Protocol

1. Effects of exercise intervention on aging-related motor decline

(Short title: Exercise & Motor Decline)

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2. Abstract:

The present study investigates the effects of an exercise intervention on neurological correlates of aging with sedentary older adults. Whereas chronological age measures the number of days a person has been alive, biological age refers to an individual's immediate state of health in relation to the average individual across age groups. As progressive biological aging in sedentary adults is associated with declines in the neurological motor system, an important application of aging-related research is the identification of treatment interventions that may slow, stop or even reverse the biological aging process.

Aerobic exercise may exert such anti-aging effects on the neurological system, particularly in relation to patterns of interhemispheric communication. Recent studies by the principal investigator have indicated that aerobic activity level might maintain upper extremity motor performance. This study extends the previous cross-sectional research using a direct exercise intervention to measure effects on upper extremity function and patterns of motor activity within the brain.

The current study proposes to use modern neuroimaging (functional magnetic resonance imaging [fMRI] and diffusion tensor imaging [DTI]) and neurophysiological (transcranial magnetic stimulation [TMS]) techniques to investigate the effects of aerobic exercise on aging related changes of the neurological system. We will recruit 40 older, sedentary individuals. Our previous research has indicated that aging is associated with a loss of interhemispheric inhibition present in TMS (duration of the ipsilateral silent period) and during the performance of unimanual tasks in fMRI. We hypothesize that, initially, the sedentary adults will show patterns of motor activity in TMS and fMRI consistent with that of older adults, whereas after training, brain activity will be more reflective of patterns typically seen in younger adults.

Outcome measures will include changes in the battery of upper extremity motor function tests which target dexterity, psychomotor speed and reaction time. For experimental design, we will examine the effects of both aerobic exercise and dexterity training. This will be a cross-over design whereby some participants receive aerobic exercise training prior to dexterity training and others will receive dexterity training prior to aerobic exercise training. This will allow us to differentiate the effects of each type of training on motor performance and neurophysiological function.

3. Introduction and Background:
Aging, Interhemispheric Communication and Motor Performance

Older adults frequently complain of increased clumsiness and slowing reaction times in the upper extremity. Motor performance measures such as reaction time (Salthouse, 1984), psychomotor speed (finger tapping) (Salthouse, 1996; Cullum et. al, 1989), fine dexterity (Poston et al., 2003; Carmeli et al., 2003;) and reach accuracy (Sarlegna, 2006; Christou, 2011; Kornatz et al, 2005) all show decreases with advancing age. While some individuals find such loss of function a mere annoyance or slight embarrassment, many individuals suffer more severe consequences including loss of job responsibilities or social withdrawal. Behavioral interventions have been shown to improve motor performance (Poston et al., 2003; Christou et al., 2011), but the retention of these improvements tend to be short-lived (Carvalho et al., 2009).

Recent evidence has indicated that upper extremity performance declines in older age and may be related to changes in levels of interhemispheric inhibition of the primary motor cortices (Langan et al., 2010; Fling & Seidler, 2011; Bernard & Seidler, 2011). During volitional movement of a single upper extremity, the contralateral primary motor cortex exhibits an increase in metabolic activity consistent with increased synaptic function. However, in young adults in particular, the activity level of the opposite primary motor area tends to decrease as compared to baseline levels (inactivity). FMRI has been used to probe patterns of motor cortical recruitment during unimanual activity and increasing evidence shows that older adults tend to show increases in BOLD activity in bilateral primary motor areas during such movements (Riecker et al., 2006; Talleli et al., 2008a; Ward et al., 2008; McGregor et al., 2009, 2011). However, the use of fMRI in the description of interhemispheric and intracortical patterns of communication is a relatively recent development for investigation of the human neural motor system, however. Over the past twenty years, the standard technique for motor systems inquiry has been the use of TMS, which directly investigates neural response while offering excellent temporal resolution. Interestingly aging-related studies of interhemispheric communication have employed this modality and yielded analogous findings to investigations involving fMRI. For example, using a paired-pulse TMS paradigm, Peinemann et al., (2001) reported decreases in levels of interhemispheric inhibition across increasing age, a finding later replicated by Talelli et al. (2008a; 2008b). Using a separate, but related measure called the ipsilateral silent period, Sale and Semmler (2005) reported that their elderly volunteers reported a significantly shorter duration of ipsilateral inhibition; a finding recently replicated by Fujiyama et al., (2009) and previous work by the PI (McGregor et al., 2011).

In aging adults, recent work has shown that the decline in unimanual motor performance in older adults is associated with decreased duration of the ipsilateral silent period (McGregor et al., 2011; Fling et al, 2011) and changes in excitability of the ipsilateral hemisphere (Bernard & Seidler, 2011; Langan & Seidler, 2010). The implication of these findings is that aging is associated with decreased interhemispheric inhibition which may be driving declines in motor performance. For example, Bernard & Seidler used transcranial magnetic stimulation to measure motor evoked potentials in the ipsilateral cortex of younger and older adults. The group found that older adults had larger motor evoked potentials in the ipsilateral cortex indicating a decrease in interhemispheric inhibition as compared to younger adults. Importantly, the group also found a somewhat proportional increase in reaction time in the older group respective of the group differences in evoked response amplitude. In our own lab, we have recently shown that duration of ipsilateral silent period, also associated with interhemispheric inhibition, is negatively correlated with age in sedentary older adults (McGregor et al., 2011). Further, work from a recent project has shown that sedentary individuals even in mid-life (40-60) show decreases in duration of ipsilateral silent period and these decreases are correlated with performance on reaction time and dexterity measures (9-hole pegboard test). Further, evidence from cases of motor pathology indicates that a loss of interhemispheric inhibition is associated with increases in performance detrimental mirror movements. For example, individuals with Klippel-Feil (Leinsinger et al., 1997) or Kallman's syndrome (Verstynen et al., 2007) have an increased incidence of mirror movement concomitant with impaired levels of interhemispheric inhibition. These disorders tend to result in debilitating mirror activity during intended unimanual activity making activities such as driving or typing difficult or impossible.

Recent evidence suggests that chronological age may not be the ultimate cause of these changes in interhemispheric communication (McGregor et al., 2011; McGregor et al., 2009; Talelli et al., 2008b; see also Manson et al., 2006). For example, sampling across multiple age groups, Talleli et al. (2008b) reported that while chronological age did predict a pattern of fMRI activity that is consistent with a potential loss of ipsilateral inhibition (i.e. – positive BOLD in bilateral motor cortices), the best predictor of such fMRI activity was actually measures of cortical reactivity during TMS stimulation. In their sample, numerous participants in middle age showed fMRI data that were more characteristic of patterns found in elderly populations. Additionally, these same investigators reported cortical reactivity measures typically associated with elderly brains (e.g. – TMS recruitment curve assessment). As such, the age-related changes in motor activity and upper extremity performance described above may not be inevitable consequence of age, however, and, importantly, may not be immutable in individuals. There is increasing evidence that aerobic exercise and physical fitness may be a critical component in not only slowing the onset of these changes, but also reversing them.

Exercise, Aging and Interhemispheric communication

Aerobic activity has long been shown to have promise in the mitigation of age-related declines in neurophysiology. While the beneficial effects of aerobic exercise on learning and memory are well known (Vaynman & Gomez-Pinilla, 2005; Winter et al.,

2007), the use of advanced neuroimaging techniques has begun to provide valuable information about the positive effect exercise has on brain structural neuroanatomy. For example, aerobic exercise has been associated with increases in overall brain volume (Colcombe et al., 2006; Chaddock et al., 2010a; 2010b), increases in gray matter density (Chaddock et al., 2010a; Colcombe et al., 2003) and may contribute increased density in brain white matter (Marks et al., 2007, 2010). Erickson et al., 2011 recently produced a very exciting recent report of the beneficial effects of physical fitness on neural health that described changes to the size of the hippocampus of individuals enrolled in a longitudinal exercise program. Individuals were enrolled in either a long-term exercise program or a stretching control. Those in the long term exercise (walking) program showed increases in the size of the hippocampus and improved cognitive performance at study conclusion as compared to age-matched individuals in a stretching control group.

Some of the most exciting research exploring aerobic fitness' effects on the aging brain has come from functional neuroimaging methodologies. Functional MRI has been recently used to investigate differences in brain activity on tasks involving prefrontal areas between individuals of varying aerobic fitness. In a recent cross-sectional study, Prakash et al., (2011) reported that older participants (>55 years) who were aerobically fit tended to show brain activation consistent with proper task performance during an interference task, while sedentary older adults, who exhibited lower performance behavioral testing and showed patterns of recruitment in the prefrontal cortex more similar to adults. A previous longitudinal fMRI investigation was completed by Colcombe et al. (2005), and targeted the direct effects of exercise intervention on sedentary populations during an executive function task. In this study, participants were either divided into a group engaging in an aerobic exercise program or a control group that engaged in anaerobic stretching. Older adults who engaged in the exercise intervention program tended to show speed and accuracy improvement in memory search tasks. Further, exercising older adults revealed changes in brain activity during fMRI of the same task that more closely resembled activation patterns characteristic of younger adults.

Importantly, there is recent evidence that indicates that regular engagement of physical activity can alter age-related changes in patterns of motor neural activity as described above. Our lab has recently reported (McGregor et al., 2011) that elderly adults who engage in regular physical activity have shown decreased bilateral recruitment of the primary motor cortex during unimanual task performance in fMRI. Importantly, these fMRI changes were correlated with duration of ipsilateral silent period providing strong evidence of change in interhemispheric communication during unimanual tasks. In a recent project, preliminary data analysis shows that middle age (40-60 years), sedentary adults begin to exhibit patterns of neural activity associated with a loss of ipsilateral inhibition (see Preliminary Studies). Voelcker-Rehage et al., (2010) recently reported that elderly adults who engage in aerobic activity show decreased activity in the ipsilateral motor cortex during unimanual movement in fMRI. However, to date, we know of no investigation that has attempted to investigate the direct effects of an exercise intervention on older adults on motor cortex excitability.

From the cited findings and the PI's previous research program, the impetus behind the PI's current research program has evolved. A previous study compared the effects of long-term physical fitness on neurophysiological measures of interhemispheric inhibition that may be diagnostic of biological motor health. This study has shown that elderly adults who are physically fit may mitigate a loss of interhemispheric inhibition prevalent in sedentary older adults (McGregor et al., 2011). However, the project was limited realized that like most cross-sectional aging research using advanced neuroimaging, the interpretation of findings from this study was somewhat limited due to the use of age extremes. To address this issue, the PI completed a project investigating the effects of aerobic fitness on interhemispheric inhibition in middle aged individuals. Preliminary data analysis shows that physical fitness level in middle age is associated with higher levels of motor performance (McGregor et al., 2012).

The goal of the current project is to investigate the direct effect of an aerobic exercise intervention on levels of interhemispheric communication in sedentary older Veteran's and subsequent changes in motor performance. We hypothesize that an aerobic intervention will improve upper extremity motor performance and decrease or reverse losses in interhemispheric inhibition in these participants.

4. Objectives

The short term objective of this proposal is to take the next logical step of our research program and identify the effects of aerobic exercise in addition to dexterity training on upper extremity motor function using an intervention approach. Prior to any intervention, participants will undergo a self-monitored 12-week stretching program of the upper extremity. We will inquire as to physical activity level on a bimonthly basis. After this period participants will be randomized into either a 12-week exercise or 12-week behavioral training sessions (depending on group placement) both of which are expected to facilitate physiological changes.

For the 12-week aerobic exercise component of this group, participants will follow the guidelines provided by the American College of Sports Medicine for optimizing cardiovascular fitness. Drs. McGregor and Nocera will monitor the implementation of these guidelines. Participants will exercise 3 times a week on a stationary cycle ergometer in a group setting. We chose a lower extremity intensive exercise task to decrease potential confounds with upper extremity motor practice (such as the use of an arm cycling ergometer). The 12-week behavioral training component of the study is an adaptation of a motor training program previously shown increase dexterity, coordination and decrease motor output variability in older adults (Kornatz et al., 2005; Christou et al., 2011).

The following specific aims and hypotheses are proposed:

1. To determine the relative effectiveness of a behavioral motor training regimen versus an aerobic exercise regimen respective of upper extremity motor performance.

Consistent with the findings from previous work we expect dexterity gains in upper extremity function from behavioral training. We also expect dexterity gains after the aerobic exercise intervention.

2. To assess the effects of the aerobic activity on interhemispheric inhibition using fMRI and TMS.

We expect that aerobic activity intervention can mitigate or reverse aging-related loss of interhemispheric inhibition as measured by fMRI and TMS. We expect that behavioral training of unimanual activity acts to alter interhemispheric communication dynamics and this is a potential mechanism of improvement in motor performance.

3. To determine if role of exercise duration in maintaining or enhancing motor performance and levels of interhemispheric inhibition.

We anticipate that groups that are enrolled in the regular aerobic exercise will show maintenance or enhancement of potential gains from the other interventions. This group (n=20) will show maintained or better upper extremity performance in conjunction with increased levels of interhemispheric inhibition as compared to 9 month assessments and the no-intervention group. The no-intervention group will still show improvement as compared to their enrollment assessments.

5. Study Design and Methods:

Specific Aim 1) To determine the relative effectiveness of a behavioral motor training regimen versus an aerobic exercise regimen respective of upper extremity motor performance.

Introduction. A critical aspect for rehabilitation of motor pathology is the understanding of the neural mechanisms at work in the healthy brain. Sedentary aging is associated with changes in interhemispheric communication within the brain that likely denote a loss of interhemispheric inhibition (McGregor et al., 2011; McGregor et al., 2009; Riecker et al., 2006; Fling et al, 2011; Bernard & Seidler, 2011; Fling & Seidler, in press). There is evidence that these changes are associated with declines in motor performance of the upper extremity including dexterity, reaction time and psychomotor performance (Langan et al., 2010; Fling & Seidler, in press; McGregor, unpublished CDA1 data). Data from previous work (McGregor et al., 2011) and preliminary data from a recently completed project (McGregor et al., 2012) shows that aerobic activity may serve to mitigate losses of interhemispheric inhibition and improve motor performance. It is unknown whether aerobic activity can reduce loss of interhemispheric inhibition. In the current project, we test the hypothesis that an aerobic activity regimen can be effective in mitigating losses of interhemispheric inhibition and improve motor performance.

Research Design. The proposed study will examine 40 older, sedentary Veterans from the greater Atlanta area. Participants will be randomly assigned to one of two15-month intervention conditions involving aerobic fitness training and behavioral training. The study is a reversal design in which both groups are assessed pre-treatment with both fMRI and TMS measures, as well as behavioral measures at study enrollment. As part of the design, both groups will undergo a 3-month waiting period during which they will engage in an organized stretching routine with no aerobic exercise component. After this waiting period, the participants again undergo behavioral, fMRI and TMS assessments. At this point, the participants will be assigned to either a behavioral treatment group involving a motor coordination protocol previously shown to improve upper extremity motor performance in older adults (Christou et al, 2011) or a supervised aerobic training group at the Atlanta VA Medical Center. The design of the study is depicted in the in Figure 6 below. The primary outcomes of this aim are changes in fMRI and TMS measures of interhemispheric communication and motor performance tests.

Research Plan:

Healthy right-handed participants of older age (60+) will be recruited for this study from the greater Atlanta community. Groups will be matched for gender. The inclusion of the younger participants is important for statistical symmetry in group comparisons.

Aerobic Exercise & Behavioral Motor Training. Prior to any intervention, participants will undergo a self-monitored 12-week stretching program of the upper extremity. We will inquire as to physical activity level on a bimonthly basis. After this period participants will be randomized into either a 12-week exercise or 12-week behavioral training sessions (depending on group placement) both of which are expected to facilitate physiological changes.

For the 12-week aerobic exercise component of this group, participants will follow the guidelines provided by the American College of Sports Medicine for optimizing cardiovascular fitness. Dr. Joe Nocera will monitor the implementation of these guidelines. Participants will exercise 3 times a week on a stationary cycle ergometer in a group setting. We chose a lower extremity intensive exercise task to decrease potential confounds with upper extremity motor practice (such as the use of an arm cycling ergometer). Exercise intensity will begin at low levels (50% of maximal heart rate reserve) measured by the Karvonen method. This method calculates target exercise HR by subtracting the persons age from 220. Resting heart rate is then subtracted from this number. The answer is then multiplied by the target percent (50% for example) and the product is added back to resting heart rate to provide the target exercise session heart rate. Intensity will be increased by 5% every week (as tolerated by the participant) to a maximum of 80% of maximal heart rate. Exercise time will progress from an initial 20 minutes per session to a maximum of 45 minutes by increasing 5 minutes each week. Each session will be monitored by a cardiopulmonary resuscitation (CPR) certified research associate training in the fitness testing. Additionally, to maximize safety, all participants will be cleared by a physician prior to beginning any portion of the study. Further, the intervention site is located at the Atlanta VAMC and portable defibrillators

are available at the intervention site. Similar protocols have been used in other studies involving effective change in behavioral measures after aerobic exercise.

The 12-week behavioral training component of the study is an adaptation of a motor training program previously shown increase dexterity, coordination and decrease motor output variability in older adults (Kornatz et al., 2005; Christou et al., 2011). Training involves the regular engagement in contraction and relaxation of the first dorsal interosseous (FDI) muscle response recorded via surface EMG. Training session will occur three times per week consistent with the exercise intervention. The training begins with a 1 week familiarization period over three experimental sessions. In the familiarization sessions, subjects will receive written and oral descriptions of the project, watch a visual demonstration of the protocol, and are allowed initial practice trials of the experimental task. The experimental task is an adaptation of an anisometric abduction-adduction task of the index finger (Kornatz et al., 2005). Participants will lift and lower the same load identified by the recruitment threshold by using abduction-adduction movements of the index finger that are produced with shortening and lengthening contractions of the first dorsal interosseus (finger bending with a weight). Participants will attempt to match index finger displacement to a template shown on a computer monitor; they will be required to produce slow, constant-velocity (1.7°/s) abduction-adduction movements over a 10° range of motion. Each subject will raise the load during 6 s of abduction (shortening contraction) and lowered the load during 6 s of adduction (lengthening contraction). Participants will repeat this movement five times for an exercise trial. We will carefully monitor the hand to ensure movement is limited to abduction of the index finger about the metacarpophalangeal joint. The weekly experimental sessions include 5 weeks of light-load training (50% of maximum), and an additional 6 weeks of heavy-load training (75% of maximum). This design will allow each subject to serve as their own control in addition to allowing for assessment of group differences at the end of the project. Four motor training activities will be made in these sessions: recruitment threshold, determination of the minimal weight load that had to be supported by an isometric contraction of the FDI for the isolated motor unit to discharge action potentials repetitively; anisometric task, shortening and lengthening contractions of the first dorsal interosseus muscle to lift and lower a light inertial load; and one repetition maximum load, i.e.- identification of the maximal load that could be lifted by a shortening contraction of the first dorsal interosseus muscle. The discharge of motor units will be recorded during the anisometric task. Each experimental session will last 45 minutes.

After the each group has completed the first component of the study, behavioral and neuroimaging (fMRI/TMS) assessments will be completed. The groups will then switch from the exercise intervention to the behavioral or vice-versa, depending on initial assignment. The interventions will proceed as previously described within these groups for another 12-week timeframe after which imaging and behavioral assessments will again be taken to test for effect of training type and effect of reversal.

Fitness Assessments:

Fitness level will be assessed using four measurements:

- Physical Activity Inventory: During phone screening we will ask the participant about their current aerobic activity.

- Direct Assessment: Following written consent, the participant will be asked to complete a direct measure of aerobic fitness using a submaximal assessment commonly conducted to estimate aerobic capacity.

- Daily Log: During the study, the participant will complete the Physical Activity Inventory to log their daily exercise activity.

The direct fitness assessment is to be completed at the VA Atlanta Center of Excellence Aerobic Fitness Testing Laboratory. The direct fitness assessment will consist of a sub-maximal testing protocol performed with trained research staff following ACSM (American College of Sports Medicine) testing guidelines and under the direction of an exercise physiologist. Participants will perform a sub-maximal exercise test on a cycle ergometer. We will adhere to the YMCA sub-maximal test protocol. This sub-maximal test will be used to estimate the participant's maximal oxygen uptake (VO_2max). This test uses and "extrapolation" method in which heart rate workload values are obtained at 2-4 points and extrapolated to predict workload at the estimated maximum heart rate (MHR) (e.g. 220-age). VO_2max is then calculated from the predicted maximum workload. Participants will be asked to ride a stationary bicycle for 4 three-minute stages. The first stage will be a warm-up at 50 revolutions per minute (RPM) at a power level of 25 watts. During all testing stages, heart rate will be continuously monitored and will not exceed 85% of age-predicted maximum heart rate. For the analysis, average heart rate during the final 30 seconds of the 2nd and 3rd minutes will be plotted against workload. Three minute trial workloads, shown in Table 1 below will be chosen based on the participants' heart rate at the end of the warm-up period. The fourth 3-minute stage will be a cool down period added to the end of the test. Participants will be allowed to stop the test at anytime for any reason and will be instructed to inform the researcher if they feel faint, dizzy, short of breath during the test, at which time the researcher will terminate the testing session.

Table 1. YMCA Sub-maximal Cycling Protocol				
	< 80 bpm*	80-90 bpm*	90-100 bpm*	> 100 bpm*
Stage 1	125 watts	100 watts	75 watts	50 watts
Stage 2	150 watts	125 watts	100 watts	75 watts
* beats per minute				

Immediately prior to and following the fitness test, subjects will have their heart rate and blood pressure (BP) evaluated. Individuals with a resting heart rate above 120 beats per minute, a systolic BP above 180 or a diastolic BP above 100 will not be permitted to perform the test. Heart rate and blood pressure will also be monitored throughout the assessment. Physician's clearance, for those 40 years of age or older, will be obtained from the potential subject's physician or nurse prior to conducting the fitness test with the subject's oral permission. All lab personnel present during the fitness test will be CPR certified and trained in emergency contact procedures (i.e. – VA Code Blue).

Measures for Specific Aim 1.

Motor performance

1. *Fitness Level:* We will assess physical fitness level using a cycle ergometry assessment of maximal volume of oxygen consumption. This is described in detail below.
2. *Purdue Peg Board Task*: The Purdue Pegboard measures unimanual motor dexterity. The test consists of two parts: 1) placing pins in a column of holes and 2) an assembly task using three components (pin, washer, collar). The participant is asked to place as many items as possible in 30 or 60 seconds, respectively.
3. *Nine Hole Peg Board Task*: A standard dexterity assessment, this task ask participants to place and remove pegs on a nine-hole pegboard as quickly as possible.
4. *Pinch and Grip Strength:* FDI and hand strength will be assessed using standard dynamometer (JAMAR) squeeze tests.
5. *Coin rotation task:* Another dexterity assessment, this task asks participants to rotate a coin (U.S. nickel) as quickly as possible for 20 rotations.
6. *Halstead finger tapping:* The Halstead finger tapping test is a standard test for testing psychomotor speed. The participant is asked to press a lever attached to a counter as many times as possible in 10 second trials.
7. *JebsenTaylor hand function test:* The Jebsen Taylor hand function test is a timed 7-part assessment of common motor abilities performed with the dominant hand.
8. *Hand steadiness test:* A standard occupation therapy test measuring hand steadiness over a brief time interval (10 seconds).
9. *Poffenberger Crossed-Uncrossed Difference Test:* The Poffenberger paradigm is a test of transcallosal transfer time during reaction time responses. This 200-trial test compares reaction time based on hand of response and spatial presentation of stimulus cue.
10. *Target force matching:* The target force matching task is similar to the anisometric FDI muscle training used in the behavioral intervention. A computer presents a target force marker and the participant's task is to match the cued lift load target and hold for a set period of time. EMG is of the FDI taken during this assessment.
11. *Motor tracking:* The motor tracking task is a computer based presentation of a stimulus (vertical bar) moving laterally. The participant is instructed to track the

movement of the stimulus using a computer cursor controlled by a touchpad input device.

Specific Aim 2) To assess the effects of the aerobic activity on interhemispheric inhibition using fMRI and TMS.

Introduction. The PI's previous research line has indicated that increased aerobic activity level is associated with decreases in aging-related loss of interhemispheric inhibition (McGregor et al., 2009; McGregor et al., 2011). In a previous project, we have noted that in fMRI, highly-fit elderly and middle age adults show lower bilateral positive BOLD activity in the primary motor cortex. Further, highly fit individuals show patterns of activity more similar to younger adults in that they show a negative BOLD signal in the ipsilateral primary motor area likely indicating an active suppression. In TMS, highly fit elderly and middle age adults show a longer duration of the ipsilateral silent period, a putative measure of transcallosal inhibition (Meyer et al., 1995).

We will test the hypothesis that an aerobic activity intervention can mitigate or reverse aging-related loss of interhemispheric inhibition as measured by fMRI and TMS. We will also test the hypothesis that behavioral training of unimanual activity acts to alter interhemispheric communication dynamics and this is a potential mechanism of improvement in motor performance.

Research Design and measures. fMRI and TMS paradigms are discussed below.

fMRI tasks. Two block-design right-hand motor tasks will be used to evaluate interhemispheric cortical activation patterns. Both tasks have been reported sensitive to aging-related differences in interhemispheric inhibition (McGregor et al., 2009; Ward et al., 2008). Blocks will consist of seven images (28 seconds) for both rest and active conditions and six block cycles (alternating between 7 rest images and 7 active images) will comprise each run (5 minutes 36 seconds). In the scanner, participants will engage in two runs of each motor task and all performance data (accuracy, reaction times) will be saved for later analysis. Participants will train on the both tasks outside of the scanner and again inside the scanner immediately prior to data acquisition. Mirror movements (symmetric movement of the opposite hand) are of concern for unimanual digit manipulations. To monitor for such movements, force output of the left first dorsal interosseous muscle (FDI) will be recorded during practice sessions.

Finger opposition motor task: The first motor task will be a block presentation of a repeated button press using opposition of the index finger and thumb. This task has been shown to exhibit a negative BOLD response in ipsilateral primary motor cortex (M1) in younger adults at spatial resolutions comparable to those planned for the present investigation. Performance of similar tasks in sedentary elderly samples, however, shows positive BOLD responses in ipsilateral M1 (McGregor et al., 2011). Participants will fixate gaze on a central point of a computer screen throughout each of 2 runs. Blocks will be cued by the change of fixation shape varying between a red octagon (rest condition) and a green circle (movement condition). During the movement condition, participants will be instructed to time button presses with a flashing visual

stimulus (2 Hz). Participants are instructed to press a button until a tactile click is obtained (at 3N of force) before repeating the task.

Force matching task. A second motor task will be employed to potentially differentiate the effect of muscle recruitment on patterns interhemispheric activity. As previous studies (e.g. - Ward et al., 2008) have indicated that increasing muscle recruitment may influence levels of ipsilateral inhibition, the inclusion of a target force matching task of varying intensities may quantitatively inform such activity. In this task, we will ask participants to match grip force to a target level set at a percentage of the participants' maximum voluntary contraction (MVC). During scanning, participants will perform a series of dynamic isometric pinch squeezes with the dominant right hand using a MRI-compatible manipulandum built by the applicant. Continuous visual feedback about the force exerted (represented as a vertical bar) will be provided throughout the scanning session. Prior to scanning, but while lying in the scanner, participants will use a pinch squeeze of the manipulandum to generate their MVC for each hand. Within trial blocks, target force for the hand grip will be visually cued using horizontally presented line parallels with vertical offset equal to +/-3% (relative to MVC). Target forces for active response blocks for each of two runs will be 25% and 35% of MVC, respectively.

TMS. Both single and paired pulse TMS measures will be taken for the current study to assess cortical excitability and interhemispheric communication. Paired-pulse TMS requires the use of multiple stimulators, to which Dr. Butler has granted unfettered access for the duration of the study. All TMS procedures will be completed in Dr. Butler's VA laboratory.

Single pulse measure. Four single-pulseTMS measures will be taken: size and latency of Motor Evoked Potential (MEP) in right FDI muscle at resting motor threshold, duration of ipsilateral silent period (iSP), and MEP recruitment curves (RC). Electromyography (EMG) will be taken from the FDI muscle on both hands. Muscle activation will be monitored with a real-time oscilloscope software package (Scope; ADInstruments Ltd). A Magstim 200 magnetic stimulator (The Magstim Company Ltd, Carmarthenshire, UK) and a focal figure of 8 coil will be used to stimulate the left primary motor cortex during the initial mapping procedure. The coil will be placed tangential to the scalp with the handle pointing backwards and 45° away from the midline for stimulation. The scalp site corresponding to the lowest stimulator output sufficient to generate a magnetic evoked potential of at least 50 mV in 6 out of ten trials will be defined as the area of lowest motor threshold (LMT), also known as the "hotspot". The MEP size measure will be taken using ten stimulations of the left primary motor area FDI hotspot at LMT. The right FDI motor hotspot will be assessed using the same procedure, but with the left hand and stimulation delivered to the right motor cortex.

The ipsilateral silent period will be determined using a longstanding method (Wasserman et al. 1991). For ipsilateral silent period, the left FDI muscle will be contracted via pinch grip at 25% maximal voluntary contraction (determined by grip dynamometer) and an 80% LMT stimulus will be delivered to the left primary motor area

FDI "hotspot", previously determined by an initial sensitivity assessment. Stimulator output equivalent to 150% LMT is delivered to the left FDI hotspot.

FDI recruitment curves, a measure of cortical excitability, will be generated by stimulation at the LMT hotspot over progressively increasing intensities. Testing proceeds by placing the coil at the hotspot and recording 5 stimuli in 10% increments beginning at an intensity of 10% below LMT threshold. Data collection for the RC will be terminated when a plateau of the sigmoidal curve is observed. The slope of the RC will be generated from the data using linear regression.

Paired-Pulse measures. The paired pulse procedure) interhemispheric inhibition assessment (Ferbert et al., 1992 requires a second Magstim 200 magnetic stimulator and attached figure of 8 coil. In this procedure, hotspots on both motor cortices are target for stimulation in a paradigm assessing the effects of stimulation of one laterality on the opposite cortex's output MEP after its stimulation. For this procedure, a "conditioning" TMS pulse is applied to the right motor cortex at either 10 or 40 milliseconds prior to a "test" pulse's administration to the left motor cortex. As a result of the conditioning stimulation, the test MEP's response amplitude (in the right FDI muscle) is lowered due to interhemispheric inhibitory processes. Both 10 and 40 ms durations will be tested in this protocol. Magnitude and duration of IHI increase with the intensity of the conditioning stimulus (Ferbert et al., 1992). As such, the intensity of the conditioning stimulus will be varied from 100 to 150% of resting motor threshold in 10% steps (i.e., six different intensities) to obtain a range of IHI magnitudes from threshold to maximum. Test pulse intensity will be adjusted to produce an unconditioned MEP of on average 1 mV in peak-to-peak amplitude. Testing will be comprised of eight trials per intensity and eight unconditioned test stimulus trials (i.e., total of 56 trials), applied in pseudo-randomized order. The intertrial interval will vary randomly between 4 and 6 s to reduce anticipation of the next trial. Conditional averages of the single-trial MEP peak-to-peak amplitudes will be calculated.

Functional Imaging and Neurophysiological Analyses. Functional images will be analyzed and overlaid onto structural images with the Analysis of Functional Neuroimaging (AFNI) program (Cox, 1996). To minimize the effects of head motion, time series images will be spatially registered in 3-dimensional space. The average functional intensity of each acquired slice for every subject will be normalized to the mean intensity of all slices for all subjects to control for differences in functional intensity between subjects. A subject's data will be excluded from further analyses if any time series of a subject is judged from visual inspection to contain a significant number of images with gross artifacts or residual motion.

FMRI Analysis. The current study is concerned with the response of the ipsilateral primary motor cortex during actions performed by right hand. It is hypothesized that sedentary aged adults will initially show an increase in metabolic activity (i.e. – a positive BOLD response) in this area. To assess this, an analysis of average hemodynamic (impulse) response functions in the right primary motor cortex will be performed. These impulse response profiles will be tested across session using repeated measures ANOVA. Significant differences will be assessed at the individual

subject and group level.

For group analysis of intervention effects, anatomic and functional images will be interpolated to volumes with 1 mm^3 voxels, co-registered, and converted to stereotaxic space of Talairach and Tournoux (1988) using AFNI. Estimates of hemodynamic response functions (HRFs) generated from a deconvolution procedure will be spatially smoothed using a 5 mm full-width half-maximum (FWHM) Gaussian filter to compensate for variability in structural and functional anatomy across participants. Area under the curve (AUC) of the HRF's will be entered into a voxel-wise two-way mixed-design ANOVA (age grouping as the between subjects factor, session as the within-subjects factor). Additional pair-wise comparisons of age groupings will be performed using Student's t-test ($p<.05$, False Discovery Rate corrected) on a voxel-by-voxel basis with left and right primary motor cortices selected as regions of interest (ROI). Pairwise comparisons will be computed for all motor response conditions.

TMS Analysis. Six measures will be computed and analyzed from TMS acquisitions: Right FDI MEP size, right FDI MEP latency, recruitment curves (RC), ipsilateral silent period (iSP), interhemispheric inhibition at 10ms (IHI10) and 40ms (IHI40). The size of left FDI MEP evoked by TMS will be measured from peak-to-peak between TMS stimulation (first peak) and subsequent muscle response (second peak). The latency of MEPs will be measured from the onset of the stimulus presentation to the onset of the MEP. Recruitment curves will be evaluated based on slope generated from linear regression. Onset and end latency of the iSP will be taken at the initial and final intersections of the averaged signal with baseline indicating 80% of the background EMG level; the duration of EMG suppression between these two points will be computed. For each of the stimulation intensities, IHI will be expressed by (1 - mean conditioned MEP/mean unconditioned MEP) x 100%. Accordingly, IHI = 0%, IHI = 100%, and IHI < 0% will indicate no inhibition, complete inhibition, and interhemispheric facilitation, respectively. For within subjects comparison, repeated measures ANOVA with subsequent repeated measures pairwise t-tests will be generated. Group comparisons of TMS measures will be analyzed by between-subjects ANOVA and subsequent t-test pairwise comparisons. For all test, p-values < 0.05, corrected for multiple comparisons where applicable, will be considered statistically significant.

BDNF Evaluation. Fasting whole blood will be collected, by a certified phlebotomist, via venipuncture or peripheral venous line into pre-cooled plasma EDTA tubes and serum separator tubes (~40 ml per visit). Following collection, tubes will be transferred in sealed biohazard bags and in according with blood borne pathogens training standards to the Molecular Biology Lab (4A 178/4A 167) of the Atlanta VAMC Center of Excellence.

Plasma volume will be measured using methods described by Dill and Costill. Lactate will be measured by the Accusport Lactate Analyzer (Roche Molecular Biochemicals, Mannheim, Germany). Serum samples will be allowed to clot for 30 minutes before centrifugation at 1000*g* for 15 minutes. Plasma samples will undergo centrifugation within 30 minutes of collection at 1000g for 15 minutes at 2-8ºC. Plasma aliquots will be separated and will then undergo an additional centrifugation "Super-

Spin" at 10,000*g* for 10 minutes at 2-8ºC in order to completely remove platelets. Serum and plasma samples will be stored at –80°C. Serum and plasma aliquots will be analyzed for measures of interest as determined by ELISA (R&D Systems, Minneapolis, MN). All assays will be performed in duplicate and in a single run.

The procedure below describes the blood collection timeline during the 12-week intervention period and follow up:

Baseline: 4 measurements over 1 hour, taken every 15 minutes, to demonstrate reliability of the measures. (Assessment 1, Day 1)

Week 1: Taken immediately prior to exercise, immediately post, 15 min post, 30 min post. (Intervention 1)

Week 4: Taken immediately prior to exercise, immediately post, 15 min post, 30 min post. (Intervention 1)

Week 8: Taken immediately prior to exercise, immediately post, 15 min post, 30 min post. (Intervention 1)

Week 12: Taken immediately prior to exercise, immediately post, 15 min post, 30 min post. (Intervention 1)

Follow-up: 1 measurement taken at 12-week follow-up. (Assessment 5, Day 1)

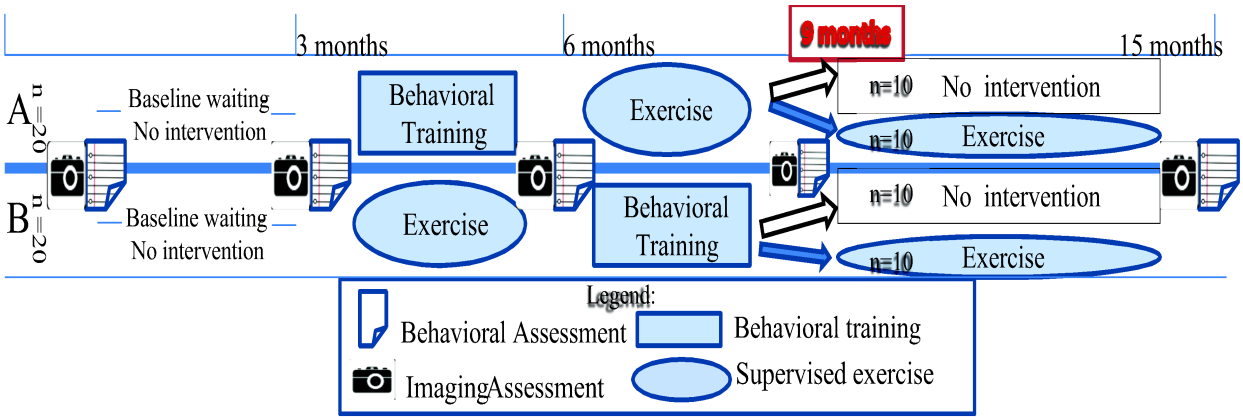
All blood draws will be conducted in the Movement Studies Laboratory (Intervention site) at the Atlanta VA Medical Center. Tubes will be coded and thus have no identifiable patients information. The master code list will be maintain in a locked file cabinet in the office of the PI (12C115).

Specific Aim 3) To determine if role of exercise duration in maintaining or enhancing motor performance and levels of interhemispheric inhibition.

Introduction: Behavioral interventions (motor strength and coordination) have been shown to be effective in improving upper extremity motor performance in older adults (Christou, 2011; Engler et al., 2011), however the duration of these gains appear to be short-lived (Toraman, 2004; Ivey et al., 2000). After as little as a few weeks of detraining, motor strength and coordination in the upper extremity rapidly begins to return to pre-intervention levels (Carvalho et al., 2009; Toraman, 2005; Toraman & Ayceman, 2005;). Evidence from exercise interventions assessing gait and locomotion have shown that exercise programs over a longer term (>6 months) are associated with improved proprioception, fewer falls and better balance (Arnold et al., 2008). However, the comparison of outcomes of upper extremity function in elderly adults respective of exercise duration remains largely unexplored. In addition to comparing the effects of short-term exercise (3 months) versus behavioral training (3 months) on upper extremity function, the current proposal will evaluate if a longer-term (6 months) exercise program

can maintain or enhance upper extremity function and associated levels of interhemispheric inhibition. Further, we will inquire as to how the long-term exercise program has affected the general quality of life in our sample.

Research Design: As shown in the figure below, the study groups will be split after the second intervention. Participants will then be either enrolled in 26 week exercise program or placed in no treatment group with no physical activity requirements other than a light stretching regimen. Participants will be given elastic Therabands during the waiting phase and asked to engage in light stretching exercises of the large muscle groups of the arms, trunk and legs (prescribed in the Theraband training manual and instructed by research associates at first assessment). During this six-month period, participants enrolled in the exercise group will continue to adhere to the exercise regimen described in detail above (participants will be provided with an exercise bike for home use). Participants in the no aerobic exercise intervention will asked to engage in the Theraband stretching training similar to the waiting period protocol. At the end of the six months, all participants will return for a final/followup behavioral and neuroimaging assessment. At this time, participants in the no exercise intervention group will be given an exercise bicycle for home use as compensation.



Measures for Specific Aim 3.

1. Cycle ergometer test VO2max assessment (described in detail below)
2. Upper extremity performance measures as listed in Specific Aim 1.
3. Imaging measures as listed in Specific Aim 2.
4. Daily Activity Log. All participants will track exercise activity over the course of each day while in the study until the final 12 week follow-up. At this time, they will receive a summary of their activity reports and physical fitness progress.
5. Physical Activity Readiness Questionnaire. The questionnaire, inquires about perceived difficulties in general physical activities involving voluntary exercise. .
6. Quality of Life Survey: We will also administer the standardized SF-36v2 Health Survey questionnaire that assesses an individual's quality of life in comparison with previous points of remembrance.
7. Epworth Sleepiness Scale- self report used to determine the level of daytime sleepiness.

8. Pittsburgh Sleep Quality Index- 10 item questionnaire examining participants sleep habits over the past month.
9. Sleep Profiler: These monitors will provide pre and post-intervention objective data on sleep quality, patterns, and continuity that will supplement the Pittsburg Sleep Quality Index, Epworth Sleepiness Scale. They are non-invasive wearable recorders with 3 EEG electrodes housed with a headband placed just below the hairline that provide study-validated information including total sleep time, REM sleep, sleep efficiency, snoring intensity and frequency, and arousal. Participants will be provided a brief orientation and instruction on how to properly wear the device. The device requires minimum setup, and the wearable headband is similar to a headband that might be worn at the gym. It is battery powered, with up to 16 hours of recording between charges, and data is stored and easily downloaded via USB from the device.

Additional measures for Specific Aim 3:

Fitness Assessments: Initial fitness level will be assessed using three measurements. The initial assessment is the participant's self-report on the two phone-administered exercise inventories. Secondly, during the first participation session a direct test of the participant's aerobic fitness level is to be completed at the Atlanta VAMC aerobic fitness testing center. This test will also be used as an outcome measure for aerobic fitness for the duration of the study. The direct fitness assessment will consist of cycling ergometer assessment with trained research staff following ACSM (American College of Sports Medicine) testing guidelines (listed under Human Subjects Protection) and under the direction of an exercise physiologist, Dr. Nocera. Dr. Nocera will also assist with the training of participants on self-directed cycling and theraband exercise.

Possible Discomforts and Risks:

Behavioral Testing:

Patients may experience some frustration if they have trouble with cognitive testing. Researchers will be trained on how to handle frustration by taking breaks, allowing patients to express frustration, and offering encouragement. As noted above, we will also include items that the participants can answer correctly to minimize frustration. In the vast majority of patients, these techniques are adequate to deal with frustrations.

Magnetic Resonance Imaging (MRI):

More than 150 million diagnostic magnetic resonance studies have been performed worldwide. The vast majority of these procedures were completed with no sign of patient injury (Schenck, 2000). There is a high degree of patient safety with an fMRI because of the miniscule value of magnetic susceptibility and lack of ferromagnetic

components of human tissue. Studies have ranged in magnetic field intensities from 1.5 to 8T. No negative cardiac, physiological, or cognitive effects were noted (Kangarlu & Robitaille, 2000). Therefore, long-term effects on human health from magnetic resonance imaging are unlikely. Those at risk for injury include those with indwelling ferromagnetic material (e.g. foreign object in eye, surgical implant) or an implanted bioengineering device (e.g. pacemaker, infusion pump), due to the possible interaction with a magnetic field. Subjects identified as at risk from the screening checklist will be excluded from the study.

Another potential hazardous effect is related to the high level of noise produced by the machinery during imaging. Unprotected, patients can experience hearing loss. For this reason, individuals will be given foam earplugs to wear to minimize this risk.

Additionally, some individuals are perceptible to experiencing distress during the fMRI process. The small, closed-in space may trigger anxiety. Participants will be screened for claustrophobia, generalized anxiety disorder, post-traumatic stress disorder, or obsessive-compulsive disorder.

Further, persons who are pregnant (or could be pregnant) or those with a seizure disorder should not undergo magnetic resonance imaging, and will be excluded.

Subjects may have early stages of disease, not previously diagnosed, detected through the use of MRI. A trained neurologist is available to the investigators to evaluate the findings and determine if there is pathology present or a normal variant should the investigators suspect that the imaging shows abnormalities. If pathology is present or suspected, subjects will be counseled about what the findings are and what should be the next steps for clarification of ambiguous findings or seeking help with pathological findings. This is not a risk in the conventional sense of physical harm or disease, but does pose a potential psychological risk to the patient.

Transcranial Magnetic Stimulation (TMS):

There may be some discomfort associated with application (skin preparation) and removal (tape removal) of the EMG surface sensors or reflective markers during TMS testing. Some slight irritation is possible, although measures are taken to reduce the likelihood of this occurrence, which in any case should disappear in a few days.

Subjects sometimes report low level, but easily tolerable scalp discomfort from TMS. Occasionally, subjects report headaches from TMS that are relieved by common over-the-counter pain medications. However, both of these issues are less of a problem in the particular scalp areas that we will be stimulating.

Possible effects on hearing have been described so participants will be asked to wear earplugs during TMS. As with any electronic device or appliance, using it the wrong way could result in electric shock. While this is very, very unlikely, it cannot be completely left out as a possibility. To mitigate risk, all participants will remove any metal objects on their person prior to TMS stimulation.

Possible Benefits:

There are substantial potential benefits of participation in this study. All participants may benefit from the pre intervention evaluation, which may detect unknown or inadequately treated medical problems. In addition, we expect fitness improvement in the participants, which confers secondary health benefits.

Potential benefits for adults who exercise include:

- improvement of physiologic indicators of health (e.g., balance, blood pressure);
- full or partial elimination of impairments in ability to do daily activities;
- improvement in mental and/or social health status;
- reduction of risk of falls and fall-related injuries;
- reduction in risk of mortality; and
- reduction in medical care costs.

Potential benefits for adults who participate in the exercise testing include:

- improved awareness of their cardiovascular fitness level;
- greater confidence in the knowledge of their cardiovascular fitness level;
- greater knowledge of their fitness changes and the relationship between those measures and their ADLs.
- improvement in mental and/or social health status;
- reduction of risk of falls and fall-related injuries;
- reduction in risk of mortality; and
- reduction in medical care costs

As a service to the participants, anatomic scans will be read by a qualified physician if unexpected abnormalities arise. The major benefit of the study is the advancement of scientific knowledge about brain systems. Such knowledge may one day be used to increase the efficiency of rehabilitation techniques after brain injury, and might even have some implications for the way we teach skilled movement or other functions to neurologically normal individuals.

Participants may be compensated (250 US dollars) for participation in the project. Additionally, participants will be allowed to retain the exercise cycle used for the intervention.

6. Participant selection:

The study will enroll between 60 and 80 older, sedentary adults. Participants will be randomly assigned to one of two 12-week interventions conditions: aerobic exercise or dexterity training. The pre and post evaluations will be conducted over 3 days during a 1-week period. For example, Monday-dexterity Evaluation, Wednesday-fMRI and

TMS, Friday-Physical Evaluation. Each day will last no more than 2 hours. The investigators expect a dropout rate of anywhere from 10-15% of participants. To ensure completion of study by 40 participants, as many as 60 subjects may be enrolled.

Inclusion/Exclusion Criteria. *Prior to beginning any portion of this study we must receive a physician's written medical approval for participation in the fitness assessment and/or exercise intervention.* We will provide the physician a letter to sign if the participant is cleared explaining the study protocol. The key inclusion criteria and final participant pool will consist of right-handed English speaking individuals aged 60 to 89. Participants will be sedentary as defined by < 120 min/week of aerobic exercise over prior 3 months. Additionally, participants will be non-demented (MMSE <24). Further, those with severe diabetes requiring insulin will also be excluded. However, those with less severe, controlled diabetes that meet our physical and cognitive function inclusion criteria will be allowed to participant.

Recruitment. Individuals will be recruited from the Atlanta VA Rehab R&D Subject Registry (IRB00000159) based on the inclusion and exclusion criteria. The Subject Registry study staff will query the data base of all patients meeting the inclusion and exclusion criteria who have consented to be contacted for research purposes and will print out their contact information. The Principal Investigator, or his research colleagues, will contact these individuals and will summarize the study procedures. If the potential participant is interested we will mail them a copy of the informed consent and will schedule an orientation session that describes the study goals and provides an opportunity to go over the informed consent.

Healthy subjects will also be recruited via the Emory University Alzheimer Disease Research Center (ADRC) Registry. Written permission from all patients is recorded on a signed informed consent form before inclusion in the ADRC Registry. This consent requires that the patients be willing to complete detailed histories, undergo comprehensive neurological and neuropsychological evaluations on an annual basis; submit blood for ascertainment of genetic information and establishment of lymphoblastoid cell lines; and agree to be contacted regarding participation in research projects of Emory investigators.

The PI and the Research Coordinator(s) will be added to the ADRC Registry protocol (IRB #133-98). Following this, the ADRC request form will be filled out in order to identify an appropriate list of potential participants.

Additionally, participants may be recruited from word of mouth and via print advertisements.

Individuals will be provided a copy of the informed consent and will be scheduled for an orientation session that describes the study goals and provides an opportunity to go over the informed consent. The PI/Co-PI or their trained and qualified colleagues will go over and describe the consent in a private office of the PI/Co-PI.

7. Statistical Analysis

Statistical Analysis for Specific Aim 1. We will model each of the outcome measures in Aim 1 (upper extremity) jointly using a mixed linear model to determine whether the mean responses of the groups are significantly different across the time points. We expect that modeling the outcomes jointly, using subject as a random factor, will allow for some gains in power. Our fixed factors will be time, treatment group and their interaction. This model will enable us to estimate the overall effects of time and treatment, and most importantly, how the effects of treatment differ with time.

Statistical Analysis for Specific Aim 2. For each of the outcome measures in Aim 2 (changes in fMRI and TMS activity during unimanual tasks) we will use a mixed linear model to determine whether the mean responses of the groups are significantly different across the time points. Our fixed factors will be time, treatment group and their interaction. This model will enable us to estimate the overall effects of time and treatment, and most importantly, how the effects of treatment differ with time.

Statistical Analysis for Specific Aim 3. For each of the outcome measures in Aim 3 we will use a mixed linear model to determine whether the mean responses of the groups are significantly different across the time points. Our fixed factors will be time, treatment group and their interaction. This model will enable us to estimate the overall effects of time and treatment, and most importantly, how the effects of treatment differ with time. Level of interhemispheric inhibition will be characterized by proportion of ipsilateral motor cortex recruited during fMRI tasks, ipsilateral silent period in TMS, and level of diminution of MEP size in the interhemispheric inhibition protocol in paired-pulse TMS.

Power Analysis and Sample Size. We powered this study on the previous cross-sectional studies assessing interhemispheric communication and motor performance. These studies had large effect sizes (1.33 to 1.67) as aerobic activity was related to ipsilateral motor cortical activity. As the proposed work is an intervention, we have applied a conservative estimate of these effects based on previous work with behavioral performance training over a similar timeframe (Kornatz et al., 2005) and those in the neuroimaging literature (Erickson et al., 2011). Further, data from our ongoing CDA-1 pilot study has shown a somewhat linear relationship between VO2max and levels of interhemispheric inhibition. While this data does not provide enough information to enable us to estimate a required sample size under the mixed model that will be used in the final analysis, we can conservatively estimate the sample size assuming a two-sample t-test would be performed on the post-pre differences for each group. Under this simpler model, a sample size of 20 per group will give us an approximate effect size of .83 for the fMRI and TMS measures. While this effect sizes is relatively large, we will have more power under the mixed model that we actually will use to analyze the data. Equally important, from the standpoint of our sample size, is that the data gathered on the other outcome measures would be invaluable in fully powering future study. Lastly, based on the retention rates in previous exercise studies we anticipate a 20 percent

drop-out rate among groups. As such, we will plan to enroll 24 per group to reach the needed sample size of 20 per group.

The research staff will use REDCap issued through the Atlanta Veterans Health Care System. Only deidentified data will be entered into REDCap by VA credentialed research personnel to run data analysis.

8. Adverse event reporting:

In the case of a reportable event, the Principal Investigator will complete the form "Reportable Protocol Event Form for VA sites," attach supporting documentation, and provide it to the VA Science Information Office. The SIO will then route the report to the Emory IRB. The Emory IRB will be notified within five days of any adverse event occurring.

9. Data and safety monitoring plan (DSMP)

This is a minimal risk protocol and therefore data will not be reviewed by a Data Safety Monitoring Board (DSMB) or Data Monitoring Committee (DMC).

This study will only recruit healthy older adults. Prior to beginning any portion of this study we must receive a physician's written medical approval for participation in physical activity. We will contact the physician and inform him/her of their patient's interest in participating in this study and explain the study criteria for participation detailing the intervention and testing procedures. Additionally, the pre/post testing carries minimal risks due to the sub-maximal nature of the fitness testing. As per the YMCA protocol, "physician supervision is not necessary with sub-maximal testing in low to moderate risk adults." (ACSM's Health Related Physical Assessment Manual, 2007). Although any exercise program carries with some possibility of exercise-induced cardiovascular events, we will minimize this by including those who have been cleared by a health care professional. While the risk of a cardiac emergency is increased when a person is exercising, these events are rare and usually occur during high-intensity activities. This study will utilize a moderately intense exercise regiment for all participants. The cardiovascular benefits of exercise have been consistently shown to outweigh the acute cardiovascular risk during the act of exercising. Moreover, a person beginning a moderately intense exercise program is actually at a lower overall risk of sudden death than their sedentary peer.

Protection Against Risk

Several precautions will be made to reduce the risk of exercise-related injuries:

- The exercises are carefully designed with warm-up, stretching and cooling down components to minimize injuries;
- Written instructions highlighting some fundamental guidelines all study participants should do to ensure that they are exercising safely will be provided to all study participants;
- For those individuals who are extremely de-conditioned, frequent rest periods and exercise from a sitting position will be offered at the beginning of the training period;
- At each exercise session, participants will be questioned about the presence of musculoskeletal symptoms;
- If a participant experiences a significant illness requiring mobility restriction, surgery or hospitalization, the project coordinator will contact the participant's primary physician for clearance to return to the intervention classes.
- If adverse symptoms develop a 1-week hiatus from the exercise session will be enforced, and during this time the participant will perform static stretching exercises. A slow reintroduction of the exercise program will follow all hiatuses;
- The instructors/evaluators will be instructed to be alert for the emergence of symptoms of angina and shortness of breath. Participants will be instructed to discontinue exercise if there is significant pain, weakness, or joint swelling after exercise.
- We will ensure that that the site has immediate access to a phone and that provision of interventions and evaluations are undertaken in the immediate vicinity of a phone. All trainers and evaluators will also be encouraged to carry cell phones;
- All activities will occur inside an air-conditioned facility because environmental extremes are poorly tolerated;
- Every precaution will be taken to provide fluids, rest and other measures to insure each participant is comfortable, safe and secure in the testing environment.

Confidentiality of data is maintained by using research identification numbers that uniquely identify each individual. Safeguards are established to ensure the security and privacy of participants' study records. The information collected from participants in this study has a low potential for abuse, since the data do not address sensitive issues. Nevertheless, appropriate measures are taken to prevent unauthorized use of study information. The research ID number is used. The research records are kept in a locked cabinet in the locked office of the PI. The files matching participants' names and demographic information with research ID numbers are kept in a separate locked room and are stored in a locked file that uses a different key from that of all other files. Only study personnel have access to these files. Electronic data will be stored in a password-protected file on a secure network. After the study is completed, procedures for long-term storage of VA data will be followed.

FMRI data will be stored in a secure electronic environment in a locked office (6205 A&B) at the Woodruff Memorial Research Building, 1639 Pierce Dr. Atlanta, GA 30322, without identifiers.

10. Pharmaceutical, biologic, and device information:

N/A

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