Protocol

1. Project Title

Effects of Aging on Cortical Excitability During Motor Learning

(Short title: Aging and Cortical Excitability)

2. Investigators:

Principal Investigator: Joe Nocera, PhD

Co-Investigators: Lisa Krishnamurthy, PhD

Camille Vaughan, MD, MS

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3. Abstract:

Aging is associated with changes in brain excitability that has direct impacts on behavior. Brain excitability is modulated by either selective increases in firing rates (excitation) or selective decreases in firing rates (inhibition). Our brains maintain cortical function by balancing excitability with inhibition largely through inhibitory interneurons that use the neurotransmitter gamma aminobutyric acid (GABA). It is currently unknown if total GABA levels in the brain increase or decrease as a function of age, but recent studies have shown that GABA can be selectively modulated during cortical processing including motor learning. That is, GABA levels change during selective engagement in repetitive task practice (Floyer-Lea et al., 2006) and this change is related to functional performance (pilot data). Specifically, in younger adults, GABA levels tend to decrease during the entrainment of a motor task and return to baseline when the task is performed automatically. This selective decrease in GABA levels is consistent with long term potentiation, as cortical excitability is acutely increased with the reduction in GABA. These data have been shown in younger adults, however, little is known about the function of GABA during motor learning in older adults. This is particularly important because behavioral neuroplasticity is dependent on training induced changes in cortical excitability likely mediated by alteration of GABAergic function. We do know that older adults learn motor tasks at a much slower rate and with greater errors than younger adults (Salthouse, 1996). Interestingly, our pilot data show that GABA levels increase during this errorful learning phase in training with aging adults. The cause of this increase is yet unknown and unconfirmed. The purpose of the current project is to attempt to understand changes in the aging brain respective of motor learning by focusing on the neural mechanisms responsible for changes in cortical excitability states. Importantly, we expect that there are individual differences in performance in aging adults as has been reported widely in previous literature (D'Esposito & Aguirre, 2003). We will employ magnetic resonance imaging, magnetic resonance spectroscopy, transcranial magnetic stimulation and behavioral testing to attempt to quantify the neural factors associated with GABAergic function that may account for individual differences in skill acquisition and retention. Interestingly, aerobic exercise has been shown to improve motor skill performance and learning performance in older adults (McGregor et al., 2018). To test the potential mechanism of how aerobic exercise changes cortical excitatory balance, we will be randomizing participants into either a 12-week aerobic exercise (cycling) or a balance and stretching condition. Aerobic exercise is believed to increase blood flow, increase neurotrophic factors and increase metabolic efficiency at the cellular and receptor level. Using transcranial magnetic stimulation and magnetic resonance imaging and spectroscopy, we will test the effects of exercise on both GABA levels and receptor function in conjunction with measuring levels of serum neurotrophic factors and cardiovascular response. The current project represents a considerable leap forward in terms of combining leading edge neurophysiological techniques in a human model to understand the mechanisms responsible for aging-related changes in motor performance and in future work, rehabilitation of disease. This has strong implications to rehabilitation after motor pathology, as most motor diseases affect the aged brain. By understanding the mechanisms of learning in this age cohort,

we can directly translate the current study's findings to rehabilitation programs focused on using brain stimulation to up-regulate or down-regulate cortical excitability.

4. Background:

Over 56% (11.8 million) of Veterans are over the age of 60; a percentage which will be maintained for the next 30 years (VetPop). For comparison, 28% of the general American population is over 60 (CDC data). Increased lifespan within the Veteran population carries with it an expected increase in motor pathologies requiring rehabilitation. Clinical trials have employed non-invasive brain stimulation (NIBS) to increase or decrease cortical excitability for rehabilitation of upper extremity dysfunction, but with varied results (Vancleef et al., 2017; Rose et al., 2014). A critical assumption of rehabilitation involving motor retraining with NIBS is that practitioners can quantify the excitatory/inhibitory states that best promote motor learning. Based on the variability of the results to date, we argue that this assumption is unmet. A major component of motor disorders (e.g. – Parkinson's disease, stroke, etc.) are that they are layered on top of a brain that is aging, the effects of which likely contribute heavily towards changes in inhibitory cortical tone (Crosson et al., 2015; McGregor et al., 2017). Our previous work has shown that sedentary older adults exhibit a loss of inhibition in primary motor areas, which has a deleterious effect on motor control (McGregor et al., 2009; 2011; 2013; 2016). However, our work has also shown that aerobic exercise can improve inhibition of the primary motor cortex with coincident improvements in dexterity (McGregor et al., 2012; McGregor et al., 2017). Using recent advances in transcranial magnetic stimulation (TMS) and magnetic resonance spectroscopy (MRS), we now have the unique opportunity to identify how motor training modulates neurotransmitter systems associated with aging-related loss of inhibition due to: a) neurotransmitter receptor function (using TMS) and b) levels of neurotransmitter concentration changes during motor training (using MRS). Moreover, using the effective aerobic exercise platform created by Dr. Joe Nocera (Co-I) and implemented at the Atlanta RR&D Center for Visual and Neurocognitive Rehabilitation (CVNR), we can explore the neuromodulator effects of aerobic training on previously sedentary older adults to understand how aerobic exercise alters cortical excitability and inhibition using the measures. The current proposal seeks to understand the mechanisms underlying agingrelated changes in cortical excitability during motor learning by comparing motor performance to a) neurotransmitter levels assessed with MRS and b) neurotransmitter receptor function as assessed by TMS. As explained in more detail below, these two measures of cortical inhibition (MRS and TMS) describe the GABA neurotransmitter system from different physiological aspects: MRS indexes GABA concentration while TMS measures GABA receptor function. In addition, as aerobic exercise is being widely incorporated in almost all types of treatments for motor pathology, our lab can immediately test the effects of an effective aerobic exercise intervention in partnership with our CVNR's Aerobic Exercise Core (led by Joe Nocera, Co-I).

Importance of GABA

The primary inhibitory neurotransmitter system in the brain is gamma-amino butyric acid (GABA). It is highly likely that alteration of this neurotransmitter system is, at least in part, responsible for the aging related changes in function. However, a critical gap in our

understanding is that we do not yet know how the aging process alters the GABA system. It is possible that older adults have less of the neurotransmitter available. Regional studies of GABA tone across age groups have shown some discordance with respect to the specific brain regions that exhibit aging related decrease in GABA signal as assess using magnetic resonance spectroscopy (MRS). Given this heterogeneity in findings, there is an opportunity for more systematic exploration using targeted MRS, and the present study intends to employ this technology in a focal, hypothesis driven approach. Beyond differences in the amount of GABA in aged adults, it is highly likely that the aging process is associated with changes in the metabolism of the neural system. As such, it is probable that these metabolic changes manifest in alteration of GABA receptor function. We now have the capability to systematically and rigorously address this question with the application of two technologies. That is, we can assess how overall GABA tone is associated with cortical function in the motor system respective of interhemispheric transfer with a study that employs combined magnetic resonance imaging and transcranial magnetic stimulation.

The two non-invasive technologies with which we can index the effects of aging on the GABA system are magnetic resonance imaging (MRI) and transcranial magnetic stimulation (TMS). We have employed both technologies to assess the effects of aging on interhemispheric communication. As interhemispheric communication in the primary motor cortices is largely inhibitory in nature, the motor system offers a great deal of possibility for our investigation into the effects of aging on GABA function. As such, we will be employing both MRI and TMS with a specific focus on the effects of aging in the primary motor cortex and the dynamics of GABA function that sub serve interhemispheric communication.

However, GABA is widespread throughout the brain and exerts potent effects on neural regions that have been associated with cognitive functions. Language function is believed to be altered by the loss of cortical inhibition in aging and disease (Meinzer et al., 2011; 2013). In our own lab, we have recently replicated findings by Naeser et al., 2004, that investigated the effects of the right lateral inferior frontal cortex on object naming. That is, the exogenous inhibition (via rTMS) of pars triangularis exerts a beneficial effect on naming performance in older adults. This indicates that aging related increases in activity of this area is detrimental to behavioral language performance. However, the neural mechanisms that underlie this effect have yet to be probed. Given the strong effect that GABA has on local tissue via interneurons (Buzsacki et al., 2007) it is likely that properties of this neurotransmitter system underlie some of the changes associated with the aging process.

Magnetic Resonance Imaging

Beginning with our previous experience with MRI, we have established and replicated protocols that can reasonably measure levels of interhemispheric communication during functional magnetic resonance imaging. This somewhat simple protocol uses paced finger tapping of the dominant hand to assess degree of ipsilateral recruitment or inhibition during the tapping task. We have refined this methodology over several studies (McGregor et al., 2009; McGregor et al., 2011; McGregor et al., 2013; McGregor

et al., 2014). Our projects have used fMRI to assess the amount of interhemispheric inhibition present during a finger movement task in younger and older adults. This can be indexed by the presence or absence of an fMRI signal called negative blood oxygenated level dependent response (BOLD) in the motor cortex ipsilateral to the moving hand. During volitional movement of a single extremity, the contralateral primary motor cortex exhibits an increase in metabolic activity consistent with increased synaptic function (Logothetis, 2001). However, in young adults, the activity level of the opposite primary motor area tends to decrease as compared to baseline levels (inactivity). This decrease as compared to baseline is deemed “negative BOLD” and appears to show a drop in the amount of neural activity in areas where present (Shmuel et al; 2006; Klingler et al., 2011; Kastrup et al., 2010. Functional magnetic resonance imaging 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).

Magnetic Resonance Spectroscopy

A central question that has not been well addressed in the literature respective of aging and GABA function is a simple one. As people age, does the brain produce less of the GABA molecule? While somewhat simplistic in terms of the highly complex neurochemical interactions that exist within the human central nervous system, this question is nevertheless a starting point. That is, per unit volume, do older brains contain less GABA in brain regions that have been shown to evidence tonic levels of GABA across age groups? We can address this question by the implementation of magnetic resonance spectroscopy, an in vivo technique to assay and quantify specific molecules within the brain.

Recently, in collaboration with magnetic resonance physicists at Georgia State University and Emory University, we have successfully gained the capability to acquire MRS data using a vetted MRI sequence called MEGA-PRESS. We will utilize this sequence during the present study to answer the question of how much GABA per unit volume exists in posterior cingulate and primary motor areas of the brain, two regions previously shown to evidence tonic levels of GABA at rest. We now have the capability to use magnetic resonance spectroscopy to index the amount of GABA present in a given area. As such, this is a great leap forward for our capabilities to assay the function of the central nervous system as we can now link GABA levels in the brain with both behavioral performance and regional task activity during functional MRI. The effect of aging on the quantity of GABA in the central nervous system, particularly in the motor cortex is currently unknown. In the current project, we will be measuring GABA levels using MRS during a motor sequence learning task, which has been shown to modulate GABA levels in younger adults. Importantly, we will also be acquiring MR measures of cortical glutamate-GABA metabolism using a MRI sequence called glucoCEST. This affords us the capability of indexing the energy exchange within the brain. With the assistance of Dr. David Reiter, PhD, we will also be indexing energy metabolism in skeletal muscle. Specifically, participants will be repositioned in the MRI magnet after a short break and we will then acquire Phosphorus-31 MRS of the quadriceps. These

data will be correlated to data from the cortical acquisition using the glucoCEST sequence. Importantly, these data will give us an index of muscular energy metabolism (production of adenosine triphosphate) prior to and after engaging in a 12-week exercise program that has been shown repeatedly to increase oxygen metabolism (Nocera et al., 2017; McGregor et al., 2018). As an exploratory aim, we will relate the changes in glucoCEST metabolism within the brain with changes in muscular energy capacity. We believe this is the first project to ever acquire such data.

Transcranial Magnetic Stimulation

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 (IHI) across increasing age, a finding later replicated by Talelli et al. (2008a; 2008b). Using a separate, but related measure called the ipsilateral silent period (iSP), Sale and Semmler (2005) reported that their elderly samples reported a significantly shorter duration of ipsilateral inhibition; a finding recently replicated by Fujiyama et al., (2009) and our own lab (McGregor et al., 2011; 2013). Recent work has shown that in older adults declines in unimanual motor performance are 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 and motor performance in sedentary older adults (McGregor et al., 2011; 2013). We plan to employ transcranial magnetic stimulation both inside and outside of the MRI environment to assess the effects of aging related alteration of GABA receptor function on measures of TMS (iSP, IHI) that are believed to reflect GABAergic activity. Using a battery of assessment TMS measures (i.e. – no cortical modulation – NOT repetitive TMS), we will assess changes in a GABAergic receptor function due to motor learning and exercise.

Aerobic Exercise

Exercise, Aging and Interhemispheric communication

Aerobic activity has long been shown to have promise in the mitigation of agerelated 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 produced a very exciting 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.

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 reported (McGregor et al., 2011; 2013) 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. This provided the first evidence of a possible link between two very disparate measures of cortical function (MRI and TMS). Voelcker-Rehage et al., (2010) reported that elderly adults who engage in aerobic activity show decreased activity in the ipsilateral motor cortex during unimanual movement in fMRI.

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; 2013). Moreover, an aerobic exercise intervention was effective in increasing the duration of the ipsilateral silent period while improving motor performance (McGregor et al., 2018). We believe this was the first study to show that an aerobic exercise intervention has direct effects on TMS measures of cortical excitability. However, we do not yet have data as to the mechanism causing these changes. The present study seeks to address this gap in knowledge by integrating leading edge MRI/MRS, TMS, behavioral and bioassay techniques to improve our understanding of how exercise changes neurochemical, metabolic and intracortical patterns of activity associated with motor learning.

5. Specific Aims:

Specific Aim 1) To determine the relationship of motor performance on two different measures of cortical inhibition (TMS and MRS).

GABA levels in left primary motor areas will be lower in older adults who perform more poorly on upper extremity behavioral assessments.

Specific Aim 2) To determine the effect of an aerobic exercise regimen on motor learning and related GABA concentration changes using MRS.

Aging-related changes in GABA function can be mitigated with an aerobic exercise intervention.

Specific Aim 3) To determine the effects of motor learning prior to and after an aerobic exercise intervention on GABA receptor function using TMS.

Motor learning will modulate cortical inhibition at the receptor level causing decreased intracortical inhibition, but preserved interhemispheric inhibition. Older adults that are enrolled in the regular aerobic exercise will show maintenance or enhancement of interhemispheric inhibition and increased GABA efficiency during motor learning resulting in increased modulation of GABA receptors.

Specific Aim 4) To compare measures of cortical energy metabolism with measures of skeletal muscle metabolism using MRI and MRS measures prior to and after an aerobic exercise intervention.

Older adults who are more physically active at the study outset will have more similar measures of energy metabolism in skeletal muscle as compared to cortical metabolism. The aerobic exercise intervention will promote a stronger relationship between skeletal muscle metabolism and cortical metabolism.

6. Design/Methods

Video and Telephone Consenting Process

To limit face-to-face interactions with participants, the study will be using Emory and VAapproved video or audio communication to screen and consent. Some VA-approved virtual methods include Microsoft Teams Version 1.3+, Zoom 5.0 or greater, and Cisco Webex version 40+. Recordings of the audio or visual communication will not be permitted. If the participant meets initial eligibility criteria, we will mail or email through VA Outlook using encryption two unsigned copies of the ICF/HIPAA. We will ensure the individual has enough time after receiving the document to read it before scheduled phone/video call. Trained staff will perform consenting process including speaking with the individual to discuss the study and highlighting each section of the consent form, allowing the participant an opportunity to ask questions before providing consent, and giving the participant enough time to consider being in the study. Study team will inform the individual that if they would like to take more time to consider the study, another telephone call can be scheduled. If the individual would like to participate, the participant

will sign and date the document and return it to study team via mail or via email to the VA Outlook email address. If the participant chooses to return the signed consent via email, they are required to send the consent as a reply to the original VA encrypted message sent by study staff. The study team will write a "Note to File" documenting everything about the interaction including:

1. When and how the consent form was sent
2. When the video/telephone call was made
3. What was discussed during the call
4. When the signed consent form was received
5. When the signed consent form was signed by the person obtaining consent
6. When a copy of the consent form signed by both subject and study team was given to the participant
7. A description of why signature dates are different (if applicable)

Once the study team receives the signed consent form, the person obtaining consent should sign the form and date it for the day it was signed. Study procedures will begin once the signed copy is received. A copy of the fully signed consent form will be given to the participant via mail or in person at the next scheduled visit.

Executive Function Measures - We will be acquiring a battery of cognitive outcomes associated with changes in aging. These data are important to help classify cognitive status, as well as offering potential prediction of neurocognitive declines (and reversal of declines through aerobic exercise).

Language Function

1. *Letter Verbal Fluency*: Participants produce as many words (F, A, S, or P, R, W) as they can that begin with that letter in 60 seconds. Clinical findings are in line with numerous functional magnetic resonance imaging studies, demonstrating that letter verbal fluency is associated with extensive activation in the left frontal cortex. Further, age-related decline in letter fluency have been demonstrated.

2. *Semantic Verbal Fluency:* Participants produce as many words as they can that fit a category (animals or fruits/vegetables) in 60 seconds. This is generally considered a measure of a verbal component of executive function and has been demonstrated to decline with age.

3. *Switching Verbal Fluency:* The switching condition evaluates the examinee's ability to generate exemplars while alternating between two different semantic categories (i.e. fruits and furniture) in 60 seconds. Switching requires the ability to engage in strategic search processes such as initiation, cognitive flexibility and mental set shifting. Because it is related to fronto-executive functioning, impaired performance is seen among patients with frontal lobe lesions and older adults.

Response Inhibition

4. *Color-word Interference Test*: Participants say the color in which another color word is printed in (e.g., for BLUE printed in red ink, the answer is 'red'). This is a commonly-used executive function task. Additionally, the interference effect, caused by difficulty inhibiting over-learned word reading, is often more pronounced in older adults.

Working Memory

5. *Digit Span forward/backward*: Participants must recall increasingly long strings of digits in order (forward) or reverse order (backwards) of presentation. Central executive component of working memory has been demonstrated to play a key role in digit backward span performance and age-related decline has been reported.

6. *Hopkins Verbal Learning Test*: Assess verbal learning and memory (immediate recall, delayed recall, delayed recognition).

7. *Digit Symbol-Coding:* a neuropsychological test sensitive to brain damage, dementia, age and depression. It consists of digit-symbol pairs followed by a list of digits. Under each digit the subject should write down the corresponding symbol as fast as possible. The number of correct symbols within the allowed time (e.g. 90 or 120 sec) is measured.

Non-Verbal Executive Function

8. *Trail Making Test A and B:* Easily administered tests measure attention, visual searching, mental processing speed, and the ability to mentally control simultaneous stimulus patterns.

Crystallized Word Knowledge

9. *American National Adult Reading Test (ANART)*: reading test used to help assess intelligence.

Video Conferencing for Assessments

To limit face-to-face interactions with participants, the study will be using Emory and VA-approved video or audio communication to screen and consent. Some VA-approved virtual methods include Microsoft Teams Version 1.3+, Zoom 5.0 or greater, and Cisco Webex version 40+. Recordings of the audio or visual communication will be permitted as described previously to facilitate scoring of the assessment data in an off-line manner. During appointment scheduling, the participant will be asked if they have access to a computer with video, microphone, and speaker capabilities with access to high-speed internet that supports this level of communication. Once equipment access is confirmed the participant will be eligible to participate in tele-assessment, instructions for accessing the tele-assessment session will be sent via encrypted email through the VA outlook server. Any test that can be administered via video should be administered at this time. If an assessment cannot be accomplished remotely, it may be administered on the day of an in-person visit.

Study Visits

Participation in this study will take up to 4 months. A full description of the activities follows this table. Sessions will meet at the Exercise Lab of the Atlanta VA Rehab R&D Center or the Geriatric Research Education and Clinical Center (the GRECC), an Atlanta VA Healthcare System affiliate. You can also choose to have your sessions held remotely.

Physical Function Assessment

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

2. *400 Meter Walk:* Participants will be asked to walk at their usual pace, without over-exerting. They can stop for up to 1 min for fatigue or other symptoms. A time limit of 15 minutes to perform the test has been established based on the following considerations. First, individuals who complete the walk in >15 minutes have an extremely slow pace (<0.44 m/sec), which would make their walking capacity of little utility in daily life. Second, selecting a higher cut-point, such as 30 or 60 minutes makes the objective assessment impractical and does not add to the clinical significance of the outcome. Participants will be allowed to use a cane, but not a walker, to complete the 400m walk. Procedurally, we will first request that participants attempt the walk without the use of a cane. Those who feel unsafe will be allowed to attempt the walk with their cane.

3. *Walking and Walking While Talking:* Participants will be asked to walk across an electronic walk way which captures walking speed and foot placement in a quiet well-lit hallway wearing comfortable footwear. Start and stop points 10 meters apart will be marked by lines on the floor and include 3 feet from the walkway edge for initial acceleration and terminal deceleration. Eight walking trials will be randomly conducted; 4 single task walking only trials and 4 dual-task walking while talking trials. For the walking while talking trials participants will be asked to recite alternating letters of the alphabet. The order of the initial letter will randomly vary between "A" and "B" to minimize practice effects and/or count backwards by 3's.[37]

4. *Short Physical Performance Battery (SPPB):* The SPPB is based on a timed short distance walk, repeated chair stands and a balance test (as described by Guralnik et al.[38]). The battery will be administered by a trained and certified examiner.

5. *The Activities-specific Balance Confidence (ABC) Scale:* Indicates self-report of participants' level of confidence in doing daily activity without losing their balance or becoming unsteady. Self-Report Function Questionnaire. We will use a modified version of the disability instrument that was used in LIFE-P, now called the Pepper Assessment Tool for Disability (PAT-D)[44]. The questionnaire inquires about perceived difficulties in general activities of daily living during the last month. For each item, the response categories include: 1) no difficulty, 2) a little difficulty, 3) some difficulty, 4) a lot of difficulty, or 5) unable to do. Answers are averaged across the items, in order to better assess the overall perceived disability burden by a person. The questionnaire consists of 5 subscales: mobility, transferring, upper extremity, instrumental and basic ADLs. In addition to being a valid measure, the disability questionnaire has been shown to be responsive to change in previous exercise intervention studies among various disease populations [45].

6. *Leg Press*: An assessment of bilateral leg movement function will be completed using the Keiser Leg press.

7. *Sit and Reach Test*: An assessment to measure flexibility in the lower back and hamstrings.

8. *Functional Reach Test*: Will be used to assess participants' balance while reaching forward from a fixed position.

Direct Assessment: Following written consent, the participant will be asked to complete a direct measure of aerobic fitness using a Maximal Treadmill Exercise Test or a Submaximal Treadmill Exercise Test.

Submaximal Treadmill Exercise Test: Submaximal exercise testing will be performed using an exercise protocol validated for this purpose (e.g., Naughton, modified Bruce, or similar). For graded protocols where participants progress through a series of stages in which speed, incline, or both are increased in a stepwise manner, the progression will be such that it allows the participant to adapt to a specific workload before advancing onto a more difficult stage. Termination of the test occurs if the participant requests to stop, the submaximal threshold is reached, other symptoms prohibit further exercise, or there is an absolute indication for termination of exercise testing (**VO2 Peak).**

Maximal Treadmill Exercise Test: Unless contraindicated, participants will undergo maximal exercise testing using an exercise protocol validated for this purpose (e.g., Bruce, Balke, or similar, or using a ramp protocol). Participants will progress through a gradual increase in work rate based on the specific protocol being used until they either become fatigued and decide to stop, develop other symptoms that prohibit further exercise (e.g., musculoskeletal pain), or there is an absolute indication for termination of exercise testing (Table 2). Vital signs, including blood pressure and heart rate, will be recorded at regular intervals throughout the test, and participants will be monitored with continuous electrocardiography. Participants may also be asked to report their rate of perceived exertion (RPE) at various points during the test. After testing, participants will be monitored until their heart rate and perceived exertion have normalized. A registered nurse (RN) or exercise physiologist (EP) will be present for the test duration.

Participants will be randomly placed in an aerobic exercise intervention or a stretching control intervention.

Aerobic Exercise Intervention: 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 has assisted in the implementation of these guidelines. To this end, this project will utilize the VA CVNR's Aerobic Exercise Core's (directed by Dr. Joe Nocera) vetted 'spin' exercise training program. This program has been shown to improve cognitive and motor functions across a variety of older populations including individuals with motor pathology (Nocera et al., 2016; Nocera et al., 2015). 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 (Target HR = 220 – Age). Resting heart rate is then subtracted from this number for heart rate reserve (HRR). The answer is then multiplied by the target percent (e.g. 50%) 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 90% of maximal heart rate reserve. Exercise time will progress from an initial 20 minutes per session to a maximum of 60 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 at the VAMC. Additionally, to maximize safety, each participant's primary care physician, physician assistant, or nurse practitioner will sign a letter approving study participation. 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). Similar protocols have been used in other studies involving effective change in behavioral measures after aerobic exercise.

Interval Exercise Group: For 12-weeks participants will engage in progressive whole body stretching, toning and balance exercises designed for individuals 60 years and older. Participants will meet for the same total duration time as the aerobic exercise group. Heart rate will be monitored to ensure participants maintain exercise levels below aerobic training zone.

Remote Exercise Monitoring: As an alternative, participants that are uncomfortable with receiving face-to-face training within the hospital will be given the option to continue their exercise intervention visits remotely. For participants that have been randomly assigned to the 'spin' exercise training program, will be provided a stationary cycle ergometer that has been sanitized properly to use during the 12-week period. All subjects will be monitored and trained through VA and Emory approved remote access sites.

Control Group: A random subset of participants will be in a 12-week wait-list control. These participants will undergo the same pretesting procedures as described above. After 12-weeks of no activity with the study team, these participants will return and repeat the pre-assessment, 12-week exercise intervention and post assessment as described above.

3. Electronic Activity Data: Electronic activity data will be generated by Apple Watches functioning in two possible modes. Each mode has unique requirements for data security. The Watch will collect the participants' daily total activity level.

1. *Stand-alone*. Some Apple Watches loaned to participants will be paired with VA-furnished iPhones. When not loaned out to participants, these iPhones will remain at the VA in a locked and secure environment (12C-173b). Because the watches will be functioning at a remote location, they will be unable to sync the data they generate to an iPhone or a network cloud. Instead, our team has written code to preserve this data in formatted files on the watch until the watch is returned to our possession. The data stored on passcode protected Apple Watches is secured with AES-256 encryption.

Once the watch is returned to the VA, it will be synced to the VA-issued iPhone. (iCloud backup will be disabled for apps handling Apple Watch generated data.) The data transferred will be devoid of participant identifiers except participant study ID, year of birth, timestamps, and Apple Watch device identifiers. It should be noted that although the timestamps contain dates, these dates are not directly related to significant life events (e.g. birth, death, hospital admission) but rather are continuously catalogued while the watches are on and not charging. Also, Apple Watch device identifiers are not perpetually unique to a participant because these watches are VA property and will be worn by numerous participants over time. Regardless, data stored on passcode-protected watches and phones will remain AES 256-bit encrypted.

Once data files are located on a VA iPhone, they will be backed up to a studycontrolled Apple iTunes account that is linked to a study-provided Apple User identification (UID) and transferred to a disk-encrypted, password-protected VA laptop. (Local iTunes encryption is optional by default but will be enabled for our purposes.)

2. *Deployed with VA-issued iPhone*. Some Apple Watches will be loaned to participants along with the iPhones to which they are paired. This mode is preferable to the Stand-alone mode; however, Stand-alone mode may be necessary if access to VA-issued iPhones is limited. Most data handling procedures and precautions used for Stand-alone mode apply to this use-case as well, including use of participant Study ID, AES 256-bit encryption, and disabled iCloud backup. One exception is that data generated by the Apple Watch will be synced directly to the paired VA-issued iPhone (i.e. - our code writing files to the Apple Watch will not be used). The iPhones loaned to participants will not have data plans. Therefore, unless participants have Wi-Fi access, data will be retrieved from the iPhones as described for Stand-alone mode after the phones are returned to the study team (i.e. study-controlled iTunes encrypted backup, and ultimately, transfer to a VA password-protected computer). If participants have Wi-Fi access, study data will be transferred to a remote server (hosted by Emory University in a secure data center under a VA Data Usage Agreement) via SSL encrypted connections. Terms specified in the handling of research data in the Data Usage Agreement will apply.

To increase data security, de-identified user data will be encrypted using Apple's Secure Enclave coprocessor embedded in Apple's A9 series processors (in the iPhone SE and Apple Watch). A FIPS-140-2 FISMA High environment, the Secure Enclave runs the L4 microkernel which is signed by an ephemeral key and cnonce entangled to the unique ID (UID) of the user with antireplay initiated at login. Antireplay revokes all data back transformation of encrypted data without express sign-on through direct passcode authentication. This means that all data is encrypted throughout transmission until authenticated by the UID on an iOS device. The encryption scheme uses a

dedicated AES-256-XNT engine. No software or firmware can read this data directly. Only after decryption by the silicon based cryptographic engine using the UID key can the data be accessed and this is further restricted to devices sharing a group id (GID). This can only be completed on another iOS device that has the UID (which is controlled by the study coordinator) and the Secure Enclave decryption engine on a device with at least an A7 series encryption coprocessor. Apple synchronizes this encrypted, deidentified data on its secure Health environment located on cloud services. These data cannot be decrypted or read by any party that does not have access to the UID, generated at the outset of the study and resides with the study staff. Only study staff can read files from the Health environment due to this encryption. Even if the physical chips are removed from the device to access the data, the device UID that created would maintain encryption of the data rendering the files inaccessible. Apple's cryptographic architecture received NIST validation as FIPS-140-2 validated with Certificate #3148 (3/8/2018 - 3/9/2023).

For all modes, filenames contain dates and times indicating when the files were created for transmission/export. These dates/times do not necessarily reflect date and times that the data were generated.

Motor performance - Motor assessments taken in the present study have been shown to previously be sensitive to aging and changes in aerobic fitness (McGregor et al., 2018). Primarily based on dexterity, these are a wide-ranging assessment of general upper extremity motor function.

1. *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.

2. *Coin rotation task:* Another dexterity assessment, this task asks participants to rotate a coin (U.S. nickel) as quickly as possible for 20 rotations.

3. *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.

4. *9 Hole Pegboard Task:* Participants place plastic pegs as quickly as possible into a nine-hole pegboard.

5. *Grip Strength:* Participants will squeeze a Jamar-brand pinch and hand-grip dynamometer to assess total force output.

6. *Motor learning*: Participants' right hand will be placed on a joystick controller connected to a laptop computer. The participant will be asked to follow the direction

of movement as indicated on the computer screen, which will be displayed in a sequence of motions. This sequence is a repetitive pattern that the participant will have the opportunity to train to automaticity (i.e. learning), followed by the opportunity to recall the motor sequence. The participant's movement of the joystick will be recorded by the computer for post-analysis of error rates and reaction times.

Additional behavioral measures:

In addition to executive function testing and motor function testing, we will also be assessing the general well-being of the participant at the outset of the study and after the intervention. We will be using a battery of behavioral surveys to assess activities of daily living and general well-being. These include:

1) Beck Depression Inventory
2) Godin Leisure Time Exercise Questionnaire
3) Montreal Cognitive Assessment
4) Personal Wellness Survey
5) ADLQ – Activities of daily living questionnaire
6) Patient Health Questionnaire-9
7) Global Physical Activity Questionnaire
8) MAC-Q
9) Activities-specific Balance Confidence Scale
10) Pittsburgh Sleep Quality Index
11) Epworth Sleepiness Scale
12) MAI
13) Pelvic Floor Distress Index

TMS Measures

Single pulse measures: Four single-pulse TMS 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 (BrainSight 2.3; Rogue Research). A MagVenture x100 magnetic stimulator (MagVenture, Denmark) 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 procedures for interhemispheric inhibition assessment (Ferbert et al., 1992) require a second MagVenture magnetic stimulator (R30 series) and attached 60mm 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 (SIHI) or 40 (LIHI) 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).

Motor learning TMS

In addition, we will be executing a motor learning paradigm during TMS. Participants will view a screen with the picture of a line-figure hand. At the tip of each finger, a color will appear to indicate that this is the finger to be moved. The sequence progresses through 12 finger movements that are repeated throughout the acquisition. Over time, participants learn the finger movements to automaticity, but do so at different rates. During this time, we will be using assessment TMS to measure GABA receptor function using the SICI/LICI/SIHI/LIHI protocols as stated above. This will inform us if receptor function changes as a result of the motor learning paradigm.

Magnetic Resonance Imaging:

Structural Imaging: High-resolution structural imaging will be obtained as an anatomical reference for functional data. This data can be used for Voxel Based Morphometry analysis used to compare white and gray matter intensities. Additionally, magnetic resonance spectroscopy will be acquired using the MEGA-PRESS imaging sequence.

Resting State fMRI: Resting-state fMRI is based on spontaneous low frequency fluctuations (0.1 Hz) in the BOLD signal (Biswal et al. 1995). Studying correlations between variations of the BOLD signal, it can identify regions which activate synchronously with each other (Lee et al. 2012). Network modeling techniques can then be used to identify which brain areas were interrelated during resting state. The participant will be asked to lie still with eyes closed during echo planar MRI acquisition. The scan duration is 8 minutes.

Motor Learning Task: Motor learning is operationally defined as the ability to perform a movement progressing to 100% performance over a set number of trials. The motor learning task used in the present study will be adapted from a previous project involving learned sequence finger movements (McGregor et al., 2009). Participants will view a screen with the picture of a line-figure hand. At the tip of each finger, a color will appear to indicate that this is the finger to be moved. The sequence progresses through 12 finger movements that are repeated throughout the acquisition. Over time, participants learn the finger movements to automaticity, but do so at different rates. We will be acquiring magnetic resonance spectroscopy during these times to test if GABA levels in the motor cortex affect the skill acquisition (see Floyer-Lea et al., 2006).

7. Possible Discomforts and Risks:

Behavioral/Cognitive Testing:

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

Magnetic Resonance Imaging (MRI):

More than 150 million diagnostic magnetic resonance studies have been performed worldwide. Most 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, subjects can experience hearing loss. For this reason, individuals will be given foam earplugs to wear to minimize this risk.

Additionally, some individuals are susceptible 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.

The MRI may detect early stages of disease(s) that had not been previously diagnosed amongst participants. 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 overthe-counter pain medications. However, both issues are less of a problem in the scalp areas that we will be stimulating.

Possible effects on hearing have been described so participants will 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.

Blood Draw

The risks of drawing blood from a vein include discomfort at the site of injection; possible bruising and swelling around the injection site; rarely an infection; and, uncommonly fainting from the procedure. A trained phlebotomist will collect all blood samples following standard protocol.

Saliva will be collected at study assessment visits in a 15mL sterilized tube; roughly 3mL. Once collected, saliva will be immediately placed on ice and stored at -80 degrees until subsequent analysis,

All blood and saliva collections will occur at week 1 and week 12.

Physical function and Exercise Intervention

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. 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 at a lower overall risk of sudden death than their sedentary peer. Neither an ECG nor a stress electrocardiogram will be included in the screening process due to the lack of evidence to support their usage in detecting those who will have an adverse exercise outcome.

Some participants will experience exercise-related injuries and possibly muscle soreness and fatigue as a result of the testing and the intervention. There is a small risk for loss of balance and injury from a fall while walking and during balance testing. However, walking and balance measurements will only be a few minutes at a time, and research personnel will be with the participant at all times. Most injuries will be selflimited, though it is possible for permanent injuries to occur, including broken bones or joint problems. There are minor risks of musculoskeletal problems associated with the performance evaluation measures of the study.

Adverse event reporting:

In the case of a reportable event, the Principal Investigator will follow the current Atlanta VA Research and Development operating procedure for reporting. This includes notifying the Emory IRB within the appropriate time period of any adverse event occurring.

All study staff will be trained on proper reporting procedures of adverse events (i.e.- contact emergency medical services for immediate medical emergency and later the Principal Investigator, if not present). Dr. Nocera will notify the IRB and VA SIO of any reportable events.

8. Possible Benefits:

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. As a responsibility to the participants, anatomic scans will be read by a qualified physician if unexpected abnormalities arise.

9. Participant selection:

The study will recruit 40 healthy adult participants between the years of 60 and 80. The study will also recruit 20 healthy adults ages 18-35 as a comparator group for the MRI scans. Groups will be matched for gender, if possible, given that a large majority of Veterans are male.

Inclusion/Exclusion Criteria. Participants will be initially screened via phone for inclusion. The key inclusion criteria and final participant pool will consist of right handed English speaking individuals. All participants will be strongly right-handed (Edinburgh Handedness Inventory, Oldfield, 1971). Cortical activation characteristics in primary motor cortex are altered by repeated digit movement practice (piano playing, expert typists, etc.).

Participants will be sedentary as defined by < 120 min/week of aerobic exercise over prior 3 months. This is required, as increased level of physical activity has been shown to alter these aging-related changes of cortical activity. Participants will be enrolled based on self-report from physical fitness inventories (Godin Leisure Time Activity Questionnaire and Aerobic Exercise Readiness Questionnaire) administered over the telephone.

Participants will be non-demented and will have no cognitive-executive function deficit (MoCA < 24).

Participants must complete an MRI and TMS screen and will be excluded if exhibiting medical or behavioral conditions where exposure to strong magnetic fields is contraindicated (pregnancy, metal implants, etc.).

As motor systems change with repeated practice, individuals will be screened for expert use of single or dual handed motor movements (e.g., typing, piano, other instruments). As such, we will screen out participants from participation if they report engaging in repeated skilled finger movement practice (specified as at least four weekly one-hour training sessions).

Potential participants will also be excluded if they have a history of chronic motor disease (e.g. - Essential Tremor, Palsy), significant cardiac or cardiovascular complications (e.g. - arrhythmia, uncontrolled hypertension, angina, myocardial infarct), neurological disease, major psychiatric disturbance, substance abuse, are taking psychoactive prescription medication or antiretroviral therapy medications, are HIV positive, or have poor visual acuity unable to be corrected which will be reviewed during pre-screening of participant's medical chart as well as through the medical history questionnaire after Informed Consent. Current medications and all disclosed medical conditions will be recorded for each participant and a board-certified medical doctor will

be consulted if concerns or questions arise. Additionally, the Atlanta VA RR&D Center for Visual and Neurocognitive Rehabilitation will internally review all fitness testing procedures to ensure safety of participants.

Recruitment. This study seeks to enroll Veterans who are putatively healthy. Given the demographics of the VA, the majority of the older Veterans will be male. To increase the impact of the research, we will seek to match the same number of females as males in the study and, as such, will recruit from the greater Atlanta community. This should not impact the study results as the literature does not currently support that GABA function will be significantly differentially impacted when comparing gender.

Importantly, this study will target United States Veterans; however, we will enroll participants as they present to us and meet the inclusion/exclusion criteria. While we will not target non-veterans, to meet criteria for human subject research, it would be unethical to treat them differently from any other potential participant and not enroll them in the study if they expressed interest in the study and meet study criteria. This is especially true since the study interventions have the potential to result in a benefit to the participant, although directs benefits cannot be guaranteed.

Individuals will be recruited from the Center for Visual and Neurocognitive Rehabilitation (CVNR) Participant Registry (IRB00000159) based on the preliminary inclusion and exclusion criteria. The CVNR Participant Registry study staff will query the database of all members meeting the inclusion and exclusion criteria who have consented to be contacted for research purposes and will provide their contact information to the study team. The Principal Investigator, or his research colleagues, will contact these individuals and will summarize the study procedures.

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 to identify an appropriate list of potential participants.

CDW/VINCI data will be used to identify potential research participants. Veterans who are age 65 or older and who live within 20 miles of the Atlanta VA Health Care System will be identified and contacted via mail and then subsequently by phone. Addresses and phone numbers will be used for recruitment only and the information will be verified as current and accurate in CPRS prior to use.

Additionally, participants may be recruited by word of mouth, at education and outreach events, and via print and/or broadcast advertisements. Advertisements will be submitted to the IRB, R&D and PAO for approval prior to their use in recruitment. We will give educational outreach interactions at Veterans Service Organizations and Veteran's functions on the benefits of health activity throughout the lifespan. Over the last few years, we have developed a relationship with the YMCA, the Atlanta Civitan Group, local senior centers and will recruit from these organizations and others like them. These outlets allow for flyers and handouts of ongoing studies to be disseminated.

When a potential participant is interested in enrolling, we will provide them a copy of the consenting documents and will schedule an orientation session to describe the study goals and provide an opportunity to go over the informed consent and answer questions. This will be done by the PI, Co-Is or study-approved research associates in a private office or lab at the Atlanta VAMC.

In order to decrease burden of the potential recruitment pool of participants, cognitive, motor, and physical function measurements will be shared between this study Effects of Acute Exercise on Functional Magnetic Resonance Spectroscopy Measures of GABA in Aging and Chronic Stoke (IRB00001334), and Effects of Aging in Cortical Excitability During Motor Learning (IRB00104274) as both studies administer the same assessments.

Participants will receive compensation for attendance of testing sessions. Participants will be compensated $50 after completion of each visit during Assessment 1 and Assessment 2. Participants in the comparator group will receive $50 per visit. Payments will be completed by check. Compensation will be prorated if the subject withdraws early.

10. Power Analysis and Sample Size:

Our overarching specific aims are to link upper extremity motor performance with changes in GABA physiology that are affected by aging. We will be powering on our least sensitive comparison, which is likely change in MRS after aerobic exercise intervention. Given that we expect greater variability in older adults respective of motor performance and cortical plasticity, we set a moderate effect size for group comparisons at baseline (d=.5) and will recruit 40 healthy adult participants. For the aerobic intervention, we are afforded a large effect sizes (1.33 to 1.67) based on the demonstrated efficacy of the aerobic intervention (Nocera et al., 2017). Further, data from our recent work has shown a somewhat linear relationship between $VO2_{max}$ and levels of interhemispheric inhibition (McGregor et al., 2017). Under this model, a sample size of 20 per group will give us an approximate effect size of .83 for the MRS and TMS measures. 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 work. Dropout is a concern for any intervention. As such, we will plan to enroll an additional 8 older participants (as needed) to reach the needed sample size of 20 per group.

REDCap

This study will utilize REDCap issued through the Atlanta Veterans Health Care System. Only deidentified data will be entered in REDCap by VA credentialed research personnel to run data analysis.

Protection Against Risk

Protection against risks introduced due to cognitive testing, MRI and TMS involved have been presented above in Section 7 – Possible Discomforts and Risks.

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 and/or laboratory 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 accessed in a locked office (6205 A&B) at the Woodruff Memorial Research Building, 1639 Pierce Dr. Atlanta, GA 30322, without identifiers.

11. Conflict of Interest:

There are no real or potential conflicts of interest associated with this research project.

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