ABSTRACT

Title of Dissertation: THE IMPACT OF ACUTE EXERCISE AND SLEEP QUALITY ON EXECUTIVE FUNCTION: THE POTENTIAL MEDIATING EFFECTS OF FUNCTIONAL CONNECTIVITY IN OLDER ADULTS

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Over the last decade, the prevalence of dementia has increased exponentially. With 9.9 million new cases per year, dementia is one of the fastest growing chronic diseases in the world. Insufficient sleep and physical inactivity occur more often in older adults and have been associated with cognitive impairment. Aerobic exercise not only mitigates many of the cardiometabolic risk factors for dementia but also facilitates better sleep and enhanced cognition. Although understanding the interrelationship between sleep, exercise, and cognition is important for healthy aging, no single study has used multi-modal neuroimaging and actigraphy to examine how these variables interact. Thus, this study sought to identify the joint impact of acute exercise and sleep quality on executive function in older adults. We also aimed to determine the degree to which exercise-induced changes in resting state functional connectivity (rsFC) influenced the relationship between sleep quality and exercise-altered executive function performance and functional activation. Older adults (age 55-85) were recruited to participate in this study. Written informed consent was obtained, and all participants were deemed safe for moderate intensity exercise and magnetic resonance imaging (MRI). For at least three consecutive days, preceding the first experimental condition, eligible participants wore...
an actigraphy device to objectively measure sleep quality. Participants were then exposed to 30-minutes of rest and exercise on separate days (using a within-subjects, counterbalanced design). Directly after each condition, participants underwent both resting state and task-based functional MRI to assess prefrontal rsFC and executive function, respectively. Repeated measures ANOVA and multi-variant linear regression were used to identify individual, interactive, and mediating effects. Results demonstrated that acute exercise increased prefrontal rsFC and functional activation in long sleepers (> 7.5 hours/night), while decreasing these parameters for individuals with less total sleep time. Moreover, these results corresponded to behavioral data demonstrating that acute exercise and adequate sleep improved select aspects of executive function performance, while decreasing inhibitory control in short sleepers alone (< 7.5 hours). Findings suggest that the effects of acute exercise on prefrontal rsFC are similar, or even related, to the effects of acute exercise on conflict-dependent functional activation, and that this relationship may depend on sleep duration. Moreover, our results imply that although acute exercise elicited improved executive function for those with adequate sleep, it may weaken already vulnerable, and perhaps fatigued, executive function networks among short sleepers.
THE IMPACT OF ACUTE EXERCISE AND SLEEP QUALITY ON EXECUTIVE FUNCTION: THE POTENTIAL MEDIATING EFFECTS OF FUNCTIONAL CONNECTIVITY IN OLDER ADULTS

by

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Dedication

I would like to dedicate this dissertation to the people in my life who have been touched by Alzheimer’s disease; you are and will continue to be an ongoing source of inspiration. I would also like to dedicate this manuscript to my family, specifically my parents Dr. William and Cheryl Alfini, for their unconditional love and support, know that this work could not have been completed without you. And finally I would like to dedicate this work to my Grandfather, William R. Alfini Sr., for his encouragement and unyielding love for family and education.
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Chapter 1: Organization and Introduction

1.1 Document Organization

This document has been organized to serve three main purposes. The first is to inform the reader of the author's research interests. The second is to demonstrate both the author's work and progress during his tenure as a graduate student at the University of Maryland, and the third is to provide the reader with a detailed account of the dissertation experiment's execution and findings. Chapters 1, 2, and 3 contain background information and rationale for the dissertation experiment and Chapter 4 is the dissertation manuscript.

1.2 Public Health Significance

Over the last decade, the number of people living with dementia has increased exponentially\(^1\). Today, 46.8 million people suffer from dementia, making it one of the most common non-communicable diseases in older adults around the world\(^1\). Without a cure or successful treatment strategy the prevalence of dementia is projected to increase threefold, exceeding 131 million cases by 2050\(^2\). Although dementia is pandemic, the WHO describes the incidence of dementia in developing countries as a rapidly growing epidemic\(^3,4\). As the aging population increases globally, many new cases are being concentrated in poor communities with inadequate, if any, dementia-related resources\(^4\).

The global economic impact of dementia is approximately $818 billion per year, and continues to rise\(^1,5\). At the current rate, dementia will be the most
expensive disease society has ever faced. Healthcare services (paid and unpaid services) for one dementia patient in the US costs almost $49,000 per year, which is more than three times the cost of healthcare for a healthy older American. In 2015, dementia-care alone cost more than 20% of the entire federal healthcare budget.

Alzheimer's disease (AD) accounts for approximately 60% of all dementia. AD is characterized by a progressive decline in cognitive and social ability, and is accompanied by a loss of functional independence. More than half of the estimated 9.4 million cases of dementia in the US result from AD. The incidence of AD increases with age and, therefore, primarily affects older adults (≥ age 65) with almost half of the oldest adults (≥ age 85) in the US believed to have the disease.

Sleep is a fundamental component of neuropsychological function across the cognitive domains. Poor sleep quality and the accumulation of sleep fragmentation critically impair memory, attention, and emotional regulation. Disrupted sleep occurs more frequently and severely with AD than in normal aging, and chronic sleep disturbances likely contribute to the development of Mild Cognitive Impairment (MCI) and conversion to AD.

Exercise has a profound effect on brain health and sleep. Research has consistently shown that higher levels of physical activity are associated with reduced age-related and pathological cognitive decline. Additionally, evidence from randomized controlled trials has demonstrated that exercise training may protect against cerebrovascular damage and facilitate structural
changes associated with enhanced cognition\textsuperscript{22–24}. In humans, aerobic exercise is related to many sleep indices, including extended total sleep time (TST) and improved sleep efficiency (SE; minutes asleep/minutes in bed)\textsuperscript{18,25,26}. Similarly, evidence from animal models has demonstrated that exercise is highly involved in the homeostatic sleep drive and regulation of the sleep-wake cycle\textsuperscript{27,28}. Adequate sleep and exercise improve cerebrovascular health\textsuperscript{29,30}. Sufficient sleep and exercise not only preserve brain volume but also improve neural efficiency, and decrease markers of neuropathology\textsuperscript{26,31–34}. Altered resting cerebral blood flow (rCBF) facilitates physiological changes in the brain that increase microvascular density and volume\textsuperscript{24,35,36}. While a singular mechanism remains elusive, exercise-altered rCBF is related to synaptic potentiation and angiogenesis\textsuperscript{23,24,36}. Although rCBF naturally decreases with age\textsuperscript{37}, exercise training in older adults has been shown to mitigate and even reverse this decline\textsuperscript{29,38}. Moreover, evidence suggests that chronic exercise induces changes in rCBF that are often accompanied by improved cognitive performance\textsuperscript{23,38}.

In addition to perfusion-weighted MRI, resting state functional connectivity (rsFC), another fMRI analysis technique, has proven to be an important method for examining the aging brain\textsuperscript{39}. Resting state functional connectivity measures low frequency oscillations in the blood-oxygen-level dependent (BOLD) signal while at rest\textsuperscript{40}. While these oscillations often occur in anatomically distinct brain regions, reliable patterns of correlated activity are believed to reflect functionally and/or structurally connected neural networks. Similar to rCBF, rsFC, between brain regions (e.g., hubs and nodes) and within particular neural networks,
declines with age and becomes increasingly dysregulated in cognitive decline\textsuperscript{39}. Interestingly, several studies suggest that exercise training improves rsFC within networks known to be most susceptible to age related and pathological decline\textsuperscript{41}.

1.3 Research Question

This study sought to identify the effects of acute exercise on executive function and resting state functional connectivity (rsFC) in older adults. We also aimed to determine the degree to which both sleep quality and exercise-induced changes in rsFC influenced executive function performance. To accomplish these goals, healthy older adults (aged 55-85) were recruited to participate.

1.4 Specific Aims

Aim #1: To identify the main and interactive effects of acute exercise and sleep quality on executive function.

H\textsubscript{1}: Acute exercise, compared to rest, would be associated with decreased functional brain activation in the anterior cingulate cortex (ACC) and increased functional activation in the superior and medial frontal gyri (SFG/MFG) and superior parietal lobule (SPL); and better behavioral performance during the executive function task.

H\textsubscript{2}: Worse sleep quality would be associated with increased functional brain activation (incongruent activation > congruent activation) in the ACC and decreased functional activation in the SFG/MFG and SPL, and poorer behavioral performance during the executive function task.
H₃: Individuals with worse sleep quality would garner greater exercise-induced improvements in functional brain activation and better behavioral performance during the executive function task.

**Aim #2: To assess the main and interactive effects of acute exercise and sleep quality on prefrontal rsFC.**

H₄: Acute exercise would be associated with decreased salience (SN) and central executive network (CEN) rsFC.

H₅: Worse sleep quality would be related to increased SN and CEN rsFC.

H₆: Individuals with worse sleep quality would experience greater exercise-induced decreases in SN and CEN rsFC.

**Exploratory Aim #3: To evaluate the mediating effects of exercise-altered rsFC on the relationship between sleep quality and exercise-induced changes in executive function.**

H₇: Exercise-altered prefrontal rsFC would mediate the relationship between sleep and exercise-induced changes in executive function activation and performance.

In order to test these hypotheses, healthy older adults (age 55-85) were recruited to participate in this study. For at least three consecutive days, preceding the first experimental condition, eligible participants wore an actigraphy device to objectively measure sleep quality. Participants were then exposed to 30-minutes of rest or exercise on separate days, using a within-subjects counterbalanced design. Directly after each condition, participants underwent both resting state and task-based functional MRI to assess rsFC and
executive function, respectively. Repeated measures ANOVA were used to evaluate the main and interactive effects of exercise and sleep quality on executive function and rsFC. Multivariate linear regression was used to assess the mediation effects of rsFC on the relationship between exercise and executive function.

Chapter 2: A Review of the Literature

2.1 Cognitive Decline and the Trajectory of AD

Cognitive decline typically occurs later in life and is among the major risk factors for AD\(^9,\,42\). Cognitive decline can affect any of the cognitive domains (memory, language, visuospatial ability, executive function)\(^9,\,43\). While these domains govern a range of cognitive and functional skills, they are not mutually exclusive, but rather work cohesively to complete complex tasks (conflict resolution, problem solve, inhibition of prepotent responses)\(^44\).

Executive function guides goal-oriented behavior\(^44,\,45\). As the supervisory cognitive faculty, executive function is believed to coordinate the actions of the other domains in an organized context-dependent way\(^45\). Executive function can be broadly divided into three constituent groups\(^46\) including set-shifting (the ability to shift between tasks and mental states); updating (the capacity to monitor and manipulate items in working memory); and inhibition (the ability to suppress habitual responses)\(^44,\,45\).

The prefrontal cortex (PFC) and complementary neural networks provide the infrastructure for executive function\(^46\). Despite some debate, most scientists
now concede that the frontal lobes are highly involved in, if not the seat of executive function. While this was not always the case, evidence-based research from both electrophysiological and neuroimaging studies have provided relatively strong support for this theory. The PFC composes a significant portion of the human brain (approximately 35%), which has been sub-divided into smaller anatomical regions. Executive function is supported by many of these areas including the dorsal and ventral aspects of the lateral PFC, the dorsomedial PFC (including anterior cingulate cortex), and orbitofrontal cortex. The PFC also shares cortico-cortical connections with the temporal, sensory, and parietal cortices and projects to many subcortical areas including the basal ganglia, thalamus, striatum, amygdala, and hippocampus. The diversity and density of these connections likely reflect the complex processes involved in goal-oriented behavior.

The central executive network (CEN) and salience network (SN) synergistically regulate executive function. Recent evidence suggests that discrete neural networks within (although not limited to) the PFC regulate higher-level cognitive control including many aspects of executive function. Namely, the CEN (key nodes: dorsolateral prefrontal and posterior parietal cortices) and salience network (key nodes: anterior insula and anterior cingulate cortices) exhibit strongly correlated functional activation during cognitive tasks. While these networks engage at the same time, they likely have disparate roles and responsibilities. Many neuroscientists believe the SN responds to environmental salience (sensory input of greatest importance) and transmits...
regulatory control signals\textsuperscript{48}. These signals are received by the CEN and help to guide and prioritize its actions. The CEN is believed to play a more direct role in executive function processes (working memory, shifting, inhibition)\textsuperscript{48}.

Age-related cognitive decline often manifests as executive dysfunction\textsuperscript{50}. Several studies have demonstrated that older adults consistently perform worse on tasks requiring attention and inhibitory control, compared to their younger counterparts\textsuperscript{44,45}. Morphological studies have shown that even in the absence of disease, brain atrophy begins early in life (~age 30), continues throughout adulthood (0.2\% per year), and is accelerated in old age\textsuperscript{50}. Cortical gray matter makes up the majority of this tissue loss and includes subtle yet progressive deterioration of the PFC and prefrontal neural networks (SN and CEN)\textsuperscript{50,51}. While age-related cognitive changes are problematic, they are one of the most frequent grievances in older adults, and are not necessarily pathologic or intractable\textsuperscript{52}.

The pathophysiological processes involved in AD likely begin decades before the clinical onset of dementia\textsuperscript{53,54}. The process of cognitive decline can be broadly divided into two stages, latent and prodromal AD\textsuperscript{42,55}. During the latent stage, the affected individual remains symptom-free, while in the prodromal stage, mild cognitive impairment (MCI) and behavioral changes begin to emerge\textsuperscript{56}. MCI is a clinical diagnosis defined by both subjective and objective impairment in at least one cognitive domain and is accompanied by functional independence\textsuperscript{9}. Individuals with MCI have an increased risk of progressing to dementia, with 40\% converting to AD over a four-year period, and 60\% showing AD-related pathology after death\textsuperscript{57,58}. Many pathological biomarkers start to
appear during the pre-dementia stages, including beta-amyloid (Aβ) aggregation, tau protein phosphorylation, increased neuroinflammation, and decreased rCBF and metabolism. These processes ultimately lead to brain tissue deterioration, severe memory impairment, and a complete loss of independence.

Increased Aβ and neurofibrillary tangles (NFTs) are the two most commonly cited hallmarks of AD. According to the Amyloid Cascade Hypothesis, amyloid plaque resulting from an over-production or insufficient clearance of Aβ, is the primary factor underlying AD etiology. Extracellular Aβ is composed of amino acids chains (peptides) that are derived from the amyloid precursor protein (APP). Dysfunctional APP processing (regulated by the presenilin gene) is thought to play a major role in the extracellular aggregation of Aβ. The accumulation of cortical amyloid is a common characteristic of AD in older adults, but is also consistently observed in individuals with Down’s syndrome and early-onset AD; each of which invariably leads to dementia.

These findings suggest that disrupted Aβ homeostasis likely plays a major role in the pathophysiological process of AD. Although the Amyloid Cascade Hypothesis has received much-warranted notoriety and support over the last two decades, many researchers still believe it is incomplete.

NFTs may promote neuronal death and lead to structural deterioration in AD. NFTs are composed of abnormally shaped phosphorylated microtubule-associated tau protein. This tau protein typically provides structural support for cytoskeletal microtubules, but in the case of NFTs, these microtubules essentially lose their structural integrity (during post-translational modification) and lead to
oddly shaped and misfolded protein tangles. The majority (95%) of AD-related tau pathology is found in unmyelinated gray matter, where brain tissue is primarily composed of neuronal cell bodies and dendrites. Tau pathology likely mediates neurodegeneration through a loss of microtubule regulation or increased toxic function via aberrant NFTs. In both scenarios tau pathology results in dysfunctional synaptic transmission and ultimately cell death.

Chronic inflammation exacerbates AD biomarkers. Although inflammation is the body’s natural immune response to injury and disease, recent evidence suggests that neuroinflammation may help drive the pathophysiological processes of AD. Glial cells (which make up the majority of brain tissue), regulate and support the neuronal environment. Microglia (the primary immune cells of the CNS) mediate the immune response by scavenging and removing foreign invaders. In AD, microglia respond to Aβ aggregates by assuming various conformational changes, which prepares them to confront and clear pathology. Microglia possess various receptors to which Aβ fibrils bind (CD14, CD36, CD47). When Aβ binds to these receptors, microglia release pro-inflammatory cytokines including Interleukin-1 (IL-1), IL-6, I-12, and tumor necrosis factor-α (TNF-α). Under normal conditions, microglia can remove Aβ through phagocytosis (metabolic removal). However, Aβ cannot be sufficiently cleared when it is over produced, such as the case in AD. This leads to a toxic environment sustained by the perpetual release of pro-inflammatory cytokines. Chronic inflammation not only impairs microglial function but also affects
neighboring neural cells (neurons and glia) and potentially disrupts microtubule tau protein phosphorylation, which accelerates the development of NFTs.\textsuperscript{63}

Altered patterns of rCBF may be a viable biomarker for risk and detection of AD.\textsuperscript{55} While Aβ and tau protein assessment techniques seem to be sensitive to the earliest signs of AD,\textsuperscript{55} a number of studies have demonstrated that altered patterns of cerebral perfusion (particularly regional hypoperfusion) also provide a reliable index of AD pathology.

Cortical rCBF is reduced in AD compared to healthy controls.\textsuperscript{70} In one of the first studies of its kind, Alsop and Detre (2000) compared rCBF in AD patients vs. healthy age-matched controls using a non-invasive measuring technique called arterial spin labeling (ASL). They found that cortical perfusion, particularly in the posterior cingulate cortex, was significantly lower among AD patients. Additionally, regional hypoperfusion was associated with the severity of cognitive impairment, assessed by the Mini Mental Status Exam (MMSE).\textsuperscript{70} Since this time ASL sequencing techniques and signal-to-noise ratio have been significantly improved.\textsuperscript{71} Continuous (CASL) and pseudo continuous (pCASL) labeling schemes, in addition to background suppression and 3D readouts have greatly enhanced the sensitivity and application of this imaging method.\textsuperscript{71}

Altered patterns of rCBF are associated with MCI and may be moderated by the APOE (apolipoprotein-E) genotype.\textsuperscript{72} In a similar study, Wierenga and colleagues (2012) used ASL to examine the interrelationship between the APOE genotype, rCBF, and cognition in MCI and healthy older adults. APOE is a polymorphic gene that helps regulate cholesterol transport and metabolism within
the CNS\textsuperscript{73}. The APOE gene has three alleles, E2, E3, and E4; with E4 being the strongest genetic risk factor for AD\textsuperscript{73}. Their findings showed a significant interaction between the APOE E4 genotype and cognition on rCBF in three brain regions. Interestingly, compared to healthy E4 carriers, those with MCI had decreased rCBF in the left parahippocampus/fusiform gyrus. Conversely, this trend was reversed in the right middle and left medial frontal gyri, as those with MCI had greater rCBF in these areas. These results suggest that specific patterns of cerebral perfusion may be indicative of; 1) the pathological time course of MCI, 2) a neurovascular compensatory mechanism during MCI, and 3) the potential for therapeutic intervention during MCI\textsuperscript{72}. Exercise has been shown to play an important role in bolstering the compensatory mechanisms that may be involved in MCI, and is likely a viable intervention strategy to prevent and delay the onset of dementia\textsuperscript{74}.

Reduced regional rCBF is associated with MCI and AD\textsuperscript{75}. In a recent study, Binnewijzend (2015) collected AD related-physiological data (ASL, Aβ-40 and 42, and CSF-tau) in individuals with subjective cognitive complaints. The study sample was divided into three groups: healthy control, those with MCI, and AD patients using both cognitive (MMSE) and physiological assessments (Aβ-42, CSF-tau). Their results revealed that AD severity was correlated with lower rCBF in all cortical areas, with the posterior cingulate and parietal cortices displaying the most notable diminutions. Moreover, rCBF in MCI was significantly lower than controls but greater than rCBF in AD\textsuperscript{75}. The posterior cingulate and lateral parietal cortices are components of the DMN. While the DMN is particularly
vulnerable to AD, it is also receptive to the beneficial effects of aerobic exercise.\textsuperscript{41}

Altered patterns of rCBF may present a unique and perhaps diagnostic neural signature for MCI and AD.\textsuperscript{55,70,76} ASL, which uses endogenous blood water as a magnetic contrast medium (rather than radioactive ligands), measures rCBF as accurately as more expensive (and invasive) techniques like positron emissions tomography (PET) and single photon emissions computed tomography (SPECT). Assessing cerebral perfusion is a promising method for both identifying those in the early-stages of AD and rapidly expanding our understanding of the physiological changes that occur during AD progression.\textsuperscript{71} Aerobic exercise training and acute exercise have been shown to alter patterns of rCBF and could potentially help reregulate the perfusion alterations that occur along the trajectory of AD.\textsuperscript{77–79}

In summary, increasing age is the greatest risk factor for cognitive decline and AD. However, even in the absence of disease cognitive deficits can occur. These deficits are often exhibited by executive dysfunction and are preceded by cerebral perfusion and structural changes. The pathophysiological biomarkers of AD (including Aβ, NFTs, neuroinflammation, and altered patterns of rCBF) begin decades before the clinical onset of dementia. ASL-MRI is a valid and economically feasible method for assessing cerebral perfusion. Due to the fact that altered patterns of rCBF become apparent in MCI, ASL-MRI should be used to identify and treat individuals at greatest risk for developing dementia.
Resting state functional connectivity (rsFC) may also be a promising biomarker for the detection of MCI and risk of AD\textsuperscript{39,80}. This non-invasive technique measures correlational patterns of low frequency brain activity and several studies have repeatedly demonstrated distinct and predictable neural patterns of activity in both aging and diseased populations\textsuperscript{81,82}. The following classic studies (among others) have helped expand our understanding of the role for rsFC in cognitive decline and AD.

Wang and colleagues (2007) examined whole brain rsFC in 17 healthy older adults and 17 individuals with AD (~70 years old). Their objective was to identify discrete rsFC activity patterns between the two groups. Their findings revealed that individuals with AD exhibited decreased connectivity between the medial prefrontal and parietal cortices (default mode network; DMN), and that this anterior-posterior disconnection separated those with AD from the age-matched controls\textsuperscript{82}.

In a similar study, Greicius et al. (2004) used a processing technique called independent components analysis to specifically compare connectivity changes within the DMN in 13 individuals with mild AD and 13 healthy age-matched controls. However, this investigation examined deactivation patterns in the presence of a task. The data unveiled three primary findings: 1) strong connectivity was observed between the hippocampus and the posterior cingulate cortex (PCC), implying that these two brain regions are functionally connected and are likely involved in similar cognitive processes; 2) individuals with AD had notably decreased connectivity between the hippocampus and PCC; and 3) DMN
functional connectivity may be a useful diagnostic tool for individuals at increased risk of AD

In 2005, Rombouts et al., also measured functional connectivity (in the presence of a task) in three groups: 1) older adults with MCI (n = 28), 2), older adults with mild AD (n = 8), and 3) age-matched controls (n = 41). Results demonstrated between group differences in DMN deactivation. Healthy controls showed greater DMN deactivation than those with MCI, and individuals with MCI showed greater DMN deactivation than AD, suggesting an incremental deficit corresponding to degree of cognitive decline.

In summary, both rsFC and task-based functional connectivity are valid and reliable markers of intrinsic patterns of neural activity. While rsFC analysis techniques are relatively new, a growing body of research has increased our understanding about the brain’s functional organization. Functional connectivity is a promising biomarker of both health and disease and may soon be used as a diagnostic tool for identifying individuals at increased risk of AD.

2.2 The Interaction between Exercise and Cognition

Over the last two decades a large (and still accumulating) body of evidence supports the notion that acute and chronic exercise improve cognitive performance and brain health. While many studies have shown that increased aerobic fitness positively affect response time and goal-oriented behavior, exercise training appears to exert a greater influence on more long-term cognitive processes. In addition, and perhaps more importantly, increased physical activity and exercise training have been shown to mitigate the
incidence of both age-related and pathological cognitive decline. In the following section a number of studies are summarized, examining young and older adults, to introduce the reader to this literature. At the end of the section, several potential mechanisms by which exercise may impact the brain are described, and directions for future research are discussed.

Acute moderate intensity exercise improves response time and inhibition in young adults. In 1996, Hogervorst examined the effects of acute moderate intensity aerobic exercise (70% of $\dot{V}O_2_{\text{max}}$ for one hour) on select aspects of cognition in 15 highly trained young adults (age ~24). Before and immediately after the exercise session, participants underwent three cognitive assessments including the Finger Tap Task (motor function), a Simple Response Time Task, and the Stroop Color-Word Interference Task (executive function). Acute exercise improved performance on the Simple Response Time and Stroop Tasks, while showing no effect on Finger Tapping. The authors concluded that improvements in performance might have been the result of increased sympathetic activation (leading to elevated arousal and heart rate). While these are interesting and important findings, the physiological mechanisms underlying these effects on the brain are unclear. Employing a neuroimaging technique during this experiment would have provided more mechanistic insight into this relationship.

Acute moderate intensity exercise improves performance and resource allocation during the Flanker Task in young adults. Using electroencephalography (EEG), Hillman (2003) assessed the effects of acute
exercise on event related potential (ERP-P3 waveform) and performance during the Erikson Flanker Task (executive function). Twenty young adults (age ~20) participated in the study. Both before and immediately after a single session of moderate intensity treadmill exercise (~84% of HR$_{\text{max}}$ for 30 minutes) participants engaged in the Flanker Task, while simultaneously being assessed by EEG. The results demonstrated that acute exercise increased P3 amplitude (response confidence) but not P3 latency (stimulus evaluation) during incongruent trials of the Flanker Task. These findings suggest that acute exercise may improve executive function performance by perhaps altering the allocation of executive function-related neural resources. However, this experiment was conducted in young individuals only and it is not clear if the same effects would be observed in an older population.

Acute moderate intensity exercise selectively improves delayed memory performance, while showing minimal effects on executive function. In 2008, Coles and colleagues tested the effects of acute moderate intensity bicycle exercise (60% of VO$_{2\text{max}}$ for 40 minutes) on cognitive performance in 18 young adults (age ~22). Using a counterbalanced repeated measures design, participants underwent tests of executive function (set-shifting, working-memory) and delayed-memory. Participants were tested before and after three different conditions including: bicycle exercise, rest on a bicycle, and rest in a chair. Their findings showed that exercise had no significant effect on the two executive function tests, but selectively improved delayed-memory performance; implying that memory might be preferentially enhanced by acute exercise. While these
results are in opposition to the hypothesis that acute exercise preferentially enhances executive function, it may be that some aspects of memory overlap with executive function\textsuperscript{94}, especially working memory. Additionally, the results from this study must be interpreted cautiously, as the sample size was small and the study was likely under powered (n=18).

The electrocortical underpinnings of exercise-induced improvements in Flanker Task performance may be age-dependent\textsuperscript{95}. In 2009, Kamijo explored the effects of acute exercise on executive function across the lifespan. This study included 12 older (age 60-74) and 12 younger (age 19-25) adults and evaluated task performance at baseline and immediately after exercise on two separate occasions. In a counterbalanced order, participants were exposed to both low (30\% of VO\textsubscript{2}\textsubscript{max}) and moderate (50\% of VO\textsubscript{2}\textsubscript{max}) intensity exercise. Akin to the Hillman (2003) study, electrocortical activity was assessed using EEG during the Flanker Task. Moderate, but not low, intensity exercise improved P3 latency in both groups. However, significant improvements in P3 amplitude were only observed in the young adults. These results suggest that although acute exercise may enhance executive function across the lifespan, the electrocortical underpinnings of these improvements might differ by age\textsuperscript{95}. This is an important study as very few acute exercise investigations have been conducted in older adults. Although the temporal resolution of EEG is very good (on the order of milliseconds), a neuroimaging technique with greater spatial resolution (functional MRI, positron emission tomography) would improve our understanding of these potential age differences. However, like the previous
study, these results must be interpreted carefully as both groups have a very small sample size.

Acute moderate intensity exercise has persistent and preferential effects on executive function. In a recent randomized controlled study (2015), Basso measured the effects of acute moderate to high intensity exercise (85% HRmax for 50 minutes) on cognition in 85 younger adults (age ~22). Participants were randomly assigned to either the exercise or seated rest control group. After the intervention participants underwent a battery of cognitive tests at random intervals (30, 60, 90, or 120 minutes). Tasks were chosen to specifically elicit and measure prefrontal cortex mediated executive function (a composite score of: Stroop Color-word, Symbol Digit Modalities, Digit Span, Trails Making, Verbal Fluency) and hippocampal-mediated memory (a composite score of: Visual Retention and Hopkins Verbal Learning). Results revealed that exercise selectively improved performance in executive function, while showing no significant effect on memory. Moreover, these effects were significant at all time-points. The authors conclude that acute exercise preferentially enhances executive function performance. This was a well-executed study, but could have been more informative if it incorporated some form of neuroimaging technique. Both perfusion-weighted and functional (task-based) MRI would have been ideal modalities to employ, as they provide useful insight into the neurovascular changes associated with improved behavioral performance.

Higher aerobic fitness in older adults is associated with better performance and more efficient functional brain activation during the Flanker
Task. In a two-part study, Colcombe (2004) and colleagues assessed 1) the effect of fitness on cognitive function in older adults, and 2) the effects of exercise training on cognition in older adults. During part one (cross-sectional), 41 healthy older adults (age 55-79) were classified as either high or low fitness using an estimate of their VO2\text{max} (Rockport 1-mile Walk). Participants then performed the Flanker Task while undergoing a functional MRI scan. In addition to having better behavioral performance on the Flanker Task, higher fit individuals, compared to less fit individuals, displayed greater functional brain activation (incongruent > congruent) in the superior frontal and medial frontal gyri, while demonstrating less functional brain activation in the anterior cingulate cortex. This study used fMRI to elucidate brain activation during the functional task, however it remains unclear if differences in rCBF between the two groups precipitated differences in functional brain activation.

Aerobic exercise training improves executive function performance and enhances functional brain activation during conflict resolution. During part two (longitudinal) of this study, 29 older adults (age 58-77) were randomly assigned to an exercise or stretching control group. During the intervention both groups met three times per week for six months. Exercise training was designed to increase aerobic fitness and began at an intensity of 40-50% of heart rate reserve (HRR), lasting just 10-15 minutes. Training intensity and duration gradually increased until participants were exercising at an intensity of 60-70% of HRR for 40-45 minutes. Using the same fMRI protocol as study one, participants were scanned before and immediately after the 6-month intervention. Results
revealed a significant increase in aerobic fitness (10.2% of VO₂ max) for the exercise group. Similar to the high fit subjects in the cross-sectional study (part one), exercise-trained individuals experienced greater improvements in Flanker Task performance than those in the stretching group. Additionally, exercise-trained individuals also demonstrated patterns of functional brain activation resembling the higher fit group, with greater activation (incongruent > congruent) in the superior frontal and medial frontal gyri, and less activation in the anterior cingulate cortex. The authors concluded that these findings expanded the literature by elucidating exercise-induced neurovascular alterations, which parallel improved cognitive performance. While increased fitness and chronic exercise appear to have similar effects on executive function performance and patterns of functional activation, little is known about how a single bout of exercise or variable sleep quality might affect these outcomes.

Consistent and prolonged aerobic exercise training increases hippocampal volume and is associated with better spatial memory in older adults. In 2009, Erickson and colleagues randomly assigned 120 older adults (age 50-80) to participate in either 12-months of aerobic exercise (walking) or stretching and toning. Exercise intensity and duration gradually increased throughout the program, beginning with light exercise (50-60% of HRR lasting just 10 minutes) and increasing each session until participants were able to exercise at 60-75% of HRR for 40 minutes. Before, during, and after the intervention, both groups underwent structural MRI (hippocampal volume assessment); blood draws for brain derived neurotrophic factor (BDNF), and
spatial memory assessments. In addition to improving aerobic fitness (7.78% of \( \dot{V}O_2\text{max} \)) results revealed that the exercise intervention significantly increased the size of the left (2.12%) and right hippocampus (1.97%), while hippocampal volume decreased in the stretching control group (1.40% and 1.43%, for left and right hippocampus, respectively). The researchers also found a greater change in serum BDNF to be associated with a greater increase in hippocampal volume. Moreover, baseline fitness was associated with better spatial memory performance. These results suggest that the hippocampus, a brain area that is highly involved in learning and memory, remains modifiable across the lifespan. This is a provocative study demonstrating the protective effects of exercise on brain function. However, many neuroscientists would agree, that alterations in rCBF and cerebral metabolism precede structural decline. Therefore, an important next step is to measure rCBF in older adults before and after exercise training, and also before and after acute exercise.

Consistent resistance training improves executive function in older women. In 2012, Liu-Ambrose tested the effects of resistance training on cognitive function in 155 older women (age 65-75). In this 12-month randomized controlled trial, participants were assigned to one of three groups: 1) resistance training one time per week, 2) resistance training two times per week, or 3) balance and toning two times per week. Before, during, and after the exercise intervention executive function was assessed (Stroop Color-word, Trails Making, and Digit Span). Interestingly, results showed that both one and two days of resistance training per week were associated with a 12.6% and 10.9%
improvement in executive function, respectively. These results suggest that resistance training, which can often be more feasible for an older and sedentary population, significantly improved executive function\textsuperscript{97}. This is an important study as most empirical investigations of exercise training have exclusively focused on aerobic exercise. While more studies should focus on this exercise modality, new studies should also consider the effects of single session of resistance or aerobic exercise on executive function.

Low intensity exercise training may improve executive function in those with MCI\textsuperscript{98}. In one of the first studies of its kind, Scherder (2005) randomly assigned 43 older adults (age 76-94) with MCI to one of three experimental groups: 1) self-paced (assisted) walking, 2) hand/face exercises, 3) and sedentary control. The two intervention groups met three times weekly (30 minutes per session) for six weeks. The hand/face intervention involved bending/stretching fingers and performing facial expression (rehabilitative exercises for paralysis). All participants underwent cognitive assessments before and after the intervention. Assessments included: executive function (Verbal Fluency, Trails Making, and Digit Span) and memory (Visual Memory Span, Verbal Learning and Memory, Face Recognition, and Picture Recognition). While the effects of exercise favored executive function, the improvements were not significant. These results suggest that exercise may be beneficial for those with MCI\textsuperscript{98}. Interestingly, MCI is also associated with increased risk for sleep impairments\textsuperscript{30}. Evidence suggests that exercise may lead to improved sleep
quality\textsuperscript{99}, which in turn may lead to improved cognitive functioning\textsuperscript{100}, however mediation models have yet to explore these relationships.

Aerobic exercise training improves memory performance and neural efficiency in MCI and healthy older adults\textsuperscript{33}. In another recent study, Smith and colleagues (2013) examined the effects of a 12-week aerobic exercise (walking) intervention on cognitive function in individuals with MCI (n=17) and healthy older adults (n=18) between the ages of 60-88. The exercise intervention consisted of four 30-minute sessions of moderate intensity (50-60\% of HRR) exercise per week. Over the course of the intervention, both groups significantly improved their aerobic fitness (\textasciitilde10\% of \(\dot{V}O_2\text{peak}\)). Before and after the exercise intervention all participants underwent fMRI scans during which they were exposed to a semantic memory discrimination task. Interestingly, 11 brain areas that showed semantic memory-related activation at baseline demonstrated significant decreases in activation after the intervention, with no change in memory performance. Moreover, those with MCI also displayed a significant improvement in a list-learning task. These findings suggest that exercise training may preserve cognitive resources by improving semantic memory-related neural efficiency, and that these improvements prevail even in the face of cognitive decline\textsuperscript{33}. These are important results as they elucidate the possibility that less functional activation may indicate more efficient neural activity. Similar to the study above, understanding the influence of sleep on the relationship between exercise and cognition is an important next step, particularly for those at risk for MCI and dementia.
Exercise-related research in animals has greatly expanded our understanding of the cellular and molecular mechanisms that drive the neurobiological effects of exercise\textsuperscript{101}. In 1996, Neeper and colleagues examined the short-term effects (2, 4, and 7 nights) of voluntary wheel running on BDNF and nerve growth factor (NGF) in young (age 3-4 months) mice. Their results demonstrated that short-term exercise increased neurotrophic (BDNF and NGF) mRNA expression in the hippocampus (CA1 and CA4) and cortex (layers II and III), with NGF also being upregulated in the dentate gyrus\textsuperscript{102}. In line with these findings, Kesslak (1998) showed that augmented BDNF mRNA in the hippocampus was associated with better spatial memory performance (water-maze)\textsuperscript{103}. Moreover, van Praag found that exercise training increased neurogenesis (creation of new neurons) in the dentate gyrus, while also improving spatial memory and long-term potentiation (the strengthening of synaptic connections)\textsuperscript{104}. In addition to BDNF and NGF, insulin-like growth factor (IGF-1) and vascular endothelial growth factor (VEGF) also mediate the effect of exercise on the brain and its microvasculature\textsuperscript{105}. In 2007, Cotman described how neurotrophins (specifically BDNF, VEGF, and IGF-1) work together to facilitate learning, neurogenesis, and angiogenesis\textsuperscript{106}. In a two-part study, Pereira and colleagues (2007) provided support for this finding. First, they showed that two weeks of exercise training in mice selectively increased cerebral blood volume (CBV) and cellular proliferation in the dentate gyrus. They then tested this model in humans (age 21-45) and demonstrated that exercise training (1 hour, 4 times per week, for 12 weeks) increased hippocampal CBV (using
optimized MRI) and also improved memory performance (Rey Auditory Verbal Learning Task). Suggesting that increased CBV may be necessary for neurogenesis (and microvascular changes), and that these processes combined influence cognitive performance.

While much progress has been made, many questions about the impact of exercise on cognition remain unanswered. First, it appears that moderate intensity exercise generally provides a beneficial effect on brain function, however the optimal duration and type of exercise remains unclear. Second, many laboratories have focused on the effects of exercise on cognitive behavioral performance in humans, while neglecting to delve into the potential neural underpinnings that support better performance. Third, very few studies have examined the potential mediating role that sleep may play in the exercise and cognition relationship. Fourth, an effort must be made to better understand the effects of acute exercise in older adults, as these individuals may respond differently to exercise (e.g. hemodynamic and neurotrophic response). Finally, we must utilize multi-modal neuroimaging techniques (functional MRI, perfusion-weighted MRI, EEG) to gain a better understanding of the potential mechanisms that drive the effects of exercise on cognition.

In summary, over the last twenty years a large body of evidence supports the hypothesis that exercise (chronic and acute) improves cognitive performance. While acute exercise has been shown to improve response time and executive function, exercise training appears to benefit long-term brain health and memory processes. EEG and MRI have helped elucidate some of the neural mechanisms
underlying these effects. Specifically neuroimaging studies examining exercise-training interventions in older adults have been shown to increase hippocampal volume and improve spatial memory. Aerobic exercise also improves memory and neural efficiency in those MCI. In addition to high and improved levels of aerobic fitness, resistance training has also been found to improve executive function. Exercise-induced increases in neurotrophic factors and neurotransmitters likely mediate the relationship between exercise and cognition, but much remains to be learned.

2.3 Sleep Across the Lifespan

Sleep is a fundamental component of life for all animal species\textsuperscript{107}. Human beings spend almost a third of their lives asleep\textsuperscript{108}. Despite the vast amount of time we dedicate towards this behavior, a single function for sleep remains elusive\textsuperscript{108}. This is likely because sleep does not serve a single purpose, but rather multiple functions, ranging from tissue repair to cognitive performance\textsuperscript{108,109}.

Sleep can be divided into two distinct types, non-rapid eye movement (NREM) and rapid eye movement (REM)\textsuperscript{110}. The former can be further separated into four stages (stages one, two, three, and four), with each stage being characterized by specific electroencephalographic/polysomnographic (EEG/PSG) waveforms\textsuperscript{111}. The arousal threshold (awakening point) increases as waveforms progress from theta (low amplitude high frequency, stage one and two sleep) to delta waves (high amplitude low frequency, stage three and four)\textsuperscript{112}. During NREM sleep the brain maintains regulatory function, showing only periodic bursts
of higher frequency activity called K-complexes and sleep spindles\textsuperscript{112}. Although the body (skeletal muscle) retains muscle tonus and voluntary mobility during NREM, limb and body movement is normally low\textsuperscript{110}. Conversely, REM sleep is defined by dreaming, bursts of rapid eye movements, partial paralysis of skeletal muscle, and cardiorespiratory irregularities\textsuperscript{110}.

Sleep patterns cycle throughout the night\textsuperscript{113}. A young healthy adult with a normal sleep schedule typically sleeps eight hours per night\textsuperscript{114}. These individuals will enter sleep through NREM, descend successively through each of the four stages, and enter REM sleep approximately 80 minutes after sleep onset\textsuperscript{110}. NREM and REM then cycle throughout the night (every ~90 minutes), with stage one acting as a transitional period between these two types of sleep\textsuperscript{110}. Stage one sleep is short and light with a very low threshold for arousal. Stage two sleep is longer and\textsuperscript{111} contains sleep spindles and K-complexes, which have been associated with memory processing\textsuperscript{113,115}. Stage three and four contain slow wave sleep (SWS), and are defined by low frequency electrocortical activity\textsuperscript{111}. SWS is also known as deep sleep as the arousal threshold is much greater than stages one and two\textsuperscript{110}. SWS has also been strongly implicated in memory consolidation and cerebral metabolite clearance\textsuperscript{116,117}. Small movements and muscle twitches normally indicate the ascension through NREM back towards REM. NREM sleep makes up approximately 75\% of TST with REM making up the remaining 25\%. During the early part (first third) of the sleep period NREM composes the greatest portion of the sleep cycle, whereas in the
later part (last third) of the sleep period REM constitutes the greatest portion of the cycle\textsuperscript{110}.

Sleep architecture and duration change considerably throughout the night\textsuperscript{118}. At the beginning of the night REM sleep is short (~5-10 minutes), but increases successively with each cycle\textsuperscript{15}. As the sleep cycles oscillate throughout the night, REM sleep expands, while levels of SWS shrink\textsuperscript{110}. This shrinkage of SWS is related to an increase in stage two. The duration of each sleep cycle increases as the night progresses, growing from approximately 80 minutes in the first cycle to 90 minutes in subsequent cycles\textsuperscript{110}. Slow wave sleep is most densely concentrated in the beginning of the night and is believed to be a consequence of previous wakefulness\textsuperscript{119}. Wakefulness increases the accumulation of metabolic waste (adenosine, A\textbeta)\textsuperscript{120,121}, which increases sleep pressure and triggers the homeostatic sleep drive to maintain balance (which helps eliminate metabolite waste and “clean” the cerebral environment)\textsuperscript{117,120,121}. REM sleep dominates the latter portions of the night and is thought to reflect circadian rhythmicity, which parallels body temperature and troughs in the early morning hours\textsuperscript{110}. Circadian rhythms regulate both sleep and wakefulness according to an ~24 hour clock (solar day-night cycle), and are controlled by the suprachiasmatic nucleus (SCN) near the anterior hypothalamus\textsuperscript{122}. The homeostatic sleep drive and the circadian system govern the sleep/wake cycle. The circadian sleep gates are believed to regulate both the wake-maintenance (2-3 hours before sleep) and sleep-maintenance zones (2-3 hours before awakening)\textsuperscript{123–125}, essentially helping to maintain wakefulness during the
afternoon lull and to stay asleep during the early morning hours. These two sleep systems work together to regulate sleep patterns, structure, and composition\textsuperscript{122,124,125}.

In summary, sleep is a fundamental part of life and is essential to brain and body health. There are two broad types of sleep; NREM and REM. NREM sleep is further divided into stages one through four. Stages one and two are light sleep, while three and four are considered deep sleep, which is characterized by slow oscillating neocortical waves. Human sleep contains 90-minute cycles composed of both NREM and REM sleep. Sleep stages and composition oscillate throughout the night with greater SWS in the first third of the sleep period and REM becoming denser in the last third of the sleep period.

\textbf{2.4 Sleep, Cognition, and the Aging Brain}

Older adults report greater sleep problems than any other age group, with the most commonly reported issues being the inability to initiate or maintain sleep, decreased TST, and greater wake after sleep onset (WASO)\textsuperscript{126–128}. Moreover, older adults have the highest rates of sleep disordered breathing (SDB; or sleep apnea is characterized by periodic respiratory cessations often accompanied by reduced blood oxygen saturation), advanced circadian rhythmicity, and decreased amounts of SWS\textsuperscript{116,129,130}.

Empirical evidence suggests that poor sleep quality (e.g., short sleep duration and frequent awakenings) is a major risk factor for cognitive decline in older adults\textsuperscript{12,30,131}. In fact, community-dwelling individuals with poor sleep quality performed worse on tests of working memory and abstract problem solving\textsuperscript{132}.
Additionally, prospective studies have shown poor sleep quality to be associated with two to four times the risk of cognitive impairment\textsuperscript{30,133–136}. SDB has also been linked to accelerated cognitive decline\textsuperscript{16,30,129,137}. Findings from a structural neuroimaging study revealed that individuals with moderate to severe SDB, not only had decreased hippocampal brain volume, but also concomitant deficits in recall, inhibition, and working memory compared to healthy controls\textsuperscript{138}. Moreover, cross-sectional studies examining circadian rhythms have found increased sleep fragmentation to be associated with decreased processing speed, and executive function\textsuperscript{123,130,139}. While another study recently showed disruption of the sleep maintenance zone to be mainly associated with MCI\textsuperscript{140}.

Sleep fragmentation increases the risk of cognitive decline and AD in older adults\textsuperscript{12}. In 2013, Lim et al. conducted a study to determine if sleep fragmentation (measured by 10 consecutive days of actigraphy) at baseline would be associated with cognitive decline and/or AD at follow-up approximately 3.3 years later. Data were collected from 737 participants (age ~82) and included annual neuropsychological testing. At the end of the study 97 individuals developed AD, and those with AD also had greater chances of having fragmented sleep. In fact, those individuals who were above the 90\textsuperscript{th} percentile for sleep fragmentation had 1.5 times the risk of developing AD, compared to those in the 10\textsuperscript{th} percentile. These are important findings as sleep fragmentation increases with age. Separately, exercise has been shown to reduce sleep disruptions and improve cognitive performance but few studies have examined these exposures together. Understanding this interrelationship is an important next step.
Poor sleep quality, but not quantity is associated with increased AD pathology\textsuperscript{141}. In a similar study, Yo-EI (2013) evaluated whether or not pre-clinical AD was associated with poor sleep quality or quantity in 142 older adults without cognitive decline or AD (age 45-75), half of which had a family history of late-onset AD. The primary outcome measures were: SE (sleep quality), TST (sleep quantity), and CSF-42 (marker of Aβ burden). Almost a quarter of the study sample (22.5\%) was determined to have increased Aβ deposition. This group also had significantly lower SE, compared to those with less Aβ deposition. TST was not different between the two groups. Both sleep and exercise have been shown to reduce the Aβ burden\textsuperscript{117,142}. Future studies examining sleep and Aβ burden in older adults should also explore the data for differences in daily activity, or at least inquire about physical activity level as these main variables likely work together to improve brain health\textsuperscript{123}.

Subjective and objective measures of sleep are not perfectly correlated\textsuperscript{143}. In 2008, van den Berg examined 969 older participants (age 57-97) enrolled in the Rotterdam Study to determine the association between self-reported sleep and actigraphy measured sleep over six consecutive nights. Subjective and objective TST were the primary outcome variables. Approximately 34\% of participants self-reported a TST that was at significantly different from their objective TST (≥ 1 hour), and this discrepancy was most apparent in older men and those with cognitive and functional impairment. It was also the case that those with poor subjective sleep quality also had longer actigraphy measured TST. Additionally, those with poor subjective sleep quality also reported shorter
TST than was measured by actigraphy\textsuperscript{143}. The authors conclude that whenever possible, subjective and objective sleep data should be collected, especially in older adults. In general, people (cognitively intact or impaired) tend to over or under report their TST, which leads to non-differential misclassification and reduces the chance of detecting true effects.

Subjective sleep measurements are more prone to error in older adults with cognitive impairment\textsuperscript{144}. In a similar study, Anderson (2013) compared subjective sleep (measured by the PSQI and the Epworth Sleepiness Scale) to objectively measured sleep-wake patterns (actigraphy 5-7 days) in 421 older adults (age 87-89). Their findings demonstrated significant differences between subjective and objective measures of sleep and these differences were greater in individuals with cognitive and functional impairment\textsuperscript{144}.

Obstructive sleep apnea (OSA) may accelerate brain atrophy and decrease cognitive performance\textsuperscript{145}. In 2011, Torelli and colleagues explored the interrelationship between obstructive sleep apnea, brain volume, and cognitive performance in 16 older adults (age ~ 56) with clinical obstructive sleep apnea and 14 healthy older adults (58). Using structural MRI, their results demonstrated that individuals with obstructive sleep apnea had significant atrophy of the right hippocampus, bilateral caudate, and total gray matter, compared to controls. Patients also performed markedly worse in tests of memory (Rey Auditory Verbal Learning Test; RAVLT) and executive function (Stroop and Digit Span Backwards)\textsuperscript{145}. Again, it would have been interesting to know if rCBF or physical activity differed between the two groups, as increases in these variables have
been associated with better brain health and cognitive outcomes\textsuperscript{38}. This dissertation project begins to address these questions by examining the interrelationship between exercise-induced alterations in rsFC (which likely precede structural decline) and executive function older adults with variable objective sleep quality.

Sleep disordered breathing and nocturnal hypoxia increase the risk of cognitive decline in older adults\textsuperscript{129,137}. Blackwell (2015) conducted a study of 2,636 cognitively normal older men (~age 76) to determine the associations between SDB and cognitive decline over 3.4 years. PSG was used to determine oxygen saturation index and apnea-hypopnea index and the modified MMSE and Trails-Making Test B were used to determine cognitive decline. Hypoxia during sleep was moderately associated with cognitive decline (1\% of total sleep time at < 90\% $\text{SaO}_2$)\textsuperscript{129}. Recently, a randomized controlled trial showed that combined aerobic and resistance exercise training (moderate intensity four times per week for 12 weeks) in young and middle aged adults (18-55) significantly reduced OSA severity\textsuperscript{146}. With this knowledge, it would have been interesting to know if baseline physical activity levels were different from the follow-up time-point, and if decreased activity was related to the development of OSA.

Reduced SWS activity and prefrontal atrophy mediate the relationship between increasing age and impaired sleep-dependent memory consolidation\textsuperscript{147}. In 2013, Mander and colleagues explored the complex relationship between age-related brain atrophy, decreased slow wave activity during non-rapid eye movement sleep, and sleep-dependent episodic memory consolidation. Eighteen
healthy older adults (age ~72) and younger adults (age ~20) were trained and tested in a memory consolidation task prior to sleep. Participant sleep was then monitored for eight hours using PSG. Within two hours of waking, participants underwent structural and functional MRI. While in the scanner, they were presented with the memory task from the night before to measure sleep-dependent episodic memory. Compared to younger individuals, older adults had significant brain atrophy in the medial prefrontal cortex. Additionally, brain atrophy was related to decreased levels of neural activity during SWS, and poorer sleep-dependent memory consolidation. Mediation analyses demonstrated that SWS activity mediated the relationship between brain atrophy and impaired memory consolidation\textsuperscript{147}. Exercise training increases whole brain and hippocampal volume in older adults, and these effects are paralleled by improvements in memory performance\textsuperscript{51,148}. However, this study did not explore the effects of exercise or fitness in either the younger and older groups. This is an important next step as increased physical activity and exercise have been shown to improve objective sleep quality in older adults\textsuperscript{99}

\textit{Aβ} is likely regulated by the sleep-wake cycle\textsuperscript{120}. Kang (2009) used microdialysis to examine fluctuating \textit{Aβ} levels in the interstitial fluid of mice in vivo. Findings revealed that \textit{Aβ} levels were positively associated awake time and negatively associated with sleep time. They also found that sleep deprivation caused substantial increases in \textit{Aβ}, which were immediately reversed with the reintroduction of sleep\textsuperscript{120}. Although this is a very important finding, it will be important to understand how insufficient \textit{Aβ} clearance during sleep directly
affects cognition, and whether or not better clearance enhances cognitive performance.

Sleep may restore brain function by “cleaning” the cerebral environment\textsuperscript{117}. In 2013, Xie also found that the sleep-wake cycle regulates metabolite clearance from the brain’s interstitial fluid. CSF influx around the cerebrovasculature has been found to increase during sleep and promote the clearance Aβ and Tau protein oligomers. Using two-photon imaging, they found that natural sleep and anesthesia increase interstitial space by as much as 60\% allowing for a convective transfer of metabolic waste between the interstitial fluid and the CSF\textsuperscript{117}.

Insufficient sleep is associated with impaired Aβ production and/or clearance in older adults\textsuperscript{121}. In a cross-sectional analysis of 70 older adults (age \textasciitilde 76) from the Baltimore Longitudinal Study of Aging, Spira and colleagues (2013) examined the association between subjective sleep quality (Women’s Health Initiative Insomnia Rating Scale) and Aβ burden (carbon 11–labeled Pittsburgh compound B positron emission tomography). Results demonstrated that self-reported short sleep was associated with increased Aβ deposition in the cortex and precuneus\textsuperscript{121}.

Until recently, few studies have employed rsFC to examine the effects of variable sleep quality on intrinsic patterns of functional connectivity. However, these imaging methods will likely improve our understanding of the impact that sleep disturbances have on brain health and function. Below are several studies that have used rsFC in the context of sleep.
Samman (2010) employed rsFC to examine the acute impact of partial sleep deprivation during a single night. Two rsFC analysis techniques were used. Independent components analysis and seed-based correlation in 16 healthy adults were performed both after a single night of normal sleep and partial sleep deprivation, respectively. Findings showed that rsFC within regions of the DMN and salience network were decreased after a night of partial sleep deprivation compared to normal sleep, indicating that adequate sleep has a significant influence on the connectivity patterns of two prominent brain networks\textsuperscript{149}.

In 2012, Huang and colleagues explored the relationship between amygdala rsFC and primary insomnia. A seed-based correlation was used to examine rsFC in both insomnia patients (n = 10) and healthy controls (n=10) with normal sleep. Compared to controls, those with primary insomnia had decreased rsFC between the amygdala and insula, with compensatory increases in rsFC between the amygdala and premotor cortex. Post hoc analyses revealed that compensatory connectivity was positively correlated with worse subjective sleep quality\textsuperscript{150}.

Li et al. (2015) performed an rsFC analysis comparing individuals with obstructive sleep apnea (OSA) (n = 25) to healthy controls (n = 25). Results revealed that individuals with OSA exhibited less local connectivity within the PCC/precuneus and greater connectivity in the IFG than healthy controls. These findings suggest that OSA likely disrupts DMN connectivity, and that increases in the IFG may reflect a compensatory mechanism for underlying deficits\textsuperscript{151}.
In summary, sleep disruptions and disorders significantly alter rsFC within the DMN and salience network. These impairments may also cause the brain to develop compensatory connections with other cortical areas in order to maintain cognitive and functional performance. These findings suggest that inadequate sleep leads to intrinsic changes with deleterious and potentially long-term consequences.

Many theories have described sleep's complex role in maintaining and enhancing brain health and cognition. In 2003, Tononi and Cirelli described the Synaptic Homeostasis Hypothesis. Simply, this hypothesis postulates that synaptic potentiation (the strengthening of synapsis through repeated activation) increases during wakefulness. Wakefulness is associated with increased stimulation, which elicits synaptic firing and potentiation. Synaptic potentiation while awake is directly correlated with synaptic downscaling during sleep. Synaptic downscaling is linked to the homeostatic sleep drive and SWS activity during NREM sleep, which is concentrated early in the sleep period and progressively declines. SWS activity emanates from the medial frontal cortex and appears to be directly related to cognitive performance the subsequent day\textsuperscript{152}.

*Active Systems Consolidation* is another well-recognized sleep theory. This hypothesis states that events that take place while an individual is awake are encoded in the neocortex and hippocampus, concurrently. Particularly during SWS, these newly encoded memories are reactivated through hippocampal-neocortical communication, which move these memory traces from the
hippocampus to the neocortex. Once in the neocortex they are integrated into stronger and more permanent knowledge representations\textsuperscript{116}.

Additionally, evidence suggests that REM sleep may play an important role in information extraction and processing, and could be an extension of Active Systems Consolidation. Walker and Stickgold (2010) describe three nuanced forms of memory processing, which likely occur after memory consolidation and may be facilitated by REM sleep. These forms are 1) unitization (encoding similar bits of information together), 2) assimilation (weaving newly encoded information into existing networks), and 3) abstraction (applying pre-existing rules and patterns to the newly integrated knowledge)\textsuperscript{14}. In this way REM sleep enhances long-term memory storage and strengthens the cortical knowledge networks.

Changes in the sleep-wake cycle, and alterations in sleep quality and quantity begin at midlife and worsen with age\textsuperscript{15}. Many sleep-scientists believe that dysregulation of homeostatic sleep system and circadian rhythm contribute to these disruptions, but a clear cause remains unknown\textsuperscript{124,130}. The PFC, hippocampus, and SCN all play integral roles in our daily lives. Structural deterioration of these brain regions, not only impairs sleep quality and composition (increased fragmentation, decreased SWS, less REM) but also decreases cognitive function and biological rhythmicity (hormone release, body temperature control, appetite)\textsuperscript{125,130,145,147}. Gaining a better understanding of the interrelationship between exercise, sleep, and cognition could advance our capability to promote better brain health and prevent the onset of dementia\textsuperscript{10,30,123}. 
In summary, sleep architecture changes with age, and older adults have less TST, more sleep fragmentation, less SWS, and greater circadian dysrhythmia. Insufficient sleep and sleep disruptions are associated with decreased executive function performance, memory impairment, cognitive decline, and increased Aβ burden. SDB is also common in older adults and likely contributes to cognitive deficits. Better sleep quality appears to improve Aβ clearance, and may mediate enhanced cognition. While there are many theories about sleep, many neuroscientists agree that sleep is integrally involved in memory consolidation, synaptic plasticity, and cerebral metabolite clearance.

2.5 The Effect of Exercise on Sleep

Exercise, perhaps more than any other factor, has a tremendous influence on an individual's perceived sleep quality\textsuperscript{153}. Acute exercise and exercise training have been positively associated with a number of objectively measured sleep indices, including aspects of sleep architecture and composition. The following section is designed to introduce the reader to a handful of studies that examine the impact of exercise on sleep. We will then present a number of viable mechanisms, which influence this complex relationship.

Fitness, but not acute exercise, is associated with better objective sleep\textsuperscript{154}. In a 1993, Edinger examined the effects of both fitness and acute exercise on 24 older (age ≥ 60) men. Twelve aerobically fit (\(\dot{V}O_2\text{max}\) of ~21 ml/kg/min) and 12 sedentary individuals (\(\dot{V}O_2\text{max}\) of ~16 ml/kg/min) underwent two nights of objective sleep monitoring (PSG). Sleep was assessed once...
following a single session of exercise (~40 minutes of incremental exercise to exhaustion) and once following a day without exercise. Compared to the sedentary group, aerobically fit individuals had shorter sleep onset latency (SOL), less WASO, greater SE, and larger quantities of SWS. However, acute exercise had no significant effect on the above sleep indices\textsuperscript{154}. These results imply that increased fitness could improve sleep in older adults. While these results demonstrate the effects of acute exercise on subsequent sleep quality, the influence of sleep quality on exercise-altered cognitive performance has not been explored.

Moderate intensity acute exercise may be a successful treatment strategy for insomnia\textsuperscript{25}. Passos (2010) studied the effects of acute exercise on sleep quality in people with primary chronic insomnia. In this study thirty-eight adults (age ~44) were randomly assigned to one of four groups: 1) moderate intensity aerobic exercise (50 minutes), 2) high intensity aerobic exercise (3 sets of 10-minute sprints partitioned by 10-minute rest periods), 3) moderate intensity resistance training (8 exercises, 3 sets of 10 reps), or 4) non-exercise control. Objective sleep (PSG) and pre-sleep anxiety (State-Trait Anxiety Inventory) were assessed during the nights before and after the intervention. Compared to the reference group, moderate intensity aerobic exercise significantly reduced total wake time, and SOL, while increasing TST and SE\textsuperscript{25}. Moderate intensity exercise also decreased pre-sleep anxiety, although this finding may not be clinically relevant. These results suggest that exercise may improve sleep in people with
chronic primary insomnia. However, how acute exercise affects healthy older adults is not well understood.

Moderate intensity exercise training has been shown to enhance sleep quality in older adults with sleep complaints\textsuperscript{155}. In a study analyzing the effects of exercise training on sleep, King (1997) randomly assigned 43 older adults (age 50-76) with sleep complaints to either moderate intensity exercise (60-75\% of HRR) or a waitlist control. The 4-month exercise intervention met four times a week for 30-40 minutes. Subjective sleep quality was assessed with the PSQI. Compared to controls, those who exercised had significantly improved global sleep quality, including estimates of SOL and TST. These results demonstrate that in older adults with sleep complaints, exercise training can be used as a successful treatment strategy to improve subjective sleep quality\textsuperscript{155}. While these are intriguing findings, it would be interesting to know how this intervention affected either objective indices of sleep and cognitive performance.

Moderate intensity exercise training also improves objective sleep indices in those with sleep complaints\textsuperscript{99}. In a more recent study, King and Colleagues (2008) explored the effects of 12-month exercise intervention of 66 older (age $\geq$ 55) adults with moderate sleep complaints. Participants were randomly assigned to moderate intensity exercise (60-85\% of $HR_{\text{max}}$) for 12 weeks (2 supervised session and 3 home-based sessions/week for 35-40 minutes) or an educational control. To objectively measure sleep, participants underwent PSG monitoring at baseline, during, and after the intervention. PSQI was also used to assess ratings of subjective sleep. The results revealed that those who participated in
exercise had fewer awakenings during the first third of the night, a reduction in stage one sleep, and spent more time in stage two sleep. In addition, those in the exercise group reported better ratings of sleep quality and restfulness. These results demonstrate that moderate intensity exercise training may improve some aspects of sleep in those with sleep complaints\(^9\). This study complements the study above and expands our understanding of exercise on sleep, however how these exercise-induced improvements in sleep translate to improved cognitive and/or behavioral outcomes still has not been established.

Exercise training improves objective sleep quality and levels of systemic inflammation in older adults\(^\text{156}\). Santos and colleagues (2012) examined the interplay between exercise training, objective sleep, and a number of inflammatory cytokines. Twenty-two older men (age ~71) participated in a 6-month moderate intensity exercise-training program (≤ ventilatory threshold) one hour per day, three days per week. At baseline and after the intervention, sleep and inflammatory cytokines (IL-6, IL-10, TNF-α, and TNF-α/IL-10 ratio) were measured with PSG and blood draws, respectively. Six months of moderate intensity aerobic exercise effectively increased fitness (\(\text{VO}_2\text{max}\) increased ~15%), while also reducing REM latency, TWT, and markers of systemic inflammation. These findings imply that moderate intensity exercise training can improve sleep and may be effective at reducing age-related and (perhaps) pathologic inflammation\(^\text{156}\). As systemic inflammation is an important risk factor for AD, this study is a valuable addition to the literature. Understanding how reduced inflammation translates to cognitive or behavioral changes is a crucial next step.
Moderate intensity exercise training may be a successful treatment strategy for obstructive sleep apnea (OSA)\(^{146}\). In 2011, Kline and colleagues examined the effect of exercise training on sleep quality and OSA. Forty-three adults (age 18-55) with varying degrees of OSA (apnea-hypoxia index ≥ 15) were randomly assigned to participate in either a 12-week moderate intensity (60% of HRR) exercise program (four days/week, totaling 150 minutes/week) or stretching control. Additionally, those individuals assigned to the exercise group completed a bout of resistance exercise twice a week immediately following aerobic exercise. Obstructive sleep apnea and blood oxygen saturation was measured with PSG, and sleep was assessed (with PSG, actigraphy, and PSQI) both before and after the intervention. At the end of the 12-weeks, those in the exercise group experienced a significant reduction in apnea-hypopnea index and improvements in oxygen saturation. Moreover, PSG measured sleep showed that exercise was also associated with increased SWS. Actigraphy data also demonstrated significant improvements in the following sleep parameters: TST, SOL, WASO, SE, and sleep fragmentation in addition to better self-reported sleep quality on the PSQI. These findings suggest that exercise training may be a viable treatment strategy for OSA\(^{146}\). Again it would be interesting to know how these improvements in OSA are related to rsFC and how they translate to cognitive performance.

Exercise and sleep may share a bidirectional relationship\(^{157}\). Dzierzewski (2014), collected sleep (diary) and exercise (Leisure Time Exercise Questionnaire) data from 79 older (age ~64) adults once a week for 18 weeks.
The sleep diary also included questions related to SOL, WASO, and sleep quality. Additionally, a sub-set of participants wore actigraphy devices to measure objective activity and sleep. Their results demonstrated that increased levels of persistent exercise were associated with less WASO. Interestingly, they also found that better sleep on any given night was significantly related to more exercise the following day. These effects were not different when controlling for whether or not exercise was performed indoors or out. These findings suggest a bidirectional relationship may exist between exercise and sleep\textsuperscript{157}.

While a precise purpose for sleep remains unknown, evidence supports a number of plausible ways in which exercise may influence sleep. As previously stated, body temperature and circadian rhythm tend to oscillate in unison, whereby decreases in body temperature are related to sleepiness and fatigue, and increases are related to wakefulness\textsuperscript{158}. Although it may seem counterintuitive, increased body temperature (passive body heating or exercise) prior to bed have been found to improve sleep quality and composition (including SWS)\textsuperscript{159}. This likely occurs as the cessation of both heating and exercise result in a precipitous drop in body temperature, thus initiating sleep. The SCN is also highly involved in thermoregulation\textsuperscript{160}, suggesting that alterations in circadian rhythms (or gating of the homeostatic sleep drive) could be achieved by manipulating body temperature\textsuperscript{123}. Evidence for this hypothesis has been further supported by studies showing that older adults and people with insomnia have poor nocturnal temperature regulation\textsuperscript{15}. Energy conservation is another plausible (although likely incomplete) hypothesis, which postulates that living
beings have a limit of available energy, and to conserve and restore these energy supplies sleep must occur. Under this idea, exercise would prompt sleep by exhausting the available energy supplies\textsuperscript{158,161}. Tissue restoration is also another popular (yet overly simplistic) theory, which proposes that exercise wears down and causes damage to the body’s systems, creating a need for healing and an impetuous for sleep\textsuperscript{158,161}. Light is the most powerful regulator (zeitgeber) of circadian rhythm. Daylight governs our sleep and activity patterns. Exposure to bright light (e.g., the sun, blue light) at habitual times synchronizes this activity\textsuperscript{162}. Older adults often suffer from circadian phase advancements, making them feel sleepy earlier in the evening and wakeful later at night\textsuperscript{139}. Exercise, when timed correctly, can shift the circadian phase back and reinstate healthful patterns of sleep\textsuperscript{163}. Increased levels of anxiety and depression, which often co-occur with disrupted sleep\textsuperscript{164}, can be successfully treated with exercise\textsuperscript{165}. Exercise provides both antidepressant and anxiolytic effects that have been found to be as effective as pharmacological treatments\textsuperscript{166}. Wakefulness and activity, particularly physical activity, produce increased levels of adenosine throughout the body\textsuperscript{27}. Adenosine is directly involved in energy utilization and neurotransmission. The build-up of adenosine is thought to contribute to increasing sleep pressure and is primarily cleared during sleep. Therefore many researchers believe it is through this path that exercise improves sleep\textsuperscript{27}.

While our understanding of the relationship between exercise and sleep continues to grow, a number of areas remain unexplored. First, the majority of
exercise-related sleep studies have been conducted in healthy populations (including those without sleep complaints), it is important to identify whether or not the effects of exercise on sleep are uniform, or if they are sleep-quality dependent (both subjective and objective). Second, we must expand our knowledge about how sleep quality affects the cerebrovascular response to acute exercise, specifically in areas like the SCN and PFC, where sleep waves and circadian rhythms originate. Third, the majority of studies examining the effects of exercise on sleep have seemed uninterested in how this relationship may affect cognition. Considering the evidence in support of main effects of exercise on sleep, exercise on cognition, and sleep on cognition; a next logical step is to determine the potential mediating effects of sleep quality on the exercise-cognition relationship, and or the moderating effects of sleep (or exercise) on the effects of exercise (or sleep) on cognition. Finally, if acute exercise is influencing the hemodynamic response during an executive function task, we should understand if and how this is being influenced by sleep quality.

In summary, exercise, perhaps more than any other factor, has a beneficial effect on perceived sleep quality. Randomized controlled trials have demonstrated that exercise improves indices of subjective and objective sleep quality in older individuals with and without sleep complaints. Additionally, exercise training has been shown to mitigate the severity of SDB in individuals with diagnosed, but untreated OSA. While a single mechanism remains elusive, there are a number of plausible theories about how exercise improves sleep. Among these hypotheses are altered core body temperature, tissue healing and
restoration, energy conservation, increased (day) light exposure, decreased symptoms of depression and anxiety, and the accumulation of adenosine.

2.6 Exercise-Induced Alterations in rCBF

Although acute exercise and exercise training have been shown to benefit cognitive performance, the physiological mechanisms underpinning these relationships remain poorly understood. Aerobic exercise likely improves cerebrovascular health by enhancing endothelial function and vascular conductivity. Consistent exercise in animals has been shown to elicit increased microvascular density, and in humans brain volume changes are likely paralleled by altered patterns of cerebral perfusion. While several studies have examined the effects of exercise training on the cerebrovascular system, few studies have analyzed cognition and perfusion after acute exercise, at rest. In the section below the author has reviewed a portion of the literature related to these effects.

Acute low-moderate intensity aerobic exercise leads to elevated CBF at both rest and during simple motor activity. Smith (2010) examined the effects of 30-minute of acute low-moderate intensity bicycle exercise (Borg’s Rating Scale of Perceived Exertion; RPE = 13) on five young (age ~25) healthy adults. Using a within subjects repeated measures design, rCBF was assessed before exercise and immediately afterwards, during rest and a simple motor task (finger tapping). Results demonstrated a 20% increase in global CBF at rest, and in the motor cortex during the finger-tapping task. These findings suggest that low-moderate intensity aerobic exercise leads to elevated CBF at both rest and during
subsequent simple motor activity\textsuperscript{79}. While this is an important study, questions regarding how previous sleep quality impact these effects remain unanswered. Since sleep may moderate the relationship between exercise and rCBF, incorporating sleep as a potential predictor variable is an important next step.

In opposition to previous results from acute and chronic aerobic exercise studies, a single session of exercise may decrease rCBF\textsuperscript{77}. In 2014, MacIntosh explored the effects of acute moderate intensity bicycle exercise in healthy young (age 20-35) adults. Pseudo-continuous ASL was used to assess rCBF before and after exercise. Post-exercise rCBF was assessed at two time-points, 10-minutes and 40-minutes post. Task-based fMRI was also used to assess functional brain activation during the Go/No Go Task to measure executive function. Results revealed, compared to baseline, that gray matter rCBF at the 10-minutes post-exercise was 11\% lower. At the 40-minutes post-exercise rCBF was not significantly different than baseline levels. On the other hand, white matter rCBF was significantly greater during both post-exercise assessments. Additionally, region of interest analyses demonstrated decreased perfusion in the hippocampus and insula. Functional brain activation during the executive function task was similar, but was significantly lower after exercise in the left parietal operculum. These effects are opposite from those shown by Smith and colleagues and suggest that acute exercise may affect rCBF differently than chronic exercise training\textsuperscript{77}. Again sleep may be an important intermediate variable in these effects and should be included in the analysis model.
Acute exercise-induced changes in rCBF may be intensity dependent\textsuperscript{78}. Robertson (2015) studied the effects of acute bicycle exercise (20-minutes), at two different intensities (40-50\% and 60-70\% of HRR) on rCBF in 13 older stroke survivors (age \textasciitilde64). Using a within-subjects repeated-measures design, participants underwent perfusion-weighted MRI before and immediately after exercise. Low and moderate intensity exercise were performed on separate days. While their findings were convoluted, in general rCBF decreased after low intensity exercise, and was elevated after moderate intensity exercise\textsuperscript{78}.

Fitness is positively associated with rCBF in the DMN\textsuperscript{170}. Using a cross-sectional design, Johnson (2016) examined the interrelationship between myocardial function, fitness, and rCBF in healthy older adults (\textasciitildeage 64). Resting state fMRI and perfusion-weighted MRI were used to determine, functional regions of the DMN, and perfusion within these regions, respectively. Using linear regression, results revealed that myocardial function was correlated with perfusion in the DMN. Mediation effects were also detected, such that fitness accounted for the relationship between myocardial function and rCBF in the DMN. While these are interesting findings, future studies should examine the effects of directly manipulating rCBF within the DMN using acute exercise. In addition, rCBF in the executive function networks (central executive and salience network) should be examined, as these networks are important in aging and executive function\textsuperscript{170}.

Higher levels of aerobic fitness in older women are associated with preserved cerebrovascular conductance and better cognitive performance\textsuperscript{171}. In
another cross-sectional study, Brown (2010) explored the relationship between fitness and many physiological, cerebrovascular, and cognitive variables including mean arterial pressure (MAP), CVC (an index of brain reserve), and cerebral blood volume (CBV), and a comprehensive neuropsychological assessment. The study sample consisted of 42 older women (age 50-90). Results demonstrated strong associations between fitness and CVC, fitness and MAP, and fitness and global cognition.

Short-term exercise interventions in older adults can improve cerebrovascular function and cognition. In 2013, Chapman et al. conducted a randomized controlled trial examining the effects of aerobic exercise training on 37 healthy older adults (age 57-75). Half of the study sample performed exercise for one hour three times per week for three months, while the other half served as a waitlist control. Fitness, rCBF, and cognition were assessed at three-time points throughout the trial. After three months, their findings demonstrated that exercise increased rCBF in the ACC and improved performance in immediate and delayed memory. Although these results expand our understanding of the exercise-induced effects on rCBF and cognition in older adults, the hypothesis that sleep mediates these relationships remains untested.

Consistent exercise training throughout life protects the older brain from decreases in rCBF such that absolute rCBF remains comparable to younger adults. Using a unique sample of highly trained older athletes, Thomas (2013) performed a cross-sectional analysis examining the effects of life-ling aerobic exercise on rCBF and cerebrovascular reactivity (CVR). Ten master athletes
(age ~75), 10 sedentary older adults (age ~75), and nine younger adults participated in the study. Results demonstrated that life-long exercise preserved rCBF in the posterior cingulate and precuneus, which are regions of the DMN and known to be susceptible to the AD pathology. However, CVR was decreased in the master athletes compared to their sedentary counterparts. This is an important study as it demonstrates the protective effects of a healthy and aerobically fit lifestyle on cerebrovascular health. However, it remains poorly understood if these effects translate to cognitive performance. Additionally, this study provides no insight into the effects of this lifestyle on sleep quality, or vice-versa.

Increased hippocampal blood volume is positively correlated with hippocampal neurogenesis. In 2007, Pereira and colleagues performed a two-part study (across species) to better understand exercise-induced neurogenesis and its effects on the cerebral microvasculature. First, 46 mice were randomly assigned to exercise (wheel running) or sedentary control. Active mice had access to running wheels for two weeks. MRI was used to assess CBV in the dentate gyrus of the hippocampus at baseline and again after two, four, and six weeks. During the second week BrdU was injected and used as a marker of neurogenesis. At the end of the six weeks, both groups were sacrificed and immunohistochemistry was used to measure and compare levels of neurogenesis in the dentate gyrus. Second, 11 healthy young humans (age ~33) were assigned to aerobic exercise training for one hour, four times per week, for three months. Using the same (although optimized) MRI sequence that was used
for the mice, hippocampal CBV was assessed before and immediately after the exercise intervention. Additionally, the RAVLT was use to assess short and delayed memory at both time-points. Results demonstrated that exercise mice had increased CBV in the dentate gyrus, which paralleled neurogenesis in the same regions. Additionally, exercise increased CBV in the human dentate gyrus, which paralleled improved memory performance. Together these results suggest that exercise not only increases neurogenesis and CBV in the hippocampus but also that these changes likely translate to improved cognitive performance. While this is a landmark study, it does not explain how sleep quality might influence these effects.

Running elicits compensatory angiogenesis in the cerebellar cortex. In 1990, Black randomly assigned 38 rats to aerobic exercise, acrobatic training (climbing, balancing, hanging), or sedentary behavior for 30 days. After the intervention all rats were sacrificed and electron microscopy was used to assess cerebrovascular and synaptic remodeling. Results showed that the acrobatic group had more synapses per perkinje cell in the cerebellum (brain area known for its role in physical movement) compared to the aerobic and sedentary groups. However, those rats that exercised had increased blood vessel density in the cerebellar cortex. The authors conclude that both acrobatic and exercise training cause physiological changes in the rat cerebellum, and that the repetitive nature of aerobic exercise (repeated synaptic firing) elicits compensatory angiogenesis in the cerebellar cortex.
Exercise training increase cerebrovascular volume and cognitive performance, while sedentary behavior reverses these effects\textsuperscript{24}. In 2010, Rhyu and colleagues explored the effects of exercise training/detraining in 24 older (age 15-17) and younger (age 10-12) monkeys. The monkeys were randomly assigned to either aerobic exercise or sedentary behavior for one hour, five times a week, for five months. At the end of the 5-month period all but a sub-sample of runners were sacrificed. The sub-sample of runners engaged in three months of sedentary behavior to assess the effects of detraining. Additionally, all monkeys were trained and tested in the Wisconsin General Testing Apparatus (WGTA). Results revealed that regardless of age, runners learned the WGTA significantly faster than sedentary monkeys. Additionally, at the end of the 5-month intervention runners had significantly greater cerebrovascular volume in the motor cortex than their sedentary counterparts. However, this increase was not maintained after 3-months of detraining. The authors conclude that aerobic exercise training can improve cerebrovascular health and likely enhances cognitive performance in humans\textsuperscript{24}.

Aerobic exercise increases rCBF, CVR, and increases microvascular density in the rat motor cortex\textsuperscript{36}. In 2003, Swain examined the effects of exercise on rCBF, CVR, and angiogenesis by randomly assigning 18 rats to either 30 days of exercise training or sedentary behavior. Using MRI and immunohistological methods, their findings demonstrated that exercise training significantly increased rCBF and CVR in the motor cortex. Additionally, the immunohistological assessment showed that exercised mice had greater
angiogenesis and microvascular reconstruction in the motor cortex than their sedentary counterparts.\(^{36}\)

In summary, exercise has been shown to improve cognitive performance but the physiological underpinnings of this relationship remain unclear. Resting CBF has a propensity to decrease with age, but higher levels of aerobic fitness and exercise training ameliorate this effect. The effects of acute aerobic exercise in older adults have been understudied and appear to be more ambiguous. In animals, exercise-induced increases in blood flow and volume have been associated with structural changes in the hippocampus, motor cortex, and cerebellum;\(^{23,35,36}\) and in monkeys physical inactivity facilitates the dissipation of these improvements.\(^{24}\) While exercise may boost cognition, the role of CBF in this relationship remains unclear.

### 2.7 Exercise-Induced Alterations in rsFC

Resting-state fMRI is a relatively new method for exploring the communication patterns of functionally connected brain regions in the absence of an explicit task.\(^ {172} \) Unlike traditional task-based fMRI, resting-state paradigms examine correlated low frequency neurovascular oscillations while at rest. While few studies have examined the relationship between rsFC and exercise training (or acute exercise), those that have employed this technique have unveiled provocative results. Below are several studies that have specifically explored the impact of exercise on intrinsic patterns of rsFC in older adults.

Chirles and colleagues (2017) explored the effects of a 12-week aerobic exercise intervention on rsFC in older adults with and without MCI. The exercise
intervention included 30 minutes of moderate intensity walking 4x/week (50-60% HRR). Resting state functional connectivity was measured both before and immediately after the exercise intervention. Findings demonstrated significantly increased rsFC in the PCC/precuneus in individuals with MCI that were not observed in healthy older adults, suggesting that exercise training may strengthen DMN connectivity, thus preferentially benefiting individuals at increased risk of AD\textsuperscript{173}.

In a similar investigation, Voss et al. (2012) sought to determine the effects of a yearlong exercise-training program of rsFC in healthy older adults (n = 65, ~age 66.4). Additionally, this study aimed to examine the relationship between altered levels of BDNF, VEGF, and IGF-1 on effective rsFC. Using a seed-based analysis, results revealed that exercise-induced increases in rsFC were positively associated with increased levels of the above neurochemicals\textsuperscript{41}.

Finally, Weng (2016) examined the effect of a single session of exercise (30 minutes, moderate intensity) on both young (n = 12) and older (n = 13) adults. Interestingly, and in line with previous research, findings demonstrated increased rsFC in several brain networks, including those believed to be involved in reward, executive function, and memory\textsuperscript{174}.

In summary, the effects of both acute and chronic exercise appear to enhance rsFC connectivity in several prominent networks, including those considered task-positive (salience, executive function, reward-processing) and negative (default mode network). Moreover, these effects do not seem to be limited to younger or strictly healthy adults. In fact, compelling findings from at
least one study suggest exercise may preferentially enhance rsFC in those with increased risk for AD$^{173}$. 
Chapter 3: Hippocampal and Cerebral Blood Flow After Exercise Cessation in Master Athletes

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Hippocampal and Cerebral Blood Flow After Exercise Cessation in Master Athletes

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Abstract

While endurance exercise training improves cerebrovascular health and has neurotrophic effects within the hippocampus, the effects of stopping this exercise on the brain remain unclear. Our aim was to measure the effects of 10 days of detraining on resting cerebral blood flow (rCBF) in gray matter and the hippocampus in healthy and physically fit older adults. We hypothesized that rCBF would decrease in the hippocampus after a 10-day cessation of exercise training. Twelve master athletes, defined as older adults (age ≥ 50 years) with long-term endurance training histories (≥ 15 years), were recruited from local running clubs. After screening, eligible participants were asked to cease all training and vigorous physical activity for 10 consecutive days. Before and immediately after the exercise cessation period, rCBF was measured with perfusion-weighted MRI. A voxel-wise analysis was used in gray matter, and the hippocampus was selected a priori as a structurally defined region of interest, to detect rCBF changes over time. Resting CBF significantly decreased in eight gray matter brain regions. These regions included: (L) inferior temporal gyrus, fusiform gyrus, inferior parietal lobule, (R) cerebellar tonsil, lingual gyrus, precuneus, and bilateral cerebellum (FWE p < 0.05). Additionally, rCBF within the left and right hippocampus significantly decreased after 10 days of no exercise training. These findings suggest that the cerebrovascular system, including the regulation of resting hippocampal blood flow, is responsive to short-term decreases in exercise training among master athletes. Cessation of exercise training among physically fit individuals may provide a novel method to assess
the effects of acute exercise and exercise training on brain function in older adults.

**Keywords:** Aerobic Fitness, Arterial Spin Labeling, Athlete, Cerebral Blood Flow, Cerebrovascular Health, Exercise, Healthy Older Adults, Hippocampus, MRI

### 3.1 Introduction

Endurance exercise training (exercise) produces physiological adaptations that enhance aerobic fitness and cardiovascular health\textsuperscript{175}. Consistent exercise effectively augments the maximal rate of oxygen consumption (\(\dot{V}O_2\max\)) centrally, by increasing cardiac output, and/or peripherally by widening the arterial-venous oxygen (A-\(\dot{V}O_2\)) difference\textsuperscript{176}. \(\dot{V}O_2\max\) is the gold-standard index of cardiorespiratory fitness and is highly correlated with both morbidity and mortality\textsuperscript{177,178}, with greater fitness status associated with a reduced risk of chronic disease and a longer lifespan. In addition to enhancing the function of the cardiovascular system, exercise has been shown to increase bone density, improve muscle quality, and protect against metabolic dysfunction\textsuperscript{175}.

Conversely, when the exercise stimulus is removed many of these systemic adaptations rapidly dissipate\textsuperscript{179–181}, thereby increasing the potential for adverse health effects. For example, 20 days of bed rest immobilization resulted in a substantial 28% decrease in \(\dot{V}O_2\max\)\textsuperscript{182}; a prolonged detraining period reduced muscle fiber capillarization and oxidative enzyme activity\textsuperscript{183}; and a 10-day period of physical inactivity was related to the development of impaired glucose tolerance and insulin resistance\textsuperscript{184}. 
A growing body of empirical evidence supports the notion that exercise also robustly affects the human brain. Multimodal neuroimaging studies, including both structural and functional MRI, have helped elucidate the brain’s complex neurobiological response to exercise. These exercise-induced effects include cytoarchitectonic modifications\(^{185-187}\), altered patterns of neural activity\(^{188}\), and improved performance across the cognitive domains\(^{189-192}\). The hippocampus, a subcortical brain structure well known for its role in learning and memory, has shown neurotrophic effects as the result of exercise training in humans and animal models\(^{187,193,194}\). Exercise interventions in humans have been shown to affect hippocampal-dependent cognition and to increase hippocampal blood perfusion\(^{193}\) and volume\(^{187}\). While the effects of detraining have been reported in peripheral physiological systems, the effects of detraining on brain function, and on cortical and hippocampal blood flow, have not been reported.

A key unanswered question, and the primary aim of this study, was to determine how short-term exercise cessation impacts cerebrovascular function in healthy highly physically active and physically fit older adults. To accomplish this goal we measured the resting cerebral blood flow (rCBF) of master athletes both before and immediately after 10 days of exercise cessation. To quantify rCBF we employed pseudo-continuous arterial spin labeling (pCASL), a perfusion-weighted MRI technique. Our hypotheses were twofold, we predicted 1) that 10 days of physical inactivity would alter rCBF in areas known to be susceptible to
age-related decline\textsuperscript{195,196}, and 2) that detraining would decrease hippocampal blood flow, which we chose as an \textit{a priori} region of interest (ROI).

### 3.2 Methods

#### Participants

Our unique study sample consisted entirely of master athletes, which were defined as a sub-group of highly trained healthy older adults who regularly engaged in endurance exercise. Master athletes ranged in age between 50 and 80, and were recruited from local Washington D.C. area running clubs. These individuals had an endurance exercise history of at least 15 years and had recently competed in regional and national endurance events. Personalized training regimens must have entailed at least four hours of high intensity endurance training per week.

This study was approved by the Institutional Review Board, and written informed consent was obtained from all participants, in accordance with the Helsinki declaration. A telephone screen was used to determine eligibility. Those who met inclusion/exclusion criteria (see below) and agreed to participate completed the following: anthropometric measurement, a maximal intensity treadmill/electrocardiography test, DEXA (Dual-energy X-ray absorptiometry) body composition assessment, neuropsychological testing, and fMRI scanning (Table 3.1 shows descriptive data for variables examined at baseline only).

#### Inclusion and Exclusion Criteria

Individuals were excluded if they had a BMI $\geq 30$, reported being a smoker (within the past 5 years), or had a history of heart attack, stroke, lung disease,
chronic obstructive pulmonary disease, peripheral vascular disease, heart
disease, liver disease, kidney disease, anemia, or diabetes. Individuals taking
prescription medication for hypertension were also deemed ineligible. Women
participants must have been post-menopausal for at least two years and must
not have used hormone replacement therapy during the previous year.
Additionally, individuals were excluded if they presented any absolute
contraindications to MRI. During the initial telephone screening 21 persons were
deemed ineligible to participate in this study. Of the 12 individuals who qualified
for participation, nine (7 men, ~89% Caucasian) were included in the final
analysis. One individual was removed due to irregular ECG activity during the
graded treadmill test; another because of dental work that severely distorted the
MR signal; and a third for failure to achieve VO2max during the graded treadmill
exercise test.

Neuropsychological Testing

Prior to the baseline MRI scan, all participants were administered the Mini
Mental State Exam (MMSE)\textsuperscript{197}, which is a 30-point questionnaire used to screen
for global cognitive impairment and dementia. Additionally, after both the
baseline and follow-up MRI scan all participants were administered the semantic
verbal fluency test\textsuperscript{198}. This test required participants to list as many words as
possible from a given category in 60-seconds (fruits and animals, order
counterbalanced).

\textbf{VO2max Testing}
Cardiorespiratory fitness was determined by assessing \( \dot{V}O_2 \text{max} \) during a graded treadmill test with indirect calorimetry (Quark, Cosmed USA). Exercise tests were conducted utilizing a protocol we have used numerous times previously in older athletes, and included standard ECG monitoring\(^{175,184} \). The exercise test continued until maximal effort or exhaustion was achieved. For exercise tests to be considered maximal, participants had to reach both a plateau in \( \dot{V}O_2 \text{max} \) with increasing workload and a respiratory exchange ratio > 1.1. The highest \( \dot{V}O_2 \) attained during the test was recorded as \( \dot{V}O_2 \text{max} \). The maximal exercise test was conducted several hours after the baseline MRI scan.

**Body Composition Assessment**

Body composition was measured using dual energy x-ray absorptiometry (DEXA) (DXA; Prodigy, LUNAR Radiation Corp).

**Exercise Cessation Period**

MRI scanning occurred at two time points, baseline and immediately after the 10-day exercise cessation. Participants were asked to refrain from exercise during the 12 hours preceding the baseline assessment. The exercise cessation commenced 72 hours after the baseline scan, at which time all exercise training was stopped. During this period participants were asked to refrain from exercise and all other forms of vigorous physical activity. The abstention from exercise during the 10-day period was verified frequently by telephone conversations with the participants and during their final testing. The second MRI scanning session occurred on the morning after the last day of the exercise cessation period, and before participants resumed their training regimens.
MRI Acquisition

All MRI data were acquired with a Siemens 3.0 Tesla MR system (Magnetom Trio Tim Syngo, Munich, Germany). A 32-channel head coil was used for radio frequency (RF) transmission and reception. Foam padding was positioned within the head coil to minimize patient motion. A high-resolution T1-weighted anatomical image was acquired for co-registration with the following sequence parameters: Magnetization Prepared Rapid Acquisition of Gradient Echo (MPRAGE), matrix = 256, field-of-view (FOV) = 230 mm, voxel size = 0.9 x 0.9 x 0.9 mm, slices = 192 (sagittal plane, acquired right to left), slice thickness = 0.9 mm, repetition time (TR) = 1900 ms, echo time (TE) = 2.32 ms, inversion time (TI) = 900 ms, flip angle = 9°, sequence duration = 4:26 min. The pCASL data were acquired using the following sequence parameters: single-shot gradient echo planar images, matrix = 64, FOV = 210 mm, voxel size = 3.28 x 3.28 x 6.0 mm, slices = 20 (axial plane, acquired in ascending order), slice thickness = 5.0 mm, gap between slices = 1 mm, single slice acquisition time = 48 ms, label duration = 1500 ms, post-label delay = 1000-1912 ms, TR/TE = 4000/19 ms, volumes = 140, number of label/control pairs = 70, flip angle = 90°, RF blocks = 80, RF pulses = 20, gap between pulses = 360 µs, bandwidth = 3004 Hz/Px, and sequence duration = 9:28 min. Additionally, a concatenated series of control volumes stacked in the time dimension was used as the proton density (PD) image for perfusion calibration.

MRI Data Preprocessing
The pCASL and PD images were realigned to the first volume of the image time series for motion correction. Using pairwise subtraction, a perfusion-weighted image was derived from the motion-corrected interleaved (control - tag) volumes, and this image was corrected for slice-timing delay.

Using FSL’s BASIL (Bayesian Inference for Arterial Spin Labeling, FMRIB Software Library v5.0, Oxford, UK), a reference mask from the T1-weighted anatomical image was used to isolate and sample the cerebral spinal fluid (CSF) within the ventricles of the PD image. The CSF sample was used to compute the magnetization equilibrium (M0) of tissue, which was further used to obtain the magnetization equilibrium of arterial blood (M0a).

Buxton’s general model for kinetic inversion was used to estimate absolute CBF (ml/100g/min), and included both Bayesian inferences and the following parameters: \( \Delta M \) (proportional magnetization change represented by the perfusion-weighted image), \( T_1^{\text{blood}} \) (longitudinal relaxation time of blood = 1650 ms), \( T_1^{\text{tissue}} \) (longitudinal relaxation time of tissue = 1300 ms), \( \alpha \) (labeling efficiency = 0.85), \( M_0^{a} \) (magnetization equilibrium of arterial blood), \( \text{BAT} \) (bolus arrival time = 1300 ms), \( \text{Bolus} \) (label duration = 1500 ms), \( \text{PLD} \) (post label delay = 1000 ms), and \( \lambda \) (blood/brain partition coefficient of GM = 0.98 ml/g). The rendered CBF map was spatially smoothed using an adaptive technique, which combined neighboring voxel signals on an intensity-dependent basis, while preserving the non-linear kinetics where smoothing was unnecessary. Due to the inherently low spatial resolution of the perfusion image, partial volume error correction was performed to improve the accuracy of CBF estimation.
This process effectively calculated separate gray matter and white matter perfusion using tissue specific partial volume estimates in each voxel\textsuperscript{205,206}. Co-registration and normalization was performed with SPM8\textsuperscript{207} (SPM, University College, London, UK), using the PD image as the reference to which the CBF map was aligned. The output parameters were used to transform the CBF map and gray matter estimates to standard space, which effectively up-sampled the images to a 2 mm\textsuperscript{3} isotropic voxel resolution. Normalized gray matter estimates were merged together and used as a mask for the voxel-wise analysis.

**Gray Matter Voxel-Wise Analysis**

A voxel-wise analysis, restricted to voxels within the gray matter mask, was conducted to explore the effect of exercise cessation on rCBF. Statistical parametric maps were produced\textsuperscript{199}, indicating where rCBF had significantly changed over time. We used AFNI’s 3dClustsim program (on the 2 mm\textsuperscript{3} data) to control for the effects of multiple comparisons and reduce the likelihood of a Type-I error. This analysis tool used Monte Carlo simulations to establish a family-wise error (FWE) corrected probability threshold at both the voxel (\(p < 0.05\)) and cluster (\(\alpha < 0.05\)) level\textsuperscript{199}. Using first-order nearest neighbor clustering, we maintained results at a minimum cluster size of \(\geq 480\) mm\textsuperscript{3}. To further illustrate the results of the voxel-wise analysis, the mean rCBF from each significant ROI was extracted from all subjects, at both time points.

**Hippocampal Analysis**

To examine the effect of exercise cessation on hippocampal blood flow, we conducted an \textit{a priori} analysis that was restricted to voxels within the
hippocampus. To isolate and examine hippocampal rCBF, we used FreeSurfer’s (version 5.3.0) automated subcortical processing stream\textsuperscript{208}. This procedure segmented the T1-weighted anatomical image and rendered a segmentation map based on both atlas probabilities and subject-specific tissue intensities. The segmentation map was normalized using non-linear transformation to maintain both the accuracy and integrity of the labeled subcortical anatomy. The non-linear transformation parameters were then used to warp the co-registered CBF map to standard space. The normalized bilateral hippocampal regions were extracted, merged together, and used as a mask for the voxel-wise analysis. Using the same clustering procedures as described above, Monte Carlo simulations were run over the hippocampal volume to establish a FWE threshold of $p < 0.05$ with a minimum cluster size of $\geq 200$ mm$^3$.

3.3 Results

The Master Athlete Profile

The master athletes who volunteered for this study are a unique population and should not be considered equivalent to older adults who engage in regular moderate to vigorous intensity leisure-time physical activity. Our participants had a mean continuous endurance training history of approximately 29 years, and on average were running 59 km per week and training 5 days per week just prior to the baseline testing. They also regularly participated in regional and national endurance competition. Moreover, as a group these master athletes had a $\dot{V}O_2\text{max}$ above the 90$^{th}$ percentile for their age and sex.

Gray Matter rCBF
Results of the gray matter voxel-wise analysis demonstrated that the 10-day exercise cessation period significantly reduced absolute rCBF in eight brain regions (Figure 1). Of note are the Pre > Post comparisons, shown in blue on the ∆ rCBF maps in Figure 1, revealing significantly decreased rCBF in each ROI (total volume = 5,560 mm³) that remained after correction for multiple comparisons using the False Discovery Rate (see Table 3.2). These regions included: (L) inferior temporal gyrus, fusiform gyrus, inferior parietal lobule, (R) cerebellar tonsil, lingual gyrus, precuneus, and (L/R) cerebellum. No statistically significant change in whole brain absolute CBF in gray matter was detected (mean (±SD) baseline = 69.4 (±10.4 ml/100g/min), post-cessation = 67.2 (±12.6 ml/100g/min)).

**Hippocampal rCBF**

The a priori hippocampal analysis also revealed significantly decreased blood flow in both the left and right hippocampus from before to after the cessation of exercise training (see Figure 2).

**Verbal Fluency**

Verbal fluency performance did not significantly change from before to after the cessation of training period (mean (±SD) baseline = 19.9 (±4.9 words), post-cessation = 17.4 (±5.8 words), t(8) = 0.91, p = 0.39).

**3.4 Discussion**

In the present study, we examined the relationship between short-term exercise cessation and resting rCBF in master athletes. Exercise cessation was associated with reduced rCBF within eight gray matter regions, including bilateral
regions of the hippocampus. Importantly, these significant changes were regionally specific, and not the result of global CBF changes after the 10-day period of exercise cessation.

In one of the first studies of its kind, Saltin and colleagues (1968)\textsuperscript{182} demonstrated the deleterious effects of bed rest immobilization on the cardiovascular system. Comparable to decades of biological aging, this extreme sedentary behavior reduced \( \dot{V}O_2\text{max} \) approximately 27\% in just 20 days\textsuperscript{209}. Using biopsy samples from the vastus lateralis, Klausen (1981) examined the effects of exercise cessation on skeletal muscle. After an 8-week detraining period, both muscle fiber capillarization and oxidative enzyme activity declined\textsuperscript{183}. Finally, in a study of master athletes, Rogers (1990) examined the effects of a short-term period without exercise on insulin-regulated glucose metabolism. After 10 days, approximately 29\% of these older adults developed signs of impaired glucose tolerance or insulin resistance\textsuperscript{184}. Here, we have extended this literature to demonstrate that not only does exercise cessation among physically fit older adults affect markers of peripheral metabolic function, but also appears to affect brain cortical and hippocampal blood flow.

Exercise training has been shown to robustly affect the structural and functional integrity of the hippocampus in animals and humans, producing neurotrophic effects leading to neurogenesis and angiogenesis in rodents\textsuperscript{193,194,210}, and to increased structural volume in healthy older adults\textsuperscript{187}. Twelve weeks of exercise training in healthy younger adults was shown to increase the blood volume within the dentate gyrus of the hippocampus and to
improve episodic memory performance. Our findings of reduced rCBF bilaterally in the hippocampus suggest that training-induced changes in hippocampal blood flow may be reversed with 10 days of exercise cessation. Our participants, however, did not show any changes in cognitive function over the 10-day cessation of exercise period. Nevertheless, it is not known if cellular adaptations, undetectable using MRI, occur when exercise training is stopped temporarily.

Although several studies have used perfusion-weighted imaging to probe for exercise-induced alterations in rCBF, differences in exercise intensity, study design, and data analysis methods have likely led to conflicting results. In a randomized controlled trial, Chapman and colleagues (2013) demonstrated that a 12-week aerobic exercise intervention significantly increased rCBF in the anterior cingulate cortex of previously sedentary older adults\textsuperscript{189}. Likewise, a cross-sectional study by Thomas (2013) showed that master athletes had significantly greater rCBF in the posterior cingulate cortex than age-matched sedentary controls\textsuperscript{211}. While these findings seem to corroborate one another, the effects of acute exercise on rCBF have proven to be complex. Although Smith and colleagues (2010) found rCBF in the motor cortex to be significantly greater after a session of moderate-intensity exercise\textsuperscript{212}, MacIntosh (2014) demonstrated, in a similar study, that acute exercise decreased rCBF in the hippocampus and insula\textsuperscript{213}.

Until now, the effects of exercise cessation on cerebrovascular function have been virtually unexplored. This study has extended the literature by showing that a short-term period of physical inactivity among master athletes
reduces rCBF in the hippocampus and several gray matter regions. While these effects may have implications for brain function in older adults, it is also possible that these effects represent changes in arterial transit time or cerebral blood volume, neither of which we were able to measure. A decrease in arterial transit time, for instance, while using the same post-label delay parameter, could result in an apparent decrease in rCBF. In addition, total blood volume has been shown to increase in response to exercise training and to decrease after detraining. These are important points to consider; however, the regional specificity of the effects we observed suggests these changes were not an artifact of a global flux in blood flow or volume. Although increases in blood volume have been documented in the dentate gyrus after 12 weeks of exercise in younger adults, the impact of exercise cessation on cerebral blood volume and arterial transit time has not been established.

Animal research suggests the most viable mechanism for exercise-induced perfusion alterations may be structural modifications to the cerebrovasculature. One such study established these effects by examining the association between increased cerebral blood volume and post-mortem neurogenesis in exercised mice. The results indicate that local increases in hippocampal blood volume coincide with the cellular proliferation and reconfiguration within the dentate gyrus. In another investigation, Ryhu (2010), provided further support for this proposition by evaluating the effects of both exercise training and exercise cessation on the non-human primate cerebrovascular system. During this study, animals were randomized to either
treadmill training or sedentary behavior. After five months, a sub-set of primates from the exercise group were subjected to an additional three months without exercise. At the 5-month time point, the exercised animals had significantly greater cerebrovascular volumes than their sedentary counterparts. As for the effects of exercise cessation, the increases in vascular volume induced during the exercise-training period were reversed after three months of detraining. It is plausible that the cessation of exercise involves a reversal of these effects, which would need to be confirmed in additional animal models.

There are several limitations to the current study. We had a small \( (n = 9) \) and homogeneous (7 men, \( \sim 89\% \) Caucasian) study sample, in which all participants exhibited a high level of aerobic fitness, long history of competitive endurance training, and normal BMI, which limits the generalizability of our findings. While our sample was small, the effects we observed were substantial and all in the same direction. We may have lacked statistical power to detect smaller effects and may have underestimated the decreases in CBF that occurred. In addition, because we only measured rCBF at two time points, we do not know the time course of the changes we observed. It is possible reduced rCBF may have been evident prior to day 10. Further, we cannot speculate regarding whether or not these effects would have increased, decreased or remained stable with a longer period of exercise cessation. We made a priori predictions of changes in hippocampal blood flow based on an extensive literature, a method also used by others\(^{193}\), which avoided adjustment for whole brain voxel-wise comparisons and prevented a possible Type-II error.
Nevertheless, future studies should confirm these effects in a larger sample. Additionally, aerobic fitness was assessed only at baseline, so we do not know the magnitude of the fitness change over time. Several studies have documented that in trained individuals substantial decreases in fitness occur after short-term cessation from exercise training\textsuperscript{183,184,220}. Finally, the nature of our imaging sequence (single-delay) limited our ability to estimate changes in cerebral blood volume, and possible changes in arterial transit time. Our post label delay values began at 1000 ms and increased at each slice, which does not precisely correspond to the ISMRM Perfusion Study Group recommended guidelines for ASL scanning among older adults\textsuperscript{203}. This could contribute to CBF quantification challenges, including arterial transit time artifacts. Measuring cerebral blood volume and arterial transit time with multi-delay ASL is an important next step\textsuperscript{203}.

Approximately two-thirds of the ROIs that showed altered rCBF after exercise cessation are considered part of the brain’s default mode network (DMN), which is known to be disrupted with age-related cognitive decline and Alzheimer’s disease\textsuperscript{221–223}. Exercise training in older adults has been shown to augment functional connectivity within the DMN in healthy older adults\textsuperscript{224}. While our findings suggest that short-term cessation from endurance training in highly trained older adults may lead to decreased rCBF within the DMN, there was no indication that these effects were detrimental to cognitive function, as measured by a semantic verbal fluency task (which activates brain regions that overlap with the DMN)\textsuperscript{225}, or to the integrity of these neural networks. Future exercise detraining studies should examine participants after resuming their training.
schedules to document whether or not these effects would be reversed. It is also not known if the decreased rCBF we observed solely reflects changes in the rate of blood flow within parenchymal regions, or additionally reflects changes in arterial transit time and/or blood volume. Nevertheless, the exercise cessation paradigm may provide useful information to probe the durability of the effects of exercise training and physical activity on brain function. Just as the effects of long-term endurance exercise training on cardiovascular and metabolic function wane considerably after a short period of detraining, so also may hippocampal and gray matter rCBF be sensitive to exercise cessation.

Author Contributions

Substantial contributions to the conception or design of the work (JH, JS). The acquisition, analysis, or interpretation of data for the work (AA, BL, LW, TS, JS, JH). Drafting the work or revising it critically for important intellectual content (AA, JS, BL, LW, TS, JH).

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Disclosure
This research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Table 3.1 Descriptive Participant Data Collected at Baseline

<table>
<thead>
<tr>
<th>Measure</th>
<th>Mean (SD)</th>
<th>Maximum</th>
<th>Minimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>61 (7.76)</td>
<td>71</td>
<td>51</td>
</tr>
<tr>
<td>( VO_2\text{peak} ) (ml/kg/min)</td>
<td>46.16 (5.47)</td>
<td>53.95</td>
<td>36.1</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>25.18 (3.60)</td>
<td>29.9</td>
<td>18.55</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>17.84 (4.03)</td>
<td>23.7</td>
<td>11.99</td>
</tr>
<tr>
<td>Lean Mass (kg)</td>
<td>50.32 (8.98)</td>
<td>63.44</td>
<td>34.49</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>23.35 (3.49)</td>
<td>29.4</td>
<td>19.5</td>
</tr>
<tr>
<td>SBP (mm/Hg)</td>
<td>117.56 (16.48)</td>
<td>146</td>
<td>104</td>
</tr>
<tr>
<td>DBP (mm/Hg)</td>
<td>70 (8.88)</td>
<td>82</td>
<td>60</td>
</tr>
<tr>
<td>MAP (mm/Hg)</td>
<td>85.85 (10.35)</td>
<td>103.3</td>
<td>74.7</td>
</tr>
<tr>
<td>MMSE</td>
<td>28.66 (1.11)</td>
<td>30</td>
<td>27</td>
</tr>
</tbody>
</table>

\( VO_2\text{max} \), maximal oxygen consumption; BMI, body mass index, SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; MMSE, Mini Mental State Exam.
Figure 3.1 Results of The Gray Matter Voxel-Wise Analyses
Results of the gray matter voxel-wise analyses reveal eight brain regions (A-H), which demonstrate significant rCBF changes over time. Adjacent bar graphs represent the mean rCBF difference within each region, and include brain area, LPI coordinates, and cluster volume in mm$^3$. The color bar represents the mean absolute CBF difference (post-detraining minus pre-detraining) within each region, expressed in ml/100g/min. Corrected $p$-values reflect the contrast between the Pre and Post time points.
Table 3.2 CBF Results from Gray Matter Voxel-Wise Analysis

<table>
<thead>
<tr>
<th>#</th>
<th>Side</th>
<th>Region</th>
<th>BA</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>vol</th>
<th>Pre CBF (ml/100g/min)</th>
<th>Post CBF (ml/100g/min)</th>
<th>p</th>
<th>( \eta^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>L</td>
<td>Cerebellum</td>
<td>22, 70</td>
<td>-58</td>
<td>1032</td>
<td></td>
<td>22.8 (14.6)</td>
<td>13.7 (10.5)</td>
<td>&lt;0.000046</td>
<td>0.031</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>R</td>
<td>Lingual Gyrus</td>
<td>18, 19, -15</td>
<td>51</td>
<td>-9</td>
<td>512</td>
<td>58.4 (12.4)</td>
<td>35.2 (14.7)</td>
<td>&lt;0.000023</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>L</td>
<td>Inferior Temporal Gyrus</td>
<td>20, 40</td>
<td>1</td>
<td>-45</td>
<td>872</td>
<td>31.8 (15.2)</td>
<td>13.6 (9.6)</td>
<td>&lt;0.00058</td>
<td>0.044</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>L</td>
<td>Inferior Parietal Lobule</td>
<td>40, 55</td>
<td>25</td>
<td>25</td>
<td>488</td>
<td>123.3 (21.5)</td>
<td>84.3 (20.7)</td>
<td>&lt;0.00108</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>L</td>
<td>Fusiform Gyrus</td>
<td>16, 42, 54, -11</td>
<td>792</td>
<td></td>
<td>50.1 (17.3)</td>
<td>30.7 (12.6)</td>
<td>&lt;0.00056</td>
<td>0.038</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>R</td>
<td>Cerebellum</td>
<td>-16, 58, -24</td>
<td>496</td>
<td></td>
<td>43.8 (11.2)</td>
<td>28.7 (6.7)</td>
<td>&lt;0.00015</td>
<td>0.013</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>R</td>
<td>Cerebellar Tonsil</td>
<td>-36, 49, -48</td>
<td>752</td>
<td></td>
<td>18.9 (5.9)</td>
<td>10.3 (5.3)</td>
<td>&lt;0.00008</td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>R</td>
<td>Precuneus</td>
<td>7, -17, 56, -21</td>
<td>696</td>
<td></td>
<td>55.0 (25.8)</td>
<td>28.7 (17.5)</td>
<td>&lt;0.00036</td>
<td>0.025</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

-Values and effect sizes reflect the change from baseline (pre) to after detraining (post) based on the average CBF within each ROI at each time point \( \eta^2 \), partial eta squared (effect size); # corresponds with regions shown in Figure 3.1; BA, Broadmann areas; LPI coordinates, positive, left (x), posterior (y), inferior (z); volume in mm\(^3\); CBF, cerebral blood flow (ml/100g/min); p, family wise (FWE) corrected p < 0.05 significance level.
Figure 3.2 Results of the A Priori Hippocampal Analysis

(A) Results of the a priori hippocampal analysis demonstrating significant CBF changes over time in the left and right hippocampus. The color bar represents the mean absolute CBF difference (post-detraining minus pre-detraining) within each ROI, (B) Scatter plots showing hippocampal CBF for each participant at both time points. Additionally, scatter plots indicate LPI coordinates and cluster volume in mm³. Corrected p-values were 0.0011 and 0.0041 for left and right hippocampus, respectively.
Chapter 4: The Joint Impact of Total Sleep Time and Acute Exercise on Prefrontal Resting State Functional Connectivity and Functional Activation.

Abstract

**Background:** Although, improved longevity is a major public health accomplishment, the prevalence of chronic disease, including cognitive impairment, increases with age. Insufficient sleep and physical inactivity exacerbate chronic disease and may accelerate the onset of dementia. While a cure remains elusive, a growing body of evidence demonstrates that exercise training facilitates better sleep and enhanced cognition. Exercise-altered patterns of neural activity, including resting state functional connectivity (rsFC) and task-based functional activation, likely coincide with and may facilitate cognitive improvements in the aging brain.

**Purpose:** This study sought to examine the joint impact of acute exercise and sleep quality on executive function in older adults. We also aimed to determine the degree to which exercise-induced changes in prefrontal rsFC influence the relationship between sleep and executive function performance/functional activation.

**Methods:** Using a within subjects counter-balanced design, 21 participants (aged 55-85) underwent at least three days of objective sleep monitoring (actigraphy), followed by two experimental visits on separate days. During each visit, participants engaged in 30-minutes of rest or exercise followed immediately by resting state and task-based functional MRI. After the MRI scanning session,
participants completed several executive function assessments. Neuroimaging and behavioral data were processed using AFNI (version 17.1.06) and SPSS (version 23), respectively.

**Results:** Repeated measures ANOVA and multivariate linear regression revealed two significant voxel-wise interactions in the (L) precuneus. Our findings demonstrated that acute exercise increased prefrontal rsFC and functional activation in long sleepers (> 7.5 hours/night), while decreasing these parameters for individuals with less total sleep time. Moreover, these results correspond to behavioral data demonstrating that acute exercise and adequate sleep improved select aspects of executive function performance, while decreasing inhibitory control in short sleepers alone (< 7.5 hours).

**Conclusion:** These findings suggest that the effects of acute exercise on prefrontal rsFC are similar, or even related, to the effects of acute exercise on conflict-dependent functional activation, and that this relationship may depend on sleep duration. Moreover, our results imply that although acute exercise elicited improved executive function for those with adequate sleep, it may weaken already vulnerable, and perhaps fatigued, executive function networks among short sleepers.

**4.1 Introduction**

Individuals over age 60 compose one of the fastest growing populations in the world\(^1\). While improved longevity is a major public health accomplishment, the prevalence of chronic disease, including cognitive impairment, increases with age\(^2\)\(^6\). Insufficient sleep and physical inactivity exacerbate chronic disease and
may accelerate the onset of dementia\textsuperscript{30}. A growing body of evidence demonstrates that exercise training not only facilitates better sleep and enhanced cognition, but also mitigates the cardiometabolic risk factors for dementia\textsuperscript{146,148}.

The prevalence of sleep disruptions, including insomnia and circadian dysrhythmia, increase with age\textsuperscript{16,227}. These conditions collectively result in less total sleep time (TST) and increased daytime fatigue\textsuperscript{30}. Neuroimaging studies have revealed that moderate levels of wake after sleep onset (WASO) and sleep disordered breathing are not only associated with decreased hippocampal volume, but also slower processing speed and poorer executive function performance\textsuperscript{228}. Likewise circadian dysrhythmias alter the sleep-wake cycle and have been implied in age-related cognitive decline\textsuperscript{123,227}.

Independently, increased physical activity and exercise have been shown to improve sleep and cognition\textsuperscript{21,153}. Resting-state functional connectivity (rsFC), particularly within networks of the prefrontal cortex, may mediate the relationship between sleep and exercise-induced improvements in executive function\textsuperscript{41,101,229}. Although rsFC has been shown to deteriorate with age, evidence suggests that exercise training may mitigate this decline\textsuperscript{29,38,41}, and enhance cognitive performance in older adults\textsuperscript{84,159}. While a comprehensive and thorough understanding of this relationship would seem necessary to assure healthy aging, no single study has combined multi-modal MRI and actigraphical sleep monitoring to carefully examine the complex relationship between acute exercise, sleep quality, and executive function. To this end, we conducted a within-subjects crossover design experiment to evaluate the joint impact of acute exercise and
sleep quality on executive function performance in older adults. We also explored whether or not the relationships between acute exercise and sleep quality on executive function performance and functional activation were mediated by exercise-induced changes in rsFC within neuronal hubs of two established prefrontal networks, the salience (SN; bilateral insula) and central executive (CEN; inferior frontal gyrus) networks.

**Research Objectives**

Based on findings from previous neuroimaging studies\textsuperscript{19,97} that examined the effects of exercise training on conflict interference and inhibitory control, we hypothesized that acute exercise would result in similarly altered patterns of functional brain activity.

**Primary:** To identify the main and interactive effects of acute exercise and sleep quality on executive function. **Secondary:** To assess the main and interactive effects of acute exercise and sleep quality on prefrontal rsFC.

**Exploratory:** To evaluate the mediating effects of exercise-altered rsFC on the relationship between sleep quality and exercise-altered executive function.

**Research Hypotheses**

**Primary:** $H_1$: Acute exercise, compared to rest, would be associated with decreased functional activation in the anterior cingulate cortex (ACC) and increased functional activation in the superior and medial frontal gyri (SFG/MFG) and superior parietal lobule (SPL); and better behavioral performance during the executive function task.
Primary H2: Worse sleep quality would be associated with increased functional activation (incongruent activation > congruent activation) in the ACC and decreased functional activation in the SFG/MFG and SPL, and poorer behavioral performance during the executive function task.

Primary H3: Individuals with worse sleep quality would garner greater exercise-induced improvements in functional activation and exhibit better behavioral performance during the executive function task.

Secondary: H4: Acute exercise, compared to rest, would be associated with decreased salience (SN) and central executive network (CEN) rsFC.

Secondary H5: Worse sleep quality would be related to increased SN and CEN rsFC.

Secondary H6: Individuals with worse sleep quality would experience greater exercise-induced decreases in SN and CEN rsFC.

Exploratory: H7: Exercise-altered prefrontal rsFC would mediate the relationship between sleep and exercise-induced changes in executive function activation and performance.

4.2 Methods

Participants

Our study sample consisted of healthy, physically active older adults between the ages of 55 and 81. Participants were primarily recruited from in-person informational sessions at senior fitness classes and recreation centers, but were also solicited through posted study fliers at local YMCAs, yoga studios, and running clubs. Additionally, study advertisements were published online
(e.g., the university event calendar, faculty listserves, and master athlete email threads) and in local regional newspapers. All participants were initially screened using a structured telephone interview (~15 minutes) to identify potential contraindications and determine study eligibility. If individuals met general study criteria, all three study visits were scheduled, including the in-person screening visit (~1.5 hours) and both experimental sessions (~3.5 hours each).

Individuals were considered ineligible if they reported a history of heart attack, stroke, transient ischemic attack; or a current diagnosis of coronary artery disease, cardiovascular disease, diabetes, hypertension, atrial fibrillation, or obstructive sleep apnea (STOP-Bang score ≥ 4). Additionally, individuals were ineligible if they reported a current diagnosis of psychiatric disorders including attention-deficit/hyperactivity disorder, clinical depression, clinical anxiety, post-traumatic stress disorder; or neurological disorders including, seizures, brain tumor, epilepsy, Parkinson’s disease, Alzheimer’s disease, or closed head injury. Individuals who demonstrated less than normal cognitive status (Mini-Mental Status Exam; MMSE score < 27), less than normal vision, limited English language proficiency, preferential left-handedness (Edinburgh Handedness Inventory score > 50), or presented any absolute contraindications to MRI were also ineligible for study participation.

Eligible participants completed the in-person screening visit prior to the experimental sessions at the participant’s selected location (common sites included the participant’s home, workplace, or recreation center). During the screening visit, written informed consent was obtained and participants were
administered the Mini-Mental Status Exam (MMSE)\textsuperscript{231}, to measure global
cognitive status and screen for possible cognitive impairment. Participants also
completed the STOP-Bang Questionnaire\textsuperscript{232}, to screen for possible obstructive
sleep apnea and the Pittsburgh Sleep Quality Index (PSQI)\textsuperscript{233} to evaluate
subjective sleep. At the end of the screening visit, eligible participants began
wearing a wrist-worn actigraphy device to obtain quantifiable indices of objective
sleep. Figure 4.1 illustrates the enrollment process from initial recruitment to
study completion. During the initial telephone screening, 53 persons were
deemed ineligible to participate in the study. Of the 32 individuals who qualified,
21 completed the entire study protocol (16 women, \~76\% white). All eligible
participants obtained physician approval for moderate intensity exercise. This
study was approved by the Institutional Review Board of the University of
Maryland, College Park (IRB# 347139-1).

**Neuropsychological Measures**

During each experimental visit, participants completed the following five
executive function tests: 1) the Erikson Flanker Task (Flanker Task)\textsuperscript{234}; 2) the
Controlled Oral Word Association Test (COWAT)\textsuperscript{235}; 3) the Symbol Digit
Modalities Test (SDMT)\textsuperscript{236}; 4) the Paced Auditory Serial Addition Test
(PASAT)\textsuperscript{237}; and 5) the Stroop Color and Word Task (Stroop Task)\textsuperscript{238}. With the
exception of the SDMT, alternate test versions were used for each experimental
visit. For habituation purposes, each of the five executive function tests included
a short practice session that consisted of an abbreviated version of the actual
test. Except for the Flanker Task, all practice sessions occurred immediately
before the true assessment. In an effort to conserve imaging time, the practice session for the Flanker Task occurred in a testing room outside the MRI scanner at the onset of each experimental visit. All executive function tests were completed either during (Flanker Task only) or after the MRI scan. Figure 4.2 depicts the time course of neuropsychological testing.

**Flanker Task**: During the functional MRI scan, and approximately 40 minutes after the experimental condition, participants engaged in a computerized Flanker Task to test attention and inhibitory control. The task lasted approximately 10 minutes and included 80 flanker trials interspersed by 40 null displays. Flanker trials were presented for 500 milliseconds (ms) and consisted of five arrows aligned in a horizontal array (one center arrow flanked by two pairs of adjacent arrows). For each trial, the flanking and center arrows were oriented in the same (congruent) or opposite direction (incongruent), with the center pointing both left and right equally throughout the task. To make a response, participants held an ergonomically fitted button box in their right hand, with the “left” and “right” buttons positioned under their index and middle fingers, respectively. Participants were instructed to focus on the center arrow and press the button corresponding to its direction as quickly and accurately as possible. Response time (RT) and accuracy were recorded for each trial and only accurate responses were included in the analysis. The interference score was our primary measure of inhibitory control and was calculated using the following equation:

\[
\frac{(\text{Incongruent RT} - \text{Congruent RT})}{\text{Congruent RT}} \times 100
\]
**Controlled Oral Word Association Test**\(^{235}\): The COWAT is a short phonemic verbal fluency test specifically designed to elicit executive function. It was administered immediately after the MRI scan and approximately 75 minutes after the experimental condition. Each test session involved three discrete letters. Participants were presented one letter at a time and were given 60 seconds to generate as many words beginning with that letter as possible. Except for repeated words and proper nouns, all real words generated were scored. Performance was evaluated by summing the total number of words produced for all three letters. Alternate test versions included letters CFL and PRW.

**Symbol Digit Modalities Test**\(^{236}\): The SDMT is a measure of processing speed and working memory. It was administered immediately after the COWAT and approximately 80 minutes after the experimental condition. Participants were presented a symbol-digit key and a sequence of symbol-only trials. During the test, participants were given 90 seconds to sequentially match the symbol-only trials with the appropriate number from the key. Performance was determined by summing the total number of correct trials. Participants completed both the written and oral forms of the test, consecutively, at each experimental visit. Only one version of the SDMT was used throughout the experiment.

**Paced Auditory Serial Addition Test**\(^{237}\): The PASAT assesses aspects of auditory attention, processing speed, and mathematical ability. It was administered immediately after the SDMT and approximately 85 minutes after the experimental condition. The participant was instructed to listen and respond to an audio recording. The audio recording consisted of single digit numbers spoken at
a rate of one every three seconds. The participant’s objective was not to give a running total, but rather to add the most recent number to the one heard immediately before. A second version of the task consisting of numbers spoken at a rate of one every two seconds was administered afterwards. Both versions contained 60 possible responses.

**Stoop Color and Word Task**: The Stroop Task was administered immediately after the PASAT and approximately 100 minutes after the experimental condition. This computerized task consisted of 56 color-word trials (e.g., red, green, blue, and yellow) presented one at a time on a black computer screen. Word text was either the same (congruent) or a different color (incongruent) as the word meaning. Utilizing a modified keyboard with four buttons representing each color, participants used their index and middle fingers on both hands to indicate the color of the word on the screen. Participants were instructed to respond as quickly and accurately as possible and only accurate trials were included in the analysis. Like the Flanker Task, the interference score was our primary outcome of interest and was calculated with the following equation:

\[
\frac{\text{Incongruent RT} - \text{Congruent RT}}{\text{Congruent RT}} \times 100
\]

**Actigraphy Data Acquisition**

At the end of the in-person screening visit, participants were fitted with, and provided instructions for wearing an actigraphy device (MotionLogger® Watch; Ambulatory Monitoring, Inc., Ardsley, New York, USA). Participants wore the actigraphy watch on their non-dominant wrist and were instructed to remove
it prior to water submersions only (e.g., swimming and bathing). In addition to
wearing the actigraphy watch, participants were provided two other methods for
recording sleep and wake times. The first method was the Consensus Sleep
Diary\textsuperscript{239}, which inquired about the times the participant got in bed, went to sleep,
awoke at night, awoke in the morning, and got out of bed each day. The second
method was to press an event-marking button on the watch to time-stamp the
actigraphy data. Participants were instructed to press the button when attempting
to initiate sleep and upon their final awakening in the morning.

Although actigraphy provides a valid and reliable measure of motion, it
does not assess sleep directly; therefore, sleep was quantitatively inferred from
low levels of recorded motion\textsuperscript{240}. A sensor within the internal circuitry of the
actigraphy device produced an electrical charge every time the watch was
moved\textsuperscript{241}. This voltage was transformed and optimized into a conditioned signal,
and processed by two distinct modes of operation: the Zero Crossing Mode
(ZCM) – which assesses signal frequency\textsuperscript{242}; and the Proportional Integral Mode
(PIM) – which assesses both signal intensity and acceleration\textsuperscript{243}. ZCM produces
a count score by quantifying the number of times the signal crosses an
established motion threshold, while PIM quantifies the area under the curve of
the rectified signal\textsuperscript{240}. For both modes of operation, the signal was collected
continuously and scored over 1-minute intervals. Our analysis relied exclusively
on the PIM score, as several studies have suggested it provides the most
accurate index of sleep in older adults\textsuperscript{243–246}. 

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Once the actigraphy data were downloaded from the watch, they were processed using Action-W Software (Ambulatory Monitoring, Inc., Ardsley, New York, USA). All data were visually inspected for quality assurance, and distorted or missing data were removed from further analysis. Sleep windows were manually identified according to the following four criteria (in all cases at least two of the four indices were aligned): 1) event-marker placement, 2) self-reported sleep/wake times, 3) activity counts per epoch, and 4) ambient light intensity. The sleep analysis was conducted using the UCSD Sleep Estimation Algorithm and was restricted to sleep windows only. This generated several parameters of objective sleep, including mean total sleep time (TST), wake after sleep onset (WASO), and sleep efficiency (SE).

Total Sleep Time, WASO, and SE were independently examined to identify the sleep parameter having the strongest association with exercise-induced changes in executive function performance. Using a bivariate correlation analysis, we found that TST was the only sleep parameter that shared a significant relationship with exercise-altered executive function performance ($r = -0.511$, $p = 0.018$). As a result, two binary sleep groups were created based on the TST distribution (median split = 7.5 hours). Accordingly, individuals sleeping greater than and less than 7.5 hours per night were classified as long and short sleepers, respectively. Actigraphy was used to collect activity data on a minute-by-minute basis continuously for a mean ($\pm$SD) of 6.57 (2.5) solar days.
Study Protocol and Timeline

Acute Exercise Session

Throughout the exercise protocol, the participant wore a heart rate (HR) monitor (Polar RS800CX) and max HR was determined using the age-predicted equation for older adults (max HR = 208 – 0.7*age)\(^{247}\). To establish each participant’s target HR, the Karvonen equation was used to calculate HR reserve (HRR = max HR – resting HR)\(^{248}\). Exercise was performed on a Monark 828 E (Vansbro, Sweden) mechanically braked cycle ergometer located in a testing room inside the neuroimaging facility. The exercise protocol consisted of a 5-minute warm-up (self-selected intensity), followed by 20-minutes of moderate intensity exercise (Borg’s rating scale of perceived exertion (RPE)\(^{249}\); RPE = 15), and a 5-minute cool-down (self-selected intensity). Before engaging in exercise, participants were provided standardized instructions for RPE, PAIN (e.g., leg pain intensity), and emotional valence and arousal ratings (Self Assessment Manikin; SAM-V and SAM-A)\(^{250}\). During moderate intensity exercise, participants were instructed to maintain a pedal cadence of 60 to 70 RPMs and to select a flywheel resistance corresponding to an RPE of 15. Before, during (at the fourth minute of each 5-minute interval), and after the exercise session, HR, RPE, PAIN, emotional valence, and arousal were assessed. Throughout the session, water was provided ad libitum and the participant was given a towel and change of clothing (for excess perspiration) before entering the MRI scanner. The MRI scan session began approximately 10 minutes after the completion of the exercise session.
Seated Rest Condition

Seated rest was performed in the same room as the exercise condition and lasted a total of 30 minutes (equivalent to the duration of the exercise condition). During the rest condition, the participant remained seated, wearing the HR monitor. Before, during (at the fourth minute of each 5-minute interval), and after the rest session, HR, RPE, PAIN, emotional valence, and arousal were assessed and water was provided ad libitum. While resting, the participant was prevented from using digital devices (including laptops, phones, MP3 players, etc.), reading, writing, or talking excessively. The MRI scan session began approximately 10 minutes after the completion of the rest session.

MRI Data Acquisition

At the beginning of each MRI scan session, high-resolution T1-weighted brain images were collected using a 3D Magnetization Prepared Rapid Gradient Echo imaging (MPRAGE) protocol for coregistration and normalization. The MPRAGE sequence lasted 4 min 26 sec and utilized single shot, multi-slice parallel acquisition with the following scan parameters: matrix = 256, field-of-view (FOV) = 230 mm, voxel size = 0.9 x 0.9 x 0.9 mm, axial slices = 192 (ascending order), slice thickness = 0.9 mm, repetition time (TR) = 1900 ms, echo time (TE) = 2.3 ms, inversion time (TI) = 900 ms, and flip angle = 9°. All scans were performed on a (3T) Siemens Trio Tim MRI scanner (Erlangen, Germany) using a 32-channel head coil for radio frequency transmission and reception.

Perfusion-weighted brain images were collected using a multi-delay 3D Gradient and Spin Echo (GRASE) pseudo-continuous arterial spin labeling
imaging (PCASL) protocol for perfusion quantification. The PCASL sequence lasted 6 min and 28 sec and utilized single-shot, background-suppressed acquisition with the following scan parameters: matrix = 64, FOV = 200 mm, voxel size = 3.1 x 3.1 x 5.0 mm, axial slices = 26 (ascending order), slice thickness = 5.0 mm, slice oversampling = 7.7%, label duration = 1500 ms, post-label delay (PLD) = 1400/1800/2200/2600 ms, TR/TE = 4000/22.6 ms, volumes = 96, number of label/control pairs = 12/PLD, flip angle = 180°, RF blocks = 80, block duration = 18.5 ms, and bandwidth = 3004 Hz/Px. Additionally, a short (55 sec) calibration sequence, with a long TR (5000 ms) and no background suppression, was used to acquire a proton density (PD) image for equilibrium calibration. During the PCASL and calibration sequences, participants were instructed to remain still with their eyes open and to look at a small fixation cross (white cross on black background) positioned directly above their eyes.

T2*-weighted functional brain images were collected using a fast Echo Planar Imaging (EPI) protocol. The EPI sequence utilized Blood-Oxygen-Level Dependent (BOLD) contrasts to measure low frequency oscillations and functional activation during the resting-state and task-based fMRI scans, respectively. The EPI sequences employed the following scan parameters: matrix = 64, FOV = 192 mm, voxel size = 3.0 x 3.0 x 3.0 mm, axial slices = 36 (interleaved), slice thickness = 3.0 mm, TR/TE = 2000/24 ms, volumes for resting-state = 240, volumes for task-based = 288, flip angle = 70°, and bandwidth = 2232 Hz/Px. The first EPI sequence was the resting-state scan, which lasted 8 min and 6 sec. Like before, participants were instructed to remain
still, with their eyes open and to look at a small fixation cross (white cross on black background) positioned directly above their eyes. The second EPI sequence was the task-based scan, which lasted 9 min and 42 sec. During this scan, participants engaged in the Flanker Task (see Figure 4.3). Immediately, before the scan, participants were again prompted to press the button that corresponded to the direction of the center arrow.

**MRI Data Processing**

**MPRAGE Data:** T1-weighted anatomical images were initially processed using FreeSurfer’s (version 5.3.0) reconstruction processing stream. Utilizing tissue-specific intensities and atlas probabilities, this automated procedure rendered both cortical parcellation and sub-cortical segmentation maps\(^{251}\). These high-resolution maps served several key purposes during the functional image analysis, including coregistration, nuisance regression, gray matter isolation, and normalization.

**Resting-State Data:** EPI mosaic files were sorted and concatenated to form a 3D image time-series using AFNI’s Dimon program\(^ {252}\). Once converted to 3D space, the first four image volumes were discarded to avoid possible artifact from magnetization disequilibrium. The resulting truncated time-series was realigned using a slice-wise motion correction algorithm (SLOMOCO)\(^ {253}\), which uses both in- and out-of-plane motion parameters to correct for misalignment between consecutive slices. The motion corrected time-series was coregistered with the FreeSurfer rendered anatomical brain map and together these datasets
were visually inspected for proper alignment, and entered into AFNI’s proc.py processing stream\textsuperscript{252}.

Because resting state data has increased susceptibility to physiological noise, an outlier fraction for each image volume was computed and tested for significant outliers\textsuperscript{254}. Volumes containing voxels outside the fraction threshold (> 10\%) were censored from the image time-series. The remaining volumes were despiked\textsuperscript{255} to attenuate high intensity transients within the BOLD signal, and time-shifted to adjust for the interleaved order of slice acquisition. Image volumes with a frame-wise displacement greater than 0.2 mm were also censored to reduce the spurious effects of head motion\textsuperscript{256}. Of the affected datasets (n = 17) the average censor fraction never exceeded 6\%. To preserve the heterogeneity of the individual brain anatomy, the T1-weighted image and anatomical followers (FreeSurfer rendered gray matter, white matter, and ventricular segmentations) were warped to MNI space using non-linear transformation\textsuperscript{257}. The resulting transformation matrices were then used to normalize the functional image time-series to standard space, effectively resampling the dataset to 2 mm\textsuperscript{3} isotropic voxel resolution. To further reduce physiological noise, signal from the normalized white matter, ventricles, and motion parameters were regressed\textsuperscript{258}, and the residual time-series was bandpass filtered to retain only signal frequencies between 0.01 - 0.1 Hz.

**Seed-Based Analysis:** To accurately identify key nodes of two well-known brain networks, we used the open-source interactive Neurosynth website\textsuperscript{259}. Given an appropriate search term (e.g., a psychological construct
such as anxiety), Neurosynth automatically processes hundreds of neuroimaging articles (provided the article contains at least one set of readable coordinates and is published in HTML format online), extracts the activation coordinates associated with the term, and generates a downloadable standardized statistical inference map (Figure 4.4). This statistical map can then be used to find areas of generalized and peak activation associated with a particular construct. For the purposes of this experiment, we used the term “conflict” for its pertinence to executive function and inhibitory control. This term prompted an automatic meta-analysis of 273 relevant articles. The peak activation coordinates generated from the analysis were used to seed bilateral regions of the central executive network (L and R Inferior Frontal Gyrus; ±46, 8, 26) and salience network (L and R Insula; ±34, 22, -2). Spherical seed masks (diameter = 10 mm) were plotted at these coordinates and used to extract the mean bilateral time-series from each functionally defined region (see Figure 4.5). These mean time-series were then cross-correlated with the time-series of every voxel in the functional image, effectively producing a correlation coefficient value for each. Using a Fisher’s Z-transformation, voxel-wise correlation coefficients were converted to z-scores for between-subject parametric comparisons.

**Task-Based Data:** The initial processing steps for the task-based data were the same as the resting-state analysis (see methods above). Briefly, the truncated motion corrected functional image time-series was coregistered with the T1-weighted image. Together, they were visually inspected for proper alignment and entered into AFNI’s proc.py processing stream. Image volumes
were despiked and time-shifted to attenuate signal fluctuations and adjust for the interleaved order of slice-acquisition. Volumes with a frame-wise displacement greater than 0.3 mm were censored to reduce the spurious effects of head motion. Using this threshold, the average censor fraction of the affected datasets (n = 3) never exceeded 1%. The functional time-series was then warped to standard space using the non-linear transformation matrices from the normalized T1-weighted image, effectively resampling the dataset to 2 mm$^3$ isotropic voxel resolution. After scaling the time-series to reflect percent signal change$^{260}$, the motion parameters were residualized and a deconvolution analysis was used to isolate and compare the hemodynamic response during incongruent and congruent flanker trials (incongruent – congruent contrast). Irrespective of the overall high accuracy rates during the task, only accurate trials were included in the analysis.

**Gray Matter Voxel-Wise Analysis:** Using the FreeSurfer rendered gray matter segments, single-subject gray matter maps were merged to create a group-level gray matter mask. Using a linear mixed effects model (AFNI’s 3dLME program)$^{261}$, a voxel-wise analysis restricted to the gray matter mask was performed to determine the main and interactive effects of acute exercise and TST on both rsFC and task-based functional activation. To control for the effects of multiple comparisons, and help protect against Type-1 error, we used AFNI’s cluster simulation program to establish a family-wise error corrected probability threshold. Combined with first order nearest neighbor clustering criteria, we retained statistical results from the rsFC analysis surviving $p < 0.01$ ($\alpha = 0.01$)
and from the Flanker analysis surviving \( p < 0.05 \) (\( \alpha = 0.01 \)). To further confirm and elucidate our results, we applied the resultant group-level region of interest (ROI) mask to each participant individually and extracted the mean Z-score (resting state data) and percent signal change (task-based data). ROI values were used to confirm the voxel-wise results and determine the extent and directionality of the Group x Condition effects.

**Task-Based ROI Analysis:** A large number of fMRI studies have demonstrated discrete, yet reliable patterns of functional activation associated with conflict interference (e.g., incongruent-congruent contrasts during the Flanker Task, Stroop Task, etc.\textsuperscript{262,263}. Based on this knowledge, and our a priori hypothesis that exercise would alter these patterns, we used AFNI’s 3dTtest++ program to specifically isolate and threshold conflict-dependent functional activation after the exercise and rest conditions. Significant activation clusters surviving a family-wise error corrected probability threshold of \( p < 0.05 \) (\( \alpha = 0.01 \)) for each condition were combined to create one functional ROI mask containing nine brain regions. This conjunction mask was applied to every participant individually and the mean percent signal change was extracted from each ROI. The extracted data were then entered into SPSS (version 23) for statistical analysis. Using a 2 x 2 repeated measures ANOVA we identified six regions exhibiting a significant main effect of acute exercise on conflict-dependent activation (\( p < 0.05 \)). To protect against the inherent risk associated with multiple-comparisons, we used a rank-order False Discovery Rate (FDR) correction technique\textsuperscript{264} to reduce the incidence of chance findings. Five of the six regions
survived FDR correction and post hoc t-tests were used determine the degree and directionality of the exercise effects.

**Neuropsychological Assessment Statistical Analysis**

All executive function assessment data were analyzed using a 2 x 2 repeated measures ANOVA (SPSS version 23) to identify the main and interactive effects of acute exercise and TST on executive function performance. Although the study sample consisted entirely of older adults, stratifying the sample by sleep duration created an age imbalance between the two groups. Moreover, because the experimental conditions were not perfectly counterbalanced, we used a post hoc method of covariate selection to adjust for these imbalances. Using previously described methods\textsuperscript{265–267} we used correlations (for continuous independent variables) and one-way ANOVA (for categorical independent variables) to explore the potential confounding effects of three variables: age, exercise order, and test version order. Covariates were only included in the model if they were significantly correlated with the outcome parameter (executive function difference score). Post hoc t-tests were used to examine both the extent and direction of notable findings (p < 0.1).

**Exploratory Mediation Analysis**

To explore the potential mediation effects of rsFC on the relationship between TST and Executive Function (Flanker Task performance and functional activation), we utilized PROCESS (version 2.16), an SPSS plugin tool. We used a single mediation model (Figure 4.6) with PROCESS’s bootstrapping capabilities to assess the indirect effects of CEN rsFC (\textit{Mi} = mediator) on TST (\textit{X})
= predictor) and functional activation/performance during the Flanker Task (Y = outcome)\textsuperscript{268}.

4.3 Results

Participant Demographics by Sleep Group

Long (> 7.5 hours) and short (< 7.5 hours) sleepers did not significantly differ by sex, race, education, BMI, self-reported physical activity, mental status, or resting heart rate (Table 4.1). Additionally, groups were not different by subjective sleep quality, risk of sleep apnea, or objective measures of SE or WASO. However, groups significantly differed by age (p = 0.014), age-predicted max HR (p = 0.014), and TST (p < 0.001).

Experimental Condition Parameters by Sleep Group

As expected, 30 minutes of moderate intensity aerobic exercise (compared to rest) elicited significantly greater ratings of perceived exertion (p < 0.001), higher emotional valence (p = 0.001), greater ratings of pain (p = 0.001), higher HR (p = 0.001), and greater HR intensities (p < 0.001) (see Table 4.2). These variables did not significantly differ by sleep group with the exception of emotional arousal, where short sleepers reported significantly higher ratings of arousal during the exercise condition (p = 0.007).

Task-Based Executive Function Behavioral Performance

As shown in Table 4.3, no significant interactive or main effects were found for Flanker Task performance. Mean percent accuracy for incongruent and congruent trials were not significantly different by group or condition. Similarly, incongruent and congruent trial response time (RT) between and within groups
was not different. However, follow-up analyses of notable results ($p < 0.1$) revealed significant within group differences by condition in incongruent RT – congruent RT ($p = 0.017$, Exercise > Rest) and interference score ($p = 0.029$, Exercise > Rest) in short sleepers only.

**Neuropsychological Assessment Performance**

As shown in Tables 4.4 and 4.5 and Figure 4.7, no significant interactive effects were found for COWAT, SDMT, PASAT, or Stroop Task performance. However, a significant main effect of group was found for SDMT written (Long > Short) and a main effect of condition for SDMT oral (Exercise > Rest). Additionally, follow-up analyses of notable results ($p < 0.1$) revealed a significant within group effect of condition on COWAT performance in short sleepers ($p = 0.017$, Exercise < Rest) and a significant effect of exercise on Stroop interference score ($p = 0.021$, Exercise < Rest).

**Voxel-Wise Flanker Task Analysis**

The voxel-wise Flanker Task analysis revealed a significant Group x Condition interaction confined to one brain region. As seen in Figure 4.8 and Table 4.6a, significant interactive effects were found within the (L) precuneus alone, with long-sleepers demonstrating exercise-induced increases in functional activation and short-sleepers showing exercise-induced decreases in activation.

**Voxel-Wise Resting State Functional Connectivity**

The voxel-wise rsFC analyses also revealed a significant Group x Condition interaction (CEN analysis) in addition to a main effect of exercise (SN analysis). As seen in Figure 4.9 and Table 4.6b, significant interactive effects
were confined solely within the (L) precuneus, with long-sleepers demonstrating exercise-induced increases in rsFC and short-sleepers exhibiting exercise-induced decreases in rsFC. Figure 4.10 and Table 4.6c shows the main effect of exercise (Exercise < Rest), with both groups exhibiting significantly decreased SN rsFC with (L) amygdala/hippocampus, middle temporal gyrus, and amygdala.

**Exercise-Induced Functional Changes: A Bi-Modal Association**

In view of the striking regional and directional similarities between exercise-altered CEN rsFC and exercise-altered voxel-wise functional activation, we performed a *post hoc* correlation analysis to further elucidate this relationship. Difference scores (Exercise – Rest) were computed for both rsFC and functional activation within the (L) precuneus. The difference scores were then stratified by sleep (Long and Short) and analyzed to determine the association between the bi-modal exercised-induced functional changes. As shown in Figure 4.11, results revealed an overall significant positive correlation ($r = 0.462, p = 0.035$) between exercise-induced changes in brain activity that differed by sleep duration (Long > Short).

**Conflict-Dependent Activation: Functional ROI Analysis**

Analysis of the mean signal intensities extracted from the conflict-dependent ROI mask resulted in a significant main effect of condition in six brain regions, five of which survived FDR correction (significant ROIs can be seen in Table 4.6d and Figure 4.12). Of the five regions, three exhibited decreased functional activation after exercise including: (R) anterior cingulate cortex, (L) putamen, and (L) middle temporal gyrus, while two showed significant increases
after exercise including: (L) inferior temporal gyrus and (L) inferior parietal lobe. However, after further examination, significant within group differences were found in four of the five regions. Namely, significant changes were found for short sleepers in the (R) anterior cingulate cortex, (L) inferior temporal gyrus, and (L) middle temporal gyrus, but not for long sleepers, while significant changes in long sleepers underpinned effects in the (L) Putamen.

**Exploratory Mediation Analysis Results**

As shown in Figures 4.13 and 4.14, CEN rsFC did not mediate the relationship between TST and exercise-altered Flanker Task performance (total indirect effect $p = 0.6549$) or TST and exercise-altered functional activation (total indirect effect $p = 0.2351$).

**4.4 Discussion**

The current study evaluated the interaction between acute exercise and TST on both behavioral and neurovascular indices of executive function in older adults, while also exploring the potential mediating effects of prefrontal rsFC on the relationship between TST and exercise-altered executive function performance and activation. Neuropsychological assessments revealed significant main effects of acute exercise on SDMT oral and Stroop interference scores, and a main effect of sleep on SDMT written scores, suggesting that exercise and adequate sleep, individually, enhanced processing speed, attention, and inhibitory control. While no significant Group x Condition interaction was found, post hoc analyses demonstrated that short sleepers performed significantly worse after exercise than their well-rested counterparts on the
COWAT and Flanker Task, respectively. These findings suggest that acute exercise, at least in the short-term, may have a detrimental effect on executive function performance in older adults who sleep less during the night.

Neuroimaging analyses, including assessments of prefrontal rsFC and Flanker Task functional activation, uncovered two significant voxel-wise interactions (both within the precuneus region), one voxel-wise main-effect of exercise (affecting three areas of the limbic system), and one ROI-based main-effect of exercise (within five areas associated with conflict interference). Additionally, a post hoc correlation analysis revealed a significant association between exercise-induced changes in CEN rsFC and exercise-altered Flanker Task functional activation that differed by sleep group. These findings are further discussed within the next section, which explores how well our predicted outcomes aligned with our findings.

Hypothesis Testing and Interpretation

\( H_1: \) Acute exercise, compared to rest, would be associated with decreased functional activation in the anterior cingulate cortex (ACC) and increased functional activation in the superior and medial frontal gyri (SFG/MFG) and superior parietal lobule (SPL).

Findings from the conflict-dependent functional ROI analysis partially supported this hypothesis. Specifically, acute exercise was associated with decreased functional activation in the ACC and increased activation in the parietal lobule, but was not significantly related to functional activation within the other hypothesized regions. While we predicted this type of exercise-altered
pattern of functional activation, it is important to remember that the *a priori*
regions (ACC, SPL, SFG/MFG) we selected were based on results from
exercise-training intervention studies\textsuperscript{19,97} that examined the effects of long-term
exercise and aerobic fitness on conflict-dependent patterns of functional
activation\textsuperscript{19,97}, not acute exercise. Specifically, these studies showed decreased
conflict-dependent ACC activation and increased activation within the parietal
cortex\textsuperscript{19}. To the best of our knowledge, only one other acute exercise study has
demonstrated this type of activation alteration\textsuperscript{269}. Using a within subjects design,
Li (2014) measured functional activation during a working memory task after 20
minutes of both rest and exercise, independently. Similar to the current study,
their results demonstrated decreased ACC activation after exercise without
concomitant changes in task performance. Although more research is necessary,
these findings, together, suggest that short-term changes in task-based
activation precede and might be indicative of the executive function performance
improvements associated with long-term exercise adherence.

\textbf{H\textsubscript{2}: Worse sleep quality would be associated with increased functional
activation (incongruent activation > congruent activation) in the ACC and
decreased functional activation in the SFG/MFG and SPL, and poorer behavioral
performance during the executive function task.}

This hypothesis was not supported by the current results as no significant
main effect of sleep was found in either the voxel-wise or conflict-dependent
Flanker Task analysis.
**Hypothesis:** Individuals with less TST would garner greater exercise-induced improvements in functional activation and better behavioral performance during the executive function task.

The voxel-wise Flanker Task analysis unveiled a significant Group x Condition interaction (including significant between group differences), with localized effects in the (L) precuneus, which supported the above hypothesis. Moreover, short sleepers displayed significantly decreased activation within this region, suggesting that short sleepers may receive less of an exercise-induced increase in cortical resources than long sleepers\(^1\),270. Given that exercise decreased ACC activation across the entire sample, while increasing activation in the parietal lobule in long-sleepers may signify that short-sleepers have a reduced capacity to reallocate neural resources or utilize compensatory neural networks. Perhaps, explaining why short-sleepers exhibited worse exercise-induced Flanker Task performance.

This reallocation of neural resources is a phenomenon that has been observed in studies of acute exercise before. In 2003, Hillman and colleagues used EEG to examine the effects of acute exercise on Flanker Task performance\(^9\),24. Their results demonstrated that exercise-altered patterns of cortical activity paralleled improvements in task performance. Although our findings demonstrated reduced functional activation in the ACC, we cannot conclude that this was a signature of improvement in neural network function, since Flanker Task performance remained unchanged (long sleepers) or significantly decreased (short sleepers) after exercise. However, several
established theories suggest that decreased activation in the ACC may be an index of improved conflict interference and conflict monitoring during information processing\textsuperscript{271}. This interpretation of ACC activation during conflict monitoring is inconsistent, however, with the current behavioral findings, which showed that short sleepers performed significantly worse on the Flanker Task after exercise than rest, despite having reduced ACC activation after exercise. Therefore, our data align well with previous findings demonstrating that exercise decreases functional activation within the ACC, but the impact of this change on executive function remains less clear when sleep history is taken into consideration.

\textbf{H\textsubscript{4}}: Acute exercise, compared to rest, would be associated with decreased rsFC within the SN and CEN.

To test this hypothesis, bilateral nodes of two established prefrontal networks were used to map brain areas with functionally correlated brain activity (during rest). Findings from the SN rsFC analysis partially supported this hypothesis, demonstrating a significant main-effect of exercise, whereby exercise decreased rsFC with three regions of the limbic system: the (L) amygdala/hippocampus, (L) middle temporal gyrus, and (L) amygdala – which are important sub-cortical components of the SN. However, these results contradict findings from a previous study, which demonstrated exercise-induced increases rsFC in several brain networks, including those believed to be involved in reward, executive function, and memory\textsuperscript{174}. Nonetheless, decreased intrinsic activity in the limbic system could reflect the anxiolytic effects of exercise that have been demonstrated throughout the animal literature\textsuperscript{272,273}. Moreover, these
decreases coincided with ancillary data (positive and negative affect schedule; PANAS scores) that we collected both before and immediately after the exercise session, which demonstrated significant exercise-induced increases in positive affect ($p = 0.014$) and near significant decreases in negative affect ($p = 0.062$). These findings imply that exercise-induced decreases in SN rsFC likely reflect (and may underpin) improvements in emotional affect and mood that result from exercise.

**H5:** Less TST would be related to increased rsFC within the SN and CEN.

This hypothesis was not supported by the current results as no significant main effect of sleep was found in either the SN or CEN rsFC voxel-wise analysis.

**H6:** Individuals with less TST would experience greater exercise-induced decreases in rsFC within the SN and CEN.

The Group x Condition interaction resulting from the CEN rsFC voxel-wise analysis do support the above hypothesis. The interactive effects revealed that short sleepers experienced the greatest exercise-induced decreases in intrinsic connectivity, which were localized in to the (L) precuneus. Possibly suggesting that exercise is acting to reduce intrinsic activity related to anxiety.²⁷⁴

Although, our findings did not demonstrate a significant Group x Condition interaction in SN rsFC, a larger sample size could have helped elucidate these effects. Particularly, since insufficient sleep and sleep deprivation have been associated with altered intrinsic connectivity within the insula and other areas of the salience network¹⁴⁹ and because we did find exercise-induced decreases in SN rsFC across the sample as a whole. In 2010, Sämann et al. examined the
effects of a single night of partial sleep deprivation on DMN and SN rsFC. Their findings demonstrated that increased sleep pressure, resulting from the partial sleep deprivation, was associated with decreased cortico-cortical connectivity between the two networks\textsuperscript{149}. While we did not observe this relationship, we did find that exercise significantly altered functional integration between these networks in ways that differed by sleep status, suggesting that sleep may partially determine the brain’s receptiveness to exercise. Moreover, disrupted sleep and is strongly associated with higher levels of anxiety\textsuperscript{275}, which may help drive dysfunctional connectivity within the SN\textsuperscript{274}. Specifically, recent findings suggest that patients with clinical anxiety, and perhaps even persons with high levels of trait anxiety may exhibit a unique neural signature of functional activity including increased rsFC in the SN\textsuperscript{274}. Considering these results, our data potentially underscore the anxiolytic effects of exercise in short-sleepers without, but at greater risk for, anxiety disorders\textsuperscript{275}.

**Exploratory H\textsubscript{7}: Exercise-altered prefrontal rsFC would mediate improvements in functional activation and behavioral performance during the executive function task.**

Central Executive Network rsFC did not mediate the relationship between TST and exercise-induced changes in Flanker Task performance, or TST and exercise-induced changes in Flanker Task functional activation. However, while we did not find a significant mediation effect, we did find that exercise-induced changes in functional activation were positively correlated with exercise-altered
rsFC within the (L) precuneus, which differed significantly by sleep duration. This suggests that the effects of acute exercise on CEN rsFC are similar (or even related) to the effects of acute exercise on conflict-dependent functional activation, and that this relationship may depend on habitual sleep duration.

Limitations

There are several limitations to this study, including a small (n = 21) and homogenous sample (16 women, ~76% white). All participants were highly educated (~95% had a college degree or higher), self-reported regular physical activity (engaged in at least 30 minutes of moderate activity ≥ 3x/week), had normal BMI, and had arguably high indices of objective sleep quality (SE = ~93.4%; age-norm = 80–85%, WASO = ~25 min; age-norm = 40-60 min). Additionally, participants were extremely healthy for their age, with none presenting a diagnosis of cardiovascular disease (including hypertension, diabetes, or severe obesity), sleep apnea, or psychiatric disorder (including taking medication for depression or anxiety). Moreover, due to the nature of our study design (long experimental visits, multi-sequence scanning sessions, and population of interest), mental and physical fatigue may have influenced some outcome measures. While we do not have data to support or refute the possibility of fatigue, experimental visits were scheduled at the same time of day for each participant to control for these potential effects. Due to our experimental design, executive function was not assessed immediately after exercise, but rather incrementally over the following two hours (Flanker Task = ~40 min-post, COWAT = ~75 min-post, SDMT = ~80 min-post, PASAT = ~85 min-post, Stroop
Task = ~100 min post), suggesting the possibility we could have missed some short-term effects of acute exercise on executive function. Together, these factors restrict the generalizability of our findings to very healthy older adults and extrapolation of findings is cautioned outside of this population.

Considering the evidence that increased age is associated with poorer sleep quality, worsening executive function performance, and altered patterns of neurovascular activity, we attempted to account for age as a potential confounder in the following ways: 1) exclusionary criteria restricted our study sample to older adults (age 55-85), 2) we used a repeated measures study design to control for within-subject differences, and 3) we used correlation analyses to test whether the relationship between sleep and executive function, and sleep and neurovascular activity were confounded by age \(^{265–267}\). Although the sleep groups significantly differed by age (short-sleepers were older), results from the correlation analyses demonstrated that age was not associated with executive function performance (in most cases) or neurovascular activity (in all cases), and therefore, was not a confounding factor \(^{265–267}\). In cases where age was found to be significantly associated with the executive function parameter (Stroop Task: incongruent accuracy, and Flanker Task: congruent accuracy), it was included as a covariate in the model.

**Future Directions**

This study is novel for a number of reasons including its focus on sleep, acute exercise, and their joint impact on neuropsychological performance and functional connectivity/activation. However, more high quality research is needed
to gain a comprehensive understanding of this complex relationship. The following examples are investigative ideas/questions that will help provide insight and practical guidance to an aging and increasingly physically inactive population struggling with inadequate sleep: 1) Can acute exercise facilitate improved sleep architecture, and do exercise-induced changes in sleep architecture effect cognitive performance, specifically executive function? 2) What are the neurovascular effects (e.g., as measured by rsFC and task-based functional activation) of acute exercise on older adults with sleep apnea or severe insomnia? 3) What are the acute effects of exercise on markers of β-Amyloid and do exercise-induced improvements in sleep facilitate enhanced β-Amyloid clearance during the sleep window? 4) What are the effects of acute exercise on default mode network rsFC, and do acute exercise-induced changes in this network facilitate better cognitive performance, specifically executive function? 5) Does acute exercise differentially affect cerebral perfusion in those with good and poor sleep quality, and how do exercise-induced alterations in cerebral perfusion (either regional or global) affect cognitive performance, specifically executive function? These relevant questions are a good platform from which to extend the current study and would significantly expand our understanding of the complex interplay between sleep, aerobic exercise and brain function.

**Conclusion**

Until now, few studies have explored the relationship between acute exercise and sleep in older adults. To the best of my knowledge, none have combined multimodal MRI with actigraphy to objectively examine the impact of
this relationship on both cognitive and neurovascular function. This study adds to
the literature by revealing several key findings: 1) 30-minutes of moderate
intensity acute exercise and higher levels of TST (> 7.5 hours) likely facilitate
faster processing speed and improved attention; 2) Acute exercise enhances
inhibitory control and reduces conflict interference; 3) Individuals with less TST (<
7.5 hours) may perform worse after a single session of acute exercise on tasks of
executive function than individuals with greater levels of TST (> 7.5 hours); 4)
Exercise-induced improvements in executive function appear relatively long
lasting (≥ 2 hours); 5) Acute exercise is associated with decreased rsFC between
prefrontal regions and the precuneus (a major hub of the default mode network),
and several additional key areas of the limbic system; 6) Acute exercise elicits
altered patterns of conflict-dependent functional activation; and leads to
alterations within the precuneus that differ by TST; and 7) The effects of acute
exercise on CEN rsFC are similar (or even related to), the effects of acute
exercise on conflict-dependent functional activation, and this relationship likely
affects long and short sleepers differently. Given the rapidly aging population in
the US, this work is timely and significant. Future research is warranted to
confirm the findings presented here and to elucidate the mechanisms by which
sleep and exercise work together to improve executive function for older adults.
Table 4.1 Demographic Variables by Group.

<table>
<thead>
<tr>
<th></th>
<th>Total Sample (n = 21)</th>
<th>Long Sleepers(^a) (n = 10)</th>
<th>Short Sleepers(^a) (n = 11)</th>
<th>Group Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.6</td>
<td>8.0</td>
<td>61.3</td>
<td>5.2</td>
</tr>
<tr>
<td>Sex (n, %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5.0</td>
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<td>16.0</td>
<td>76.2</td>
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<tr>
<td>Race (n, %)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
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<td>16.0</td>
<td>76.2</td>
<td>7.0</td>
<td>70.0</td>
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<tr>
<td>Other</td>
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<td>23.8</td>
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<tr>
<td>Education (n, %)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ College</td>
<td>7.0</td>
<td>33.3</td>
<td>4.0</td>
<td>40.0</td>
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<tr>
<td>≥ Graduate</td>
<td>14.0</td>
<td>66.7</td>
<td>6.0</td>
<td>60.0</td>
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<tr>
<td>BMI (kg/m(^2))</td>
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<td>Physical Activity</td>
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<td></td>
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<tr>
<td>7-Day PA Recall (kcal/kg/wk)</td>
<td>221.2</td>
<td>33.2</td>
<td>216.7</td>
<td>34.3</td>
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<tr>
<td>Sleep</td>
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<td></td>
</tr>
<tr>
<td>TST (hours)</td>
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<td>1.1</td>
<td>7.9</td>
<td>0.5</td>
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<tr>
<td>SE (%)</td>
<td>93.4</td>
<td>2.6</td>
<td>94.0</td>
<td>1.7</td>
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<tr>
<td>WASO (minutes)</td>
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<td>11.1</td>
<td>25.8</td>
<td>10.1</td>
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<td>Actigraphy Monitoring (days)</td>
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<td>PSQI</td>
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<td>STOP-Bang</td>
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<td>MMSE</td>
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<td>29.6</td>
<td>0.7</td>
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<tr>
<td>HR (BPM)</td>
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<tr>
<td>Age-Predicted HR(^{\text{Max}})</td>
<td>162.1</td>
<td>5.6</td>
<td>165.1</td>
<td>3.6</td>
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<tr>
<td>HR(^{\text{Resting}})</td>
<td>65.6</td>
<td>8.1</td>
<td>64.8</td>
<td>9.2</td>
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</table>
P-values and effect sizes reflect differences between the two sleep groups. SD, standard deviation; BMI, body mass index; TST, total sleep time; SE, sleep efficiency; WASO, wake after sleep onset; PSQI, Pittsburgh Sleep Quality Index; MMSE, Mini-Mental Status Exam; HR, heart rate; BPM, beats per minute. \(^a\)Long sleepers defined as > 7.5 hours of sleep and short sleepers < 7.5 hours. \(^b\)Continuous variables use eta squared as the effect size; categorical variables use Cramer's V. Bold indicates \(p < 0.05\).
### Table 4.2 Experimental Variables During Intervention by Group and Condition.

<table>
<thead>
<tr>
<th></th>
<th>Total Sample (n = 21)</th>
<th>Long Sleepers* (n = 10)</th>
<th>Short Sleepers* (n = 11)</th>
<th>Interaction Effect</th>
<th>Group Main Effect</th>
<th>Condition Main Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exercise</td>
<td>Rest</td>
<td>Exercise</td>
<td>Rest</td>
<td>Exercise</td>
<td>Rest</td>
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<tr>
<td>RPE</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td></td>
<td>15.0</td>
<td>0.9</td>
<td>6.0</td>
<td>0.2</td>
<td>15.0</td>
<td>1.1</td>
</tr>
<tr>
<td>SAM-V</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>2.0</td>
<td>7.0</td>
<td>2.2</td>
<td>5.5</td>
<td>1.7</td>
</tr>
<tr>
<td>SAM-A</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>2.0</td>
<td>4.4</td>
<td>2.2</td>
<td>4.9</td>
<td>2.0</td>
</tr>
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<td>PAIN</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td></td>
<td>1.6</td>
<td>1.4</td>
<td>0.2</td>
<td>0.5</td>
<td>1.4</td>
<td>1.3</td>
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<tr>
<td>HR (BPM)</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td></td>
<td>140.5</td>
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<td>65.6</td>
<td>8.1</td>
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<td>Intensity (%)</td>
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<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td></td>
<td>77.3</td>
<td>19.2</td>
<td>0.0</td>
<td>0.0</td>
<td>81.4</td>
<td>15.7</td>
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</table>

P-values and effect sizes reflect between and within group differences. SD, standard deviation; RPE, rate of perceived exertion; SAM-V, Self Assessment Manikin-valence; SAM-A, Self Assessment Manikin-arousal; HR, heart rate; BPM, beats per minute; Intensity, percentage of heart rate reserve; η²p, partial eta squared. *Long sleepers defined as > 7.5 hours of sleep and short sleepers < 7.5 hours. Bold indicates p < 0.05.
Table 4.3 Flanker Task Results by Group and Condition.

<table>
<thead>
<tr>
<th></th>
<th>Total Sample (n = 21)</th>
<th>Long Sleepers s (n = 10)</th>
<th>Short Sleepers s (n = 11)</th>
<th>Interaction Effect</th>
<th>Group Main Effect</th>
<th>Condition Main Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Post-Exercise</td>
<td>Post-Rest</td>
<td>Post-Exercise</td>
<td>Post-Rest</td>
<td>p-value (η²p)</td>
<td>p-value (η²p)</td>
</tr>
<tr>
<td>Congruent response</td>
<td>Mean 566.5 94.9</td>
<td>Mean 569.0 96.1</td>
<td>Mean 581.3 109.6</td>
<td>Mean 553.0 82.4</td>
<td>0.975 (0.000)</td>
<td>0.495 (0.025)</td>
</tr>
<tr>
<td>time (ms)</td>
<td>SD 569.0 96.1</td>
<td>SD 569.0 96.1</td>
<td>SD 581.3 109.6</td>
<td>SD 553.0 82.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incongruent response</td>
<td>Mean 645.2 121.7</td>
<td>Mean 634.2 114.1</td>
<td>Mean 649.9 131.1</td>
<td>Mean 640.9 118.8</td>
<td>0.231 (0.075)</td>
<td>0.633 (0.012)</td>
</tr>
<tr>
<td>time (ms)</td>
<td>SD 634.2 114.1</td>
<td>SD 634.2 114.1</td>
<td>SD 655.3 136.2</td>
<td>SD 614.9 92.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incongruent</td>
<td>Mean 78.7 48.1</td>
<td>Mean 68.6 57.0</td>
<td>Mean 87.9 38.8</td>
<td>Mean 98.9 1.3</td>
<td>0.055 (0.181)</td>
<td>0.856 (0.002)</td>
</tr>
<tr>
<td>minus</td>
<td>SD 65.2 38.9</td>
<td>SD 71.9 48.8</td>
<td>SD 59.0 28.1</td>
<td>SD 99.0 3.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congruent accuracy</td>
<td>Mean 99.2 1.2</td>
<td>Mean 99.5 1.1</td>
<td>Mean 98.9 1.3</td>
<td>Mean 99.0 3.4</td>
<td>0.102 (0.142)</td>
<td>0.760 (0.005)</td>
</tr>
<tr>
<td>b (%)</td>
<td>SD 99.2 2.6</td>
<td>SD 99.5 1.6</td>
<td>SD 99.0 3.4</td>
<td>SD 99.0 3.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incongruent accuracy</td>
<td>Mean 96.5 6.4</td>
<td>Mean 97.5 3.9</td>
<td>Mean 95.6 8.2</td>
<td>Mean 94.9 7.3</td>
<td>0.529 (0.022)</td>
<td>0.320 (0.055)</td>
</tr>
<tr>
<td>a (%)</td>
<td>SD 96.0 6.2</td>
<td>SD 97.1 4.8</td>
<td>SD 94.9 7.3</td>
<td>SD 94.9 7.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interference score (%)</td>
<td>Mean 13.7 7.1</td>
<td>Mean 11.9 8.8</td>
<td>Mean 15.3 5.1</td>
<td>Mean 10.5 5.3</td>
<td>0.093 (0.141)</td>
<td>0.739 (0.006)</td>
</tr>
</tbody>
</table>

P-values and effect sizes reflect between and within group differences. SD, standard deviation; ms, millisecond; η²p, partial eta squared. aAdjusted for experimental condition order. bAdjusted for age. cAdjusted for executive function test version order. sLong sleepers defined as > 7.5 hours of sleep and short sleepers < 7.5 hours. $ Indicates a significant within-group difference. Bold indicates p < 0.05.
Table 4.4 Stroop Task Results by Group and Condition.

<table>
<thead>
<tr>
<th></th>
<th>Total Sample (n = 21)</th>
<th>Long Sleepers (n = 10)</th>
<th>Short Sleepers (n = 11)</th>
<th>Interact-ion Effect</th>
<th>Group Main Effect</th>
<th>Condition Main Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Post-Exercise</td>
<td>Post-Rest</td>
<td>Post-Exercise</td>
<td>Post-Rest</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean  SD</td>
<td>Mean  SD</td>
<td>Mean  SD</td>
<td>Mean  SD</td>
<td>p-value (η²p)</td>
<td>p-value (η²p)</td>
</tr>
<tr>
<td>Congruent RTa (ms)</td>
<td>1165.9 305.3</td>
<td>1125.5 321.1</td>
<td>1047.1 183.1</td>
<td>1045.0 245.8</td>
<td>0.346 0.059</td>
<td>0.137 0.141</td>
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<tr>
<td>Incongruent RT (ms)</td>
<td>1296.7 313.5</td>
<td>1284.1 282.0</td>
<td>1165.7 198.4</td>
<td>1180.5 200.3</td>
<td>0.508 0.028</td>
<td>0.081 0.178</td>
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<td>Incongruent-Congruent (ms)</td>
<td>130.8 104.9</td>
<td>158.6 143.0</td>
<td>118.6 97.5</td>
<td>135.4 127.0</td>
<td>143.0 116.3</td>
<td>181.8 161.6</td>
</tr>
<tr>
<td>Congruent accuracy (%)</td>
<td>99.8 0.8</td>
<td>99.6 1.2</td>
<td>99.6 1.2</td>
<td>100.0 0.0</td>
<td>100.0 0.0</td>
<td>99.2 1.6</td>
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<td>Incongruent accuracy (%)</td>
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<td>97.6 4.7</td>
<td>96.0 5.2</td>
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<td>16.86 15.4</td>
<td>11.8 11.4</td>
<td>15.0 13.7</td>
<td>12.6 11.5</td>
<td>18.6 17.6</td>
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RT= Response Time. P-values and effect sizes reflect between and within group differences. SD, standard deviation; ms, millisecond; η²p, partial eta squared. aAdjusted for experimental condition order. bAdjusted for age. cAdjusted for executive function test version order. dLong sleepers defined as > 7.5 hours of sleep and short sleepers < 7.5 hours. eIndicates a significant within-group difference. Bold indicates p < 0.05.
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P-values and effect sizes reflect between and within group differences. SD, standard deviation; COWAT, Controlled Oral Word Association Test; SDMT, Symbol Digit Modalities Test; PASAT 3 & 2, Paced Auditory Serial Addition Test, 3 = one word every three seconds, 2 = one word every two seconds η²p, partial eta squared. *Adjusted for experimental condition order. **Adjusted for age. ***Adjusted for executive function test version order. #Long sleepers defined as > 7.5 hours of sleep and short sleepers < 7.5 hours. Bold indicates p < 0.05.
Table 4.6a. Flanker Task Voxel-Wise Analysis.

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Table 4.6b. Resting State Functional Connectivity Voxel-Wise Analysis.

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<th>z</th>
<th>vol</th>
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<th>Post-Rest</th>
<th>Short Sleepers$^a$ (n = 11)</th>
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<th>Post-Rest</th>
<th>Interaction Effect</th>
<th>Group Main Effect</th>
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<td>p-value (η$^2_p$)</td>
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Region: P= Precuneus, H=Hippocampus, MTG= Middle Temporal Gyrus, A=Amygdala. P-values and effect sizes reflect between and within group differences. SD, standard deviation; # corresponds with regions shown in figure; BA, Broadmann areas; RAI coordinates, positive, left (x), posterior (y), inferior (z); vol, volume in mm³. Long sleepers defined as > 7.5 hours of sleep and short sleepers < 7.5 hours. Common superscript symbols ($,#$,ø,å) indicate a significant between or within group difference. Bold indicates p < 0.01.
Table 4.6d. Flanker Task Functional Activation ROI Analysis.

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Region: MOO= Middle Occipital Gyrus, ACC= Anterior Cingulate Cortex, ITG Inferior Temporal Gyrus, PT= Putamen, MTG= Middle Temporal Gyrus, IPL= Inferior Parietal Lobe, D= Declive, IFG= Inferior Frontal Gyrus. P-values and effect sizes reflect between and within group differences. SD, standard deviation; # corresponds with regions shown in figure; BA, Broadmann areas; RAI coordinates, positive, left (x), posterior (y), inferior (z); vol, volume in mm³. *Long sleepers defined as > 7.5 hours of sleep and short sleepers < 7.5 hours. Common superscript symbols (¢,#,ø,å) indicate a significant between or within group difference. Bold indicates p < 0.05.
Figure 4.1 Derivation of The Final Study Sample

N = ~385 Exposed to In-Person Solicitation for Study Participation
- n = 360 recruited from local senior fitness classes (n = 40/class x 9 classes) (93.51%)
  - n = 20 recruited from a local brain fitness program (5.19%)
  - n = 5 recruited from a local retirement center (1.30%)

n = 153 Responded to Study Solicitation and Advertisements
- n = 19 could not be contacted at follow-up (12.41%)
- n = 49 did not meet inclusion/exclusion criteria (31.37%)

n = 85 Completed Initial Phone Screen
- n = 53 did not meet inclusion/exclusion criteria (62.35%)
  - n = 3 for left handedness (3.52%)
  - n = 10 for taking excluded medication (11.76%)
  - n = 18 for MRI contraindications (21.17%)
  - n = 22 for medical contraindications (25.88%)

n = 32 Scheduled for Experimental Sessions
- n = 7 withdrew for lack of time or loss of interest (21.88%)
- n = 1 could not obtain physician consent for exercise (3.12%)

n = 24 Signed Informed Consent
- n = 2 disqualified for previously unidentified MRI contraindications (8.33%)

n = 22 Completed Screening Visit
- n = 1 withdrew due to an unrelated medical event (4.45%)

n = 21 Completed Entire Study Protocol
- n = 5 men (23.81%)
- n = 16 women (76.19%)

Figure 4.1 demonstrates the participant enrollment process beginning with all persons solicited (n = ~385) and ending with the total number of participants who completed the experiment (n = 21). Enrollment was ongoing and stretched from mid-November 2016 to late April 2017.
Figure 4.2 reflects the overall study design (repeated measures and counterbalanced). Horizontal arms (upper and lower) represent the two experimental days. Colors demonstrate both the type of assessments and the repeated measures nature of the design. Exercise (red) and rest (gray) conditions were the only factors that differed between the two experimental days.
Figure 4.3 The Flanker Task

Adapted from Colcombe et al. (2004) and Eriksen et al. (1974). The figure above shows the four screen types that were displayed during the functional MRI scan. Using an event-related design, participants were presented with a blank fixation screen (1500-9500 ms), which was followed by a pre-cue (500 ms) to notify participants that a stimulus was about to appear. When the stimulus appeared the participants pressed the button (left/right) that corresponded to the direction of the center arrow. The flanker stimuli remained on the screen for 500 ms and were followed immediately by a 1500 ms response window (blank screen). Response time and accuracy were recorded at any point after stimulus onset and during the response window. The participants completed a total of 80 trials (40 congruent/incongruent)\textsuperscript{19}. Flanker task behavioral performance was computed using the following equation\textsuperscript{19,97}, and depicts the percent increase in response time (RT) for incongruent trials over the average response time for congruent trials: This scoring method reflects inhibitory control unbiased by differences in base response time\textsuperscript{19,97}. Only correct responses were used in the analysis.

\[
\left[ \frac{\text{Incongruent RT} - \text{Congruent RT}}{\text{Congruent RT}} \right] \times 100
\]
Figure 4.4, panel (A) reflects the inference map generated by the online processing tool Neurosynth. Using the term “Conflict,” Neurosynth performed an automated meta-analysis using results from 273 published papers. The green and orange clusters denote conflict-dependent functional activation, with orange representing areas of peak activation. Panel (B) shows the conflict-dependent maps generated from the current experimental data. Activation clusters reflect the Incongruent – Congruent contrasts (Flanker Task T-test results) collapsed across each subject and condition. Clusters survived a family-wise error corrected probability threshold of $p < 0.05$ (voxel-wise = $p < 0.05$, cluster-wise = $\alpha = 0.01$).
Figure 4.5 Bilateral Insula and IFG Seeds and Corresponding rsFC Map

Figure 4.5, panel (A) demonstrates the seeded regions (bilateral insula) used to elucidate the salience network. The mean time-series from each seed (diameter = 10 mm, composed of 81 voxels) was extracted, averaged, and cross-correlated with every other voxel in the brain to create a functional connectivity map (correlation map positioned to the right of the seed map). Panel (B) demonstrates the seeded regions (bilateral inferior frontal gyrus) used to elucidate the central executive network. The mean time-series from each seed (diameter = 10 mm, composed of 81 voxels) was extracted, averaged, and cross-correlated with every other voxel in the brain to create a functional connectivity map (correlation map positioned to the right of the seed map). The color bar indicates the strength of the correlation.
Figure 4.6 is a general representation of a single mediation model. The arrow between $X$ and $Y$ represents the direct effect of the predictor on the outcome variable. $M_i$ represents the intermediate variable and the product of the arrows between $X$ and $M_i$ and $M_i$ and $Y$ reflects the indirect effect of $M_i$ on the relationship between $X$ and $Y$. 
Figure 4.7 Neuropsychological Assessment by Condition and Group

A.

1. **Symbol Digit Modalities Test (written)**
   - (Main Effect of Sleep)
   - \( p = 0.035 \)

   ![Graph showing comparison between Long-Sleepers and Short-Sleepers for Symbol Digit Modalities Test (written).]

2. **Symbol Digit Modalities Test (oral)**
   - (Main Effect of Exercise)
   - \( p = 0.020 \)

   ![Graph showing comparison between Post-Exercise and Post-Rest for Symbol Digit Modalities Test (oral).]

3. **Stroop Interference Score**
   - (Main Effect of Exercise)
   - \( p = 0.021 \)

   ![Graph showing comparison between Post-Exercise and Post-Rest for Stroop Interference Score.]

B.

1. **Controlled Oral Word Association Test**
   - (Post-Hoc Analysis)
   - \( p = 0.017 \)

   ![Graph showing comparison between Long-Sleepers and Short-Sleepers for Controlled Oral Word Association Test.]

2. **Flanker Incongruent – Congruent RT**
   - (Post-Hoc Analysis)
   - \( p = 0.017 \)

   ![Graph showing comparison between Long-Sleepers and Short-Sleepers for Flanker Incongruent – Congruent RT.]

3. **Flanker Interference Score**
   - (Post-Hoc Analysis)
   - \( p = 0.029 \)

   ![Graph showing comparison between Long-Sleepers and Short-Sleepers for Flanker Interference Score.]

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Figure 4.7, panel (A) demonstrates the main effect of both sleep and exercise on executive function performance. Panel (B) depicts the results from post hoc analyses showing that exercise decreased executive function performance in short sleepers alone.
Figure 4.8 depicts the significant Group x Condition interaction revealed during the Voxel-Wise Flanker Task Analysis. Results indicate that exercise increased functional activation in the (L) precuneus in long-sleepers, while reducing functional activation in the (L) precuneus for short-sleepers. These results suggest that the effects of exercise on functional activation depend on previous sleep duration.
Figure 4.9 Voxel-Wise CEN Resting State Functional Connectivity

Figure 4.9 depicts the significant Group x Condition interaction revealed during the Voxel-Wise Central Executive Network (CEN) Resting State Analysis. Results indicate that exercise increased functional connectivity between bilateral nodes of the CEN and the (L) precuneus in long-sleepers, while reducing functional connectivity for short-sleepers. These results suggest that the effects of exercise on CEN functional connectivity depend on previous sleep duration.
Figure 4.10 Voxel-Wise SN Resting State Functional Connectivity

Figure 4.10 depicts the significant main effect exercise revealed during the Voxel-Wise Salience Network (SN) Resting State Functional Connectivity Analysis. Results indicate that exercise decreased functional connectivity between bilateral nodes of the SN and three areas of the limbic system. These results suggest that, irrespective of sleep, exercise reduces SN functional connectivity.
Figure 4.11 demonstrates the relationship between exercise-induced changes in CEN functional connectivity and exercise-induced changes in functional connectivity within the (L) precuneus. As illustrated by the figure, the direction of these effects depends on sleep duration.
Figure 4.12 reflects the results of the Flanker Task Region of Interest (ROI) Analysis. Of the nine ROIs used, six revealed a significant main effect of exercise. The top row shows three brain areas that experienced exercise-induced decreases in functional activation and the bottom row shows three brain areas that experienced exercise-induced increases in functional activation. The brain map that has been crossed out did not survive FDR correction. Noteworthy, are the exercise-induced decreases in the anterior cingulate cortex and increases in the parietal lobule.
Figure 4.13 shows the results of the exploratory mediation analysis. Results demonstrated that exercise-induced changes in prefrontal resting state functional connectivity (rsFC) did not mediate the relationship between total sleep time (TST) and exercise-induced changes in Flanker Task performance.
Figure 4.14 shows the results of the exploratory mediation analysis. Results demonstrated that exercise-induced changes in prefrontal resting state functional connectivity (rsFC) did not mediate the relationship between total sleep time (TST) and exercise-induced changes in Flanker Task functional activation.
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