Rhythm Experience and Africana Culture Trial (REACT)
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Approved Protocol

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1.0 SUMMARY:

African Americans are almost two times more likely than whites (i.e., Caucasians) to experience Alzheimer's disease or other forms of dementia (1). Higher prevalence translates to 45% higher Medicare costs for African Americans compared to whites (2). A critical public health question emerges from these statistics that we intend to address in this study: Is there an effective method for reducing or eliminating the race disparities in cognitive and brain health?

Fortunately, physical activity (PA) interventions may be effective at improving neurocognitive function and reducing risk for dementia. Despite these promising results, prior PA interventions have had few African Americans making it difficult to stratify results by race to determine whether African Americans respond to PA in a similar manner and magnitude as whites. In addition, the terms 'physical activity' and 'exercise' are often considered unpleasant, painful, and fatiguing, which can negatively influence interest, enrollment, and long-term adherence to PA interventions.

Here, we propose an innovative and culturally sensitive method of increasing PA in older (60-80 yrs.) African Americans. We will conduct a ***randomized intervention*** where 180 older African Americans are assigned to either a moderate intensity ***African Dance*** group 3 days per week (N=90) or to an ***Africana Culture*** group 3 days per week (N=90) for 6-months. Both before and at the completion of the intervention, we will collect a comprehensive neuropsychological battery, physiological health metrics, and MRI metrics of brain health and function to identify biological pathways by which PA influences neurocognitive health in an African American population.

We have assembled a highly creative, productive, and interdisciplinary team with a long history of collaboration and experience conducting exercise interventions in older adults to test the following aims:

Aim 1. Examine whether a 6-month African Dance intervention improves cognitive performance compared to the culture control group. H1: The dance group will show cognitive improvements in a domain-specific fashion such that executive and memory functions will be enhanced more than other cognitive domains.
Aim 2. Examine whether African Dance influences brain morphology, task-evoked neural responses, cerebral blood flow, and resting state connectivity. H1: We predict that African Dance will increase volume, white matter integrity, perfusion, and functional activation/connectivity in a regionally-specific fashion such that prefrontal and hippocampal areas will be more sensitive to the intervention than other brain regions.
Aim 3. Explore potential physiological and socioemotional mechanisms of the dance intervention. We will collect measures of physical and psychosocial health such as waist circumference, blood pressure, blood glucose and lipid levels, mood, anxiety, depression, and loneliness and examine whether intervention-related changes to these measures mediate improvements in cognitive performance.

1.1 BACKGROUND AND RATIONALE:

1.1.1 Health Disparities and Age-Related Neurocognitive Decline.

When compared to whites, African Americans are 2-3 times more likely to develop dementia (1, 3). Such striking health disparities do not appear to be driven by genetic factors but by increased rates of vascular diseases, obesity, and hypertension in African Americans (4–6). *Despite such remarkable disparities, African Americans are routinely underrepresented in both longitudinal studies and clinically randomized trials for prevention or treatment of cognitive decline.*

Cognitive performance typically declines across the adult lifespan with executive and inhibitory control processes, working memory, and episodic memory displaying early and disproportionate decline (7). Brain atrophy precedes cognitive decline in a graded fashion with prefrontal and medial temporal lobes showing earlier and more rapid decline (8–10). Although the biological bases for these changes remain unclear, cardiometabolic risk factors predict and possibly contribute to accelerated loss (11, 12). For example, our group demonstrated that obesity is associated with diffuse reductions in white matter integrity (13).

Increased prevalence of vascular and metabolic risk factors in African Americans is thought to contribute to their increased risk for neurocognitive deficits (4, 5, 14). For example, 72% of African Americans over the age of 55 have high blood pressure compared to 54.0% of whites (15) and, after adjusting for age, 46.55% of African Americans meet criteria for obesity compared to 33.8% of whites (16). Because of elevated vascular risks in African Americans, decline in brain integrity and function may occur earlier in the lifespan than for whites (10, 12, 17). As such, *interventions that target cardiovascular health in African Americans could have significant consequences on neurocognitive function*.

1.1.2 Physical Activity (PA) Restores Brain Function. PA has emerged as a promising, low-cost method for improving neurocognitive function in older adults (18–21). Prospective epidemiological studies indicate that physical inactivity is a significant risk factor for dementia and potentially contributes to 21% (over 1.1 million) of AD cases in the US (22). Randomized interventions have shown restorative effects of PA on cognitive and brain outcomes in older adults (18, 20). For example, moderate intensity exercise, in the form of brisk walking, improves cognitive function in as little as 3-months, primarily in domains of executive function and memory (23). In addition, our research group has found that higher fit adults have larger hippocampal volumes than less fit counterparts (24) and that a one-year walking intervention reverses age-related atrophy of the hippocampus (18). Our research has also found that walking about 1 mile per day was sufficient to spare gray matter volume in prefrontal and hippocampal areas and that greater volume in these regions cut the risk of cognitive impairment in half (25). These changes have downstream effects on brain function. For example, we have demonstrated increased activity in prefrontal and parietal regions during inhibitory control after 6-months of exercise training (26). Furthermore, we

found that exercise training increased functional brain connectivity between the posterior cingulate and hippocampal and frontal medial cortices relative to the control group (27).

There are three important take-home messages from this research: 1.) *Diminution*: a sedentary lifestyle leads to cognitive and brain decline, 2.) *Plasticity*: the brain remains modifiable throughout the lifespan and PA has the capacity to take advantage of this property, 3.) *Intervention*: PA interventions enhance brain integrity. Despite these well-established principles, evidence for all of them comes from studies with predominantly Caucasian participants, even though African Americans could equally gain from such an intervention.

1.1.3 Dancing as a method for increasing PA. Dancing is an engaging form of PA that attracts cross-generational groups of all races and ethnicities. In particular, older adults prefer activities involving music, which is why dance is often used in senior centers and nursing homes as a therapeutic tool to elevate activity (28). Randomized interventions have demonstrated that dancing is effective at increasing PA and improving physical fitness. For example, traditional ballroom, tango, or salsa, improves mobility, balance, motor skills and coordination, and resting heart rate in older adults (29, 30). Dancing also improves self-confidence, motor skills, mood (31) and cognitive function (e.g., 32) while epidemiological studies find that dancing reduces risk for cognitive impairment (33). Preliminary data from our lab suggests that dancing also improves balance, gait, and cognitive function (34).

African Dance refers to a loose constellation of dance practices derived from Africa. In part because of the large African diasporic population in the US, African Dance is surging in popularity in many cities and has helped revitalize the roots and traditions of African culture by educating both the black and white communities in African heritage. African dance differs from almost all other dance cultures in one primary way. Whereas in the majority of dancing around the world the body is treated as a single "block," in African dance the body has multiple, semi-independent centers (35). In African dance, the torso, shoulders, pelvis, and legs are relatively independent – each region of the body follows a different rhythm and gestural pattern. Because of African dance's "polycentricity" (35), total body articulation is heightened and therefore requires significant movement, coordination, and endurance. Hence, African Dance is engaging while also being a moderately intense form of PA. *In sum, African Dance is an effective and engaging method of increasing PA and is culturally sensitive and appealing to an African American community (see below for preliminary data).*

1.1.4 Innovation

Our primary goal is to conduct a study that will transform scientific-based policy and health care recommendations for improving cognitive function and reducing health disparities in older African Americans. We have assembled a highly productive and interdisciplinary team with a long history of conducting this type of research to provide answers to important unsettled scientific questions that have critical public health

consequences. Ours will be the first study of its kind, is highly innovative, and will add to the scientific literature in several important ways:

(1) *For the first time, we will examine whether systematically increasing PA through a randomized African Dance intervention enhances neurocognitive function in African Americans.* Our research group has demonstrated the benefits of PA on brain and cognition (13, 18, 24, 25, 27, 36–38), but these studies have almost exclusively studied majority Caucasian samples. Hence, there is an immediate need to expand these results to minority, and more vulnerable, populations.
(2) *We will examine whether African Dance is effective at improving brain morphology and function using cutting-edge MRI sequences and analytical approaches*. Our group has previously described the effects of PA on brain morphology and function (18, 20, 27), but these studies have had only small numbers of African Americans.
(3) *For the first time we will examine whether increasing PA through African Dance could serve as a platform to assess health disparities.* As such, our innovative design could be molded into future interventions targeting other biological systems and to assess reductions in health disparities.
(4) *By taking advantage of a culturally sensitive activity* we will be able to target a minority population that has traditionally been underrepresented in clinically randomized trials of brain health.

1.2 PRELIMINARY DATA:

We describe three areas of preliminary data that demonstrate effects of exercise on cognitive and brain outcomes including results from our pilot African Dance study that formed the basis for this trial.

1.2.1 Effects of exercise on cognitive function: Our group was one of the first to demonstrate the favorable effects of a 6-month aerobic exercise RCT on cognitive outcomes (39, 10). In a meta-analysis of 18 RCTs in older adults, we found that exercise broadly influenced cognitive function (Hedge's g=.47), but improved executive function more than other cognitive domains (Hedge's g=.67), with the largest effect sizes for study durations of 6 months or longer (23). These encouraging effects have now been replicated by our group and others (18, 27, 40). *Implications:* We have significant experience conducting PA interventions. Our results demonstrate that >6-months of PA improves cognitive function and that we have been successful at recruiting and retaining older adults in PA intervention trials.

1.2.2 Effects of exercise on brain outcomes: Our research group has published >100 papers on the effects of exercise and fitness on brain morphology, white matter integrity, resting state connectivity, and task-evoked functional MRI activity. In 120 cognitively normal adults between 60-80 yrs of age (N=60/group), moderate intensity exercise was effective at increasing hippocampal volume, which was correlated with improvements in spatial memory (18). In addition to brain

morphology, changes in cardiorespiratory fitness resulting from an exercise RCT were correlated with increased white matter integrity in PFC and temporal lobes in 70 cognitively normal adults (41). Our results also extend to resting-state connectivity and task-evoked activation. For example, in 65 cognitively normal older adults exercise increased functional connectivity (27) and these changes were correlated with improvements in executive function, memory, and increases in serum BDNF and IGF-1 (37). *Implications for this project*: Our team is well-versed in the methods, challenges, and analytical approaches for examining MRI outcomes in PA interventions. We have been successful in coordinating neuroimaging efforts across sites and maintaining participants in MRI studies that are >1 year in duration.

1.2.3 Results from a pilot African Dance intervention. We have preliminary data from a pilot sample of 33 older African Americans tested across 2 study waves with an average age of 69.21 (standard deviation = 3.60). One-third of the sample reported a family history of cognitive impairment. The data from this study was collected as part of an Alzheimer's Association-funded study on non-pharmaceutical interventions to reduce disparities in cognitive decline (PI: Erickson; NPSASA-14-321093).

We randomized participants into one of two groups – an African Dance group or an Africana Education control group. Participants in both groups completed three 1-hour sessions per week for 6 months. Participants (N=19) in the Dance group learned traditional dances from Guinea and South Africa. The dance class started with ~5 minutes of warm-up and dynamic stretching followed by ~50 minutes of instructor-led dance sequences while continuously moving, and finally ~5 minutes of cool down and static stretching. Resting blood pressure and resting heart rate measurements were taken at the beginning and end of class. Heart rate was monitored continuously throughout the class and recorded every 15 minutes. Rating of Perceived Exertion (RPE) from each participant was also recorded at the end of each class. A trained staff member monitored participants' heart rates to ensure participants remained in the target heart rate zone (60-75% of their age-predicted maximal heart rate (APMHR)) for the duraton of the class.

Participants in the Education class (N=14) learned about Africana History in 3 sections organized by time period: pre-colonial, colonial, and post-colonial. The topics covered in each section included culture/way of life/customs, spirituality, ethnicity, language, family structures, rites of passage, political structures, and economy. The format of the class was discussion-based and was led by staff originating from Africa. For each topic, instructors typically lecture or show an educational film for part of the hour. The remaining class time is spent in an instructor-led discussion.

Sixty-six African American older adults (53 female; Mean age = 69.4 $\pm$ 4.9 years) were screened and scheduled for baseline testing in Pittsburgh. Of this group, 48 (~90%) successfully completed the baseline measurements and were subsequently randomized and enrolled. First, we successfully maintained heart rate in our prescribed heart rate zone. On average, participants in the Dance group maintained a heart rate (HR) of 68.3% of their APMHR throughout the intervention classes. Thus, our dance intervention was effective at maintaining a HR intensity

in the target range and can be considered a form of moderate-intensity PA. Second, enrollment and adherence continued to improve with each wave of the intervention so that attendance rates in the last wave were >70% for both groups. Attendence rates improved from the first to the second cohort, and enrollment rose from 7 participants in the Dance group and 3 in the Education group in wave 1, to 12 and 11 participants in these two groups, respectively, in wave 2. Importantly, we learned successful advertising methods and tactics to improve attendance. We plan to implement several strategies to further promote attendance in the present study (e.g., transportation support, raffles, performance summaries). We found that participants were overwhelmingly enthusiastic about both interventions. On average, participants rated the enjoyability of the Dance and Education interventions as 5.0 and 4.6, respectively, on a scale of 1-5. In fact, when asked what they would improve about the intervention, many participants suggested that we make the intervention duration longer.

1.3 STATISTICAL ANALYSIS:

1.3.1 General Approach. We will analyze data in an intent-to-treat (ITT) manner such that all participants, regardless of whether they complete the intervention, will be invited to return for the follow-up sessions and will be included in primary analyses. Secondary analyses will be conducted using a per-protocol approach, which restricts the analysis to the participants that completed the intervention. All cognitive and neuroimaging data will be interrogated for normality, and symmetry, and any non-normal data may be transformed based on the distribution of error terms. Any data that cannot be transformed will be analyzed using non-parametric tests. Non-parametric regression models will be used for continuous variables when necessary. We will examine the data for potential outliers. If it is determined that outliers are due to misreporting of data or administrative error, we will delete them from the analysis. If the outliers are not due to misreporting of data or adminstirative error we will conduct the analysis with and without outliners included. Outlier de-weighting will be conducted on voxel-wise MRI data to remove the influence of anomalous voxels in the analysis. All analyses will be two-tailed. We will first summarize baseline demographic and brain characteristics of the sample and intervention groups using descriptive statistics and statistical hypothesis testing. Descriptive statistics will include mean, median, standard deviation, range for continuous variables, and frequency for categorical variables. Statistical tests will be conducted using t-test, ANOVA F-test, Chi-square test, correlations (Pearson or Spearman) as appropriate. Our primary goals are to examine whether the intervention improves cognitive and brain health. To test these hypotheses, we propose to use a General Linear Mixed Model (GLMM) in standard neuroimaging and statistical packages. Effects of Time (baseline, 6-month), Group (Dance, Education), and Time by Group interactions will be examined. Post-hoc comparisons using least square means will decompose any significant interactions and will be plotted to illustrate the direction of the effect. Mean differences between groups for all cognitive and neuroimaging outcome measures will be derived using linear contrasts and multiple comparisons will be corrected by standard protocols. Differences in baseline characteristics will be accounted for by using covariance

adjusted models We will conduct multiple sensitivity analyses to examine and determine how to handle missing data. Based on a Missing at Random assumption, the distributions of the missing data will be examined and will be compared. The potential effect of missing data on the primary hypothesis tests will be adjusted by including covariates that are significantly associated with missing data in the regression models.

We will describe differences in baseline characteristics between participants with complete versus incomplete follow-up to assess any bias (e.g., age) in those who do not return for follow-up. However, according to ITT analyses, all participants including those that do not complete the intervention will be analyzed as part of the trial. Using hierarchical regression models, we will be able to explore whether changes in any cognitive or neuroimaging metric (e.g., white matter integrity) varies as a function of improvements in PA or fitness independently of assigned group. We also appreciate that there will be significant individual variability in factors such as body mass, blood pressure, fitness level, or SES. Instead of modeling this variation as 'noise' we plan to take advantage of the variability to further explore, independently of group assignment, the associations between cognitive and brain imaging metrics and initial levels and individual change trajectories. Main effects and interaction terms will be modeled in these regression analyses and time-dependent changes will be explored and illustrated by scatter and spaghetti plots. These analyses will be useful for generating future hypotheses and questions.

Aim 3 proposes to explore physiological and socioemotional mechanisms of the dance intervention. We plan to explore our hypotheses using several different approaches including simple mediational models applying the change score method and will estimate whether the dance intervention (predictor) on *changes* in cognitive function outcomes are mediated through *changes* in the suspected mediators. Goodness-of-fit will be assessed through standard summary indices (e.g., RMSEA, CFI) and model assessment via residual analyses will performed. Total, indirect and direct effects will be estimated. Additionally, we will explore mediation applying the half-longitudinal approach proposed by Cole and Maxwell as well as an autoregressive mediational approach. Finally, we will explore recent advancements in repeated measures mediation analyses developed by Preacher and Hayes. Depending on the results from simple mediational modeling, we may also combine mediators into a multiple mediator model to develop a more comprehensive picture of these pathways.

1.3.2 Power Analyses. Our planned sample size of 180, with randomization of 90 participants in each group, is based on effect sizes from preliminary data. Even when accounting for attrition and retention of ~70% of participants (65 in each group), we are sufficiently powered to detect effects in cognitive and brain outcomes and to test for mediation.

1.3.3 Cognitive performance. Our prior PA interventions have found moderate sized effects on cognitive function after 6-months with a Cohen's d ranging from 0.40 to 0.80 depending on the outcome (23). In our analysis of the pilot data from the African Dance intervention with only 19 participants (9 in the Dance group) we estimate similar or greater effect sizes of ~.70. If we estimate an effect of 0.70, 65 participants per group after attrition will provide us with >91% power to examine our primary hypotheses.

1.3.4 Brain Volumetrics. Our prior 6-12 month PA interventions have used between 30 and 60 subjects per group with Cohen's d ranging between 0.40 and 0.50 using similar analysis approaches (18, 20, 27, 41, 42). Cross-sectional research has reported significant effects of PA on brain volume with as few as 32 subjects and effect sizes between 0.50 – 0.60 (24, 40, 42–44). Preliminary data on 37 African Americans from one of our prior studies suggest that cross-sectional differences between African Americans engaging in PA compared to those not engaging in PA are larger than for whites (Cohen's d = 1.83). These studies argue for a moderate-to-large effect of PA on brain volume depending on the sample, the measure of PA and fitness, and the brain metric used. If we estimate a conservative effect size of 0.50, 65 participants per group will be sufficient to detect differences in brain volume at 81% power.

1.3.5 Diffusion tensor imaging. Changes in white matter have been detected in PA interventions in as little as 6-months with only 50 subjects (45). In a 12-month PA intervention, we found increased FA with only 35 subjects in each group with an estimated effect size of 0.70 (41). Based on an estimated effect size of 0.75, we should have >85% power with 65 participants per group to test our main hypotheses as well as enough power for exploratory analyses.

1.3.6 Task-evoked activity. Task-evoked brain imaging studies of PA have found statistically significant effects with 30 - 50 subjects per group with effect sizes ranging between 1.0 and 1.3 depending on the region examined and the task employed (26). Cross-sectional studies of fitness on brain activity report even larger effects up to 1.70 (36, 46, 47). These patterns indicate a large effect size of PA on brain function, so 65 participants per group will provide us with substantial power to test our main aims and hypotheses.

1.3.7 Connectivity. With only 65 people, we have shown that a PA intervention is effective at enhancing functional connectivity in prefrontal-inhibitory networks (27). The difference between the groups for prefrontal cortex connectivity was .15 with a standard deviation of .10 suggesting an effect size of 1.5 (Cohen's d). Therefore, similar to the effect sizes and power for the other measures, 65 participants per group should be sufficient for detecting changes as a function of the intervention with >85% power.

1.7 PROCEDURES:

Before the collection of baseline data, all staff will have had ethics training and appropriate certification of research training modules. All staff will also be a part of a training certification to ensure that all data collection practices will be conducted in the same way by all study staff.

1.7.1 Phone Screening

During the phone screen, numerous medical history questions will be asked to verify that the subject meets inclusion criteria (see Chapter 2): Inclusion and Exclusion and below for more details). Medical record retrieval will not be used,

nor will be PCP approval be required for participation unless the study staff identifies a potential medical issue that warrants such approval (e.g., a previous cardiac event). Information about safety to exercise will be obtained over the phone (e.g., history of falls). Finally, information regarding metal in the body and history of claustrophobia for MRI safety will be obtained including information about any metallic objects in their body and claustrophobia.

1.7.2 Inclusion and Exclusion Criteria.

Inclusion Criteria:

- African American men and women (race self-identified)
- Between 60-80 years of age at enrollment
- Ambulatory without pain or the assistance of walking devices
- No history of falls or balance problems
- Able to speak and read English
- Available during the times that classes are offered, and able to make at least 80% of offered classes (e.g., no long-term travel plans)
- Reliable means of transportation or willingness to let us develop a transportation plan for them
- Willingness to be randomized to *either* group
- Scores on the TICS or MOCA that indicate no more than mild cognitive impairment (scores 26 or above or 21 and above, respectively)
- No diagnosis of a neurological disease that primarily affects central nervous system function (e.g., dementia, multiple sclerosis, Parkinson's disease).
- No current diagnosis of a psychiatric condition (e.g., a current depression or anxiety disorder).
- Eligible to undergo MRI (not claustrophobic and no metal or history of injury involving metal that could be a safety concern for the MRI)
- Able to complete a submaximal fitness test

Exclusion Criteria:

- Not self-identified as African American
- Not between the ages of 60 and 80 at the time of enrollment
- Not fluent in English
- Not available for at least 80% of times classes are offered
- Not willing to be randomized into either group
- Does not have reliable means of transportation and not open to us assisting them with a transportation plan
- Scores below 26 on the TICS, or below 21 on the MOCA (indicating moderate or severe cognitive impairment)
- Metal in body or history of injury involving metal that isn't approved by an MRI safety committee
- Claustrophobia
- History of severe head trauma, major stroke, or brain tumors

- History of psychiatric disorders other than past major depressive episodes or anxiety
- History of falls or other balance problems
- History of heart or cardiovascular disease, including heart failure, angina, or hypertension not currently controlled by medication (i.e., exceeding 190/100).
- Unable to complete submaximal fitness test

1.7.3 Baseline Testing.

There are several outcome measurement sessions at baseline (Cognitive, Fitness/Blood, and MRI sessions) which must be completed prior to randomization. It will be recommended that all assessments happen within a 6-week window that starts the day the informed consent document is signed at session 1. However, given challenges with scheduling and safety clearances that may be necessary in older adults, we will work to collect all baseline data on participants, even if the session exceeds the 6-week window.

Session 1: Participants will come to the laboratory and complete a battery of cognitive and neuropsychological tests (see below for a description of the tests). Compliance with the test instructions will be monitored by a study certified administrator. Participants will be allowed to take breaks between tests on a regular basis to help reduce fatigue. The cognitive tasks to be completed during the cognitive session are described in more detail below. The tasks can be grouped into several broad cognitive categories:

- *General Cognition:*
  1. MOCA (Montreal Cognitive Assessment): was designed as a rapid screening instrument for mild cognitive dysfunction. It assesses different cognitive domains: attention and concentration, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation. Time to administer the MoCA is approximately 10 minutes. The total possible score is 30 points; a score of 26 or above is considered normal.
  2. Wechsler Test of Adult Reading (WTAR): estimates pre-morbid intellectual functioning (approximate intelligence). This test takes approximately 5 minutes to administer and will only be administered at the baseline testing session.

- *Working Memory:*
  1. Spatial Working Memory (SWM): measures spatial memory functions and takes about 10 minutes. It requires that subjects attend to and retain the location of several dots presented simultaneously on a computer display. They are requested to press buttons on a keyboard that correspond to whether a probe dot appeared in one of the same locations as the previous dots.

2. List Sort Working Memory: (10 minutes) This task is from the NIH Toolbox and assesses working memory and requires the participant to recall and sequence different visually and orally presented stimuli. Pictures of different foods and animals are displayed with both an accompanying audio recording and written text that names the item. The participant is asked to repeat the items back to the examiner in size ordered from smallest to largest.

- *Executive Function:*
  1. Trail Making B (5 min): This task measures executive control (part B). Both parts of this test consist of 25 circles distributed over a sheet of paper. In Part A, the circles are numbered 1-25, and the subject is instructed to draw lines to connect the numbers in ascending order. In Part B, the circles include both numbers (1-13) and letters (A-L); as in Part A, the patient draws lines to connect the circles in an ascending pattern, but with the added task of alternating between the numbers and letters (i.e., 1-A-2-B-3-C, etc.). The task is discontinued if the subject has not completed both parts after five minutes have elapsed.
  2. Dimensional Card Sort: (6 minutes) This task is part of the NIH Toolbox and measures cognitive flexibility and attention. Two target pictures are presented that vary along two dimensions (e.g. shape and color). Participants are asked to match a series of bivalent test pictures (e.g., yellow balls and blue trucks) to the target pictures, first according to one dimension (e.g., color) and then, after a number of trials, according to the other dimension (e.g., shape). The relevant dimension for sorting is indicated by a cue word (e.g., "shape" or "color") that appears on the screen for all participants.
  3. Flanker: (4 minutes) This is a measure of inhibitory control and attention and is part of the NIH Toolbox. The task requires the participant to focus on a particular stimulus while inhibiting attention to the flanking stimuli.
  4. Color-Word Stroop (10 minutes): The Stroop Task measures selective attention, cognitive flexibility and processing speed. This task is administered on a computer and the participants are asked to respond to the colors of the ink of printed words on the display as quickly as they can while ignoring the meaning of the word. The task takes less than 10 minutes to complete.

- *Processing Speed*:
  1. Trail Making A (5 min; see description of Trails Part B above): This task measures processing speed (part A). As with Part B, the test consists of 25 circles distributed over a sheet of paper. In Part A, the circles are numbered 1-25, and the subject is instructed to draw lines to connect the numbers in ascending order. The task is discontinued if the subject has not completed both parts after five minutes have elapsed.

2. Digit Symbol Substitution Test Coding (3 min; DSST): This task is part of the RBANS. Digit Symbol Substitution asks participants to match symbols with their corresponding digit. It consists of 9 digit symbols matched with their corresponding numerical digit and only takes approximately 3-4 minutes to administer.

- *Episodic Memory:*
  1. Word List: This is part of RBANS. A list of 10 semantically unrelated words is orally presented, and the examinee is asked to recall as many words as he or she can remember. This process is repeated over 4 learning trials.
  2. Story Recall: This is part of RBANS. A short story is orally presented, and the examinee is asked to retell the story from memory. The same story is presented a second time, and the examinee is again asked to retell the story from memory.
  3. Word List Recall: The examinee is asked to recall the list of 10 words learned in the List Learning subtest.
  4. List Recognition: The examinee is read 20 words (10 targets, 10 distractors) and asked to indicate whether each word was on the word list.
  5. Story Memory: The examinee is asked to retell the story they learned earlier.
  6. Figure Recall: The examinee is asked to draw the figure shown earlier from memory.

- *Visuospatial/Constructional:*
  1. Figure Recall: This is part of the RBANS. The examinee is shown a multipart geometric drawing and is asked to make an exact copy while the drawing remains on display.

  2. Line Orientation: The examinee is presented with the drawing that consists of 13 equal lines radiating out from a single point to form a semicircular fan-shaped pattern. All lines are numbered (1-13). Below this drawing are two lines that match two of the lines from the array above. The examinee is asked to identify which two lines they match. Ten trials are given, with different sets of test lines on each trial.

- *Language:*
  1. Semantic Fluency test: This is a semantic fluency test that is part of the RBANS. In this task, the examinee is given one minute to name as many exemplars as possible from a given semantic category (e.g., Fruits and Vegetables).

2. Picture Naming: The examinee is presented with a series of pictured objects and is asked to name each one. A semantic cue is provided only if an object is obviously misperceived.

The following questionnaires will also be completed either during the outcome sessions or taken home by participants to complete. They are administered at baseline and after completion of the intervention:

- Demographics, Health History, and Lifestyle:
  1. Health History
  2. Demographic Questionnaire

We also plan to collect a number of other questionnaires at both time points, including, but not necessarily limited to:

3. MacArthur SES
4. Positive and Negative Affect Schedule (PANAS)
5. Satisfaction with Life Scale (SWLS)
6. UCLA Loneliness Scale
7. The Pittsburgh Fatigability Scale
8. Center for Epidemiology Studies Depression Scale (CES-D)
9. Everyday Cognition Questionnaire (ECOG)
10. McGill Pain Questionnaire
11. PROMIS – Physical and Mental, as well as Global Health items

Session 2: The second session will consist of the fitness assessment.
Staff will begin by obtaining a resting blood pressure and heart rate. As long as blood pressure and heart rate are within the permitted safety guidelines (link to Fitness MOP chapter), the participant will begin the Senior Fitness test (link to Senior Fitness test in MOP chapter). Note that the Senior Fitness Test must be completed prior to the submaximal exercise test in order to limit effects of fatigue on these measures. The participant will rest for a brief period and complete some brief questionnaires related to physical activity behaviors and feelings before moving on to the submaximal exercise test.

Participants will complete a submaximal graded exercise test on a motor-driven treadmill to assess cardiorespiratory fitness (VO_2). The participant will be fitted with a chest-worn Polar Heart Rate monitor and three ECG electrodes. The test will follow a Modified Balke Protocol in which the speed of the test will be mutually agreed upon by the participant and exercise physiologist. Treadmill speed will remain constant while grade will increase every two minutes.

Before beginning the submaximal test, a second resting blood pressure will be obtained. Only participants with safe blood pressure readings will be allowed to continue.

During the fitness test, the participant's expired air will be collected while walking on the treadmill. The participant will wear a fitted facemask which is connected to a metabolic cart in order to analyze all respiratory gases. This VO_2 test usually takes about 15 - 30 minutes to complete depending on the starting fitness level of the participant. These procedures follow guidelines recommended by the American College of Sports Medicine for graded exercise testing. During the test, heart rate is continuously monitored via the Polar Heart Rate monitor chest strap. Blood pressure readings and Rating of Perceived Exertion (RPE) will also be recorded during every two-minute stage. When the subject reaches the endpoint of the exercise test (85% age-predicted maximal heart rate, RPE ≥15 for those prescribed beta-blockers, safety concerns, or participant request), the facemask will be removed and they will undergo a four-minute active cool-down in which they will walk at a slower rate with zero incline grade. After these four minutes, the participant will be helped off of the treadmill and undergo a passive cool-down seated in a chair. The participant's heart rate and blood pressure will be recorded every two minutes throughout the cool-down period. The participant will not be allowed to leave until vital signs return to normal patterns (systolic and diastolic blood pressure within 20 mmHg of resting values and heart rate within 20 beats per minute of resting values).

Each submaximal exercise test will be administered by a trained exercise physiologist and assistant. The assistant will record the required values of each stage and aid with test administration. The assistant will be another exercise physiologist/technician, nurse, or staff with a medical background. All exercise testing staff must complete the REACT II certification standards. The exercise testing will be used to measure changes in fitness levels after the 6-month intervention. The testing site will have the room set at a comfortable temperature (60 – 70 degrees Fahrenheit), have necessary fans in place and have access to water for the participants.

At the conclusion of the fitness visit, participants will be fitted with a commercially available physical activity monitoring device (i.e., Actigraph) around the non-dominant wrist and/or hip (as instructed by the fitness staff member) that will record objective physical activity data over the duration of approximately one week. Participants will be provided with detailed instructions regarding wearing of the device as well as the option to remove the device if it becomes problematic.

Once the VO_2 session and Actigraph fitting is complete, the research staff member will email the participant a link to complete the diet history questionnaire (NCI). Participants will be asked to click on the link and complete the questionnaire on a home computer. If the participant does not have access to a home computer, they will be permitted to complete this assessment on a computer within the lab.

Session 3: The third session will generally consist of the blood draw and an MRI scan (exact session content may be changed as needed – e.g., based on MRI availability). This session will be scheduled in the morning because the blood draw must be done in a fasted state. Both before randomization (at baseline) and after the intervention we will collect approximately 60 cc of blood for a total of 120 cc for each subject during the duration of the study. Blood collection will be postponed in subjects experiencing an acute infection (e.g., respiratory or flu-like symptoms). Another session may be scheduled for the blood draw in the case that a participant forgets to fast or is experiencing acute illness. After blood is collected, the subject will be provided with a light snack and water or juice. They will also be reminded (in pre-session reminder call) to drink plenty of water before and up to the actual blood draw and to take medications on their normal schedule.

Following the blood draw, practice for the MRI session will begin in the cognitive testing room. It is anticipated that the entire length of the MRI session (from the beginning of blood draw to the scan conclusion) will take about 2.5 hours. This includes the blood draw itself and brief snack break, transitions to and from the scanner, tech metal screening, practice of tasks outside scanner, and scan time. Participants will be scheduled well in advance for the MRI scan and standard sequences will be collected that will consist of images of brain structure and function. To assess brain function, we will use an n-back paradigm that measures working memory. Before entering the MRI machine, participants will undergo an additional safety screen by the MRI center technicians and will be instructed about the task and be allowed to practice the tasks that they will be performing along with completing any questionnaires that weren't completed in prior sessions. All data collected during this session will then be interrogated for quality control.

During the N-Back, the subject will be presented with a sequence of stimuli, and the task consists of indicating when the current stimulus matches the one from n steps earlier in the sequence. The load factor n can be adjusted to make the task more or less difficult. For older adults, the maximum load is usually 2 (i.e., the most difficult condition asks participants to make 2-back judgments).

MRI Sequences:

- Scout
- MPRAGE
- Resting State fMRI
- N-Back
- Hippocampal subfield sequence
- DSI (Diffusion Spectral Imaging)
- FLAIR
- pCASL

If the research team discovers an incident or abnormality (e.g., tumor) as a part of the MRI scan, it is important for the study subject to be informed (a) that these scans were not designed for clinical purposes and so a clear diagnosis and prognosis cannot be made, but (b) that if an incidental finding or abnormality is found in the scan that we will follow standard procedures of the MRI center which includes the consultation of a board certified neuroradiologist at the University of Pittsburgh. The consultation would involve an assessment of the scan and the neuroradiologist would provide feedback to the Principal Investigator to pass along to the subject. This may require the Principal Investigator to recommend that the subject inform their personal physician about the abnormality and request follow-up procedures that are outside the purview of the research study.

1.7.4 Post-Randomization Tasks. The self-efficacy questionnaire will be completed during the third week of the intervention. In addition, the physical activity monitoring device will be worn for one week, every 6 weeks for the duration of the study.

1.7.5 Follow-up Testing (6 months). After 6-months of the intervention sessions, the participants will complete one cognitive assessment similar to that of the baseline visit. The tasks will be the same as those described above but will not include the WTAR or the Health History questionnaire. This time point also includes a blood draw to collect the same biomarkers as the Time point 1 blood draw.

1.7.6 Blinding of Study Staff. All study staff administering the outcomes assessment sessions will be blinded to participants' group assignments. Given this, special planning is in place to ensure adequate staffing for both the pre- and post- assessments.

1.9 RANDOMIZATION:

After the completion of the measures and sessions described above the participants will be randomly assigned to one of two groups: (a) The Dance condition will consist of approximately 180 minutes per week of moderate intensity African Dance, or (b) an Africana Culture control condition that will consist of 180 minutes per week of mixed-programming related to African Culture, including art projects, cooking, and films. We will use a computer randomization protocol that will incorporate a verification that all session visits have been completed and data entered into the database. We will use a stratified permuted block randomization algorithm with equal allocation to one of the two groups. The use of this strategy will ensure treatment balance on the two factors of age at study entry (=<70, >70) and gender. Once randomized, an assigned staff member will contact the subject via phone with the group information. On this contact, the staff

member will give information such as date, day, time, and location of the subject's first intervention session.

1.10 EXERCISE INTERVENTION GROUPS:

1.10.1 African Dance Group. Participants in the Dance condition will begin by dancing at reduced intensity during the first week of the program and will gradually increase their dancing intensity each subsequent week until they reach 40-60% of their heart rate reserve for all of the dance class. All dance sessions start and end with 5-10 minutes of active stretching for the purpose of warming up and cooling down. As the participants will be low active at baseline, the prescribed intensity will be 50–65% of the maximum heart rate reserve for weeks one to four and 65–80% for the remainder of the program. Participants will be fitted with a Polar Bluetooth Smart heart rate sensor around their chest and be encouraged to dance within their target heart rate zone by fitness staff. Continuous heart rate readings of all participants will be displayed in real-time via the Polar Team app on an iPad or other tablet. Certified exercise instructors will closely monitor attendance, intensity, frequency, and safety. Dance sessions will occur three days/week at one of two study sites for the duration of the intervention.

1.10.2 Africana Culture Group. The control group will focus on learning about and experiencing Africana culture through interactive class discussions, lectures, and hand-on demonstrations. This goal for this group is 150 minutes of contact per week so the social component of the Dance intervention is matched. However, crucially, the control group will not participant in any aerobic activities; the majority of their activities will be conducted in a sitting position. Adherence is also tracked in this group, and study participants are encouraged to attend as many classes as possible of the offered sessions (3 classes per week). Each class is expected to last 60 minutes with 5-10 minutes for taking attendance and making administrative announcements at the beginning/end of the class.

Session Compliance. Participants in the study will be completing the African Dance classes or Culture classes under supervised conditions. We will monitor compliance during the classes by having trainers or staff monitor attendance, duration of the dance, ratings of perceived exertion, and heart rate intensity throughout the class period.

Based on the above, we have operationally defined compliance to the intervention as those participants meeting the prescription of either 150 minutes per week of moderate intensity Dance (Group 1), or 150 minutes per week of Africana Culture class (Group 2). Compliance to these prescriptions will be based on the information gathered from the supervised sessions.

1.11 ANALYSIS PLAN

Our analysis plan has proposed an intent-to-treat analysis where all participants, regardless of whether they complete all sessions, will be invited to return for follow-up assessments. However, there will likely be some participants that refuse to return for follow-up assessments. "Lost to follow up" will be defined here as missing data that is contributing to the primary outcomes (executive functioning and hippocampal volume). The reason will be recorded (i.e., missed visit due to traveling, illness). These criteria and documentation will also be applied for any discontinued subjects with the reasons for discontinuation being recorded (i.e., moving out of state).

We propose to use all available data in the planned analysis for testing our primary outcomes. We will assess the missingness of data consistent with the assumptions of the modeling approach described above. That is, we will examine the randomness of missing data and examine if the data is missing completely at random (MCAR) or missing at random (MAR). Parameters of the GLMM will be estimated using the maximum likelihood estimation or restricted maximum likelihood methods that are known to produce consistent and efficient estimates under MAR.

> **Imaging data analysis.** We will first analyze each imaging outcome (e.g., volume) separately from every other imaging outcome (e.g., fMRI). But, once voxel-wise associations related to the intervention are established, we could test, using hierarchical regression, the relative significance of each imaging measure to explain improvements in cognitive function with the intervention.

1.12 PARTICIPANT SAFETY:

The safety of study participants will be monitored throughout the study. Study participants will undergo an extensive screening prior to entering the trial to determine that it is safe for them to participate in the intervention. Safety is continuously monitored during the intervention during three supervised sessions per week. We are aware of the potential for serious adverse events (AEs) to occur with any type of exercise training. As such, the trial will take measures to reduce the risk of any adverse events or serious adverse events from occurring. All individuals involved in human subject's research where exercise training is involved, are trained and certified in CPR and First Aid. Included in the exercise stress testing room is an automated external defibrillator (AED), should it be deemed necessary. Lastly, protocols are currently in place to respond to any adverse events that include contacting emergency response personnel and facilitating their arrival to the correct rooms within the buildings at each intervention site.

The REACT II primary investigator has full responsibility for the safety of study participants. The Data Safety Monitoring Board (DSMB), is responsible for objectively monitoring study data for evidence of adverse effects attributed to the intervention. The DSMB also has the authority to recommend changes or pausing the trial until problems are resolved. All AEs experienced by the participant during the study (starting from consent signing until the end of study outcome collection) are to be documented and reported. Serious adverse events will be reported to the DSMB and the IRB. The REACT II trial will track the occurrence of:

- Serious Adverse Events (SAE)
- Unexpected events
- Unfavorable medical events that occur during intervention sessions

The trial has created two forms for adverse event reporting. These documents have been created based on the belief that trainers and staff should not be responsible for determining whether the event is related to the intervention or whether/how it should be reported to the IRB and DSMB. Only the PI will determine if the event is (a) expected or unexpected, and (b) related to the intervention. Based on responses to these questions, regardless of seriousness or relatedness, the trainer or dance instructor will complete a form documenting an adverse event. We have created a brief form for trainers and instructors (REACT II Adverse Event Form for Trainers) for this purpose. Trainers or staff would first document the event on this form. This form allows staff to circle the reported event (or provide an event not listed on the form). This completed document would be sent to the project coordinator who would then speak to the trainer and/or participant to find out more information about the event. The project coordinator would then complete the REACT II Event Evaluation Form based on this communication. This form has several sections with boxes available for documenting the chronicity (single occurrence, intermittent, persistent), whether the event was resolved or not, and whether it was related to the intervention and expected or unexpected. In particular, these forms are intended to clarify for the investigators, the IRB, and the DSMB, whether the adverse event was related to the intervention, and whether it was expected or unexpected. An example of an expected adverse event from the dance intervention would be muscle soreness which would be listed as a known risk in the IRB. An unexpected adverse event related to the intervention would be if someone falls during a class and injures an ankle. The PI will oversee the determination of these events and if there is any question about whether the event was related to the intervention, and its expectedness, the event will be discussed during weekly staff meetings. For DSMB purposes, all adverse events that are reported will be recorded and separated based on whether the event was related to the intervention, expectedness, severity, chronicity, and resolution.

1.13 DATA MANAGEMENT:

We appreciate the challenges associated with the organization, execution, management, and analysis of a clinical trial of this kind and recognize the importance of frequent communication between staff, students, analysts, and investigators. Protection of subject privacy and safety, meticulous QC, and prudent organization is the bedrock for success of a study like this, which will require a sizeable team of staff and students. For this study, Dr. Erickson will maintain overall responsibility for DM and QC. Dr. Kang (biostatistician) will monitor database integrity for missing data, errors, outliers, and distributions.

IT staff will control permissions to the database to ensure proper access and DM by staff,

students, and investigators. We will store and link all behavioral and assay data on a HIPAA secure cloud-based server (REDcap) and may use other applications (e.g., the Flywheel application) for securely storing and accessing imaging data from the imaging center.

Data entry to the REDcap database will be completed within the BACH Lab that is directed by PI Erickson. The data forms in REDcap are similar to the paper version of the forms that will be completed by participants and staff. All participant data will be stored and archived on a secure server. The server will be set for safe mode that allows two copies of each data to be saved. The data server will have a hard drive array, which is composed of a few hard drive disks. If one or two of the disks fail, the rest of the disks will back up the data of the failed disks automatically. By having the database on REDCap, all data is secure in case of a natural disaster or other calamity.

Missed Visits: All outcome collection must be reported and stored in the database. Therefore, if an outcome visit is missed or not collected, a missed visit form must be completed in REDCap. Once a missed visit form is completed, the data management team will indicate in the database that the outcome data for the specific measure is missed to follow up. All missing data in the database will require additional documentation regarding whether the data are "lost to follow up" or has been collected but there was a problem with transferring the data to the lab.

The missed visit form has three sections that must be completed:

- Time point of the missed visit
- Outcome that was missed
- Reason the outcome was missed

If a study participant misses multiple outcome measures during the same time point, one missed visit form can be completed if the reason missed is the same for each measure. Thus, staff do not have to complete a new form for each missed outcome for the same time point. For example, at follow-up, if the study participant missed the cognitive session and blood draw because the participant moved out of the area, this information could be completed on one missed visit form. If this study participant would also miss the baseline physical activity monitoring, a new form must be generated since this is a different time point.

1.14 QUALITY CONTROL:

This RCT demands rigorous QC to ensure consistency of the intervention procedures and protocols. Staff will be trained early in the first year to standardize protocols. We will also hold weekly meetings with all staff to ensure continuation of all protocols. Reports of QC will be a standard agenda item for Data Safety and Monitoring Board (DSMB) meetings. During the course of the study, we anticipate a turnaround of staff, graduate and undergraduate assistants, and these QC procedures will ensure compliance and uniformity of study protocol for the duration of the project.

1.14.1 Cognitive and MRI Quality Control. To maintain QC of the cognitive data, all possible administrators will receive QC training in Year 01 to administer the tests in a standardized way. These certification sessions will be conducted annually for maintenance of QC.

MR sequences will be stablished and piloted before the trial begins. The new joint Pitt-CMU Imaging Center is anticipated to be the location for the REACT II scans, which will have a research dedicated Siemens 3T machine. We will have monthly QC checks using phantoms and QC will be monitored for any changes over time. Before the start of the intervention, several staff members will be scanned to ensure equivalent contrast and image quality for each sequence. All imaging data will be uploaded to a server and will be examined by Dr. Erickson's laboratory to assess QC, proper sequences, and site- specific effects.

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Statistical Analysis Plan

Power and Statistical Analysis*:* We will analyze in an intent-to-treat (ITT) manner such that all participants, regardless of whether they complete the intervention, will be invited to return for the follow-up sessions and will be included in primary analyses. Secondary analyses will be conducted using a per-protocol approach, which restricts the analysis to the participants that completed the intervention. All cognitive and neuroimaging data will be interrogated for normality and any non-normal data will be transformed. Any data that cannot be transformed will be analyzed using non-parametric tests. Non-parametric regression models will be used for continuous variables when necessary. Outlier de-weighting will be conducted to remove the influence of anomalous data. All analyses will be two-tailed. We will first summarize baseline demographic and brain characteristics of the sample and intervention groups by conducting Pearson, Spearman, and Chi-square statistics as appropriate. Our primary goals are to examine whether the intervention improves cognitive and brain health. To test these hypotheses, we propose to use a General Linear Mixed Model (GLMM) in standard neuroimaging and statistical packages. Effects of Time (baseline, 6-month), Group (Dance, Education), and Time by Group interactions will be examined. Post-hoc comparisons using least square means will decompose any significant interactions and will be plotted to illustrate the direction of the effect. Mean differences between groups for all cognitive and neuroimaging outcome measures will be derived using linear contrasts and multiple comparisons will be corrected by standard protocols. Differences in baseline characteristics will be accounted for by using covariance adjusted models.

We will describe differences in baseline characteristics between participants with complete versus incomplete follow-up to assess any bias (e.g., age) in those who do not return for follow-up. However, according to ITT analyses, all participants including those that do not complete the intervention will be analyzed as part of the trial. Using hierarchical regression models, we will be able to explore whether changes in any cognitive or neuroimaging metric (e.g., white matter integrity) varies as a function of improvements in PA or fitness independently of assigned group. We also appreciate that there will be significant individual variability in factors such as body mass, blood pressure, fitness level, or SES. Instead of modeling this variation as 'noise' we plan to take advantage of the variability to further explore, independently of group assignment, the associations between cognitive and brain imaging metrics and initial levels and individual change trajectories. Main effects and interaction terms will be modeled in these regression analyses and time-dependent changes will be explored and illustrated by scatter plots. These analyses will be useful for generating future hypotheses and questions.

Power Analyses: Our proposed sample size of 180, with randomization of 90 participants in each group, is based on effect sizes from preliminary data. Even when accounting for attrition and retention of ~70% of participants (65 in each group), we are sufficiently powered to detect effects in cognitive and brain outcomes and to test for mediation.

Cognitive performance: Our prior PA interventions have found moderate sized effects on cognitive function after 6-months with a Cohen's d ranging from 0.40 to 0.80 depending on the outcome. In our analysis of the pilot data from the African Dance intervention with only 19 participants (9 in the Dance group) we estimate similar or greater effect sizes of ~.70. If we estimate an effect of 0.70, 65 participants per group after attrition will provide us with >91% power to examine our primary hypotheses.

Brain volumetrics: Our prior 6-12 month PA interventions have used between 30 and

60 subjects per group with Cohen's d ranging between 0.40 and 0.50 using similar analysis approaches. Cross-sectional research has reported significant effects of PA on brain volume with only 32 subjects and effect sizes between 0.50 – 0.60. Preliminary data on 37 African Americans from one of our prior studies suggest that cross-sectional differences between African Americans engaging in PA compared to those not engaging in PA are larger than for whites (Cohen's d = 1.83). These studies argue for a moderate-to-large effect of PA on brain volume depending on the sample, the measure of PA and fitness, and the brain metric used. If we estimate a conservative effect size of 0.50, 65 participants per group will be sufficient to detect differences in brain volume at 81% power.

Diffusion tensor imaging: Changes in white matter have been detected in PA interventions in as little as 6-months with only 50 subjects. In a 12-month PA intervention, we found increased FA with only 35 subjects in each group with an estimated effect size of 0.70.Based on an estimated effect size of 0.75, we should have >85% power with 65 participants per group to test our main hypotheses as well as enough power for exploratory analyses.

Task-evoked activity: Task-evoked brain imaging studies of PA have found statistically significant effects with 30 - 50 subjects per group with effect sizes ranging between 1.0 and 1.3 depending on the region examined and the task employed. Cross-sectional studies of fitness on brain activity report even larger effects up to 1.70. These patterns indicate a large effect size of PA on brain function, so 65 participants per group will provide us with substantial power to test our main aims and hypotheses.

Connectivity: With only 65 people, we have shown that a PA intervention is effective at enhancing functional connectivity in prefrontal-inhibitory networks. The difference between the groups for prefrontal cortex connectivity was .15 with a standard deviation of .10 suggesting an effect size of 1.5 (Cohen's d). Therefore, similar to the effect sizes and power for the other measures, 65 participants per group should be sufficient for detecting changes as a function of the intervention with >85% power.

Given the continued challenges of navigating the COVID-19 pandemic, we requested to modify our recruitment/randomization accrual and targeted sample size for the study titled "Rhythm Experience and Africana Culture Trial (REACT II)" on August 29th, 2023. We have determined that we will have sufficient statistical power to test our primary aims with a reduction in the targeted sample size from 180 to 150.
The recommended changes were approved by the meeting of the Data Safety and Monitoring Board on August 29, 2023.