will be delivered using the method described by Kujirai et al [10]. The conditioning stimulus (CS) will be established at 70% aMT [11]. The test stimulus (TS) will be established as the SI that produces an MEP of ~1 mV at power grip at 10% MVC. The TS will be delivered 2.5 ms after the CS, an inter-stimulus interval (ISI) that has been determined as optimal to avoid overlap of the two recognized phases of SICI [12]. Ten unconditioned and ten conditioned stimuli will be delivered, in random order, while participants produce constant isometric power grip at 10% MVC. MEPs will be analyzed offline, as described above (4.a). The mean Conditioned MEP_{area} will be expressed as a ratio of the mean Unconditioned MEP_{area}[13, 14] to determine the magnitude of SICI.

4.e. Experimental Protocol – Aim 1

Following clinical assessments, behavioral function will be tested using three repetitions of the BBT[1] with the paretic side to obtain a stable baseline level of motor performance. Maximal isometric power grip force will be tested in both P and NP sides by performing six maximal effort contractions with each hand. Forces will be recorded using an analog force transducer (Interface, Inc., Phoenix, AZ) integrated into our data acquisition system. Visual feedback and loud, verbal encouragement will be provided to promote maximal effort. Following baseline behavioral measures, participants will be tested with TMS measures to assess MEP amplitude and and SICI in both CL and IL hemispheres. Following baseline TMS assessment, participants will perform repeated submaximal (30% age-referenced maximum[15]) isometric contractions with the **NP hand to task failure**. Submaximal contractions will be performed in blocks of 10 repetitions (7s hold, 3s rest) followed by a single maximal effort trial (i.e., MVC following every 10^{th} submaximal repetition). This sequence will be repeated until **task failure**, defined as: i) 30% reduction in MVC force, ii) inability to reach and/or maintain the submaximal criterion for 3 consecutive trials, or iii) completion of 200 blocks. Immediately following NP hand task failure, the BBT will be retested in the P hand after which the TMS measures will be repeated. Behavioral tests (maximal grip force – both hands, BBT – P hand) and TMS assessments will be repeated every 45 minutes for 4 hours (i.e., 45, 90, 135, 180, and 225 minutes post-task failure). All procedures for this aim will be completed in a single, extended session.

4.f. Experimental Protocol – Aim 2

Aim 2 investigates the consistency of neural and behavioral facilitation over repeated sessions. The protocol enumerated for Aim 1, including induction of task failure, will be repeated twice weekly for four weeks. Repeated sessions will be performed at the same time of day, separated by at least 72 hours (e.g., Monday – Thursday; Tuesday – Friday) to assure complete recovery from task failure. Behavioral and TMS assessments will be performed twice at each session: baseline and immediately following task failure.

D5. Analysis Plan

D5.a. Statistical Analysis for Specific Aim 1

Repeated-measures ANOVA will be performed to test acute neural (i.e. SICI, MEP) and behavioral (i.e., BBT) adaptations over 7 time points. In the presence of a significant F-test, polynomial trends will be tested. Post-hoc pairwise comparisons will be conducted to compare each time point vs. baseline. Bonferroni's correction will be applied for multiple comparisons. Statistical significance for F-tests will be established at $p < 0.05$.

D5.b. Statistical Analysis for Specific Aim 2

Repeated-measures ANOVA will be used to analyze *pre-exercise* neural (i.e. SICI, MEP) and behavioral (i.e., BBT) outcome measures over 8 sessions. In the presence of a significant F-test, polynomial trends will be tested. Post-hoc pairwise comparisons will be conducted to compare each session vs. Session 1. Bonferroni's correction will be applied for multiple comparisons. Statistical significance for F-tests will be established at $p < 0.05$.

D5.c. Power Analysis and sample size justification

Using a combination of data obtained in our studies and published literature[1], requisite sample sizes to detect changes in all parameters at $p < .01$ and power = .9 are noted in **Table 1**. Neurophysiologic and behavioral

parameters to be monitored over time in Aim 2 require a sample size ranging from 10-12 to detect changes relative to baseline. Therefore, we will study 12 subjects in each sub-aim. All 24 subjects will be included in the analysis for Aim1 affording ample statistical power to detect the anticipated effects.

Table 1.	**Mean (SD)**	**Mean change**	**E.S.**	**Aim1**	**Aim2**
Paretic grip force (N)	245.9 (188.2)	32.2 (32.5)	0.99	N = 17	N = 12
Paretic BBT (blocks)	30 (13.1)	4.5 (3.9)	1.15	N = 12	N = 10
IL iSP duration (ms)	36.3 (12.7)	12.17(14.1)	1.54	N = 14	N = 11
IL MEP_{area} (% change)	73 (24)	68.1 (33.8)	2.02	N = 7	N = 10
SICI (CL) (% inhibition)	50 (5)	18(15)	1.0	N = 16	N = 12

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