Priming Upper Extremity Motor Practice with Aerobic Exercise (Pump-Ex): A feasibility and pilot study

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PROTOCOL TITLE:

Priming Upper Extremity Motor Practice with Aerobic Exercise (Pump-Ex): A feasibility and pilot study

PRINCIPAL INVESTIGATOR:

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1.0 Objectives / Specific Aims

The purpose of this project is to establish initial feasibility and tolerability of a combined aerobic exercise (AEx) and upper extremity motor practice intervention on upper extremity (UE) function in chronic stroke survivors. This novel intervention pairs AEx with a virtual reality-based upper extremity rehabilitation game, Duck Duck Punch (DDP). While response to UE rehabilitation interventions, such as DDP, involves a multitude of factors, neuroplastic changes are a primary mechanism underlying functional recovery. [1] AEx has been shown to improve overall brain function [2]and promote a neuroplastic environment [3,4]; thus it may serve as an effective 'primer' and enhance the effects of DDP. Movement-based priming for neurorehabilitation involves performing movement or exercise before, or concurrent to, a therapeutic intervention with the goal of improving the efficacy of the therapeutic intervention. [5] Emerging evidence supports AEx as a potential priming tool for UE stroke rehabilitation. AEx combined with UE task training can improve UE function and self-reported health status in chronic stroke survivors. [6-8] Despite the promising results, there are gaps in the literature involving: 1) the clinical applicability of an AEx priming session; and 2) mechanisms contributing to changes in UE functions in response to AEx-primed UE rehabilitation. Addressing these gaps will be necessary to develop an AEx primer that is potent and time efficient, with respect to current clinical models. Therefore, the purpose of this pilot proposal will be to establish initial feasibility and tolerability benchmarks for the AEx + DDP intervention.

Aim 1: Demonstrate the feasibility of pairing AEx + DDP in stroke survivors with UE hemiparesis.

> *Outcomes for Aim 1: The primary objective of this project is to demonstrate feasibility and tolerability of this novel intervention. Metrics including recruitment, adherence, acceptability, retention, and adverse events will be examined to assess the feasibility and tolerability of AEx +DDP. Recently Valkenborghs and colleagues (2019) reported rates of 93% adherence, 95% retention, and 100% acceptability among 9 stroke survivors participating in a similar dual intervention. [8] We will use these benchmarks to determine the feasibility and tolerability of AEx+DDP.*

Aim 2: Quantify the magnitude of the effect of AEx + DDP intervention on UE impairment and function.

> *Outcomes for Aim 2: Two objective outcome measures, Fugl-Meyer Upper Extremity Assessment (FMA-UE) and Wolf Motor Function Test (WMFT), will be used to assess upper extremity impairment and function. This data will compared to historical controls from previous DDP trials from Dr. Woodbury's (Co-I) lab.*

Aim 3: Examine the relationship of biomarkers of neuroplasticity (BDNF and corticomotor excitability) and response to AEx + DDP.

> *Outcomes for Aim 3: We have selected transcranial magnetic stimulation (TMS)-based and blood-based measures to assess the neuroplastic potential of each subject as well as the acute effects of AEx+DDP on biomarkers of plasticity. These assessments will provide important data describing the neuroplastic potential of each subject (potential responders vs. non-responders) and the acute effect of AEx and DDP on indices of neuroplasticity.*

2.0 Background

With a surviving cohort of nearly 7 million individuals, stroke is the leading cause of long-term disability in the United States. [9] Of the ~795,000 new strokes occurring annually, approximately

2/3rds of survivors will have some degree of long term disability. [9,10] The prevalence of post-stroke disability coupled with the fact that more people are surviving stroke reflects an increasing need to develop effective rehabilitation strategies aimed at reducing disability and improving quality of life for the millions of stroke survivors, their families and caregivers.

The overwhelming majority of stroke survivors, >75%, exhibit upper extremity (UE) hemiparesis, and only 15% will recover fully. [11,12] Furthermore, residual UE impairment is closely linked to long-term disability [13] and reduced quality of life. [14] Current meta-analytic evidence supports virtual reality stroke rehabilitation interventions for improving UE function suggesting that this is a promising area for further therapeutic development. [15] Our team (Co-I: Woodbury) has developed an innovative virtual reality stroke rehabilitation game, Duck Duck Punch (DDP). DDP is an interactive computer game deliberately designed to enhance UE movement quality via individualized progressive movement practice along with an array of performance metrics allowing for within-session feedback on movement performance. Although response to UE virtual reality rehabilitation interventions, such as DDP, involves a multitude of factors, neuroplastic changes are a primary mechanism underlying functional recovery. [1] Thus, pairing DDP with a priming intervention to facilitate a 'neuroplastic-friendly' environment may make the CNS more amenable, and enhance response to DDP rehabilitation and ultimately improve outcomes.

Aerobic exercise (AEx) training has positive benefits on overall brain function including enhanced global cognition, executive function, and processing speed and attention in healthy, older adults. [2] Additionally, a single session of AEx acutely improves motor memory and learning in younger, healthy adults. [3,16] Although AEx has been used to improve cardiovascular function following stroke, [17] its neurofacilitatory effects in stroke have yet to be tested empirically. Candidate mechanisms through which AEx enhances brain function and motor learning include changes in circulating brain-derived neurotrophic factor (BDNF) and corticomotor excitability. BDNF is believed to play an integral role in several neuroplastic processes and promotes the strengthening of synaptic connections, i.e. long-term potentiation (LTP) and current research indicates that AEx can acutely [4,18] and chronically [19] increase circulating BDNF. Corticomotor excitability (CME) is often used as an indicator of LTP-like neuroplasticity and may underlie improvements in motor memory and learning. [20] Similarly to BDNF, AEx can acutely enhance corticomotor excitability in control and chronic stroke subjects. [3,21,22] Facilitating central nervous system function provides rationale to determine the role of AEx in 'priming' the brain for a subsequent intervention to maximize neuroplastic potential.

Preliminary Data:

Aerobic exercise enhances indices of neuroplastic potential. Single sessions of AEx can enhance neuroplastic potential [3,21] and serum BDNF [4,23,24] suggesting that it may 'prime' the brain for a subsequent rehabilitation intervention. Work from our lab indicates that, in neurologically intact subjects, AEx increases neuroplastic potential (Figure 1.a) and serum BDNF [4] in an intensity dependent manner. Currently, we have an ongoing project investigating the acute effect of AEx on neuroplastic potential in chronic stroke survivors (Fig 1.b).

Exploring a bout of priming AEx that fits into current clinical model. Current AEx priming models in UE stroke rehabilitation have incorporated priming bouts that have exceeded 30 minutes. [6,8] This could be problematic for stroke survivors lacking endurance to complete a 30-minute bout of AEx before rehabilitation. Additionally, extended priming bouts may be clinically burdensome if resources or staff is limited or if it incurs greater cost to the stroke survivor. Thus we are seeking to find a potent, yet time efficient, priming bout of AEx. Our previous work indicates that 15 minutes of AEx was sufficient in enhancing neuroplastic potential (Figures 1.a and 1.b) for up to 60 minutes post-AEx.

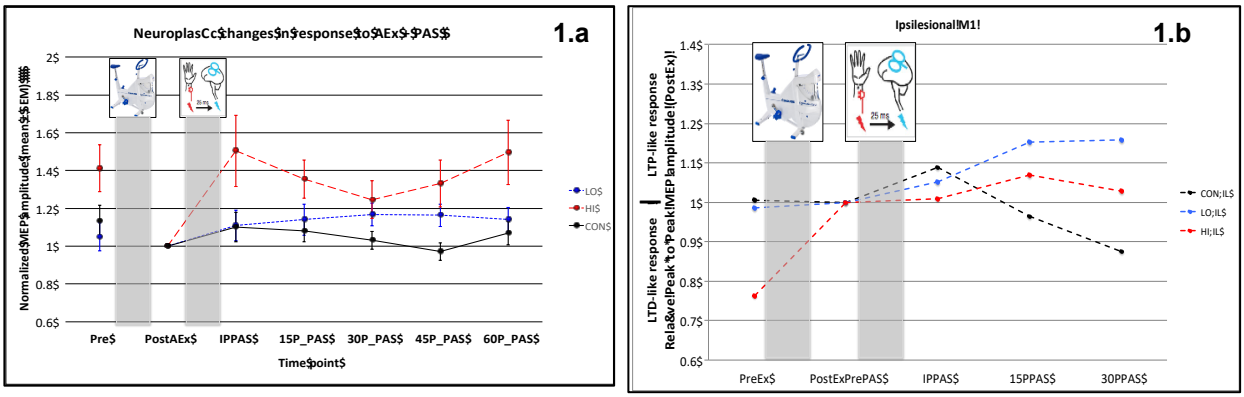

Figure 1. The acute effects of AEx on neuroplastic potential in control subjects (1a, n=38) and stroke subject (1b, n=1). Note that AEx (red and blue lines) enhances neuroplastic potential compared to sitting (black line).

3.0 Intervention to be studied

Figure 2. A subject playing Duck Duck Punch

This project involves the study of the effects of the combination of AEx and an UE motor practice intervention (DDP) on UE function in chronic stroke survivors. A preceding 15-minute bout of AEx will serve as a 'primer' to enhance the effects of the DDP intervention on UE function. Duck Duck Punch (DDP) is an interactive game with an old time carnival theme. In 2015 DDP received FDA clearance for use as a rehabilitation device for post-stroke upper extremity rehabilitation. The patient sits (we prioritized safety so that the player sits vs. stands) in front of the Microsoft Kinect and controls a virtual arm with his/her physical arm; reaching forward to "punch" virtual ducks (Figure 2). The game can be customized, i.e., made easier or harder, so that patients with all levels of impairment can play. Success motivates continued play, so that the user engages in high-repetition UE movement practice. Importantly, DDP was deliberately designed by therapists so that all aspects of the game elicit beneficial therapeutic arm and postural movements. These beneficial movements are ensured via:

Trunk Constraint: Trunk motions will be constrained by fixing the shoulder avatar in space meaning that the avatar arm does not respond to attempts to use compensatory trunk motion to "punch" the target rather than reach forward with the arm. As shown in the photos, the avatar hand moves forward only when the patient reaches forward using shoulder flexion-elbow extension not when he/she leans the trunk forward. Therefore, when a therapeutically beneficial (i.e., "therapist approved") strategy is used, the patient experiences the reward of successful game play to reinforce the movement strategy.

Avatar Elbow Extension Scaling: During set-up, the Kinect maps the patient's actual hemiparetic arm movement abilities and displays it on the screen. The user (therapist or patient) can scale, i.e., exaggerate, the patient's actual arm motion so that the avatar arm has more movement than the hemiparetic arm. This enables patients who have limited range of motion in the real-world, to have full range of motion in the virtual world which allows them to successfully play the games. The importance of this feature is that it enables the difficulty of game play sessions to match the patients' levels of ability. A task-difficulty to patient-ability match is required to facilitate post-stroke motor skill (re)learning that underlies recovery. [25] If game play is too difficult, i.e., if the patient does not have enough skill to achieve the task goal, compensatory trunk and/or arm movement strategies will be used. [26] Atypical, poor quality motions (learned bad-use [27]) may inhibit overall recovery. Matching

game difficulty to players' ability allows a combination of both errors and success during repetitive practice [28] thereby providing implicit and explicit feedback to challenge the nervous system and promote learning of good quality motions.

4.0 Inclusion and Exclusion Criteria/ Study Population

Eligibility screening: Subjects (male and female), ages 21-90, will be screened and recruited for the study six months to ten years post-stroke, allowing for natural recovery during the first 6 months post-stroke. The pool of candidates for the study will be recruited from the database registry supported by the Clinical and Translation Tools and Resources (CTTR) Core of the NIH-funded Center of Biomedical Research Excellence (COBRE) in Stroke Recovery at MUSC.. Eligible participants will be screened for participation and if appropriate will be accepted into the study.

Inclusion criteria: 1) experienced unilateral stroke at least 6 months, but no more than 120 months prior; 2) voluntary shoulder flexion of the affected arm ≥20° with simultaneous elbow extension ≥10°; 3) moderate arm movement impairment (UE Fugl-Meyer Assessment > 21 but < 52 points; 4) passive range of motion in paretic shoulder, elbow, wrist, thumb and fingers within 20 degrees of normal; 5) 21-90 years of age; 6) ability to communicate as per the therapists' judgement at baseline testing; 7) ability to complete and pass an exercise tolerance test.

Exclusion criteria: 1) lesion in brainstem/cerebellum as these may interfere with visual-perceptual/cognitive skills needed for motor re-learning; 2) presence of other neurological disease that may impair motor learning skills; 3) orthopedic condition or impaired corrected vision that alters reaching ability (e.g., prior rotator cuff tear without full recovery); 4) paretic arm pain that interferes with reaching; 5) unable to understand or follow 3-step directions; 6) severe cognitive impairment (Montreal Cognitive Assessment score <22); 7) severe aphasia; 8) inability to read English, 9) history of congestive heart failure, unstable cardiac arrhythmias, hypertrophic cardiomyopathy, severe aortic stenosis, angina or dyspnea at rest or during ADL's; 10) Severe hypertension with systolic >200 mmHg and diastolic >110 mmHg at rest; 11) history of, or current, depression; **and for brain stimulation procedures only:** 12) women of child-bearing potential; 13) electronic or metallic implants; 14) history of seizures.

5.0 Number of Subjects

A total of 10 chronic stroke survivors will be accrued locally.

6.0 Setting

All study procedures will take place at MUSC.

Functional Neurostimulation Laboratory (FNL): This laboratory provides state-of-the-art brain stimulation resources to support research activities. The bulk of research procedures for this proposal will take place in this laboratory. The FNL is physically located in the College of Health Professions Research building although researchers from various disciplines, including neurology, psychiatry, and rehabilitation, regularly collaborate in this lab. This lab offers state-of-the-art brain stimulation equipment that can be utilized for a multitude of research protocols. The primary brain stimulation tools are a MagStim BiStim^2 and MagStim Rapid^2 transcranial magnetic stimulator modules (The Magstim Company Limited; Whitland, UK). The stimulators can be outfitted with a figure-of-eight, double-cone, or air-cooled coil. The combination of these brain stimulation tools allow researchers to perform a variety of protocols including single pulse, paired pulse, and repetitive pulse to assess either upper or lower extremity neurophysiology. The lab is also equipped

with a stereotaxic passive marker and camera system as well as neuro-navigation software. Magnetic resonance images can be uploaded to the neuro-navigation software, which allows researchers to identify and accurately stimulate brain structures of interest. Neurophysiological data is collected using a multi-channel data acquisition and analysis package consisting of Spike2 v7.12 software (Cambridge Electronic Design; Cambridge, UK), a CED 1902 amplifier (Cambridge Electronic Design; Cambridge, UK), and a CED Micro 1401-3 data acquisition unit (Cambridge Electronic Design; Cambridge, UK). Additionally, the lab is equipped with a constant current stimulator and train/delay generator for paired peripheral nerve and brain stimulation protocols (i.e. paired associative stimulation). The myriad of tools available in this lab allows researchers to perform and analyze mostly all brain stimulation protocols. Specific to this proposal, there is ample space to perform the aerobic exercise protocol within the laboratory. Additionally there is a laboratory space adjacent to the FNL, which provides ample space for pre-participation screening. This reduces travel time between laboratories, and provides convenience for both study personnel and participants.

Upper Extremity Motor Function Laboratory: This MUSC supported laboratory features resources directed to generating and implementing innovative and scientifically-based rehabilitation interventions to improve recovery of UE motor function after neurological injury/disease. This lab features a virtual environment (enabling DDP intervention) and a multitude of assessment tools used in UE rehabilitation trials.

Locomotor Rehabilitation Laboratory: This 800 ft^2 laboratory is a shared resource supported in part by the Department of Health Sciences and Research, and features equipment capable of collecting kinematic, and electromyographic data during walking. Additionally, a COSMED Quark Cardio-Pulmonary Exercise Metabolic Analyzer is housed in this lab and provides the ability to assess respiratory gas exchange and electrocardiographic activity during exercise. Various exercise equipment (3 recumbent bikes, 2 treadmills, 1 recumbent step trainer) with appropriate safety apparatuses (overhead support system) are available in this lab.

7.0 Recruitment Methods

Subject recruitment:

Since the proposed research investigates stroke recovery it will be supported by the Clinical and Translation Tools and Resources (CTTR) Core of the NIH-funded Center of Biomedical Research Excellence (COBRE) in Stroke Recovery at MUSC. The CTTR Core provides subject recruitment resources through a bioinformatics-enabled database registry. Currently there are more than 900 post-stroke subjects in our database registry and ongoing recruitment is expected to add 1-3 more subjects each week. Additionally, Co-I's on this project have successfully recruited subjects for trials as part of the South Carolina Center for Stroke Recovery Research. Thus, we are confident that recruitment goals will be met without difficulty.

8.0 Consent Process

Informed consent will be obtained from participants prior to participation. Participants will first be informed of the purpose of the experiments and possible risks. The PI or an authorized study team member will then review the Informed Consent form with the potential participant, ensuring they are given adequate time to review the document. The potential participant will be asked if they have any questions about the study, and asked if they agree to participate. The Informed Consent and HIPAA forms will be signed by the participant. Copies of the signed forms will be given to the participant. The consent process will take place in a private room in the MUSC College

of Health Professions Research Building. There will be no set period between informing the prospective participant and obtaining the consent. In every session, participants will be reminded that they may end their participation in the study at any point.

9.0 Study Design / Methods

Project Overview. Subjects will attend 3 initial visits consisting of informed consent, screening, and assessments. All assessments and AEx + DDP sessions will take place in the College of Health Professions Research Building. The AEx + DDP intervention will consist of 18 sessions to be completed within 7 weeks. Subjects will complete 15 minutes of AEx prior to DDP. Sessions will be scheduled up to three times weekly separated by at least 24 hours to allow for adequate rest. After 18 sessions have been completed, post-testing will be scheduled. Any sessions that are not completed within 7 weeks of the first session will be considered missed visits. After completion of the intervention subjects will complete 2 assessment visits. It is expected that the entirety of the study will take

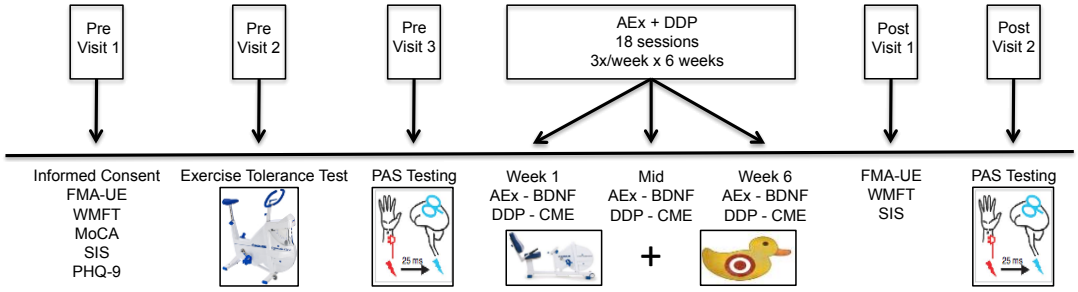

Figure 3. Study timeline

approximately 8 weeks to complete (See Figure 3 for study timeline).

Screening and assessment visits

Screening Assessments: Following informed consent procedures we will perform screening assessments. Participants will be screened for cognitive function and depression with the Montreal Cognitive Assessment and Patient Health Questionnaire.

- **Montreal Cognitive Assessment (MoCA):** The MoCA is 30-point test used for assessing cognitive impairment. It assesses short-term memory recall, attention and concentration, executive function, language, visuoconstructional skills, orientation, and calculations. Subjects demonstrating cognitive impairment, indicated by a MoCA score < 22 will be excluded. [29]
- **Patient Health Questionnaire (PHQ-9):** The PHQ-9 is a 9-item instrument used for screening and monitoring depression. [30] Since depression has been shown to impact neuroplastic potential, [31] subjects with depression (PHQ-9 > 4) will be excluded.
- **Transcranial Magentic Stimulation (TMS) Screen:** The TMS Screen has been developed from safety guidelines [32] to ensure patients and study participants are safe to receive TMS. If the TMS screen identifies one or more contraindications to TMS subjects will be excluded from receiving TMS assessments.

Clinical Assessments: We will perform clinical assessments of UE impairment (FMA-UE), UE function (WMFT), and 'real world' arm use (SIS). This assessment battery is standard to most post-stroke rehabilitation trials. These data will help describe the functional status of participants. A licensed physical therapist, exercise physiologist, or trained study personnel through the South

Carolina Center for Stroke Recovery Research will perform all assessments. These assessments will be performed pre- and post-intervention (Pre Visit 1 and Post Visit 1).

- **Fugl Meyer Upper Extremity Assessment (FMA-UE):** The FMA-UE is a 33-item measure of UE impairment; [33] however, the 3 items testing reflex response will not be administered because they do not measure a voluntary movement construct. Assessments will be video recorded and scored from the videos by trained, blinded raters. Each item will be scored on a 3-point rating scale (0=unable, 1=partial 2=near normal performance), item ratings will be summed and reported out of 60 points so that larger numbers indicate greater UE motor ability. The FMA-UE will be video recorded to allow for scoring by a blinded reviewer.
- **Wolf Motor Function Test (WMFT):** The WMFT is a 15-item measure of UE functional ability. [34] The WMFT will be administered in a standardized manner according to lab specific procedures. Assessments will be video recorded and scored from the videos by trained, blinded raters. Performance of each item will be timed (seconds) and the average time to perform items will be reported so that lower values indicate greater UE function.
- **Stroke Impact Scale (SIS):** The SIS assesses physical function as well as other dimension of health-related quality of life: emotion, communication, memory & thinking, and social role function. [35] Specifically, the Hand and Perceived Recovery subsets of the SIS [35] will be used to assess the effect of the intervention on 'real world' arm use. The SIS-hand consists of 5-items regarding difficulty of paretic hand use during everyday tasks during the previous two weeks. Items will be rated on a 5-point scale (5=not difficult, 1=cannot do) and reported as an average item rating. The SIS-recovery subtest is a single-item in which the participant rates his/her perceived post-stroke recovery from 0%-100% recovered.
- **International Physical Activity Questionnaire (IPAQ):** The IPAQ is a 5-part self-administered physical activity seven-day recall. The IPAQ assesses physical activity frequency, duration, and intensity over the previous seven days in four domains: (1) job-related, (2) transportation, (3) housework and family caring, recreation, (4) sport and leisure. In addition, the fifth domain assesses time spent sitting at work, at home, and during leisure time. The IPAQ has been shown to produce reliable results and validity has been established against accelerometer measurement of physical activity. [36] It is also easily implemented and appropriate for broadly defining physical activity habits.
- **NeuroCom Balance Master™ Test:** Participants will take part in a balance assessment using the NeuroCom Balance Master Sensory Organization Test. This test is able to identify whether balance deficits are due to vestibular, visual, or somatosensory dysfunction. Participants will stand on a force plate, connected to a harness to prevent falls, and participate in several conditions. Participants will be asked to keep their eyes open or closed, depending on the condition, while the force plate they are standing on and/or the walls surrounding them move according to their movements. If participants stay standing still, nothing will move. However, if participants sway, the force plate and/or walls, depending on the condition, will move accordingly. There are 6 conditions: 1) eyes open, everything stable. 2) eyes closed, everything stable. 3) eyes open, force plate moving. 4) eyes closed, force plate moving. 5) eyes open, walls moving. 6) eyes open, force plate and walls moving. All 6 conditions are completed 3 times. In total, the 6 conditions completed 3 times each will take approximately 15-20 minutes.

Exercise Tolerance Testing: Prior to experimental visits, a bicycle ergometry protocol modified from the LEAPS trial [37] will be used to assess exercise tolerance prior to study inclusion. The protocol will be overseen by a physician and will commence with the subject seated quietly for two minutes. Exercise will begin with the subject pedaling at ~60 revolutions per minute (RPM) and 0 Watts (W) of workload, with workload will be increased by ~15 W every 3 minutes. If the pedal cadence drops below 50 RPM, additional reminders will be given. Testing continues until maximal effort is achieved. The test will be terminated prior to achieving maximum effort for predefined symptomatic, clinical, and electrocardiographic criteria. Symptom-related reasons for termination include angina, dyspnea, and fatigue. Fatigue is defined as either voluntary exhaustion or inability to maintain a minimum cycling cadence of 40 RPM. Clinical criteria for termination include: 1) Hypertension: ≥ 220/120 mmHg, or 2) Hypotension: a drop in diastolic blood pressure >20 mmHg, and O2 saturation <85%. Electrocardiogram criteria include: 1) ≥ 1 mm horizontal or down sloping ST segment depression, 2) sustained paroxysmal ventricular tachycardia (>30 beats), and 3) sustained paroxysmal supraventricular tachycardia (>30 beats). If the test is terminated because of electrocardiographic findings, the subject will be managed medically as needed, referred for care, and disapproved for participation. Resting blood pressure and heart rate will be obtained prior to initiation of exercise as well as after the subject has been sitting on the stationary bicycle for 1 minute. During the exercise test, blood pressure readings will be obtained every 3 minutes. Heart rate will be obtained from the 12-lead EKG. Maximal heart rate will be recorded as the highest heart rate achieved during the exercise tolerance test.

Neurophysiological Assessments

- **Assessment of neuroplastic potential:** Participants' neuroplastic potential will be assessed with a plasticity-inducing paradigm called Paired Associative Stimulation (PAS). Briefly, PAS utilizes a repeated and timed peripheral nerve stimulation combined with transcranial magnetic stimulation (TMS) of the contralateral motor cortex to induce motor cortex plasticity. Prior to- and after PAS, corticomotor excitability (CME) is assessed via motor evoked potentials (MEP) which are obtained by single pulse TMS and electromyography (EMG) of a contralateral peripheral muscle. PAS will be assessed pre- and post-intervention (Pre Visit 3, Post Visit 2). PAS will be assessed in the ipsilesional motor cortex and paretic UE.

- **Paired Associative Stimulation:** Prior to PAS, all participants will be assessed for potential contraindications to TMS using a safety-screening questionnaire. First, we will use a combination of on-line surface electromyography (sEMG) recordings to view peak-to-peak motor evoked potential (MEP) amplitude and a neuro-navigation system to identify the optimal coil position for stimulation of the abductor pollicus brevis (APB) muscle (APB 'hotspot'). The area on the hand knob of the motor cortex that produces the largest and most consistent MEP's of the APB muscle will be marked as the 'hotspot' using the neuro- navigation system. We will then assess baseline cortical excitability by collecting a 'bin' of twenty test MEP's and a standardized stimulation intensity. This stimulation intensity will remain the same throughout the experimental session in order to allow for comparisons of MEP amplitude before and after PAS. After assessment of cortical excitability participants will receive PAS. For PAS, participants will receive a combination of simultaneous peripheral nerve stimulation and brain stimulation. Stimulation of the median nerve will be delivered via constant current stimulator (DS7A; Digitimer, Hertfordshire, UK) just proximal to the wrist. Bipolar electrodes will deliver a 200μs square wave pulse at an intensity equal to 300%

sensory threshold. Single pulse TMS will be applied to the APB muscle representation on the contralateral motor cortex 25ms (ISI25) after the delivery of median nerve stimulation. A train/delay generator (DG2A; Digitimer, Hertfordshire, UK) will trigger paired peripheral nerve stimulation and brain stimulation. A total of 200 paired stimuli will be delivered at rate of .25 Hz, resulting in a total stimulation time of approximately 14 minutes. Bins of twenty test MEP's will then be collected immediately, fifteen, and thirty minutes post-PAS.

- **Electromyographic assessment:** Surface electromyography (sEMG) will be collected using 2cm by 2cm surface recording electrodes (Natus, Pleasanton, CA, USA) and will be placed perpendicular to the orientation of the fibers of the APB muscle belly. Prior to sEMG placement the site of the APB muscle will be lightly abraded and cleaned with pre-packaged alcohol swabs. All EMG signals will be collected using Spike2 v7.12 software (Cambridge Electronic Design; Cambridge, UK), amplified (x1000), bandpass-filtered (100-2000 Hz) using a CED 1902 amplifier (Cambridge Electronic Design; Cambridge, UK), and sampled at 5000 Hz using CED Micro 1401-3 data acquisition unit (Cambridge Electronic Design; Cambridge, UK). Data will be saved directly to a network drive for offline analysis. During all stimulation protocols sEMG activity will be continuously monitored in order to ensure that the APB muscle is not voluntarily active.

- **Assessment of DDP-induced CME:** The effect of DDP on CME will be assessed in the paretic UE. Before and after DDP on 3 occasions (DDP sessions 2, 10, and 17) a bin of twenty test MEP's will be collected. If reductions in impairments and improvements in function are mediated by a strengthening of the corticomotor pathway (eg. long-term potential-like response) we would expect DDP to increase CME. While PAS testing will provide information regarding the neuroplastic potential of each subject, this assessment will provide data describing the direct effect of a single and multiple sessions of DDP on the corticomotor pathway of the paretic UE.

Blood specimen collection

- **BDNF assessment:** Blood specimens will be obtained immediately before and after AEx on three separate occasions (sessions 1, 9, and 18). A nurse from the South Carolina Clinical and Translational Research Institute (SCTR) will conduct all blood specimen collections. Briefly, an intravenous catheter will be placed in a superficial forearm vein at the beginning of the experimental session and will be maintained patent using an isotonic saline solution. Baseline blood samples will be drawn after a 30-minute equilibration period and will be drawn immediately before exercise commences. Immediate post-exercise blood samples will be taken within sixty seconds of exercise completion while the participant remains seated in the cycle ergometer. Blood samples will be collected into serum-separating tubes (SST) containing gel and clot activator and Ethylenediametetraacetic acid (EDTA) tubes and will be processed by The South Carolina Clinical and Translational Research Institute's Laboratory and Bio-repository. The purpose of collecting samples into SST and EDTA tubes will be to allow for assays for serum and plasma BDNF to be performed. Samples will be processed according to manufacturers recommendations. Enzyme-linked immunosorbent assays (Quantikine, R&D Systems, Minneapolis, MN) will be performed, in duplicate, to determine the concentration of serum and plasma BDNF. Serum and plasma BDNF will be assayed so that we may more completely describe

exercise-induced changes in BDNF. Recent work suggests reporting both serum and plasma to account for the potential of platelet activation to overestimate the effect of exercise on serum BDNF. [38,39]

Intervention visits

The AEx + DDP intervention will be a total of 18 sessions to be completed within 7 weeks. Each week, subjects will attend up to 3 sessions of AEx + DDP on non-consecutive days (M, W, F). It is expected that AEx + DDP sessions will last approximately 90 minutes. Post assessment visits will occur after the completion of 18 AEx + DDP session or 7 weeks, whichever occurs first.

AEx: Aerobic exercise will be performed on a recumbent stationary cycle (Monark 837e). During AEx, heart rate, blood pressure, and rate of perceived exertion (RPE) [40] will be assessed at baseline, every 5 minutes during exercise (or sitting), immediately post-exercise, and then 5 minutes post-exercise. Exercise intensity will be determined using the Karvonen equation. [41] The Karvonen equation uses an individual's maximum heart rate (MHR) and resting heart rate (RHR) to calculate heart rate reserve (HRR) using the equation: HRR = (MHR) – RHR. The MHR for each subject will be determined during his/her exercise tolerance test. An exercise physiologist will oversee each AEx session. The target intensity of each AEx session will be 70% HRR and will remain constant throughout the intervention. This intensity was selected for the 'priming' bout of AEx as preliminary results from our lab have shown it is has a substantial impact on neuroplastic potential (See Figure 1.a) and serum BDNF [4] in control subjects.

DDP: A therapist will oversee the patient's safety and progress during DDP by: (1) monitoring patient compliance and performance via metrics provided by the Microsoft Kinect system, (2) determining session parameters for all DDP sessions, and (3) continuously monitoring the patient to assess fatigue/pain, answer questions, and problem solve any issues. DDP will be dosed based on the number of repetitions performed. A repetition is recorded when the player moves his/her arm so that the avatar leaves a start position. The goal dose for subjects will be 200 repetitions per DDP session. The number of repetitions was selected because subjects enrolled in our previous studies have averaged 200 self-selected repetitions per day, achieved in ~1 hours without adverse event. Birkenmeir et al (2010) demonstrated that subjects with chronic stroke could safely obtain 200-300 arm movement repetitions in a 1-2 hour therapy session. [42] The therapist overseeing DDP will be permitted to reduce the dose if a subject reports substantial fatigue or pain.

Participant remuneration: Study subjects will be paid $25 per study visit. Subjects may attend up to a total of 23 study visits thus may receive up to $575 of total compensation for participating in the study. Payments to subjects will be made via ClinCard. Subject will have $25 added to their ClinCard for each study visit attended.

10.0 Specimen Collection and Banking

Blood specimens will be collected as part of the study procedures. The specimens collected will be used to assess serum and plasma BDNF. Blood samples will be processed and stored by The South Carolina Clinical and Translational Research (SCTR) Institute's Laboratory and Bio-repository at MUSC. The specimens will be transported to the SCTR Research Laboratory in a clearly marked biohazard container by study staff or a SCTR nurse. The PI and SCTR Research Laboratory staff will have access to the specimens and associated data. Specimens will not be banked for future use after processing and analysis is completed.

11.0 Data Management

Power Analysis: Although the primary goal of this proposal is to demonstrate feasibility and tolerability of AEx+DDP, the sample size is based on change in FMA-UE scores from recent work from Linder and colleauges.[6] These data, for individuals with moderate UE impairment (the target sample of this study) yielded a FMA-UE mean change score of 12.3 for AEx + UE task practice and a FMA-UE mean change score of 4.4 for UE task practice only. Assuming the difference in mean FMA-UE change scores of 7.9 (pooled SD of 4.25), a sample size of n=5 for each group (AEx+DDP and DDP only) achieves 80% power with alpha = 0.05. Although we will not enroll a DDP-only group, we will be able to compare AEx+DDP to historical controls from the Co-I's lab. Two trials involving DDP have data from large samples (n=66 and n=103) providing ample data for comparison of AEx+DDP to DDP. Considering recruitment rates from the Co-I's previous trials (2-5 subjects/month) and anticipating a 20% dropout rate will inflate the sample size to n=10. Given recruitment rates and the timeline of this proposal (1 year) achieving this sample size appears feasible. This sample size is comparable to published literature [6,8] and is sufficient to detect a large effect of AEx+DDP on FMA-UE compared to historical controls receiving DDP only. Additionally, this sample will provide a robust pilot data set for calculating effect sizes to power future trials examining AEx as primer for UE motor rehabilitation.

Statistical Analysis Plan: Feasibility and tolerability metrics of adherence, retention, and acceptability will be assessed (Aim 1). FMA-UE, WMFT, and SIS data will be compared to historical control data from previous DDP trials from Dr. Woodbury's lab (Aim 2). We will utilize ANOVAs to test the effect of adding AEx to DDP on the mean FMA-UE, WMFT, and SIS-hand item rating and the average SIS-recovery scores (PROC GLM, SAS v9.4). Additionally, data describing the effect of AEx+DDP on UE impairment and function will be used to generate effect sizes for future research proposals. Pre- and post intervention PAS assessment will be tested with a student's T-test or Wilcoxon rank-sum test based on distributional assessment. The acute (within session) and training (over the course of intervention) effect of AEx on BDNF and DDP on CME will be assessed with a generalized mixed model analysis (PROC MIXED). Relationships of biomarkers of plasticity (PAS, BDNF, CME) and response to AEx+DDP (change in FMA-UE or WMFT) will be explored with Pearson or Spearman-rank order correlational analysis, depending on distributional assessment (Aim 3).

Confidentiality: All records regarding participation in this study will be kept in locked file cabinets in the appropriate laboratories and/or offices, and stored on MUSC network storage. Access to linked identifiers is limited to research personnel intimately involved with the human subjects. All data and records acquired from subjects is for research purposes only and will be kept confidential and maintained in a secure database identifiable only by subject code. The results of the study may be published for scientific purposes; however, subjects' identities will not be revealed and data will not be traceable to any individuals in any resultant publications. The information gathered during this study will be kept confidential to the extent permitted by law.

12.0 Provisions to Monitor the Data to Ensure the Safety of Subjects (if applicable)

An exercise physiologist or occupational therapist will be present during all treatment sessions. In addition, the research staff will closely monitor the subject to ensure their comfort. Any adverse events will be recorded and monitored as required by our Institutional Review Board. In the event of an adverse medical event, standard facility emergency procedures will be followed and proper personnel notified. The PI on this proposal is trained in exercise physiology and the PI's mentors on this project include a physical therapist and an occupational therapist.

Blood Specimen Collection Data Safety and Monitoring Plan: A registered nurse will conduct all blood specimen collections and be present during each testing session. All specimens will be given unique identifiers and will be stored in the SCTR Research Nexus Biorepository.

Brain Stimulation Data Safety and Monitoring Plan: We will implement a screening tool for all participants undergoing TMS that is currently used in the FNL at MUSC. In addition, the PI's mentor will personally supervise the first several participants. Stimulation sessions will be stopped immediately with any complaints of pain or burning at the stimulation sites or for any complaints of dizziness or light-headedness. All brain stimulation techniques utilized in this project have been demonstrated to have minimal risk to participants and all parameters will be within the published guidelines. All participants will be receiving TMS while in a recumbent position. In the event of a seizure the participant will remain in the TMS chair and all study procedures will cease. The immediate surrounding area will be cleared of all potential hazardous materials or objects. If the seizure persists for 5 minutes or longer, emergency personnel will be called. Participants experiencing seizures that last for shorter periods of time will be observed for 20 minutes after the seizure has ended. If a participant experiences a second seizure or remains confused or disoriented during this time frame then emergency personnel will be called. Under no circumstances will study procedures continue after a participant has experienced a seizure.

13.0 Risks to Subjects

Screening: This activity will involve answering a few questions that could cause the participant to become upset, emotionally distressed, or embarrassed. To reduce this risk, screening will occur in a private office with only study personnel present.

NeuroCom Balance Master™ Test: The participant will wear a safety harness attached to the top of the machine so that a fall cannot occur (weight limit 500 lbs), and a research assistant will be in close proximity to provide assistance if a loss of balance does occur. If a participant is unable to complete conditions due to loss of balance or dizziness, the assessment will be stopped.

Aerobic Exercise: The risks of exercising at submaximal intensities on a cycle ergometer (as proposed in this study) are minimal but could include fainting, abnormal blood pressure response, irregular heartbeat, dizziness, and muscle soreness. However to ensure safety, all participants will complete an exercise tolerance test evaluated by a physician. The risk of serious adverse response to exercise testing, which is a greater intensity than will be prescribed in this study, has been reported to be less than 1 in 2500 cases. Study personnel will monitor participants' vital signs (heart rate and blood pressure) before, during, and after exercise. Given the low likelihood of an adverse response to submaximal exercise and screening prior to participation we believe the risk of exercise to be low. In addition, professional staff (exercise physiologist, physical therapist) will be present and available throughout each session.

Duck Duck Punch: The treatment session duration and number of movement repetitions (200/session) may induce fatigue or pain, primarily in the paretic arm. Subjects in Dr. Woodbury's DDP studies have averaged ~200 repetitions per session and empirical evidence suggests that chronic stroke survivors can safely achieve 200-300 arm movement repetitions per in a 1-2 hour therapy session. [42] To assess and monitor pain and fatigue, subjects will be instructed in how to use the Borg Rating of Perceived Exertion [40] scale and a 10-point Pain Rating Scale. [43]

Transcranial Magnetic Stimulation (TMS): There is a very low risk of a seizure during or after TMS. The risk of seizure induction by this protocol has been thoroughly assessed and the TMS parameters have been chosen to be well within published safety guidelines for the conduct of TMS studies in human subjects. The risks of non-invasive brain stimulation via TMS are minimal when proper screening is conducted prior to participation. Those with absolute contraindications (eg. history of seizures, metal implants) to TMS will be excluded from participating in the study. Headaches and complaints of short-term hearing difficulties have also been reported following TMS. Headaches are temporary and manageable with common over-the-counter pain remedies and all subjects will wear foam earplugs for protection during TMS sessions. To reduce the risk of an adverse event, all participants will be required to pass a TMS screen, wear foam ear plugs and will be continuously monitored for any abnormal responses to TMS. Additionally, the TMS device is equipped with an automatic shut-off switch in case the coil delivering the stimulation begins to overheat.

Muscle Activity Testing: There is a slight risk of skin irritation with the use of surface EMG electrodes and tape. To reduce this risk, study personnel will perform visual inspection of the participant's skin before and after testing to ensure that the participant did not have an adverse reaction to the electrode.

Paired Associative Stimulation (PAS): The risks associated with PAS are the same as the risks for TMS, however some irritation of the skin at the site the peripheral nerve stimulation may occur.

Blood Sample collection: A nurse will perform all blood sample collections. The risks of taking blood include pain, a bruise at the point where the blood is taken, redness and swelling of the vein and infection, and a rare risk of fainting. To reduce this risk, only a nurse will be permitted to take blood sample collections. Proper anti-septic procedures will be followed in order to minimize the risk of infection at the site of the puncture of the vein.

Unforeseen risks: The study may also have risks that are unforeseeable at this time.

Protection against risks

An exercise physiologist, occupational therapist, or physical therapist will be present during all testing and intervention sessions and a nurse will perform all blood draws. All participants will be screened prior to participating in exercise or brain stimulation procedures to ensure safety. Any adverse events will be recorded and monitored as required by our Institutional Review Board. The PI on this proposal is an exercise physiologist, a certified strength and conditioning specialist and has approximately 10 years experience in the development and implementation of exercise interventions and three years experience delivering TMS. In the event of an adverse medical event, standard facility emergency procedures will be followed and proper personnel notified. Any adverse events will be recorded and monitored as required by the IRB. Subjects will be able to terminate the training or testing sessions at their request at any time without prejudice.

Aerobic exercise: During all exercise sessions the research staff will closely monitor the subject to ensure their comfort. Minimization of risk will be accomplished by monitoring vital signs within prescribed criteria for termination of aerobic exercise. We will follow the American College of Sports Medicine criteria for terminating an exercise session, which includes: subject complaints of light-headedness, confusion, or dyspnea; onset of angina; excessive blood pressure changes (systolic BP greater than 220 mmHg, diastolic BP greater than 110 mmHg); and inappropriate bradycardia (drop in heart rate >10 beats per minute).

Duck Duck Punch: We will document subjects' perceived exertion and pain before, during and after each therapy session in a written log. Ratings of 12-14 on the Borg scale (6-20) suggest that the activity is being performed at a moderate intensity level. If a Borg rating ≥14, or if a pain rating ≥5 (moderate; 0-10 scale), then subjects will rest and we will reduce DDP game difficulty. Subjects' ratings will be reviewed by study personnel and adjustments to the individual subject's program will be made as needed.

TMS testing: The primary safety concern with TMS is the induction of seizures; however, the incidence of seizures is very low and mostly associated with high frequency *repetitive* TMS (rTMS). We will use single-pulse TMS, which is safer yet, and we will follow published safety guidelines for diagnostic TMS to minimize the risk of inducing a seizure. Hearing protection for all subjects during TMS will be provided. Stimulation sessions will be stopped immediately with any complaints of discomfort or for any complaints of dizziness or light-headedness. There are no lower-risk methods available to gain the same scientific information. In addition the PI has also been trained in TMS through a training course provided by the Brain Stimulation Laboratory (BSL) at MUSC. The PI, or study personnel that has been appropriately trained, will perform all TMS procedures.

Confidentiality: All records regarding participation in this study will be kept in locked file cabinets in the appropriate laboratories and/or offices, and stored on password-protected computers/servers in the offices and laboratories of the PI's research team. There will be no direct link to participant identifying information (other than subject code) without access to a password-protected computer containing the identifying information linking information to a given subject. Access to linked identifiers is limited to research personnel intimately involved with the human subjects. All data, video and records acquired from subjects is for research purposes only and will be kept confidential and maintained in a secure database identifiable only by subject code. The results of the study may be published for scientific purposes; however, subjects' identities will not be revealed and data will not be traceable to any individuals in any resultant publications. The information gathered during this study will be kept confidential to the extent permitted by law.

14.0 Potential Benefits to Subjects or Others

Subjects who participate in this study may see improvements in UE function, but any benefit cannot be guaranteed. Others may benefit from advancement of scientific knowledge. Given the minimal risks involved and the potential for short-term benefits and advancement of scientific knowledge, the potential benefits of participation make the potential risks reasonable.

While AEx has established functional and health benefits for stroke survivors, its ability to enhance concurrent UE motor rehabilitation programs, such as DDP, has largely been theorized. Combining AEx with UE motor rehabilitation has the potential to foster a perpetual cycle of improved health and physical function which stimulate improvements in other aspects of stroke recovery (eg. activity limitations, participation restrictions, recurrence of subsequent stroke). Given the minimal risks involved and the potential to add to the limited base of scientific knowledge describing this problem, the potential risks are reasonable.

15.0 Drugs or Devices

Transcranial Magnetic stimulation, specifically repetitive TMS (rTMS), is an FDA approved device to be used for the treatment of depression. Although, in this project only single pulse TMS will be employed. The TMS device will be stored and dispensed in the Functional

Neurostimulation Laboratory in the College of Health Professions Research Building at MUSC. The PI will be designated to store the device and the PI, Co-I's, or appropriately trained study staff will be permitted to dispense the device. TMS will only be dispensed according to the published guidelines and safety measures. [32]

Duck Duck Punch (DDP) has FDA clearance for use as a rehabilitation device for post-stroke upper extremity rehabilitation. DDP will be stored in the Upper Extremity Motor Function Laboratory in the College of Health Professions Research Building at MUSC. The PI will be designated to store the device and the PI, Co-I's, or appropriately trained study staff will be permitted to dispense the device.

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