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EXPLORING THE IMPACT OF PHYSICAL ACTIVITY ON THE HEALTHY AGING BRAIN: A VOLUMETRIC STUDY

Dissertação no âmbito do Mestrado em Investigação Biomédica, no ramo de Neurobiologia orientada pela Prof. Doutora Otília da Anunciação Cardoso d'Almeida e pela Doutora Anabela Silva Fernandes e apresentada à Faculdade de Medicina da Universidade de Coimbra

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"Success is not final, failure is not fatal:

It is the courage to continue that counts."

Winston Churchill

ABSTRACT

Age-associated shrinkage in gray matter volume, particularly in prefrontal and medial temporal areas, has been associated with cognitive deficits in several domains. A growing body of evidence has suggested that modifiable lifestyle behaviors may alter the trajectory of cognitive decline associated with healthy aging. Physical activity is one of the most promising behavioral interventions associated with beneficial effects on brain structure and cognitive function. However, the mechanisms underlying these effects remain unclear. The current physical activity guidelines from the World Health Organization emphasize that adults aged 65 or above should engage in, at least, 30 minutes per day of moderate-to-vigorous physical activity (MVPA) to ensure overall health. The present work aims to explore whether meeting the current physical activity guidelines, as compared to those who are not meeting them, would impact on morphometric brain measures, specifically in cortical prefrontal and core hippocampal structures such as dentate gyrus (DG) and cornu ammonis (CA), and cognitive functioning.

Twenty neurologically healthy older adults underwent a 3T structural magnetic resonance imaging acquisition, an objective assessment of daily physical activity pattern through wearable accelerometers, and a comprehensive cognitive assessment. Volumes of cortical and subcortical gray matter of regions-of-interest (ROI), controlled to total intracranial volume were estimated through FreeSurfer.

Participants that met the 30 minutes per day of MVPA had significantly larger volumes in several cortical and subcortical brain regions, including the ventral lateral prefrontal and motor regions, the thalamus and in hippocampal structures, specifically in the CA4 and DG subfields, compared with those who did not. Those above the guidelines also demonstrated better performance in processing speed and executive-function related tasks.

The current cross-sectional findings reinforce the literature about the beneficial effects of the daily physical activity on mental health, demonstrating that even modestly increases in daily MVPA could prevent age-related volume decline in frontal regions and hippocampal subfields, which may also have repercussions on delaying the age-related cognitive decline.

Keywords: Aging, Brain Imaging, Cognition, Gray Matter, Physical Activity

RESUMO

A redução do volume de substância cinzenta relacionada ao envelhecimento, particularmente nas áreas pré-frontais e temporais mediais, tem sido associada a declínios cognitivos em vários domínios. Evidências sugerem que a modificação de comportamentos associados ao estilo de vida podem alterar a trajetória do declínio cognitivo associado ao envelhecimento saudável. A atividade física é uma das intervenções comportamentais mais promissoras associada a efeitos benéficos na estrutura cerebral e na função cognitiva. No entanto, os mecanismos subjacentes a esses efeitos permanecem por clarificar. As atuais recomendações para a atividade física da Organização Mundial de Saúde enfatizam que adultos com idade igual ou superior a 65 anos devem praticar, pelo menos, 30 minutos por dia de atividade física moderada-a-vigorosa (AFMV) no intuito de promover a saúde. O presente trabalho tem por objetivo explorar se o cumprimento das atuais recomendações de atividade física, em comparação com aqueles que não as cumprem, tem um impacto em medidas morfométricas do cérebro, especificamente em estruturas pré-frontais e regiões centrais do hipocampo, tais como o giro denteado (GD) e *cornu ammonis* (CA), e no funcionamento cognitivo.

Vinte idosos neurologicamente saudáveis foram submetidos a uma aquisição de ressonância magnética cerebral estrutural a 3T, uma avaliação objetiva dos padrões diários de atividade física através de acelerómetros, e a uma avaliação cognitiva abrangente. Volumes de regiões-de-interesse de substância cinzenta cortical e subcortical, controladas para o volume total intracraniano foram estimados através do FreeSurfer.

Os participantes que realizaram os 30 minutos diários de AFMV tinham volumes significativamente superiores em várias regiões corticais e subcorticais, incluindo as áreas ventrolateral do córtex pré-frontal e motoras, o tálamo e estruturas do hipocampo, especificamente os subcampos CA4 e GD, em comparação aos participantes que não atingiram os mínimos recomendados. Os participantes acima das recomendações mínimas também demonstraram melhor desempenho na velocidade de processamento e em tarefas relacionadas com a função executiva.

Os resultados deste estudo transversal reforçam os dados da literatura sobre o efeito benéfico da atividade física quotidiana na saúde mental, demonstrando que mesmo um ligeiro aumento na AFMV diária poderá retardar o declínio do volume de áreas frontais e de sub-regiões do hipocampo, com repercussões no atraso do declínio cognitivo relacionado com a idade.

Palavras-chave: Envelhecimento, Imagiologia Cerebral, Cognição, Substância Cinzenta, Atividade Física

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LIST OF ABBREVIATIONS

ACC	Anterior Cingulate Cortex
AFMV	Atividade Física Moderada-a-Vigorosa
BDNF	Brain-Derived Neurotropic Factor
BMI	Body Mass Index
CA	Cornu Ammonis
cpm	Counts per minute
CSF	Cerebrospinal Fluid
DAN	Dorsal Attention Network
DLPFC	Dorsolateral Prefrontal Cortex
DMN	Default Mode Network
DTI	Diffusion Tensor Imaging
EPI	Echo Planar Imaging
eTIV	Estimated Intracranial Volume
FA	Flip Angle
fMRI	Functional Magnetic Resonance Imaging
FOV	Field of View
GAI	Geriatric Anxiety Inventory
GC-DG	Granule Cell layers of the Dentate Gyrus
GD	Giro Denteado
GDS	Geriatric Depression Scale
GM	Gray Matter
GTX	Graded Exercise Test
HATA	Hippocampal Amygdala Transition Area
IGF-1	Insulin-Growth Factor
LTC	Lateral Temporal Cortex
MET	Metabolic Equivalent Task
MNI	Montreal Neurological Institute
MoCA	Montreal Cognitive Assessment
MPRAGE	Magnetization-Prepared Rapid Gradient-Echo
MRI	Magnetic Resonance Imaging
MVPA	Moderate-to-Vigorous Physical Activity
MWM	Morris Water Maze
NBS	Network-based Statistics
PFC	Prefrontal Cortex
RER	Respiratory Exchange Ratio
ROI	Region-of-Interest
rs-fMRI	Resting-State fMRI
RSNs	Resting-State Networks
SD	Standard Deviation
T1	Longitudinal relaxation time

T2	Transverse relaxation time
TBS	Tensor-based Morphometry
TE	Echo Time
TI	Inversion Time
TMT-A	Trail Making Test – Part A
TMT-B	Trail Making Test – Part B
TR	Repetition Time
TrkB	Tyrosine Kinase B Receptor
VBM	Voxel-Based Morphometry
VEGF	Vascular Endothelial Growth Factor
VLPFC	Ventral Lateral Prefrontal Cortex
VO_2	Oxygen Uptake
VO_2max	Maximal Oxygen Uptake
VO_2peak	Peak Oxygen Uptake
WM	White Matter
WMS	Weschler Memory Scale

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INTRODUCTION

Modern societies are experiencing an unprecedented worldwide phenomenon of aging. According to a 2017 United Nations report on world population aging, the number of people aged 65 or older is projected to double by 2050, reaching nearly 2.1 billion people[1]. The progressive improvement of education, living conditions, and health and social services, aligned with advanced medical research and scientific discoveries have ameliorated the quality-of-life substantially. There is no doubt that this demographic shift, mainly driven by reduced birth rates and increased life expectancy, is accompanied by the exponential increase of the social and economic burdens of physiological consequences of the aging process. These challenges are even more concerning given that healthy (or nonpathological) aging is a complex biological process[2] characterized by structural, functional and physiological brain changes associated with a decline in cognitive functioning[3].

In fact, for many older adults, the cognitive decline becomes extremely severe, preventing them from managing their lives and from living independently. However, the deleterious effects of aging do not impact all individuals in the same way. Even facing a decline in cognitive abilities, evidence show that the older human brain still has some capacity to adapt to physical and cognitive demands[4]. The brain is not only the source of the behavior but can also be affected and modified by the behaviors that it produces[5]. Thus, the range of early and lifelong activities across the lifespan, such a highly active lifestyle, can help to build and maintain brain tissue or network flexibility, explaining some individual differences in cognitive outcomes observed during late-life[6,7]. This remarkable capacity of constantly adapting its function and structure throughout life, can either delay or compensate for structural brain changes that occur during healthy aging or even delay the onset and progression of neurodegenerative diseases such as Alzheimer's disease[8]. Despite growing interest in the use of physical activity as a nonpharmacological intervention, only recently the mechanisms and neural correlates underlying this relationship began to be examined. Exploring the dynamic relationship between brain plasticity and physical activity may help

us to gain a better understanding of how and why differences in lifestyle influence the course of healthy or pathological aging.

1.1. Age-related changes in the healthy aging brain

One of the clear hallmarks of the aging process is brain atrophy. This atrophy is linked to shrinkage in gray matter (GM) volume and a decrease in white matter (WM) integrity[9] and expansion of the cerebrospinal fluid (CSF) spaces[10]. From the third decade onwards, the human brain gradually loses tissue, and it is estimated that up to 90 years, the volume decreases around 14% in the cerebral cortex, most significantly in the GM volume of the prefrontal cortex, caudate nucleus and medial temporal lobes[10-12], 35% in the hippocampus, and 26% in the cerebral WM[3]. The rate and the trajectories of change differ among the brain regions and individuals, although the reasons for these differences are not fully understood[13-15]. These neurophysiological changes are thought to precede and lead to cognitive deficits in several domains, in which the degree of age-related structural alterations in specific brain areas was associated with the magnitude of behavioral deficits[16].

The aging process is coupled with progressive declines in performance in multiple cognitive domains including episodic memory, processing speed, and executive functions[6,17,18]. However, the cognitive decline may not be immediately apparent, since commonly changes are gradual and subtle. Typical aging (even with no sign of neurodegenerative disease) is characterized by slower cognitive processing speed[19], which has recently been associated with loss of WM integrity[20]. The slowed processing speed is the most critical predictor of driving cessation in the elderly[21]. Longitudinal studies have found a significant decrease in episodic memory (the conscious recollection of memories related to personally experienced events[22]) after the age of 60 years[23]. Substantial age-related differences have also been seen in tasks that involve working memory (the ability to maintain and manipulate several pieces of information in mind simultaneously), attention and task-switching (switching from one task to another)[24] processes. All these cognitive functions are considered executive functions which rely mainly on the prefrontal cortex, both known to be particularly affected by an age-related decline[25]. Performance differences between younger and older adults tend to be minimal on simple span tasks (e.g., digit span) but differences are noticeable when executive functions such as memory updating, reordering, or inhibition are added to the task. These differences have been associated with reduced task-related activation in frontal areas of the cerebral cortex[17].

1.2. Neural mechanisms underlying the effects of a physically active lifestyle in brain-related outcomes

Before describing the benefits of physical activity, exercise, or cardiorespiratory fitness, it is essential to define each precisely since they are related but represent distinct outcomes related to a physically active lifestyle (Table 1). The **physical activity** comprises all types of muscular activity that increase energy expenditure substantially[26]. In older adults, physical activity comprehends a variety of activities as leisure time (e.g., walking, gardening, swimming), transportation, occupational (if the individual is still engaged in work), households activities, sports or planned exercise[27]. Based on how much energy expenditure increases relative to rest, physical activity can be categorized into light-, moderate- or vigorous-intensity physical activity. The World Health Organization[27] and the American College of Sports Medicine[28] recommend that in order to benefit from regular physical activity, adults over 65 years should engage in, at least, 150 minutes of moderate-intensity exercise or 75 minutes of vigorous exercise per week, or a combination of both, preferably distributed throughout the week (e.g., 30 min, 5 times per week). Unfortunately, most older adults do not meet public health recommendations for physical activity[29]. Typically, the relationship between physical activity (or sedentary behavior) and health outcomes in population-based studies is based on self-reported questionnaires. This subjective method increases the odds of misclassification of the type, intensity, and duration of the physical activity due to social desirability, being susceptible to overestimation or imprecise recall[30]. To counteract these weaknesses related to self-reported activity, objective assessments of physical activity have become an alternative method. The duration and the intensity of the physical activity can be measured by devices, such as accelerometers, without the need for participants to self-report their activities.

Exercise can be defined as a subcategory of physical activity that is planned, structured, repetitive and has the purpose of improvement or maintenance of one or more components of the physical fitness[26]. Physical exercise is characterized by a precise frequency, duration, and intensity. Thus, **physical activity** and **exercise** refers to bodily movement and can be understood as a behavior, while **physical fitness** is a "set of attributes that people have or achieve"[26], which enables the execution of daily activities with the least effort possible. Physical fitness includes attributes related to performance (abilities associated with athletic performance) or related to health (traits associated with reduced risk of diseases such as obesity, type-II diabetes or cardiovascular disease). Research on the relationship between physical fitness and brain health has focused on health-related fitness, mainly in **cardiorespiratory fitness** (also referred to as aerobic fitness or aerobic capacity). As a

modifiable health factor, cardiorespiratory fitness refers to "the ability to perform large muscle, dynamic, moderate-to-vigorous intensity exercise for prolonged periods of time"[31] and reflects the combined integrity of the cardiovascular, respiratory, and skeletal muscle systems. Even though the cardiorespiratory fitness could be deeply determined by several factors such as age, sex, health status, and genetics, it can also be optimized through regular engagement in physical activity of moderate-to-vigorous intensity[32,33]. The gold standard to assess cardiorespiratory fitness is the measure of oxygen consumption during a graded treadmill test. Relevant measures include VO_2peak [highest volume of oxygen value attained on a graded exercise test (GTX)] and VO_2max (value at which VO_2 levels fails to increase further or increases minimally despite increased workload on GTX). The VO_2max reflects the maximal capacity of the cardiorespiratory systems to deliver oxygen to the skeletal muscle and the ability of the muscle to extract and use oxygen[34]. Cardiorespiratory fitness maintenance is important for functional independence throughout aging, but the decline experienced in aerobic capacity could be a consequence of the physical inactivity[35].

Table 1. Key definitions of fitness-related terms.

Term	Concept
Physical activity	Any bodily movement produced by skeletal muscles that require energy expenditure. Physical activity is a behavior that can potentially improve cardiorespiratory fitness.
Metabolic Equivalent Task (MET)	A MET is defined as the resting metabolic rate, that is, the amount of oxygen consumed while sitting at rest and is equal to 3.5 mL per kg per minute or 1 kcal (4.2 kJ) per kg per hour.
Cardiorespiratory fitness	The ability of the body to transport and use oxygen during sustained physical activity.
Maximal oxygen uptake (VO_2max)	The measurement of the maximum amount of oxygen that a person can utilize during exercise, used to determine a person's aerobic capacity.
Peak oxygen uptake (VO_2peak)	The measurement of the highest value of VO_2 attained upon an incremental exercise test, directly reflective of VO_2max.
Exercise	Exercise is a subcategory of physical activity that is planned, structured, repetitive, and purposeful in the sense that the maintenance or improvement of physical fitness is the objective.

1.2.1. Exercise-induced neuroplasticity and the aging brain

Throughout the lifespan, the Central Nervous System is in constant remodeling via dynamic processes that involve molecular, structural, and functional mechanisms[36]. Researchers have been focused on identifying lifestyle, behavioral, and/or biological factors that best characterize individuals with less cognitive impairment or that show fewer markers of brain dysfunction than their age-matched counterparts[37]. Consistently, aged brains exhibit alterations that are linked to neurodegeneration, such as the progressive loss of structure, function, and number/density of neurons[38]. Even in advancing ages, the human brain still retains the remarkable capacity to constantly adapt its function and structure in response to environmental changes and demands[39]. The understanding of the impact of these modifiable agents that could improve neuroplasticity may provide insights about novel therapeutic strategies to overcome the deleterious effects that occur during the aging process[40,41]. Therefore, convergent evidence from both human and animal studies suggests that physical activity promotes neuroplasticity of certain brain structures, resulting in a reduction of cognitive decline in aging. Rodents studies have proposed that the beneficial effects of exercise on cognitive functioning are mediated by an enhancement of neurogenesis, synaptogenesis, angiogenesis and the release of neurotrophins[42]. In humans, while neuroimaging research has been conducted to investigate the exercise-induced neuroplasticity changes in the brain, studies integrating different neuroimaging measures and investigating the association between them and cognitive improvement are lacking. Increases in brain volume should be interpreted within the context of behavioral outcomes to address fundamental issues of functional significance[43].

1.2.2. Biological mechanisms of exercise on brain and cognition

Human and animal studies have converged on the exciting evidence for the beneficial effects of physical activity and cardiorespiratory fitness on brain function in older adults[8,44]. However, the molecular and cellular mechanisms for these benefits are not fully understood. The translational studies with animal models have been crucial for elucidating the biological mechanisms by which exercise can improve brain function[45]. The use of animal models (mainly mice or rats) allows to reduce some of the inherent confounding factors that are often present in human studies, along with a deep exploration of neurobiological mechanisms (changes at the molecular, cellular and neuronal circuits levels) by which physical activity protects and restores the brain[45].

Animal studies have focused mainly in three mechanisms by which physical exercise is capable of inducing a cascade of molecular and cellular processes which promote **neurogenesis**, or the proliferation and survival of new neurons; **angiogenesis**, or the proliferation and survival of new vasculature; and elevated levels of **neurotransmitters and neurotrophic factors**[46].

Neurogenesis, the development of new neurons, and **angiogenesis**, the development of new blood vessels, are complex cellular changes that result from increased growth factor production and up-regulated molecular cascades. Both processes act as mediators of improvements in cognition[47]. It has been suggested that changes in brain vascularization precede neurogenesis in rodents, especially in the hippocampus[48]. Thus, improvements in cognition following exercise could be mediated, at least partially, through increased growth of blood vessels, which in turn stimulates cell proliferation and survival[49]. Angiogenesis could promote neurogenesis by strengthening or expanding the cerebral vasculature, enhancing the neurogenic niche. Consequently, the increased demand for nutrients to support the new neural architecture is provided through increased brain vascularization.

Research in neurogenesis has tried to identify stimuli that can modulate the proliferation and/or survival rate of the newly formed neurons. To address the effects of physical activity as a lifestyle change on neuronal systems, neurogenesis is one of the most replicated cellular changes linked with it[48]. In animal studies, exercise is typically manipulated by given free access to a running wheel (i.e., voluntary exercise) to animals in the exercise group, while those in the control group are deprived of a running wheel to ensure they are comparatively inactive. All other environmental conditions remain equal to both groups. Experimental studies employing rodents have established that physical activity improves cognition, particularly in cognitive domains dependent on the hippocampus, such as spatial or relational learning tasks, often evaluated through Morris Water Maze (MWM) test[50]. In this behavioral test, the animal is placed in a large circular pool of water in which it must find a submerged platform just beneath the surface that allows it to escape. The platform remains in the same position on all trials, but the animal is placed into the pool at variable locations and must learn the location of the submerged platform in presence of extra-maze cues[51]. Using this paradigm, Fordyce and Wehner[52] reported that rodents that had voluntary access to the running wheel in their cage, even though having swim speeds similar to their sedentary counterparts, demonstrated faster learning of the location of the submerged platform and consequently a reduction in the time needed to find the platform. Using MWM in aged rodents, van Praag and colleagues[53] demonstrated that the rodents engaged in exercise not only performed better on MWM but also had more newborn neurons in the

dentate gyrus (DG) than age-matched sedentary controls. Onwards these pioneering studies, consistent findings within this line have been reported, supporting the idea of exercise-induced cell proliferation in the hippocampal DG of young and also aged animals[53-57]. Furthermore, the inhibition of the integration of these newborn cells into the existing hippocampal structure prevented the learning and memory improvements that generally was observed following such manipulations[58]. Along with these lines, animal models enable insight into the importance of the integration of new neurons into cellular networks to perceive cognitive improvements following exercise, particularly in learning and memory domains. The cellular mechanisms of physical activity in humans are difficult because we cannot use brain tissue samples as we can do with animal models. However, it was also demonstrated that the human hippocampus retains its ability to generate neurons throughout life in regions previously identified as neurogenic niches in adult rodents[59] suggesting the remarkable plasticity of the brain throughout the lifespan.

The benefits of exercise on learning and memory are exerted by modulating key growth factors cascades responsible for energy maintenance and synaptic plasticity[49]. Animal models have provided insights and allowed to identify several neurochemicals by which exercise exerts its effect, including the brain-derived neurotrophic factor (BDNF), insulin-growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF)[47,60].

BDNF, a member of neurotrophin's family, is an important molecular mediator of the neuroplasticity of the brain that supports survival, differentiation and neuronal growth[61]. The BDNF is highly concentrated in the hippocampus[62,63] and cortex, and it is known that BDNF concentrations decrease with aging[64]. Neeper and colleagues observed that physical activity affects BDNF production in the brain with the greatest effects of exercise on BDNF mRNA occurring in regions not directly related to the motor system, but associated with a cognitive function such as the hippocampus and frontal cortex[65]. A study with rodents showed that voluntary exercise increases mRNA and protein levels of BDNF in the hippocampus, cerebellum and frontal cortex. However, when the binding of BDNF to the tyrosine kinase B receptor (TrkB) was blocked, the benefit of exercise-induced performances was abolished, which suggests that BDNF influences brain functions as learning and memory[66]. Also, in humans, the circulating BDNF levels increase following acute and chronic exercise[67-70].

Exercise also increases the production and secretion of molecules involved in the formation of new blood vessels including **IGF-1**[71,72] and **VEGF**[73] that are thought to mediate angiogenesis and also neurogenesis[47]. Blocking the entrance of IGF-1 and VEGF into the brain has been shown to suppress the exercise-induced neuron proliferation in the

hippocampus[73,74]. IGF-1 is emerging as a key growth factor that also modulates synaptic plasticity, synapse density, neurotransmission, and even adult neurogenesis[75,76]. Furthermore, IGF-1 is also crucial for exercise-induced angiogenesis in the brain, since it is critically involved in vascular maintenance and remodeling. Lopez-Lopez et al.[71] reported that blocking IGF-1 abolished the secretion of VEGF, which produced a significant decrease in the appearance of new capillaries. Thus, IGF-1 might induce new blood vessel formation indirectly through the synthesis of VEGF, a molecule that plays an essential role in the growth of blood vessels. The levels of IGF-1 levels decrease with age which is associated with decreased cerebral vascular density and blood flow[77]. In animal studies, it has been consistently reported that after exercise there is an increase in vascularization in several different brain regions including the cerebellum, motor cortex, hippocampus and frontal cortex[78-80]. Interestingly, exercise-induced changes in hippocampal vasculature are associated with adult neurogenesis and may be mediated by VEGF and IGF-1, both produced in the periphery.

BDNF and IGF-1 signaling could be considered causal pathways underlying exercise-related neurocognitive improvements because both are necessary to observe exercise-induced cellular effects. As described above, blocking signaling in these pathways (e.g., with receptor-blocking ligands) eliminates or attenuates the beneficial effects of exercise on cellular and molecular pathways related to cognition[47]. Also, there is evidence that blocking the BDNF attenuates behavioral learning and memory improvements following exercise[66]. Blockade of the IGF-1 receptor during exercise inhibits the ability of exercise to enhance the expressions of pro-BDNF and BDNF in the hippocampus, which suggests that these two pathways converge at a certain point in their cascades[72,81].

Briefly, the increasing of neurotrophins and supporting factors levels supported by physical activity lead to the delivery of these factors to critical brain areas through new brain blood vessels that are formed by adaptations to regular physical activity. These factors enhance neurogenesis and brain plasticity leading, consequently, to cognitive benefits[4].

1.2.3. Effects of physical activity on human cognitive function

The relationship between increased physical activity and cognitive ability has been conjectured for centuries, but only recently have the mechanisms underlying this relationship began to emerge.

Spirduso[82] was the first to empirically study the relationship between physical activity and cognitive aging, suggesting that active older adults had better cognitive function than inactive older counterparts, particularly in processing speed of a cognitive-related task. Since this pioneering work, several studies published in recent years indicated that physical activity may contribute to delay typical age-related decline in cognition[83,84]. Nonetheless, there are also studies that have reported that exercise interventions do not offer benefits in any cognitive domain in older adults[85].

Smith and colleagues reported modest improvements in attention, processing speed, executive function, and memory, with less consistent effects on working memory[86]. Executive function is a higher-order cognitive ability (which includes scheduling, planning, working memory, multi-tasking and dealing with ambiguity) that has been shown to have the greatest improvements with physical activity[87]. Furthermore, the relationship between physical activity and executive functioning seems to be bidirectional since better executive functioning also leads to the maintenance of physical activity[88]. Similar significant associations between cardiorespiratory fitness and performance on executive function-related tasks have also been found[89], despite some counterevidence[90]. Previous studies were mainly based on subjective assessments of physical activity and often translated the results of an isolated task reflecting an effect in a cognitive domain, which may partially explain the ambiguous results.

Previous observational work indicated that higher-intensity physical activity provides greater benefits to cognitive health than low-intensity exercise[91,92]. In the study of Angevaren and colleagues[91], it was found that healthy individuals aged 45-70 years with higher self-reported intense weekly physical activity had significantly better processing speed, memory, mental flexibility, and global cognition. Even though these previous findings are encouraging, it is crucial to address the methodological issues related to inaccuracies in self-reported levels of habitual physical activity[30]. Objective assessments of physical activity can provide more reliable insights about the dose-response effects of different intensities of physical activity on cognitive performance. However, so far, a yet small number of studies have used motion sensors such as accelerometers to explore the associations between objectively measured physical activity profiles with cognition in the elderly[92-95].

Kerr et al.[95] showed that moderate-to-vigorous physical activity (MVPA), measured with waist accelerometers, was associated with better processing speed and cognitive function performance in older adults. Interestingly, when the total amount of physical activity was combined without distinguishing between time spent in each intensity, no associations were found with cognitive function. Another cross-sectional study reported a significant

association between physical activity intensity, but not quantity, and better performance on digit symbol and verbal fluency test[92]. Zhu et al.[96], showed that higher levels of MVPA, but not the proportion of time spent in light physical activity or sedentary behavior, were associated with better performance in memory and executive function in older adults. In contrast, a study by Johnson et al.[97] found that light physical activity, but not MVPA or sedentary behavior, is associated with higher executive functioning in the elderly. Most of the studies that examined physical activity and brain health failing to provide an objective and accurate estimation of time spent in MVPA and sedentary behavior, which perhaps could explain the contradictory findings. Together, these findings suggest that intensity (rather than amount) of physical activity may play a more important role in the achievement of benefits for cognitive functioning.

1.3. Neuroimaging studies of physical activity in older adults

1.3.1. Basic principles of Magnetic Resonance Imaging (MRI)

Magnetic Resonance Imaging (MRI) is a non-invasive neuroimaging technique based on the magnetization properties of atomic nuclei. The atomic nuclei have an intrinsic property called spin, due to thermal energy, which allows to measure the interaction between some atoms/molecules with magnetic fields (e.g., water). When a strong and uniform external magnetic field (commonly of 1.5T or 3T) is applied, the water protons, randomly oriented, tend to align to this field allowing the tissue to be examined in an MRI scanner. This alignment can be switched by the introduction of radiofrequency pulses, at the proton resonance frequency, leading to flips in the proton's spins. When the external stimulus is removed, some nuclei will return to their original state by releasing this additional energy, emitting the previously absorbed photons at a rate proportional to the time taken to relax[98].

The changes in the MR signal over time are known as relaxation, and two kinds of relaxation can characterize tissue: T1- longitudinal relaxation time, is the time taken for spinning protons to re-align with the external magnetic field and T2- transverse relaxation time, is the time taken for spinning protons to lose phase coherence among the nuclei spinning perpendicular to the main field. Thus, molecules that relax at different rates, such as fat and water, emit a different amount of energy. Fourier transformation is used to convert the frequency information contained in the signal from each location in the imaged plane to corresponding intensity levels, which are then displayed as shades of gray in a matrix arrangement of pixels. Depending on the pulse sequence applied by the scanner, it is

possible to differentiate tissue compositions in the created images, such as the fat-rich myelinated WM and neural cell bodies of GM.

Repetition time (TR) is defined as the amount of time between successive pulse sequences applied to the same slice. Echo Time (TE) represents the time between the delivery of the sequence pulse and the receipt of the echo signal. Thus, the quantity being measured is different for each of these image types.

For anatomical analysis of brain images, the scan sequences used are the T1-weighted sequences since they give a good contrast between GM and WM. In T1-weighted images, the contrast between light and dark is a measure of the relative difference in the T1 property of the tissues, in which fluid appears as black, GM as dark gray, and the WM appears as light gray (Figure 1). These images can be used to estimate morphometric measures such as the total brain, GM and WM volumes, GM thickness, surface area, deep GM volumes, and regional brain volumes.

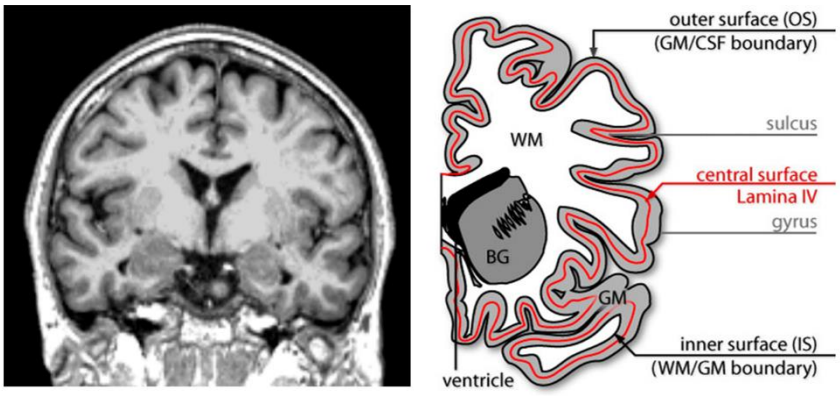

Figure 1. Representative images of (left) an anatomical brain section of a participant showing the high contrast of the GM, WM and CSF tissues (coronal section) and (right) a schematic (coronal) view of the highly folded sheet of GM and the boundaries of that define the three main brain tissues (GM/CSF and WM/GM boundaries). The highly convoluted surface of the cerebral cortex and the intensity differences between tissues allows the tissue segmentation and quantification of morphometric measures. Abbreviations: GM, Gray Matter; WM, White Matter; CSF, Cerebrospinal Fluid. Adapted from[99].

Human brain imaging has been playing a fundamental role in the exploration of the neurophysiologic mechanisms underlying the neuroprotective effects of physical activity, at a more macroscopic level[49]. The initial cross-sectional studies using MRI techniques attempted to address the association between cardiorespiratory fitness and brain volume and, more recently, the associations with different levels of physical activity. There are several methods to analyse brain images, all with strengths and limitations. However, in the following section, neuroimaging findings will be discussed regardless of the specific methodology used.

1.3.1.1. Effects of *cardiorespiratory fitness* and *physical activity* in brain structures

It is known that aging affects not only the organization of connections within specific functional networks but also the communication between several functional networks[100], namely the Default Mode Network (DMN, a system of areas involving the medial prefrontal cortex, the precuneus/posterior cingulate, the inferior parietal cortex/angular gyrus and hippocampal regions)[101,102]. In fact, age-related reductions in functional connectivity between key nodes of the DMN such as the medial prefrontal cortex and posterior midline structures has been postulated as a hallmark of aging[103].

Preserved cerebral connectivity patterns seem to sustain healthy brain functioning, and lifestyle factors as physical activity appear to modulate connectivity and may provide useful insights into age-related connectivity changes. A growing body of evidence indicates that aerobic fitness promotes efficient functional connectivity within brain networks for executive function (frontoparietal network), attention and learning (dorsal and ventral attention networks), and memory (DMN)[104,105]. In a 12-months follow-up study with a supervised aerobic walking program, Voss et al.[104] found increased functional connectivity in parts of the Default Mode and Frontal Executive Networks in the experimental group, compared to an active control group that did non-aerobic stretching and toning exercises. The changes in functional connectivity patterns in these crucial networks to brain dysfunction in aging were also behaviorally relevant, with greater improvement in executive function. It has been suggested that retained, or even improved, functional connectivity in DMN in elderly is associated with improved executive function, particularly in anterior prefrontal cortices[103,106]. Additionally, a cross-sectional study of Resting-State Networks (RSNs)[107] found that older adults who engaged in higher levels of physical activity over 10 years had stronger integrity of the DMN, particularly in posterior cingulate regions. Furthermore, the decrease in connectivity within DMN has been associated with deterioration in cognitive performance on processing speed and working memory tasks[108-111].

Thus, evidence shows that there is a complex functional neuronal reorganization process underlying aging that may be associated with the well-known structural brain changes that occur through life. Interestingly, by using diffusion tensor imaging (DTI, structural connectivity) and functional magnetic resonance imaging (fMRI, functional connectivity) data, Horn A. and colleagues[112] showed that the highest structure-function connectivity agreement was settled within the DMN areas. In fact, the pattern of brain atrophy is not

uniform in late life and evidence show that some brain areas (e.g., prefrontal cortex) are more sensitive to the effects of aging than other regions.

Also, previous studies proposed that late life was associated with widespread losses in GM tissue, most accentuated in the prefrontal, temporal, and parietal regions[3,113,114]. Interestingly, the brain regions that showed the most accelerated age-related losses in volume might also be the region's most sensitive to the effects of exercise[41]. A seminal meta-analysis of randomized trials of aerobic exercise interventions in older adults revealed that higher fitness levels attenuated the age-related loss in the brain regions supporting executive functions (i.e., prefrontal cortex), suggesting some degree of regional specificity for cardiorespiratory fitness[89]. The brain areas that support executive functions may be more influenced by participation in exercise than other areas not so critically involved in this cognitive domain[86,89]. However, the results emphasized the idea that the protective effects of higher fitness levels may only be detected after a certain age when losses in tissue volume are more accentuated.

The first published studies with neuroimaging techniques addressing this topic examined the cross-sectional association between cardiorespiratory fitness and GM volume without considering the effects of physical activity levels on brain volume. Colcombe and colleagues[115] demonstrated, using voxel-based morphometry (VBM), that higher fitness levels (estimated VO_2 from Rockport one-mile protocol) were associated with attenuated age-related loss in tissue density in frontoparietal WM and GM regions including the prefrontal cortex, superior parietal cortex, and temporal regions in healthy older adults (Figure 2A). Following studies using GTX in the treadmill to determine VO_2max have also reported positive associations between fitness and GM volume in the prefrontal cortex, lateral parietal regions[116,117], and medial-temporal areas[117], as illustrated in Figure 2.

Several years of rodent research have demonstrated that exercise impacts the morphology and function of the hippocampus[47,60]. Erickson et al.[118] addressed this question in older adults and found that higher levels of aerobic fitness were associated with greater hippocampal volume. In turn, greater volume was associated with better memory performance on a spatial memory task, independent of sex, age and education levels. However, the authors have not considered other factors associated with lifestyle that could impact cognitive functioning, such as physical activity[119] and body composition[120]. In a study with obese older adults, Bugg et al.[121] reported that higher aerobic fitness levels (VO_2peak) were also associated with larger hippocampal volumes.

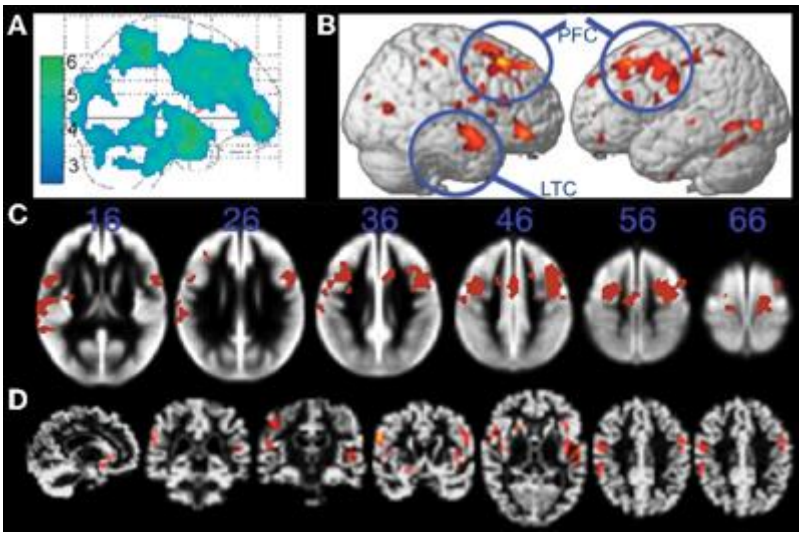

Figure 2. Structural MRI studies showing positive relationships with brain volume and fitness. (A) GM density in prefrontal cortex, temporal and parietal regions, showed fitness-related GM preservation in older adults[115]; (B) Greater physical activity predicted greater volumes of prefrontal, occipital, entorhinal, and hippocampal regions in older adults[122]; (C) GM volume in the prefrontal cortex, motor cortex, cingulate gyrus, anterior parietal lobe, and the temporal lobe showed a positive relationship with fitness (after controlling for age, gender, and education) in older adults. The blue numbers represent *Montreal Neurological Institute* (MNI) coordinates in the axial (z) plane[116]; (D) Brain regions such as medial-temporal, parietal and frontal areas showed also a positive relationship with fitness (after controlling for age, education, and gender) in older adults[117]. Abbreviations: GM, Gray Matter; LTC, Lateral Temporal Cortex; PFC, Prefrontal Cortex. Adapted from[123].

There is growing evidence that brain structure also mediates the positive association between cardiorespiratory fitness and cognition. Weinstein et al.[116] demonstrated that GM volume in the dorsolateral prefrontal cortex (DLPFC) mediated the positive association between aerobic fitness levels and executive functions, particularly inhibitory control and spatial working memory. In another study, Szabo et al.[124] reported that hippocampal volume in older adults was associated with reductions in the frequency of self-reported forgetting episodes.

Yet, investigation in other brain regions beyond the hippocampus and prefrontal cortex is scarce. Verstynen and colleagues[125] studied the association between cardiorespiratory fitness levels and the size of the basal ganglia, a region critically involved in motor and executive functions that showed significant age-related atrophy. Increased aerobic fitness was associated with a greater volume of the caudate nucleus and nucleus accumbens in older adults which were also positively associated with accuracy rates in a Task Switching paradigm. Caudate nucleus volume mediates the relationship between cardiorespiratory fitness and performance on this task of cognitive flexibility.

So far, the evidence suggests that aerobic fitness has differential effects in specific regions of the cortex. Thus, aerobic fitness could play an important role in other regions of the brain in which the relationship with aerobic fitness is far from clear.

The consistently reported findings between higher cardiorespiratory fitness levels and greater hippocampus and prefrontal cortex volume led to the hypothesis that engagement in greater amounts of physical activity might be also associated with increased GM volume.

Cardiorespiratory fitness has often been used as a proxy indicator of regular physical activity, without direct measurement of physical activity levels. Most older adults are sedentary, and their physical activity is rarely intensive and lengthy enough to improve aerobic fitness. Thus, the relationship between physical activity and fitness may not be meaningful and even modestly lifestyle physical activities may modify brain structure.

In a sample of 75 cognitively healthy older adults, Floel and colleagues[126] assessed self-reported levels of physical activity. Using VBM methods, these researchers reported that greater amounts of activity were associated with greater GM volume in the prefrontal cortex, cingulate cortex, temporal lobes, and cerebellum.

Another study by Bugg and Head (2011)[127], in a sample of 52 older adults between 55 and 79 years of age, without dementia, used a self-reported history of engagement in exercise over the past 10 years. MRI brain images were analyzed with FreeSurfer, an automated labelling technique[128,129], that estimates regional volumes, similar to those derived from manual morphometry. Despite the authors finding that higher levels of physical activity engagement were associated with larger superior frontal cortex volume, the major finding was that a greater amount of physical activity had a selective moderating effect on age-related decline in medial temporal lobe volume, as opposed as those who engaged in low levels of exercise. A limitation of this study was not including direct measures of cardiorespiratory fitness and the inaccuracies of the elderly participants to report physical activity engagement over the past 10-years which may limit even more the sensitivity of the self-report measure. However, a study assessing daily walking activity using a step activity monitor on older adults found that a greater amount, duration, and frequency of total daily walking activity were associated with greater hippocampal volume among older women, but not among men[130].

Other studies have reported contradictory results, showing that physical activity is not significantly associated with GM volume in older adults[131]. Smith et al.[131], using VBM methods and self-reported levels of physical activity in sixty-eight older adults, reported that higher amounts of physical activity were not associated with greater GM volume. In line with these results, another study conducted by Ho and coleagues[132] using tensor-based morphometry (TBS) methods on 226 adults between 73 and 84 years of age without dementia, reported that association between greater brain volume and levels of physical

activity was eliminated when including body mass index (BMI) as a covariate. The heterogeneity of the findings could indicate that cross-sectional assessments of physical activity are confounded by other unmeasured variables, such as BMI[133]. Besides, the inaccuracies in self-reported assessments of physical activity are contributing to noise and reducing the likelihood of finding reliable effects. Most of the studies relied on assessments of self-reported physical activity which are subject to measurement error. Few studies examined the relationship between objectively measured physical activity and brain structure[130,134,135]. A study using data from United Kingdom Biobank found a link between physical activity and higher GM volume in older participants, as well as with both left and right hippocampal volumes[134]. However, a meta-analysis reported a positive effect from exercise on the left hippocampus, but not in the total volume of the hippocampus[136].

The findings of the relationship between cardiorespiratory fitness and brain structure seem more consistent than the results reported by physical activity. However, when examining both fitness and physical activity, the regions that are reported in these studies are highly overlapping which suggests that both may be tapping into similar mechanisms to influence brain health and integrity.

1.3.2. Aims of the current study

Aerobic fitness has been associated with increased regional brain volumes and improved cognition in elderly adults[41], but almost no data are available regarding the relationship between habitual physical activity levels, regional GM volumes, and cognition. Engaging in physical exercise has been considered one of the most effective interventions to slow down the aging process and to promote a healthy quality-of-life, but it can be difficult for older adults to engage and maintain a physical activity program on a regular basis. Nonetheless, possibly even a moderately active lifestyle may be beneficial and buffer the decline of brain structures, preserving several cognitive domains (Figure 3).

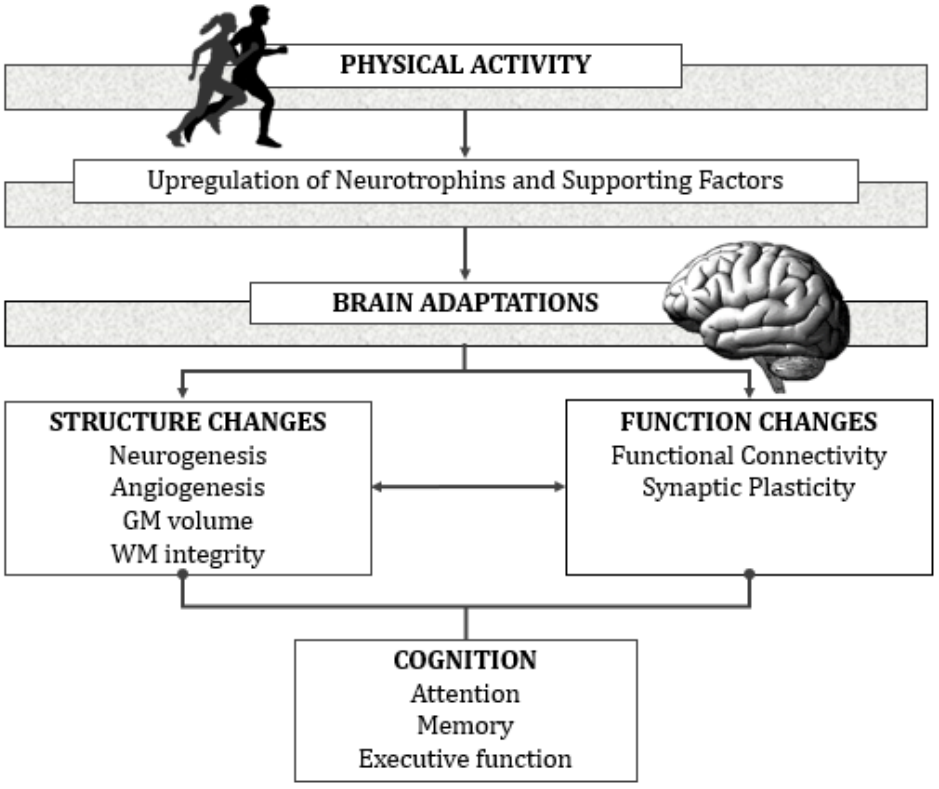

Figure 3. Relationship between physical activity, brain and cognition. Abbreviations: GM, Gray Matter; WM, White Matter.

Physical activity includes all the daily tasks that increase energy expenditure above the resting level. Understanding which characteristics of physical activity are important to modify brain structure and function represents a crucial step to determine how exercise can improve outcomes in cognitive healthy aging.

Thus, this study aims to provide a more accurate overview of the possible relationship between daily physical activity patterns and protection against age-related brain atrophy

and cognitive decline. Prior studies relied mainly on self-reported physical activity assessments. To overcome the inherent limitations associated with this method, objective measures of daily physical activity will be explored. Specifically, we aim to provide further insight into the effects of meeting or not the physical activity guidelines as a preventive strategy. The relationship between structural brain changes and the multiple cognitive domains in the elderly will be also explored as a proxy of structure-function relation mediated by physical activity levels.

To sum up, the main purpose of the current study was to examine the cross-sectional association between **objective measures of daily physical activity**, **cortical and subcortical brain volumes**, and **cognitive function** in neurologically healthy older adults.

2

MATERIALS AND METHODS

2.1. Participants

Independent community-dwelling older adults were recruited before enrollment in a senior physical exercise program at Faculty of Sports at the University of Porto, entitled "*Mais ativos, mais vividos".* Recruitment took place in the North area of Portugal through community advertisements and personal invitation. All participants were invited to fill in a demographic and medical history questionnaire to determine their participation in this study. Eligible participants had to meet the following inclusion criteria: age above 60 years, be able to perform activities of daily living independently, have a normal or corrected-to-normal vision, absence of clinical depression or severe anxiety symptoms and demonstrate strong right-handedness (scoring 75% or more on the Edinburgh Handedness Inventory[137]). Exclusion criteria included: detection of cognitive impairment [assessed by the Montreal Cognitive Assessment (MoCA)[138]], contraindications to MRI scanning, history of neurological disease, history of head injury resulting in loss of consciousness, history of psychiatric illness, alcohol, drug or substance abuse; presence of depressive or severe anxiety symptoms [as measured by the Geriatric Depression Scale (GDS)[139] and Geriatric Anxiety Inventory (GAI)[140], respectively], a relevant visual, auditory, or language impairment that would negatively affect the ability to understand test instructions or complete tests; BMI ≥ 40 kg/m^2 (thus, avoiding co-morbidities associated with obesity) and severe respiratory or cardiovascular diseases. Older adults currently prescribed with beta-blockers for hypertension management were not excluded from the study. After excluding individuals with performance in MoCA assessment below the normative range (n=2), severe anxiety or depressive symptoms (n=6), missing data on accelerometer (n=2), no brain MRI scanning (n=7) and abnormalities in MRI anatomical images (n=1), a total of twenty participants were included in the present study.

The study was conducted in accordance with the Declaration of Helsinki. The nature, benefits, and risks of the study were explained to the volunteers and all participants hold the opportunity to ask to investigator questions related to the study before providing written informed consent. All methods and procedures were reviewed and approved by the Ethics Subcommission of Life and Health Sciences (SECVS 120/2016) as well as by the Ethics Committee of Coimbra University, Faculty of Medicine (CE-044/2019). All participants gave their written informed consent.

2.2. Procedure

The participants included in the current study were recruited for an interventional physical exercise study that aims to examine the specific changes that occur in the brain and cognition with 10-months of multicomponent training, followed by 3-months of detraining time. The present study focused on participants that had high-resolution MRI acquisition, accelerometer data validated and completed the cognitive assessment described in detail below. The purpose was to evaluate the results from the baseline neurocognitive assessment and MRI acquisition, according to the different patterns of habitual physical activity of the participants. At baseline inclusion in the study, participants completed a demographic and medical history questionnaire to identify potential study candidates. The participants who meet the criteria were invited for a session in which the objectives and procedures of the study were explained. All individuals that manifested interest in voluntary participating in the study provided written consent. Subsequently, the neurocognitive and cardiorespiratory fitness assessment was scheduled, as well as the MRI acquisition. Participants took part in these evaluations in separate study sessions within a 1-month interval. Between these evaluation sessions, participants were fitted with an accelerometer and instructed to use it for one week in order to record their habitual physical activity and returned the equipment in the next session.

2.3. Measures

2.3.1. Cognitive assessment

All the neuropsychological and mood state assessments were administered in a single session by a trained neuropsychologist, and none lasted for more than two hours. More than

one test was included for each cognitive construct, allowing to investigate cognition in a broad sense. The neuropsychological assessment included measures of general cognition, verbal fluency, speed processing/attentional skills, executive functions and memory. Each task will be described under its respective cognitive domain.

Depression and Anxiety symptoms assessment

Depression and anxiety are two prevalent mental health problems among the elderly. The **Geriatric Depression Scale (GDS)** and the **Geriatric Anxiety Inventory (GAI)** are two reliable screening tools validated for the Portuguese population, designed for detecting these disorders in older individuals. The **GDS** is a self-reported scale, containing 30 items in the form of yes/no responses, which evaluates the symptomatology of depression[139]. On the other hand, the **GAI** is an instrument that consists of 20 "Agree/Disagree" items designed to assess the severity of anxiety symptoms in the elderly[140,141]. Based on normative data for the Portuguese population, severe anxiety symptoms were considered for scores higher than 8 points in GAI[140] and depressive symptoms for scores above 10 points in GDS[139].

Global cognitive performance

The MoCA is a brief cognitive screening instrument specifically developed to detect cognitive impairment[142]. It was used as an assessment of the global cognitive function of the participants, which assesses various cognitive functions such as short-term memory, executive functions, language, attention, among other domains. Age and level of education are significant predictors for the MoCA scores. Thus, inclusion criteria were based on the mean score of the normative data for the Portuguese population (provided for age and educational level) and 2 standard deviations (SD)[138].

Verbal fluency

Verbal fluency was assessed in semantic and phonemic fluency tasks[143]. In the **semantic fluency test** (sometimes called **category fluency**), the participants were asked to produce as many names of species of animals as possible within 60 seconds. Any repetition of the same animal species (including name variations according to gender or age) was not credited. The total number of species named corresponds to the total score, with higher scores revealing better performance[144].

On **phonemic fluency test** (sometimes called **letter fluency**), participants were instructed to produce orally as many words as possible beginning with specific letters. In Portugal, the chosen letters for standardization of the phonemic fluency were "M", "R", and "P", because these letters had different levels of difficulty. The test consists of three trials, of 1-minute each. The participants were instructed to produce orally as many words as possible beginning with the letter "M", then with the letter "P" and at last with the letter "R". Participants were asked to avoid producing names of people or places. When the participant provided several responses with the same derivation (variations of gender or number) referring to the same object, action, or concept, only the first response was credited. The number of words correctly generated in 60 seconds corresponds to the trial score. The total score corresponds to the sum of the three trials, with higher scores representing better performance[144].

Performance on these tasks is related to indicators of non-motor processing speed (lexical access speed), language production and executive function[144].

Speed Processing/Attention

A hallmark of age-related decline is a decrease in the rate at which people perform perceptual, motor and decision-making tests[145]. **Cognitive speed processing** is defined as the ability to process information quickly and it is closely associated with the capacity to perform higher-order cognitive tasks[146]. Thus, it is assumed as one of the strongest predictors of performance across cognitive tasks in aging populations[19]. **Trail Making Test – Part A** (TMT-A)[147] and **Symbols Search** subtest[148] were used to assess information on visual search and speed of processing.

The **TMT-A** is generally assumed as a test of visual search and motor speed skills[149] where participants use a pencil to connect a series of 25 encircled numbers in numerical order, as fast as they can. In the **Symbol Search subtest**, participants were asked to indicate whether one of two target symbols on the left of a row was also included among an array of five symbols printed on the right. Participants were asked to mark "yes" if one of the symbols were present and "no" if none of them were present. The total score is the number of correct answers subtracted by the wrong answers in 120 seconds. This subtest allows the recording of visual stimuli searching speed.

Executive functions

Executive functions refer to a family of cognitive processes that are necessary for the cognitive control of behavior. There are three core executive functions: working memory, inhibition, and cognitive flexibility[150].

Trail Making Test – Part B (TMT-B) was used to assess mental flexibility. In the **TMT-B** task, the participants are asked to draw lines connecting 25 encircled numbers and letters in numerical and alphabetical order, alternating between the numbers and letters (i.e., 1-A-2-B-3-C, etc), as fast as possible[149]. Despite this task provides information about mental flexibility by measuring the ability to task-switching, the TMT-B reflects mainly working memory function[151,152].

Digit span subtest[148] was used to assess working memory. **Working memory** refers to the ability to store and manipulate information in an easily accessible state over brief periods (several seconds to minutes)[153,154]. The Digit Span task (from the Wechsler Adult Intelligence Scale, Third Edition[148]) is a measure of attention, highly associated with verbal working memory. There are two parts in this subtest: Digits Forward and Digits Backward. In the first task (**Digit Span Forward**), the examiner says a series of numbers at the rate of about one per second and the participants were asked to repeat them back to the examiner, in the order of verbal presentation. In the second part (**Digit Span Backward**), subjects were also asked to listen to a series of numbers, however, it was required to repeat them to the examiner in the reverse order. The difficulty of the test increases as the length of the numerical sequence increases as well.

Memory

In the elderly population, impairments in memory are a common cognitive complaint. To assess memory performance, two subtests from the Weschler Memory Scale (WMS)[155] were used: **Logic memory II** and the **Verbal Paired Associates II**. The **logic memory II subtest** is composed of two brief stories with only a few sentences, which are read by the neuropsychologist. Following a filled delay (nearly 30 minutes that was filled with other unrelated cognitive tasks), the participants were asked to recall each story with many details as they could remember. The **Verbal Paired Associates II** consists of memorizing eight unrelated paired words and after a delay of approximately 30 minutes, the participant was asked to recall the paired word list.

2.3.2. Anthropometric measures

Height and weight of each participant were measured while wearing light clothing and without shoes. BMI was calculated using the standard formula of weight/height^2 (kg/m^2).

2.3.3. Cardiorespiratory fitness assessment

Aerobic fitness was estimated from a GTX on a calibrated treadmill performed by an experienced tester at the *Centro de Investigação em Actividade Física, Saúde e Lazer* (CIAFEL) at *Faculdade de Desporto da Universidade do Porto* (FADEUP). The participants were asked to not engage in intense physical activity for at least 24 h before the fitness test. Prior testing, participants were fitted with a Cosmed K4b^2 portable metabolic analyzer (Cosmed, Rome, Italy). This system utilizes a breath-by-breath measurement of gas exchange through a rubberized facemask (Hans Rudolph, Inc.) and a turbine for gas collection.

Figure 4. Apparatus of the cardiorespiratory fitness assessment. Adapted from[156].

All the participants performed a warm-up period on the motor-driven treadmill to familiarize them with the apparatus. Next, the Bruce protocol was conducted, a multi-stage test lasting 3 minutes each[157]. The initial workload was set at 2.7 km/h and 10% inclination. After 3 minutes, the inclination increased by 2%, and the speed of the treadmill was set at 4.0 km/h (stage 2). The test proceeded in stages of 3-min each with gradual increases in speed and inclination (see Table 2, for details). Strong verbal encouragement was given throughout the test. To ensure participant security, heart rate was constantly monitored (Polar Electro Inc.) and the rate of self-perceived exertion was collected at the end of each

3-minute stage. It was used the modified Borg Scale, a simple scale of intensity exercise, range from 0 to 10, being 5-6 considered moderate-intensity exercise and 7-8 being vigorous-intensity exercise[158]. It was aimed to continue the cardiorespiratory fitness test until exhaustion at the maximal volitional work rate unless there were medical indications for termination[31].

Table 2. Description of the Bruce protocol.

Stage	Stage length (minutes)	Speed (km/hour)	Grade (%)
1	3’	2.7	10
2	3’	4.0	12
3	3’	5.4	14
4	3’	6.7	16
5	3’	8.0	18
6	3’	8.8	20
7	3’	9.6	22

None of the fitness tests were interrupted due to medical complications in the current study. A post-exercise cool-down period was provided. Oxygen uptake (VO_2), carbon dioxide production (VCO_2), and respiratory exchange ratio (RER; a ratio of metabolic gas exchange calculated by VCO_2 divided by VO_2) were collected. The gas-exchange variables were reported as 30-seconds averages. The maximal effort of the participant was defined when two of the following criteria were met: i) a plateau in VO_2 with further increases in workload; ii) a RER ≥ 1.0, and iii) maximal heart rate ≥ 85% of the age-predicted maximal heart rate (i.e., 220-age). Most of the participants did not achieve full exertion since objective outcomes previously mentioned or subjective measures (Borg Scale > 8) were not met. Consequently, aerobic capacity was defined as VO_2peak in milliliter per kilogram body weight per minute (ml/kg/min) rather than VO_2max. VO_2peak was reported as the 30-seconds mean VO_2 of the highest complete performance level achieved by the participants.

2.3.4. Physical activity levels assessment

Daily Physical Activity Monitoring

The physical activity was recorded using the monitor GT3X, manufactured by Actigraph (Pensacola, Florida), illustrated in Figure 5. The Actigraph GT3X uses a capacitive accelerometer capable of detecting accelerations produced by the human body, consisting of piezoelectric transmitters that are stressed by accelerative forces. These forces lead to the production of an electrical signal which indicates the frequency, intensity, and duration of body movement[159]. The detection of the Actigraph is restricted to accelerations between 0.05 to 2 G, ensuring that only normal body motion is recorded, avoiding under- or over-estimations[160]. Actigraph devices are validated to quantify physical activity and activity-related energy expenditure[29].

Monitoring Protocol

The participants were instructed to wear the accelerometer for 7 consecutive days and to perform their daily physical activities as they normally would. They were informed to wear the monitor during all waking hours and only take it off to sleep and during showers, bathing, or other water activities[29]. The monitor was mounted on an adjustable waistband over their right hip (close to the iliac crest) that the participants could wear either over or under a shirt (Figure 5). At the end of the 7-day activity assessment period, participants returned their monitors.

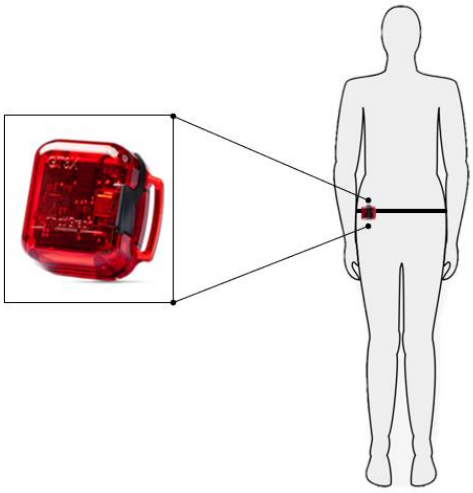

Figure 5. Representation of the instructions of use of the Actigraph GT3X monitor device.

Validation criteria and accelerometer-related outcomes

Raw data collected from movements registering on the vertical axis were integrated into 60 second increments periods (epochs). Accelerometer data were downloaded, reviewed and processed with the ActiLife v6.0 software (Pensacola, Florida). The GT3X monitor quantifies accelerations assigning each one a value measured in "counts" over a specified amount of time, with higher counts indicating a higher intensity of movement. The monitors were programmed to record data as *counts per minute* (cpm) throughout the entire data-recording period. A day of accelerometer wear was considered valid if the wear time lasted at least 8 hours. The wear-time was checked, and the participants with less than four valid days over the week (three days of the week and one weekend day) were excluded from the analysis. Non-wear time intervals were set as a period of ≥60 consecutive minutes with zero accelerometer counts, during which no activity was detected. If the wearing-criteria were not met, participants were asked to re-wear the device as previously described. It was necessary to request a new record of daily physical activity to one participant.

The number of minutes per day spent in physical activity of different intensity levels was categorized using count-based intensity threshold values of cpm developed by Troiano for older adults[161]: Sedentary behavior (total time spent sitting or lying) was set as 0-99 cpm; light-intensity physical activity as 100-2019 cpm and MVPA as > 2020 cpm[29]. The amount of time that participants spent engaged in each of these intensity levels were averaged per day.

To assess the influence of adherence to current recommendations of MVPA for older adults[28], participants were divided into two groups with reference to currently recommended volume of physical activity: (1) MVPA<30 min/day ("MVPA<30") or (2) MVPA≥30 min/day ("MVPA≥30").

2.3.5. MRI acquisition

All participants were examined on a 3T Siemens Magnetom Spectra scanner (Erlangen, Germany) with a standard head coil. For structural analysis, a high-resolution T1-weighted Magnetization-Prepared Rapid Gradient-Echo (MPRAGE) sequence was performed with the following scanning parameters: repetition time (TR) = 2.7 s, echo time (TE) = 2.33 ms, inversion time (TI) = 1000 ms, flip angle (FA) = 7°, 240 slices, slice thickness of 0.8 mm, voxel size 0.8 x 0.8 x 0.8 mm^3 and field of view (FOV) = 256x256 mm^2.

2.4. Neuroimaging analysis

2.4.1. Structural imaging

Cortical surface reconstruction and volumetric segmentation were performed using the neuroimaging package FreeSurfer, version 6.0 (http://surfer.nmr.mgh.harvard.edu) in a Linux (Ubuntu 14.04 LTS) platform. The procedures of automated image processing are described elsewhere[162,163]. Briefly, this processing includes motion correction and averaging[164] of volumetric T1 weighted images, removal of non-brain tissue using a hybrid watershed/surface deformation procedure[165], automated Talairach transformation, volumetric segmentation of subcortical WM and GM structures (including hippocampus, amygdala, caudate, putamen, ventricles)[129,166], intensity normalization[167], tessellation of WM and GM boundaries, automated topology correction[168,169], and surface deformation following intensity gradients to optimally place the GM/WM and GM/CSF borders at the location where the greatest shift in intensity defines the transition to the other tissue class[163,170]. Further data processing and analysis included surface inflation[162], registration to a spherical atlas using individual folding patterns to match cortical geometry across subjects[171], and sulcal and gyral based cortical parcellation[128,172]. The cortical labelling of the brain was based on the Desikan-Killiany atlas. All images were visually inspected to ensure that they were accurately reconstructed and without topological defects. Whenever necessary, manual edits were made, and the subsequent steps re-executed. A summary measure for each ROI was derived by averaging the values from the right and left hemispheres and then expressed as a percentage of estimated intracranial volume (eTIV) to account for differences in overall head size. Thus, to obtain normalized volumes, subcortical GM, cortical GM, and WM volumes were divided by eTIV and then multiplied by 100 to produce percentage volume fractions.

Regions-of-interest

The regions-of-interest based on the Desikan-Killiany cortical atlas[128], depicted in Figure 6, were further combined in 4 lobes: **Frontal** (superior frontal, rostral and caudal middle frontal, pars opercularis, pars triangularis, pars orbitalis, lateral and medial orbitofrontal, precentral, paracentral and frontal pole), **Parietal** (superior parietal, inferior parietal, supramarginal, postcentral and precuneus), **Temporal** (superior, middle, and inferior temporal, banks of the superior sulcus, fusiform, transverse temporal, entorhinal, temporal

pole and parahippocampal) and **Occipital** (lateral occipital, lingual, cuneus and pericalcarine).

The frontal cortex is one of the brain regions particularly vulnerable to the deleterious effect of aging. Thus, it was further analysed the effects of MVPA levels on frontal lobe regions, defined as all regions anterior to the precentral gyrus using the Desikan-Killiany parcellation. DLPFC, ventral lateral prefrontal cortex (VLPFC) and anterior cingulate cortex (ACC) are three ROIs that are not parcellated directly by the FreeSurfer software since they are not singular regions of the brain. As such, DLPFC volume was estimated by combining the fraction of GM volume of the frontal middle gyrus and sulcus from the Destrieux et al. atlas[173], the VLPFC was obtained by combining the pars opercularis, pars triangularis, and pars orbitalis and ACC volume was obtained by combining the rostral and caudal anterior cingulate cortices from the Desikan-Killiany atlas[128]. Similar to the study of Anderson-Hanley et al. (2018)[174], the chosen combination for the DLPFC estimates is one of the most conservative and non-controversial groupings, despite some authors have included more medial or caudal regions in their studies[175].

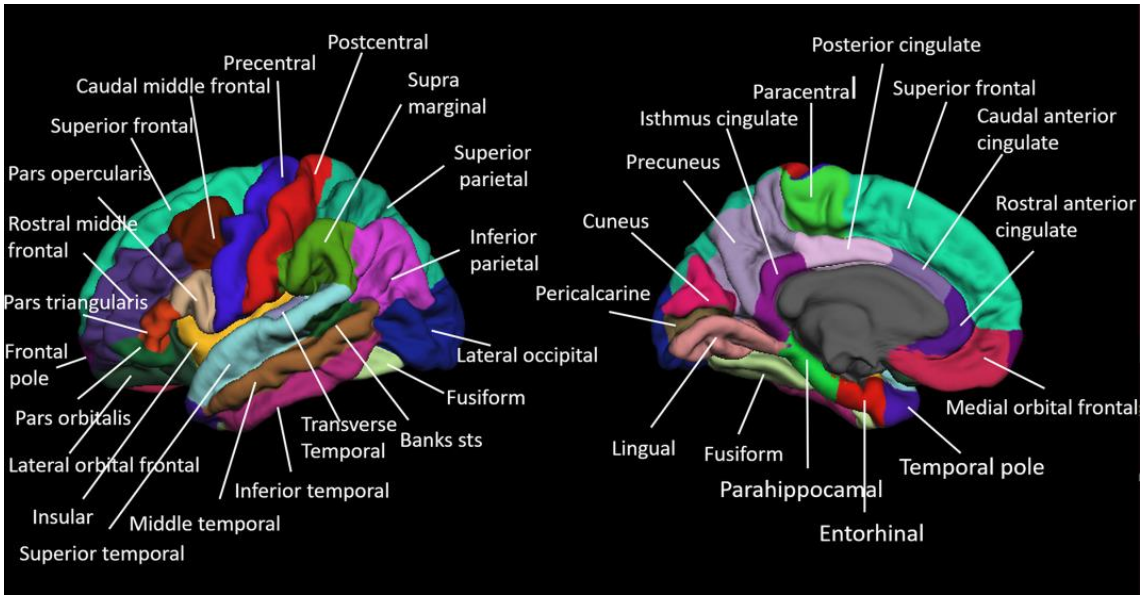

Figure 6. Regions-of-interest in the cortex according to the Desikan-Killiany atlas. Adapted from[176].

2.4.2. Hippocampal subfield volume estimation

Automatic labeling and volumetric estimations of hippocampal subfields were guided by the segmentation of the whole hippocampus done in the previous step and performed using the adaptive segmentation technique in FreeSurfer combined with MATLAB Runtime,

described by Iglesias et al.[177]. This algorithm relies on a computational atlas of the hippocampal formation, which was constructed from the manual delineation of the hippocampal subfields in ultra-high-resolution T1-weighted MRI scans. The right and left hippocampus were automatically segmented including the subfields: Cornu Ammonis (CA) 1, CA2/3, CA4, fimbria, the hippocampal fissure, presubiculum, subiculum, hippocampal tail, parasubiculum, the molecular and granule cell layers of the dentate gyrus (GC-DG), the molecular layer of the subiculum and CA fields, and the hippocampal amygdala transition area (HATA)[177], as shown in Figure 7. Since the contrast between the subfields CA2 and CA3 could not be distinguished on MRI, they were combined[177]. In this study, the fimbria and the hippocampal fissure were not included in the subsequent analysis because they are the smallest subfields that are considerably the less reliably segmented[178]. Noteworthy, the hippocampal tail is not a histologically distinct region, but instead represents a conglomeration of CA1-CA4 and dentate gyrus, where subfields were not discernable, and for this reason, was disregarded in this study, as well as three extrahippocampal structures (the parasubiculum, presubiculum, and the HATA). The hippocampal subfield segmentation and corresponding T1-weighted structural images for each participant were visually inspected using FreeSurfer's Freeview interface, however, the precise visualization of the boundaries that define each hippocampal subfield is not possible due to the limited spatial resolution provided by 3T MRI. Percent of hippocampal subfields was calculated by dividing each hippocampal subfield by the eTIV and multiplying it by 100.

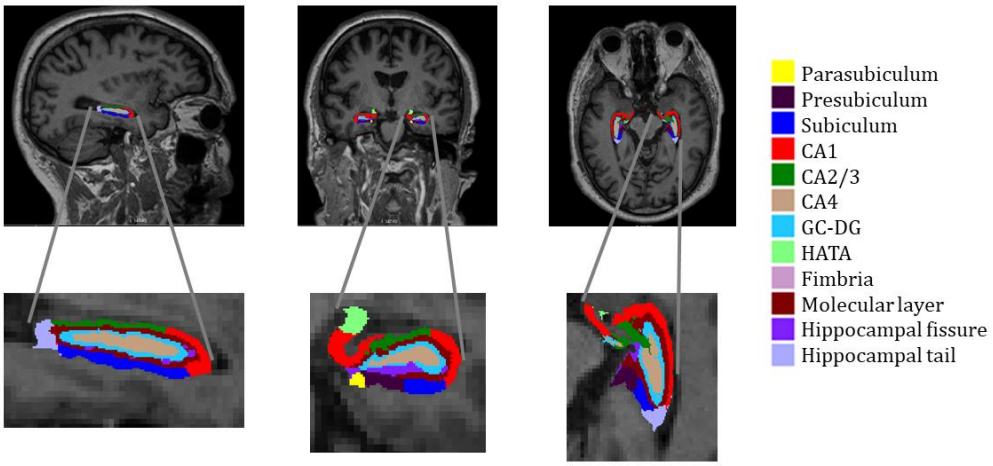

Figure 7. Hippocampal subfields segmentation displayed in the sagittal, coronal, and axial planes.

2.5. Statistical Analysis

The total number of minutes spent in each intensity (sedentary behavior, light-intensity physical activity, and MVPA) measured by the accelerometer was divided by the considered valid days to obtain an average of minutes per day. Participants who had, on average, at or above 30 minutes per day of MVPA were considered to meet guidelines. Thus, participants were divided into two groups: MVPA<30 (group below guidelines) and MVPA≥30 (group at or above guidelines).

Statistical analyses were conducted with the Statistical Package for Social Sciences (SPSS), version 25.0 (IBM, Chicago, USA) for Windows. At a first step, descriptive statistics were performed and are reported as mean ± standard deviation for all participants and displayed stratified by physical activity group.

The effects of meeting or not the current MVPA guidelines on brain structure and cognitive function were further explored. Assumptions for normality were tested for all continuous data with Kolmogorov-Smirnov tests and by visual inspection. Group comparisons were performed using the t-test for independent samples, or the nonparametric equivalent Mann-Whitney test, when appropriate. A p-value below 5% was considered statistically significant. Effect size (Cohen's d) was calculated for each t-test.

Further analyses were restricted to those cognitive domains and regional brain volumes for which significant differences between groups were observed. Partial correlations adjusted for age, sex, and years of education were conducted between physical activity levels and cognitive tests performance. Pearson's correlation coefficients, or Spearman correlations when appropriate, were performed between duration of physical activity divided into sedentary behavior, light- and MVPA with cortical and subcortical brain regions volumes. Correlations between variables were interpreted by guidelines of 0.1, 0.3, and 0.5 as small, moderate, and large, respectively.

3

RESULTS

3.1. Demographic and sample characteristics

A total of 20 older adults (9 men, 11 women) enrolled in this study. This cohort had an age range of 64 to 77 years (mean ± SD = 68.20 ± 3.71), and on average had 9.95 ± 4.69 years of education. BMI of the sample ranged from 23.7 to 36.3 (29.25 ± 3.45), indicating that the mean BMI was in the overweight range. MoCA cognitive scores for all participants were within the expected range confirming that all participants were cognitively normal. Participants included in the study reported low levels of depression (mean GDS score ± SD: 2.40 ± 1.57) and anxiety symptoms (mean GAI score ± SD: 2.00 ± 2.25). Examination of the participants by sex revealed that females had significantly higher levels of education when compared to males [11.9 vs. 7.6 years of education; t(18) = -2.286, p = 0.035], are shorter and weight less than males, but did not differ in the BMI [t(18) = 1.312, p = 0.201], neither in the other demographic and clinical variables (statistics not shown). More details on the participants' clinicodemographic characteristics and mean scores of the neuropsychological tests used for screening are summarized in Table 3.

Table 3. Demographic and clinical profile of the participants.

		Group		
	All Participants (n=20)	**MVPA<30 (n=7)**	**MVPA≥30 (n=13)**	**p-value**
Gender (F/M)	11/9	3/4	8/5	-
Age	68.20 ± 3.71	68.86 ± 4.26	67.85 ± 3.51	0.575
Years of Education	9.95 ± 4.69	9.00 ± 4.76	10.46 ± 4.75	0.520
Weight (kg)	72.95 ± 12.15	76.79 ± 16.05	70.88 ± 9.58	0.313
Height (cm)	157.75 ± 8.94	157.79 ± 10.90	157.72 ± 8.19	0.989
BMI (kg/m^2)	29.25 ± 3.45	30.66 ± 3.81	28.49 ± 3.13	0.186
MoCA score	24.15 ± 3.15	24.57 ± 2.37	23.92 ± 3.57	0.673
GDS score	2.40 ± 1.57	1.86 ± 0.90	2.69 ± 1.80	0.267
GAI score	2.00 ± 2.25	0.43 ± 0.79	2.85 ± 2.34	**0.004**

Results are reported as mean ± SD. P-values refer to the statistical comparison between MVPA<30 and MVPA≥30 groups.
Abbreviations: BMI, Body Mass Index; MoCA, Montreal Cognitive Assessment; GDS, Geriatric Depression Scale; GAI, Geriatric Anxiety Inventory.

The participants included in our analysis were more likely to report ≥ 150 minutes of MVPA per week. However, the participants spent most of their time in sedentary behavior (mean ± SD: 383.47 ± 90.90 min/day). In order to understand the possible differential effect of two different levels of daily physical activity, these participants were further split into two groups, according to the current guidelines for physical activity: MVPA<30 (min/day) and MVPA≥30 (min/day). Seven participants failed to reach current MVPA recommendations (averaged 14.96 ± 5.47 min/day), and thirteen exceeded them (averaged 59.30 ± 23.80). Importantly, the MVPA groups were matched in age with a similar age range (MVPA<30: 65 to 77; MVPA≥30: 64 to 76), years of education, BMI, overall cognitive function (MoCA) and depressive symptoms (Table 3). The groups were only significantly different in the GAI scores, in which the MVPA≥30 group obtained higher values than the MVPA<30 group (MVPA<30: 0.43 ± 0.79; MVPA≥30: 2.85 ± 2.34, p = 0.004). However, normative data for the Portuguese population set up scores higher than 8 points for severe anxiety symptoms[140], so the between-group differences were not clinically relevant.

Independent samples t-test, or Mann-Whitney when appropriate, were conducted to compare physical activity levels, aerobic capacity and neurocognitive performance between the two groups (Table 4). The MVPA<30 and MVPA≥30 groups were statistically different in minutes per day spent in MVPA [t(18) = -6.410, p > 0.001], but not in sedentary behavior [t(18) = 1.853, p = 0.080] neither in minutes per day spent in light-intensity physical activity [t(18) = 0.358, p = 0.725]. Also, the VO_2peak measured in the cardiorespiratory fitness assessment was not significantly different between groups [t(18) = 0.400, p = 0.695]. Despite males and females did not differ in the time spent in each intensity of physical activity, as expected males had higher cardiorespiratory fitness estimates than females as assessed by VO_2peak [t(16) = 3.479, p = 0.003].

Regarding the cognitive performance, the MVPA≥30 group outperformed the individuals engaged in low levels of MVPA in measures of visual search and information processing speed such as TMT-A [t(18) = 2.520, p = 0.021] and Symbols Search [t(18) = -2.462, p = 0.024]. The MVPA≥30 group also had a higher performance in a cognitive flexibility task, spending less time to complete the TMT-B task [U = 16.00, n = 20, p = 0.019] compared to the MVPA<30 group.

Table 4. Physical activity patterns and statistical comparison between MVPA<30 and MVPA≥30 groups for physical fitness measures and neuropsychological evaluation.

	All participants	MVPA<30	MVPA≥30	p-value
Physical activity levels				
Sedentary (min/day)	383.47 ± 90.90	431.80 ± 101.01	357.45 ± 76.72	0.080
LIPA (min/day)	341.81 ± 91.01	351.96 ± 94.36	336.34 ± 92.58	0.725
MVPA (min/day)	43.78 ± 28.95	14.96 ± 5.47	59.30 ± 23.80	**<0.001**
Cardiorespiratory fitness				
VO_2peak (ml/min/kg)	24.65 ± 4.68[a]	25. 29 ± 4.86[b]	24.33 ± 4.78[c]	0.695
HRmax (beats/min)	135.56 ± 12.51[a]	131.67 ± 13.01[b]	137.50 ± 12.35[c]	0.367
RER (VCO_2/VO_2)	1.04 ± 0.12[a]	1.09 ± 0.14[b]	1.01 ± 0.10[c]	0.605
Cognitive performance				
Verbal Fluency				
Category fluency	14.53 ± 5.18	11.86 ± 3.98	16.08 ± 5.30	0.086
Letter Fluency	32.75 ± 12.59	26.43 ± 10.98	36.15 ± 12.45	0.100
Speed Processing/Attention				
TMT-A	46.15 ± 22.31	61.29 ± 13.73	38.00 ± 22.10	**0.021**
Symbols Search	16.90 ± 9.71	10.43 ± 5.38	20.38 ± 9.85	**0.024**
Executive Functions				
Digits Span Forward	9.00 ± 1.81	8.29 ± 1.70	9.38 ± 1.81	0.202
Digits Span Backward	5.75 ± 2.40	5.29 ± 0.76	6.00 ± 2.94	0.422
TMT-B	165.50 ± 81.49	209.71 ± 87.60	141.69 ± 70.17	**0.019**
Memory				
Verbal Paired Associates	4.30 ± 2.56	3.00 ± 2.24	5.00 ± 2.52	0.096
Logical Memory	23.80 ± 8.35	23.71 ± 9.79	23.85 ± 7.89	0.974

Results are reported as mean ± SD.
[a]n=18; [b]n=6; [c]n=12
Abbreviations: LIPA, Light-Intensity Physical Activity; MVPA, Moderate-to-Vigorous Physical Activity; VO_2peak, Peak Oxygen Uptake; HRmax, Maximal Heart Rate; RER, Respiratory Exchange Ratio; VCO_2, Carbon Dioxide Production; VO_2, Oxygen Uptake; TMT-A, Trail Making Test-Part A; TMT-B, Trail Making Test-Part B.

3.2. Evaluation of global gray and white matter volume estimates

To understand the impact of engagement in higher levels of MVPA on total GM and WM volume, the participants with less than 30 minutes of MVPA per day (MVPA<30) and the individuals that met the current guidelines (MVPA≥30) were compared (Table 5). The normalized volume of all analysed structures was calculated by dividing individual volume values by the eTIV and then multiplied by 100, obtaining a percentage of volume fractions.

Considering the fraction of global GM and WM volume, the analysis revealed no differences between them [GM: $t(18) = -1.262$, $p = 0.223$; WM: $t(18) = -0.772$, $p = 0.450$]. There was only

a marginally significant difference on subcortical GM volume [t(18) = -2.061, p = 0.054] suggesting that the MVPA≥30 group have a greater volume of deep GM (mean ± SD = 3.78 ± 0.24) when compared to individuals with low levels of MVPA (mean ± SD = 3.55 ± 0.24). Results from volumetric analyses of frontal, temporal, parietal and occipital lobes are also presented in Table 5. No significant differences were found between MVPA groups in the fraction of GM volume of frontal [t(18) = -1.032, p = 0.316], parietal [t(18) = -0.705, p = 0.490], temporal [t(18) = -1.571, p = 0.134], and occipital [t(18) = -1.433, p = 0.169] lobes.

Table 5. MRI data of global volumes in neurologically healthy older adults, and comparison between MVPA<30 and MVPA≥30 groups.

	All participants	MVPA<30	MVPA≥30	p-value	Cohen's d
Total gray matter volume	40.12 ± 2.11	39.33 ± 2.36	40.56 ± 1.92	0.223	0.57
Subcortical gray matter volume	3.70 ± 0.26	3.55 ± 0.24	3.78 ± 0.24	0.054	0.96
Cerebral Spinal Fluid volume	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.781	1.00
White matter volume	30.60 ± 1.77	30.18 ± 1.47	30.82 ± 1.93	0.450	0.37
Cerebellum Cortex volume	29.95 ± 1.73	3.22 ± 0.44	3.16 ± 0.30	0.705	0.16
Frontal lobe volume	5.50 ± 0.35	5.39 ± 0.27	5.55 ± 0.37	0.316	0.49
Parietal lobe volume	3.47 ± 0.25	3.41 ± 0.23	3.50 ± 0.27	0.490	0.36
Temporal lobe volume	3.38 ± 0.24	3.27 ± 0.26	3.44 ± 0.21	0.134	0.72
Occipital lobe volume	1.55 ± 0.17	1.48 ± 0.20	1.59 ± 0.14	0.169	0.64

Data are given as mean ± SD.
Volumes were normalized and are reported as volume fractions (in percentage) of the estimated total intracranial volume.

Further, to address the possible association between greater intensity levels of physical activity and global brain volumes, bivariate correlations were conducted. The analyses showed that, although MVPA minutes per day was not significantly associated with total GM volume [r(18) = 0.129, n = 20, p = 0.588], increased MVPA was positively associated with subcortical GM volume [r(18) = 0.462, n = 20, p = 0.040] (Figure 8). This association remains significant after controlling for covariate effects, such as sex and age.

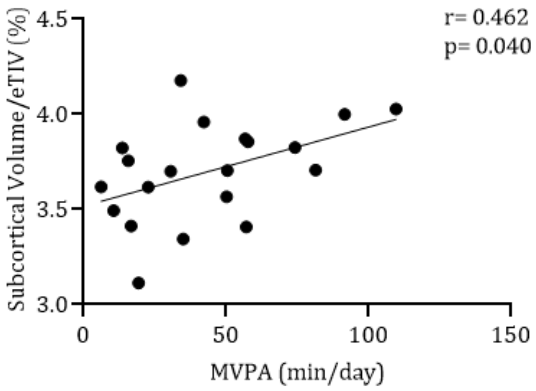

Figure 8. Linear correlation between the average of minutes per day spent in moderate-to-vigorous physical activity (MVPA) and subcortical GM volume. Abbreviations: eTIV, estimated total intracranial volume; GM, Gray Matter.

3.3. Cortical and subcortical parcellation volumes

Even the brains of apparently healthy individuals undergo several cortical and subcortical changes with aging. To explore the possible role of engagement in higher levels of MVPA to attenuating declines in brain volume observed in frontal and subcortical regions with aging, an exploratory analysis focused on these regions was conducted.

3.3.1. Regional cortical volumes

Exercise-related changes may be carried by specific brain regions in the frontal lobe. Thus, the impact of MVPA levels on age-related decline in the frontal lobe could be diluted when analysing the total volume of these regions. Table 6 presents the comparison between frontal lobes ROIs volumes of MVPA<30 and MVPA≥30 groups. As aforementioned, some of them are not parcellated directly by the FreeSurfer software since they are not singular regions of the brain. In Figure 9, are displayed the combination of regions used to estimate DLPFC, VLPFC and ACC.

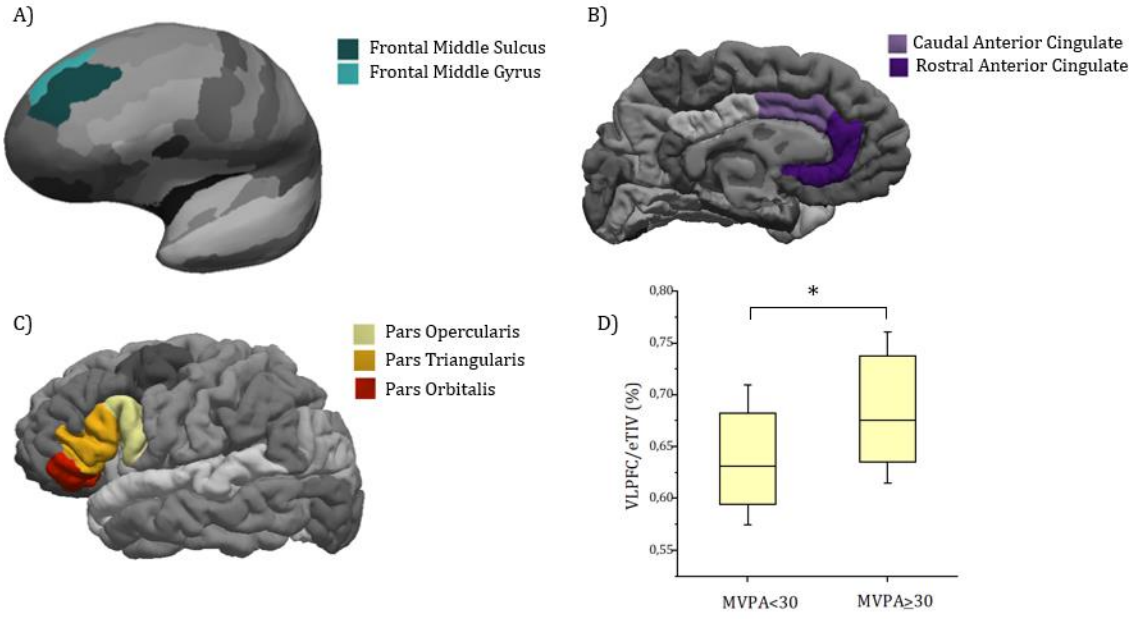

Figure 9. Representation of the region's combinations used to estimate Dorsolateral Prefrontal Cortex (DLPFC), Anterior Cortex Cingulate (ACC) and Ventrolateral Prefrontal cortex (VLPFC). A) DLPFC= Frontal Middle Sulcus + Frontal Middle Gyrus; B) ACC= Caudal Anterior Cingulate + Rostral Anterior Cingulate; C) VLPFC= Pars Opercularis + Pars Triangularis + Pars Orbitalis; D) Comparison of the VLPFC volumes between groups.

Contrary to expectations, independent samples t-test revealed that physically MVPA<30 and MVPA≥30 group did not display any significant difference neither in the DLPFC (p =

0.699), nor in the ACC (p = 0.811). However, the MVPA≥30 group had significantly larger volumes in the VLPFC (p = 0.049) and in the Precentral gyrus (p = 0.026), with effect sizes over 1.

Table 6. Differences between groups in regions-of-interest in the frontal lobe.

	MVPA<30	MVPA≥30			
Frontal Lobe ROIs	**Mean ± SD**	**Mean ± SD**	**t-test for volume**	**p-value**	**Cohen's d**
Superior Frontal gyrus	1.389 ± 0.093	1.408 ± 0.122	-0.376	0.711	0.18
Middle Frontal gyrus	1.379 ± 0.010	1.373 ± 0.152	0.098	0.923	0.06
VLPFC	0.638 ± 0.044	0.687 ± 0.051	-2.108	**0.049**	1.03
Orbitofrontal cortex	0.836 ± 0.054	0.845 ± 0.054	-0.356	0.726	0.17
Frontal Pole	0.064 ± 0.008	0.072 ± 0.011	-1.648	0.117	0.83
Precentral gyrus	0.844 ± 0.068	0.916 ± 0.060	-2.431	**0.026**	1.12
Paracentral lobule	0.238 ± 0.020	0.253 ± 0.022	-1.548	0.139	0.71
DLPFC	0.803 ± 0.069	0.785 ± 0.104	0.393	0.699	0.20
ACC	0.277 ± 0.041	0.281 ± 0.035	-0.243	0.811	0.10

Bold values indicate statistically significant (p ≤ 0.05).
Volumes were normalized and are reported as volume fractions (in percentage) of the estimated total intracranial volume.
Abbreviations: VLPFC, Ventrolateral Prefrontal Cortex; DLPFC, Dorsolateral Prefrontal Cortex, ACC, Anterior Cingulate Cortex.

3.3.2. Regional subcortical volumes

Deep GM nuclei volumes, significance of between groups tests and effect sizes (Cohen's d) are depicted in Table 7. The analysis of regional subcortical volumes revealed that the physical activity engagement groups did not differ in the subcortical nuclei that compose the basal ganglia, such as the caudate nucleus [t(18) = 0.224, p = 0.825], the putamen [t(18) = -1.279, p = 0.217], the globus pallidus [t(18) = -0.631, p = 0.536] and the nucleus accumbens [U = 35.00, n = 20, p = 0.405].

Table 7. The difference in subcortical volumes between MVPA<30 and MVPA≥30 individuals.

Regional Subcortical Volumes	**MVPA<30**	**MVPA≥30**	**p-value**	**Cohen's d**
Thalamus	0.415 ± 0.031	0.455 ± 0.035	**0.020**	1.21
Caudate nucleus	0.246 ± 0.022	0.244 ± 0.027	0.825	0.08
Putamen	0.302 ± 0.037	0.319 ± 0.025	0.217	0.54
Globus pallidus	0.131 ± 0.016	0.135 ± 0.016	0.536	0.25
Nucleus accumbens	0.033 ± 0.008	0.036 ± 0.006	0.405	0.42
Hippocampus	0.244 ± 0.023	0.265 ± 0.030	0.139	0.79
Amygdala	0.105 ± 0.008	0.115 ± 0.011	**0.038**	1.04

Data are presented as mean ± SD.
A p-value less than 0.05 was considered significant (significant p-values are indicated in bold).
Volumes were normalized and are reported as volume fractions (in percentage) of the estimated total intracranial volume.

Contrary to expectations, the hippocampus volume was not significantly different between physical activity groups [t(18) = -1.547, p = 0.139]. However, individuals who engaged in average in 30 minutes or more of MVPA per day had greater thalamus [t(18) = -2.555, p = 0.020] and amygdala [t(18) = -2.240, p = 0.038] volume when compared to the MVPA<30 group, with large effects.

3.4. Comparisons of hippocampal subfield volumes

Surprisingly, no group differences were found on overall hippocampal volume at baseline. Since the MVPA could have an impact in specific subfields of the hippocampus, and this effect could be diluted when measuring the total volume, it was further conducted an automatic segmentation of the hippocampal subfields (Figure 10A).

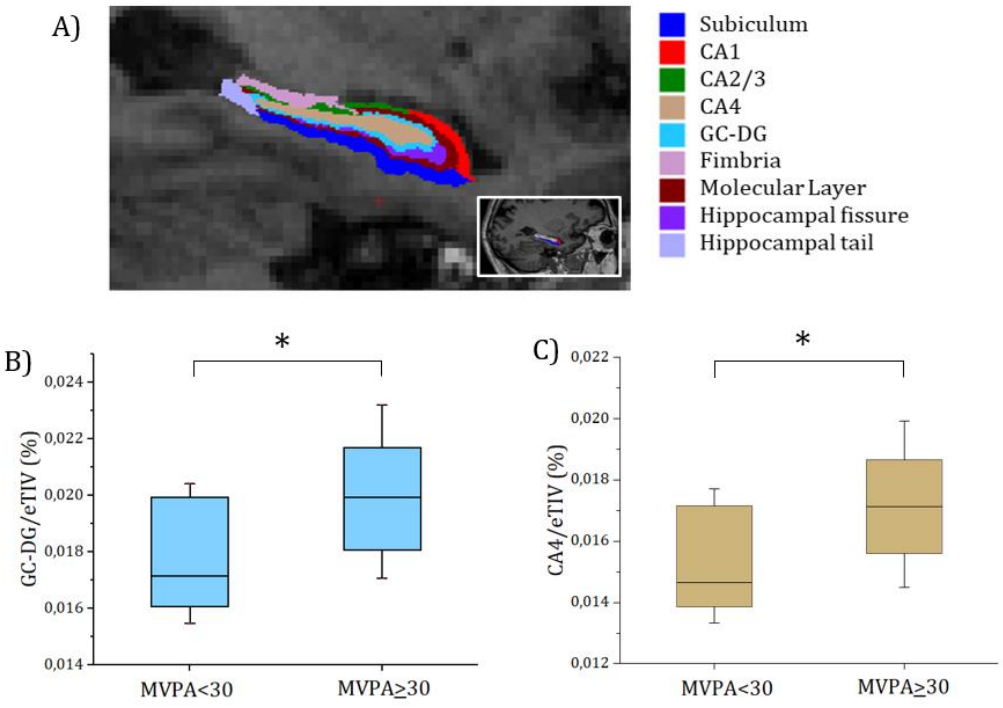

Figure 10. Estimation of hippocampal subfields volumes. A) Hippocampal subfields segmentation, shown in the sagittal plane; B) Comparison of the GC-DG volume between the two physical activity groups; C) Comparison of the CA4 volume between the two physical activity groups. Abbreviations: CA, Cornu Ammonis; GC-DG, Granular Cells layer of the Dentate Gyrus.

The statistical results of the hippocampal subfields measurements between groups are shown in Table 8. Even though no significant difference was found at the whole hippocampal level, there were significant differences in subfields volumes between the two groups. An independent t-test revealed that the group engaged in higher levels of MVPA had significantly larger volumes in the CA4 [t(18) = -2.193, p = 0.042] and in the GC-DG [t(18) =

-2.158, p = 0.045] subfields compared with those engaged in few minutes of MVPA (Figure 10B and Figure 10C, respectively).

Table 8. The difference in hippocampal subfields volumes between MVPA<30 and MVPA≥30 groups.

Hippocampal subfields	MVPA<30	MVPA≥30	p-value	Cohen's d
Subiculum	0.0277 ± 0.0029	0.0284 ± 0.0023	0.560	0.27
CA1	0.0397 ± 0.0035	0.0426 ±0.0051	0.184	0.66
CA2/3	0.0128 ± 0.0016	0.0142 ± 0.0017	0.087	0.85
CA4	0.0155 ± 0.0017	0.0171 ± 0.0015	**0.042**	1.00
GC-DG	0.0180 ± 0.0019	0.0199 ± 0.0018	**0.045**	1.03
Molecular Layer	0.0349 ± 0.0030	0.0375 ± 0.0037	0.119	0.77

Data are presented as mean ± SD.
Abbreviations: CA, Cornu Ammonis; GC-DG, Granular Cells layer of the Dentate Gyrus.
A p-value lower than 0.05 was considered significant (significant results are indicated in bold).

3.5. Associations between physical fitness measures, cognitive performance, and regional brain volumes

An exploratory analysis was conducted to understand the association between physical measures and cognitive performance. Partial correlations adjusted for age, sex and years of education revealed no significant associations between VO_2peak and neuropsychological tasks assessed. Regarding the association between cognitive performance and objectively measured physical activity, the time spent in MVPA was positively correlated with information processing speed assessed by Symbols Search task (r(18) = 0.493, n = 20, p = 0.044), as depicted in Figure 11.

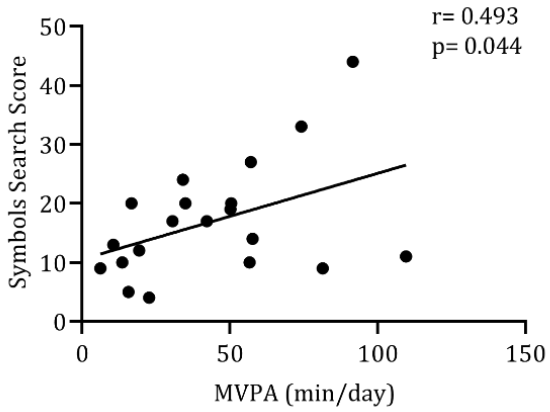

Figure 11. Correlation between the average of minutes spent in moderate-to-vigorous physical activity (MVPA) per day and symbols search subtest performance. Sex, age, and years of education were set as confounding factors.

In further exploratory analyses, the specific influence of different intensity levels of physical activity and/or cardiorespiratory fitness on GM volumes was examined. First, it was inspected the association between cardiorespiratory fitness, different physical activity

levels and demographic and health variables that may act as sources of noise in the analyses. Since none of these variables (such as age and BMI) were correlated with GM volumes in this sample, they were not included as covariates in all subsequent analyses.

The regions that were significantly different between groups, were then correlated with different physical activity levels and cardiorespiratory fitness. Associations between higher levels of MVPA and greater GM volume were found in cortical and subcortical brain regions (Figure 12). Specifically, there were positive significant associations between time spent in higher intensity of physical activity and the VLPFC ($r(18) = 0.470$, $n = 20$, $p = 0.036$), the thalamus ($r(18) = 0.623$, $n = 20$, $p = 0.003$) and the amygdala ($r(18) = 0.474$, $n = 20$, $p = 0.035$). No associations were found between precentral gyrus volume and minutes per day spent in MVPA ($r(18) = 0.317$, $n = 20$, $p = 0.174$). Regarding hippocampal subfields volumes, there were no significant associations between CA4 and GC-DG volumes and time spent in MVPA. The possible association between these ROIs and cardiorespiratory fitness, light-intensity physical activity, and sedentary behavior were examined, but any association was found in this sample.

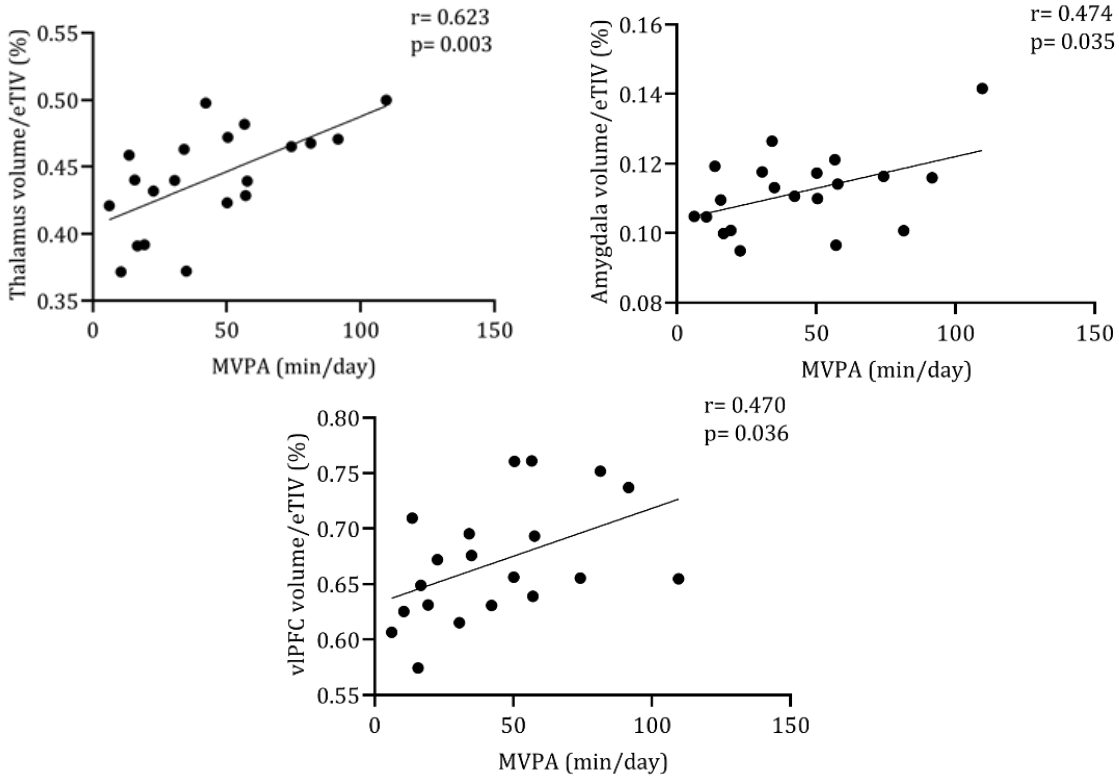

Figure 12. Associations between regional brain volumes and the average of minutes in moderate-to-vigorous physical activity (MVPA) per day. Abbreviations: VLPFC, Ventral Lateral Prefrontal Cortex; eTIV, estimated Total Intracranial Volume.

4
DISCUSSION

In this study, it was explored the association between physical activity, regional brain volumes and cognitive function within a sample of community-dwelling older adults. Previous studies have focused mainly on brain structural benefits related to cardiorespiratory fitness, structured aerobic exercise, or subjective assessments of physical activity[118,125]. However, this study was designed to quantify daily physical activity accurately. The primary finding was the association between engagement in MVPA (min/day), but not light physical activity or sedentary behavior, and the volumes of cortical and deep GM structures. Specifically, higher volumes were found in several brain regions such as the thalamus, amygdala, precentral gyrus, VLPFC, and CA4 and CG-DG subfields of the hippocampus in the individuals that met the current physical activity guidelines, compared to those who did not. Those above the guidelines also demonstrated better performance in information processing speed tests and executive function-related tasks, specifically in the working memory and cognitive flexibility.

Since this is a cross-sectional assessment, causality cannot be inferred from these results. Physical activity daily habits could impact positively cognitive function, or conversely, the integrity of cognitive function could influence individuals for engaging in higher physical activity levels. However, it was demonstrated that among different levels of physical activity, only MVPA was significantly associated with changes in regional brain volumes or cognitive performance tasks, which supports the need of high-intensity levels for observing changes in the volume of brain structures.

Patterns of physical activity between groups

There is strong evidence documenting the benefits of higher levels of moderate-to-vigorous physical activity, not only by reducing the risk of developing several disabling medical conditions but also by improving health-related quality-of-life[179]. Despite several reports finding an association of physical activity and cardiorespiratory fitness with GM volume in older adults[41], the research investigating whether meeting physical activity guidelines is

indeed effective for attenuating declines in brain volume and cognitive function is still lacking.

Since our main goal was to evaluate the potential distinctive effect of meting or not the physical activity guidelines for older adults, the cohort was divided into two groups, based on an average MVPA threshold of 30 minutes per day. Regarding the physical activity pattern of both groups, they did not differ neither in time spent in light-intensity physical activity nor in time spent in sedentary behavior. The cardiorespiratory fitness levels assessed through the VO_2peak were also similar between groups. Thus, the main difference between the two physical activity groups was only the daily time spent in MVPA. In this sample study, 65% of the participants met the physical activity guidelines which are higher than the reported average in Portugal, in which, unfortunately, only 35% of older adults performed at or above 30 minutes per day of MVPA[180]. This active sample cohort allows identifying the potential effects of meeting the 30 minutes per day of MVPA on the brain health, structurally and cognitively. Noteworthy, despite spending more time in MVPA, the group above guidelines did not spend less time in sedentary behavior than their peers. This is similar to the reported in the previous studies[181], exemplifying the possibility that one can get daily MVPA recommendations and be relatively sedentary for the rest of wakeful hours, and thus experience some negative physiological effects of sedentary behavior[182].

Physical activity intensity and cognition

Physical activity has been suggested as a positive intervention to support the preservation of cognitive performance in late life[183,184]. However, most of the attempts to understand the relationship between physical activity and cognitive benefits rely on subjective assessments of regular physical activity, looking for the quantity, and disregarding its intensity. In this study, to remove many of the issues of recall and response bias, accelerometers were used to objectively quantify the daily pattern of physical activity intensities and sedentary behavior.

Our results provide supporting evidence that meeting MVPA guidelines is associated with better cognitive functioning. By comparing the cognitive performance of the two physical activity groups, we found that 30 minutes or more per day of MVPA was associated with better attention/processing speed (TMT-A and Symbols Search subtests) and executive functions (TMT-B), compared with those who did not meet the 30 minutes per day of MVPA. Despite Part A and Part B of the trail making test require attentional processes for successful performance, they provide information about different domains. While the TMT-A provides an assessment of visual search speed, the TMT-B relies on cognitive flexibility and task-

switching ability, both known to decline with age. Attaining 30 minutes per day of MVPA was associated with better performance in both parts of the TMT tests, similar to the cross-sectional study of Kerr and colleagues[95]. Accordingly, 30 minutes per day of MVPA is associated with greater processing speed in executive control processes. Here, no relationship was found between light-intensity physical activity and TMT performance. These results highlight the importance of a *dose-response* relationship between physical activity intensity and cognitive functioning since no associations were found between them if considering the total physical activity duration disregarding intensity levels.

Physical activity and GM volume of frontal and primary motor regions

The process of brain aging is typically characterized by a widespread but spatially heterogeneous decline in GM volume[14]. By hypothesis, higher daily MVPA levels may lead to cognitive benefits in the elderly through a decrease in the GM atrophy rate of critical brain regions that modulate cognitive functions. Since executive functions were the most significantly different cognitive measures between groups, relying primarily on the frontal regions, one of the most affected with the advancing age[150], an analysis of the regions of the frontal lobe was further conducted.

Neuroimaging studies have suggested that cognitive flexibility and set-shifting, as required in the TMT test, are supported (among others) by the GM volume in the DLPFC and the VLPFC[185,186]. A cross-sectional study using FSL-Voxel Based Morphometry procedure found that higher fitness levels are associated with better executive function mediated through the increased GM volume in the DLPFC[116]. In our study, no difference was found regarding DLPFC GM volumes between the two physical activity groups. In fact, some studies consider middle frontal gyrus and superior frontal gyrus to be part of the DLPFC and some include the anterior or posterior extension of the gyri. Here, DLPFC was defined as a combination of the middle frontal gyrus and the middle frontal sulcus, which is a more conservative approach[174]. The inconsistent anatomical definitions of DLPFC among studies may be a potential source for the controversial findings. Regarding the VLPFC, the MVPA≥30 group had significantly larger GM volumes in comparison with those who did not meet the guidelines.

Additionally, the precentral gyrus volume, or primary motor cortex, was larger in the MVPA≥30 group. Morphometric studies reported that primary cortices decline later in age[187], and the involvement of the precentral cortex in motor functions could possible explain the motor slowness observed with advancing age[188].

Importantly, volume differences found between groups cannot be attributed to variation in intracranial volume, which can reflect sex or cohort effects, and brain volumes measures were corrected for the effects of estimated intracranial volume to avoid possible confounds. The underlying mechanisms by which exercise affects brain structure are not fully understood, but the effects of neurotrophic factors and neuroplasticity have been demonstrated to play a critical role. Most studies have argued that greater GM is beneficial[115,118], especially to the elderly for whom atrophy is relatively widespread. Nevertheless, it remains unclear how and why GM volume leads to better cognitive status. However, since aging is characterized by brain atrophy, larger volumes between groups are often indicative of less atrophy and/or slower rate of atrophy and better cognitive performance[189].

Physical activity and deep GM volumes

In this study, evidence for the positive impact of MVPA levels on subcortical structures was found. Consistent with the regional variability of age on brain volume, the findings suggest that physical exercise has differential effects on subcortical brain regions. The individuals who performed at or above 30 minutes per day of MVPA, had a larger thalamic, amygdala, and hippocampal subfields volume.

Cross-sectional studies have reported smaller thalamic volumes with aging[190,191], also reporting associations between the thalamus volume and cognition, specifically related to attentional, working memory, and executive functions. These cognitive functions were also the affected domains in which MVPA≥30 group displayed a better performance. The thalamus is connected to cortical, subcortical, and cerebellar structures and it is a critical node in these interacting and interconnecting networks[192].

Exercise-induced increase of hippocampus volume has been consistently described in animal studies, however, this observation in humans is not so clear[136]. Accumulating data primarily generated from animal studies, support evidence that physical activity has been correlated with increased neuronal proliferation in the hippocampus, mainly in the DG subregion[54,193,194]. In humans, this effect may be only observed indirectly such as by manual or automatic segmentation of T1-weighted anatomical images, and the differences observed in imaging studies likely reflect multiple processes and not just changes in neurogenesis[195]. Human studies exploring the relationship between physical activity and sub-regional structural volumes of the hippocampus have mainly focused on structured physical exercise and cardiorespiratory fitness[118]. Regarding the associations of physical performance and hippocampal volume, findings have been heterogeneous. If on the one hand, cross-sectional

studies[118,124] reported a relationship between physical activity and brain volume, on the other hand, others failed in finding such associations[196,197]. A recent study with older adults found a sex-dependent association, in which daily walking activity was associated with larger hippocampal volumes in older women, but not in men[130]. Another study indicated that increased structured exercise may be associated with anterior hippocampal volume, a region that includes, among others, the DG[195,198]. While most of the prior investigations have explored the influence of physical activity on global hippocampus volume, more specific associations have been explored here, analyzing hippocampal subfields volumes. A recent clinical trial focused on the impact of a standardized progressive aerobic exercise program on sedentary healthy participants on hippocampus and CA4-DG subfields volume[199]. These participants were also compared to a control (without intervention) group. They found an increase in the overall left hippocampus and CA4-DG volumes in the interventional group. Also, the older adults in the control group had significant age-related reductions in the CA4-DG subfields volume. By contrast, older adults in the exercise group did not show volume decline. In this line, in the present study, the group that met the current physical activity guidelines had larger GC-DG and CA4 subfields volumes in comparison with those who did not.

Despite the histological and chemical basis for volumetric changes in humans remaining unknown, the effects observed with physical exercise on the hippocampus in rodents suggest that engagement in higher levels of physical activity may preserve hippocampal volume in elderly humans. The findings of this study suggest the existence of physical activity-related increased capacity for neuronal proliferation in neurologically healthy older adults. The intensity of physical activity does not influence all brain regions uniformly, having a minimal impact on certain brain regions.

Associations between cardiorespiratory fitness, regional brain volumes and cognitive function

Regarding the relationship between aerobic fitness and cognitive functioning, previous cross-sectional and longitudinal studies have linked higher levels of cardiorespiratory fitness and preserved cognitive performance, particularly in tasks of attention and executive function[118,200]. However, a relationship between regional brain volumes, cognitive function, and aerobic capacity was not apparent in this study.

There are several possible explanations for the absence of such association. All participants were encouraged to exercise to exhaustion. It is only the maximum oxygen uptake achieved during severe-intensity large muscle mass exercises such as running that allows measuring

the upper ceiling of the O_2 transport and utilization system. However, most of the participants did not achieve maximal effort in cardiorespiratory fitness testing, due to the level of motivation, perceived exhaustion, and muscular weakness[201]. Thus, an absolute VO_2max could not be determined, and here it was used the term VO_2peak as the highest VO_2 level reached on the fitness test. Unfortunately, this procedure cannot discriminate among subjects who terminated the exercise test due to lack of motivation or muscular weakness. Cardiorespiratory fitness levels of each participant might have been underestimates since the VO_2peak did not reflect necessarily the maximal rate of O_2 transport/utilization of the participants. This, in addition to the small number of participants, make the results more challenging to interpret and may reduce the analysis power as it may conceal relationships with cognition-related outcomes. Another possible explanation is that despite cardiorespiratory fitness and MVPA are related, they represent potentially dissociable aspects of physiology[202]. Cardiorespiratory fitness is, in part, an outcome of engaging in MVPA, whereas MVPA is best considered a health-related behavior[202]. Since there is a range of heritability of cardiorespiratory fitness, even individuals engaged in similar levels of MVPA, differ in values of cardiorespiratory fitness, as a reflection of genetic differences[203]. Thus, there is a growing body of evidence suggesting that cardiorespiratory fitness and MVPA have dissociable effects on brain structure[204,205]. Actually, like in our study, a recent cross-sectional study found that MVPA, but not cardiorespiratory fitness, was associated with hippocampal volumes, which suggests that the engagement in MVPA may have beneficial effects on the brain beyond those related with cardiorespiratory fitness levels.

Limitations and Strengths of the present study

There are certain limitations in the current study that should be considered. First, the inclusion of participants who have voluntarily enrolled in an exercise program is biased in favor of those with an active lifestyle, predominantly motivated and cognitively high-functioning individuals. However, a large selection of cognitive tests was selected and should be sensitive to cognitive differences, even in this high-performing cohort. Second, only the data of the baseline collection was assessed, and cross-sectional studies prevent establishing the direction of the reported associations. Even though the suggestive evidence that physical activity contributes to better cognitive function, previous studies have suggested that individuals with better executive abilities tend to engage in a more physically active lifestyle over time in comparison to those who perform poorly on executive function-related tasks[88]. Noteworthy, the characterization of habitual physical activity of the participants by accelerometers does not provide information about the type of physical

activity performed, neither about the nature of sedentary behavior. Also, even though objective measurements are more accurate than subjective measurements, it is known that hip-worn accelerometers fail to detect some upper body movements during activities such as weightlifting. Finally, the sample size was relatively small and therefore more vulnerable to random errors in sampling and measurement. Further studies including also other MRI modalities are necessary to investigate the direction of the observed relationships in larger samples.

Despite the limitations aforementioned, this study has several strengths in comparison to previous research. The most important is the quantification of physical activity through objective measures that allows analyzing the amount and proportion of time spent in different intensity levels of physical activity. This allows overcoming the subjectivity associated with physical activity questionnaires especially in older adults with difficulty recalling past events. Secondly, this is one of the first studies to examine the association between objectively measured physical activity and volumetric brain estimates. Specifically, the high-resolution MRI scanning allows a detailed assessment of the hippocampus sub-regional volumes. Furthermore, it was explored simultaneously the relationship of physical activity and sedentary behavior within specific cognitive domains including the verbal fluency, memory, and executive functions. A wide range of covariates, including age, sex, education, depressive and anxiety symptoms, and BMI were also evaluated.

5
CONCLUSION

In this preliminary study with neurologically healthy older adults, objectively measured minutes per day engagement in MVPA, but not in light physical activity, was associated with greater cortical and deep GM volumes as well as improved processing speed information.

Individuals who met the current physical activity guidelines of 30 minutes per day of MVPA had larger VLPFC, precentral gyrus, thalamus, and hippocampal subregions volumes. Specifically, there were found larger volumes in hippocampal subfields CA4 and GC-DG, both known to be central to core hippocampal functions, in individuals who met the guidelines compared to those who did not. To the best of our knowledge, this is the first study reporting the association between hippocampal subfields and high intensity daily physical activity, at baseline, and according to the health guidelines. Notably, using objective assessment of daily physical activity patterns, this investigation suggests that the engagement accordingly to the physical activity recommendations is not only beneficial for preserving frontal regions and hippocampal subfields volumes, but also an important approach for older adults to delay the cognitive decline.

6
FUTURE WORK

The current study reports the benefits of habitual physical activity engagement for the preservation of cortical and subcortical brain regions and cognitive domains in a cohort of healthy older adults. Importantly, we aimed to quantitatively and accurately describe the main study variables. Cardiorespiratory fitness and physical activity should be properly assessed and standardized in the literature to facilitate comparisons between studies and thus reduce methodological variability. Also, in this study, the MRI acquisition was performed with a standard T1 sequence resulting in a voxel size of 0.8 mm^3. A higher resolution and complementary T2-weighted images would allow a more precise segmentation of hippocampal subfields[177], increasing the sensitivity for detecting reliable differences in hippocampal volume.

Studies with a larger sample size and physical activity dose manipulation and/or interventional programs could help to determine an accurate threshold of the optimum intensity of physical activity to preserve specific regional brain volumes and obtain the maximum benefits to cognitive health. These studies are needed to fully validate current physical activity guidelines. Future longitudinal investigation within physical exercise intervention, such as the *Mais ativos, mais vividos* program, from which this sample was acquired, will provide better understanding of whether modest increases in physical activity may have sub-regional specific effects on the hippocampus, and other brain regions, with subsequently (or independent of) impact on cognitive function.

The longitudinal studies should also evaluate the serum levels of BDNF, IGF-1, and VEGF since they are putative markers of exercise-induced benefits on brain structure and function. This would elucidate whether the concentrations of these growth factors are associated with alterations in regional brain volumes following exercise.

Also, the functional connectivity between RSNs has been suggested to be critical in complex cognitive processes[206], playing an important role in the maintenance of healthy brain functioning[207,208]. Some RSNs and even between-network connections are influenced by

aging and such alterations are associated with the age-cognitive decline in the elderly[109,209]. Thus, exploring age-related changes within or between RSNs is a key towards the understanding of how lifestyle factors could modulate the cognitive trajectory of aging. Examining functional connectivity between brain regions associated with high-order cognitive functions, such as the PFC, along with structural connectivity using diffusion tensor imaging, would give a broader overview of the complex neural network mediating the link between physical activity and executive function. To better understand the way as structural changes of thalamic volume translates into age-related changes in cognition, further investigations must examine how GM and WM structural integrity affects the function of brain networks[210].

Further interventional research should target lifestyle physical activity, which is generally chosen and facilitate engagement among the elderly population. Animal research suggests that the combination of exercise and environmental enrichment may best stimulate neurogenesis in the hippocampus[48,211]. Since this study evaluates the daily physical activity, it is possible that social interactions and experience of novel environments could also contribute to the effects observed in this study. In the future, it would be interesting to address these interactive effects of cognitive training and physical exercise as an important direction for future human studies and improvement of quality-of-life.

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