Protocol Title: Robots Paired with Transcranial Direct Current Stimulation in Stroke Recovery

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Introduction

Cerebral ischemia, stroke, is the third leading cause of death in the U.S. and the leading cause of permanent disability (1). Traditionally, rehabilitation medicine treatment for stroke patients consists of one-on-one treatment and group therapy with physical, occupational and speech therapists who focus their treatment on both compensatory strategies to regain independence and a variety of techniques that can be described as neuromuscular re-education. For example, one learns to brush one's teeth with the non-preferred hand. There are also labor-intensive motor training exercise protocols that focus on the stroke-affected limb using hand-over-hand techniques to move the impaired limb. Prompted by work in non-human primates and other basic experiments, it has become routine in the rehabilitation medicine programs for stroke patients with paralyzed or weakened limb motor function to increase the intensity of activity-based therapy (2-4).

Two promising modern approaches to stroke rehabilitation are the highly reproducible and intense activity-based robotic training and the modulation of brain function by non-invasive brain stimulation (NBS); specifically, trans-cranial magnetic stimulation (TMS) and trans-cranial direct current stimulation (tDCS). This protocol will test whether the addition of the tDCS treatment to the standard robotic treatment protocol positively influences the motor outcome in patients with chronic hemiparesis after stroke. TMS will be used to enhance the characterization and measurement of the motor performance.

Robotic Therapy

Recently, the invention of robotic therapy has been investigated as a potentially superior technique to maximize motor recovery after stroke. Interactive motors with low impedance and driven by smart controllers has led to a revolution in treatment of motor impairment. These devices move a patient's limb when the patient cannot move and then as a patient's motor function improves, the device allows the patient to execute voluntary movement. A robot delivers reproducible movement without tiring and can render the level of training intensity required to alter impairment.

It is important to know that the robot control system is an impedance controller that modulates the way the robot reacts to mechanical perturbation from a patient or clinician and ensures a gentle compliant behavior. Impedance control has been the central contribution of Hogan's engineering research since the early eighties and has been extensively adopted by other robotics researchers concerned with human-machine interaction (60). At present the MIT-MANUS impedance controller is implemented using coupled nonlinear position and velocity feedback structured to produce a constant isotropic end-point stiffness and damping. High-bandwidth current-controlled amplifiers produce motor output torques directly proportional to commanded input. These facts make the robot a stable device.

For this application, the most important feature of the controller above is that its stability is extremely robust to the uncertainties due to physical contact (61-63). The stability of most robot controllers is vulnerable when contacting objects with unknown dynamics. In contrast, dynamic interaction with highly variable and poorly characterized objects (to wit, neurologically impaired patients) will not de-stabilize the impedance controller above; even inadvertent contact with points other than the robot end-effector will not de-stabilize the controller. This is essential for safe operation in a clinical context.

The robot control architecture is implemented on a standard personal computer (presently a 66 MHz 486CPU) with 16-bit A/D and D/A I/O cards, as well as a 32-line DIO card. Besides its primary control function, this computer displays the exercise to be performed to both the operator (clinician) and patient via a video-splitter with dedicated monitors. The neuro-rehabilitation workstation also includes a second personal computer (presently a 25 MHz 386CPU) to display on-line video and audio information. Communication between computers is through a serial port at transmission rates that allow video update above 30 frames/sec. Thus important aspects of the patient's sensory and motor experience can be controlled at the same time.

Transcranial Magnetic Stimulation (TMS)

TMS as it is used today, was introduced by Barker et al in 1985 (7), and has been applied in numerous clinical and physiologic investigations using single and paired pulse techniques (8). The magnetic stimulator comprises an insulated coil wire connected to a large electrical capacitance, and induces electric currents in brain tissue proportional to the rate of change of the magnetic field, when a high current (9) (peak ~several thousand amps) runs through the coil extremely briefly (~200micro sec) . The induced currents are thought to activate corticospinal cells trans-synaptically in the relaxed subject, resulting in waves of descending corticospinal volleys which recruit a portion of the spinal motoneuron pool (probably monosynaptically) and are detected via the surface EMG response, the amplitude of which is one measure of the corticospinal excitability. Motor threshold represents the stimulus intensity needed to activate the most excitable corticospinal elements and motoneurons. A motor-evoked potential (MEP) is evoked only when a cortical stimulus produces a volley of impulses in the corticospinal tract which is of sufficient size to bring the spinal motoneurons to their firing threshold (10). The threshold for producing a local effect in the brain is much lower. The probability of evoking a response is the most logical way of defining motor threshold, and one commonly used method is to increase the stimulus intensity in 5% increments until reaching a level which induces reliable responses in 50% of stimuli. Threshold curves may be used to detect small changes in motor threshold (11).

Paired-pulse experiments are used for investigations into the nature of the cortical circuitry activated by TMS, and a variety of different methods exist to examine cortico-cortical connections, or connections to the motor cortex from other parts of the nervous system (12, 13). The precise mechanisms by which TMS elicits these effects are not yet fully understood, but the modulations are believed to occur in the cortex, as they are accompanied by parallel changes in the descending corticospinal volleys recorded over the cervical cord (14). With paired-stimuli delivered over the motor cortex target muscle area, stimulus intensity and interval parameters can be manipulated to preferentially activate inhibitory or facilitatory cortical circuits (13), although modulation of the test response probably reflects the balance between inhibitory and excitatory effects. A small subthreshold conditioning stimulus is known to reduce the response to a suprathreshold test stimulus if the inter-stimulus interval is between 1-6ms (15). The mechanism of this reduction is thought to be the activation of short-latency intracortical inhibitory networks by the conditioning stimulus that inhibits later I-waves (12, 15, 16). The inhibition is thought to be mediated by GABAa, with the observation of inhibition with administration of GABA enhancing drugs, although other neurotransmitters such as dopamine and acetylcholine might also be involved (12, 17-19). GABA and glutamate are considered the main inhibitory and excitatory neurotransmitters in the cerebral cortex, and animal studies have shown that both types of neurons receive cholinergic inputs that are capable of modulating the efficacy of synaptic transmission (20). Refractoriness of pyramidal neurons is not considered to substantially contribute to short latency intracortical inhibition (21). A suprathreshold stimulus delivered 50-200ms before the same intensity test stimulus, can

reduce the amplitude of the second response, probably as a result of GABAb receptor activation due to activation of intracortical inhibitory networks at long latency (12). Intracortical facilitation may occur as a result of activation of cortico-cortical pyramidal cells and their axons, via excitatory glutamatergic synapses (22).

The mechanism and interneuron pools may be different between intracortical facilitation and inhibition, between short and long latency intracortical inhibition, and between different latencies of inter-stimulus interval in the short-latency inhibition range (23, 24). Recent evidence has shown that brain stimulation might be beneficial in stroke recovery. Indeed epidural stimulation of the motor cortex induces an improvement in motor function in acute stroke (25). Although invasive brain stimulation has shown positive results, this technique is costly and associated with adverse effects. Therefore the field of non-invasive brain stimulation has developed rapidly. TMS applied repetitively is known to induce lasting effects and has been studied more recently as a tool for neuromodulation, where the aim is to promote return of normal cortical excitability following the disruption caused by neurological damage. Long-term changes in corticomotor excitability can be induced using repetitive TMS that are largely dependent on the frequency, duration and intensity of stimulation (26, 27). We are aware of the gaps in the detailed and complete understanding of these mechanisms clinically available to influence motor performance, and we are equally aware of their current interest and possible usefulness (28).

Transcranial Direct Current Stimulation (tDCS)

tDCS modulates the excitability of a targeted brain region non-invasively by altering neuronal membrane potentials (29, 30). Hence this technique can be used to increase or decrease the excitability of neurons in a brain area, in order to determine if that region plays an integral role in a specific motor / cognitive function. Unlike TMS, tDCS does not cause neurons to fire. tDCS only alters the likelihood that neurons will fire by hyperpolarizing or depolarizing brain tissue. The prolonged effects of tDCS have been attributed to long-term potentiation (LTP) (59, 60) and long-term depression (LTD) (31, 32). Dextromethorphan, an NMDA antagonist suppressed post-tDCS stimulation effects of both anodal and cathodal stimulation strongly suggesting the involvement of NMDA receptors in both types of DC-induced neuroplasticity. In contrast, Carbamazepine selectively eliminated anodal effects. Since Carbamazepine stabilizes the membrane potential voltage-dependently, the results reveal that after-effects of anodal tDCS require a depolarization of membrane potentials. This study by Liebetanz et al., provided pharmacological evidence that induction of the after-effects of tDCS requires a combination of glutamatergic (excitatory) and membrane mechanisms, similar to the induction of established types of short- and long-term neuroplasticity (33).

In animals, anodal cortical stimulation of 5-30 minutes has been shown to cause excitability increases lasting for hours after the stimulation, primarily through modulation of the resting membrane potential (29, 30, 34-36).

In humans, 13 minutes of tDCS resulted in an increase in excitability up to 150% and lasting 90 minutes (37). Research with tDCS has revealed that anodal stimulation can induce transient (on the order of 30 minutes) improvements in performance on cognitive, motor and linguistic tasks (38, 39). For example, Hummel et al. found that anodal tDCS delivered to the primary motor area in the lesion hemisphere elicited significant improvements in motor control of the paretic limb. The effect lasted for more than 25 minutes after stimulation. In a recent study, Fregni et al. also verified that anodal tDCS to the affected hemisphere and cathodal tDCS to the contralesional hemisphere improved motor function (40). Other examples highlighting the efficacy of anodal tDCS include: anodal tDCS to dorsolateral prefrontal cortex elicited an

improvement in working memory (41, 42); stimulation to primary motor cortex improved motor learning (43); tDCS delivered to primary motor area or to visual area V5 induced improvements in visuo-motor coordination (44); anodal stimulation of fronto-polar regions improved probabilistic classification learning (45); and left prefrontal cortical stimulation lead to increased verbal fluency. Cathodal stimulation decreases cortical excitability in humans - i.e., affected neurons will be less likely to fire (46). The above studies attest to the efficacy and safety of tDCS in stroke patients, as well as its potential for therapeutic applications in stroke recovery.

It remains to be determined if non-invasive brain stimulation could be used to further enhance the effects of behavioral training such as robotic therapy. Anodal tDCS, applied at rest over the primary motor cortex can raise corticomotor excitability and transiently improve motor function in healthy participants, and chronic stroke patients (38, 47). No reported studies of tDCS have investigated the physiological interactions of tDCS and highly controlled motor training, like robotic training, and additionally reported detailed kinematic changes. Again we are aware of the flux in this field and of the scientific details that are not currently available, but the procedures with the appropriate constraints are safe and may be clinically effective (48).

In this protocol we will test the effectiveness of adding tDCS to the standard robotic training. Despite the best efforts of current standard clinical rehabilitation, stroke survivors are left with significant and seemingly permanent motor impairments. New clinical data in the past decade supports the use of intensive re-training efforts to abet motor recovery. The rationale for intensive re-training, as in this robotic protocol, derives from experiments in pre-clinical and clinical work in which activity-dependent plasticity leads to improved motor outcome.

Specific Aims

SPECIFIC AIM 1: To evaluate whether multiple sessions of combined tDCS and robotic upper limb training in patients with chronic hemiplegia after stroke, leads to a sustained clinical improvement in upper limb motor function.
In patients with chronic stroke (>6months post-injury, stable unilateral motor deficit) using a within-subjects repeated-measures design we will evaluate the effects of 12 weeks of robotic upper limb training (3x/week, 36 sessions, shoulder/elbow/wrist in alternating sessions) with real or sham tDCS before the robotic training. Functional improvement will be determined by a change in upper-limb Fugl-Meyer (primary), Wolf Motor Function Test, Barthel Index, and Stroke Impact Scale (secondary) outcome measures following the training, and assessed again six months later.

SPECIFIC AIM 2: To identify and compare kinematic performance characteristics between intervention groups.
Quantitative measurements obtained from robotics are highly sensitive, precise and reliable. These data will provide important contributions to understanding the components of motor control that underlie improvements in clinical function. Previously published studies and new pilot data from our group support the hypothesis that a relationship may exist for five key parameters (mean speed, peak speed, smoothness, aim, deviation) (50), however confirmation in a larger number of patients needs to be established. This information may additionally be useful as a clinical predictor of motor recovery. Since movement smoothness was shown to improve with just one session of tDCS and robotics, we predict that there will be a strong and sustained effect on this parameter. Movement smoothness is associated with more

advanced stages of motor learning, and has high correlation with functional clinical scales, and as such should be mirrored with change in Fugl-Meyer score.

SPECIFIC AIM 3: To identify and compare the neurophysiological characteristics between intervention groups.
The relationship between clinical improvement and neurophysiological measures pertaining to robotic motor training following stroke are presently not described in the literature. By measuring accepted, quantitative and reliable EMG response (wrist and elbow flexor/extensor muscles) to TMS (cortical inhibition and excitation) we will establish: (i) the plasticity associated with training, and (ii) the neurophysiological characteristics of patients (and muscles within patients) who respond to training. By understanding how brain excitability changes underpin motor dysfunction, and motor recovery, interventions can be more effectively prescribed and prognoses established.

SPECIFIC AIM 4: To evaluate whether multiple sessions of anodal tDCS to the motor cortex of the affected hemisphere in patients with chronic aphasia after stroke leads to sustained clinical improvements in verbal expression.
In patients with chronic aphasia after stroke (>6months post-injury, stable expressive language deficit) using a within-subjects repeated-measures design we will evaluate change in verbal expression scores across 12 weeks of anodal stimulation to affected motor cortex with real or sham tDCS before the robotic training. Improvement in verbal expression will be determined by a change in verbal fluency as measured by the Apraxia Battery for Adults (ABA-2, primary), Philadelphia Naming Test short form (PNT), Action vs. Non-action word naming task, generative naming task, “Repetition” subtest of the Aphasia Diagnostic Profiles (ADP), picture description and connected speech sample, the and “Symbol Cancellation” subtest of the Cognitive Linguistic Quick Test (CLQT) (secondary) following the training, and assessed again six months later. The Western Aphasia Battery Revised (WAB-R) will additionally be given upon admission, discharge, and at follow up six months after training for a comprehensive profile of language function.

SPECIFIC AIM 5: To identify and compare cytokine levels between stroke rehabilitation intervention groups.
We hypothesize that persistent HMGB-1 serum levels in patients with stroke retard functional recovery. A first step in the test of this hypothesis is to gather serum data in study participants to longitudinally assess if cytokine levels correlate with responsiveness to the robotic rehab interventions, and to determine if that response is affected by intervention group (sham vs. real tDCS).

Preliminary Data

A recent multi-center study has demonstrated the effectiveness of robotic training in patients recovering from hemiparesis after stroke, and the robot training has become a standard of care (5, 6).

We recently demonstrated that the combination of the NBS technique of transcranial direct current stimulation (tDCS) and upper-arm robotic therapy improves motor performance after stroke, but only when tDCS is used to prime the therapy; that is, prior to robotic training (49). When tDCS is applied during or after robotic therapy it confers no additional advantage. The interpretation is that tDCS increases cortical excitability and neural plasticity for a time period, and that robotic therapy is more effective when applied at that time.

Having established that tDCS cortical excitability after-effects could be sustained during robotic upper-limb training, we tested if this combination of tDCS and robotic training applied over multiple practice sessions might translate to improved motor function. In six right-handed patients with chronic stroke (>6 mo since ictus), two with right hemiparesis and four with left hemiparesis (3/3 Male/Female, mean 4.7 yrs since stroke; and 67. 7± 12.7 years of age) received a full course of 36 weeks of robotic training on the shoulder-elbow and wrist devices. They were considered to be at a stable plateau for their upper limb function. They received 2 weeks (6 sessions) of combined tDCS and robotic wrist training. Four patients received real tDCS and training, while 2 patients received sham stimulation and training (randomly assigned). The results demonstrated that in the group that received tDCS and robot training, but not the sham (no tDCS and robot training), a clinically meaningful improvement occurred over this short training period. Motor power at the wrist improved to 112±6% pre-training in the real-treatment group (pre-training = 19.5, to post training 22 points (out of 30) and no change in the sham group (pre training=19.5, post training=19.5). The wrist-hand Fugl-Meyer improved by 3 points (mean pre=16.83, post=19.83 out of 30) and improved 1 point in the sham condition (mean pre=18, post=19). Preliminary analysis of the kinematic data also revealed that the group that received tDCS had smoother movements (fewer jerks) than controls receiving sham tDCS. While this preliminary study was in a small number of patients, the results suggest that tDCS followed by motor training may be advantageous over training alone.

These studies support investigations aiming to understand the mechanisms and clinical benefit of combined tDCS and robotic motor training in chronic stroke.

Research Design and Methods

Inclusion/Exclusion Criteria

Inclusion Criteria:

1. ≥ 18 years of age
2. First single focal unilateral lesion with diagnosis verified by brain imaging (MRI or CT scans) that occurred at least 6 months prior;
3. Cognitive function sufficient to understand the experiments and follow instructions (Mini-Mental Status Score of 24 or higher or interview for aphasic subjects);
4. Fugl-Meyer assessment 7 to 58 out of 66 (neither hemiplegic nor fully recovered motor function in the muscles of the shoulder and elbow and wrist).

Exclusion Criteria:

1. Botox treatment within 6-weeks of enrollment;
2. Fixed contraction deformity in the affected limb;
3. Complete and total flaccid paralysis of all shoulder and elbow motor performance;
4. History of hemorrhagic stroke
5. Ongoing use of CNS-active medications
6. Ongoing use of psychoactive medications, such as stimulants, antidepressants, and anti-psychotic medications
7. Presence of additional potential tDCS / TMS risk factors:
    - Damaged skin at the site of stimulation (i.e., skin with ingrown hairs, acne, razor nicks, wounds that have not healed, recent scar tissue, broken skin, etc.)
    - Presence of an electrically, magnetically or mechanically activated implant (including cardiac pacemaker), an intracerebral vascular clip, or any other electrically sensitive support system

- Metal in any part of the body, including metal injury to the eye (jewelry must be removed during stimulation)
- A history of medication-resistant epilepsy in the family
- Past history of seizures or unexplained spells of loss of consciousness

Visit Schedule

In this double-blind research study, 66 participants will be randomized to receive real or sham tDCS prior to receiving an established interactive upper limb (shoulder/elbow and wrist) robotic training regimen. During the lead-in period, each subject will attend 3 weekly visits to complete TMS screening and have outcome measures (see below) assessed. Following the lead-in period, each subject will attend 36 90-minute sessions (3 visits/week) over a 12-week period (or up to 14-weeks to allow for missed appointments) comprising the training period. These visits will include further TMS screening and outcome measures, TMS, tDCS and robot training. Subjects will undergo further outcomes measures upon discharge and during a final 6-month follow-up visit. All study procedures will be administered and supervised by a therapist. All visits will be conducted in either the robot and TMS suites at the Feinstein Institute for Medical Research or at the Burke Medical Research Institute (Fig. 1). Participants will additionally undergo five blood draws longitudinally during the study to determine if cytokine levels change over the course of rehabilitation intervention, and if those changes are correlated with improved functional outcomes and treatment group (sham vs. real tDCS).

<u>Lead-in Period</u>

- Week 1, Visit 1 (approximately 30 minutes)
  - TMS screening questionnaire
  - Outcome measures
- Week 2 & 3, Visit 2 & 3 (approximately 60 minutes each)
  - Outcome measures
  - One time 10cc DNA sample and 2 separate 4cc baseline blood draws (2 total draws)

<u>Training Period</u>

- Week 4-15, Visits 4-39 (approximately 90 minutes)
  - TDCS risk questionnaire (visit 4 only)
  - TMS
  - tDCS
  - Robotic training
  - 4cc blood draw at midpoint, session 18 (1 total)

- Weeks 16 Visit 40 (approximately 2.5 hours)
  - tDCS

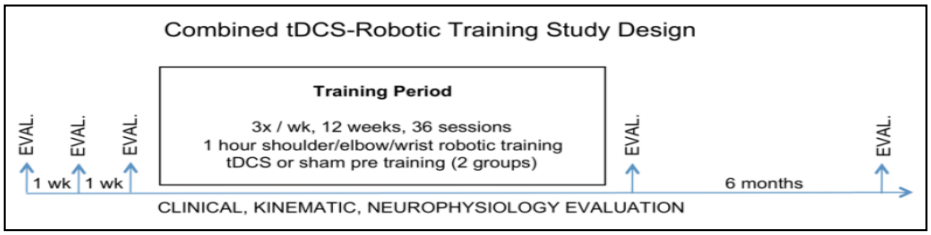

Fig 1: Diagram of study design showing the relationship between the robotic training program with or without tDCS and the evaluation schedule

- Robotic training
- Outcomes measures
- 4cc discharge blood draw (1 total)

Final Visit

- Week 40, Visit 41 (approximately 60 minutes)
    - Outcome measures
    - 4cc follow up blood draw (1 total)

Clinical Outcome Measures

All outcome measures will be recorded three times prior to the training period separated by one week to ensure reliability and stability of measures, then again following the 12-week training program, and six months later. In general, study visits will take place at the institution of recruitment (e.g. subjects recruited through Feinstein will be treated at Feinstein). However, occasionally TMS and clinical assessments may take place at the other institute. Individual patient assessments will always be performed by the same evaluator to ensure internal consistency.

Fugl-Meyer (Primary): The Fugl-Meyer scale is a valid and reliable evaluation instrument used for measuring performance-based impairment in stroke patients (54, 55).

Medical Research Council motor power score (MRC): The MCR is a valid and reliable score that measures strength in isolated muscle groups of the involved shoulder and elbow on an ordinal scale (scale range: 0, no muscle contraction; 5, normal strength) .

Wolf Motor Function: The Wolf Motor Function Test is a valid and reliable assessment of upper extremity function by asking the patient to complete 15 motor-based tasks and two strength-based tasks (56).

Barthel Index (BI): The BI is a valid and reliable index of independence widely used in scoring improvement in the rehabilitation of stroke population (57). The BI consists of 10 items assigned various weightings: 15 points for the subcategories of walking and transfers; 10 points for the subcategories of feeding, bowel, bladder, toileting, dressing and stairs; 5 points for the subcategories of bathing and grooming. The BI is a cumulative score achieved by summating the score for all sections. The BI scores are recorded in multiples of 5, ranging from 0 (completely dependent) to 100 (independent in basic activities of daily living).

Stroke Impact Scale (SIS): The SIS is a valid and reliable index that assesses changes in impairments, disabilities and handicaps following a stroke and has internal consistency and established test-retest reliability (58, 59). We have chosen the Stroke Impact Scale (SIS) Version 3.0 to measure physical abilities, as well as other dimensions that contribute to quality of life and participation in everyday life. This is a 59-item self-reported questionnaire that asks persons with stroke to rate perceived problems in eight domains using a 5-point scale: strength, hand function, mobility, activities of daily living, emotion, memory, communication and social participation. This self-report assessment is unique in that it addresses motor impairments of the paretic limbs in addition to other key variables that are important to patients and their caregivers. This assessment will not only offer data concerning perceived changes in motor abilities following involvement in the planned study, but will also help to identify

other incidental changes in emotional status, memory and thinking, or activities of daily living following participation in robot training.

Verbal Expression Battery for Aphasia: A set of brief subtests from larger aphasia batteries will be used to asses changes in verbal expression and fluency in subjects with chronic expressive aphasia. Audio/Video recordings will be made of speech-language assessments for review and scoring by approved clinicians.

"Diadochokinetic Rate," "Increasing Word Length," and "Inventory of Articulation Characteristics" subtests of the Apraxia Battery for Adults (ABA-2): The ABA-2 is a valid and reliable measure of changes in motor speech across isolated speech sounds, multisyllabic utterances, and connected speech samples. Together, these subtests take approximately 5 minutes to administer and derive an apraxia impairment profile score (75, 78, 79).

Generative Naming: Generative Naming is a commonly used clinical measure of verbal fluency and semantic memory in individuals with aphasia. It has been used frequently in the tDCS literature as a sensitive metric of changes in verbal fluency (71, 72, 76).

Philadelphia Naming Test short form (PNT): The PNT is a valid and reliable measure of changes in expressive naming across time in stroke patients with expressive aphasia. Performance on the PNT short form has been statistically correlated to PNT long form performance for individuals with aphasia (73, 74, 75).

Verbal production of Action vs. Nonaction words: Network models of language suppose that motor acts and the words used to describe them are a part of the same neural network. tDCS stimulation of the motor cortex may produce enhanced verbal production of action vs. nonaction words (69, 70).

Connected Speech Sample including the "Cookie Theft" or "Birthday Party" picture descriptions: Connected speech samples are frequently used metrics of changes in rate, fluency, and language formulation in individuals with aphasia. Audio/visual recordings of naturalistic language samples allow for independent quantitative scoring of language function by clinicians (78, 79).

"Repetition" subtest of the Aphasia Diagnostic Profile (ADP): The ADP is a valid and reliable measure of changes in repetition and verbal facility for simple sentences and basic reflexive speech (78, 79).

"Symbol Cancellation" subtest of the Cognitive Linguistic Quick Test (CLQT): is a valid and reliable non-verbal, visual cancellation task. It is frequently used by clinicians in aphasia literature as a non-verbal control task of attention and visual memory (76).

Western Aphasia Battery Revised (WAB-R): The WAB-R provides an aphasia quotient for type and severity of aphasia, and is commonly used in the aphasia literature. Changes of >5 points are clinically significant (75, 77, 78).

TMS Evaluation

Screening: A questionnaire to screen for TMS risk factors, published for use with TMS research, will be completed by every participant prior to undergoing TMS (67). An answer of "yes" to any of the listed questions will necessitate further inquiry by the investigator, but not necessarily exclusion from the TMS study.

Motor cortex stimulation and electromyography: Patients will sit comfortably in a chair and the investigator will gently apply the TMS wand against the skull. There will be a clicking noise that indicates the stimulator is generating a magnetic field and the sound will be followed by a muscle twitch in the opposite arm, forearm or hand.

We will use Brainsight™ neuronavigation to deliver stimuli on a grid in 1-cm steps in the coronal and sagittal planes, over the region of the primary motor cortex in the affected hemisphere. TMS will be delivered using a MagPro X100 stimulator with a 5 cm diameter figure-of-eight coil, held tangential to the skull and aligned in the para-sagittal plane with the handle rotated 45° lateral. Surface electromyographic (sEMG) recordings will be made from electrodes positioned over the muscle belly of the right flexor carpi radialis (FCR) muscle. EMG signals will be amplified (x1000) and band-pass filtered between 20 and 1000 Hz, before being digitized at 2000 Hz for 100 ms following each stimulation, using a Cambridge Electronic Design (CED) acquisition system. The optimal site of stimulation for FCR will be determined from initial exploration, and used throughout the experiment.

Resting Motor Threshold (RMT): Motor evoked potentials (MEP) recorded via surface EMG during the torque recordings, will be analyzed for peak-to-peak amplitude. The lowest stimulus intensity evoking a MEP of 50 μV in at least five of ten trials in the relaxed muscle will be used. We will use 2% increments in stimulator output starting just below the intensity determined from initial exploration. Motor threshold is considered to reflect membrane-related intrinsic neuronal excitability. Ten MEP responses with single stimuli delivered at 120% RMT intensity will be recorded, to determine if a change in amplitude is observed at each time point.

Intracortical Inhibition: A single pulse TMS stimulus can be inhibited or facilitated if preceded by a subthreshold conditioning stimulus (Short Interval Cortical Inhibition SICI, 3msec inter-stimulus interval) is an accepted measure to determine if changes in corticospinal output are related to altered intracortical excitability. We will record 10 test MEPs, and 10 conditioned MEPs (80%RMT conditioning TMS intensity, test intensity adjusted to give 1mV amplitude MEPs) for SICI. Conditioned and unconditioned pulses will be randomly presented.

The frequency of TMS stimulation will not exceed 0.2 Hz, as this will avoid cumulative effects of the stimulation during assessment.

Transcranial Direct Current Stimulation (tDCS) Application
Patients will sit in a comfortable chair and a plastic band will encircle the skull. An electrode – a flat 6cm X 6cm plate – will be ensheathed in a disposable cotton sponge and held in place by the band. The electrode (cathode) covered by the pad will be placed above the eye and on the forehead contralateral to the affected limb and another electrode (anode) will be placed over the skull site that has been determined by TMS to produce a maximal motor contraction in the affected limb (33).

A 2mA current will be delivered using the surface rubber-carbon electrodes (35cm^2) with surrounding saline soaked sponges (0.9% NaCl) by a battery driven, constant current stimulator (maximum output 10mA) (51). Participants will receive stimulation for 20 minutes while seated (prior to robotic motor training), with the anode over the optimal site for flexor carpi radialis (FCR) as identified using TMS, and the cathode on the contralateral supraorbital area (37). Sham tDCS: comparable set-up to real tDCS, 30 seconds real current ramping to 2mA at commencement, then after 5 seconds a slow decrease but to no current sustained for 20 minutes.

Robotic Training
We will use existing robots at the Feinstein Institute which are FDA-approved robotic devices that move the shoulder-and-elbow or wrist-and-forearm (used in a previous IRB protocol 11-121B). Following tDCS, the patient will be seated in a chair facing a video screen and the robot. The patient will hold onto the end of the robotic arm, and if this is not possible, then the patient's

affected arm will be placed in a foam-lined trough attached to the robot handle. Their hand and forearm will be held in place by Velcro® straps. The patient will move the robot arm as best he/she can through a series of exercises guided by a visual display on the video screen. The patient will see their moving arm and hand and see their movements recorded on the video screen. If a patient cannot move within 1.5 seconds, the device will move his/her arm through the exercises. These sessions will alternate the treatment of shoulder and elbow with wrist in successive sessions.

The standard patient robot interaction involves visuomotor tasks, moving the robotic manipulandum according to targets on a computer screen mounted at eye level. The force required to move the robotic arm is minimal, comparable to moving unrestricted, and if a patient cannot move the robot arm, it will guide the limb to provide an adaptive sensorimotor experience. The robot program will always be tuned to the so-called progressive algorithm which blends the features of aiming or path trajectory correction and speed of movement.

Blood Sampling

As we are interested in assay of HMGB-1 and other cytokines longitudinally for the duration of the rehabilitation intervention, we will collect two baseline admission samples during admission evaluations 2 and 3. We will then collect additional samples at midpoint, treatment 18 (end of week 6), discharge, treatment 36 (end of week 12), and follow up, 6 months following the end of the rehabilitation intervention. Given the challenges of collecting samples within a specific 24 hour time period, the collection window will be extended such that all samples collected during the intervention will be obtained plus or minus one treatment day of the target (e.g. between treatments 17-19, 35-36-final evaluation day, respectively), and follow up samples will be collected plus or minus 7 days of the 6 month follow up target. Each of the 5 samples will be 4 cc each (green top tube). We will also obtain a one-time DNA sample, via the collection of a 10cc lavender tube (EDTA) at the time of the first sample collection. If this is not possible we will collect this sample at the time of a subsequent research blood sample collection. The total volume of blood drawn for each study subject will be no more than 30 cc over the duration of the study. All blood sampling will take place at the CRC during scheduled intervention and evaluation visits.

The blood will be centrifuged and prepared for HMGB-1 analysis by NS-LIJ Biorepository staff. If we miss sample collection across the eight time points the available collected samples from a subject will be analyzed. We will measure HMGB-1 and if the levels are elevated (>20ng/ml) we will return to the banked samples in the bio-repository to repository to assay samples for IL-1, IL-6, IL-8, IL-10, TNF and BDNF, all molecules known to be stimulated by HMGB-1 secretion [4, 5].

Randomization

Previous studies indicate that stroke patients may differentially respond to non-invasive motor cortex stimulation protocols according to whether the lesion includes cortex, or is confined to white matter (52). Accordingly, we will use blocked stratified randomization to ensure our two comparison groups are balanced with respect to this known prognostic factor (cortical versus subcortical classification). Classification of this grouping will be assessed using CT or MRI with 'subcortical' defined as a stroke without involvement of cortical motor areas, and 'cortical' as infarction of cortical primary or secondary sensorimotor areas in addition to subcortical infarction.

Randomization of patients is performed by a statistician and epidemiologist, Dr. Jessica Elder. She will communicate the status to Dr. Volpe. The switch is covered so that the therapist and the PI will not know the group assignment.

Blinding
The study is double-blind. All investigators interacting with patients will be unaware which patients are receiving real or sham stimulation. This includes the principal investigator and research fellows conducting robotic, neurophysiology or clinical assessments. The tDCS device has a coding option to program sham or real stimulation. Ms. Roseann Berlin, a histopathologist in Dr. Volpe's lab who is not involved with the robot project and has agreed to perform this toggling task will set the tDCS device for any particular patient. She knows Dr. Elder and we will establish communication between Dr. Elder – who will determine the randomization and Ms. Berlin so that Dr. Volpe and the Research Associate to be named will not have the patient's code. It is important to note that once the machine to deliver the trans cranial direct current stimulation is toggled no further manipulation of the device is needed. The tDCSM operates automatically to deliver current or not. We will receive the coding option from the collaborating institution, and will reveal this information after analysis, unless there is a need to inform the PI in case of an adverse event. Patients will be asked at visit 9 and visit 15 (mid-point and end of the intervention period), whether they think they received real or sham stimulation.

Statistical Considerations and Data Analysis

Specific Aim 1 (Clinical): Determine if there is a statistically significant difference in the change in upper limb Fugl-Meyer and Wolf Motor Function score between subjects trained with and without tDCS prior to robotic training.

Methods to address specific aim 1: We will compare demographic, clinical, kinematic, and tDCS variables between intervention groups using t-tests to compare means and chi-squared tests to compare proportions; where continuous data is non-parametric as defined by Shapiro-Wilk or Q-Q plots, we will use Kruskall Wallis tests. To assess the univariate relationship between treatment group and upper limb Fugl-Meyer score, repeated measures ANOVA will be used in order to account for longitudinal evaluations. Multivariate methods will include linear mixed models and ANCOVA to adjust for interactions or other covariates potentially contributing to the association between treatment and outcome. Further, Pearson correlation coefficients will be assessed to identify any linear association between kinematic, clinical and tDCS data points. The Spearman Rank correlation coefficient will be used for non-normally distributed variables. Like the Fugl-Meyer, secondary outcome measures, Wolf Motor Function, Barthel Index and Stroke Impact Scale are scored on a continuous scale and thus, will be analyzed as above. The MRC is scored on a scale from 0-5; Fisher analyses will be used to look for differences in strength between groups.

Specific Aim 2 (Kinematic): Determine if there is a statistically significant difference in a) mean speed, b) peak speed, c) smoothness, d) aim, and e) deviation from the straight line between subjects trained with and without tDCS prior to robotic training. This will be determined for both the trained tasks, as well as for the untrained, circle-drawing task. We will also include a static and dynamic quantitative measure of strength using the robotic device.

Methods to address specific aim 2: To assess the univariate relationship between treatment group and outcome, repeated measures ANOVA will be used in order to account for longitudinal evaluations. Multivariate methods will include linear mixed models and ANCOVA to adjust for interactions or other covariates potentially contributing to the association between treatment and outcome.

Specific Aim 3 (Neurophysiology): Determine if there is a statistically significant difference in: a) RMT, (b) MEP amplitude at 120% RMT, and c) SICI between subjects trained with and without tDCS prior to robotic training, both immediately following the training regimen, and 6 months later.

Methods to address specific aim 3: To assess the univariate relationship between treatment group and outcome, repeated measures ANOVA will be used in order to account for longitudinal evaluations. Multivariate methods will include linear mixed models and ANCOVA to adjust for interactions or other covariates potentially contributing to the association between treatment and outcome.

POWER: Based on the previous study by Lo et al., we can expect twelve weeks of upper limb robotic training to result in a 2.88 change in the FM score (5). We estimated that tDCS plus robotics would result in a FM change score of 4.33. Using a two-sided alpha and a standard deviation of approximately 1.5, enrolling 56 subjects (28 per group) would give us 90% power to detect a difference in Fugl-Myer score of 1.45 between the intervention groups. To account for potential subject attrition, we aim to increase our sample size by 15% thus resulting in an enrollment goal to 66 (33 subjects per group).

Specific Aim 4 (Clinical): Determine if there is a statistically significant difference in the change in verbal expression scores on the Apraxia Battery for Adults (ABA-2) and Philadelphia Naming Test Short Form (PNT short form) between subjects with aphasia trained with and without tDCS prior to robotic training.

Methods to address specific aim 4: We will compare demographic, clinical, and tDCS variables between intervention groups with aphasia using t-tests to compare means and chi-squared tests to compare proportions; where continuous data is non-parametric as defined by Shapiro-Wilk or Q-Q plots, we will use Kruskall Wallis tests. To assess the univariate relationship between treatment group and ABA-2 score, repeated measures ANOVA will be used in order to account for longitudinal evaluations. Multivariate methods will include linear mixed models and ANCOVA to adjust for interactions or other covariates potentially contributing to the association between treatment and outcome. Further, Pearson correlation coefficients will be assessed to identify any linear association between clinical and tDCS data points. The Spearman Rank correlation coefficient will be used for non-normally distributed variables. Fisher analyses will be used to look for differences in strength between groups.

Specific Aim 5 (Biological): Examine whether blood levels of HMGB-1, and TNF,IL-1, IL-6, IL-8, IL-10, and BDNF change across rehabilitation intervention, and if those changes are correlated to functional recovery.

Methods to address specific aim 5. **The statistical analysis for this pilot study will be primarily descriptive in nature. The main statistical analysis will employ repeated measures analysis to describe the patterns of change in HMGB-1 and the cytokine values over time. Other techniques will be explored if the data warrant it; such as examining**

pairs of outcome variables (HMGB-1 and TNF, HMGB-1 and IL-1, or HMGB-1 and stroke severity; as pioneered at Feinstein by Dr. O Bloom and as in [57]).

Protection of Human Subjects

RISKS TO SUBJECTS

Human Subject Involvement and Characteristics: We anticipate enrolling 66 human subjects. Inclusion and exclusion criteria are stated above in the Research Design and Methods section. Subjects will be enrolled into one of two sites: the Feinstein Institute for Medical Research or the Burke Medical Research Institute.

Sources of Material: Sources of research material for participants from Feinstein will be the hospital records providing demographic and medical information including CT or MRI imaging studies, and clinical examinations performed at outpatient facilities run by the Department of Physical Medicine and Rehabilitation of the North Shore University Hospital (NSUH) and LIJ Medical Center (LIJMC). Sources of research material for study participants at Burke Medical Research Institute will be obtained through hospital records at the Burke Rehabilitation Hospital in White Plains, NY, and overseen by the Burke IRB.

Potential Risks:
TMS Risks: TMS is a technique that has been used widely and in a growing number of laboratories since 1985. A consensus was reached at the International Safety Conference on Transcranial Magnetic Stimulation, held at the National Institutes of Health in June of 1996, that single-pulse TMS and slow rTMS (with rates of stimulation less than one Hz) are safe. Subjects may experience mild headaches or neck pain, which are believed to be due to muscle tension and from the straight posture of the head and neck during the application of TMS. A rare possibility of activating a seizure in susceptible subjects has been documented.

tDCS Risks: tDCS is a safe technique that poses a non-significant risk to participants. The safety of this technique has been addressed and tested by multiple researchers (38, 40-42, 45, 46) who have concluded that tDCS, as applied in a manner similar to our proposed protocol has no long-term negative side effects. More than 30 research studies involving hundreds of participants have been published using tDCS. Hundreds more participants have undergone tDCS for unpublished pilot research (46). No undesirable or long-lasting effects have been reported, nor have any participants reportedly abandoned a study due to discomfort.

Researchers at the National Institute of Neurological Disorders and Stroke (NINDS) conducted a safety study on tDCS, investigating 20-minute sessions of 1 mA and 2 mA current stimulation with healthy controls (n=103) (45). No negative effects were identified. Nitsche and colleagues (2004) found no measurable structural changes in brain tissue due to tDCS (64). Additionally, studies have shown that tDCS can be used safely in stroke patients (38, 40, 41, 47). Thus, a growing body of research from different laboratories has shown that tDCS is a safe, non-invasive and painless technique for modulating neural excitability, with measurable but only transient effects. The protocol described here uses stimulation levels that fall well within safety limits established by basic research investigating neural tissue damage, as well as numerous studies applying tDCS with human participants (64-66). tDCS has the potential to cause erythema – redness of the skin that is uniform or mottled around the area of stimulation. The reddening has been found to be transient for levels of stimulation proposed in this protocol (45). 2005).

Nitsche and colleagues (2003) reported that, in unpublished research, stimulation at electrical current levels above 1 mA can be uncomfortable for subjects. No such discomfort was reported in the NINDS safety study conducted by Iyer and colleagues (2005), in which 103 participants were stimulated at levels higher than those used by Nitsche and colleagues (2003).

Robot Risks: There are no known risks associated with the use of robotic training for stroke rehabilitation. Some patients have pain in the shoulder after a stroke. Our experience has demonstrated a comparable incidence of shoulder pain in groups that were or were not treated by the robot.

Blood Draw Risk: Minimal risks of blood withdrawal include pain from the needle being inserted through the skin into the vein, bruising, clot formation under the skin, lightheadedness, possible fainting and rarely infection.

Confidentiality Risk: One additional risk concerns the risk to confidentiality incurred with any collection of medical data.

ADEQUACY OF PROTECTION AGAINST RISKS

Recruitment and Informed Consent: Stroke subjects who meet inclusion criteria and do not meet exclusion criteria will be recruited at two sites by consenting professionals: the Burke Rehabilitation Hospital and the Department of Physical Medicine and Rehabilitation of NSUH and LIJMC.. Recruitment will be done with direct contact, flyers and letters sent to patients who have been enrolled on other research projects.

Recruitment of patients at Burke Rehabilitation Hospital will be primarily based on screening of the Burke outpatient list, as well as inpatient medical records for identifying future candidates, physician referral, advertising within the Burke network, on the Burke Website and collaborator websites. Participants enrolled at the Burke Medical Research Institute study site will be overseen by the Burke IRB, and its associated, approved study protocol and consent form.

Northwell Health physicians who have appropriate patient populations will be made aware of the research study protocol and procedures, and given an overview of the study through contacts with the study personnel. The physician will identify potential study participants. If the patient expresses interest in participation, the physician will either 1) obtain informed consent (if they are listed as an investigator on this study) or 2) provide the patient with the study coordinator's contact information or 3) provide the patient's contact information to study personnel with the patient's permission, which will be documented in the medical record.

After a discussion about the study with a potential subject and a potential subject's legally authorized representative (LAR)/next of kin, interested parties will be given a copy of the consent form by one of the study investigators. The investigator will review and explain the consent form. All information about the study will be provided. Ample time will be given for individuals to ask questions regarding participation and to have questions answered prior to signing the consent form. If so desired, those interested will be given a copy of the consent form so that they may have the opportunity to discuss participation further with family and/or advisors. Only those investigators listed in the study protocol will obtain informed consent. If an individual chooses to enroll, the consent form will be signed before participation begins. Once an individual joins the study and informed consent is obtained, the subject will receive a signed

copy of the consent form. The subject may withdraw from the study at any time without repercussions to subsequent care.

If the patient is awake, alert, and oriented to person, place, and time, and demonstrates appropriate cognitive and communicative abilities as determined by the treating physician, the patient will be deemed to have the appropriate capacity to consent; however, given that borderline cognitive dysfunction and/or aphasia may not be easily distinguishable, the patient's LAR/next of kin will be routinely included when consent to participate is being obtained for all subjects.

If it is determined that a patient is unable to consent for him/herself, due to a lack of capacity or lack of comprehension, consent will be sought from the patient's LAR/next of kin. Assent of the adult subject with LAR/next-of-kin will be obtained as appropriate. If such a subject regains his/her ability to make healthcare decisions, he/she will be given the opportunity to provide consent. This consent will be documented using the Addendum to Consent by Research Proxy for Continuing Participation in a Research Study form.

If the patient provides the consent delegate with assent to participate in the research but, due to a physical disability, is unable to sign the consent form, the patient will provide verbal consent and a witness and the patient's LAR/next of kin will sign the document affirming their presence during the consent process and the patient's physical disability as reason for an absent signature.

A study investigator will obtain informed consent, in person, from interested persons. After a discussion about the study with a potential subject, interested persons will be given a copy of the consent form by one of the study investigators. The investigator will review and explain the consent form to the person. All information about the study will be provided. Ample time will be given for persons to ask questions regarding participation and to have questions answered prior to signing the consent form. If so desired, those interested will be given a copy of the consent form so that they may have the opportunity to discuss participation further with family and/or advisors.

Investigators may contact (or be contacted by) a potential subject's LAR/next-of-kin by telephone to discuss participation in this research protocol. The investigator will provide subject's LAR/next-of-kin with all the information contained in the written consent form. The investigator will answer any questions regarding the research and give the subject's LAR/next-of-kin ample time to consider participation in the study which may require a follow-up phone conversation.

If the subject's LAR/next-of-kin agrees to allow the decisionally incapacitated patient to participate in the research study, the investigator will provide his/her contact information. The investigator will explain (and repeat) the next steps necessary for the LAR/next-of-kin to provide informed consent, which include the following processes. A written consent form will be sent to the LAR/next-of-kin as an email attachment, or left at the nurses' station on the unit where the potential subject is an inpatient. The LAR/next-of-kin must read the consent form and call or email the investigator if he/she to discuss research and resolve issues/questions. If subject's LAR/next-of-kin agrees to participation in the protocol, the investigator will direct him/her to sign the consent form and return it to the investigator by mail or fax. Another option would be to scan the signed consent form to a PDF file and return it to the investigator as an email attachment. An enrollment note must be written by the investigator documenting all phone conversations with the LAR/next-of-kin. Printouts of any email correspondence must be placed in the subject's

research chart. After the signed consent form is received, investigator will sign the consent form. A copy will be made and sent to the LAR/next-of-kin for his/her records. In cases where consent is obtained by e-mail/mail, investigators request a waiver for the need for a witness signature.

In the event that a subject and/or LAR/next of kin arrives for the first evaluation and then requests to take the consent form home for review prior to signing it, minimal risk clinical measures of the subject's upper extremity function may still be collected during the initial study visit, prior to the signing of the consent, in order to reduce the burden on the disabled subject and their family who would otherwise be required to come for an additional study visit. If that subject and/or their LAR/next of kin then decline further participation in the study, the data for that subject's first evaluation will be destroyed.

Only those investigators listed in the study protocol will obtain informed consent. If a person chooses to enroll, the consent form will be signed before participation begins. Once an individual joins the study and informed consent is obtained, the subject will receive a signed copy of the consent form. The subject may withdraw from the study at any time without repercussions to subsequent care.

Protection Against Risk:

Protection against TMS-related risk: All participants will be screened twice for seizure-related risk factors (visit 1 & visit 4). Seizure-susceptible patients will be excluded from the study by using this screening questionnaire. sEMG has no know risks. We will monitor subjects continually during the stimulation period, and will be in constant contact with the subjects. The study can be immediately stopped at the subject's request.

Protection against tDCS-related risks: If any redness is apparent where the electrodes were placed, a cold compress will be offered to the subject. We will monitor subjects continually during the stimulation period, and will be in constant contact with the subjects. The study can be immediately stopped at the subject's request.

Protection against Robot-related risk: The practical issues regarding the robot control system and patient safety include that the software that runs the robot device continuously checks the force, speed and position of the robot armed and on the status of the power. This software can brake the robot if the force, speed, or position is beyond set ranges. There are two clearly visible "shut-down" switches that are always in reach of the technician trainer. In the event of software failure, motion beyond the specified range, loss of electrical power, or activation of the "shut-down" switch the device stops (brakes) within 2 milliseconds. The robotic arm will only move the patient's upper limb, and no other body part will be within the active range of this movement (the head for example is well out of the possible range of the movement of the robotic arm). The patients hand is attached to the robotic arm with a magnetic clasp, which can be released with a sudden pull from the patient or therapist. The entire apparatus is ground-fault protected to exceed clinical standards. We will monitor subjects continually during robotic training, and will be in constant contact with the subjects. The study can be immediately stopped at the subject's request.

Protection of Confidentiality: To protect subjects' confidentiality, each subject will be assigned a number, and all data will be stored with the subject number only and not the subject's name. Data will be stored on a password-protected computer and on the cloud data sever, REDcap, which is encrypted and password protected Subject charts with medical history and assigned subject numbers will be kept in locked file cabinets stored at either the Feinstein robot suite for patients enrolled through Northwell Health or the Burke robot suite for patients enrolled through

Burke Rehabilitation Hospital. Access to charts will be granted only to study investigators at Feinstein and Burke, and CRC staff. Charts will be kept confidential and will not be shared with any third parties without permission from the subject. Any study data containing PHI that is transferred between investigators at Feinstein and collaborating institutes will be shared via encrypted email or encrypted storage drives.

Of note, Dr. Krebs is a sub-investigator from MIT and will be assisting in the analysis of data only. Subject data they receive from this research study will be de-identified.

Data and Safety Monitoring: To protect both the integrity of the data and the safety of all study participants, study data review in aggregate will occur every 4 months by the Principal Investigator.

POTENTIAL BENEFIT TO SUBJECTS AND OTHERS

The risk/benefit ratio is very low in the proposed study due to the established safety of the protocol and to the great potential for using the findings to improve rehabilitation methods.

SCIENTIFIC VALUE

The results of this study may help to improve stroke recovery.

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