Protocol for: **The Entorhinal Cortex and Aerobic Exercise in Aging**

NCT#: **NCT02775760**

IRB Number: H-34579

Sponsor: NIH/National Institute on Aging (NIA)

Principal Investigator: Karin Schon, Ph.D.
brainex@bu.edu
72 East Concord St., L-1004
Boston, MA 02118

August 17, 2020

Statistical Plan: pages 17-18

Background

Exercise has many health benefits. Some of these benefits are increased physical fitness and reduced risk of developing heart disease. Researchers currently do not know whether exercise also benefits the human brain. These benefits may include cognitive (thinking) processes, such as learning, memory, and navigating through unfamiliar environments. Exercise may be an effective and low-cost way to promote brain health and healthy aging.

Aerobic exercise consistently appears as one of the most effective interventions to attenuate cognitive decline in Alzheimer's disease (AD) (Burns et al., 2010; Burns et al., 2008), which is characterized by a profound hippocampal and entorhinal pathology (Braak and Braak, 1997). In humans, the neurophysiological mechanisms through which exercise improves cognition are unknown, with most studies focusing on prefrontal-mediated executive and memory functions (Colcombe et al., 2004), or hippocampal volume (Erickson et al., 2009; Erickson et al., 2011). Recent research from our laboratory illuminated a striking association between entorhinal cortical (EC) volume and aerobic fitness in young volunteers such that greater fitness was associated with a prominent increase in EC volume (Whiteman et al., 2015). Within the medial temporal lobes (MTL), the EC uniquely displayed this effect. The EC sits at the epicenter of inputs to the hippocampus (HC), serving as a gateway for cortical-hippocampal communications (Hoesen et al., 1975). The EC has direct projections to the dentate gyrus (DG) (Hoesen et al,. 1975; Witter et al., 1989) the neurogenic zone of the HC. EC and HC respond strongly to aerobic exercise in animal models by enhancing neurogenesis (van Praag et al., 1999; Pereira et al., 2007) and increasing spine density (Head et al., 2012), respectively, and in clinical trials and cross-sectional studies in humans by showing increased volume (Erickson et al 2011; Honea et al., 2009; Maass et al., 2014). Thus, it is plausible that aerobic exercise may improve memory formation by enhancing communication between the EC and the HC. Based on the strong response of patients with mild cognitive impairment and, to a lesser extent, of healthy seniors to aerobic exercise, we hypothesize that the EC-HC memory system in healthy seniors will respond well to exercise, but the response might vary depending on the degree of atrophy and functional impairment of the EC. For instance, participants with more severe EC atrophy or individuals with poorer EC function, such a poor allocentric spatial navigation, might exhibit a strong response to aerobic exercise.

The goal of this proposal is to establish proof-of-principle and provide evidence that the EC and EC-HC communication are affected positively by aerobic exercise, and that those seniors with a smaller EC volume relative to brain size and those who perform more poorly on behavioral tests of EC function, will benefit most strongly. Interaction between the EC and the HC memory system is vital for allocentric spatial navigation and context-dependent memory formation. Critically, loss of function of the EC is a central factor in the pathophysiology of AD. Apparently healthy older adults show impaired allocentric navigation compared to young adults (Moffat et al., 2002; Moffat et al., 2009). Spatial navigation deficits may distinguish those at risk of developing AD from normal cognitive aging (Lithfous et al., 2013). We hypothesize that the EC and EC-hippocampal functional connectivity are important functional/anatomical targets of aerobic exercise. In this study, we will examine whether the EC and other MTL areas and EC-hippocampal functional connectivity are responsive to aerobic exercise. We will also investigate whether particular subsets of participants, such as those with poor performance on tests of spatial cognition or those with greater EC atrophy, might be more responsive to aerobic exercise.

Behavioral and fMRI Pilot Studies

The purpose of the pilot studies is to develop the cognitive tasks for fMRI and adapt them for our aging sample. For fMRI we do not generally use existing, normed, tasks such as those neuropsychologists would use. Instead, we develop cognitive tasks such that they are optimized to answer our specific research question outlined above for Aims #1 and #2. The pilot studies will be optimized for the specific population studied (healthy older adults) while at the same time taking into account technical constraints of fMRI (e.g. low temporal resolution, responses most often restricted to button presses).

Specifically, with the behavioral pilots we want to determine the timing of the events that happen during the task (e.g. for how long should we show an item to the participants that they later remember the items and/or have enough time to respond?). This is critical for studies in older adults, because reaction times are known to slow with aging. Once the cognitive tasks have been optimized through behavioral piloting, we also have to make sure they work in the fMRI scanner (e.g. does a task designed to depend on a certain brain area actually result in activation of this brain area with fMRI? Does the task timing work given the technical constraints of fMRI? Due to physiological changes associated with aging a task that works well in terms of activating brain areas in young adults may not work well in older adults. Behavioral and fMRI piloting therefore makes sure the cognitive tasks work for our group of participants (healthy older adults), before we recruit participants for the experiments described for Aims #1 and #2.

There are two reasons why we propose pilot studies along with the main study:

1) The need to fine-tune the project-specific tasks before launching the actual study (described in Aims #1 and #2).

2) The pilot studies are specific to the funded project. They are not intended to generate pilot data for a future grant application.

Project-specific task piloting is a standard procedure in cognitive neuroscience / neuroimaging studies. Since the task will be designed to be used as described in Aims #1 and #2, there will be no amendment necessary when the task pilots are completed.

Study Summary

We propose to examine the effects of exercise and cardiovascular fitness on cognitive processes utilizing a parallel design randomized clinical trial. Specifically, we will investigate if exercise improves brain function in a brain area known as the entorhinal cortex. Together with the hippocampus this brain area is important for memory formation and spatial navigation. Participation in this research study will take approximately 4 months. During this time, participants will make three initial study visits. The first visit is for informed consent and screening, the second visit is for baseline fitness testing, and the third visit is for cognitive testing and a functional MRI exam. Functional MRI is a brain imaging technique that uses a magnetic field to "take pictures" of the brain while a person performs a cognitive task. It will take up to approximately three weeks to complete these initial three study visits. Following the three initial study visits, the exercise-training program will begin. Participants will be randomized to one of two training

programs: a cardiovascular endurance-training program (aerobic exercise) and a strength, balance, and flexibility training program (non-aerobic exercise). The exercise training program will last 12 weeks. There will be three one-hour exercise sessions per week. After completion of the exercise-training program, participants will attend two follow-up study visits. The first follow-up visit is for fitness testing. The second follow-up visit is for cognitive testing and an MRI exam.

Design and Procedures

Participants will be randomized into one of two groups: 1) an aerobic exercise intervention group and 2) a non-aerobic exercise control group (strength, balance, flexibility training). They will have a 50% chance to be randomized into one of the two groups.

The PI will be responsible for the randomization, because the PI's role in participant recruitment and screening is minimal. The study staff members performing the recruiting, informed consent, and screening will not know which group each participant will be randomized to at the time of recruitment, informed consent, or screening.

1) Study Visit 1: Informed Consent and Screening

Duration of Study Visit 1: Approximately 2 hours. These numbers include informed consent, general screening, breaks and neuropsychological testing. The duration of the neuropsychological testing is approx. 60-80 minutes (including breaks).

Location: Cognitive Neuroimaging Center or Cognitive Neuroimaging Laboratory at Boston University Charles River Campus

Procedures:

1. Informed consent (approx. duration: 40 min)

After initial pre-screening over the phone (see attached phone and email pre-screening protocol) and/or email, we will invite potential participants for a study visit with a study staff member trained in human subject protection and consent procedures at the Cognitive Neuroimaging Center or Cognitive Neuroimaging Laboratory at Boston University Charles River Campus. During this visit, the study staff member will first allow the participant to read and review the consent form independently. If requested by the potential participant, the study staff member will go over any specific paragraph of the consent form with the potential participant. The study staff member will ensure that the potential participant understands the purpose of the study, how many visits will be required, and the approximate study duration and duration of each visit. In addition, the study staff member will ensure that the potential participant understands the purpose of each visit and what payments participants will receive for what part of the study and when. For each section of the consent form, the study staff member will ask the participant whether he or she has any questions. Once the participant and study staff member have signed and dated the consent form, the participants of Specific Aims #1 and #2 will undergo all screening procedures. Participants will be consented for all procedures (cardio-respiratory fitness and strength assessments, cognitive testing, functional and structural MRI) at the initial study visit, but will have the opportunity to receive a copy of the consent form before coming in for Study Visit 1 (by mail or email).

In general during the consenting process, the potential participant will be reassured that his or her participation is voluntary and that he or she can withdraw consent at any time, even after signing the consent form, as long as it is safe for the participant to do so (e.g., the

participant may need to stay on the treadmill for a cool down). BREAK (if needed; approx. 5 min or less)

2. Screening, part I: Interviews, questionnaires, and instruments (approx. duration: 45-60 min)

In line with new guidelines from the American College of Sports Medicine (ACSM), we will exclude participants if they have any known cardiovascular, pulmonary, or renal disease. Those who do not have any signs or symptoms, nor diagnosis, will not require medical clearance. This is also in line with new regulations from ACSM based on studies finding low predictability of adverse event by CVD risk factors, coupled with high prevalence in older adults. Only those older adults interested in participating in the research study that have any signs or symptoms of cardiovascular, pulmonary, or renal disease or mild musculoskeletal issues (such as osteopenia, osteoporosis, or arthritis) will be required to submit a medical clearance form signed by his or her physician before participating in exercise testing or training (FitRec Personal Training Medical Waiver form). In addition, we will exclude participants if they have any contraindicator of MR imaging (e.g. non-removable ferromagnetic metal in or on the body), bioelectrical impedance analysis (BIA; e.g. pacemaker), or if they have any neurological or psychiatric conditions or disorders that are known to affect the brain and cognitive functions. In addition, participants will be excluded if they are physically active, or are not considered "sedentary": The American College of Sports Medicine defines a sedentary lifestyle as a lifestyle in which a person is not participating in at least 30 minutes of moderate intensity physical activity on at least three days per week for at least three months (definition according to American College of Sports Medicine, Thompson et al., 2010). Finally, individuals will be excluded if they self-report susceptibility to severe motion sickness or claustrophobia, given that (1) the cognitive task performed in the MRI scanner may cause discomfort for individuals who are susceptible to motion sickness, and (2) being inside the MRI scanner may cause discomfort for individuals who are susceptible to claustrophobia.

Screening includes a general health screening, neuropsychological testing, screening for exercise readiness, and screening for MR compatibility. Screening will also include measurements of weight, height, waist circumference, and hip circumference. In collaboration with Dr. Storer, Ph.D., the P.I. will oversee the screening to exclude adults with medical conditions that might affect the biochemical variables of interest and will ensure that the cardiovascular fitness and strength testing is appropriate for each participant, based upon their exercise assessments. If participants respond with “Yes” to any questions of the General Screening form in sections B, C or D (Health Screening for Exercise Readiness) or to any of the questions of the HHQ, the P.I. will consult Dr. Storer, Ph.D. Participants will be screened using the attached screening forms, standard neuropsychological and neuropsychiatric tests, and questionnaires (all neuropsychological tests are described in Strauss, E., Sherman, E. M. S., and Spreen, O. (2006) A Compendium of Neuropsychological Tests. Administration, Norms, and Commentary. Third edition. New York, NY: Oxford University Press). Participants will complete the CHAMPS questionnaire, which will provide the investigators with a picture of how physically active the participant has been over the past 4 weeks. This information will be used for future analyses, not for inclusion/exclusion. Participants will also complete a Computer Experience Questionnaire; this will not be used for inclusion/exclusion, but instead, will be used as a covariate in data analysis. Next, we will assess

handedness with the Edinburgh Handedness Inventory. While we will not use this test for screening; knowledge of handedness will aid in interpreting the fMRI data.

Participants will be required to fill out the questionnaires and instruments either on paper or electronically (e.g. using REDCap software). The general screening form will be filled out by a study staff member and administered in interview format.

List of Questionnaires and Instruments (administration time: approx. 45 min):

1) General health screening: General_ScreeningForm_R21

2) General health screening for pilot studies: General_ScreeningForm_R21_short

3) Exercise readiness: Health History Questionnaire (appended HHQ_R21)

4) Exercise questionnaires: CHAMPS

5) MR compatibility: CNC_ScreenForm

6) Computer Experience Questionnaire: Computer Experience Questionnaire

7) FitRec Medical Clearance: PersonalTrainingMedicalWaiver (if applicable)

8) Handedness: EdinburghHandednessInventory

9) Anthropometric Measures and Vital Signs

Note that none of the questionnaires and instruments listed above are used routinely for standard clinical care. We noted sources on the forms when available.

BREAK (10-15 min): Once the participant has completed this part of the screening, we will offer him or her a brief break before continuing with the neuropsychological and neuropsychiatric assessment.

3. Screening, part II: Neuropsychological assessment (Exp. 2; total duration: 40-50 minutes) Cognitive domains assessed and screening for older adults includes general dementia screening, assessment of attention, processing speed, and executive functions, and assessment of motor function. Note that we selected the following tests and questionnaires from published standard tests/questionnaires (Strauss, Sherman, and Spreen, 2006). Neuropsychological assessment with older adults is necessary to screen for potential dementia and age-related cognitive deficits.

Psychiatric functioning:

1) Depression: Geriatric Depression Scale (Brink et al., 1982; Yesavage et al., 1983; administration time: 5-10 min)

2) Anxiety: Beck Anxiety Inventory (Beck and Steer, 1990; administration time: 5 min)

3) Stress: Perceived Stress Scale (Cohen et al., 1983; administration time: 5 min)

Estimated IQ / cognitive functioning:

1) General dementia screening: Dementia Rating Scale-2 (DRS-2; available at parinc.com; administration time: approx. 15-30 min). Note this standard test has not been attached here; it will be

purchased. The DRS-2 has five subscales, which provide additional information on specific abilities, include Attention, Initiation/ Perseveration, Construction, Conceptualization, and Memory.

2) Attention, processing speed, and executive functions: Trail-Making Test Versions A and B (Strauss, Sherman, Spreen, 2006; administration time: 5-10 minutes); Stroop Test, Victoria version (Strauss, Sherman, Spreen, 2006; administration time: approx. 5 min)

3) Motor function: Grip Strength test using the Smedley Dynamometer (available online from various healthcare retailers), see GripStrengthTest_Instructions (Strauss, Sherman, Spreen, 2006; administration time: less than 5 min)

Note that the neuropsychiatric and neuropsychological instruments listed above are routinely used for clinical diagnosis of neuropsychological impairments. All assessments are as stated in the Human Subject Protection pages of the attached parent NIH R21 grant.

4. Physical activity and exercise diary (instruction time: 5 min or less)

At the end of Study Visit #1, we will give the participant an exercise diary to take home with him or her. We will ask the participant to use this diary to report the type of physical activity performed each day, if any, and to provide information about duration and intensity of the physical activity. We will ask him or her to fill out the physical activity log daily for the duration of the study. For sedentary individuals, filling out this log should only take a few minutes each day. Participants will be asked to include the exercise sessions of the intervention on the physical activity diary.

2) Study Visit 2: Baseline fitness testing and body composition exam

Duration: Approx. 2-2.5 hours

Location: BU Fitness and Recreation Center

Procedures:

1. Explanation of procedures and measurement of vital signs at rest (approx. duration: 10 min) These measurements will include blood pressure, pulse and/or heart rate. We will ask participants to change into clothing appropriate for exercise.

2. Performance of the Mnemonic Similarity Task (approx. duration: 15 minutes)

While we are waiting for the participants heart rate and blood pressure to reach resting levels, the participants will use the computer to perform the Mnemonic Similarity Task (MST). The task consists of assessing recognition memory performance for objects using not only the traditional targets and unrelated foils, but also using similar lures (that intentionally vary along several different dimensions).

2. Body composition analysis using the InBody Test (approx. duration: less than 15 min) The InBody Test uses safe, low-level currents sent through the body via hand and foot electrodes. (The participant contacts these electrodes by standing on the device, which resembles a scale, and holding the cylindrical hand-held electrodes.) The impedance that the currents encounter are measured, and from there, body composition is derived. The amount of this current is slight enough that the participant cannot feel it. The InBody Test will provide an estimate of total body water, dry lean mass, body fat mass, weight, skeletal muscle mass, body mass index, and percent body fat. These estimates are printed out on the

InBody Results Sheet, which has been included with this protocol (InBody_E_Book.pdf). Each participant will be offered a copy of his or her results to compare body composition from before and after the exercise intervention. Outcome measures for the purposes of this study include weight, body mass index, and percent body fat.

3. Cardio-respiratory fitness tests (incremental work rate test) (approx. duration: 30-40 min) We will use standard submaximal clinical exercise testing procedures, including that of a submaximal, incremental work rate test, designed to ensure participant safety and measurement accuracy (Cooper and Storer, 2001; Thompson et al., 2010). We will follow the guidelines established by the American College of Sports Medicine for submaximal clinical exercise testing (ACSM; Thompson et al., 2010).

We will use a modified Balke treadmill protocol that uses a fixed walking speed and increasing increments in grade. We will choose the speeds and grades used for each participant (Cooper and Storer, 2001) so that all participants walk at a relatively comfortable pace with the grade increments calculated to provide a total test duration of 8-12 minutes (Buchfuhrer, 1983). The test will be continued until the participant reaches 85% of his or her age-predicted maximum heart rate (HRmax = 206.9 - (0.67 × age)) or 70% of heart rate reserve (HRreserve = HRmax –HRrest). While this test does not allow direct measurement of maximum oxygen uptake (VO2max),

the results of this test allow us to predict VO2max based on the known linear relationship between heart rate and VO2max. VO2max is a secondary outcome measure. Notably, participants will warm up for 3 to 4 minutes and cool down for 7 to 10 minutes before and after the test, respectively.

For this test, participants will wear a chest strap to measure heart rate and a blood pressure cuff to measure blood pressure (BP). During the test, heart rate will be continuously monitored and recorded and BP will be measured at multiple points before, during, and after the test. We will take the participant's BP at standing rest on the treadmill, at the end of the 3-minute warm-up, and after completing the first 3 minutes of the test. If the participant demonstrates any adverse response to the exercise (e.g., demonstrating a BP over 220/110), we will continue to measure BP every 3 minutes during the test. If not, we will take BP immediately after the participant reaches 85% of his or her age-predicted maximal heart rate while treadmill speed and grade are quickly returned to low warm-up levels.

At regular intervals while performing the test, we will also ask the participant to rate how hard he or she believes he or she is exercising (using the standard 15-grade scale for ratings of perceived exertion; Borg, 1982, Table 1; see attached RPE scale). If the participant fails to conform to the exercise testing protocol, experiences adverse signs or symptoms, requests to stop, or experiences an emergency situation, we will terminate the test. Symptoms such as unusual shortness of breath or leg, back, or chest discomfort will be investigated, and the test will be stopped for participant safety. We will instruct the participant to inform the technician about any discomfort he or she experiences during the test, such as dizziness, nausea, extreme shortness of breath, and/or extreme leg fatigue. Participants may ask to stop the test at any time. Trained medical staff will be available or on call for each test.

Finally, following completion of testing, there will be a recovery time including a cool-down walk on the treadmill and sitting in a chair. Throughout the duration of the recovery time, heart rate and BP will be monitored until the participant's heart rate and BP have approximately returned to pre-exercise

baseline and/or until BP is below 140/90. (Notably, as per the expertise of our study exercise physiologist, exercise induced hypertension up to 220/110 during exercise is normal.

Thus, monitoring BP to a point where it is below 140/90 ensures that the participant's cardiovascular system is returning to normal levels.)

4. Strength Assessment (duration: approx. 30 min)

Following cool-down and a brief break, participants' strength of major muscle groups in the upper body will be assessed using a chest press and a latissimus dorsi pulldown. Secondly, lower body strength will be evaluated using a leg press.

The total fitness evaluation session (Study Visit 2) will take approximately 2-2.5 hours (including review of exercise-related screening results, instructions, body composition analysis, setup, exercise tests, recovery periods, etc.).

3) Study Visit 3: Baseline MRI exam

Duration: Approx. 4 hours

Location: Cognitive Neuroimaging Center at Boston University Charles River Campus

Procedures:

1. Task Instruction and Practice (approx. duration: 30 min)

Before going into the MRI scanner, participants will be instructed in how to perform the cognitive tasks.

2. MRI procedures and cognitive testing (approx. duration: 100 min) Overview

We will give each participant an MRI (Magnetic Resonance Imaging) exam of his or her brain while he or she performs cognitive tasks in the MR scanner using structural and functional MRI (fMRI). During fMRI scanning, we may monitor the participant's heart rate, breathing rate, and

/or pulse. For all MRI procedures, we will use a Siemens 3.0T magnet at the Cognitive Neuroimaging Center at Boston University Charles River Campus.

Description of functional MRI procedures

Before the MRI, we will ask the participant about any metal in or on his or her body. The participant will be asked to remove all metal objects, regardless of whether they are ferromagnetic (e.g. hearing aids, dentures, jewelry, watches, hairpins, and makeup with metallic specs or shimmer) from his or her body. The participant will be asked to change into scrubs for the MRI scan. During the screening visit, we will ask the participant to prepare by wearing metal- free clothing/undergraments on the day of the MRI exam.

Before entering the MRI scanning room, we may take vital sign measurement, and the participant will receive task instructions. At this time, the participant will practice each task. Then, immediately before entering the MRI, we will ask the participants to wear earplugs to reduce the scanner noise.

During the MRI, the participant will be required to lie still on the MRI table. The participant will be able to hear and speak to the research staff at all times during the MRI procedures, but we will ask him or her

not to speak during any scans unless there is an emergency (for example, if he or she feels claustrophobic or sick) or he or she is prompted to speak. The researcher and/or the MR technologist will remain in contact with the participant throughout the scans, and the participant may terminate his or her participation in the study at any time (even during a scan). We can stop the procedure at any time, if necessary.

When the participant is in the MRI scanner, we will begin by taking several structural brain images. Soon after, the cognitive tasks will begin. We will instruct the participant to look at virtual environments, pictures, words and/or objects while he or she is inside the MRI scanner. He or she may be asked to listen to words and/or sounds through a headset while in the scanner. He or she may be moved passively through a virtual environment or be asked to move actively through a virtual environment using a joystick or button-box. While in the scanner, the participant may be asked to make decisions about the presented stimuli, scenes, or environments, and to indicate responses on handheld response keys or with a joystick. Between each set of stimuli, there will be a short break. We will remind the participant to keep his or her head as still as possible during task performance and the breaks in between.

We may take measurements of blood pressure, heart rate, and/or pulse immediately before entering and immediately after exiting the MRI scanner room. During scanning, we may use pulse oximetry.

Additionally, after the scan, we may ask the participant to fill out a brief questionnaire to assess motion sickness symptoms. (Motion Sickness Questionnaire: Nausea_profile). This questionnaire will be used as a covariate in our statistical analyses, not as inclusion/exclusion, given that we address susceptibility to motion sickness in our general screening procedures.

3. Post-MRI cognitive testing (approx. duration: 60-90 min)

After the scanning session, we will ask the participant to complete additional cognitive tasks at the Cognitive Neuroimaging Center. These tasks include:

1. Spatial reasoning Assessment: Triangle Completion Task104 (approx. duration: 20 min) and Virtual Y-Maze task106 (approx. duration: 5 min) (note these tasks were developed by co- investigator Scott Moffat for use with geriatric adults).

2. Spatial Cognition: Santa Barbara Sense of Direction Scale126 (approx. duration: 8

min), Rey-Osterrieth Complex Figure Test, copy condition121 (alternate version used for post- testing, counter-balanced; approx. duration: 5 min)

3. Verbal Memory: Rey Auditory Verbal Learning Test (RAVLT)121 (alternate version used for post-testing, counter-balanced; approx. duration: 10-15 min)

4. Visual-Spatial Memory: Rey-Osterrieth Complex Figure Test, delay condition121 (alternate version used for post-testing; counter-balanced; approx. duration: 5 min)

All assessments are as stated in the Human Subject Protection pages of the attached parent NIH R21 grant. Note that numbers refer to references in the Bibliography & References Cited section of the NIH R21 grant.

4) Study Visits 4-39: Exercise Training

Duration: Three 1-hour session per week for a period of 12 weeks Location: Boston University Fitness and Recreation Center

Procedures:

The study will use a randomized controlled trial. We will assign each participant by chance to one of two study groups. One group will participate in a cardiovascular endurance training program and the other group will participate in a strength, balance, and flexibility training program. We will assign participants randomly to the two training programs (50% chance). Participants will not be informed about group assignment until the first training session at the exercise facility.

Trained and experienced exercise trainers will supervise the exercise training sessions at the BU Fitness and Recreation Center. (Note: all trainers will undergo CITI training for Human Subject Protection training and HIPAA as in our previously completed clinical trial, H-32635. Trainers will then be added to the IRB protocol prior to any exercise training). The research staff, after consultation of co-investigator Dr. Thomas Storer, will monitor training progress and adherence to the exercise training program. Each exercise training group will be composed of approximately 3 to 6 participants. Morning, daytime, and/or evening hours will be available for the participant to choose from, but will depend on the number of other participants enrolled and the collective group members' availabilties.

The 12-week intervention will consist of 3 weekly, trainer-supervised exercise sessions that will gradually increase in intensity and duration. Heart rate and ratings of perceived exertion90 will be monitored and recorded for all participants. Dr. Storer will develop individualized exercise prescriptions. Note that all exercise interventions will be identical to those of our previously completed, IRB-approved clinical trial, H-32635.

1) Cardiovascular endurance training group

The aerobic exercise program will consist of three weekly 1-hour aerobic exercise sessions over a 12-week period. The participant will be required to wear a standard heart rate monitor and/or chest transmitter during each exercise session. We will provide the heart rate monitor and/or chest transmitter to the participant for use during the exercise session. Each session will include light cardiovascular warm-up exercise, such as slow walking, stretching exercises, heart-rate based aerobic exercise training on the treadmill (walking with grade adjusted depending on the participant's ability level). Each exercise session will finish with a cool-down, and stretching and

/or relaxation exercises. We will tailor exercise intensity for each participant's fitness level based on the results of the cardio-respiratory fitness evaluation during the fitness testing session (Study Visit 2). Dr. Thomas Storer will design the individualized exercise training programs.

Exercise intensity will increase gradually over the 12-week period of the aerobic exercise training program. The trainer will monitor and record intensity level, each participant's perceived level of physical exertion (using the RPE scale), heart rate, and type and duration of the exercise in an exercise diary/log.

2) Strength, balance, and flexibility training group

The strength, balance, and flexibility exercise program will consist of three weekly 1-hour exercise sessions over a 12-week period. The participants will perform strength training exercises

that target all major muscle groups. In addition, the participants may perform exercises that are designed to improve balance and flexibility. The participants will be required to wear a standard heart rate monitor and/or chest transmitter during all exercise sessions. We will provide a heart rate monitor and/or chest transmitter to the participants for use during the exercise session. This will allow us to monitor heart-rate. Strength training exercises will use progressively greater resistance over the 12-week program. We will tailor the training program to each participant's fitness level as determined during the fitness testing session (Study Visit 2). As in

the cardiovascular endurance training, Dr. Thomas Storer will design the individualized exercise training programs. The trainer will instruct each participant in proper technique in order to prevent injury. With proper instruction, injury rates in strength training are very low in the general population.

Participants will be encouraged to attend as many exercise sessions as possible by providing a 75-dollar finishing bonus and by visiting participants regularly during the exercise training program. Participants must have attended at least 30 of the 36 exercise training sessions and

they must not have missed more than 1 exercise session per week to receive the finishing bonus. In the case that a participant anticipates being unable to attend one or more of the exercise training sessions, the participant will have the opportunity to work out on his or her own if he or she informs the trainer ahead of time. The trainer will then provide the participant with "homework" and a training log. The homework will describe the exercise the participant should do while away. The participant will be reminded that he or she will need access to exercise training equipment. The trainer will instruct the participant in how to fill out the training log. We may ask the participant to wear a heart rate monitor or activity monitor or similar device during the exercise. We will provide this device to them. A study staff member may call them about their training progress while they are away. Participants will be expected to return the training log to the trainer after their return. Participants will not have the opportunity to receive homework during the first four weeks of the exercise training program. This is for the safety of the participants, given that the trainers will monitor their progress and instruct them in proper technique during the first four weeks. If a participant anticipates being unable to participate during this time, we may exclude him or her from participating in this study or place him or her on a waiting list. A participant will not necessarily be excluded if he or she is not available for the duration of the study, unless the participant anticipates that he or she will miss any of the study visits (study visits 1, 2, 3, 40, 41) and/or if he or she anticipates missing three exercise sessions in a row without fulfilling the homework and training log requirements. Make-up sessions during the same week will also be available.

Exercise diary/log. We will ask the participants to continue using the exercise diary/log until the study is completed. We will go over the exercise diary with the participant on approximately a weekly basis.

5) Study Visit 40: Follow-up fitness testing and body composition exam

Duration: Approx. 2-2.5 hours

Location: BU Fitness and Recreation Center

Procedures: Procedures are identical to Study Visit 2.

6) Study Visit 41: Follow-up MRI exam

Duration: Approx. 4 hours

Location: Cognitive Neuroimaging Center at Boston University Charles River Campus

Procedures: Procedures are identical to Study Visit 3. However, we will also include the following two neuropsychological exams, which were included at the Informed Consent and Screening Visit (Visit 1) rather than the Baseline MRI Visit (Visit 3).

Visuospatial Attention: Trail-Making Test A and B121 (baseline testing to take place during screening visit; approx. duration: 5 min)

Executive Functions: Stroop test, Victoria version121 (baseline testing during screening visit)

Procedures for Re-Screening:

Any participant who was determined eligible over three months before the exercise training program start date will need to be rescreened. The proposed timeline for eligibility and re- screening is as follows:

Eligibility timeline:

Eligibility determined at or less than 3 months before projected training start date: valid. Eligibility determined over 3 months but at or less than 12 months before projected training start date: requires phone re-screening,that will be valid for the following 3 months, detailed below.

Eligibility determined more than 12 months before projected training start date: requires a full visit Study Visit 1b (Procedures are identical to Study Visit 1) to re-assess eligibility, the will be valid for the following year.

The re-screening protocol will include a phone re-screening that consists of direct questions regarding all of the inclusion/exclusion criteria that are subject to change within 12 months or less (see attached Phone Re-Screening protocol).

After re-screening, the principal investigator and study staff member will discuss the participant's responses, and the investigator will determine whether or not the participant is still eligible for participation.

Inclusion/exclusion criteria that will NOT be directly re-screened include the following:

1. Fluent in English (must have attended elementary school and higher in English) (Inclusion Criterion #4): this cannot change from the time of initial screening to re- screening.

2. Dementia Rating Scale performance (Exclusion Criterion #4): we do not expect the participant's score to change by more than one point within the proposed 3-12-month timeline for re-screening. However, participants who originally scored a 9 on the AMSS or AEMSS at their Visit 1 will be required to come in for a full re-screening (Visit 1b) to confirm that cognitive decline has not occurred since Visit 1.

3. Impaired participant performance on the Trail Making Test or Stroop Test (Exclusio n Criterion #5): this will not be re-assessed. Other re-screening questions will be used to inform the study staff of any changes to brain structure/function (i.e., any recent diagnoses regarding neurological/psychological/psychiatric conditions). If the investigator is concerned that the participant may not perform at normative levels on neuropsychological testing, the participant will be invited in for a full re-screening (Visit 1b).

4. Grip strength test performance (Exclusion Criterion #6): this will not be re-assessed. Other re-screening questions will be used to inform the study staff of any changes to brain structure/function (i.e., any recent diagnoses regarding neurological/psychological

/psychiatric conditions) or musculoskeletal health (i.e., any recent diagnoses of musculoskeletal impairments).

Additionally, the obesity criterion (Exclusion Criterion #8) will be assessed based on the anthropometric measure obtained from the participant in his or her initial Visit 1:

a. Participants with BMI < 30: for a participant, whose BMI is below 30, we will ask during the re-screening if he or she has gained weight since the initial study visit. If the answer is yes, the previously measured height of the participant and the self-reported current weight of the participant will be used to calculate BMI. If the participant still falls below a BMI of 35 with his or her new weight, then he or she will still be considered eligible for the study. If the participant falls at or above a BMI of 35, he or she will be invited in for a full re-screening (Visit 1b). This is due to the fact that a BMI of 35 or greater is one of the exclusion criteria to be considered in the obesity criterion (exclusion criterion #8).

b. Participants with BMI ≥ 30: these participants will be invited in for a full re-screening (Visit 1b).

Procedures for Pilot studies:

For pilot studies involving only fMRI and/or cognitive testing (no neuropsychological assessment, fitness testing, or exercise training), participants will already come in for their initial study visit tentatively scheduled for behavioral testing and/or fMRI on the same day and/or on a day in the near future. However, the study staff will confirm the visit based on 1) whether they are still interested after explanation of the procedures and 2) whether they are eligible to participate based on screening.

Cognitive pilot:

The cognitive pilot requires two visits, which may be combined into a single visit. During the first visit, the investigator will review the consent form with the understanding that their choice to participate is completely voluntary. Following informed consent, the participant will undergo further screening questionnaires to determine eligibility. Visit 1 will last approximately 1 to 1.5 hours. Once fully deemed eligible, the second visit is for cognitive testing. The participant will perform the cognitive tasks on a computer screen. Breaks will be given in between runs as necessary and requested. Visit 2 will last approximately 1 to 2.5 hours.

fMRI pilot:

The fMRI pilot requires two visits, which may be combined into a single visit. During the first visit, the investigator will review the consent form with the participant to convey the understanding that his or her choice to participate is completely voluntary. Following informed consent, the participant will undergo further screening questionnaires to determine eligibility. This will include the CNC Screen Form (item 6, above), which will determine MRI safety for that subject. Visit 1 will last approximately 1 to 1.5 hours. Once fully deemed eligible, the second visit is for fMRI scanning. The participant will perform the cognitive tasks in the scanner. Breaks will be given in between runs as necessary and requested. Visit 2 will last approximately 1 to 2.5 hours. The same MRI procedures as described in study visit 3.

Screening for participants of pilot studies

Participants recruited for pilot studies (behavioral pilot/pilot of cognitive tasks only; fMRI pilot

/pilot of cognitive tasks in fMRI scanner; no neuropsychological testing, no cardio-respiratory fitness evaluation or exercise training) will be asked to fill out the short version of the General Screening Form (item 2, above) and the Edinburgh Handedness Inventory (item 8, above).

Participants recruited for fMRI piloting will additionally be asked to fill out the CNC Screen Form (item 6, above).

Description of functional MRI procedures for fMRI pilot

Before the MRI, we will ask the participant about any metal in or on his or her body. The participant will be asked to remove all metal objects, regardless of whether they are ferromagnetic (e.g. hearing aids, dentures, jewelry, watches, hairpins, and makeup with metallic specs) from his or her body. If the participant's clothing contains non-removable metals, such as metal zippers or buttons, especially near the head (e.g. on t-shirts or sweatshirts), the participant may be asked to put on a hospital gown instead (the MR technologist in charge of the scans will be consulted). During the screening visit we will ask the participant to prepare by wearing metal-free clothing on the day of the MRI exam.

Before entering the MRI scanning room, we may take vital sign measurement and the participant will receive task instructions. At this time, the participant will practice each task. Then, immediately before entering the MRI, we will ask the participants to wear earplugs to reduce the scanner noise.

During the MRI, the participant will be required to lie still on the MRI table. The participant will be able to hear and speak to the research staff at all times during the MRI procedures, but we will ask him or her not to speak during any scans unless there is an emergency (for example, if he or she feels claustrophobic or sick) or he or she is prompted to speak. The researcher and/or the MR technologist will remain in contact with the participant throughout the study, and the participant may terminate his or her participation in the study at any time (even during a scan). We can stop the procedure at any time, if necessary.

When the participant is in the MRI scanner we will begin by taking several structural brain images. Soon after this, the cognitive tasks will begin. We will instruct the participant to look at virtual environments, pictures, words and/or objects while he or she is inside the MRI scanner. S

/he may be asked to listen to words and/or sounds through a headset while s/he is in the scanner. S/he may be moved passively through a virtual environment or s/he may be asked to move actively through a virtual environment using a joystick or button-box. While in the scanner

s/he may be asked to make decisions about the presented stimuli, scenes, or environments, and to indicate responses on handheld response keys or with a joystick. Between each set of stimuli there will be a short break. We will remind the participant to keep his or her head as still as possible during task performance and the breaks in between.

We may take measurements of blood pressure, heart rate and/or pulse immediately before entering and immediately after exiting the fMRI scanner room. During fMRI scanning we may use pulse oximetry.

Outcomes

Describe anticipated primary outcome and any secondary outcomes and how they will be measured:

Primary Outcomes for Specific Aim #1:

1) Better cognitive task performance post-exercise training compared to baseline (measurements: reaction times, accuracy, etc.); effect expected to be greater for aerobic exercise training than control (strength, balance and flexibility training)

Measurements: reaction times, accuracy, and other cognitive task derived scores

2) Increased activation in and functional connectivity between brain areas associated with task performance, effect expected to be greater following aerobic exercise training than control.
Measurements: blood-oxygen level dependent fMRI scans

Primary Outcomes for Specific Aim #2:

1) Better cognitive task performance post-exercise training compared to baseline (measurements: reaction times, accuracy, path length, spatial reasoning test scores, etc.); effect expected to be greater for aerobic exercise training than control (strength, balance, and flexibility training)

Measurements: reaction times, accuracy, path length, and other cognitive task derived scores

2) Increased activation in and functional connectivity between brain areas associated with task performance, effect expected to be greater for aerobic exercise training than control; this effect of aerobic exercise training will be greater for subjects with 1) smaller compared to larger relative entorhinal cortex volume, and 2) below-median compared to above median path integration skills

Measurements: blood-oxygen level dependent fMRI scans

Secondary outcomes for all aims:

1) Increased aerobic capacity for aerobic exercise group participants

Measurements: VO2max (estimated from submaximal incremental work rate test)

2) Increased strength for control group participants

Measurements: upper body strength in lbs or kg (based on chest press and latissimus dorsi pulldown), lower body strength in lbs or kg (based on leg press)

3) Increased lean body mass

Measurements: Percent lean body mass from BIA (and/or skinfold measures)

4) Neuropsychological test performance: improved performance scores on tests of spatial cognition, verbal memory, visuo-spatial memory, visuo-spatial attention, and executive functions post-exercise training compared to baseline and compared to controls

Measurements: neuropsychological test scores

Data Analysis

Provide a description of your plan for data analysis. State the types of comparisons you plan (e.g. comparison of means, comparison of proportions, regressions, analysis of variance). Which is the PRIMARY comparison/analysis? How will the analyses proposed relate to the primary purposes of your study? If you are doing qualitative research please state how comparisons will be made.

Specific Aim #1:

Behavioral and Fitness Data Analysis. The primary measures for the primary cognitive task (Sternberg memory load task) are % correct and reaction times, and for fitness are estimated VO 2 max. The primary statistical analyses will be repeated-measures ANOVAs with the factors group (aerobic vs. control) and time (pre vs. post) to examine group x time interactions on the outcome measures. Post-hoc tests (e.g. Tukey tests) will examine if a significant group x time interaction is due to a greater improvement post-intervention in the aerobic exercise group compared to controls. Dependent measures will be tested for normality and skewed data log-transformed. We will use an alpha of 0.05, corrected for multiple comparisons.

Univariate fMRI and Functional Connectivity Analyses. We will use SPM8 and related software for statistical analysis of fMRI data. We will estimate voxel-based Statistical Parametric Maps (SPMs)

and compare them between and within groups with mixed-design ANOVAs. We will use baseline fitness as a covariate. Because the entorhinal cortex and the hippocampus are our primary brain regions of interest, we will use a seed-based functional connectivity approach. We will compare task-related functional connectivity: 1) pre versus post intervention, and 2) aerobic exercise versus control. We will use the psycho-physiological interaction (PPI) approach91 using the Generalized PPI Toolbox92 for SPM8. The time course from a seed region constitutes the physiological variable, and the contrast of interest (e.g. high memory load > low memory load) constitutes the psychological variable in the PPI model. Our search space will be restricted to the medial temporal lobe region. We will determine the PPI variable by calculating the voxel-wise product between the physiological and the psychological variables. We will also correlate SPMs and functional connectivity strength with cognitive test scores. Analyses will include nuisance regressors (e.g. head motion).

Specific Aim #2:

Data Analysis. See Aim #1. The behavioral outcome variables of the primary cognitive task (allocentric navigation task) are distance traveled and movement speed. Addicional outcome variables are neuropsychological test scores and spatial reasoning test scores. Analyses will be as described for Specific Aim #1. In addition, we will compare two groups that differ in relative EC volume or path integration ability (by median split) using descriptive statistics (means, confidence intervals) due to the exploratory nature/ small sample size.

Sample Size

Sample Size Justification

Approximately 94 participants will be recruited total for the study: For the exercise training program

/study intervention, approximately 84 participants will be recruited with the aim to enroll 42 participants, 21 per group; for the cognitive and fMRI pilot studies, an additional 10 participants will be recruited with the aim to enroll 5 participants in the cognitive pilot and 5 participants in the fMRI pilot. Thus, this totals 94 participants (84 + 5 + 5 = 94) required to complete the cognitive pilot, fMRI pilot, and exercise training program/study intervention.

For the exercise training program/study intervention, the sample size for enrollment was determined from simulation-based power predictions for fMRI (Desmond & Glover, 2002), and based on a meta-analysis on exercise training effects on cognition in older adults (Colcombe and Kramer, 2003). 1) fMRI: Given an estimated mean difference of 0.5% signal change, a between-subject variance of 0.5%, and a statistical power of 0.80, a sample size of 11-12 subject per group would be needed for $\alpha = 0.05$ and N = 21 per group for $\alpha = 0.002$. 2) Cognition: we performed sample size calculations with G*Power 3.1.7 based on effect sizes reported in the literature. Effect sizes were f = 0.26 for a short-duration exercise intervention, f = 0.30 for session durations of 45 min, f = 0.20 for cardiovascular training only, f = 0.21 for the visuospatial domain, and f = 0.35 for participants aged 65+ years. Based on an average f of 0.26, a statistical power of 0.80, and alpha = 0.05, a total N = 32 subjects will be needed. Oversampling to 42

Risks and Benefits

Risks associated with the cardiovascular fitness and strength tests

The exercise test will require a gradual increase of exercise intensity. Because it is very strenuous to reach maximum oxygen uptake even after rest it may pose a health risk for some participants. Risks and discomforts include the possibility of adverse changes during the test, including abnormal blood pressure, fainting, dizziness, severe fatigue, body aches, heart rhythm abnormalities, stroke, and very rare instances of heart attack.

Risks and discomforts associated with the strength tests include feeling tired and experiencing muscle cramps. Other, less common, risks and discomforts include muscle tear, spraining a ligament (connective tissue) and lightheadedness.

Risks associated with bioelectrical impedance analysis (BIA)

The bioelectric impedance analysis (BIA) uses a very small electrical current, which passes between electrodes placed on the hands and/or feet. The amount of this current is slight enough that the participants will not be able to feel it and poses no risk to humans. However, there is a slight risk that this low current could disrupt an artificial pacemaker or defibrillator.

Risks associated with exercise

Walking and moderate-intensity physical activities are associated with a very low risk of musculoskeletal complications, but jogging and running are associated with increased risk of injury to the muscles, bones or joints. Acute heart attacks and sudden cardiac death can be triggered by unaccustomed vigorous physical exertion, particularly in sedentary men and women who have signs or symptoms of heart disease.

MRI risks

The MRI uses a strong magnet. The magnet may affect pacemakers, artificial limbs, and other medical devices that contain iron. The magnet will stop a watch that is close to the magnet. Any loose metal object has the risk of causing damage or injury if it is pulled toward the strong magnet. The loud acoustic noise from the MRI machine may cause hearing damage. Participants may also experience claustrophobia (fear of small spaces) or anxiety while in the magnet, because the top and sides of the machine will be very close to the body. There is a chance that the person performing the MRI may notice an incidental finding. It is possible that participants will be unnecessarily worried about the incidental findings.

Risks of completing tasks

Participants may get tired during the cognitive tasks.

Questionnaire risks

Participants may feel emotional or upset when answering some of the questions.

Loss of confidentiality

The main risk of using and storing identifiable information for research is a potential loss of confidentiality.

How risks will be

Risks associated with the cardiovascular fitness and strength tests

Because it is very strenuous to reach maximum oxygen uptake even after rest, and thus, may pose a health risk for some participants, we will estimate, rather than measure, the level of maximum oxygen uptake. Therefore, participants will perform a submaximal, incremental fitness test. This test will follow established standards of the American College of Sports Medicine (ACSM). We will instruct participants to let the technician know about any discomfort, such as dizziness, nausea, extreme shortness of breath, and extreme leg fatigue. Participants may ask to stop the test at any time. The staff members who will perform the fitness testing have current certification in cardiopulmonary resuscitation (CPR). We will ask participants not to engage in strenuous physical activity for 24 hours prior to the test. Participants are advised not to participate in the fitness assessment if they have any heart or lung conditions or symptoms, or if they have any physical impairment that would preclude them from performing the test safely. We will screen them for these conditions and symptoms prior to testing. During the tests we will monitor heart rate, blood pressure and perceived exertion. Only well-trained personnel (technicians, physiologists, nurses or physicians) will conduct the exercise test.

To reduce the risks associated with strength testing, participants will perform wrap-up exercises, and trained individuals will instruct them in proper technique. Participants will be able to take breaks as

Risks associated with bioelectrical impedance analysis (BIA)

We will exclude participants with pacemakers and defibrillators from participating in this study.

Risks associated with exercise

To reduce the risk of musculoskeletal injury and complications associated with exercise, the exercise training program will be tailored to each participant's ability level and supervised by an experienced

trainer. The trainer will instruct each participant in proper technique. To reduce injury risk, each exercise session will include a light cardiovascular warm-up, cool-down and stretching, and the amount and intensity of the exercise will gradually increase over the 12 weeks of the exercise training program.

MRI risks

Participants will be excluded if they have any metal in or on their body, regardless of whether the metal is ferromagnetic, if the metal cannot be removed. This includes implanted devices, such as pacemakers and defibrillators. Participants will be screened for claustrophobia (fear of small spaces). If participants feel anxious during the procedure, they can ask us to stop the MRI at any time. The MR technologist of the Cognitive Neuroimaging Center, who is trained in magnet safety, will prepare the participants for MR imaging and will perform the scans.To prevent potential hearing damage participants will be required to wear earplugs. The MR technologist and the study staff are trained in magnet safety and will communicate with the participant during the MRI session.

It is explained to the participants that the MRI scan being done is designed to answer research questions only and does not serve as a medical scan. However, if we believe that we have found a medical problem (or serious medical problem) in the MRI scan, we will consult the director of the Cognitive Neuroimaging Center, Dr. Chantal Stern, D. Phil., and/or the center's MRI technologist, if needed. If Dr. Stern, D.Phil., and/or the MRI technologist thinks that there may be an abnormality, we will contact the participant and will help them get medical follow-up for the problem. The MRI scan will not be reviewed by a clinically-trained physician as a part of this research study. With the participant's permission, we may contact their primary care physician.

Risks of completing tasks

The testing procedures include breaks and the participants can rest at any time, except during timed tasks. For tasks performed in the fMRI scanner, we will split the tasks into several runs with brief breaks between runs.

Questionnaire risks

Participants will be instructed to let the interviewer know at any time if they want to take a break or stop the interview. Participants are not required to answer any questions that make them feel uncomfortable. However, they may be excluded if it cannot be established whether or not the participant meets the inclusion and/or exclusion criteria.

Loss of confidentiality

We will protect each participant's confidentiality by labeling identifiable information with a code and keeping the key to the code separately from the research data either in a password-protected computer or as a paper file in a locked file cabinet. The master code is limited to BUMC investigators and we will not release it to anyone outside the institution. Dr. Storer will have access to the names and birthdates of participants in addition to the research data. Information will be stored on secure servers using REDcap or similar software. If it is necessary to use a laptop's hard drive for storage of identifiable information this laptop's hard drive will be encrypted with Truecrypt or similar disk encryption software prior to storing this information. All research staff will receive training in human subject protection and the HIPAA rule.

Potential Benefits

Benefits to subjects

Participants may or may not benefit from taking part in this study. Possible benefits to subjects include improved cardiovascular health, physical fitness and/or strength, and weight loss from participating regularly in the exercise training program.

Benefits to society

Others may benefit in the future from the information that is learned in this study. These potential benefits may include improved brain function through exercise and knowledge about how exercise affects the brain and cognitive (thinking) processes.

Risk to Benefit Ratio

Assessment of Risk/Benefit Ratio

Even though the exercise interventions, exercise testing and MRI procedures performed are solely done to answer research questions, the risks are very reasonable in relation to anticipated benefits. This is so, because the risks associated with the research study are only minimal, while the anticipated benefits to subjects are expected to be more than minimal due to the well-known health-related benefits associated with regular exercise.

Selected References

Altman, J., & Das, G. D. (1965). Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *Journal of Comparative Neurology*, *124*(3), 319–335. https://doi.org/10.1002/CNE.901240303

Amrein, I., Nosswitz, M., Slomianka, L., van Dijk, R. M., Engler, S., Klaus, F., Raineteau, O., & Azim, K. (2015). Septo-temporal distribution and lineage progression of hippocampal neurogenesis in a primate (Callithrix jacchus) in comparison to mice. *Frontiers in Neuroanatomy*, *0*(June), 85. https://doi.org/10.3389/FNANA.2015.00085

Antonova, E., Parslow, D., Brammer, M., Dawson, G. R., Jackson, S. H. D., & Morris, R. G. (2009). Age-related neural activity during allocentric spatial memory. *Memory*, *17*(2), 125–143. https://doi.org/10.1080/09658210802077348

Baechle, T. R., Earle, R. W., & National Strength & Conditioning Association (U.S.). (2000). *Essentials of strength training and conditioning*. Human Kinetics,.

Barnes, D. E., Yaffe, K., Satariano, W. A., & Tager, I. B. (2003). A Longitudinal Study of Cardiorespiratory Fitness and Cognitive Function in Healthy Older Adults. *Journal of the American Geriatrics Society*, *51*(4), 459–465. https://doi.org/10.1046/J.1532- 5415.2003.51153.X

Beck, A. T., & Steer, R. A. (1990). Beck Anxiety Inventory Manual. *Evolutionary Ecology*.

Borg, G. (2008). Ratings of Perceived Exertion and Heart Rates During Short-Term Cycle Exercise and Their Use in a New Cycling Strength Test*. *International Journal of Sports Medicine*, *03*(03), 153–158. https://doi.org/10.1055/S-2008-1026080

Brotons-Mas, J. R., Schaffelhofer, S., Guger, C., O'Mara, S. M., & Sanchez-Vives, M. V. (2017). Heterogeneous spatial representation by different subpopulations of neurons in the subiculum. *Neuroscience*, *343*, 174–189. https://doi.org/10.1016/J.NEUROSCIENCE.2016.11.042

Brown, B. M., Peiffer, J. J., & Martins, R. N. (2012). Multiple effects of physical activity on molecular and cognitive signs of brain aging: can exercise slow neurodegeneration and delay Alzheimer's disease? *Molecular Psychiatry 2012 18:8*, *18*(8), 864–874. https://doi.org/10.1038/mp.2012.162

Brown, T. I., & Chrastil, E. R. (2019). Editorial: Spatial navigation: Memory mechanisms and executive function interactions. *Frontiers in Human Neuroscience*, *13*. https://doi.org/10.3389/FNHUM.2019.00202

Clark, P. J., Brzezinska, W. J., Puchalski, E. K., Krone, D. A., & Rhodes, J. S. (2009). Functional analysis of neurovascular adaptations to exercise in the dentate gyrus of young adult mice associated with cognitive gain. *Hippocampus*, *19*(10), 937–950. https://doi.org/10.1002/HIPO.20543

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior*, *24*(4), 385–396. https://doi.org/10.2307/2136404

Colcombe, S. J., Kramer, A. F., Erickson, K. I., Scalf, P., McAuley, E., Cohen, N. J., Webb, A., Jerome, G. J., Marquez, D. X., & Elavsky, S. (2004). Cardiovascular fitness, cortical plasticity, and aging. *Proceedings of the National Academy of Sciences*, *101*(9), 3316–3321. https://doi.org/10.1073/PNAS.0400266101

Daugherty, A. M., Yuan, P., Dahle, C. L., Bender, A. R., Yang, Y., & Raz, N. (2015). Path Complexity in Virtual Water Maze Navigation: Differential Associations with Age, Sex, and Regional Brain Volume. *Cerebral Cortex*, *25*(9), 3122–3131. https://doi.org/10.1093/CERCOR/BHU107

Day, D. S. (2008). Exercise physiologists talk about sex differences. *Medicine and Science in Sports and Exercise*, *40*(4), 646–647. https://doi.org/10.1249/MSS.0B013E318162136D

De Rover, M., Petersson, K. M., Van Der Werf, S. P., Cools, A. R., Berger, H. J., & Fernández, G. (2008). Neural correlates of strategic memory retrieval: Differentiating between spatial- associative and temporal-associative strategies. *Human Brain Mapping*, *29*(9), 1068–1079. https://doi.org/10.1002/HBM.20445

Devi, L., Diwakar, L., Raju, T. R., & Kutty, B. M. (2003). Selective neurodegeneration of hippocampus and entorhinal cortex correlates with spatial learning impairments in rats with bilateral ibotenate lesions of ventral subiculum. *Brain Research*, *960*(1–2), 9–15. https://doi.org/10.1016/S0006-8993(02)03699-5

Driscoll, I., Hamilton, D. A., Petropoulos, H., Yeo, R. A., Brooks, W. M., Baumgartner, R. N., & Sutherland, R. J. (2003). The Aging Hippocampus: Cognitive, Biochemical and Structural Findings. *Cerebral Cortex*, *13*(12), 1344–1351. https://doi.org/10.1093/CERCOR/BHG081

Erickson, K. I., Prakash, R. S., Voss, M. W., Chaddock, L., Hu, L., Morris, K. S., White, S. M., Wójcicki, T. R., McAuley, E., & Kramer, A. F. (2009). Aerobic Fitness is Associated With Hippocampal Volume in Elderly Humans. *Hippocampus*, *19*(10), 1030. https://doi.org/10.1002/HIPO.20547

Feinberg, D. A., Moeller, S., Smith, S. M., Auerbach, E., Ramanna, S., Glasser, M. F., Miller, K. L., Ugurbil, K., & Yacoub, E. (2010). Multiplexed Echo Planar Imaging for Sub-Second Whole Brain FMRI and Fast Diffusion Imaging. *PLOS ONE*, *5*(12), e15710. https://doi.org/10.1371/JOURNAL.PONE.0015710

Firth, J., Stubbs, B., Vancampfort, D., Schuch, F., Lagopoulos, J., Rosenbaum, S., & Ward, P. B. (2018). Effect of aerobic exercise on hippocampal volume in humans: A systematic review and meta-analysis. *NeuroImage*, *166*, 230–238. https://doi.org/10.1016/J.NEUROIMAGE.2017.11.007

Fischl, F. B. (2012). FreeSurfer. *NeuroImage*, *62*, 774–781. https://doi.org/10.1016/j.neuroimage.2012.01.021

Fred H. Gage. (2019). Adult neurogenesis in mammals. *Science*, *8*(4), 345–351. https://doi.org/10.1126/SCIENCE.AAV6885

Frodl, T., Strehl, K., Carballedo, A., Tozzi, L., Doyle, M., Amico, F., Gormley, J., Lavelle, G., & O'Keane, V. (2020). Aerobic exercise increases hippocampal subfield volumes in younger adults and prevents volume decline in the elderly. *Brain Imaging and Behavior*, *14*(5), 1577–1587. https://doi.org/10.1007/s11682-019-00088-6

Good, C. D., Johnsrude, I. S., Ashburner, J., Henson, R. N. A., Friston, K. J., & Frackowiak, R. S. J. (2001). A Voxel-Based Morphometric Study of Ageing in 465 Normal Adult Human Brains. *NeuroImage*, *14*(1), 21–36. https://doi.org/10.1006/NIMG.2001.0786

Griswold, M. A., Jakob, P. M., Heidemann, R. M., Nittka, M., Jellus, V., Wang, J., Kiefer, B., & Haase, A. (2002). Generalized autocalibrating partially parallel acquisitions (GRAPPA). *Magnetic Resonance in Medicine*, *47*(6), 1202–1210. https://doi.org/10.1002/MRM.10171

Hackert, V. H., Den Heijer, T., Oudkerk, M., Koudstaal, P. J., Hofman, A., & Breteler, M. M. B. (2002). Hippocampal Head Size Associated with Verbal Memory Performance in

Herting, M. M., & Nagel, B. J. (2013). Differences in Brain Activity during a Verbal Associative Memory Encoding Task in High- and Low-fit Adolescents. *Journal of Cognitive Neuroscience*, *25*(4), 595–612. https://doi.org/10.1162/JOCN_A_00344

Ho, S. C., Woo, J., Sham, A., Chan, S. G., & Yu, A. L. M. (2001). A 3-year follow-up study of social, lifestyle and health predictors of cognitive impairment in a Chinese older cohort.*International Journal of Epidemiology*, *30*(6), 1389–1396. https://doi.org/10.1093/IJE/30.6.1389

Holzschneider, K., Wolbers, T., Röder, B., & Hötting, K. (2012). Cardiovascular fitness modulates brain activation associated with spatial learning. *NeuroImage*, *59*(3), 3003–3014. https://doi.org/10.1016/J.NEUROIMAGE.2011.10.021

Honea, R., Thomas, G. P., Harsha, A., Anderson, H. S., Donnelly, J. E., Brooks, W. M., & Burns, J.

M. (2009). Cardiorespiratory fitness and preserved medial temporal lobe volume in Alzheimer's Disease. *Alzheimer Disease and Associated Disorders*, *23*(3), 188. https://doi.org/10.1097/WAD.0B013E31819CB8A2

Iglesias, J. E., Van Leemput, K., Augustinack, J., Insausti, R., Fischl, B., & Reuter, M. (2016). Bayesian longitudinal segmentation of hippocampal substructures in brain MRI using subject-specific atlases. *NeuroImage*, *141*, 542–555. https://doi.org/10.1016/J.NEUROIMAGE.2016.07.020

Islam, M. R., Luo, R., Valaris, S., Haley, E. B., Takase, H., Chen, Y. I., Dickerson, B. C., Schon, K., Arai, K., Nguyen, C. T., & Wrann, C. D. (2020). Diffusion tensor-MRI detects exercise- induced neuroplasticity in the hippocampal microstructure in mice. *Brain Plasticity*, *5*(2), 147–159. https://doi.org/10.3233/BPL-190090

Kramer, A. F., Hahn, S., Cohen, N. J., Banich, M. T., McAuley, E., Harrison, C. R., Chason, J., Vakil, E., Bardell, L., Boileau, R. A., & Colcombe, A. (1999). Ageing, fitness and neurocognitive function. *Nature 1999 400:6743*, *400*(6743), 418–419. https://doi.org/10.1038/22682

Kronman, C. A., Kern, K. L., Nauer, R. K., Dunne, M. F., Storer, T. W., & Schon, K. (2020). Cardiorespiratory fitness predicts effective connectivity between the hippocampus and default mode network nodes in young adults. *Hippocampus*, *30*(5), 526–541. https://doi.org/10.1002/HIPO.23169

Lautenschlager, N. T., Cox, K., & Cyarto, E. V. (2012). The influence of exercise on brain aging and dementia. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1822*(3), 474–481. https://doi.org/10.1016/J.BBADIS.2011.07.010

Lester, A. W., Moffat, S. D., Wiener, J. M., Barnes, C. A., & Wolbers, T. (2017). The Aging Navigational System. *Neuron*, *95*(5), 1019–1035. https://doi.org/10.1016/J.NEURON.2017.06.037

Lindquist, R., Wyman, J. F., Talley, K. M. C., Findorff, M. J., & Gross, C. R. (2007). Design of Control-Group Conditions in Clinical Trials of Behavioral Interventions. *Journal of Nursing Scholarship*, *39*(3), 214–221. https://doi.org/10.1111/J.1547-5069.2007.00171.X

Milham, M. P., Erickson, K. I., Banich, M. T., Kramer, A. F., Webb, A., Wszalek, T., & Cohen, N. J. (2002). Attentional Control in the Aging Brain: Insights from an fMRI Study of the Stroop Task. *Brain and Cognition*, *49*(3), 277–296. https://doi.org/10.1006/BRCG.2001.1501

Ming, G., & Song, H. (2011). Adult Neurogenesis in the Mammalian Brain: Significant Answers and Significant Questions. *Neuron*, *70*(4), 687. https://doi.org/10.1016/J.NEURON.2011.05.001

Moffat, S. D., Zonderman, A. B., & Resnick, S. M. (2001). Age differences in spatial memory in a virtual environment navigation task. *Neurobiology of Aging*, *22*(5), 787–796. https://doi.org/10.1016/S0197-4580(01)00251-2

Mueller, S. G., Yushkevich, P. A., Das, S., Wang, L., Van Leemput, K., Iglesias, J. E., Alpert, K., Mezher, A., Ng, P., Paz, K., & Weiner, M. W. (2018). Systematic comparison of different techniques to measure hippocampal subfield volumes in ADNI2. *NeuroImage : Clinical*, *17*, 1006. https://doi.org/10.1016/J.NICL.2017.12.036

Nauer, R. K., Dunne, M. F., Stern, C. E., Storer, T. W., & Schon, K. (2020). Improving fitness increases dentate gyrus/CA3 volume in the hippocampal head and enhances memory in young adults. *Hippocampus*, *30*(5), 488–504. https://doi.org/10.1002/HIPO.23166

NT, L., K, C., & EV, C. (2012). The influence of exercise on brain aging and dementia. *Biochimica et Biophysica Acta*, *1822*(3), 474–481. https://doi.org/10.1016/J.BBADIS.2011.07.010

O'Mara, S. (2005). The subiculum: what it does, what it might do, and what neuroanatomy has yet to tell us. *Journal of Anatomy*, *207*(3), 271–282. https://doi.org/10.1111/J.1469- 7580.2005.00446.X

O'Mara, S. M., Commins, S., Anderson, M., & Gigg, J. (2001). The subiculum: a review of form, physiology and function. *Progress in Neurobiology*, *64*(2), 129–155. https://doi.org/10.1016/S0301-0082(00)00054-X

Park, D. C., & Reuter-Lorenz, P. (2008). The Adaptive Brain: Aging and Neurocognitive Scaffolding. *Https://Doi.Org/10.1146/Annurev.Psych.59.103006.093656*, *60*, 173–196. https://doi.org/10.1146/ANNUREV.PSYCH.59.103006.093656

Pereira, A. C., Huddleston, D. E., Brickman, A. M., Sosunov, A. A., Hen, R., McKhann, G. M., Sloan, R., Gage, F. H., Brown, T. R., & Small, S. A. (2007). An in vivo correlate of exercise- induced neurogenesis in the adult dentate gyrus. *Proceedings of the National Academy of Sciences*, *104*(13), 5638–5643. https://doi.org/10.1073/PNAS.0611721104

Pescatello, L. S., Arena, R., Riebe, D., & Thomsen, P. D. (2014). ACSM's guidelines for exercise testing and prescription. In *ACSM's Guidelines for Exercise Testing and Prescription, Ninth Edition* (9th ed.). Wolters Kluwer/Lippincott Williams & Wilkins Health.

Poppenk, J., Evensmoen, H. R., Moscovitch, M., & Nadel, L. (2013). Long-axis specialization of the human hippocampus. *Trends in Cognitive Sciences*, *17*(5), 230–240. https://doi.org/10.1016/J.TICS.2013.03.005

Raji, C. A., Lopez, O. L., Kuller, L. H., Carmichael, O. T., & Becker, J. T. (2009). Age, Alzheimer disease, and brain structure. *Neurology*, *73*(22), 1899. https://doi.org/10.1212/WNL.0B013E3181C3F293

Raz, N., Rodrigue, K. M., Head, D., Kennedy, K. M., & Acker, J. D. (2004). Differential aging of the medial temporal lobe. *Neurology*, *62*(3), 433–438. https://doi.org/10.1212/01.WNL.0000106466.09835.46

Raz, Naftali, Gunning-Dixon, F., Head, D., Rodrigue, K. M., Williamson, A., & Acker, J. D. (2004). Aging, sexual dimorphism, and hemispheric asymmetry of the cerebral cortex: replicability of regional differences in volume. *Neurobiology of Aging*, *25*(3), 377–396. https://doi.org/10.1016/S0197-4580(03)00118-0

Raz, Naftali, Lindenberger, U., Rodrigue, K. M., Kennedy, K. M., Head, D., Williamson, A., Dahle, C., Gerstorf, D., & Acker, J. D. (2005). Regional Brain Changes in Aging Healthy Adults: General Trends, Individual Differences and Modifiers. *Cerebral Cortex*, *15*(11), 1676–1689. https://doi.org/10.1093/CERCOR/BHI044

Redila, V. A., & Christie, B. R. (2006). Exercise-induced changes in dendritic structure and complexity in the adult hippocampal dentate gyrus. *Neuroscience*, *137*(4), 1299–1307. https://doi.org/10.1016/J.NEUROSCIENCE.2005.10.050

Richards, J. E., Sanchez, C., Phillips-Meek, M., & Xie, W. (2016). A database of age-appropriate average MRI templates. *NeuroImage*, *124*, 1254–1259. https://doi.org/10.1016/J.NEUROIMAGE.2015.04.055

Shelton, A. L., & Gabrieli, J. D. E. (2002). Neural Correlates of Encoding Space from Route and Survey Perspectives. *The Journal of Neuroscience*, *22*(7), 2711. https://doi.org/10.1523/JNEUROSCI.22-07-02711.2002

Siddiqui, A. N., Siddiqui, N., Khan, R. A., Kalam, A., Jabir, N. R., Kamal, M. A., Firoz, C. K., & Tabrez, S. (2016). Neuroprotective Role of Steroidal Sex Hormones: An Overview. *CNS Neuroscience & Therapeutics*, *22*(5), 342–350. https://doi.org/10.1111/CNS.12538

Small, S. A., Chawla, M. K., Buonocore, M., Rapp, P. R., & Barnes, C. A. (2004). Imaging correlates of brain function in monkeys and rats isolates a hippocampal subregion differentially vulnerable to aging. *Proceedings of the National Academy of Sciences*, *101*(18), 7181–7186. https://doi.org/10.1073/PNAS.0400285101

Small, S. A., Schobel, S. A., Buxton, R. B., Witter, M. P., & Barnes, C. A. (2011). A pathophysiological framework of hippocampal dysfunction in ageing and disease. *Nature Reviews Neuroscience 2011 12:10*, *12*(10), 585–601. https://doi.org/10.1038/nrn3085

Sperling, R. A., Dickerson, B. C., Pihlajamaki, M., Vannini, P., Laviolette, P. S., Vitolo, O. V, & Hedden, T. (2010). Functional Alterations in Memory Networks in Early Alzheimer's Disease. *Neuromolecular Med*, *12*(1), 27–43. https://doi.org/10.1007/s12017-009-8109-7

Spiers, H. J. (2008). Keeping the goal in mind: Prefrontal contributions to spatial navigation.*Neuropsychologia*, *46*(7), 2106. https://doi.org/10.1016/J.NEUROPSYCHOLOGIA.2008.01.028

Strange, B. A., Witter, M. P., Lein, E. S., & Moser, E. I. (2014). Functional organization of the hippocampal longitudinal axis. *Nature Reviews Neuroscience 2014 15:10*, *15*(10), 655–669. https://doi.org/10.1038/nrn3785

Strauss, E., Sherman, E. M. S., & Spreen, O. (2006). A Compendium of Neuropsychological Tests Administration, Norms and Commentary. New York Oxford University Press. - References - Scientific Research Publishing. (n.d.). Retrieved August 28, 2021, from

https://www.scirp.org/(S(vtj3fa45qm1ean45vvffcz55))/reference/ReferencesPapers.aspx? ReferenceID=1343119

Suwabe, K., Byun, K., Hyodo, K., Reagh, Z. M., Roberts, J. M., Matsushita, A., Saotome, K., Ochi, G., Fukuie, T., Suzuki, K., Sankai, Y., Yassa, M. A., & Soya, H. (2018). Rapid stimulation of human dentate gyrus function with acute mild exercise. *Proceedings of the National Academy of Sciences*, *115*(41), 10487–10492. https://doi.org/10.1073/PNAS.1805668115

Thomas, A. G., Dennis, A., Rawlings, N. B., Stagg, C. J., Matthews, L., Morris, M., Kolind, S. H., Foxley, S., Jenkinson, M., Nichols, T. E., Dawes, H., Bandettini, P. A., & Johansen-Berg, H. (2016). Multi-modal characterization of rapid anterior hippocampal volume increase associated with aerobic exercise. *NeuroImage*, *131*, 162–170. https://doi.org/10.1016/J.NEUROIMAGE.2015.10.090

Thompson, C. L., Pathak, S. D., Jeromin, A., Ng, L. L., MacPherson, C. R., Mortrud, M. T., Cusick, A., Riley, Z. L., Sunkin, S. M., Bernard, A., Puchalski, R. B., Gage, F. H., Jones, A. R., Bajic, V. B., Hawrylycz, M. J., & Lein, E. S. (2008). Genomic Anatomy of the Hippocampus. *Neuron*, *60*(6), 1010–1021. https://doi.org/10.1016/J.NEURON.2008.12.008/ATTACHMENT/38D6EB88-029F-48AC- 9DFA-FABA618669A3/MMC6.PDF

Uysal, N., Tugyan, K., Kayatekin, B. M., Acikgoz, O., Bagriyanik, H. A., Gonenc, S., Ozdemir, D., Aksu, I., Topcu, A., & Semin, I. (2005). The effects of regular aerobic exercise in adolescent period on hippocampal neuron density, apoptosis and spatial memory. *Neuroscience Letters*, *383*(3), 241–245. https://doi.org/10.1016/J.NEULET.2005.04.054

van der Kouwe, A. J. W., Benner, T., Salat, D. H., & Fischl, B. (2008). Brain morphometry with multiecho MPRAGE. *NeuroImage*, *40*(2), 559–569. https://doi.org/10.1016/J.NEUROIMAGE.2007.12.025

Van Praag, H., Christie, B. R., Sejnowski, T. J., & Gage, F. H. (1999). Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proceedings of the National Academy of Sciences*, *96*(23), 13427–13431. https://doi.org/10.1073/PNAS.96.23.13427

Van Praag, H., Shubert, T., Zhao, C., & Gage, F. H. (2005). Exercise Enhances Learning and Hippocampal Neurogenesis in Aged Mice. *Journal of Neuroscience*, *25*(38), 8680–8685. https://doi.org/10.1523/JNEUROSCI.1731-05.2005

Varma, V. R., Tang, X., & Carlson, M. C. (2016). Hippocampal sub-regional shape and physical activity in older adults. *Hippocampus*, *26*(8), 1051–1060. https://doi.org/10.1002/HIPO.22586

Voss, M. W., Nagamatsu, L. S., Liu-Ambrose, T., & Kramer, A. F. (2011). Exercise, brain, and cognition across the life span. *Https://Doi.Org/10.1152/Japplphysiol.00210.2011*, *111*(5), 1505–1513. https://doi.org/10.1152/JAPPLPHYSIOL.00210.2011

Wagner, G., Herbsleb, M., de la Cruz, F., Schumann, A., Köhler, S., Puta, C., Gabriel, H. W., Reichenbach, J. R., & Bär, K. J. (2017). Changes in fMRI activation in anterior hippocampus and motor cortex during memory retrieval after an intense exercise intervention. *Biological Psychology*, *124*, 65–78. https://doi.org/10.1016/J.BIOPSYCHO.2017.01.003 Winterburn, J. L., Pruessner, J. C., Chavez, S., Schira, M. M., Lobaugh, N. J., Voineskos, A. N., Chakravarty, M. M. (2013). A novel in vivo atlas of human hippocampal subfields using high-resolution 3 T magnetic resonance imaging. *NeuroImage*, *74*, 254–265. https://doi.org/10.1016/J.NEUROIMAGE.2013.02.003

Wolbers, T., Wiener, J. M., Mallot, H. A., & Büchel, C. (2007). Differential Recruitment of the Hippocampus, Medial Prefrontal Cortex, and the Human Motion Complex during Path Integration in Humans. *Journal of Neuroscience*, *27*(35), 9408–9416. https://doi.org/10.1523/JNEUROSCI.2146-07.2007

Woodard, J. L., Sugarman, M. A., Nielson, K. A., Smith, J. C., Seidenberg, M., Durgerian, S., Butts, A., Hantke, N., Lancaster, M., Matthews, M. A., & Rao, S. M. (2012). Lifestyle and genetic contributions to cognitive decline and hippocampal structure and function in healthy aging. *Current Alzheimer Research*, *9*(4), 436–446. https://doi.org/10.2174/156720512800492477

Xu, J., Moeller, S., Auerbach, E. J., Strupp, J., Smith, S. M., Feinberg, D. A., Yacoub, E., & Uğurbil,K. (2013). Evaluation of slice accelerations using multiband echo planar imaging at 3 Tesla. *Neuroimage*, *83*. https://doi.org/10.1016/j.neuroimage.2013.07.055