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The effect of physical activity on pupil-linked arousal responses associated with decision uncertainty and feedback processing in older adults

Dissertation in the context of the Master in Biomedical Engineering, Specialization in Biomedical Instrumentation, supervised by Professors Maria Ribeiro, Luís Rama, and Ana Teixeira, and submitted to the Faculty of Sciences and Technology of the University of Coimbra / Department of Physics.

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CENTRO DE INVESTIGAÇÃO
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"Live as if you were to die tomorrow. Learn as if you were to live forever."

Mahatma Gandhi

Resumo

O declínio cognitivo associado ao envelhecimento está relacionado com a integridade estrutural do sistema noradrenérgico. O sistema noradrenérgico faz parte do sistema de *arousal* composto por vários núcleos neuromodulatórios do tronco cerebral. Estudos com modelos animais sugerem que menor atividade do sistema noradrenérgico poderá estar associada a um maior risco de desenvolvimento de doenças neurodegenerativas. No entanto, em termos funcionais, ainda não é claro como é que este sistema é afetado pelo envelhecimento. Para além disso, a prática de exercício físico resulta num aumento da libertação de catecolaminas (noradrenalina e dopamina), resultando no aumento da atividade do sistema de *arousal*, pelo que poderá ser uma intervenção capaz de potenciar a função deste sistema. Para abordar estas questões, investigámos as diferenças entre jovens adultos e adultos mais velhos na ativação do sistema de *arousal* durante uma tarefa de tomada de decisão percetual e estudámos o efeito de uma sessão única de atividade física na ativação deste sistema em adultos mais velhos. Portanto, este estudo teve dois objetivos: 1) estudar como o envelhecimento afeta o recrutamento do sistema de *arousal* durante tarefas cognitivas; 2) como uma sessão única de atividade física aeróbia moderada afeta a ativação do sistema de *arousal* em pessoas mais velhas.

Para explorar estas questões, testámos um grupo de jovens (n = 20, idade média ± desvio padrão = 23.7 ± 2.85 anos) e um grupo de adultos mais velhos (n = 20, idade média ± desvio padrão = 58.6 ± 5.92 anos) numa tarefa de perceção visual. O diâmetro da pupila foi medido e usado como indicador da atividade do sistema de *arousal*. Para testar o efeito do exercício, os adultos mais velhos realizaram a tarefa de perceção visual, em dias distintos, antes e depois de uma sessão de 30 minutos de atividade física aeróbia moderada e, como controlo, antes e depois de uma sessão de atividade mental com a mesma duração.

Tanto nos resultados comportamentais como nas respostas pupilares recolhidas durante a realização da tarefa de tomada de decisão percetual, verificámos diferenças entre grupos que sugerem alterações no processamento da incerteza no grupo de

adultos mais velhos. Ao contrário das expetativas, a atividade física não revelou quaisquer efeitos significativos no desempenho da tarefa nem nas respostas pupilares.

Em suma, estes resultados sugerem que o envelhecimento afeta o processamento da incerteza, podendo estar relacionado com alterações no recrutamento do sistema de *arousal* durante uma tarefa de tomada de decisão. Uma única sessão de exercício físico aeróbio moderado não induziu diferenças na performance da tarefa nem no recrutamento do sistema de *arousal*, o que sugere que este tipo de atividade física pode não ser suficiente para induzir alterações cerebrais com impacto na tomada de decisão percetual.

Palavras-chave: Envelhecimento, Tomada de Decisão Perceptual, Dilatação da Pupila, *Arousal*, Incerteza, Atividade Física

Abstract

Cognitive function in older people is associated with the structural integrity of the noradrenergic system. The noradrenergic system is part of the arousal system. Studies with animal models suggest that lower activity of the noradrenergic system may be linked with a greater risk of developing neurodegenerative diseases. However, it is still unclear how much aging affects this system. Physical activity results in an increase in the release of catecholamines, such as noradrenaline and dopamine, which result in increased arousal. Therefore, it could be used as an intervention to improve the function of this system. To address these questions, we investigated the differences between young and older adults in the activation of the arousal system during a perceptual decision-making task and studied the effect of a single session of physical activity on the activation of this system in older adults. Thus, this study had two main goals: 1) to study the effect of aging on the recruitment of the arousal system during cognitive tasks; 2) to examine the effect of a single session of moderate aerobic physical activity in the activation of the arousal system in older adults.

We tested a group of young (n = 20, mean age ± standard deviation = 23.7 ± 2.85 years) and a group of older adults (n = 20, mean age ± standard deviation = 58.6 ± 5.92 years) with a visual motion detection task. We measured pupil size as a proxy for activity in the arousal system. To evaluate the effect of exercise the older group performed the visual motion detection task on two different days, before and after a 30-minute session of physical activity and a session of mental activity with the same duration.

We found differences between groups in both the behavioral results and the pupillary responses collected during the performance of the perceptual decision-making task, which suggests that uncertainty processing is different in older people in comparison with young adults. Contrary to what was expected, physical activity did not induce any significant changes in task performance or in pupillary responses.

In conclusion, we found evidence that uncertainty processing changes with aging and that this is linked to changes in the recruitment of pupil-linked arousal during

a perceptual decision-making task. A single bout of moderate aerobic exercise did not strongly affect the performance of this task or task-related pupil-linked arousal responses suggesting that a single bout of exercise might not be enough to induce neural changes with an impact on perceptual decision-making.

Key-Words: Aging, Perceptual Decision-Making, Pupil Dilation, Arousal, Uncertainty, Physical Activity

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List of Abbreviations

CO_2 Carbon Dioxide. 33
O_2 Oxygen. 33
VO_2max Maximal Oxygen Uptake. 2, 26, 27, 30, 33, 34, 37, 77

ACC Anterior Cingulate Cortex. 6, 11, 12, 14, 15, 24
ANS Autonomic Nervous System. 7, 8, 12, 14, 15

BDI Beck Depression Inventory. 40
BDI-II Beck Depression Inventory – II. xv, 38, 40, 42, 56

CIBIT Coimbra Institute for Biomedical Imaging and Translational Research. 27
CIDAF Research Unit for Sport and Physical Activity. 27

EA Error Awareness. 15, 16

GLMM Generalized Linear Mixed Models. xviii, 42, 43, 44, 46, 55, 57, 58, 60

HR Heart Rate. 23, 33
HRmax Maximum Heart Rate. 22, 25, 33

LC Locus Coeruleus. 5, 6, 7, 11, 12, 14, 15, 73
LC-NE Locus Coeruleus-Norepinephrine. 5, 6, 11, 25, 73, 74, 77
LMM Linear Mixed Models. xviii, 42, 48, 50, 54, 55, 61, 62, 64, 67, 68, 74

MoCA Montreal Cognitive Assessment. xv, 38, 39
MRI Magnetic Resonance Imaging. 7

NE Norepinephrine. 5, 11, 12, 18, 24, 25, 40, 41

PES Post-Error Slowing. 15, 16
PET Positron Emission Tomography. 25
PNS Parasympathetic Nervous System. 7, 8

RDM Random Dot Motion. 16

sAA Salivary Alpha-amylase. 40, 41
SC Superior Colliculus. 11
SCi Superior Colliculus Intermediate Layers. 11, 12
SCs Superior Colliculus Superficial Visual-only Layers. 11
SNS Sympathetic Nervous System. 7, 8, 41

WAIS Wechsler Adult Intelligence Scale. xv, 39
WAIS-III Wechsler Adult Intelligence Scale - Third Edition. 38, 39

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Introduction

1.1 Motivation

Aging is by far the most significant risk factor for neurodegeneration and the development of Alzheimer's, Parkinson's, Huntington's, and other neurodegenerative diseases [2]. Furthermore, the population over 65 years old is increasing and, consequently, the incidence of age-related diseases is also increasing. Therefore, improved prevention and treatment strategies will benefit a large portion of the population [3]. To this, it is urgent to further describe the effects of age on cognition, and which changes may effectively be indicative of brain diseases and which are normal to occur [4]. In this study, we focused on investigating the relationship between activity in the brain arousal systems and cognitive aging in both younger and older healthy adults. Indeed, the preservation of the arousal system may play a protective role against neurodegeneration, which could prevent the development of neurodegenerative diseases.

Everyday decision-making abilities are essential for living independently and for maintaining physical and mental well-being. However, as people get older, these skills tend to decline, affecting their autonomy and their performance of daily tasks. Studying the neural changes underlying these problems is important to develop treatments to delay, or even inhibit, their appearance in the future [5].

Therefore, the first goal of the present thesis was to evaluate the effect of aging on uncertainty processing in a perceptual decision-making task, and its association with the activation of the arousal system. With this aim, we designed a perceptual decision-making experiment, in which we acquired data from young and older adults, and we measured the pupil size as a proxy of arousal aiming to study how pupil-linked arousal was modulated by response uncertainty and response feedback and if this modulation was affected by aging.

Moreover, it has long been known the noteworthy benefit of physical activity on several perceptual and cognitive functions [6]. In fact, during and immediately

after physical exercise there is an increase in the release of noradrenaline, associated with arousal activation [7]. Thus, the present thesis also aimed to examine whether a 30-minute bout of moderate aerobic exercise via a cycle ergometer at 70% of the participants Maximal Oxygen Uptake (VO_2max) modulates visual perception, uncertainty processing, and activation of the arousal system in this context in older adults. Maximal Oxygen Uptake (VO_2max) consumed per unit of time, commonly expressed clinically in relative units ($mL \cdot kg^{-1} \cdot min^{-1}$), is recognized as a reliable measure of cardiorespiratory fitness [8].

1.2 Effect of Aging on Cognition

Cognitive functioning refers to the mental processes and activities associated with the acquisition, storage, retrieval, and use of knowledge and information. This function is essential to understand, interpret, and interact with the environment. It allows us to live independently, take medication correctly, drive safely, communicate with other individuals, and live in a society [4]. All of these tasks require mental procedures from several domains, such as attention, memory, perception, problem-solving, language, decision-making, and visuospatial abilities [4, 9].

Cognitive decline can have a significant impact on the daily life of each individual, and can also be an early sign of a neurological disorder. Many factors lead to a decrease in cognition, including aging.

Aging refers to the biological process of becoming older. It is a complex phenomenon that is characterized by a gradual and continuous loss of physical, physiological, and cognitive functions [10]. The process of aging is influenced by a variety of factors, including genetics, lifestyle, medical conditions, and environmental factors, and it leads to complex and highly variable life changes. Thus, it is difficult to estimate the velocity and intensity of cognitive decline and to identify the transition to pathologic stages [11].

Cognitive function declines with age, and aging is the biggest risk factor for the development of neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's diseases [2]. With the larger evolution of science and health, people are living longer. Consequently, age-related neurodegenerative diseases are also increasing. Therefore, it is urgent to realize the effects of age on cognition, and which changes may effectively be indicative of brain diseases and which are normal to occur [4].

The three steps that occur in most of cognitive functions are perception of the environment, processing of the information, and action. With aging, both sensory

perception and processing speed decline, so, it may have an inevitable impact on several domains of cognition [4]. The basic cognitive abilities most affected by aging include attention, memory, and perception.

Regarding attention, the main changes are associated with the decline in the performance of complex tasks, such as selective and divided attention, while sustained attention, which is typically measured by vigilance tasks, seems to be preserved in normal aging. Selective attention is the ability to selectively attend to specific stimuli while at the same time disregarding others that are irrelevant. Divided attention is more complex and requires the processing of multiple tasks simultaneously [4, 9].

Concerning memory, which is characterized by the ability to encode, store, and retrieve information, both short-term and long-term memory decline with aging. Short-term memory, also called working memory, involves the simple maintenance and manipulation of information for brief periods. In working memory, aging mainly affects tasks that involve active manipulation of information, such as mental arithmetic. On the other hand, long-term memory requires retaining information for long periods. In this type of memory, age-related decline is most pronounced in episodic memory, especially memory for specific dates or events that happened earlier [4, 9].

Furthermore, the decrease in complex cognitive functions, such as speech and language, decision-making, and executive control is directly related to a decline in the basic cognitive functions [4].

Executive function refers to planning, organization, mental flexibility, problem-solving, and multitasking [12]. This set of abilities allows individuals to successfully live in an independent and self-serving behavior and is particularly important for novel tasks. Research has shown that the executive control tasks that most contribute to age-related cognitive decline are concept formation, abstraction, mental flexibility, and manipulation of information [13]. Aging also negatively affects the ability to inhibit an automatic response [14].

Regarding decision-making, most of the research has been done on the potential indirect impact of working memory and attention declines on decision-making, rather than on the direct effect of age on decision-making. However, most research focused on prior knowledge, emotion, motivation, and relevance, and its impact on decision-making, especially in real-life contexts [9]. Older adults tend to rely more on prior knowledge and expert opinion and less on novel information, while young adults, who probably have less knowledge about the problem domain, tend to study more alternatives and evaluate more current information before making a decision [15].

In contrast, speech and language tasks seem to remain intact with aging. Older people have a more extensive vocabulary, often tell better elaborate narratives, and

do well-structured discourses [16]. In addition, deficits that occur under this cognitive function are usually associated with working memory decline or sensory loss, and not in limitations in basic language capacities per se [17].

Overall, most studies connected aging to cognitive decline. However, this decline varies considerably between individuals and between cognitive domains, with some tasks being more susceptible than others to the age-related decline. To sum up, while some cognitive functions, such as memory, decision-making, and executive control, tend to decline with aging, others, like speech and language, can even improve with it.

1.3 Brain Structural Changes with Aging

As people get older, the changes that occur are not only functional. There are also several structural changes in the brain, that are probably the cause of functional deficits, which can affect cognitive function and might contribute to the development of disorders.

One of the most notable changes in the aging brain is a decrease in brain size. Indeed, every year the whole brain volume loses in size by 0.2 to 0.5%, as estimated in previous longitudinal studies [18]. This is caused by a combination of factors, including loss of neurons and shrinkage of remaining ones, and affects both gray and white matter regions [12].

Gray matter regions contain the cell bodies and dendrites of neurons, while white matter consists of regions with a predominance of myelinated axons and is responsible for connecting gray matter structures. Gray matter volume decrease is most prominent in the prefrontal cortex, which is involved in executive function, and also, although more moderately, in temporal lobes, particularly in the medial temporal lobe, that contains the hippocampus, an essential structure for memory [4]. White matter regions of the brain are also affected by aging. Their decreases in size are much greater than gray matter size decreases [19]. The frontal lobe and the major white matter tracts, such as the corpus callosum, are the most affected regions by white matter volume losses [19]. In addition, one of the most notable changes in white matter regions is the decline of their integrity, which is characterized by changes in the structure that surrounds the axons of the neurons and helps to transmit electrical impulses, called myelin sheath [20]. This structure can become thinner and less effective, leading to slower neural transmission. Many researchers have used diffusion tensor magnetic resonance imaging to investigate the effect of aging on white matter volume and integrity. Some studies observed that

the functional connectivity between several structures of the brain was largely affected by aging, and also that these reductions were associated with reductions in white matter integrity [21, 22]. More recently, Coelho et al. (2021) corroborated the hypothesis that age is linked to a degradation in white matter integrity [23]. In the study, it was also found significant associations between diffusion measurements and various cognitive domains, such as general cognition, memory, and executive function.

Previously, it was believed that gray matter volume loss was due to the loss of neurons. With the evolution of techniques for neuron counting, it was realized that this loss is best explained by several age-related structural changes such as the decrease in the number and length of dendrites and dendritic spines, which are the structures that neurons use to communicate with one another [24]. Also, the reduction in the number of axons, the increase in segmental demyelination's axons, and the significant decrease of the synapses contribute to the cognitive decline with aging by minimizing axonal signal transduction [18, 25]. Actually, Masliah et al. (1993) proposed that synaptic loss is an important marker of aging in the nervous system, and this has been shown in mice, humans, and non-human primates [26].

Besides the age-related changes in hippocampal and cortical regions, brainstem regions, especially the Locus Coeruleus-Norepinephrine (LC-NE) system, also play a role in cognitive decline. Locus Coeruleus (LC) is a small nucleus localized deep in the rostral pons of the brainstem in the lateral floor of the fourth ventricle that is responsible for producing and releasing the majority of the Norepinephrine (NE) [27]. Despite containing a small number of cells, LC sends projections to many brain regions, such as the cerebellum, the diencephalon, and the paleo- and neocortex [28]. It is estimated that the human LC contains about 30,000 neurons which can provide NE to approximately 100 billion neurons in the brain [29]. Accordingly, this system is a critical component of the brains neural network and plays an important role in a wide range of physiological and cognitive processes, including arousal, attention, sleep/wake states, homeostasis, and stress responses, as well as memory and learning [30, 31].

The LC-NE system has at least two modes of firing, phasic and tonic, depending on the nature of the stimuli and the brain state [32]. The phasic mode is characterized by bursts of NE release at a low baseline rate, while the tonic mode is associated with sustained release of NE without phasic responses. The phasic mode is activated in response to relevant or unexpected stimuli, such as stress, attentional demands, or changes in the environment [31]. In these situations, the release of NE in the brain is rapidly increased by the LC, which results in better performance and greater at-

tention during a task [31]. Alternatively, the tonic mode is important in response to sustained attentional demands or chronic stress and is correlated with task disengagement and poor performance and with higher levels of distraction [30, 32]. Despite resulting in worst performance on tasks that involve selective attention, the tonic mode increases behavioral flexibility, that is, it promotes the switch from the current task to other stimuli or action in a completely different context [31, 33].

According to these results, the best task engagement is achieved at a sweet spot in the middle, with high phasic activity and low tonic activity [34]. There is an inverted U-shape relationship between performance and LC-NE activity, which is usually called adaptive gain theory [35]. Therefore, this theory is an important key to control performance and further proposes that the orbitofrontal cortex and the Anterior Cingulate Cortex (ACC) are two frontal structures that play critical roles in regulating that performance [35].

Overall, the LC-NE system plays a role in arousal modulation. In order to understand the role of age-related changes in arousal modulation in age-related cognitive decline, it is important to understand how the LC function and structure change with aging and how these can be associated with the development of neurodegenerative diseases. Some studies reported an LC neuronal number decline by, approximately, 20-40% with aging with greater prominence in the rostral LC compartment [36–39]. However, some of these studies did not exclude pathology cases in the brain, used small sample sizes, or employed biased quantification methods. More recently, a research with only pathologically normal individuals has not found any differences in neuronal numbers with aging [29]. As such, there is no certainty about age-related decreases in these LC neuron counts. Apart from this, it is evident that changes in LC integrity are linked to cognitive decline in older adults [40].

Regarding understanding neuronal density changes and their impact on cognitive function, Wilson et al. (2013) analyzed a brain autopsy of the density of neurons in many regions, including the LC, the dorsal raphe nucleus, the substantia nigra, and the ventral tegmental area, from older individuals who had completed cognitive testing for six years before dying and concluded that only LC neuronal density was associated with the cognitive decline in the years before death [41]. Another research used tyrosine hydroxylase immunohistochemistry in postmortem tissues from individuals who died with no cognitive impairment, amnestic mild cognitive impairment, or Alzheimer's disease. The results showed that in the transition from no cognitive impairment to amnestic mild cognitive impairment the LC neuron number decreased by 30%, and it was found an additional 25% loss during the transition to Alzheimer's disease. Furthermore, this decrease was deeply connected

with cognitive decline in several tests of perceptual speed, visuospatial ability, and memory [42]. For instance, loss of noradrenergic LC projection neurons is a key feature of Alzheimer's disease [42].

Neuromelanin, a byproduct of noradrenaline synthesis which accumulates in noradrenergic LC cells as we get older due to its magnetic properties, can be used to study in vivo LC integrity using Magnetic Resonance Imaging (MRI) [43, 44]. To study the relationship between age-related changes in the LC integrity and cognitive performance, Hämmerer et al. (2018) acquired MRI data from young and older adults during a reversal-learning task. Data demonstrated that older adults with less neuromelanin signal intensity, and hence reduced LC integrity demonstrated worse memory performance [44]. These results are in line with the hypothesis that LC integrity is a crucial point in minimizing cognitive decline with aging.

1.4 The Role of the Autonomic Nervous System

The Autonomic Nervous System (ANS) is a complex division of the nervous system that controls several bodily processes without conscious control, such as heart and respiratory rates, and digestion. This system is composed of both preganglionic and postganglionic neurons. Preganglionic neurons lead impulses from the brain or spinal cord to postganglionic neurons located near the effector organ, and, after that, postganglionic neurons synapse with the target organ, triggering the desired response. Therefore, it involves multiple areas of both central and peripheral nervous systems, and it plays an important role in the body balance, which is called homeostasis [45].

The ANS can be divided into central ANS and peripheral ANS. Central ANS comprised several interconnected regions of the forebrain, the spinal cord, and the brainstem, which includes the midbrain, pons, and the medulla oblongata. The peripheral components of the ANS can be divided into Sympathetic Nervous System (SNS), Parasympathetic Nervous System (PNS), and enteric nervous systems (responsible for coordinating the function of the gastrointestinal tract) [45]. In a general way, SNS and PNS work in opposition to each other to maintain homeostasis in the body. The SNS is activated during times of stress and arousal and is associated with a fight-or-flight response. This response increases heart rate, blood pressure, blood sugar levels, and pupil size. On the other hand, PNS is essential to the rest and digest response, to slow heart rate, to decrease blood pressure, to conserve resources, and to maintain basic body functions. It is also linked with pupil constriction. Working together by raising the activity of one system while lowering

the activity of the other one, the SNS and the PNS are fundamental to promote a rapid and precise control of many tissues.

Furthermore, it has been demonstrated that the ANS activity varies with the level of brain arousal. In line with this, Huang et al. (2018) reported a consistent drop in ANS activity as brain arousal levels decreased over two hours of electroencephalogram record [46]. This relationship is essential to modulate brain arousal, which plays a significant role in human behavior, including how individuals interact with their environments and how they respond to external stimuli [47–49].

1.4.1 Influence of the Autonomic Nervous System on Pupil Response

As previously described, the ANS is essential to maintain the stability and internal balance of the body by controlling the activity of many tissues, including several ocular functions such as pupil size. Pupil diameter is a non-invasive way to detect autonomic activation.

The pupil is the aperture of the iris, usually with a circular shape, that enables vision by allowing light to enter in the eye, pass through the surface of the lens, and reach the retina [50]. There are two smooth sets of muscles in the iris responsible for controlling pupil size: the sphincter or constrictor muscles, responsible for decreasing its size, and the dilator muscles, which increase it [50, 51]. As mentioned above, the interaction between SNS and PNS controls these processes.

To control the sphincter muscle, the pretectal olivary nucleus receives retinal ganglion cell signals, which then are projected to the Edinger-Westphal nucleus. Thereafter, parasympathetic neurons in the Edinger-Westphal project to the ciliary ganglion and promote constriction [45, 52]. On the other hand, the dilator muscle is controlled by the sympathetic system. In this system, preganglionic fibers project to the Superior Cervical Ganglion from the hypothalamus, via the spinal cord, and postganglionic fibers, via ciliary nerves, project to the iris dilator muscles [52, 53]. Nevertheless, dilation can be evoked not only by a sympathetic mechanism but also by the parallel inhibition of the parasympathetic system [54]. Figure 1.1 illustrates the balanced activity among both sympathetic and parasympathetic circuits.

Usually, in standard light conditions, the diameter of the pupil is around 3mm [55]. However, this size can range from 1.5 to 9.0 mm due to a variety of factors, including luminance variations [56]. When the level of light or the brightness increases, the pupil size decreases as a way to reduce the amount of light that hits the eye. This phenomenon is known as pupillary light reflex and is considered the

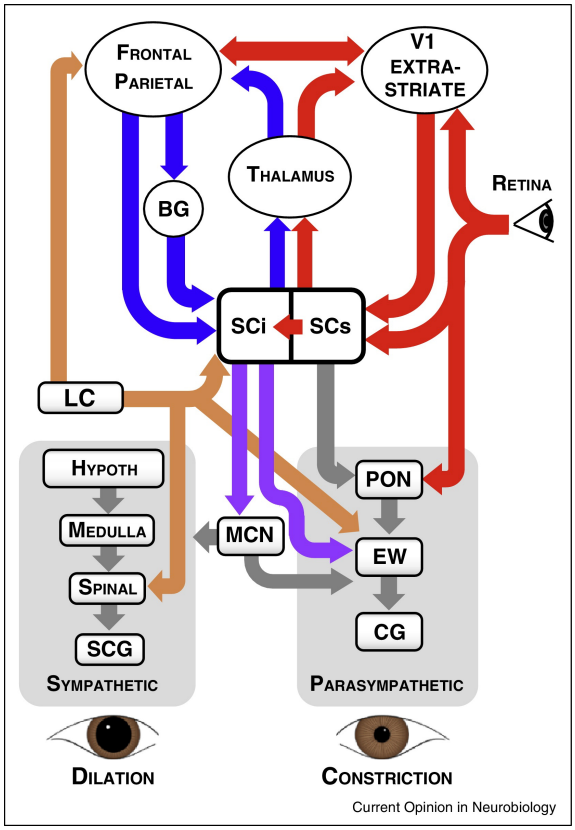

Figure 1.1: Pupil size control circuit. Abbreviations: BG, basal ganglia; CG, ciliary ganglion; EW, Edinger–Westphal nucleus; Hypoth, hypothalamus; LC, locus coeruleus; MCN, mesencephalic cuneiform nucleus; PON, pretectal olivary nucleus; SCi, intermediate layers of the superior colliculus; SCs, superficial layers of the superior colliculus; SCG, superior cervical ganglion; V1, primary visual cortex. Reprinted with permission from [52].

first and most important mechanism for adjusting to light [56]. This reflex aids in protecting the eye from excess light. Otherwise, to allow more light to enter the eye, when luminance decreases, the pupil dilates, which helps to improve visual acuity in low-light conditions. Despite resulting from a brainstem circuit in which the retina relays brightness information to the Edinger-Westphal nucleus, which subsequently instructs the pupillary sphincter to contract [57], the pupillary light reflex magnitude is not only influenced by retinal input. Ebitz & Moore (2017) discovered that this reflex is also modulated by microstimulation of the frontal eye field, a structure which is believed to connect cognition, such as attention, to brainstem circuitry [58, 59]. Thus, the findings suggest that pupillary light reflex might be modulated by attention.

The pupil also responds to different colors of light. Some studies have found that blue light induced greater pupillary constriction than other colors such as red light [60].

Furthermore, pupil size can change with cognitive load. However, these changes are rarely larger than 0.5 mm [54]. The study on changes in pupil diameter concerning cognitive function is called pupillometry [50] and it has been studied for over 50 years now [61]. Indeed, over 40 years ago Hess & Polt (1964) reported larger pupil diameter in response to increasing mental activity, more specifically, in response to increasingly difficult multiplication problems [62]. Shortly after, a memory load study where participants listen to an increasing number of digits and following a brief pause reproduce them demonstrated that pupil diameter increases incrementally during encoding and, then, decreases during recall [63]. The literature has consistently shown that pupils dilate as task demands and cognitive load increase, reflecting the complexity of the task and the greater arousal and mental effort needed to perform it [50, 51, 64]. Thus, pupil dilation is a good marker of many cognitive processes, including attention, memory, decision-making, and problem-solving tasks [50].

Nevertheless, there are two possible interpretations for this correlation: the simplest suggests that pupil dilation merely reflects the task demands, whereas the more complex one proposes that pupil dilation truly reflects the effort employed in response to these demands [62, 65]. Accordingly, as the mechanisms behind this correlation are unclear, Wel & Steenbergen (2018) reviewed many studies to study whether pupil dilation is a reliable index of effort in three specific cognitive domains: updating, switching, and inhibition [51]. Collectively, the results support the idea that pupil dilation is actually an index of the effort made in executive control tasks. Pupil size is closely related to the effort applied to each task, and, in some situations, increased dilation reflects improved task performance. Sirois & Brisson (2014) have already done a review of numerous studies concerning pupillometry across various domains within cognitive science, including language processing, memory, and decision-making [50]. Regarding language processing, pupil dilation seems to be a reliable indicator of modifications brought on by hearing loss [66], as well as an index of the difficulty of word retrieval in people who are bilingual [67]. On the other hand, regarding memory and decision-making, pupil dilation appears to be an index of the strength of the memory [68–70], and to reflect the processes associated with decision-making instead of the outcome [71].

Another factor that can modulate the pupil response is the emotional arousal. It has been shown that pupil dilation can be observed in response to arousing stimuli, positive or negative, which does not happen with emotionally neutral stimuli [72, 73]. Furthermore, pupil dilation is also enhanced by human hand touching compared to similar mechanical contact [74], and eye-to-eye interactions [75].

Fatigue, particularly mental fatigue, can also modulate pupil response. Mental fatigue is a complex state of reduced cognitive performance that can come from sustained and long-term cognitive effort and can affect several cognitive processes, motivation, and mood [76]. In this regard, studies have shown that mental fatigue is associated with decreases in stimulus-evoked pupil dilation and with noticeable fluctuations in its size [50]. Hopstaken et al. (2015a, 2015b), with two different studies, have successfully linked increases in mental fatigue with reduced pupil diameter [76, 77].

Overall, there has been increasing documentation about the brain mechanisms underlying pupil dilation and effort and most of them are tightly linked to the LC-NE system. The LC receives inputs from brain areas such as the ACC and the Posterior Cingulate Cortex [51, 78], and connects to both parasympathetic and sympathetic system pathways via efferent projections to the Edinger-Westphal nucleus and the spinal cord, respectively [53].

Acetylcholine, analogously to the NE release, has been proposed to be an important key for optimal task performance under challenging conditions. Both acetylcholine and NE systems receive projections from ACC. Acetylcholine neurons are ideally placed to control motivated behavior as they receive projections from frontal and midbrain areas, and they can also upregulate prefrontal areas in response to cognitive tasks [79]. Regarding this, Reimer et al. (2016) demonstrated that both noradrenergic and cholinergic activities are related to pupil dilation in different ways: while the activity of cholinergic axons is associated with long-time dilations, the activity of noradrenergic axons is reflected by rapid dilations [80].

Nonetheless, there is evidence of another possible circuit that can modulate the cognition-related pupil changes [52, 78]. This pathway is centered on a midbrain structure called Superior Colliculus (SC), which can be functionally subdivided into intermediate layers (SCi) that receive cognitive and motor stimulus, and superficial visual-only layers (SCs) that, as the name suggests, receive retinal and visual cortex inputs [81]. While SCs only project to the pretectal olivary nucleus, SCi receives signals from frontoparietal areas, the SCs, the basal ganglia and the LC, and activates or inhibits the parasympathetic systems by direct and indirect pathways [82, 83]. In other words, SCi can project directly to the Edinger-Westphal or can also modulate this system through efferent projections to the Mesencephalic Cuneiform Nucleus, which subsequently projects to the Edinger-Westphal [52]. Indeed, supporting this idea Joshi et al. (2016) observed effort-related pupil diameter changes in several other brain structures than the LC, including the ACC, Posterior Cingulate Cortex, SCi, and Inferior Colliculus [78]. Although it is evident the

relationship between neural activity and pupil size in other brain regions, the pupil-related changes in LC activity occurred before those in inferior colliculus and SCi. This may indicate that neuronal activity changes in these other brain areas could be influenced by LC-mediated NE release. However, such contributions could also include other mechanisms, for instance, the strong interconnections between ACC, Posterior Cingulate Cortex, and LC.

In brief, pupil size seems to be a reliable variable to measure the activity of the neuromodulatory arousal system, including the fluctuations in the LC.

1.4.2 Effect of Aging on Pupil-linked Arousal Response

As described above, the level of brain arousal is associated with changes in ANS activity. Thus, it is possible to study how aging affects brain arousal by studying changes in the ANS response, especially in the pupillary response.

Regarding this, Hämmerer et al. (2017) found that older individuals have diminished pupil dilation in reaction to negative emotional images, but no differences in pupillary responses between age groups were found during the image recognition test [73]. In a different study measuring cognitive effort (by pupil dilation) and attention (by percent fixation duration), the findings revealed a main effect of aging in percent fixation duration, but no group differences concerning pupil dilation [84].

In a working memory study, in which the memory-search task comprised two different phases, the encoding and search phase, Van Gerven et al. (2004) hypothesized that young individuals should exhibit greater pupil diameter than older individuals. In the search phase, where participants had to retrieve the numbers presented in the encoding phase, the pupillary response in older adults was significantly reduced. However, the encoding phase showed no age difference for pupil dilation, which was not in line with the hypothesis [85]. Indeed, similarly, Kim et al. (2000) already had checked no age-related differences in pupillary responses induced by novel or significant stimuli [86]. Contrary to the previous findings about the search phase of the tasks [85], Porter et al. (2010) found indistinguishable pupil dilation across three target-present/absent search tasks, suggesting that the amount of effort required during the tasks is the same for both young and older adults [87]. Equivalently, Ribeiro et al. (2019) observed no age differences in the amplitude of pupil dilation during preparatory processing, target processing, response decision, and response execution of a cued reaction time task [64]. However, by studying other parameters associated with pupillary dynamics, including the amplitude of the time derivative of pupil responses and latency of the peak pupil dilation, there were age-related changes. Concerning the time derivative of the pupillary responses in response-

locked measurements, older adults revealed substantially decreased values relative to the young group. On the other hand, the analysis of peak pupil dilation latency presented a significantly reduced interval in older adults. The amplitude of this peak was also evaluated, but the authors did not find significant age-related differences. In this regard, although there were no significant differences in pupil dilation between groups, the results indicate that as people age, the dynamics of pupillary responses change [64].

The variability in results shows that the impact of aging on task-related pupillary responses is still unclear. It is widely accepted that older adults usually show reduced pupil size and a smaller range of pupil dilation. This phenomenon is commonly called senile miosis and is the result of a degeneration of the dilator muscle of the iris, which causes an age-related linear decrease in pupil size. It can influence the older adults pupillary responses to small changes in cognitive effort, which may result in underestimation of the level of cognitive effort performed by older adults in comparison to young adults [85, 88]. Taking this phenomenon into account, Piquado et al. (2010) normalized the task-evoked pupillary responses and found that the pupillometry data was different with and without normalization. Indeed, they found increased pupil size in response to larger memory load for both age groups with raw pupil data, but, when normalizing the baseline pupil size for a limited range in pupil dilation of older adults, these individuals revealed larger pupil size than young adults [88].

1.5 Effect of Aging on Perceptual Decision-Making

Cognitive changes associated with the aging process seem to lead to a reduction in the quality of the decision or the process of receiving and dealing with information in decision-making [5]. In fact, in learning tasks that require uncertainty and changes in task conditions older adults demonstrate great difficulties, which are possible due to computational deficits in uncertainty and surprise [89, 90].

To study the hypothesis that the differences in decision quality between young and older adults are caused by age-related effects, Henninger et al. (2010) assessed two different age groups through three cognitive and decision-making tasks measuring processing speed and memory [5]. The main findings suggested that aging had systematic implications on decision quality. The same authors concluded that the reduction of cognitive capacity during aging changes how older adults process information in the decision-making process, and also that the decision of older participants highly depends on the task context. According to the previous results,

Nassar et al. (2016) also demonstrated that cognitive aging causes diminished capacity to represent and use uncertainty, which in turn results in learning deficits. These insights demonstrate how learning deficits might result from an insufficient ability to appropriately estimate how much should be learned [91].

1.6 Effect of Aging on Error Processing

The capacity to monitor our performance as well as our errors, is essential in everyday life to monitor ongoing actions, correct mistakes, and sustain safe and efficient goal-directed behavior [92, 93]. Indeed, an error is more than a simple failure, it gives us information about the necessary adjustments to adjust our behavior and to mitigate these errors in the future [94].

Nonetheless, a growing amount of evidence suggests that the aging process leads to deficits regarding error awareness, and, consequently, performance monitoring [95]. Indeed, although older adults tend to exchange speed for precision to reduce increased error rates, their capacity to notice their own errors is severely decreased [92, 95].

However, the changes in the nervous system that result in these age-related impairments are still unclear. Many studies demonstrated that the increased response of the ANS is a reliable marker of conscious error monitoring. Erroneous responses result in enhanced pupil dilation [1, 96], heart rate [96], and skin-conductance response [97], more after reported than unreported errors [96]. Therefore, phasic changes in ANS activity seem to be linked to conscious error processing during decisions. Decreased pupil dilation in older people is associated with decreased error awareness [92].

Error detection and processing require a network of brain structures and circuits that combine sensory, motor, cognitive, and emotional information. The posterior medial and lateral portions of the frontal cortex as well as the insular cortex are some of the key brain structures responsible for error processing and detection [98, 99]. The ACC, a large medial prefrontal cortex structure, plays an important role in these processes as it receives and sends input from and to multiple sources, namely the LC. Thereby, it then integrates the information and produces the error-related negativity signal. The error-related negativity signal reflects the magnitude of the error and is thought to trigger the adaptive adjustments in behavior to prevent future errors [100, 101]. As a result, the medial frontal cortex could monitor the performance and when it is needed to optimize it and adjust behavior, this structure sends information to the LC, which modulates processing in cortical areas [31]. In

addition, the insular cortex also integrates somatosensory, affective, and cognitive signals related to the error [99].

Post-Error Slowing (PES) and Error Awareness (EA) are two of the most studied events from among the processes underlying performance monitoring. PES refers to the motor slowing that occurs after errors and results in significantly longer reaction times after incorrect responses than after correct responses [102]. This phenomenon has been widely studied in several different tasks, such as Stroop, Simon, Flanker, or categorization tasks [103]. Therefore, although the functional role of PES is still not fully understood, it can be considered as an indicator of performance adaptation changes following unexpected events [92]. EA is characterized as the ability to detect committed errors [104] and, contrary to PES, gives information about the level of awareness of an error. It has been proposed that there is a relationship between PES and EA, however, this interaction is still debated. On the one hand, most researchers believe that it is EA that modulates PES, reporting that PES was larger following perceived errors than following unaware errors [96, 105, 106]. On the other hand, there has also been evidence that showed a PES effect also after unnoticed errors [107]. Given this lack of clarity, more research is required to assess the association between these two processes [93].

Regarding this, Wessel et al. (2018) showed that older adults exhibit reduced EA, and a decreased phasic ANS response to errors, measured by pupil dilation: while young adults revealed the biggest pupil dilation to reported errors and an intermediate level of dilation with unreported errors, that is, demonstrated a graded pupil response based on EA, older adults had decreased pupil dilation following both reported and unreported errors, indicating a decrease in connectivity between ACC and LC, given that after a conscious error, the pupil dilates less. Furthermore, the authors also aimed to test the hypothesis that age-related changes of PES are associated with the conscious EA. Indeed, despite predicting that PES would be higher for reported errors compared to unreported errors, the results revealed a PES effect but with no differences between both reported and unreported errors [92]. In addition, although this effect was limited to reported errors, older adults showed increased PES than young adults [92]. Harty et al. (2013) also assessed age-related online EA by comparing the performance of both young and older adults in an Error Awareness Task, a variant of the go/no-go response inhibition task [95]. As expected, the results revealed that older adults exhibited considerably poorer EA than young adults, however, in terms of overall accuracy, the two groups were matched. Similarly, also using the same response inhibition task, Masina et al. (2018) found a susceptibility of EA to the aging process, that is, this event was

reduced in older adults compared to young adults [93]. Regarding PES, the same study did not observe any age-related differences [93]. Thus, EA and PES appear to be independent processes. In line with the previous results, another research assessed the go/no-go paradigm in adults aged between 20 to 72 years old to identify age-related differences in error processing and error detection [104]. As hypothesized, higher age was accompanied by a greater amount of undetected errors. Further, Niessen et al. (2017) also observed a slower reaction time for older adults [104]. Moreover, by instructing young and older participants to perform a task in which participants had to find experimenter-introduced errors and anomalies, James et al. (2011) concluded that whether the scenes were more complex, with more errors, whether they were simpler, with fewer errors, older adults noticed fewer errors than young adults [108].

In brief, taken together the findings suggest that the ability to process and detect errors and adjust behavior is decreased in the older population. This may result in difficulty in learning new tasks and in developing progressive problems in everyday tasks [92, 95, 109].

1.7 The Importance of Response Certainty

Everyday life constantly presents us with decisions that must be made between two or more options. Such decisions seem to involve a sequential process in which evidence is steadily accumulated and stored over time until a threshold level, where a final choice is reached [110, 111]. The subjective belief that a decision is correct is referred to as decision certainty, whereas the complementary possibility of a failed result is indicated by the decision uncertainty [112]. Indeed, certainty helps to make informed and reasonable decisions in complex contexts, particularly when the subsequent decisions depend on predicted outcomes. Furthermore, it provides a basis for learning from mistakes that have been made [112, 113].

Asking the subjects to rate their degree of confidence when they are performing a decision-making task has become a standard procedure to assess decision uncertainty [114, 115]. The strength of the stimulus, which refers to the stimulus discriminability, has been connected with decision certainty, and by changing this property, by switching the presentation time or the visibility of the stimulus for instance, it is possible to modulate task difficulty. Another example of changing stimulus strength is by modifying the fraction of dots traveling in the same direction in a Random Dot Motion (RDM) task [1]. Several models of confidence are correlated with evidence strength and rely on signal detection theory. According to this idea, a correct

decision may be positively related to stimulus strength while in a wrong decision, oppositely, this correlation might be negative [116, 117]. Figure 1.2 explains this interaction.

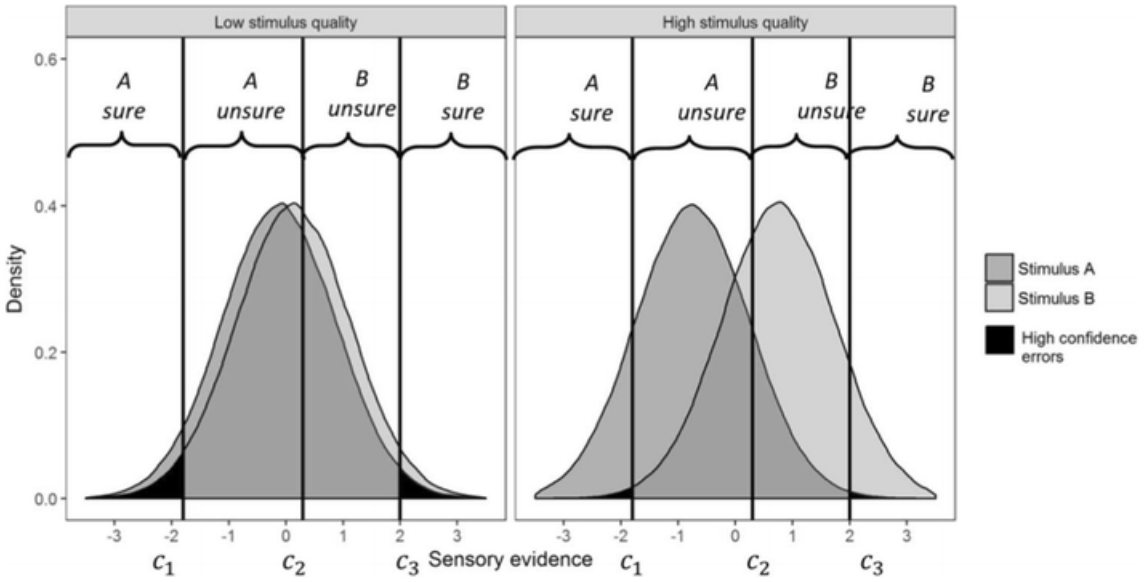

Figure 1.2: Distribution of evidence as a function of stimuli differences: low stimulus quality in the left panel and high stimulus quality in the right panel. Reprinted with permission from [117].

To make a decision with certainty, the amount of evidence must be greater than a threshold level which distinguishes between decisions with and without confidence. As shown in the left section of Figure 1.2, when two stimuli are very similar, making them virtually impossible to distinguish, the distributions of evidence almost completely overlap. Contrariwise, when the discriminability between two stimuli is better, these distributions become further separated from each other, as illustrated in the right section of Figure 1.2. As this occurs, the distance between non-overlapping regions of the evidence distribution grows, resulting in a higher possibility of the conclusion being accurate. The greater this distance, the less the proportion of the distributions that surpass the confidence criteria for incorrect decisions (black regions of Figure 1.2 located at the other side of the distributions), implying that confidence in wrong decisions will be reduced [117]. However, the models derived from signal detection theory were inconsistent for both positive and negative relationships between stimulus strength and confidence in wrong decisions [117]. Rausch et al. (2018) proposed a novel model in which confidence is not only based on evidence but also on the visibility of the stimulus, that is, the estimate of the physical quality of the stimulus. According to this model, called weighted-evidence-and-visibility, both evidence and visibility of the stimulus are weighted and

integrated into one judgment variable to determine the degree of confidence. In a general way, although the weights between evidence and visibility depend on the task and the experiment, the weighted-evidence-and-visibility model seems to be consistent with both positive and negative correlation patterns [117].

Nevertheless, both models previously described cannot explain systematic differences in decision time by themselves. Poor evidence and increased error rates are frequently linked to longer decision times. As a result, with training, the brain should learn how to use decision time as an index of stimulus strength and certainty assessment [112]. Regarding this, Kiani et al. (2014) validated their hypothesis that subjective certainty, or confidence, is affected by both decision time and the amount of evidence leading to a choice [112]. The most important finding was the inverse relationship between certainty and reaction time, even for incorrect responses, which contradicts the conventional signal detection theory-based hypothesis of certainty. In brief, the findings suggest that elapsed decision time is a proxy for task difficulty, and it is correlated with confidence level.

It is obvious that decision-making and, consequently, choice behavior, are shaped by uncertainty [118, 119]. However, the methods through which choice uncertainty is converted into subsequent adjustments are still unknown [116]. One prominent hypothesis is that the brain uses the arousal system to transmit uncertainty to the neural circuits, which, in turn, recruit neurotransmitters and modify the global state of the brain [119, 120]. Numerous steps of the decision-making process, such as stimulus encoding [121], evidence accumulation [71, 122], the threshold level of evidence archived, and the time needed to process the information and execute the response, may be strongly linked with arousal and neuromodulation [123]. The neuromodulators responsible for implementing uncertainty in the brain are acetylcholine and NE. NE is closely linked to unexpected uncertainty, whereas acetylcholine is involved in expected uncertainty [120].

In addition to the stimulus-related properties described above, decision-making under uncertainty also depends on many other limitations and potential biases that depend on each subject, such as the possibility of distraction and the difficulty of sustaining attention for a certain period of time [124].

1.7.1 Pupil Dilation as an Index of Decision Uncertainty

Uncertainty is an inherent property of most decision-making processes and it is crucial to understand how people deal with uncertainty to improve decision-making outcomes. Several eye-tracking measures have emerged as an important tool to quantify evidence accumulation during decision-making [125].

For instance, eye fixations, which are very short pauses of eye movements within a particular location, are thought to provide insights into the areas of the visual scene that are focused and brought to foveal vision. Indeed, the number of fixations increases with challenging decisions [126, 127].

Also saccades have been suggested to be linked to uncertainty. Saccades are ballistic eye movements between successive fixations that allow changing the information reaching the fovea [128]. In the visual search context, the peak velocity of saccades is a reliable proxy of difficulty-evoked arousal. Furthermore, saccadic activity provides information about the methods that are employed to weigh different choices and make decisions [129].

A third possible approach as a metric of decision-making, uncertainty, and task difficulty, is the number of eye blinks. Most eye blinks are suppressed during decisions, and those that do occur are usually short [130].

Finally, much converging literature indicates that pupil diameter also can be used as a highly sensitive proxy of arousal state, and should indirectly reflect changes in the activity of the noradrenergic system and their impact on brain processing [35, 131]. Many authors believe that uncertainty may also modulate activity in the arousal systems [35, 119]. Higher pupil dilation has been linked to uncertainty conditions [125, 132]. Regarding this, Brunyé & Gardony (2017) designed a decision-making task in which participants should indicate whether the image was a face or a house by directing their gaze and, after that, their certainty on response [125]. They examined pupil diameter during the task under different levels of uncertainty. Figure 1.3 shows the mean changes in pupil diameter as a function of three levels of certainty. During the 3 seconds of stimulus viewing, in high certainty trials, pupil diameter remained roughly constant from 0.5s until deciding. Concerning medium certainty trials, pupil dilation tends to increase approximately one second after stimulus onset. Lastly, for low certainty conditions, the pupil shows a significant dilation but only at around two seconds following stimulus onset. These increases in medium and low confidence trials could reflect the activity of the arousal system when preparing the decision and may reflect the cognitive effort employed in each condition. In other words, under difficult conditions, and, therefore, increased uncertainty, stimulus processing takes longer [125].

Urai et al. (2017) developed a study to determine, in a perceptual decision task, whether arousal indicates decision uncertainty and predicts changes in subsequent choice behavior [116]. The research revealed that there is a correlation between decision uncertainty and phasic arousal and that the dynamics of serial choice biases are shaped by this pupil-linked arousal response. The authors resorted to a statistical

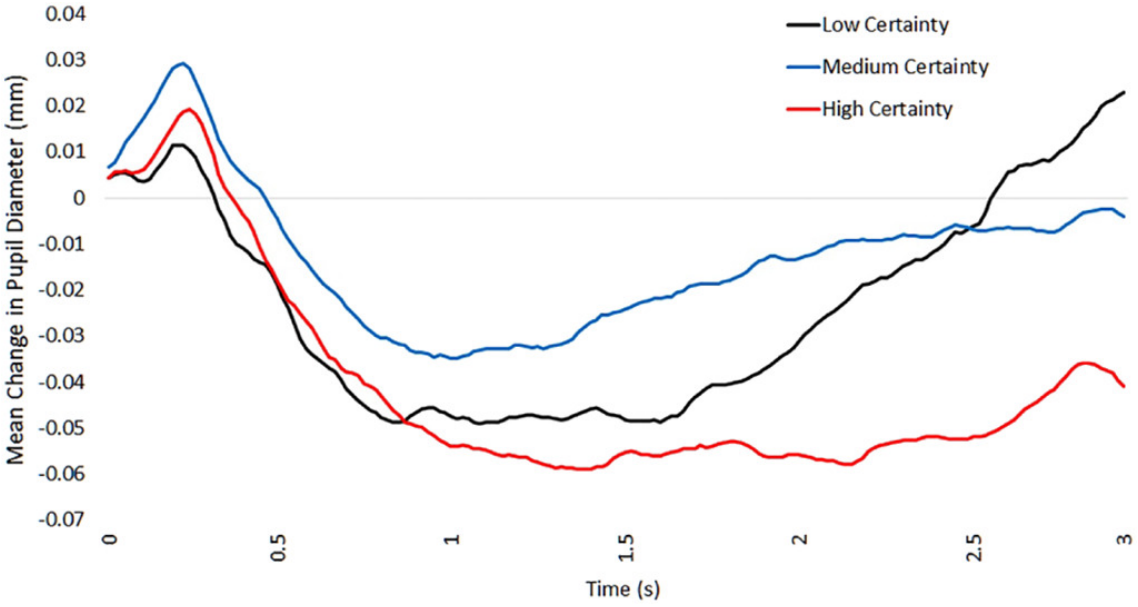

Figure 1.3: Mean change in pupil diameter (in millimeters) over time while viewing stimulus images, as a function of three certainty levels (low, medium, high). Reprinted with permission from [125].

model that predicts three signatures for decision uncertainty [116, 133]. The first one forecasts that uncertainty increases with evidence strength for wrong decisions but decreases with evidence strength for correct choices. The second signature proposes that there is a monotonic decline in response accuracy from 100% to 50% with increasing uncertainty. At last, the third signature predicts that higher uncertainty is correlated with less accuracy, even with the same evidence strength conditions. Similar to previous work [133], all three signatures of decision uncertainty were evident in changes in reaction time. Regarding pupil size, it grew throughout decision formation, reaching its maximum size soon after the answer and, then increased once again after receiving feedback, which also was in line with another study [71]. Between these two peak moments, pupil diameter also exhibited the three signatures of decision uncertainty.

In a more recent study, Colizoli et al. (2018) replicated the scaling of pupillary response with decision uncertainty found by Urai et al. (2017) and analyzed whether the same relationship was observed in the period after feedback [1]. During the pre-feedback period, they generated predictions for decision uncertainty, and, similarly, during the post-feedback interval, for the complement of the prediction error, which was defined as the difference between the predicted and actual reward-linked feedback. Both predictions are, respectively, represented in Figure 1.4.

The results showed that pupil responses during both periods were larger on error trials than on correct trials, and also it was evident an interaction between accuracy

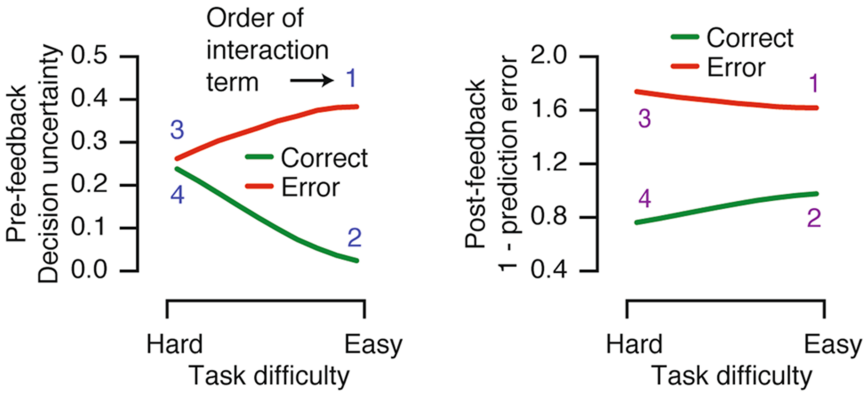

Figure 1.4: a) Decision uncertainty (complement of confidence) as a function of task difficulty during the pre-feedback interval; b) Prediction error as a function of task difficulty during the post-feedback interval. Reprinted with permission from [1].

and decision uncertainty (Figure 1.5a and 1.5b) [1]. Previous studies had already reported that feedback evoked pupillary responses are higher after negative than positive feedback [116].

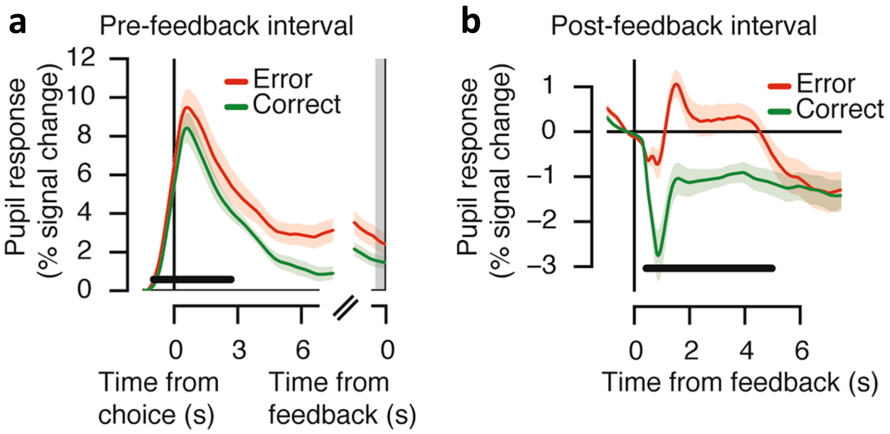

Figure 1.5: Evoked pupil responses for Correct and Error trials in the a) pre-feedback interval; b) post-feedback interval. Black bar in both figures a) and b) represents the Correct vs. Error effect. Reprinted with permission from [1].

1.8 Effect of Physical Activity on Cognition in Older Adults

An increasing amount of research has investigated the relationship between physical exercise and cognition to understand the impact of physical activity not only on physical health but also on cognitive health [134]. In fact, regarding older adults,

there are more than 1000 clinical trials, 174 systematic reviews, and 50 meta-analyses investigating the effect of physical exercise on cognitive performance [135]. Exercise intensity relative to Maximum Heart Rate (HRmax) can range from light to very hard [136]. Exercise modes can also change (resistance training, aerobic training, combination of both, and others). The duration of each exercise session and the number of sessions (regular or single-bout) can further be different. Thus, the characteristics of physical activity and the methodology used might differ substantially, yet results seem to be consistently positive [134, 135] and allow us to infer that physical activity is one of the most effective ways to influence cognitive performance favorably throughout the lifespan and lower the risk of age-related cognitive decline [137].

Physical activity is characterized by any skeletal muscle-driven movement of the body that requires energy expenditure to complete [138]. In turn, exercise is a subset of physical activity that is planned, structured, and repetitive and aims to improve or maintain physical fitness, physical performance, or health [138, 139]. According to these definitions, although most studies in this field have largely used the term *'physical exercise'*, it is important to dissociate investigations assessing *'exercise'* or *'physical exercise'*, which involves a planned and repetitive chronic physical activity engagement, from the studies assessing *'physical activity'* sessions, characterized by activities which require energy expenditure and heart rate increasing beyond resting levels [135].

In a general way, research has shown that regular physical exercise can be efficient for enhancing cognitive functions, including spatial and working memory [137], executive attention [140, 141], information processing [142], visual-spatial processing [143], and visual recognition [144], of older adults with and without cognitive impairment [145]. Furthermore, greater levels of physical activity are also linked to a lower risk of dementia [146]. Indeed, the 2018 Physical Activity Guidelines Advisory Committee in its Scientific Report emphasized that physical activity is essential for maintaining optimal levels of brain health [139]. Erickson et al. (2019), by reviewing the 2018 Physical Activity Guidelines, reported a moderate effect of long-term moderate-to-vigorous physical activity on cognition in adults aged over 50 years [137]. Another review also reported that aerobic and multimodal combined exercises led to greater benefits when compared to resistance training [147].

It has also been noteworthy the benefit of a single bout of physical activity on several perceptual and cognitive abilities. Although some studies reported better cognitive performance with very light-, light-, and moderate-intensity physical activity and no benefits for hard-, very hard-, and maximal-intensity [148], the prominent

supposition among the literature focuses on an inverted-U perspective, with greater cognitive enhancements under moderate intensities, and diminished effects under light and vigorous intensities [149, 150]. Closely linked to the intensity is the duration of the physical activity single session. Currently, most literature has used physical activity durations between 16 to 35 minutes, and indeed there should be a minimum and maximum duration for the activation of the mechanisms underlying these single bouts of exercise which induces better cognitive performance [134, 151]. Aiming to explore this best physical activity duration to improve cognitive performance, Chang and colleagues developed a study in which participants performed one of three exercise treatments, by cycling at moderate intensity for 10, 20, or 45 minutes, before doing a Stroop test [151]. The findings suggested that cycling at 65% of HR reserve for 20 minutes resulted in significantly better cognitive performance on the Stroop task than 10 and 45 min of activity at similar intensities.

Another key question that remains in the literature is the type of single-bout physical activity that induces better cognitive performance [134]. At present, the majority of studies have only looked at aerobic exercise such as walking, cycling, or running [6], and, regarding this, a meta-analysis performed by Lambourne & Tomporowski (2010) reported that cycling-based physical activity modalities had greater cognitive benefits than running-based physical activity modalities [149]. However, emerging literature also highlighted the benefits of other modalities, including resistance exercise [152, 153], dance [154], and mind-body activities [155], to improve cognitive outcomes in elderly adults. Furthermore, a growing body of research also emphasizes the advantages of multimodal exercise interventions, which combine physical activity from different components, for enhancing global cognition of older individuals [156, 157]. Overall, meta-analyses have not yet strongly suggested the most effective exercise for enhancing cognitive performance [6]. Nevertheless, the underlying characteristics of the single physical activity bout, such as the intensity and the duration, may be more important than the activity modality by itself [134].

1.8.1 Brain Mechanisms Changes Underlying the Effect of Physical Activity on Cognition

Overall, both chronic and acute exercise, defined, respectively, as a repeated amount of workout sessions over a short- or long-term period and as a single bout of exercise [158], are thought to be accompanied by improvements in cerebral blood flow [159], in the levels of brain-derived neurotrophic factor [160], and in cerebral

functions linked to cognitive performance [161]. Actually, according to the cerebral circulation hypothesis, regular exercise improves the flow of oxygen and glucose to the brain, which boosts cognitive function by increasing the amount of resources available to the cerebral environment. Aimed to test this hypothesis, Bliss et al. (2021) analyzed several studies and concluded that aerobic exercise training may enhance cerebrovascular function and that there are connections between cerebrovascular function, aerobic fitness, and cognitive performance [162]. As an example, in a study that assessed changes in cerebral blood flow and cognition in both physically trained (3 sessions of 1h per week during 12 weeks) and control older adults, the results showed increased levels of resting cerebral blood flow in the ACC, which is involved in executive function and autonomic cardiovascular control [163]. However, the same review article demonstrated that, regarding resistance training, the findings are not so obvious, possibly due to the lack of research on the effects of this training type on cerebrovascular function and cognition [162].

Furthermore, there is evidence that physical activity also increments brain-derived neurotrophic factor levels [164], which has a positive impact on cognitive performance through its capacity to promote various processes such as neurogenesis, synaptic plasticity, and long-term potentiation, all of them in the heart of learning [165]. In fact, according to a review, a single session of moderate aerobic exercise elevates brain-derived neurotrophic factor levels, and chronic aerobic exercise training boosts this increase [166].

Another factor that was reported above to contribute to age-related cognitive deficits is the degeneration of neurotransmitter systems, particularly the noradrenergic and the dopaminergic systems [167]. Regarding this, there is unequivocal evidence that during and immediately after physical exercise the release of catecholamines, such as NE and dopamine, is increased [168]. Indeed, exercise results in higher peripheral and central catecholamine concentrations due to the stress it causes (good stress) as well as the fact that we would be unable to maintain our work rate without it. This catecholamine increase is directly linked to exercise intensity increase, that is, when exercise reaches a moderate level, plasma catecholamines significantly rise, which may contribute to cognitive abilities improvement [169].

Although investigations in this field of study are few and have been hampered by technological issues [161], an animal study demonstrated that exercise has a positive impact on the activity, release, and metabolism of the dopaminergic, noradrenergic, and serotonergic systems in the brain [170]. Concerning humans, Dalsgaard et al. (2004) explored NE concentrations in cerebrospinal fluid following exercise to exhaustion and the results proved to be in line with the previously described, which

provides some evidence that acute exercise causes an increase in NE concentrations in human brains [171]. Nevertheless, a few years before, Wang et al. (2000), using Positron Emission Tomography (PET) scans, examined how dopamine release in the human brain is affected by treadmill running at a high intensity (>85% HRmax) and the findings did not show significant effects [172].

Recently, Ayala & Heath (2021) employed a study that aimed to understand whether a 20-min single bout of aerobic activity via a cycle ergometer at 80% of the participants HRmax influences pre- and immediate post-exercise cognitive function and LC-NE activity, estimated through pupil dilation [173]. The results showed that task-evoked pupil dilations and tonic baseline pupil size respectively increased and decreased from before to after exercise, suggesting that the modulation of the LC-NE system might be a mechanism behind the exercise-induced improvement in cognition. Mather et al. (2020), by developing an fMRI study using an isometric handgrip task just before an oddball task, also found that the handgrip induced increased pupil-linked arousal, with decreased tonic pupil size, strong phasic pupil responses, and increased frontoparietal network activation, which induced benefits in attention performance in the few minutes following the handgrip task [174]. Furthermore, although older women had lower frontoparietal network activation overall, the benefit of handgrip on frontoparietal network activation was similar for both older and young women, which supports the idea that, at least for the following minutes afterward, even simple exercises may improve selective attention in healthy aging [174].

Collectively, the findings allow accumulating evidence that physical activity, even if simple and of short duration, preserves brain health and leads to improved cognitive performance in several domains, and that one of the proposed mechanisms for this improvement is the activation of the arousal system during exercise [161, 164, 174].

1.9 Project Aims

The present study had two aims. The first one was to test the effect of aging on the recruitment of the arousal system during decision-making, and the second was to test whether an exercise session changes the way the arousal system is recruited during decision-making in older adults.

A growing body of evidence suggests that the activity of the arousal system increases with increasing uncertainty in a perceptual decision. Indeed, several previous studies reported that, in young adults, uncertainty is responsible for rapid

changes in pupil-linked arousal state, that is, the greater the uncertainty at the response onset, the greater the activation of the arousal system [1, 116]. Nevertheless, regarding older adults, this relationship is not so evident [5]. Therefore, the first aim of the current project is to evaluate the hypothesis that uncertainty processing, and the associated arousal system activation, is different in young and older adults.

However, uncertainty and error awareness during a decision-making process are tightly related. For example, if a subject believes their answer is correct, then he should be sure of their response, but if he thinks that it is incorrect then he may be unsure of their response. This uncertainty is important to drive the current decision as well as to guide future decisions and to modulate changes in pupil-linked arousal [116]. So, we also aimed to understand the relationship between response certainty and feedback processing.

To assess these questions, we adapted a two-alternative choice motion discrimination task with various levels of difficulty, which participants of two different age groups (young adults and older adults) performed while their pupil responses were measured as a proxy for arousal system activation. The presence of several difficulty levels was essential to understand the effect of the modulation of the response confidence in pupil-linked arousal responses and task performance.

It has long been known that physical activity enhances cognitive function. Indeed, the benefit of a single bout of physical activity on several perceptual and cognitive abilities is remarkable [6], and exercise-induced increases in pupil-linked arousal have been linked to these improvements in cognition [173]. Could exercise improve the performance of older people by enhancing the response of the arousal system during decision-making? Could exercise improve the response of the arousal system to error and low-confidence trials? The second goal of the present study is to examine whether a 30-minute bout of moderate aerobic exercise via a cycle ergometer at 70% of participants VO_2max modulates visual perception, uncertainty processing, and activation of the arousal system in older adults. If physical activity effectively improves performance and arousal system activation, it could be a feasible method to delay age-related cognitive decline and consequently the development of neurodegenerative diseases such as Alzheimer's disease.

2

Methods

2.1 Study Design

The study proposal was approved by the Ethics Committee of the Faculty of Medicine of the University of Coimbra (CE-162/2022). After explaining verbally and in writing the purpose and possible consequences of the experimental design, all participants signed the written informed consent. All measurements were performed in the Research Unit for Sport and Physical Activity (CIDAF) of the Faculty of Sports Science and Physical Education and in a laboratory of Coimbra Institute for Biomedical Imaging and Translational Research (CIBIT), both belonging to the University of Coimbra. The period of acquisition lasted from December 2022 until May 2023.

The sample comprised two distinct groups, one older and one younger. The group of older participants completed three laboratory visits that took place at the same time of the day for each participant. Visit a) aimed to perform a physical assessment in the laboratory of the Faculty of Sports Science and Physical Education of the University of Coimbra, in which it was measured anthropometry, body composition, upper limb strength, flexibility, and the VO_2max. Visit b) intended to evaluate the effect of physical activity on a perceptual decision-making task and the associated activation of pupil-linked arousal. For this purpose, individuals conducted a visual perception task with concurrent acquisition of the pupillogram and eye movements recording using an eye tracker device before and after thirty minutes of aerobic moderate-intensity exercise via a cycle ergometer at 70% of participants predicted VO_2max. To assess changes in alpha-amylase, a marker of activation of the noradrenergic system, saliva was collected three times: at baseline, after physical activity, and after the second moment of the visual perception task. To make sure that the outcomes of the physical evaluation were valid, the visit to evaluate the effect of physical activity was carried out a maximum of 120 hours, that is, five days, after the physical assessment. Lastly, the visit c) was similar to the previous one, however, instead of performing physical activity, participants completed a set

of mental activity tasks for thirty minutes between the two sessions of the task. The mental activity session was included as a sham intervention to control for test-retest effects. The physical activity and mental activity sessions occurred with at least two months interval. It is important to note that the order of the visits was counterbalanced across participants, that is, half of the participants completed first the set of both physical assessment and physical activity sessions and, after two months, performed the mental activity session, whereas the other half started with the mental activity session (see Figure 2.1).

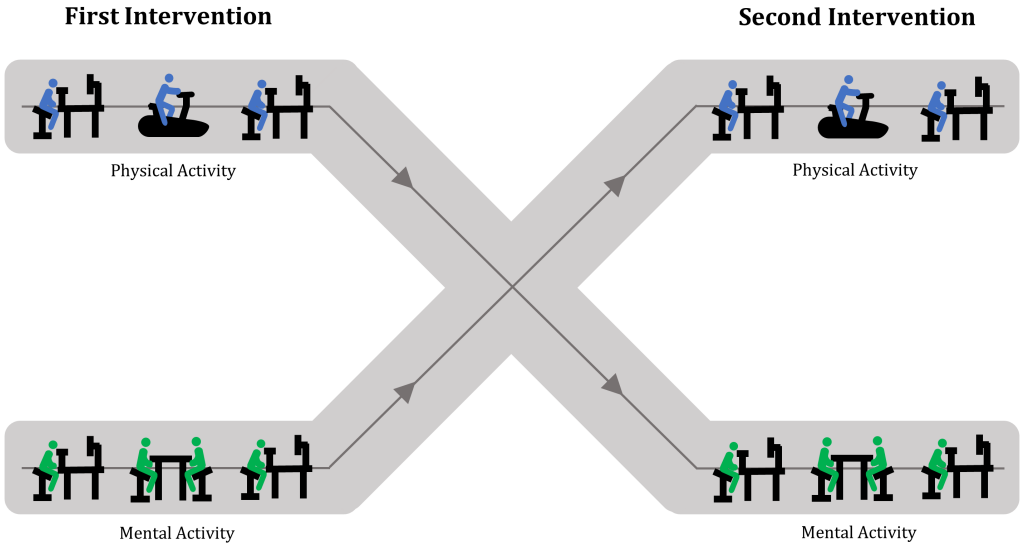

Figure 2.1: Schematic representation of the counterbalanced study design within participants. Adapted from [175].

The group of younger adults was included to compare the performance of the older group at baseline and therefore only conducted one visit, where the young participants performed the visual perception task to assess visual decision-making ability with concurrent measurement of eye tracking and pupillography data.

For both groups, a sociodemographic and clinical questionnaire was applied, in which demographic and clinical information, as well as consumption habits of tobacco, coffee, alcohol, and/or other types of psychotropic substances, were recorded. Furthermore, the short form of the International Physical Activity Questionnaire (sf-IPAQ) (Portuguese version), a handedness questionnaire, and a daily activity and consumption questionnaire for both mental and activity sessions were also used.

2.2 Participants

As described above, the sample consisted of two groups: twenty healthy young adults aged 20 to 30 years (23.7 ± 2.8, 50% women) and twenty healthy older adults aged 50 to 67 years (58.0 ± 6,3, 50% women). All participants of both groups had normal or corrected-to-normal vision, and no current or previous history of vascular, neurological, or psychiatric disease. Current medication of psychoactive substances, such as anxiolytics, antidepressants, antipsychotics, or beta-blockers, chemotherapy history, having suffered head trauma, and being pregnant (for women) were considered exclusion criteria. For inclusion, participants were requested not to ingest alcohol the night before, not to visit the dentist the twenty-four hours before, and not to eat and brush their teeth for at least 60 minutes before the session, which was important for the analyses of the saliva samples.

Due to health reasons that appeared between the two visits, two older participants did not perform the second visit (P23 and P31), one of them corresponded to the physical activity session, and the other to the mental activity session.

Initially, all comparisons between young and older adults were performed with all evaluated participants (20 young and 20 older adults). Nevertheless, after analyzing the descriptive statistics for accuracy and percentage of unsure responses (Tables 2.1 and 2.2), it was concluded that participants 24, 31, 34, and 37 were outliers. According to Table 2.1, three of the participants (P24, P34, and P37) had very low accuracies and seemed to be responding at random for every stimulus strength. Linking Table 2.1 and Table 2.2, it was noticed that participant 31 had never answered with low confidence, however, his accuracy was not high enough to justify this fact, suggesting that this person was not following task instructions. Therefore, these four older participants were excluded from all analyses. The sample, whose characteristics are described in Table 2.3, now includes 20 young participants and 16 older participants.

Regarding the comparison between physical activity and mental activity sessions, for the same reasons, the same participants were considered outliers and removed from the analyses. Participant 23 was likewise eliminated from this second analysis since, as was already mentioned, he did not perform the two sessions of the study, resulting in a sample of 15 participants.

Table 2.1: Average accuracy for each run for each participant.

Accuracy (%)									
Young Group					Older Group				
Participant	Run 1	Run 2	Run 3	Run 4	Participant	Run 1	Run 2	Run 3	Run 4
P01	81.25	89.58	93.75	85.42	P21	81.25	81.25	93.75	91.67
P02	72.92	68.75	79.17	85.42	P22	91.67	95.83	91.67	95.83
P03	73.33	72.92	89.58	91.67	P23	82.98	87.50	91.67	93.75
P04	85.11	85.42	91.67	79.17	P24	42.55	47.92	54.17	35.42
P05	89.36	87.50	85.42	83.33	P25	75.00	85.42	81.25	79.17
P06	83.33	79.17	85.42	93.75	P26	83.33	95.83	93.75	97.92
P07	77.78	80.85	91.67	91.67	P27	81.25	91.67	87.50	93.75
P08	89.58	89.58	83.33	83.33	P28	74.47	66.67	68.75	64.58
P09	83.33	89.58	95.83	91.67	P29	68.09	87.50	85.42	93.75
P10	64.58	79.17	85.42	87.50	P30	89.58	89.58	93.75	93.75
P11	93.75	87.23	87.50	89.58	P31	70.83	56.25	58.33	89.58
P12	91.67	87.50	97.92	95.83	P32	65.12	76.60	89.58	79.17
P13	72.92	91.67	89.58	89.58	P33	37.78	66.67	81.25	79.17
P14	89.13	89.58	87.50	95.83	P34	47.92	42.55	44.68	39.58
P15	77.08	76.60	95.56	80.85	P35	75.00	70.83	93.75	89.13
P16	88.89	85.42	89.36	93.75	P37	46.51	32.56	40.91	45.45
P17	83.33	85.11	87.50	89.58	P38	67.39	64.58	58.33	77.08
P18	83.33	74.47	91.67	91.67	P39	68.75	80.85	78.72	93.75
P19	93.75	97.92	95.83	95.83	P40	72.92	85.11	79.17	72.92
P20	86.96	89.58	97.92	93.75	P41	74.42	89.58	89.58	83.33

2.3 Physical Assessment

The physical assessment was subdivided into two phases. First, to characterize the physical profile of the participants, the following measurements were performed: anthropometry, body composition, upper limb strength, and flexibility. Thereafter, participants made a submaximal effort protocol on a cycle ergometer to predict the VO_2max, and, consecutively, the intensity (watts) corresponding to 70% of the predict VO_2max. This value was then used to ensure that all participants were able to complete the 30-minute bout of aerobic exercise at the specific moderate intensity.

2.3.1 Anthropometry and Body Composition

All anthropometric measures were performed by a single observer following standardized protocols [176]. Stature was measured using a portable stadiometer (*Harpenden stadiometer*, model 98.603, Holtain LTD, Crosswell, UK) to the nearest 0.1 cm. For measurement, participants were positioned straight with their back to the height rule and their arms extended laterally to the trunk and looked straight ahead

Table 2.2: Average percentage of unsure responses for each run for each participant.

Percentage of Unsure (%)									
Young Group					Older Group				
Participant	Run 1	Run 2	Run 3	Run 4	Participant	Run 1	Run 2	Run 3	Run 4
P01	56.25	56.25	58.33	52.08	P21	18.75	35.42	20.83	8.33
P02	54.17	56.25	66.67	60.42	P22	4.17	0.00	0.00	0.00
P03	68.89	64.58	60.42	52.08	P23	29.79	37.50	37.50	45.83
P04	27.66	27.08	37.50	39.58	P24	80.85	72.92	89.58	97.92
P05	12.77	22.92	25.00	10.42	P25	75.00	45.83	47.92	47.92
P06	31.25	29.17	47.92	35.42	P26	16.67	41.67	31.25	27.08
P07	37.78	51.06	35.42	27.08	P27	20.83	27.08	25.00	18.75
P08	12.50	14.58	20.83	18.75	P28	25.53	29.17	39.58	35.42
P09	33.33	16.67	22.92	18.75	P29	2.13	0.00	22.92	27.08
P10	54.17	54.17	54.17	70.83	P30	8.33	10.42	10.42	4.17
P11	18.75	27.66	27.08	27.08	P31	0.00	0.00	0.00	0.00
P12	16.67	16.67	10.42	4.17	P32	90.70	82.98	83.33	77.08
P13	60.42	62.50	45.83	50.00	P33	46.67	54.17	35.42	22.92
P14	15.22	10.42	10.42	10.42	P34	68.75	82.98	91.49	87.50
P15	2.08	21.28	6.67	10.64	P35	10.42	16.67	4.17	2.17
P16	20.00	22.92	25.53	16.67	P37	2.33	79.07	100.00	97.73
P17	52.08	42.55	54.17	29.17	P38	10.87	0.00	0.00	0.00
P18	31.25	38.30	10.42	10.42	P39	2.08	0.00	2.13	6.25
P19	41.67	27.08	4.17	0.00	P40	50.00	48.94	47.92	39.58
P20	26.09	18.75	4.17	14.58	P41	60.47	20.83	29.17	41.67

so that the nose tip was parallel with the earlobe, ensuring the orthogonality of the Frankfurt reference line. Body mass measurement was obtained with a calibrated portable scale (*SECA balance*, model 770, Hanover, MD, USA) with a precision of 0.1 kg. Both measures were made barefoot.

Table 2.3: Participants' characteristics.

	N	Age (y)			Sex		Education (y)			Dominant Eye		Smokers
		Mean	Min	Max	F	M	Mean	Min	Max	Left	Right	
Young	20	24	20	28	10	10	16	14	21	13	7	2
Older	16	58	50	67	8	8	16	12	19	7	9	5

Body composition was evaluated with a bioimpedance multifrequency method composed of six different frequencies (1, 5, 50, 250, 500, and 1000 kHz) with an *InBody770 scanner* (In-body Bldg, Seoul, Korea), a quadrupole equipment that uses eight electrodes and ensures high accuracy. Each participant removed their shoes, coats, sweaters, and metal objects, and, following the manufacturers guidelines, they stood in the anthropometric reference position for 60 seconds. The equipment re-

port provides a significant amount of data. Nevertheless, in the study, only skeletal muscle mass (kg), fat mass (kg and %), and body max index (kg/m^2) were considered.

The results of the physical assessment were also only analyzed for the participants included in the analysis of the effect of physical and mental activities on task performance and pupillary responses in older adults.

2.3.2 Upper limb strength

Upper limb isometric strength was assessed using a manual dynamometer (*Hand Dynamometer*, Lafayette, model 78010, USA). The equipment was adapted to the hand of each participant so that the second finger joint was snugly fitted under the handle and supported the weight of the tool. Participants performed the protocol standing, with their arms stretched laterally toward the trunk and spaced approximately 45 degrees. Without holding their breath, they held the instrument and squeezed it as hard as possible. Maximal handgrip strength, expressed in kilograms (kg·f) with one decimal place, was evaluated bilaterally, using two attempts for each hand, and the highest value of each one was considered. Before each measurement, the dynamometer was set to zero.

2.3.3 Flexibility

Flexibility is characterized by the capacity to move a joint freely and through its full range of motion. Particularly, hamstring flexibility is very important to carry out daily life activities [8]. However, as a result of the normal aging process, the connective tissues, including muscles, are affected, and, consequently, joint flexibility is severely reduced [177]. So, flexibility measurement is essential for the physical characterization of the group.

Individuals performed the sit-and-reach protocol, which has often been suggested to be a good marker to evaluate the lower back and, mainly, the hamstring flexibility. We assessed the flexibility of both legs as well as of each leg separately. At the beginning of the protocol, participants were sitting on the floor without shoes, and with the soles of their feet against a sit-and-reach box positioned with the zero mark at 22.5 cm. They were instructed to slowly reach as far as possible with both hands parallel, and the fingertips overlapped and in contact with the box, keeping that position for about two seconds. Ensuring that both legs were outstretched, the farthest point reached with the fingertips was measured.

Similarly, the flexibility of each leg was assessed with only one leg fully out-

stretched, and the other bent with a 90^{o} knee flexion. Participants kept the leg that was being measured, their arms, and fingers completely extended while flexing their hip joints and trunk to reach forward as far as they could.

2.3.4 Cardiorespiratory Assessment

According to The Sports Medicine Resource Manual [178], the ability of the circulatory and respiratory systems to provide oxygen to the skeletal muscle mitochondria for energy synthesis during physical activity is known as cardiorespiratory fitness. It is considered a predictor of various health indicators including mental health [179, 180] and increased values of this component are associated with many daily life health benefits and with lower risk of mortality [181].

Maximal Oxygen Uptake (VO_2max) ($mL{\cdot}kg^{-1}{\cdot}min^{-1}$) is a reliable measure of cardiorespiratory fitness [8]. Several maximal and submaximal exercise protocols can be used to assess VO_2max, and the decision to use one over the other is mostly determined by the reason for testing and the risk level for the subject. Indeed, maximal protocols demand that participants exercise until the point of exhaustion, which may not be acceptable for everyone. Furthermore, if the chosen protocol is excessively aggressive for the individual there is a propensity to overestimate VO_2max [8]. As such, taking into account the study population, a submaximal effort protocol on a cycle ergometer was chosen (*Excalibur Sport*, Lode, Groningen, Netherlands). The main goal of submaximal exercise protocols was to predict the VO_2max using the Omnia software (*Omnia Software*, version 1.6.5, COSMED, Rome, Italy) from the Heart Rate response, assuming the linear relationship between these two physiological parameters.

Before starting the protocol, participants were positioned correctly on the ergometer, that is, with the hands properly placed on the handlebars and with approximately a 25-degree bend at the knee at full leg extension. During the protocol, individuals breathed through a face mask, and Oxygen (O_2) and Carbon Dioxide (CO_2) measurements were recorded continuously breath-by-breath. The submaximal protocol began with a load of 75 watts, followed by increments of 25 watts at each 3-min stage until the participant reached 90% of their estimated HRmax, calculated by $HRmax = 208 - 0.7 \times age$ [182]. Participants pedaled at a constant cadence between 60 and 70 rpm. At the end of each stage, they were instructed to identify their perceived effort using the *Borg Cr10* scale [183] that was fixed in front of the cycle ergometer. The highest value, on a scale of 0 to 10, which was obtained in the last level of the protocol, was considered the subjective rating of perceived exertion.

Once the protocol was finished, the VO_2max was predicted. For the 30-minute bout of aerobic exercise intervention, we used the intensity (watts) at which 70% of the VO_2max was reached.

2.4 Perceptual Decision-Making Task

To investigate the modulation of arousal, measured through pupil size, during decision-making under various levels of uncertainty, we designed a two-alternative choice motion discrimination task with the Psychophysics Toolbox, version 3 [184], in *Matlab R2022b* (The MathWorks Company Ltd). The task was adapted from Gomes (2020) [124], originally developed by Colizoli et al. (2018) [1] in another study, whose code can be found here: `https://github.com/colizoli/pupil_belief_states`.

The task was shown on the monitor of a computer screen (19-inch Dell monitor) with a spatial resolution of 1440 x 1080 pixels and a refresh rate of 100 Hz. The monitor had 52.5 cm of width and 39.5cm of height. Dot motion stimuli were displayed within a central annulus with an outside diameter of 16.8° and an inner diameter of $2{,}4^{\circ}$ with borders that were not visible to the participants. The fixation target located in the center of the annulus, and consequently, in the center of the monitor, was a white combination of bulls eye and cross-hair shape, as recommended by Thaler et al. (2013) for experiments that require stable fixation [185]. Signal dots, the dots that moved coherently, moved in one of two directions (left or right) at a speed of 7.5°/s, whereas noise dots (dots that moved at random) were randomly placed within the annulus. In each frame, there were 524 white dots with 0.15° in diameter.

In a visual random dot motion task, individuals must determine which of two directions the majority of the dots are moving [186]. The proportion of dots moving coherently vs. randomly defines the motion coherence, which modulates task difficulty. For instance, if the coherence is 25%, then 25% of the dots are moving together in the same direction. In the current work, motion coherence varied in four different levels (3%, 6%, 12%, or 24%). These four coherence levels were defined after some pilot tests with various levels of coherence were conducted.

Each trial was divided into five stages: 1) baseline period (0.5 - 4.5 s); 2) stimulus interval, composed of 0.75 s of both coherent and random motion; 3) response window with a maximum duration of 2.25 s terminating immediately after button press; 4) delay period between button press and the feedback lasting between 3 s to 4 s; 5) feedback and inter-trial window with a variable duration from 1.5 s to 2.5 s. Except for the stimulus interval, random motion (0% coherence) was presented in

all phases. Stimulus onset was indicated by an 880 Hz pure tone auditory cue with a duration of 250 ms. Feedback was also presented by an auditory signal consisting of the Portuguese words *"Certo"*, *"Errado"*, or *"Não Respondeu"*, respectively for correct, wrong, or no response. All auditory sounds were broadcast via a hi-fi speaker system at a volume of about 67 dB(A), and the sounds were clearly detectable by all participants. Both the central fixation cross and the set of points had a luminance of 58 cd/m^2 and were presented on a gray background, characterized by a luminance of 43 cd/m^2.

Figure 2.2 presents a schematic representation of task design. Participants were instructed to continuously maintain fixation on the central region and to indicate the correct direction of coherent motion (left or right) by pressing a key on the computer keyboard. Furthermore, they were also instructed to use different fingers for high and low confidence responses. Participants were instructed to respond with the middle or index fingers of the left hand to indicate perception of leftwards motion and with the middle and index fingers of the right hand to indicate perception of rightward motion, with the index fingers associated with low confidence responses and the middle fingers associated with high confidence responses, as represented in Figure 2.2.

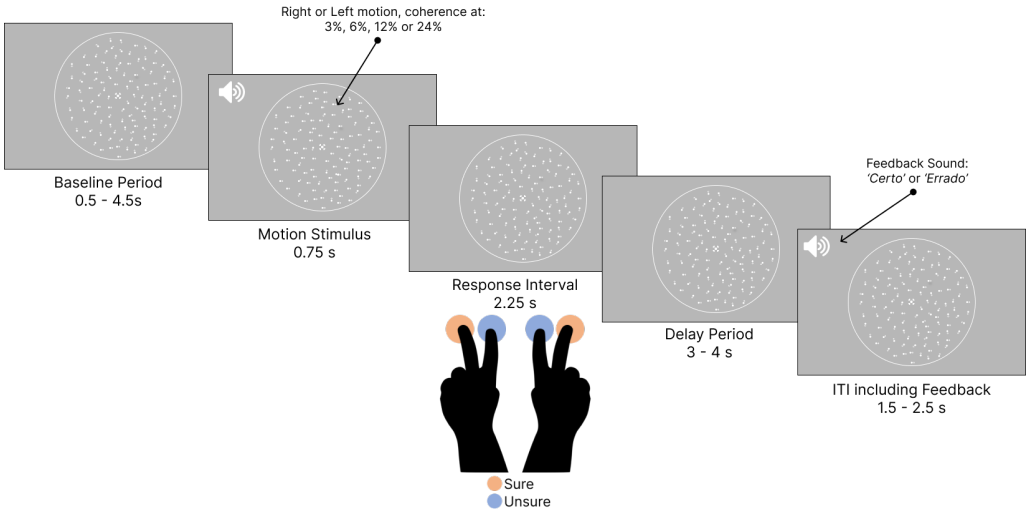

Figure 2.2: Schematic representation of motion discrimination task. Adapted from [1, 124].

In each testing moment (before and after physical or mental activity), participants completed four blocks of the task. Each block consisted of 48 trials, with 12 trials per coherence level, and lasted approximately eight minutes. Thus, participants performed a total of 192 trials per testing moment.

Before the experimental session, each participant completed a training session.

This training ensured that all participants understood the task well and that they were able to detect the high-coherence stimuli. The training consisted of the presentation of a trial with high coherence motion (24% coherence) for each direction, and following that, a sequence of three phases with eight high coherence motion trials (24% and 12% coherences), eight low coherence motion trials (6% and 3% coherences), and ten all coherence trials, in which the stimulus could assume any of the coherence levels (equal to the experimental task but with a smaller number of trials). Similar to the experimental task instructions, in the three phases, individuals should indicate the perceived direction of coherent motion and the associated response confidence.

2.5 Eye Tracking and Pupillometry Data Acquisition and Preprocessing

A stationary eye-tracking system called *EyeLink 1000 Plus* (SR Research, Ottawa, Ontario, Canada) [187] was used to record eye movement and pupilogram data. The EyeLink system has low pupil-position noise and very high resolution (0.2% of pupil diameter). A trigger pulse was generated at the onset of each stimulus, at every button press, and at every feedback moment.

The eye-tracking setup was composed of two computers. One of them was used to collect data, the Host PC, and the other one to show the experimental task, the Display PC. These computers were linked through Ethernet, allowing the transfer of pupil data and ocular events from the Host PC to the Display PC in less than three milliseconds [187].

Eye tracking can assume many configurations, however, for this experiment, it was used the Desktop Mount, in which the eye tracker was placed below the PC on which participants completed the task. Although this configuration allows for monocular, binocular, and remote (monocular) eye tracking at different sampling rates, monocular eye tracking at a sampling rate of 500 Hz was employed here as it is sufficient for measurements of pupil responses, which was the focus of this research. The equipment was positioned at 60 cm from the head support (SR Research Head Support, Ottawa, Ontario, Canada). All blocks of the experimental sessions started with eye position calibration and validation.

At the end of each block acquisition, EyeLink returns an *.edf* file that includes eye movement events and messages that were analyzed using Matlab. This file had to be converted into an *.asc* file, using a specialized program called *EDF Converter*

(SR Research).

Pupil data was analyzed with custom Matlab scripts and the EEGLAB toolbox in *Matlab R2022b*. First, we adapted a script from Urai et al. (2017) [116] to read the *.asc* EyeLink file, extract events from messages, remove and interpolate periods with blinks from pupil responses, and organize the data to be imported into EEGLAB [188]. The pupil diameter was also normalized within each run. We used the *percentage of the mean*, whose formula is:

$$\textit{percentage of the mean (pupil)} = \frac{\textit{pupil - mean(pupil)}}{\textit{mean(pupil)}}$$

After this, pupil data were imported into EEGLAB, which returns a dataset (*.set*). Finally, all datasets from the four runs of each participant testing moment were combined into a single dataset that was later used for analyses.

For analyses of the pupil response before feedback, pupil data were epoched twice. First, data was cut into epochs locked with the onset of the motion stimuli from -3 to 6 seconds after stimuli onset, and the average pupil size within the baseline of 200 milliseconds prior to the stimulus was removed. After that, the response epoch was created by cutting the existing epochs into epochs locked with the button press ranging from -3 to 3 seconds after button press. As the time of interest was from -1 to 3 seconds, in this time window, the epochs where more than half of the points were interpolated were excluded from analyses. For analyses of the pupil response after feedback, data was also cut into epochs locked with the onset of the motion stimuli ranging from -4 to 10 seconds (to include the feedback moment in all trials). Then the feedback epoch was generated by cutting the stimulus epochs into epochs locked with the onset of the feedback, ranging from -7 to 3 seconds, and the baseline was removed in this epoch, 200 milliseconds before the feedback moment. Similarly to the pupil response before feedback, the epochs where more than half of the points were interpolated in the time of interest (from -1 to 3 seconds of the feedback moment) were eliminated, and the remaining epochs were included for the analysis.

2.6 Physical Activity Intervention

In the present study, the exercise intervention involved a 30-minute bout of moderate aerobic exercise via a cycle ergometer (*Monark Ergomedic 828E*, Monark Exercise AB, Vansbro, Sweden) at 70% of participants VO_2max. In the same way, as performed in the submaximal protocol, individuals were positioned correctly and

comfortably on the ergometer with the knee bent approximately 25 degrees when the other leg was fully extended. The ergometer resistance, expressed in kilopounds (kilogram-force), was adjusted to maintain the intended moderate-intensity work rate. Throughout the intervention, heart rate was continuously monitored (*Polar V800 GPS sports watch*, Polar Electro Oy, Kempele, Finland).

2.7 Mental Activity Intervention

In the present research, mental activity was used as a control condition for the effect of physical activity. However, although the session was used as a sham intervention, to avoid biasing the participants expectations of which session should result in improved results in the perceptual task, participants were told that we wished to compare the effect of physical exercise with the effect of mental activity.

The mental intervention was applied by trained psychologists and consisted of thirty minutes of reasoning and visual perception tasks, including Montreal Cognitive Assessment (MoCA), and two Wechsler Adult Intelligence Scale - Third Edition (WAIS-III) tests, block design test, and matrix reasoning test. It is important to note that not all participants completed all of the tests, given that the goal was to remain active for 30 minutes and some participants performed the tasks faster than others. We also included Beck Depression Inventory – II (BDI-II) at the end of the mental activity session as a measure of psychiatric illness in our sample.

Heart rate was also monitored during this intervention. However, the heart rate monitors used for data collection had some problems and had to be replaced. Several other issues that came up throughout the assessment led to the loss of a significant amount of heart rate data. Therefore, there are just 13 participants with heart rate data from the mental activity session and 17 participants with heart rate data from the physical session.

2.7.1 Montreal Cognitive Assessment (MoCA)

The MoCA was used to characterize the general cognitive function of the older participants and ensure that they did not present any cognitive deficit. It is a highly sensitive tool used in both clinical practice and research to distinguish normal population from people with mild cognitive impairment or from patients with Alzheimer´s disease, with a sensitivity of 90% and 100% respectively [189, 190]. Furthermore, it is also often used to identify patients who exhibit behavioral variations of frontotemporal dementia [191], dementia linked to Parkinson's disease [192], and vascular dementia [193]. In brief, MoCA measures cognitive function across several

domains, including attention and visuospatial ability, so it can be used as a measure of overall global cognitive ability [194]. The scores of MoCA can range from 0 to 30 points, and to be considered normal it is necessary at least 21 points.

This test lasts approximately ten minutes and evaluates eight different domains in the following order: visuospatial, executive functions, naming, memory, attention, language, abstraction, and orientation. Drawing a clock and copying a three-dimensional cube are used to test visuospatial skills. To evaluate executive functions is performed an alternation task that requires drawing a line in ascending order from a number to a letter. Then, the participants are asked to name three animals (lion, camel, and rhinoceros) to assess naming ability. Memory is evaluated by two repetitions of a set of five different words at that moment and almost at the end of the test. Attention assessment is composed of several tasks, including forward and backward repetition of a list of numbers, a target detection task, and a successive subtraction task. For language evaluation, is used a fluency exercise and the repetition of two syntactically challenging sentences. Almost finishing, abstraction is assessed with a task to explain what each pair of words has in common. And finally, the test requires an assessment of orientation to time and place [195].

2.7.2 Wechsler Adult Intelligence Scale (WAIS) subtests

These tests were used to induce mental activity over 30 minutes as an active control for the 30 minutes of physical activity. The various versions of Wechsler Adult Intelligence Scale (WAIS) have been also frequently used as a gold standard for the measurement of intelligence, as well as for the assessment of several disorders, including hyperactivity disorder, learning disabilities, and other diseases in neurological conditions [196]. The Wechsler Adult Intelligence Scale - Third Edition (WAIS-III) is a very complete tool to evaluate the intellectual capacity of people over 16 years old. It consists of fourteen subtests, eleven main and three optional, divided into four different indices (verbal comprehension, perceptual organization, working memory, and processing speed). Both WAIS subtests used here, Block Design and Matrix Reasoning, evaluate the perception reasoning index. In other words, they measure nonverbal reasoning skills and visual perceptual thinking to detect the individual's capacity to accurately interpret, organize, and think with visual information.

In the block design test, participants were instructed to use a set of four or nine red and white colored blocks to replicate the two-dimensional designs presented into three-dimensional versions, in a maximum of fourteen images given in increasing complexity order with time limitation. Block design test scores range from 0 to 44

points, with additional points given for faster and correct trials totaling a maximum of 68 points, and better visuospatial performance is reflected in higher scores [197, 198].

On the other hand, in the matrix reasoning test, a matrix of abstract pictures was presented but there was one image missing. So, individuals were asked to indicate which was the number of the missing picture. Similarly, higher scores on this test also reflect increased non-verbal reasoning skills and visual intelligence. The scores of this test range from 0 to 26 points.

2.7.3 Beck Depression Inventory – II (BDI-II)

The Beck Depression Inventory – II (BDI-II) was used to characterize any depressive symptoms that might be present in our sample and could affect the results. Since its creation in 1961, the Beck Depression Inventory (BDI) has been extensively used both to evaluate a clinical patient's level of depression and to screen the general population for depression [199].

Therefore, aiming to assess the presence and severity of depressive symptomatology, at the end of the mental activity session, individuals were asked to fill in the Portuguese Version of the BDI-II [200], a revised version of the original BDI. This self-reported inventory consists of twenty-one questions in which individuals should select one of four sentences that best describe how they have been feeling during the last two weeks with regard to sadness, pessimism, sense of failure, lack of satisfaction, guilt, expectation of punishment, self-dislike, self-accusation, suicidal ideation, episodes of crying, irritability, social withdrawal, indecisiveness, worthlessness, loss of energy, agitation, concentration difficulty, insomnia, loss of appetite, fatigue, and loss of interest in sex [201]. To screen the depressive symptomatology of participants, each item was scored from 0 to 3 for severity, with a total score from 0 to 63, and the outcome was classified into: absence of depression (0 - 9), mild to moderate depression (10 - 18), moderate to severe depression (19 - 29), and severe depression (30 - 63) [202].

2.8 Saliva Collection

As previously described, saliva was collected at three different moments in both physical and mental activity sessions. The purpose of this collection was to measure Salivary Alpha-amylase (sAA) as an indirect marker of NE release. sAA is one of the most prevalent enzymes in oral fluid and was considered one of the most important enzymes in saliva [203]. It is the sympathetic branch of the ANS that regulates

alpha-amylase secretion from the salivary glands. So, this enzyme has been widely used as a reliable indicator of SNS activity [204], particularly as a well-established biomarker for NE release. For instance, Chatterton et al. (1996) [205] by measuring sAA under various stressful circumstances, including aerobic exercise, discovered that these levels were closely linked with NE levels.

Before collection participants were instructed to avoid caffeine immediately before sample collection, not to visit the dentist the twenty-four hours before, and not to eat and brush their teeth for at least 60 minutes before the session. Ten minutes before the first harvest participants rinsed their mouths with water to remove food residue.

To collect unstimulated whole saliva it was used the "passive drooling" technique [206], in which participants were instructed to collect passively all secreted saliva in the oral cavity for a period of three minutes and softly release it via the tube with their heads inclined forward. Thereafter, they were instructed to close the tube cap and put it inside an ice box. It is important to note that prior to the collection, test tubes were all weighted and labeled with the respective participant code, session, and collection moment. The three collecting tubes were frozen at -20^{o}C following each session until biochemical analysis took place [207]. Analyses of these saliva samples are still ongoing and will be reported elsewhere.

2.9 Methodological Procedure

The following steps comprised the overall protocol for the experimental sessions:

1) Rinse mouth with water at least 10 minutes before the first saliva collection;

2) Explain the purpose and possible consequences of the experimental design and sign the written informed consent (if it was the participants first session);

3) Fill in the daily activity and consumption, the medical history, and the handedness questionnaires;

4) Perform the first saliva collection (before perceptual decision-making task);

5) Carry out task training;

6) Complete the first testing moment of the cognitive task;

7a) Execute thirty minutes of mental activity (with heart rate monitoring);

7b) Execute thirty minutes of physical activity (with heart rate monitoring);

8) Identify the perceived effort using the *Borg Cr10* scale;

9) Perform the second saliva collection (after mental or physical activity);

10) Carry out the second testing moment of the cognitive task;

11) Complete the last saliva collection (at the end of the session);

12) In the case of the mental activity session, fill in the BDI-II assessment.

2.10 Statistical Analyses

Statistical analysis was performed using IBM SPSS Statistics, version 28.0.1.0 software.

Regarding the comparisons between young and older adults, and between physical activity and mental activity sessions, the statistical analyses were performed with Linear Mixed Models (LMM) to consider all factors while taking into account an unequal number of repetitions, within and between participants. Our factors of interest were considered fixed effects and the intersubject variability the random effects. Only random intercepts were taken into account when building the models. We opted for the maximum likelihood method for estimation, with absolute values for log-likelihood convergence (value = 0), parameter convergence (value = 0.0000001), and hessian convergence (value = 0). It was used a confidence interval of 95%. For the binary variables, accuracy, and percentage of unsure responses, Generalized Linear Mixed Models (GLMM) with logistic regression were used.

Regarding the comparison between young and older adults, for the LMM analysis of pupil response or reaction time, the following factors of interest were used: group, coherence, confidence, and accuracy. For the GLMM analysis of accuracy, the following factors were used: group, coherence, and confidence; and for the GLMM analysis of confidence the following factors were used: group, coherence, and accuracy. Regarding the comparison between physical and mental activities, for the LMM analysis of pupil response or reaction time, the following factors of interest were used: session (physical vs. mental), moment (before vs. after), order (first mental activity vs. first physical activity), coherence, confidence, and accuracy. Similarly, for the GLMM analysis of accuracy, the following factors were used: session, moment, order, coherence, and confidence; and for the GLMM analysis of confidence the following factors were used: session, moment, order, coherence, and accuracy.

Paired t-tests were used for heart rate analysis since there was only one dependent variable with two moments.

For all statistical tests, an alpha level of 0.05 was used.

3

Results

This chapter presents the results obtained from the previously described methodology. Section 3.1 focuses on the comparison between young adults and older adults, while Section 3.2 focuses on the effect of physical activity.

3.1 Comparison between young and older adults

First, we investigated the differences between age groups in the performance of a perceptual decision-making task with various difficulty levels, in which, by manipulating the sensory evidence, we manipulated response certainty. We also evaluated how the pupillary response was modulated by task difficulty, response uncertainty, response accuracy, and feedback, and how these modulations changed with aging.

Statistical analyses are presented in the Tables 3.1, 3.2, 3.3, 3.4, 3.5, and 3.6.

3.1.1 Behavioral Results

Accuracy

The GLMM analyses revealed that older adults had lower accuracy (81.35 ± 2.27%) than young adults (86.20 ± 1.06%) [effect of group: $F_{(1,\ 6847)} = 8.630$, $p = 0.005$] (see Figure 3.1). As expected, lower coherences were associated with reduced accuracy [effect of coherence: $F_{(3,\ 6847)} = 30.721$, $p < .001$]. The effect of group was higher for lower coherences, but this interaction did not reach significance [interaction group x coherence: $F_{(3,\ 6847)} = 3.056$, $p = 0.052$].

Accuracy was also significantly modulated by response confidence. When participants reported that they were unsure about their responses, the accuracy was lower in comparison to when they reported that they were sure about their responses [effect of confidence: $F_{(1,\ 6847)} = 102.808$, $p < .001$]. This suggests that they were following task instructions. The effect of confidence on accuracy was bigger for young

than older adults [interaction group x confidence: $F_{(1,\ 6847)} = 3.802$, $p < 0.001$] (see Figure 3.1b). For confident responses, older adults show lower accuracy than young adults meaning that when older adults report confidence in their responses, they still commit a lot of errors.

Finally, regarding the 2-way interaction coherence x confidence, the difference in accuracy for sure and unsure responses is biggest in the highest coherence [interaction coherence x confidence: $F_{(3,\ 6847)} = 11.688$, $p < 0.001$], as can be seen in Figure 3.1c), and this effect is the same for both groups [interaction group x coherence x confidence: $F_{(3,\ 6847)} = 0.921$, $p = 0.398$].

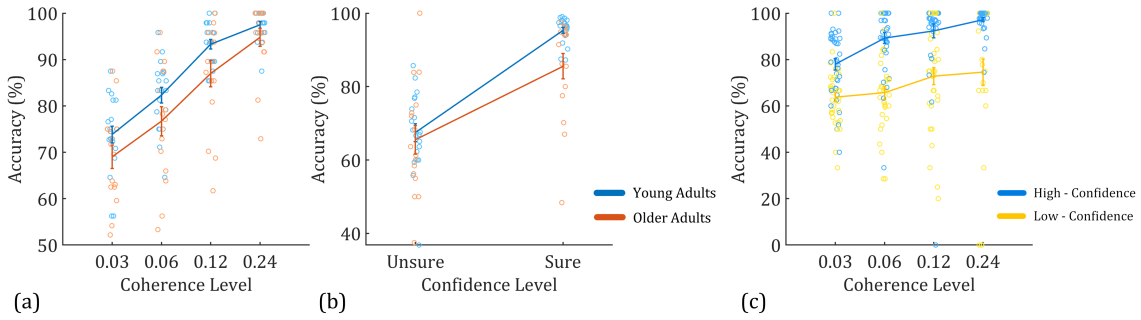

Figure 3.1: Accuracy as a function of (a) coherence level and group; (b) confidence level and group; (c) coherence level and confidence. Circles represent individual data points from each participant and line graphs represent mean ± standard error of the mean.

Table 3.1: GLMM statistical analysis of accuracy.

Variable	F	p
Group	$F_{(1,\ 6847)} = 8.630$	0.005*
Coherence	$F_{(3,\ 6847)} = 30.721$	0.000*
Confidence	$F_{(1,\ 6847)} = 102.808$	0.000*
Group x Coherence	$F_{(3,\ 6847)} = 3.056$	0.052
Group x Confidence	$F_{(1,\ 6847)} = 3.803$	< 0.001*
Coherence x Confidence	$F_{(3,\ 6847)} = 11.688$	< 0.001*
Group x Coherence x Confidence	$F_{(3,\ 6847)} = 0.921$	0.398

Confidence Level

In each trial, participants reported their responses as low- or high-confidence responses. Regarding the percentage of low-confidence responses, there were no group differences [effect of group: $F_{(1,\ 6847)} = 0.739$, $p = 0.105$]. However, there was a group x coherence interaction [interaction group x coherence: $F_{(3,\ 6847)} = 7.667$, $p = 0.006$], which can be seen in Figure 3.2a), reflecting the fact that the effect of

stimulus coherence was higher in young adults. Indeed, older adults had a lower number of low-confidence responses for the lowest coherences in comparison with young adults but had a higher number of low-confidence responses than young adults for the highest coherence. So, the probability of responding with low confidence was less dependent on stimulus strength in the older group.

The link between response confidence and accuracy was significantly reduced in older people (see Figure 3.2b). The older group had a similar percentage of low confidence in correct trials but a lower percentage of low-confidence responses in incorrect trials [interaction group x accuracy: $F_{(1,\ 6847)} = 11.789$, $p < 0.001$].

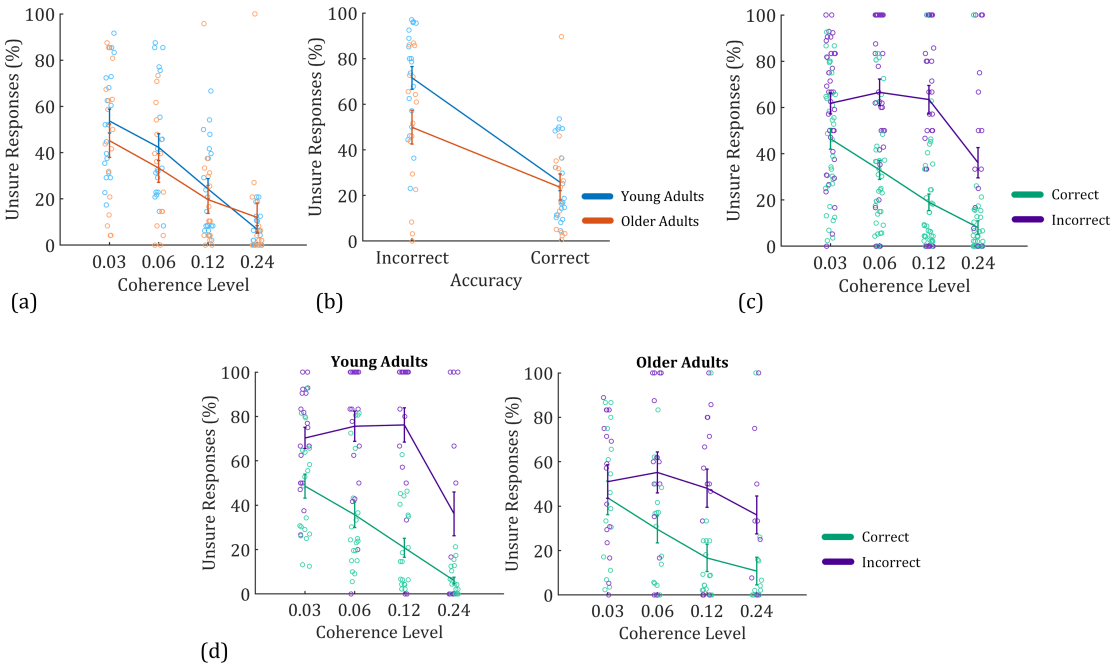

Figure 3.2: Percentage of unsure as a function of (a) coherence level and group; (b) confidence level and group; (c) coherence level and accuracy; (d) group, coherence level, and accuracy. Circles represent individual data points from each participant and line graphs represent mean ± standard error of the mean.

A 2-way interaction between coherence and accuracy was also observed in the percentage of low-confidence responses [interaction coherence x accuracy: $F_{(3,\ 6847)} = 14.883$, $p < 0.001$]. This reflects the fact that the difference in the percentage of low-confidence responses in correct and incorrect trials is lower for the lowest coherence than for the other coherences, as can be seen in Figure 3.2c). This effect is the same for both groups [interaction group x coherence x accuracy: $F_{(3,\ 6847)} = 1.045$, $p = 0.668$]. These results are presented in Figure 3.2.

Table 3.2: GLMM statistical analysis of percentage of unsure.

Variable	F	p
Group	$F_{(1,\ 6847)} = 0.739$	0.105
Coherence	$F_{(3,\ 6847)} = 29.972$	0.000*
Accuracy	$F_{(1,\ 6847)} = 87.911$	0.000*
Group x Coherence	$F_{(3,\ 6847)} = 7.667$	0.006*
Group x Accuracy	$F_{(1,\ 6847)} = 11.789$	< 0.001*
Coherence x Accuracy	$F_{(3,\ 6847)} = 14.883$	< 0.001*
Group x Coherence x Accuracy	$F_{(3,\ 6847)} = 1.045$	0.668

Reaction Time

Reaction time has been used as a continuous measure of response confidence with longer reaction times associated with lower response confidence [1, 116]. Here, we analyzed reaction time and compared this measure with explicit reports of confidence. The findings showed no differences between groups [effect of group: $F_{(1,\ 40.166)} = 0.889$, $p = 0.351$]. As expected, high-confidence responses were associated with faster reaction times [effect of confidence: $F_{(1,\ 6831)} = 311.904$, $p < 0.001$] (see Figure 3.3b). However, this effect was higher for young adults [interaction group x confidence: $F_{(1,\ 6831)} = 8.947$, $p = 0.003$]. For confident responses, older adults were slightly slower than young adults. Thus, this implicit measure of confidence suggests that when older people report high confidence these responses might be associated with a lower level of confidence than in young people.

Also, correct responses (usually associated with higher response confidence) resulted in faster reaction times than incorrect responses [effect of accuracy: $F_{(1,\ 6831)} = 323.887$, $p < 0.001$], but, in contrast to the explicit measure of response confidence, this effect was not significantly different for both groups [interaction group x accuracy: $F_{(1,\ 6831)} = 1.268$, $p = 0.260$] (see Figure 3.3c).

Reaction time analyses revealed a significant effect of coherence level [effect of coherence: $F_{(3,\ 6829.132)} = 28.287$, $p < 0.001$] (see Figure 3.3a). As expected, reaction time is slower for the lowest coherences, consistent with the idea of reduced response confidence for lower coherence stimuli. However, in contrast to the finding regarding explicit response confidence, the effect of stimulus coherence on reaction time was higher for older adults who showed faster reaction times for the highest coherence despite presenting lower response confidence [interaction group x coherence: $F_{(3,\ 6829.132)} = 10.275$, $p < 0.001$].

The effect of accuracy on reaction time further depended on coherence level

[interaction coherence x accuracy: $F_{(3,\ 6829.612)} = 14.986$, $p < 0.001$], with the effect being smaller for low coherences, but this effect did not depend on the group [interaction group x coherence x accuracy: $F_{(3,\ 6829.612)} = 1.516$, $p = 0.208$].

There was also a 2-way interaction between confidence level and accuracy on reaction time [interaction confidence x accuracy: $F_{(1,\ 6831)} = 11.757$, $p < 0.001$], and this effect was similar for both groups [interaction group x confidence x accuracy: $F_{(1,\ 6831)} = 0.05$, $p = 0.815$].

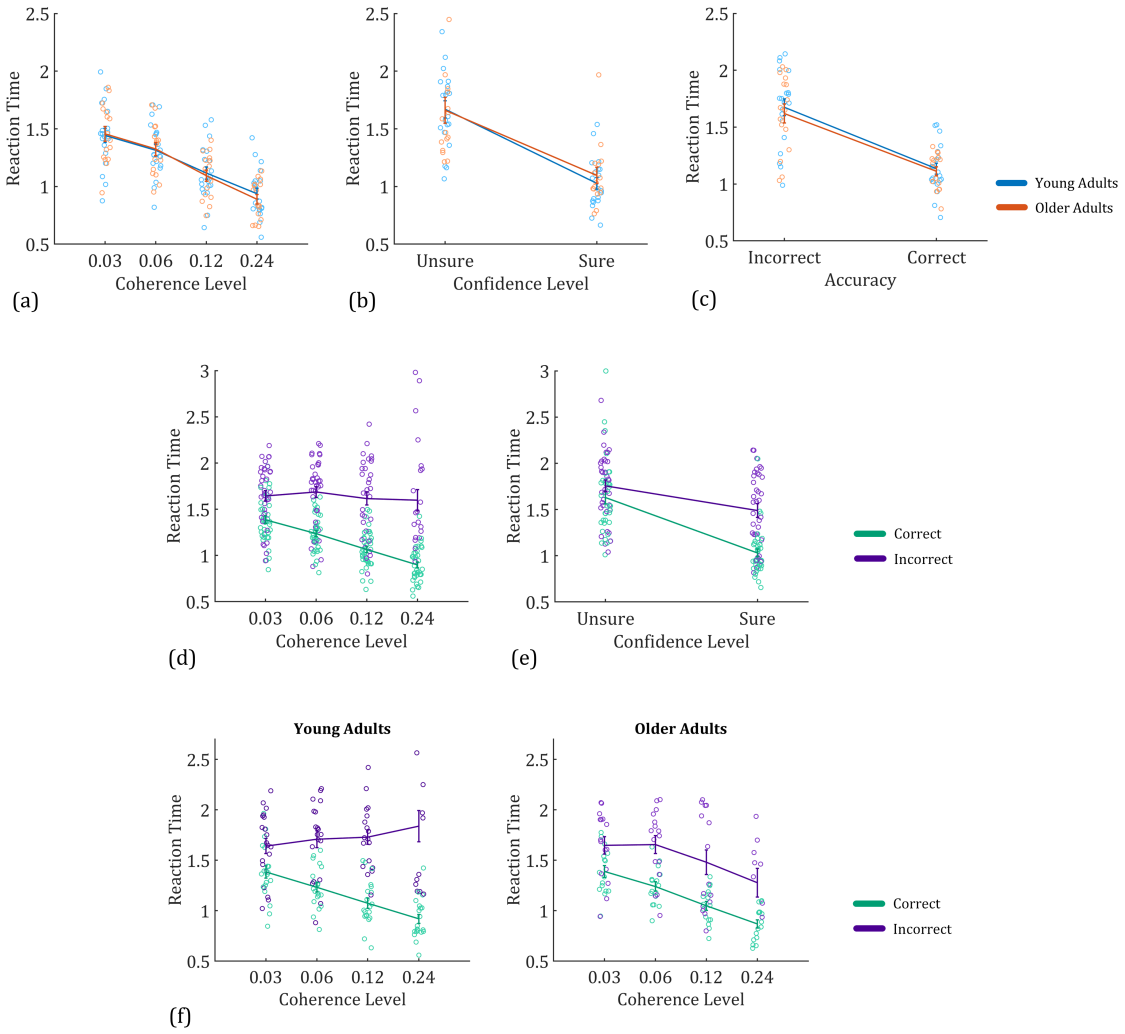

Figure 3.3: Reaction time as a function of (a) coherence level and group; (b) confidence level and group; (c) accuracy and group; (d) coherence level and accuracy; (e) confidence level and accuracy; (f) group, coherence level, and accuracy. Circles represent individual data points from each participant and line graphs represent mean ± standard error of the mean.

Table 3.3: LMM statistical analysis of reaction time.

Variable	F	p
Group	$F_{(1,\ 40.166)} = 0.889$	0.351
Coherence	$F_{(3,\ 6829)} = 28.287$	< 0.001*
Confidence	$F_{(1,\ 6831)} = 311.904$	< 0.001*
Accuracy	$F_{(1,\ 6831)} = 323.887$	< 0.001*
Group x Coherence	$F_{(3,\ 6829)} = 10.275$	< 0.001*
Group x Confidence	$F_{(1,\ 6831)} = 8.947$	0.003*
Group x Accuracy	$F_{(1,\ 6831)} = 1.268$	0.260
Coherence x Confidence	$F_{(3,\ 6829)} = 1.453$	0.225
Coherence x Accuracy	$F_{(3,\ 6829)} = 14.986$	< 0.001*
Confidence x Accuracy	$F_{(1,\ 6831)} = 11.757$	< 0.001*
Group x Coherence x Confidence	$F_{(3,\ 6829)} = 0.344$	0.794
Group x Coherence x Accuracy	$F_{(3,\ 6829)} = 1.516$	0.208
Group x Confidence x Accuracy	$F_{(1,\ 6831)} = 0.055$	0.815
Coherence x Confidence x Accuracy	$F_{(3,\ 6829)} = 1.172$	0.319
Group x Coherence x Confidence x Accuracy	$F_{(3,\ 6829)} = 1.709$	0.163

3.1.2 Pupillary Responses

Pupil response before feedback

Regarding pupil response amplitude before feedback, the time course analyses showed that the pupil response was modulated by response accuracy but was not modulated by response confidence and this was true for both groups of participants. The difference between groups emerged in the pupil response to high- or low-confidence answers for incorrect trials, in which young adults revealed a greater and longer-lasting dilation to high-confidence incorrect trials, which was not observed for older adults (see Figure 3.4c).

The statistical analysis was carried out in the average pupil response within the time window between one and three seconds after the button press. In this period, there was an effect of accuracy, with incorrect trials showing larger pupil responses [effect of accuracy: $F_{(1,\ 5980)} = 17.150$, $p = 0.008$]. This effect was similar for both groups [interaction group x accuracy: $F_{(1,\ 65980)} = 2.945$, $p = 0.086$], did not depend on coherence [interaction coherence x accuracy: $F_{(3,\ 5980)} = 1.326$, $p = 0.264$], but depended on response confidence [interaction confidence x accuracy: $F_{(1,\ 5980)} = 12.034$, $p < 0.001$]. The pupil response was larger for high-confidence incorrect responses and smaller for high-confidence correct responses, as shown in Figure 3.5d). However, this pattern was more pronounced for young adults, resulting in a 3-way interaction between group, confidence, and accuracy [interaction group x confidence x accuracy: $F_{(1,\ 5980)} = 4.717$, $p = 0.030$]. In brief, young adults had

a larger difference between incorrect and correct trials for high-confidence responses than older adults, which elicited a similar pupil response in incorrect trials for both high- and low-confidence.

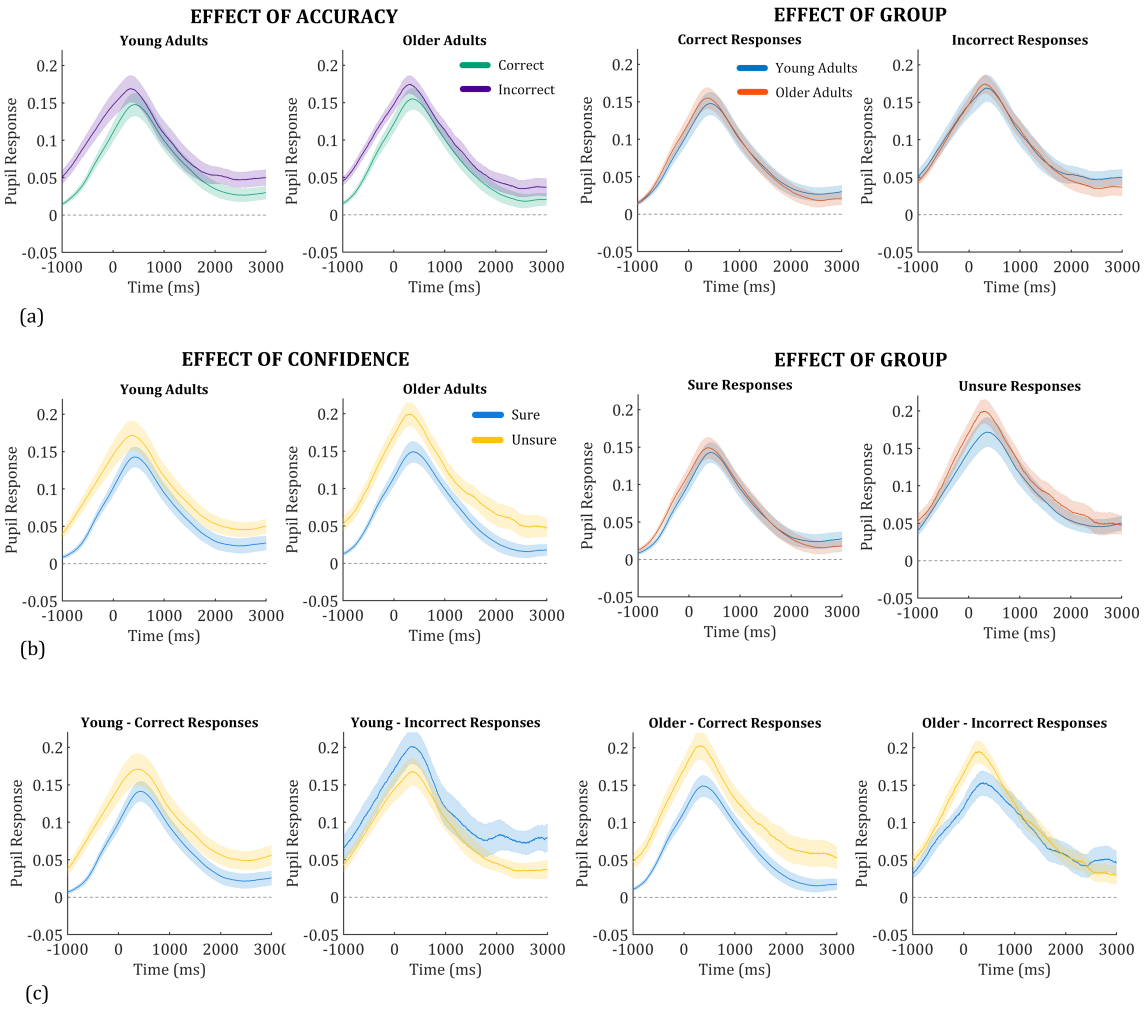

Figure 3.4: Time course of pupil response locked with response onset (a) interaction group and accuracy; (b) interaction group and confidence level; (c) interaction group, confidence level, and accuracy.

As participants might make mistakes when explicitly reporting their confidence level, we used the reaction time as an implicit measure of confidence. Incorrect trials elicited a larger pupil response than correct trials for both slow and fast reaction time (see Figure 3.5e). In slow reaction time trials, the differences in pupil size between correct and incorrect responses were similar for young and older adults, but in fast reaction time trials, there was a greater difference between correct and incorrect trials in young adults.

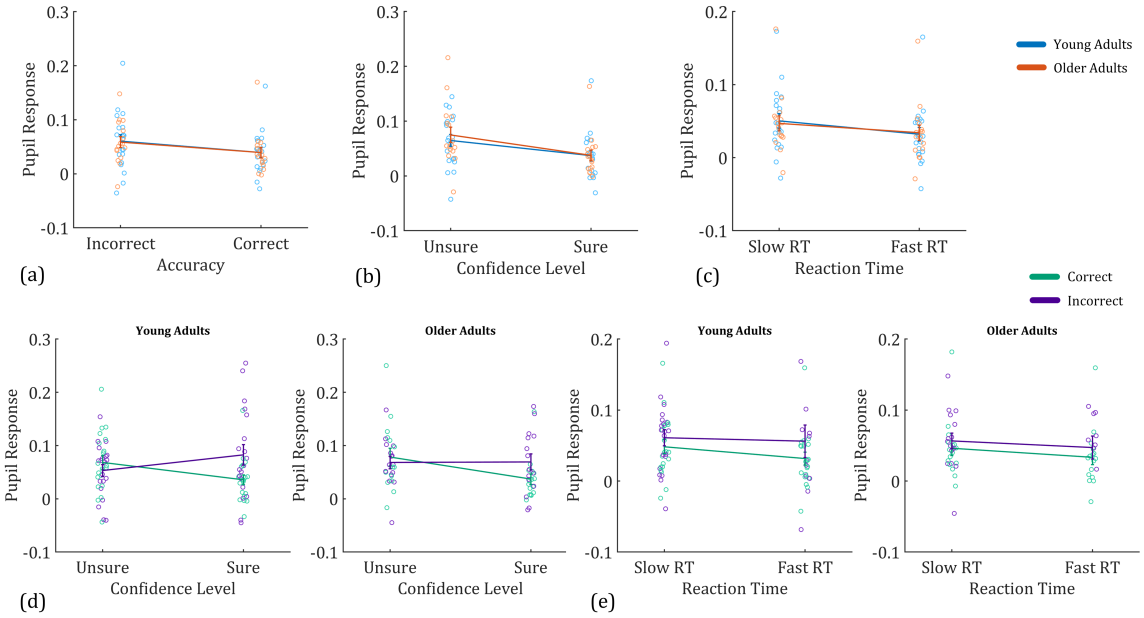

Figure 3.5: Average amplitude of pupil response evoked by response in the time window from 1s up to 3s after response as a function of (a) group and accuracy; (b) group and confidence level; (c) group and reaction time (as an indirect measure of confidence level); (d) group, confidence level, and accuracy; (e) group, reaction time, and accuracy. Circles represent individual data points from each participant and line graphs represent mean ± standard error of the mean.

Table 3.4: LMM statistical analysis of pupil response before feedback.

Variable	F	p
Group	$F_{(1,\ 53.154)} = 1.200$	0.278
Coherence	$F_{(3,\ 5980)} = 2.529$	0.055
Confidence	$F_{(1,\ 5980)} = 0.325$	0.569
Accuracy	$F_{(1,\ 5980)} = 7.150$	0.008*
Group x Coherence	$F_{(3,\ 5980)} = 1.419$	0.235
Group x Confidence	$F_{(1,\ 5980)} = 1.839$	0.175
Group x Accuracy	$F_{(1,\ 5980)} = 2.945$	0.086
Coherence x Confidence	$F_{(3,\ 5980)} = 0.616$	0.604
Coherence x Accuracy	$F_{(3,\ 5980)} = 1.326$	0.264
Confidence x Accuracy	$F_{(1,\ 5980)} = 12.034$	< 0.001*
Group x Coherence x Confidence	$F_{(3,\ 5980)} = 2.271$	0.078
Group x Coherence x Accuracy	$F_{(3,\ 5980)} = 0.481$	0.696
Group x Confidence x Accuracy	$F_{(1,\ 5980)} = 4.717$	0.030*
Coherence x Confidence x Accuracy	$F_{(3,\ 5980)} = 2.537$	0.055
Group x Coherence x Confidence x Accuracy	$F_{(3,\ 5980)} = 2.096$	0.099

Pupil response after feedback

Concerning the amplitude of the pupil response after feedback, the analysis of the time course revealed that in response to the feedback, both groups showed higher pupil response in incorrect than in correct trials. However, in the older group, there

was an earlier constriction of the pupil, which means that at the end of the trial, incorrect answers had similar pupil responses than correct answers (see Figure 3.6a). As in the pupil response before feedback, young adults also revealed higher pupil amplitude to high-confidence incorrect trials, while for older adults the time course of the pupil was similar for both high- and low-confidence incorrect responses (see Figure 3.6c).

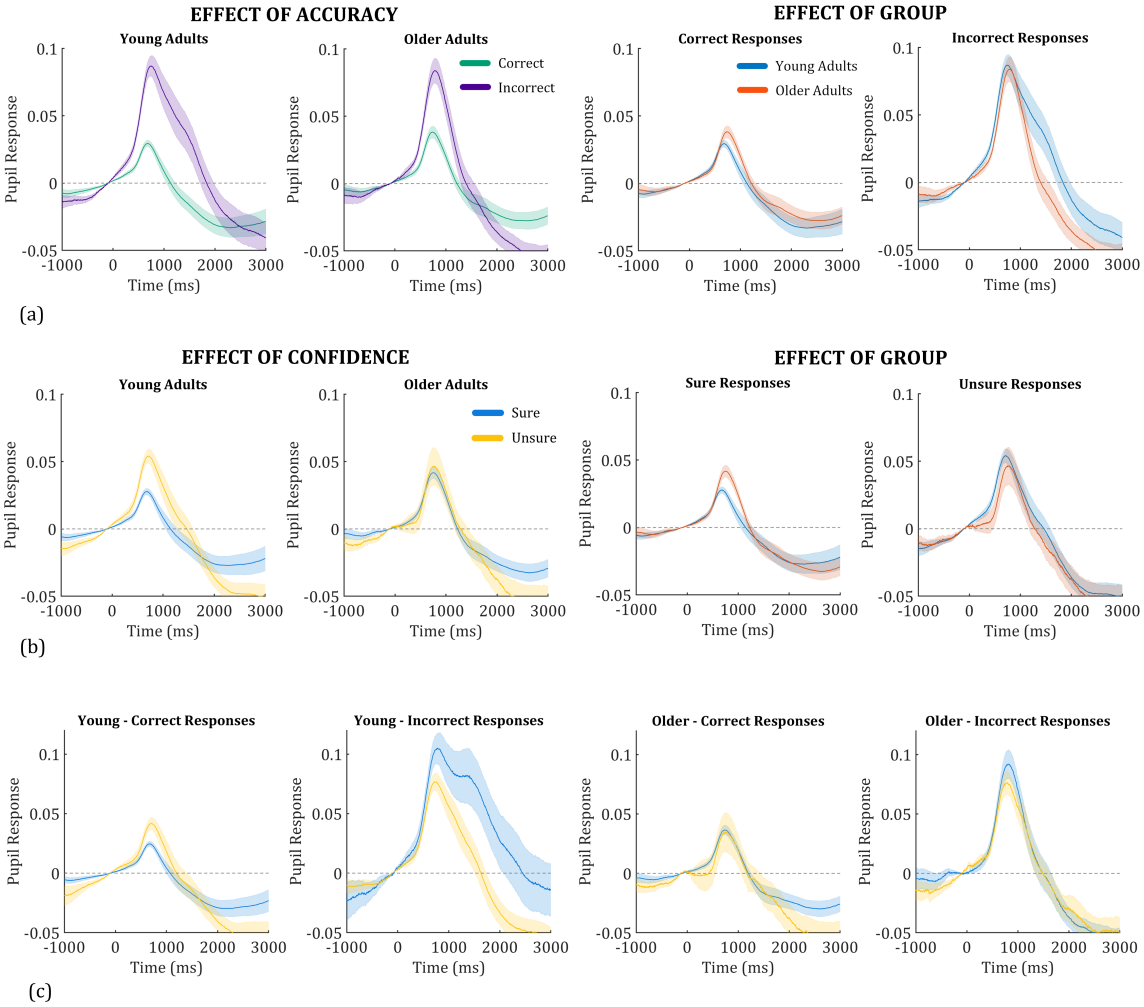

Figure 3.6: Time course of pupil response locked with feedback onset (a) interaction group and accuracy; (b) interaction group and confidence level; (c) interaction group, confidence level, and accuracy..

In the period after feedback, pupil amplitude showed large variations over the three seconds after the feedback moment. So, the statistical analysis was carried out for two different time windows, the first between zero and one and half seconds, and the second between one and half and three seconds after feedback, which corresponds to the later phase of the response to feedback.

In the earlier period of pupil response after feedback there was an effect of

accuracy [effect of accuracy: $F_{(1,\ 6096)} = 66.734$, $p < .001$], and a significant interaction between group and accuracy [interaction group x accuracy: $F_{(1,\ 6096)} = 17.859$, $p < .001$]. For incorrect trials, the response was higher in young adults, while for the correct trials, it was similar for both groups (see Figure 3.7a). The effect of accuracy was particularly strong for high-confidence responses, where incorrect trials elicited a larger pupil response than correct trials, while in low-confidence responses, correct and incorrect trials elicited a similar pupil response [interaction confidence x accuracy: $F_{(1,\ 6096)} = 15.035$, $p < .001$]. Although not statistically significant [interaction group x confidence x accuracy: $F_{(1,\ 6096)} = 0.831$, $p = 0.362$], this effect was more pronounced in young adults (Figure 3.7d).

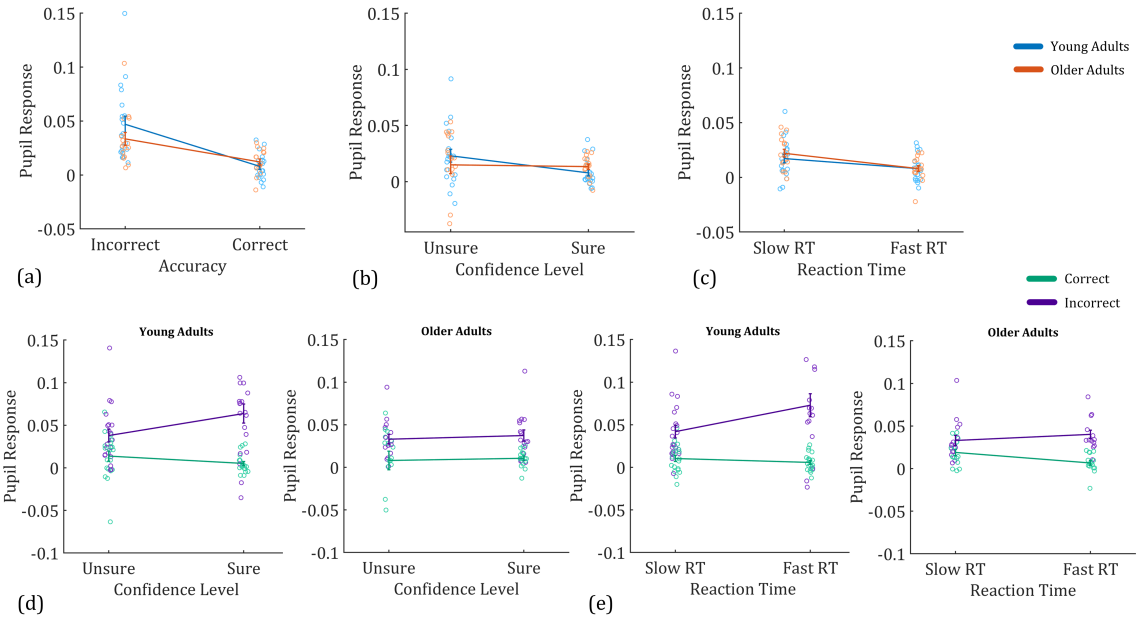

Figure 3.7: Average amplitude of pupil response evoked by feedback in the time window from 0s up to 1.5s after feedback as a function of (a) group and accuracy; (b) group and confidence level; (c) group and reaction time (as an indirect measure of confidence level); (d) group, confidence level, and accuracy; (e) group, reaction time, and accuracy. Circles represent individual data points from each participant and line graphs represent mean ± standard error of the mean.

Regarding the late period of pupil response after feedback there was a dependence on confidence level [effect of confidence: $F_{(1,\ 6081)} = 8.409$, $p = 0.004$], with the differences in pupil response between sure and unsure trials being slightly larger for young than for older adults [interaction group x confidence: $F_{(1,\ 6081)} = 7.314$, $p = 0.007$] (Figure 3.8d). The 2-way interactions between group and accuracy [interaction group x accuracy: $F_{(1,\ 6096)} = 18.646$, $p < .001$], and between confidence and accuracy [interaction confidence x accuracy: $F_{(1,\ 6096)} = 6.520$, $p = 0.011$] were still

significant.

Using the indirect measure of confidence level (reaction time), we were able to confirm the findings from the effect of confidence in both pupil after feedback time windows, as can be seen in Figures 3.7e and 3.8e. Indeed, the relationships between reaction time, group, and accuracy were very similar to the graphs that related confidence level with the same variables. In young adults, fast reaction time trials, which are supposedly associated with high-confidence trials, elicited a much larger pupil response for incorrect trials than for correct trials. In older adults, the pupil response to correct and incorrect responses was similar for both slow and fast reaction times. This pattern was similar to the one observed using the direct measure of confidence level.

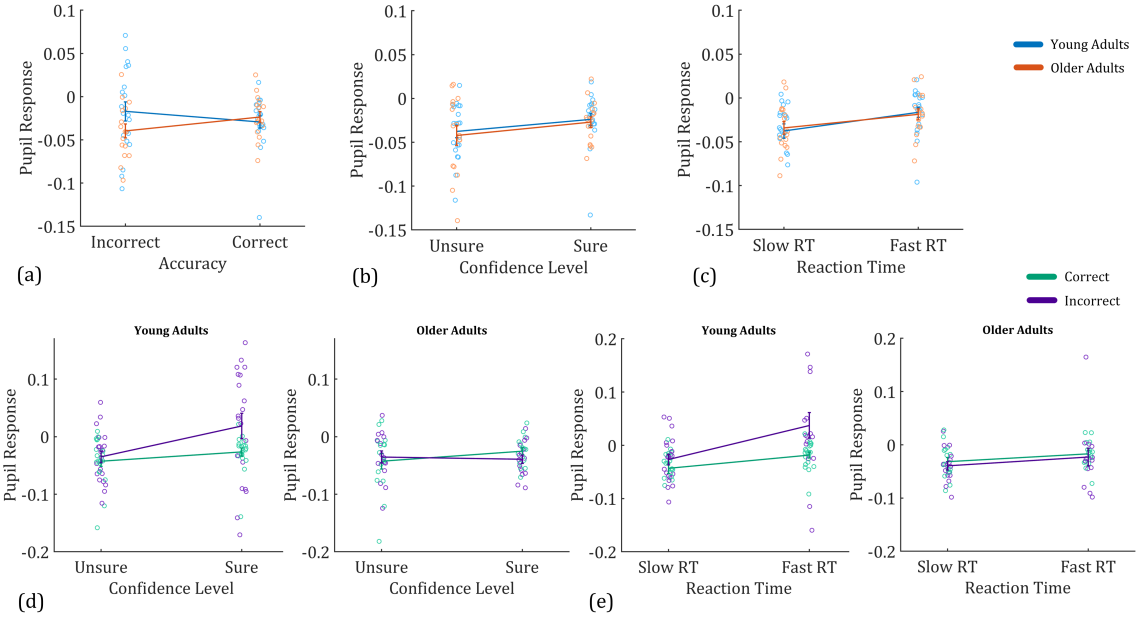

Figure 3.8: Average amplitude of pupil response evoked by feedback in the time window from 1.5s up to 3s after feedback as a function of (a) group and accuracy; (b) group and confidence level; (c) group and reaction time (as an indirect measure of confidence level); (d) group, confidence level, and accuracy; (e) group, reaction time, and accuracy. Circles represent individual data points from each participant and line graphs represent mean ± standard error of the mean.

Table 3.5: LMM statistical analysis of pupil response in the time window between 0 and 1.5s after feedback.

Variable	F	p
Group	$F_{(1,\ 74.483)} = 2.243$	0.138
Coherence	$F_{(3,\ 6096)} = 1.595$	0.188
Confidence	$F_{(1,\ 5962)} = 0.995$	0.318
Accuracy	$F_{(1,\ 6096)} = 66.734$	< 0.001*
Group x Coherence	$F_{(3,\ 6096)} = 0.747$	0.524
Group x Confidence	$F_{(1,\ 5962)} = 0.934$	0.334
Group x Accuracy	$F_{(1,\ 6096)} = 17.859$	< 0.001*
Coherence x Confidence	$F_{(3,\ 6096)} = 0.953$	0.414
Coherence x Accuracy	$F_{(3,\ 6096)} = 1.154$	0.326
Confidence x Accuracy	$F_{(1,\ 6096)} = 15.035$	< 0.001*
Group x Coherence x Confidence	$F_{(3,\ 6096)} = 0.353$	0.787
Group x Coherence x Accuracy	$F_{(3,\ 6096)} = 0.634$	0.593
Group x Confidence x Accuracy	$F_{(1,\ 6096)} = 0.831$	0.362
Coherence x Confidence x Accuracy	$F_{(3,\ 6096)} = 0.612$	0.607
Group x Coherence x Confidence x Accuracy	$F_{(3,\ 6096)} = 1.997$	0.112

Table 3.6: LMM statistical analysis of pupil response in the time window between 1.5 and 3s after feedback.

Variable	F	p
Group	$F_{(1,\ 60.002)} = 1.560$	0.216
Coherence	$F_{(3,\ 6096)} = 0.537$	0.657
Confidence	$F_{(1,\ 6081)} = 8.409$	0.004*
Accuracy	$F_{(1,\ 6096)} = 0.464$	0.496
Group x Coherence	$F_{(3,\ 6096)} = 0.218$	0.884
Group x Confidence	$F_{(1,\ 6081)} = 7.314$	0.007*
Group x Accuracy	$F_{(1,\ 6096)} = 18.646$	< 0.001*
Coherence x Confidence	$F_{(3,\ 6096)} = 1.122$	0.339
Coherence x Accuracy	$F_{(3,\ 6096)} = 1.930$	0.122
Confidence x Accuracy	$F_{(1,\ 6096)} = 6.520$	0.011*
Group x Coherence x Confidence	$F_{(3,\ 6096)} = 1.580$	0.192
Group x Coherence x Accuracy	$F_{(3,\ 6096)} = 0.682$	0.563
Group x Confidence x Accuracy	$F_{(1,\ 6096)} = 2.677$	0.102
Coherence x Confidence x Accuracy	$F_{(3,\ 6096)} = 1.157$	0.325
Group x Coherence x Confidence x Accuracy	$F_{(3,\ 6096)} = 1.180$	0.316

3.2 Effect of physical and mental activities on task performance and pupillary responses in older adults

Consistent with what was mentioned in the Introduction chapter, the findings described in the previous section (Section 3.1) revealed that older adults represent uncertainty differently from young adults, and this is reflected in the way uncertainty

is reported by older people and in the way the recruitment of the pupil-linked arousal system is modulated by uncertainty during the decision-making process. Therefore, in the present section, we aimed to understand if a single bout of physical activity could improve the performance of older adults, by enhancing the response of the arousal system.

In the following sections, the different parameters will be analyzed using GLMM or LMM, where we were particularly interested in investigating if the effect of moment (before and after the intervention) was modulated by session (physical activity vs. mental activity). If the effect of physical activity was significantly different from the effect of mental activity (our sham condition) then we should observe a significant session x moment interaction. Statistical analyses are presented in the Tables 3.9, 3.10, 3.11, 3.12, 3.13, and 3.14.

3.2.1 Participants Characterization

Physical Assessment

Table 3.7 reports the descriptive results ($mean \pm SD$) acquired in the physical assessment session, important for characterizing the physical profile of our sample.

Table 3.7: Descriptive results obtained in the physical assessment across participants ($mean \pm SD$).

	Body Composition					Sit and Reach (cm)			Upper Limbs strength (kg)	
	Height (cm)	Body mass (kg)	Body mass index (kg/m^2)	Fat mass (%)	Skeletal muscle mass (kg)	Right	Left	Both	Right	Left
All participants	166,4 ± 8,4	70,6 ± 15,0	25,3 ± 4,3	31,1 ± 6,8	26,5 ± 5,1	20,7 ± 14,0	20,8 ± 14,0	23,0 ± 10,4	29,2 ± 9,4	25,9 ± 10,3
Male participants	173,7 ± 5,1	80,4 ± 8,9	26,8 ± 3,8	30,1 ± 5,8	31,2 ± 2,1	19,9 ± 12,9	19,6 ± 11,9	21,8 ± 12,0	36,4 ± 8,2	33,7 ± 9,3
Female participants	160,1 ± 4,7	62,0 ± 14,3	24,0 ± 4,5	32,0 ± 7,9	22,4 ± 2,6	21,5 ± 15,7	21,9 ± 16,3	24,1 ± 9,6	22,9 ± 4,4	19,1 ± 5,0

According to the *ACSM's Guidelines for Exercise Testing and Prescription* [8], the Body Mass Index obtained indicates that participants were slightly overweight (25.0 - 29.9 kg/m^2), revealing some disease risk. The Body Fat Mass (%) was classified as very poor for male participants (30.1 ± 5.8 %) and as poor for female participants (32.0 ± 7.9 %). Moreover, male participants on average were classified as good in sit-and-reach with both legs (21.8 ± 12.0 cm) and as poor in both hands of upper limbs strength (right: 36.4 ± 8.2 kg, left: 33.7 ± 9.3 kg), whereas female participants on average were classified as fair (24.1 ± 9.6 cm) in sit-and-reach and also as poor in upper limbs strength (right: 22.9 ± 4.4 kg, left: $19{,}1 \pm 5.0$ kg) [8]. Thus, the physical profile of the participants may suggest that, on average, they were healthy adults, but did not regularly engage in physical activity.

Indeed, the results of the short form of the International Physical Activity Questionnaire (sf-IPAQ) showed that only three participants practice vigorous physical activity at least once a week, that is, activities that require hard physical effort and make breathing much harder than normal. Furthermore, only 10 participants reported that they practice moderate activities, that is activities that take moderate physical effort and make breathing somewhat harder than normal, for an average of 2.4 days per week, and with an average duration of 53.9 minutes per day. However, all participants said they walked regularly, for an average of 7 days per week lasting, on average, 83.3 minutes per day. These questionnaire results corroborate the findings described above regarding the physical profile of the participants.

Mental Activity Assessment

Although the mental activity session was only conducted to assess the effect of test-retest rather than the exact effect of mental activity, Table 3.8 contains all the findings from the tests developed throughout the 30 minutes.

Considering that not all participants completed every mental activity tasks and that it was essential to assess for the presence of depression in each individual, we focused our analysis on the BDI-II results. It is important to note that, as previously described in the methodology section, five participants were not taken into account for the analyses (P23, P24, P31, P34, and P37).

All participants used for analysis had ratings between 0 and 11 points, so they were all classified as no or minimal depression, as can be seen in Table 3.8.

Table 3.8: Descriptive results obtained in the mental activity intervention.

BDI-II Scores			
Absence of depression (0 - 9)	Mild to moderate depression (10-18)	Moderate to severe depression (19-29)	Severe depression (30-63)
14	1	0	0

3.2.2 Heart Rate Modulation During Physical and Mental Activity Sessions

Heart Rate was continuously monitored during the thirty minutes of both physical and mental activity interventions using a *Polar V800 GPS sports watch* (Polar Electro Oy, Kempele, Finland).

The mean heart rate during the last 25 minutes of the intervention was 76 ± 10 bpm for the mental session and 125 ± 14 bpm for the physical session. The first 5

minutes corresponded to periods of adaptation. Figure 3.9 presents some examples of heart rate variation over the 30 minutes of both interventions.

For the statistical analysis, participants who had already been previously removed from the other analyses were not used again (P23, P24, P31, and P37). Participants whose measurements were not both correctly acquired were also excluded. Therefore, statistical analysis comparing the two sessions was only performed with ten participants. As predicted, the paired samples t-test revealed that the difference between heart rate for physical and mental sessions was statistically significant [effect of session: t = *-8.591*, $p < 0.001$], with physical activity associated with much higher heart rate values than mental activity, suggesting that exercise induced the expected cardiac effect.

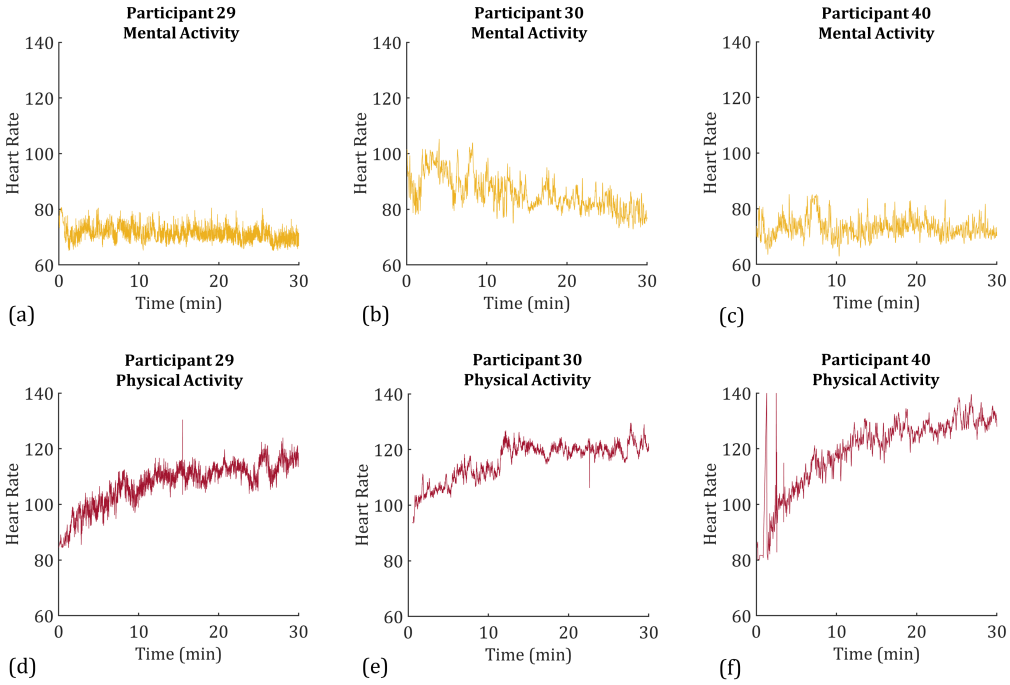

Figure 3.9: Examples of heart rate data.

3.2.3 Behavioral Results

Accuracy

Accuracy was not significantly modulated by physical activity. The GLMM analyses showed that there were no significant effects, neither session [effect of session: $F_{(1,\ 11386)} = 0.043$, $p = 0.835$], nor moment [effect of moment: $F_{(1,\ 11386)} = 0.024$, $p = 0.877$], nor 2- or 3-way interactions that include session and moment variables.

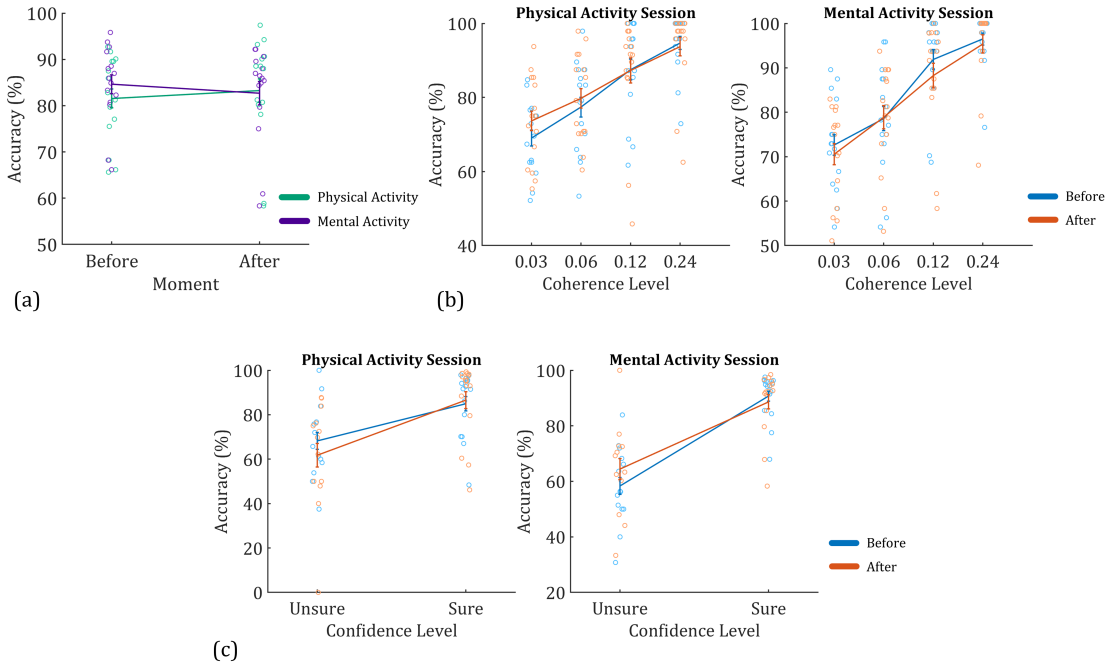

Figure 3.10: Accuracy as a function of (a) session and moment; (b) coherence level, session, and moment; (c) confidence level, session, and moment. Circles represent individual data points from each participant and line graphs represent mean ± standard error of the mean.

Table 3.9: GLMM statistical analysis of accuracy.

Variable	F	p
Session	$F_{(1,\ 11386)} = 0.043$	0.835
Moment	$F_{(1,\ 11386)} = 0.024$	0.877
Order	$F_{(1,\ 11386)} = 0.152$	0.696
Coherence	$F_{(3,\ 11386)} = 34.292$	0.000*
Confidence	$F_{(1,\ 11386)} = 1.482$	0.223
Session x Moment	$F_{(1,\ 11386)} = 0.032$	0.857
Session x Order	$F_{(1,\ 11386)} = 0.064$	0.800
Session x Coherence	$F_{(3,\ 11386)} = 0.533$	0.660
Session x Confidence	$F_{(1,\ 11386)} = 0.001$	0.975
Moment x Order	$F_{(1,\ 11386)} = 0.129$	0.719
Moment x Coherence	$F_{(3,\ 11386)} = 0.745$	0.525
Moment x Confidence	$F_{(1,\ 11386)} = 0.109$	0.741
Session x Moment x Order	$F_{(1,\ 11386)} = 0.016$	0.900
Session x Moment x Coherence	$F_{(3,\ 11386)} = 0.274$	0.844
Session x Moment x Confidence	$F_{(1,\ 11386)} = 0.125$	0.724

Confidence Level

We found no significant effect of physical exercise on the percentage of unsure responses. Again, there were also no 2-way interactions between session and moment [interaction session x moment: $F_{(1,\ 11386)} = 0.113$, $p = 0.737$], or 3-way interactions between session, moment, and coherence level [interaction session x moment x coherence level: $F_{(1,\ 11386)} = 0.104$, $p = 0.958$], or session, moment, and accuracy [interaction session x moment x accuracy: $F_{(1,\ 11386)} = 0.138$, $p = 0.711$]. Indeed, the results were very similar for both moments and both sessions (see Figure 3.11).

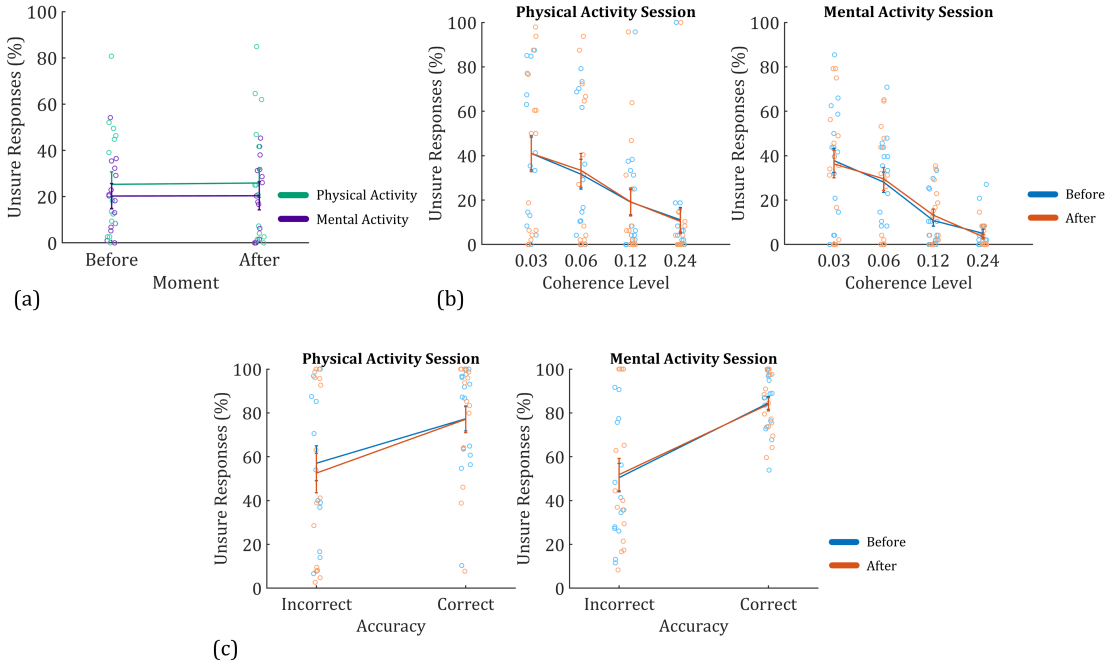

Figure 3.11: Percentage of unsure as a function of (a) session and moment; (b) coherence level, session, and moment; (c) confidence level, session, and moment. Circles represent individual data points from each participant and line graphs represent mean ± standard error of the mean.

Table 3.10: GLMM statistical analysis of confidence level.

Variable	F	p
Session	$F_{(1,\ 11386)} = 0.392$	0.531
Moment	$F_{(1,\ 11386)} = 0.094$	0.759
Order	$F_{(1,\ 11386)} = 0.292$	0.589
Coherence	$F_{(3,\ 11386)} = 48.113$	0.000*
Accuracy	$F_{(1,\ 11386)} = 1.462$	0.227
Session x Moment	$F_{(1,\ 11386)} = 0.113$	0.737
Session x Order	$F_{(1,\ 11386)} = 0.835$	0.361
Session x Coherence	$F_{(3,\ 11386)} = 1.686$	0.168
Session x Accuracy	$F_{(1,\ 11386)} = 0.067$	0.796
Moment x Order	$F_{(1,\ 11386)} = 0.084$	0.772
Moment x Coherence	$F_{(3,\ 11386)} = 0.325$	0.807
Moment x Accuracy	$F_{(1,\ 11386)} = 0.106$	0.745
Session x Moment x Order	$F_{(1,\ 11386)} = 0.114$	0.735
Session x Moment x Coherence	$F_{(3,\ 11386)} = 0.104$	0.958
Session x Moment x Accuracy	$F_{(1,\ 11386)} = 0.138$	0.711

Reaction Time

Physical exercise did not have any strong effect on reaction time. The results yielded an effect of session [effect of session: $F_{(1,\ 11323)} = 58.684$, $p < 0.001$] and an effect of moment [effect of moment: $F_{(1,\ 11323)} = 14.663$, $p < 0.001$], but not an interaction between session and moment [interaction session x moment: $F_{(1,\ 11323)} = 1.682$, $p = 0.195$].

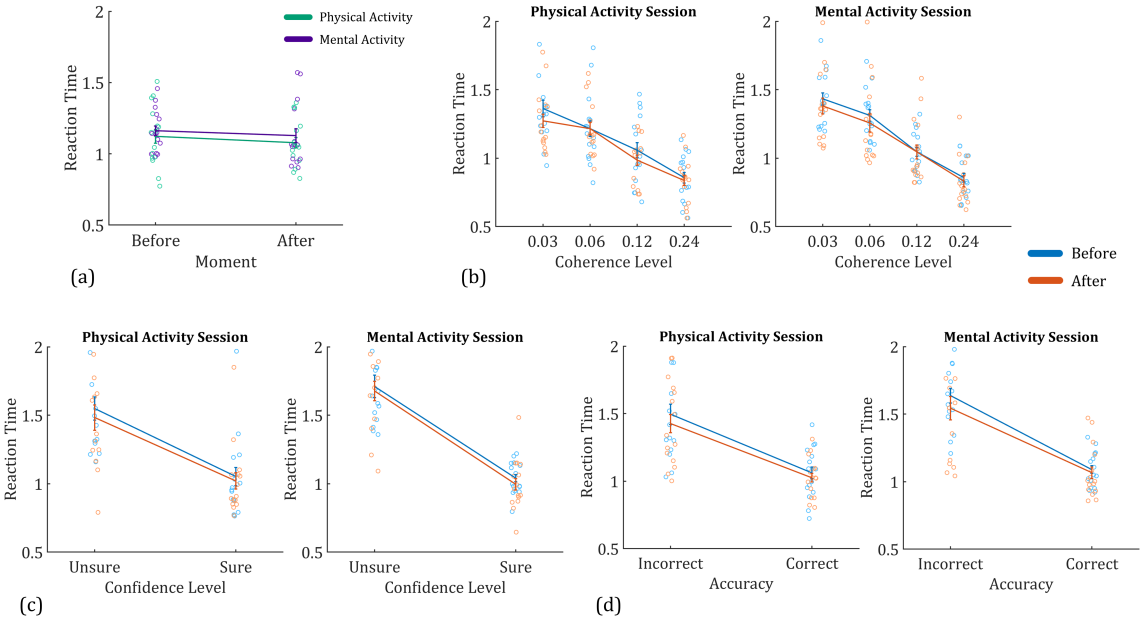

Figure 3.12: Reaction time as a function of (a) session and moment; (b) coherence level, session, and moment; (c) confidence level, session, and moment; (d) accuracy, session, and moment. Circles represent individual data points from each participant and line graphs represent mean ± standard error of the mean.

Table 3.11: LMM statistical analysis of reaction time.

Variable	F	p
Session	$F_{(1,\ 11323)} = 58.684$	< 0.001*
Moment	$F_{(1,\ 11323)} = 14.663$	< 0.001*
Order	$F_{(1,\ 15.954)} = 0.682$	0.421
Coherence	$F_{(3,\ 11323)} = 52.296$	< 0.001*
Confidence	$F_{(1,\ 11323)} = 490.020$	< 0.001*
Accuracy	$F_{(1,\ 11323)} = 234.647$	< 0.001*
Session x Moment	$F_{(1,\ 11323)} = 1.682$	0.195
Session x Order	$F_{(1,\ 11323)} = 0.373$	0.542
Session x Coherence	$F_{(3,\ 11323)} = 0.201$	0.896
Session x Confidence	$F_{(1,\ 11323)} = 15.929$	< 0.001*
Session x Accuracy	$F_{(1,\ 11323)} = 4.025$	0.045*
Moment x Order	$F_{(1,\ 11323)} = 0.065$	0.799
Moment x Coherence	$F_{(3,\ 11323)} = 0.719$	0.540
Moment x Confidence	$F_{(1,\ 11323)} = 1.606$	0.205
Moment x Accuracy	$F_{(1,\ 11323)} = 0.234$	0.628
Session x Moment x Order	$F_{(1,\ 11323)} = 1.150$	0.283
Session x Moment x Coherence	$F_{(3,\ 11323)} = 7.830$	< 0.001*
Session x Moment x Confidence	$F_{(1,\ 11323)} = 4.742$	0.029*
Session x Moment x Accuracy	$F_{(1,\ 11323)} = 0.089$	0.765

The analysis revealed a significant 3-way interaction between session, moment, and coherence level [interaction session x moment x coherence level: $F_{(3,\ 11323)} = 7.830$, $p < 0.001$]. To explore this interaction, we split the data into the four coherences and performed the LMM again. The results revealed a contradictory effect, given that for the intermediate coherences, in one coherence the effect between session and moment was larger for mental activity than for physical activity [coherence 0.06 - interaction session x moment: $F_{(1,\ 2827)} = 11.285$, $p < 0.001$], however in the other intermediate coherence the same effect was larger for physical activity than for mental activity [coherence 0.12 - interaction session x moment: $F_{(1,\ 2831)} = 7.980$, $p = 0.005$] (see Figure 3.12b). For other coherences, this 2-way interaction was not significant [coherence 0.03 - interaction session x moment: $F_{(1,\ 2819)} = 1.959$, $p = 0.162$; coherence 0.24 - interaction session x moment: $F_{(1,\ 2846)} = 1.365$, $p = 0.243$]. There was also an interaction session x moment x confidence level [$F_{(1,\ 11323)} = 4.742$, $p = 0.029$]. We divided the data into the two confidence levels and found that the interaction between session and moment was only marginally significant for unsure [unsure - interaction session x moment: $F_{(1,\ 2580)} = 2.799$, $p = 0.094$], but not for sure responses [sure - interaction session x moment: $F_{(1,\ 8743)} = 1.496$, $p = 0.221$]. Therefore, we need to be careful in over interpreting these two 3-way interactions.

3.2.4 Pupillary Responses

Pupil response before feedback

We found no significant effect of physical exercise on the pupillary responses locked with the motor responses. In general, the pupil response amplitude before feedback was slightly lower in the physical activity session than in the mental activity session [effect of session: $F_{(1,\ 8614)} = 4.322$, $p = 0.038$], particularly after physical activity (as can be seen in Figure 3.13a). But the effect of the testing moment was not statistically significant [effect of moment: $F_{(1,\ 8614)} = 2.049$, $p = 0.152$]. The interaction between session and moment was also not statistically significant [interaction session x moment: $F_{(1,\ 8614)} = 0.001$, $p = 0.981$], and no other 2- or 3-way interactions which included the session and moment variables were found.

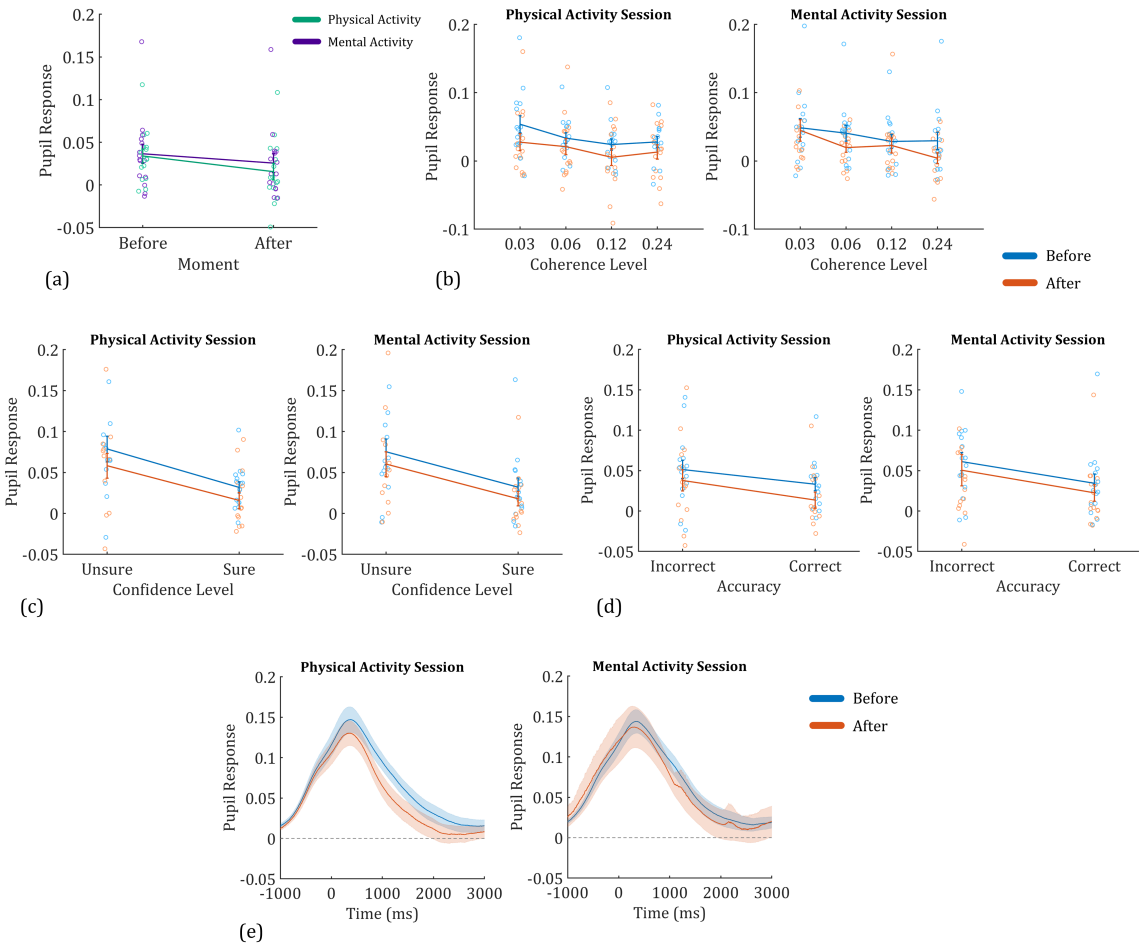

Figure 3.13: (a,b,c,d): Average amplitude of pupil response in the time window from 1s up to 3s after response as a function of (a) session and moment; (b) coherence level, session, and moment; (c) confidence level, session, and moment; (d) accuracy, session, and moment. Circles represent individual data points from each participant and line graphs represent mean ± standard error of the mean. (e): Representation of the 2-way interaction between session and moment of the time course of the pupil response locked with response onset.

Table 3.12: LMM statistical analysis of pupil response before feedback.

Variable	F	p
Session	$F_{(1,\ 8614)} = 4.322$	0.038*
Moment	$F_{(1,\ 8614)} = 2.049$	0.152
Order	$F_{(1,\ 17.546)} = 1.438$	0.246
Coherence	$F_{(3,\ 8614)} = 1.436$	0.230
Confidence	$F_{(1,\ 8614)} = 17.915$	< 0.001*
Accuracy	$F_{(1,\ 8614)} = 5.613$	0.018*
Session x Moment	$F_{(1,\ 8614)} = 0.001$	0.981
Session x Order	$F_{(1,\ 8614)} = 3.812$	0.051
Session x Coherence	$F_{(3,\ 8614)} = 1.292$	0.275
Session x Confidence	$F_{(1,\ 8614)} = 0.355$	0.552
Session x Accuracy	$F_{(1,\ 8614)} = 1.043$	0.307
Moment x Order	$F_{(1,\ 8614)} = 2.018$	0.155
Moment x Coherence	$F_{(3,\ 8614)} = 0.645$	0.586
Moment x Confidence	$F_{(1,\ 8614)} = 1.830$	0.176
Moment x Accuracy	$F_{(1,\ 8614)} = 1.070$	0.301
Session x Moment x Order	$F_{(1,\ 8614)} = 0.021$	0.885
Session x Moment x Coherence	$F_{(3,\ 8614)} = 0.395$	0.757
Session x Moment x Confidence	$F_{(1,\ 8614)} = 0.027$	0.869
Session x Moment x Accuracy	$F_{(1,\ 8614)} = 2.211$	0.137

Pupil response after feedback

The time course of pupil response after feedback revealed similar pupil variations for both physical and mental activities and for both moments of each session.

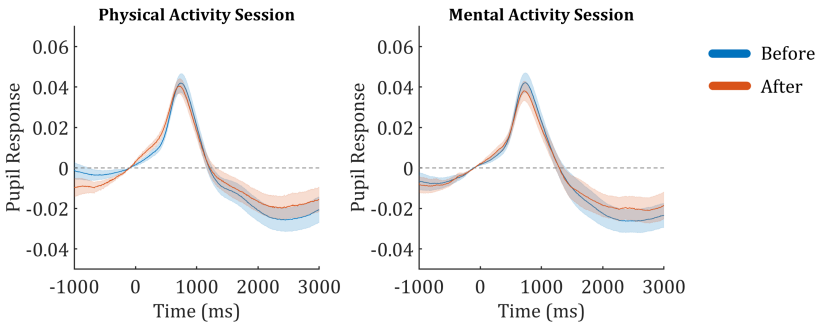

Figure 3.14: Time course of pupil response locked with feedback onset.

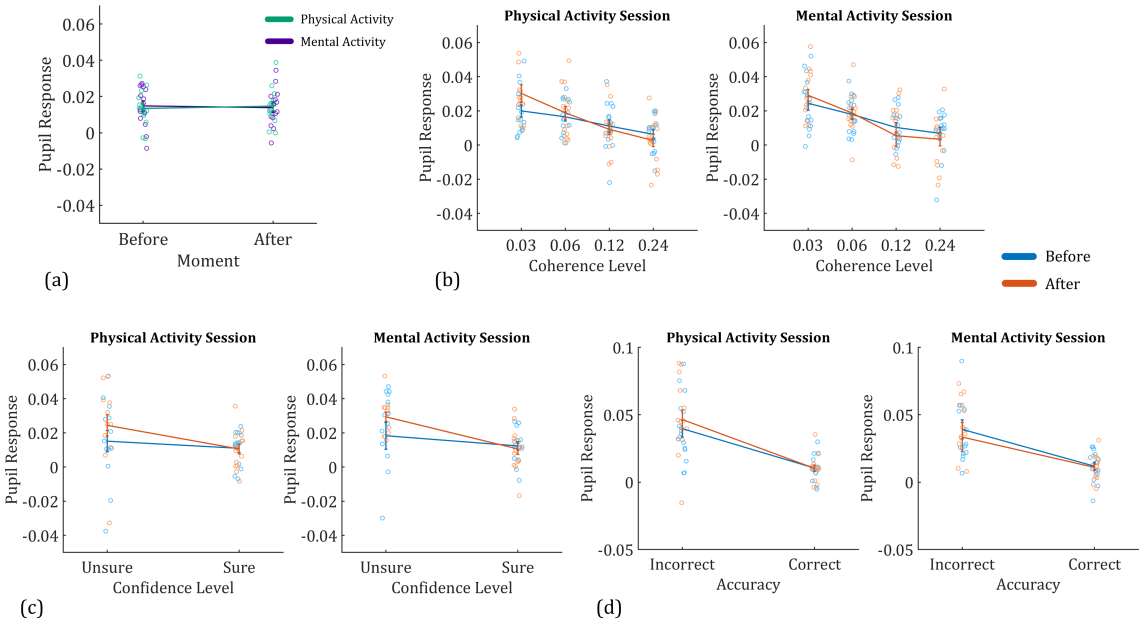

Figure 3.15: (a,b,c,d): Average amplitude of pupil response evoked by feedback in the time window from 0s up to 1.5s after feedback as a function of (a) session and moment; (b) coherence level, session, and moment; (c) confidence level, session, and moment; (d) accuracy, session, and moment. Circles represent individual data points from each participant and line graphs represent mean ± standard error of the mean.

Similar to what was done for pupil response after feedback when comparing young and older adults, the statistical analysis was developed for two different moments (early response from zero to one and half seconds after feedback, and late response from one and half to three seconds after feedback).

In the earlier period, there was no significant effect of session [effect of session: $F_{(1,\ 8793)} = 0.311$, $p = 0.577$], nor of moment [effect of moment: $F_{(1,\ 8793)} = 3.759$, $p = 0.053$], although the latter was marginally significant. It was only verified an interaction between moment and accuracy [interaction moment x accuracy: $F_{(1,\ 8793)} = 7.684$, $p = 0.006$].

In the later time window, opposite to pupil response before feedback and to the earlier period of pupil response after feedback, there was an effect of moment [effect of moment: $F_{(1,\ 8793)} = 4.532$, $p = 0.033$]. Both activities elicited higher pupil response after the interventions than before (see Figure 3.16a). Pupil response was also dependent on the interaction between moment and coherence [interaction moment x coherence: $F_{(1,\ 8793)} = 2.641$, $p = 0.048$], and on the interaction between moment and accuracy [interaction moment x accuracy: $F_{(1,\ 8793)} = 3.841$, $p = 0.050$]. However, there was no statistically significant effect of session [effect of

session: $F_{(1,\ 8793)} = 0.093$, $p = 0.761$], nor other interactions which included session or moment variables.

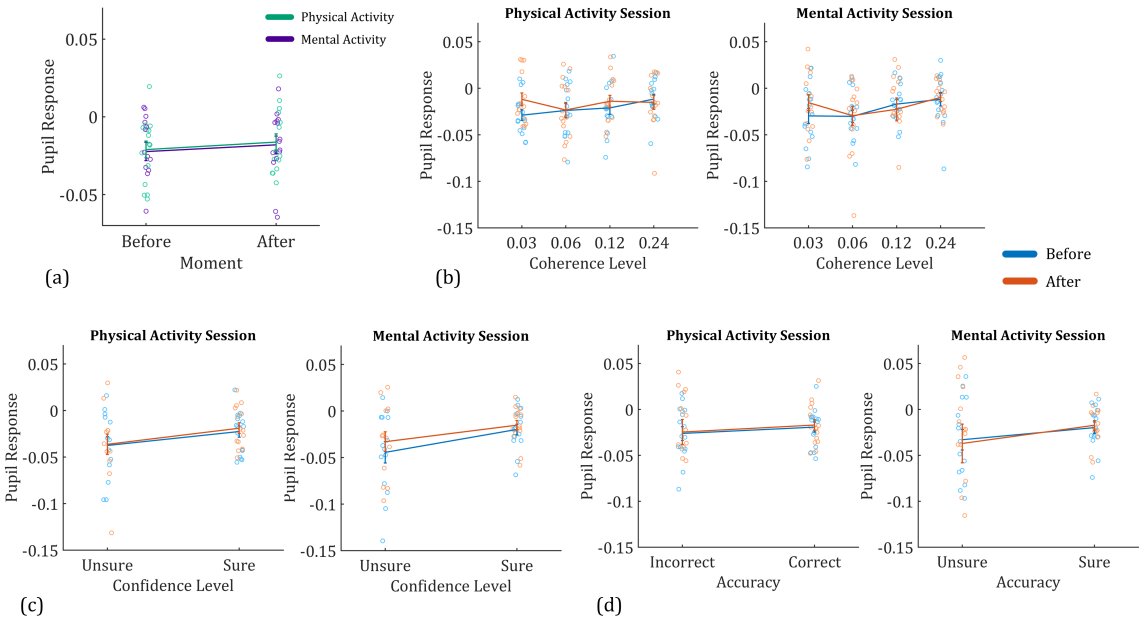

Figure 3.16: (a,b,c,d): Average amplitude of pupil response evoked by feedback in the time window from 1.5s up to 3s after feedback as a function of (a) session and moment; (b) coherence level, session, and moment; (c) confidence level, session, and moment; (d) accuracy, session, and moment. Circles represent individual data points from each participant and line graphs represent mean ± standard error of the mean.

Table 3.13: LMM statistical analysis of pupil response in the time window between 0 and 1.5s after feedback.

Variable	F	p
Session	$F_{(1,\ 8793)} = 0.311$	0.577
Moment	$F_{(1,\ 8793)} = 3.759$	0.053
Order	$F_{(1,\ 29.961)} = 0.136$	0.715
Coherence	$F_{(3,\ 8793)} = 7.487$	< 0.001*
Confidence	$F_{(1,\ 8651)} = 6.403$	0.011*
Accuracy	$F_{(1,\ 8793)} = 75.676$	< 0.001*
Session x Moment	$F_{(1,\ 8793)} = 0.609$	0.435
Session x Order	$F_{(1,\ 8793)} = 0.279$	0.598
Session x Coherence	$F_{(3,\ 8793)} = 0.115$	0.952
Session x Confidence	$F_{(1,\ 8793)} = 0.179$	0.672
Session x Accuracy	$F_{(1,\ 8793)} = 0.531$	0.466
Moment x Order	$F_{(1,\ 8793)} = 3.033$	0.082
Moment x Coherence	$F_{(3,\ 8793)} = 0.497$	0.684
Moment x Confidence	$F_{(1,\ 8793)} = 1.719$	0.190
Moment x Accuracy	$F_{(1,\ 8793)} = 7.684$	0.006*
Session x Moment x Order	$F_{(1,\ 8793)} = 1.074$	0.300
Session x Moment x Coherence	$F_{(3,\ 8793)} = 0.572$	0.633
Session x Moment x Confidence	$F_{(1,\ 8793)} = 0.144$	0.704
Session x Moment x Accuracy	$F_{(1,\ 8793)} = 0.007$	0.934

Table 3.14: LMM statistical analysis of pupil response in the time window between 0 and 1.5s after feedback.

Variable	F	p
Session	$F_{(1,\ 8793)} = 0.093$	0.761
Moment	$F_{(1,\ 8793)} = 4.532$	0.033*
Order	$F_{(1,\ 20.421)} = 0.125$	0.727
Coherence	$F_{(3,\ 8793)} = 4.363$	0.004*
Confidence	$F_{(1,\ 8793)} = 0.830$	0.362
Accuracy	$F_{(1,\ 8793)} = 0.014$	0.907
Session x Moment	$F_{(1,\ 8793)} = 0.040$	0.841
Session x Order	$F_{(1,\ 8793)} = 3.141$	0.076
Session x Coherence	$F_{(3,\ 8793)} = 0.757$	0.518
Session x Confidence	$F_{(1,\ 8793)} = 1.342$	0.247
Session x Accuracy	$F_{(1,\ 8793)} = 0.324$	0.569
Moment x Order	$F_{(1,\ 8793)} = 0.504$	0.478
Moment x Coherence	$F_{(3,\ 8793)} = 2.641$	0.048*
Moment x Confidence	$F_{(1,\ 8793)} = 0.419$	0.517
Moment x Accuracy	$F_{(1,\ 8793)} = 3.841$	0.050*
Session x Moment x Order	$F_{(1,\ 8793)} = 0.819$	0.366
Session x Moment x Coherence	$F_{(3,\ 8793)} = 1.419$	0.235
Session x Moment x Confidence	$F_{(1,\ 8793)} = 1.023$	0.312
Session x Moment x Accuracy	$F_{(1,\ 8793)} = 0.140$	0.708

3.2.5 Learning effect between sessions

As explained in subsection 2.1 of the Methodology chapter, the visits were carried out at least two months apart to reduce the amount of task knowledge that was acquired from one visit to the next. Nevertheless, to understand whether there was a learning effect between sessions, the performance in the first moment of the first visit was compared with the performance in the first moment of the second visit for all variables under study (accuracy, confidence level, reaction time, and pupil response amplitude before and after feedback).

Statistical analysis revealed a significant effect of session for almost all variables [accuracy: $F_{(1,\ 5717)} = 22.029$, $p < 0.001$; confidence level: $F_{(1,\ 5717)} = 83.966$, $p < 0.001$; reaction time: $F_{(1,\ 5710.006)} = 59.154$, $p < 0.001$; pupil response before feedback: $F_{(1,\ 5588.003)} = 59.678$, $p < 0.001$]. The effect of session is not statistically significant only for pupil response after feedback.

Overall, there was an improvement from the first to the second visit in all variables, as can be seen in Figure 3.17. These findings suggest that there may have been a learning effect that might have interfered with the outcomes. However, the

fact that visit orders were counterbalanced among participants may have attenuated these possible negative effects.

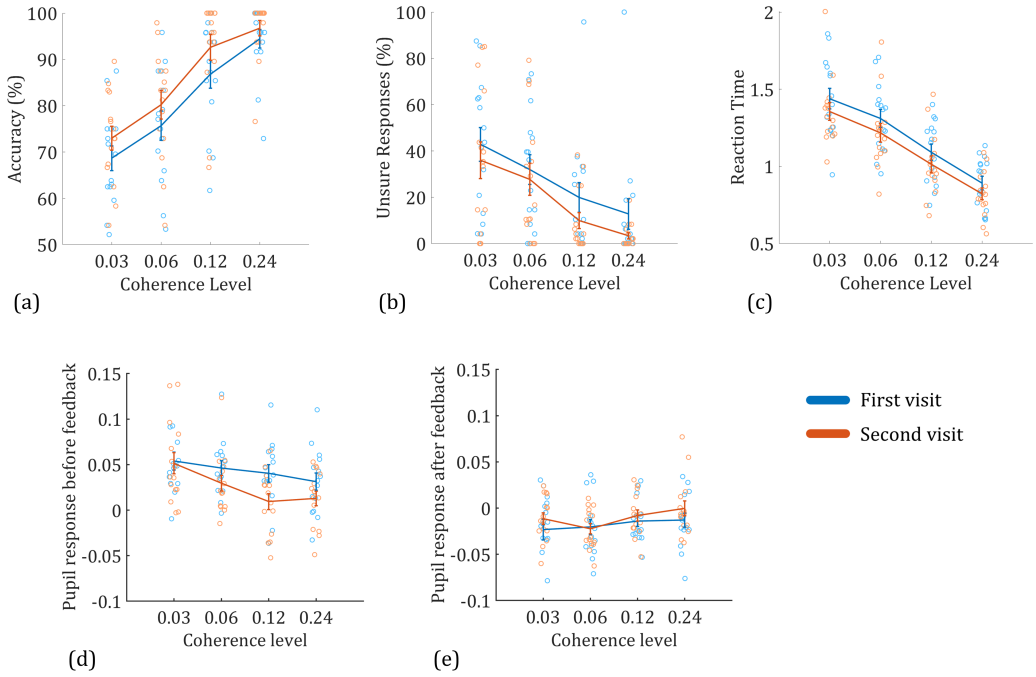

Figure 3.17: Comparison between the first moment of each visit. (a) Accuracy; (b) Percentage of unsure; (c) Reaction time; (d) Pupil response before feedback; (e) Pupil response after feedback.

4

Discussion

The present experiment used a visual motion perception task to understand the relationship between pupil-linked arousal, uncertainty processing, and error processing, and how these mechanisms are affected by aging and physical exercise. Our findings suggest that uncertainty processing is changed in older adults, as older people showed higher confidence levels for incorrect responses and higher confidence levels for more difficult stimuli than young adults. Notably, the analyses of the pupillary responses suggest that the recruitment of the arousal system associated with uncertainty is also affected in older people. In particular, pupil responses evoked by feedback showed reduced modulation with response confidence in older adults. Although physical activity has been shown to affect pupil responses in cognitive tasks, our results did not show any significant effect after 30 minutes of aerobic physical activity in older adults.

Effect of Aging

Increased stimulus evidence yielded higher accuracy, lower uncertainty ratings, and lower reaction times. These patterns are consistent with earlier research using also perceptual decision-making tasks [116, 125]. As expected, confident responses had higher accuracy than low confident responses, or, put another way, the percentage of unsure responses was lower for correct trials when compared with incorrect trials. Nevertheless, in older adults, this link between response confidence and accuracy was considerably smaller, which means that older adults are more likely to report confidence in an incorrect response. The lower number of low-confidence responses for the lowest coherence exhibited by older adults supports these findings. In sum, in older people, high-confidence responses were associated with lower accuracy, and the effect of stimulus strength and accuracy on response confidence was diminished. Moreover, older adults had lower task accuracy, and this difference increased for lower coherences, which could reflect difficulty in visual motion perception, particularly in more difficult motion levels.

In general, contrary to what was evidenced by Niessen et al (2017) [104], older participants responded as fast as young participants. However, interestingly, older adults were slightly slower when they reported high confidence in their responses, which also suggests that their high-confidence responses might be less confident than the young adults high-confidence responses. This is in accordance with the fact that they make more mistakes even when they are sure about their response. These results may indicate changes in brain processing in older adults suggesting an increased noise in their sensory system or changes in the sure/unsure threshold, leading older adults to report more confidence in their responses.

In line with previous studies [116, 133], reaction time presented all three signatures of decision uncertainty reported in Section 1.7.1 of the Introduction chapter. That is, first, reaction time tended to decrease with evidence strength for correct trials, but for incorrect trials, this effect was not verified. Further, participants were faster when they were sure about their responses, and slower reaction times were associated with lower accuracies. However, when comparing reaction times as a function of correct and incorrect trials for young and older adults, while for young participants reaction time decreased with increasing stimulus evidence only for the correct trials, for older participants this decrease happened for both correct and incorrect answers. However, this effect was not statistically significant.

It has long been known that small fluctuations in the arousal state, indexed by variations in pupil diameter, are frequent during cognitive tasks. Our findings suggest that pupil responses are modulated by decision uncertainty and error monitoring, which had already been reported by previous studies [1, 116, 208]. Indeed, we found that, for both groups of participants, pupil amplitude between the decision and feedback was larger for incorrect trials than for correct trials, and larger for unsure responses than for sure responses. However, for young participants sure incorrect responses presented a higher pupil dilation than the unsure incorrect responses, which could mean that, even before feedback, these participants already know (consciously or unconsciously) the accuracy of their high-confidence responses. However, in older adults, this effect was markedly diminished.

Concerning feedback-related pupil responses, the results revealed two different phases: an initial pupil dilation and a later pupil constriction. The early pupil dilation was higher for negative feedback and unsure responses. However, both effects were diminished in older adults. In the later phase of the pupil responses, we observed a rapid constriction, particularly for older adults, in which incorrect and unsure responses reached more negative values than correct and sure responses.

In brief, before and after incorrect feedback, young adults elicited larger and

longer-lasting pupil dilations when they reported that they were sure about their responses, which is not verified for older adults. These findings, along with what has been described for accuracy, percentage of unsure, and reaction time, corroborate the idea that older adults exhibit a reduced activation of the arousal system in response to uncertainty and to errors. This observation might be related to impaired error processing and impaired error awareness [92, 93, 95].

Effect of Physical Activity in Older Adults

Given these decreases in reduced activation of the arousal system associated with uncertainty and error processing revealed with aging, which can lead to impaired decision-making processes, the present study also aimed to investigate the extent to which a single bout of exercise induces changes in pupil-linked arousal systems, measured by changes in pupil response, and to what extent this could be considered as an attenuating factor of this age-related decline.

We did not find any strong effect of physical activity on reaction time, contrary to what has been observed in other studies [173, 175, 209]. Our study showed slightly shorter reaction times after both conditions, which suggests that there was a considerable effect of test-retest. In fact, even after the mental activity session, there was a decrease in reaction time, which was not verified in the other studies that also included a control session [175, 209].

While the previous studies reported higher pupil-linked arousal, which is a marker of LC-NE activation, by showing decreased tonic pupil size and increased task-evoked pupil dilations after physical activity [173, 174], our findings revealed that exercise did not induce any differences on task-evoked pupillometric measures. Therefore, in contrast to our a priori hypothesis that acute bouts of physical activity enhance task-evoked pupillary responses, neither the physical activity nor the mental activity modulated pupil response, and, consequently, the phasic activity in the LC. The inclusion of an active control condition, which showed a test-retest effect, was important to separate the effects of retest from the true effects of the intervention.

In brief, the current findings provide preliminary evidence that moderate aerobic physical activity does not induce significant changes in reaction time, nor in the activation of the LC nor in the modulation of the arousal system. However, our results do not exclude the possible role of the brainstem arousal systems in this relationship between physical activity and cognition. In fact, few studies have been conducted in this field, and further research is needed to understand the extent to which physical activity induces changes in the arousal system in the human

brain, and how this could be correlated with increases in the cognitive mechanisms underlying processing uncertainty and error awareness.

Study Limitations

We recognize that our study had several limitations worth considering. First, the sample size was relatively small, and, due to dropouts and outliers, the number of participants across groups was different. This could have limited the ability to detect group differences. Additionally, the reduced sample size was also an issue for pupillary measurement analyses, as some participants had some unusable data due to eye blinking, saccades, and other challenges that arise when collecting eye-tracking data. Therefore, future research to test these hypotheses using larger samples of both young and older adults is warranted. Second, the small number of erroneous responses relative to correct answers given by the majority of participants may also have influenced the findings. However, as explained before, we resorted to statistical analysis by LMM to minimize this possible interference, given that this model allows dealing with an unequal number of repetitions for the various conditions, within and between participants.

Aiming to increase the statistical power, our study was designed in a way that each participant completed a significant number of trials. So, each participant performed 192 trials before and 192 trials after the experimental condition, comprising a total of 384 trials at each visit. Thus, given the 8-14 second duration of each trial, this likely induced some fatigue over the course of the experiment.

Another limitation was related to task-evoked pupillary responses, given that the pupil reacts slowly, the reduced duration of the stimulus could have attenuated these responses. Meantime, auditory signals (that indicate the moment of the stimulus and the response feedback) seemed to give also a stimulus that could have influenced the pupillary responses.

The second moment of the task was carried out soon after the experimental condition. So, it is unclear what could happen across another post-exercise period. Future work should develop the task and assess the pupil responses across a range of post-exercise intervals to determine if there is a time frame, or what is the best time frame, at which a single bout of physical activity modulates pupil amplitude, and, consequently, the LC-NE activity.

Lastly, another limitation of our study is the learning effect that exists from one visit to the next. Effectively, despite the two-month gap between the experimental sessions, participants improved their performance in all variables from the first to the second experimental condition, which was not desired. Therefore, anticipating

the learning effect, the sessions were counterbalanced between participants, that is, half of the participants performed one of the experimental conditions first and the other half the other condition (as explained in Figure 2.1). Even so, it is possible that this effect had a significant impact on the outcomes and a follow-up study should include another session before the experimental sessions, only for training, to ensure that the participants were doing their best in the first session.

5

Conclusions

The present study aimed to examine the impact of aging on the recruitment of the arousal system associated with response uncertainty and its interaction with feedback processing. As the existing literature reported that physical activity activates the arousal system, we also aimed to test whether a 30-minute single bout of moderate aerobic physical activity via a cycle ergometer at 70% of participants VO_2max could improve the ability of older people in uncertainty processing, error awareness, by increasing the arousal system activity.

Results from the first part of the experiment presented solid evidence that older adults showed impaired regulation of the arousal system induced by response uncertainty and its interaction with response accuracy and response feedback, probably meaning that the arousal system modulation is strongly affected by the aging process. Corroborating the outcomes of earlier research, these findings support our first hypothesis that aging affects task-evoked uncertainty processing and error awareness. Nevertheless, according to previous evidence, it would be expected that physical activity would have an effect on arousal system modulation. Given that the results revealed that physical activity did not influence pupil modulation, our main hypothesis was rejected. Thereby, these findings suggest that there was no clear effect of exercise on task performance and that the activation of the arousal system is not a mechanism underlying exercise-induced improvements in cognition

Accordingly, to overcome the referred limitations, future work should include a larger number of participants in each group and a training session before the experimental sessions, aiming to have the best of the participants in both sessions and, therefore, minimizing the learning effect. Moreover, in future research, it is important to examine the potential influence of single bouts of physical activity on the arousal system and the LC-NE system activity measured by tonic pupil size and by other parameters different than pupillary responses that are sensitive to other aspects of the arousal response.

Bibliography

[1] O. Colizoli, J. Gee, A. Urai, and T. Donner, "Task-evoked pupil responses reflect internal belief states," *Scientific Reports*, vol. 8, 07 2018.

[2] S. M. A. Juan and P. A. Adlard, *Ageing and Cognition*, pp. 107–122. Singapore: Springer Singapore, 2019.

[3] Y. Hou, X. Dan, M. Babbar, Y. Wei, S. G. Hasselbalch, D. L. Croteau, and V. A. Bohr, "Ageing as a risk factor for neurodegenerative disease," *Nature reviews. Neurology*, vol. 15, p. 565—581, 10 2019.

[4] D. Murman, "The impact of age on cognition," *Seminars in Hearing*, vol. 36, pp. 111–121, 08 2015.

[5] D. Henninger, D. Madden, and S. Huettel, "Processing speed and memory mediate age-related differences in decision making," *Psychology and aging*, vol. 25, pp. 262–70, 06 2010.

[6] D. Gallardo-Gómez, J. del Pozo-Cruz, M. Noetel, F. Álvarez Barbosa, R. M. Alfonso-Rosa, and B. del Pozo Cruz, "Optimal dose and type of exercise to improve cognitive function in older adults: A systematic review and bayesian model-based network meta-analysis of rcts," *Ageing Research Reviews*, vol. 76, p. 101591, 2022.

[7] B. Winter, C. Breitenstein, F. C. Mooren, K. Voelker, M. Fobker, A. Lechtermann, K. Krueger, A. Fromme, C. Korsukewitz, A. Floel, and S. Knecht, "High impact running improves learning," *Neurobiology of Learning and Memory*, vol. 87, no. 4, pp. 597–609, 2007.

[8] A. C. of Sports Medicine, D. Riebe, J. Ehrman, G. Liguori, and M. Magal, *ACSM's Guidelines for Exercise Testing and Prescription*. American College of Sports Medicine Series, Wolters Kluwer, 2018.

[9] E. Glisky, *Changes in Cognitive Function in Human Aging*. 04 2007.

[10] J. Marmeleira, L. Galhardas, and A. Raimundo, "Exercise merging physical and cognitive stimulation improves physical fitness and cognitive functioning

in older nursing home residents: A pilot study," *Geriatric Nursing*, vol. 39, 12 2017.

[11] D. Blazer, K. Yaffe, and J. Karlawish, "Cognitive aging: A report from the institute of medicine," *JAMA*, vol. 313, 04 2015.

[12] C. Harada, M. Natelson Love, and K. Triebel, "Normal cognitive aging," *Clinics in geriatric medicine*, vol. 29, pp. 737–752, 11 2013.

[13] M. D. Lezak, D. B. Howieson, E. D. Bigler, and D. Tranel, *Neuropsychological assessment*, vol. 5. Oxford University Press, 2012.

[14] N. Wecker, J. Kramer, A. Wisniewski, D. Delis, and E. Kaplan, "Age effects on executive ability," *Neuropsychology*, vol. 14, pp. 409–14, 07 2000.

[15] A. G. Sanfey and R. Hastie, *Judgment and decision making across the adult life span: A tutorial review of psychological research.*, p. 253–273. Psychology Press, 1 ed., 2000.

[16] S. Kemper and K. Kemtes, *Aging and message production and comprehension*, pp. 229–244. Psychology Press, 1 ed., 01 1998.

[17] A. Wingfield and E. Stine-Morrow, *Language and speech*, p. 359–416. Lawrence Erlbaum Associates Publishers, 2 ed., 2000.

[18] A. Fjell and K. Walhovd, "Structural brain changes in aging: Courses, causes and cognitive consequences," *Reviews in the neurosciences*, vol. 21, pp. 187–221, 01 2010.

[19] D. H. Salat, J. A. Kaye, and J. S. Janowsky, "Prefrontal gray and white matter volumes in healthy aging and alzheimer disease.," *Archives of neurology*, vol. 56, no. 3, pp. 338–44, 1999.

[20] E. Dennis and P. Thompson, "Functional brain connectivity using fmri in aging and alzheimer's disease," *Neuropsychology review*, vol. 24, p. 49–62, 02 2014.

[21] J. Andrews-Hanna, A. Snyder, J. Vincent, C. Lustig, D. Head, M. Raichle, and R. Buckner, "Disruption of large-scale brain systems in advanced aging," *Neuron*, vol. 56, pp. 924–935, 01 2008.

[22] D. Head, R. L. Buckner, J. S. Shimony, L. E. Williams, E. Akbudak, T. E. Conturo, M. McAvoy, J. C. Morris, and A. Z. Snyder, "Differential Vulnerability of Anterior White Matter in Nondemented Aging with Minimal Acceleration in Dementia of the Alzheimer Type: Evidence from Diffusion Tensor Imaging," *Cerebral Cortex*, vol. 14, pp. 410–423, 04 2004.

[23] A. Coelho, H. Fernandes, R. Magalhaes, P. Moreira, P. Marques, J. Soares, L. Amorim, C. Portugal-Nunes, T. Castanho, N. Santos, and N. Sousa, "Sig-

natures of white-matter microstructure degradation during aging and its association with cognitive status," *Scientific Reports*, vol. 11, 02 2021.

[24] R. Terry and R. Katzman, "Life span and synapses: Will there be a primary senile dementia?," *Neurobiology of aging*, vol. 22, pp. 347–353, 05 2001.

[25] E. Pannese, "Morphological changes in nerve cells during normal aging," *Brain Structure & Function*, vol. 216, pp. 85–89, 06 2011.

[26] E. Masliah, M. E. Mallory, L. A. Hansen, R. M. DeTeresa, and R. D. Terry, "Quantitative synaptic alterations in the human neocortex during normal aging," *Neurology*, vol. 43, no. 1, pp. 192–197, 1993.

[27] R. Beardmore, R. Hou, A. Darekar, C. Holmes, and D. Boche, "The locus coeruleus in aging and alzheimer's disease: A postmortem and brain imaging review," *Journal of Alzheimer's Disease*, vol. 83, pp. 1–18, 07 2021.

[28] B. E. Jones, A. E. Halaris, M. McIlhany, and R. Y. Moore, "Ascending projections of the locus coeruleus in the rat. i. axonal transport in central noradrenaline neurons," *Brain research*, vol. 127, pp. 1–21, 05 1977.

[29] P. R. Mouton, B. Pakkenberg, H. J. G. Gundersen, and D. L. Price, "Absolute number and size of pigmented locus coeruleus neurons in young and aged individuals," *Journal of Chemical Neuroanatomy*, vol. 7, pp. 185–190, 1994.

[30] K. Schmidt, B. Bari, and V. Chokshi, "Locus coeruleus-norepinephrine: Basic functions and insights into parkinson's disease," *Neural Regeneration Research*, vol. 15, pp. 1006–1013, 06 2020.

[31] G. Aston-Jones and J. Cohen, "Adaptive gain and the role of the locus coeruleus-norepinephrine system in optimal performance," *The Journal of comparative neurology*, vol. 493, pp. 99–110, 12 2005.

[32] C. Varazzani, A. San-Galli, S. Gilardeau, and S. Bouret, "Noradrenaline and dopamine neurons in the reward/effort trade-off: a direct electrophysiological comparison in behaving monkeys," *The Journal of neuroscience: the official journal of the Society for Neuroscience*, vol. 35, pp. 7866–7877, 06 2015.

[33] G. Aston-Jones and B. Waterhouse, "Locus coeruleus: From global projection system to adaptive regulation of behavior," *Brain Research*, vol. 1645, 03 2016.

[34] J. McBurney-Lin, J. Lu, Y. Zuo, and H. Yang, "Locus coeruleus-norepinephrine modulation of sensory processing and perception: A focused review," *Neuroscience & Biobehavioral Reviews*, vol. 105, 06 2019.

[35] G. Aston-Jones and J. Cohen, "An integrative theory of locus coeruleus-norepinephrine function: Adaptive gain and optimal performance," *Annual review of neuroscience*, vol. 28, pp. 403–450, 02 2005.

[36] D. C. German, B. S. Walker, K. Manaye, W. K. Smith, D. J. Woodward, and

A. J. North, "The human locus coeruleus: computer reconstruction of cellular distribution," *The Journal of neuroscience: the official journal of the Society for Neuroscience*, vol. 8, pp. 1776–1788, 06 1988.

[37] M. Tomonaga, "Neuropathology of the locus ceruleus: a semi-quantitative study," *Journal of neurology*, vol. 230, no. 4, pp. 231–240, 1983.

[38] K. F. Manaye, D. D. McIntire, D. M. Mann, and D. C. German, "Locus coeruleus cell loss in the aging human brain: a non-random process," *The Journal of comparative neurology*, vol. 358, pp. 79–87, 07 1995.

[39] N. Vijayashankar and H. Brody, "A quantitative study of the pigmented neurons in the nuclei locus coeruleus and subcoeruleus in man as related to aging," *Journal of Neuropathology & Experimental Neurology*, vol. 38, pp. 490–497, 10 1979.

[40] H. Braak, D. Thal, E. Ghebremedhin, and K. Del Tredici, "Stages of the pathologic process in alzheimer disease: Age categories from 1 to 100 years," *Journal of neuropathology and experimental neurology*, vol. 70, pp. 960–9, 11 2011.

[41] R. S. Wilson, S. Nag, P. A. Boyle, L. P. Hizel, L. Yu, A. S. Buchman, J. A. Schneider, and D. A. Bennett, "Neural reserve, neuronal density in the locus ceruleus, and cognitive decline," *Neurology*, vol. 80, pp. 1202 – 1208, 2013.

[42] S. Kelly, B. He, S. Perez, S. Ginsberg, E. Mufson, and S. Counts, "Locus coeruleus cellular and molecular pathology during the progression of alzheimer's disease," *Acta Neuropathologica Communications*, vol. 5, 01 2017.

[43] M. Mather and C. Harley, "The locus coeruleus: Essential for maintaining cognitive function and the aging brain," *Trends in Cognitive Sciences*, vol. 20, pp. 214–226, 03 2016.

[44] D. Hämmerer, M. F. Callaghan, A. Hopkins, J. Kosciessa, M. Betts, A. Cardenas-Blanco, M. Kanowski, N. Weiskopf, P. Dayan, R. J. Dolan, and E. Düzel, "Locus coeruleus integrity in old age is selectively related to memories linked with salient negative events," *Proceedings of the National Academy of Sciences*, vol. 115, no. 9, pp. 2228–2233, 2018.

[45] C. H. Gibbons, "Chapter 27 - basics of autonomic nervous system function," in *Clinical Neurophysiology: Basis and Technical Aspects* (K. H. Levin and P. Chauvel, eds.), vol. 160 of *Handbook of Clinical Neurology*, pp. 407–418, Elsevier, 2019.

[46] J. Huang, C. Ulke, C. Sander, P. Jawinski, J. Spada, U. Hegerl, and T. Hensch, "Impact of brain arousal and time-on-task on autonomic nervous system activity in the wake-sleep transition.," *BMC neuroscience*, vol. 19, no. 1, 2018.

[47] D. Pfaff, A. Ribeiro, J. Matthews, and L.-M. Kow, "Concepts and mechanisms of generalized central nervous system arousal," *Annals of the New York Academy of Sciences*, vol. 1129, pp. 11–25, 02 2008.

[48] P. R. Murphy, J. Vandekerckhove, and S. Nieuwenhuis, "Pupil-linked arousal determines variability in perceptual decision making," *PLOS Computational Biology*, vol. 10, pp. 1–13, 09 2014.

[49] D. Pfaff, E. Martin, and D. Faber, "Origins of arousal: Roles for medullary reticular neurons," *Trends in neurosciences*, vol. 35, pp. 468–76, 05 2012.

[50] S. Sirois and J. Brisson, "Pupillometry," *Wiley Interdisciplinary Reviews: Cognitive Science*, vol. 5, 11 2014.

[51] P. Wel and H. Steenbergen, "Pupil dilation as an index of effort in cognitive control tasks: A review," *Psychonomic Bulletin & Review*, vol. 25, 02 2018.

[52] C.-A. Wang and D. Munoz, "A circuit for pupil orienting responses: Implications for cognitive modulation of pupil size," *Current Opinion in Neurobiology*, vol. 33, 08 2015.

[53] E. Szabadi, "Modulation of physiological reflexes by pain: Role of the locus coeruleus," *Frontiers in integrative neuroscience*, vol. 6, p. 94, 10 2012.

[54] J. Beatty and B. Lucero-Wagoner, "The pupillary system," p. 142–162, 10 2012.

[55] H. J. Wyatt, "The form of the human pupil," *Vision Research*, vol. 35, no. 14, pp. 2021–2036, 1995.

[56] I. Loewenfeld and O. Lowenstein, *The Pupil: Anatomy, Physiology, and Clinical Applications*. No. vol. 1 in The Pupil: Anatomy, Physiology, and Clinical Applications, Butterworth-Heinemann, 1999.

[57] P. D. Gamlin, H. Zhang, and R. J. Clarke, "Luminance neurons in the pretectal olivary nucleus mediate the pupillary light reflex in the rhesus monkey," *Experimental Brain Research*, vol. 106, pp. 177–180, 2004.

[58] B. Ebitz and T. Moore, "Selective modulation of the pupil light reflex by prefrontal cortex microstimulation," *The Journal of neuroscience: the official journal of the Society for Neuroscience*, vol. 37, p. 5008–5018, 04 2017.

[59] T. Moore and M. Fallah, "Microstimulation of the frontal eye field and its effects on covert spatial attention.," *Journal of neurophysiology*, vol. 91, no. 1, pp. 152–162, 2004.

[60] J. Oster, J. Huang, B. J. White, R. Radach, L. Itti, D. P. Munoz, and C.-A. Wang, "Pupillary responses to differences in luminance, color and set size," *Experimental brain research*, vol. 240, no. 6, p. 1873–1885, 2022.

[61] B. Laeng, S. Sirois, and G. Gredebäck, "Pupillometry a window to the preconscious?," *Perspectives on Psychological Science*, vol. 7, pp. 18–27, 01 2012.

[62] E. H. Hess and J. M. Polt, "Pupil size in relation to mental activity during simple problem-solving," *Science*, vol. 143, no. 3611, pp. 1190–1192, 1964.

[63] D. Kahneman and J. Beatty, "Pupil diameter and load on memory," *Science*, vol. 154, no. 3756, pp. 1583–1585, 1966.

[64] M. J. Ribeiro and M. Castelo-Branco, "Age-related differences in event-related potentials and pupillary responses in cued reaction time tasks," *Neurobiology of Aging*, vol. 73, pp. 177–189, 2019.

[65] D. Kahneman, "Attention and effort," 1973.

[66] S. E. Kuchinsky, J. B. Ahlstrom, K. I. V. Jr, S. L. Cute, L. E. Humes, J. R. Dubno, and M. A. Eckert, "Pupil size varies with word listening and response selection difficulty in older adults with hearing loss," *Psychophysiology*, vol. 50, no. 1, p. 23–34, 2013.

[67] J. Schmidtke, "Second language experience modulates word retrieval effort in bilinguals: Evidence from pupillometry," *Frontiers in psychology*, vol. 5, p. 137, 02 2014.

[68] A. A. Kafkas and D. Montaldi, "Familiarity and recollection produce distinct eye movement, pupil and medial temporal lobe responses when memory strength is matched," *Neuropsychologia*, vol. 50, pp. 3080–3093, 08 2012.

[69] S. C. Otero, B. S. Weekes, and S. B. Hutton, "Pupil size changes during recognition memory," *Neuropsychologia*, vol. 48, no. 10, p. 1346–1353, 2011.

[70] M. Papesh, S. Goldinger, and M. Hout, "Memory strength and specificity revealed by pupillometry," *International journal of psychophysiology : official journal of the International Organization of Psychophysiology*, vol. 83, pp. 56–64, 01 2012.

[71] J. W. de Gee, T. Knapen, and T. Donner, "Decision-related pupil dilation reflects upcoming choice and individual bias," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, p. E618–E625, 01 2014.

[72] M. Bradley, L. Miccoli, M. Escrig, and P. Lang, "The pupil as a measure of emotional arousal and autonomic activation," *Psychophysiology*, vol. 45, pp. 602–607, 08 2008.

[73] D. Hämmerer, A. Hopkins, M. Betts, A. Maass, R. Dolan, and E. Duzel, "Emotional arousal and recognition memory are differentially reflected in pupil diameter responses during emotional memory for negative events in younger and older adults," *Neurobiology of Aging*, vol. 58, pp. 129–139, 06 2017.

[74] D.-M. Ellingsen, J. Wessberg, O. Chelnokova, H. Olausson, B. Laeng, and S. Leknes, "In touch with your emotions: Oxytocin and touch change social impressions while others' facial expressions can alter touch," *Psychoneuroendocrinology*, vol. 39, p. 11–20, 01 2014.

[75] M. Honma, Y. Tanaka, Y. Osada, and K. Kuriyama, "Perceptual and not physical eye contact elicits pupillary dilation," *Biological psychology*, vol. 89, pp. 112–116, 01 2012.

[76] J. Hopstaken, D. Linden, and M. Kompier, "The window of my eyes: Task disengagement and mental fatigue covary with pupil dynamics," *Biological Psychology*, vol. 110, pp. 100–106, 07 2015.

[77] J. Hopstaken, D. Linden, and M. Kompier, "A multifaceted investigation of the link between mental fatigue and task disengagement," *Psychophysiology*, vol. 52, pp. 305–315, 03 2015.

[78] S. Joshi, Y. Li, R. Kalwani, and J. Gold, "Relationships between pupil diameter and neuronal activity in the locus coeruleus, colliculi, and cingulate cortex," *Neuron*, vol. 89, no. 1, pp. 221–234, 2016.

[79] M. Sarter, W. J. Gehring, and R. Kozak, "More attention must be paid: The neurobiology of attentional effort," *Brain Research Reviews*, vol. 51, no. 2, pp. 145–160, 2006.

[80] J. Reimer, M. Mcginley, Y. Liu, C. Rodenkirch, Q. Wang, D. McCormick, and A. Tolias, "Pupil fluctuations track rapid changes in adrenergic and cholinergic activity in cortex," *Nature Communications*, vol. 7, p. 13289, 11 2016.

[81] B. J. White and D. P. Munoz, *The Oxford handbook of eye movements*, pp. 195–213. Oxford University Press, 2011.

[82] J. K. Harting, M. F. Huerta, A. J. Frankfurter, N. L. Strominger, and G. J. Royce, "Ascending pathways from the monkey superior colliculus: An autoradiographic analysis," *The Journal of Comparative Neurology*, vol. 192, no. 4, pp. 853–882, 1980.

[83] D. Kondziella, C. Peinkhofer, G. Knudsen, and R. Moretti, "Cortical modulation of pupillary function: Systematic review," *PeerJ*, vol. 7, 05 2019.

[84] T. Li, H. Fung, and D. Isaacowitz, "The role of dispositional reappraisal in the age-related positivity effect," *The journals of gerontology. Series B, Psychological sciences and social sciences*, vol. 66, pp. 56–60, 11 2011.

[85] P. Van Gerven, F. Paas, J. J. G. Van Merrienboer, and H. Schmidt, "Memory load and the cognitive pupillary response," *Psychophysiology*, vol. 41, pp. 167–174, 04 2004.

[86] M. Kim, D. Q. Beversdorf, and K. M. Heilman, "Arousal response with ag-

ing: Pupillographic study," *Journal of the International Neuropsychological Society*, vol. 6, no. 3, p. 348–350, 2000.

[87] G. Porter, A. Tales, T. Troscianko, G. Wilcock, J. Haworth, and U. Leonards, "New insights into feature and conjunction search: I. evidence from pupil size, eye movements and ageing," *Cortex; a journal devoted to the study of the nervous system and behavior*, vol. 46, pp. 621–636, 07 2009.

[88] T. Piquado, D. Isaacowitz, and A. Wingfield, "Pupillometry as a measure of cognitive effort in younger and older adults," *Psychophysiology*, vol. 47, pp. 560–569, 05 2010.

[89] G. Samanez-Larkin, D. Worthy, R. Mata, S. M. McClure, and B. Knutson, "Adult age differences in frontostriatal representation of prediction error but not reward outcome," *Cognitive, affective & behavioral neuroscience*, vol. 14, pp. 672–682, 05 2014.

[90] M. Pietschmann, T. Endrass, B. Czerwon, and N. Kathmann, "Aging, probabilistic learning and performance monitoring," *Biological Psychology*, vol. 86, no. 1, pp. 74–82, 2011.

[91] M. Nassar, R. Bruckner, J. Gold, S.-C. Li, H. Heekeren, and B. Eppinger, "Article age differences in learning emerge from an insufficient representation of uncertainty in older adults," *Nature Communications*, vol. 7, 07 2016.

[92] J. R. Wessel, K. A. Dolan, and A. Hollingworth, "A blunted phasic autonomic response to errors indexes age-related deficits in error awareness," *Neurobiology of Aging*, vol. 71, pp. 13–20, 2018.

[93] F. Masina, E. Di Rosa, and D. Mapelli, "Intra-individual variability of error awareness and post-error slowing in three different age-groups," *Frontiers in Psychology*, vol. 9, p. 902, 06 2018.

[94] M. Ullsperger, C. Danielmeier, and G. Jocham, "Neurophysiology of performance monitoring and adaptive behavior," *Physiological reviews*, vol. 94, pp. 35–79, 01 2014.

[95] S. Harty, R. O'Connell, R. Hester, and I. Robertson, "Older adults have diminished awareness of errors in the laboratory and daily life," *Psychology and aging*, vol. 28, pp. 1032–1041, 12 2013.

[96] J. Wessel, C. Danielmeier, and M. Ullsperger, "Error awareness revisited: Accumulation of multimodal evidence from central and autonomic nervous systems," *Journal of cognitive neuroscience*, vol. 23, pp. 3021–3036, 10 2011.

[97] R. O'Connell, P. Dockree, M. Bellgrove, S. Kelly, R. Hester, H. Garavan, I. Robertson, and J. Foxe, "The role of cingulate cortex in the detection of

errors with and without awareness: a high-density electrical mapping study," *The European journal of neuroscience*, vol. 25, pp. 2571–9, 05 2007.

[98] H. Critchley, J. Tang, D. Glaser, B. Butterworth, and R. Dolan, "Anterior cingulate activity during error and autonomic response," *NeuroImage*, vol. 27, pp. 885–895, 11 2005.

[99] M. Ullsperger, H. Harsay, J. Wessel, and K. Ridderinkhof, "Conscious perception of errors and its relation to the anterior insula," *Brain structure & function*, vol. 214, pp. 629–43, 06 2010.

[100] J. G. Kerns, J. D. Cohen, A. W. MacDonald, R. Y. Cho, V. A. Stenger, and C. S. Carter, "Anterior cingulate conflict monitoring and adjustments in control," *Science*, vol. 303, no. 5660, pp. 1023–1026, 2004.

[101] T. Endrass, M. Schreiber, and N. Kathmann, "Speeding up older adults: Age-effects on error processing in speed and accuracy conditions," *Biological psychology*, vol. 89, pp. 426–432, 12 2011.

[102] P. M. A. Rabbitt, "Errors and error correction in choice-response tasks.," *Journal of experimental psychology*, vol. 71, no. 2, pp. 264–272, 1966.

[103] C. Danielmeier and M. Ullsperger, "Post-error adjustments," *Frontiers in psychology*, vol. 2, p. 233, 09 2011.

[104] E. Niessen, G. R. Fink, H. E. M. Hoffmann, P. H. Weiss, and J. Stahl, "Error detection across the adult lifespan: Electrophysiological evidence for age-related deficits," *NeuroImage*, vol. 152, pp. 517–529, 2017.

[105] S. Nieuwenhuis, K. Ridderinkhof, J. Blom, G. Band, and A. Kok, "Error-related brain potentials are differentially related to awareness of response errors: Evidence from an antisaccade task," *Psychophysiology*, vol. 38, pp. 752 – 760, 09 2001.

[106] R. Hester, J. J. Foxe, S. Molholm, M. Shpaner, and H. Garavan, "Neural mechanisms involved in error processing: A comparison of errors made with and without awareness," *NeuroImage*, vol. 27, no. 3, pp. 602–608, 2005.

[107] M. Cohen, S. van Gaal, K. Ridderinkhof, and V. Lamme, "Unconscious errors enhance prefrontal-occipital oscillatory synchrony," *Frontiers in human neuroscience*, vol. 3, p. 54, 11 2009.

[108] L. James and T. Kooy, "Aging and the detection of visual errors in scenes," *Journal of aging research*, vol. 2011, p. 984694, 01 2011.

[109] S. Harty, P. R. Murphy, I. H. Robertson, and R. G. O'Connell, "Parsing the neural signatures of reduced error detection in older age," *NeuroImage*, vol. 161, pp. 43–55, 2017.

[110] J. I. Gold and M. N. Shadlen, "The neural basis of decision making," *Annual Review of Neuroscience*, vol. 30, no. 1, pp. 535–574, 2007.

[111] M. Shadlen and R. Kiani, "Decision making as a window on cognition," *Neuron*, vol. 80, pp. 791–806, 10 2013.

[112] R. Kiani, L. Corthell, and M. Shadlen, "Choice certainty is informed by both evidence and decision time," *Neuron*, vol. 84, pp. 1329–1342, 12 2014.

[113] T. Krumpe, P. Gerjets, W. Rosenstiel, and M. Spüler, "Decision confidence: Eeg correlates of confidence in different phases of an old/ new recognition task," *Brain-Computer Interfaces*, vol. 6, 01 2020.

[114] S. Fleming and R. Dolan, "The neural basis of metacognitive ability," *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, vol. 367, pp. 1338–49, 05 2012.

[115] A. Kepecs and Z. Mainen, "A computational framework for the study of confidence in humans and animals," *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, vol. 367, pp. 1322–37, 05 2012.

[116] A. E. Urai, A. Braun, and T. H. Donner, "Pupil-linked arousal is driven by decision uncertainty and alters serial choice bias," *Nature Communications*, vol. 8, no. 1, 2017.

[117] M. Rausch, S. Hellmann, and M. Zehetleitner, "Confidence in masked orientation judgments is informed by both evidence and visibility," *Attention, Perception, & Psychophysics*, vol. 80, pp. 134–154, 01 2018.

[118] A. Pouget, J. Drugowitsch, and A. Kepecs, "Confidence and certainty: distinct probabilistic quantities for different goals," *Nature Neuroscience*, vol. 19, pp. 366–374, 02 2016.

[119] F. Meyniel, M. Sigman, and Z. Mainen, "Confidence as bayesian probability: From neural origins to behavior," *Neuron*, vol. 88, no. 1, pp. 78–92, 2015.

[120] A. J. Yu and P. Dayan, "Uncertainty, neuromodulation, and attention," *Neuron*, vol. 46, no. 4, pp. 681–692, 2005.

[121] M. Jepma, E.-J. Wagenmakers, G. Band, and S. Nieuwenhuis, "The effects of accessory stimuli on information processing: Evidence from electrophysiology and a diffusion model analysis," *Journal of cognitive neuroscience*, vol. 21, pp. 847–864, 09 2008.

[122] S. Cheadle, V. Wyart, K. Tsetsos, N. Myers, V. de Gardelle, S. Herce Castañón, and C. Summerfield, "Adaptive gain control during human perceptual choice," *Neuron*, vol. 81, no. 6, pp. 1429–1441, 2014.

[123] P. Murphy, J. Vandekerckhove, and S. Nieuwenhuis, "Pupil-linked arousal

determines variability in perceptual decision making," *PLoS computational biology*, vol. 10, p. e1003854, 09 2014.

[124] C. Gomes, "Age-related changes in arousal modulation during perceptual decision-making," 2020.

[125] T. T. Brunyé and A. L. Gardony, "Eye tracking measures of uncertainty during perceptual decision making," *International Journal of Psychophysiology*, vol. 120, pp. 60–68, 2017.

[126] S. Fiedler and A. Glöckner, "The dynamics of decision making in risky choice: An eye-tracking analysis," *Frontiers in Psychology*, vol. 3, p. 335, 2012.

[127] I. Krajbich, C. Armel, and A. Rangel, "Visual fixations and comparison of value in simple choice," *Nature neuroscience*, vol. 13, pp. 1292–1298, 10 2010.

[128] S. Liversedge and J. Findlay, "Saccadic eye movements and cognition," *Trends in cognitive sciences*, vol. 4, no. 1, pp. 6–14, 2000.

[129] L. L. Di Stasi, A. Catena, J. J. Cañas, S. L. Macknik, and S. Martinez-Conde, "Saccadic velocity as an arousal index in naturalistic tasks," *Neuroscience & Biobehavioral Reviews*, vol. 37, no. 5, pp. 968–975, 2013.

[130] D. Boehm-Davis, W. Gray, and M. Schoelles, "The eye blink as a physiological indicator of cognitive workload," *Proceedings of the Human Factors and Ergonomics Society Annual Meeting*, vol. 44, pp. 6–116–119, 07 2000.

[131] P. Murphy, R. O'Connell, M. O'Sullivan, I. Robertson, and J. Balsters, "Pupil diameter covaries with bold activity in human locus coeruleus," *Human Brain Mapping*, vol. 35, pp. 4140–4154, 08 2014.

[132] J. Geng, Z. Blumenfeld, T. Tyson, and M. Minzenberg, "Pupil diameter reflects uncertainty in attentional selection during visual search," *Frontiers in Human Neuroscience*, vol. 9, pp. 1–14, 08 2015.

[133] J. Sanders, B. Hangya, and A. Kepecs, "Signatures of a statistical computation in the human sense of confidence," *Neuron*, vol. 90, pp. 499–506, 05 2016.

[134] M. B. Pontifex, A. L. McGowan, M. C. Chandler, K. L. Gwizdala, A. C. Parks, K. Fenn, and K. Kamijo, "A primer on investigating the after effects of acute bouts of physical activity on cognition," *Psychology of Sport and Exercise*, vol. 40, pp. 1–22, 2019.

[135] J. Gomes-Osman, D. Cabral, T. Morris, K. McInerney, L. Cahalin, T. Rundek, A. Oliveira, and A. Pascual-Leone, "Exercise for cognitive brain health in aging: A systematic review for an evaluation of dose," *Neurology: Clinical Practice*, vol. 8, pp. 257–265, 06 2018.

[136] M. Pollock, G. Gaesser, J. Butcher, J.-P. Després, R. Dishman, B. Franklin, and C. Garber, "Acsm position stand: The recommended quantity and qual-

ity of exercise for developing and maintaining cardiorespiratory and muscular fitness, and flexibility in healthy adults," *Medicine & Science in Sports & Exercise*, vol. 30, pp. 975–991, 06 1998.

[137] K. Erickson, C. Hillman, C. Stillman, R. Ballard, B. Bloodgood, D. Conroy, R. Macko, D. Marquez, S. Petruzzello, and K. Powell, "Physical activity, cognition, and brain outcomes: A review of the 2018 physical activity guidelines," *Medicine and science in sports and exercise*, vol. 51, pp. 1242–1251, 06 2019.

[138] C. J. Caspersen, K. E. Powell, and G. M. Christenson, "Physical activity, exercise, and physical fitness: Definitions and distinctions for health-related research," *Public Health Reports (Washington,D.C.:1974)*, vol. 100, no. 2, pp. 126–131, 1985.

[139] P. A. G. A. Committee, "2018 physical activity guidelines advisory committee scientific report," tech. rep., Washington, DC, 2018. Available at `https://health.gov/paguidelines/second-edition/report.aspx`.

[140] R. Cardoso Cassilhas, S. Tufik, and M. De Mello, "Physical exercise, neuroplasticity, spatial learning and memory," *Cellular and molecular life sciences : CMLS*, vol. 73, pp. 975–983, 03 2016.

[141] A. F. M. de Sousa, A. R. Medeiros, S. D. Rosso, M. Stults-Kolehmainen, and D. A. Boullosa, "The influence of exercise and physical fitness status on attention: a systematic review," *International Review of Sport and Exercise Psychology*, vol. 12, no. 1, pp. 202–234, 2019.

[142] A. Kramer, S. Colcombe, K. Erickson, A. Belopolsky, E. Mcauley, N. Cohen, A. Webb, G. Jerome, D. Marquez, and T. Wszalek, "Effects of aerobic fitness training on human cortical function: A proposal," *Journal of molecular neuroscience : MN*, vol. 19, pp. 227–231, 08 2002.

[143] K. A. Shay and D. L. Roth, "Association between aerobic fitness and visuospatial performance in healthy older adults," *Psychology and aging*, vol. 7, no. 1, pp. 15–24, 1992.

[144] A. Maass, S. Düzel, M. Goerke, A. Becke, U. Sobieray, K. Neumann, M. Lövdén, U. Lindenberger, L. Bäckman, R. C. Braun-Dullaeus, D. Ahrens, H.-J. Heinze, N. G. Müller, and E. Düzel, "Vascular hippocampal plasticity after aerobic exercise in older adults," *Molecular Psychiatry*, vol. 20, no. 5, pp. 585–593, 2014.

[145] L. L. Law, F. Barnett, M. K. Yau, and M. A. Gray, "Effects of combined cognitive and exercise interventions on cognition in older adults with and without cognitive impairment: A systematic review," *Ageing Research Reviews*, vol. 15, pp. 61–75, 2014.

[146] G. Covell, C. Hoffman-Snyder, K. Wellik, B. Woodruff, Y. Geda, R. Caselli, B. Demaerschalk, and D. Wingerchuk, "Physical activity level and future risk of mild cognitive impairment or dementia a critically appraised topic," *The neurologist*, vol. 19, pp. 89–91, 02 2015.

[147] C. K. Barha, J. C. Davis, R. S. Falck, L. S. Nagamatsu, and T. Liu-Ambrose, "Sex differences in exercise efficacy to improve cognition: A systematic review and meta-analysis of randomized controlled trials in older humans," *Frontiers in Neuroendocrinology*, vol. 46, pp. 71–85, 2017.

[148] Y. Chang, J. Labban, J. Gapin, and J. Etnier, "The effects of acute exercise on cognitive performance: A meta-analysis," *Brain Research*, vol. 1453, pp. 87–101, 2012.

[149] K. Lambourne and P. Tomporowski, "The effect of exercise-induced arousal on cognitive task performance: A meta-regression analysis," *Brain research*, vol. 1341, pp. 12–24, 04 2010.

[150] T. McMorris and B. J. Hale, "Differential effects of differing intensities of acute exercise on speed and accuracy of cognition: A meta-analytical investigation," *Brain and Cognition*, vol. 80, no. 3, pp. 338–351, 2012.

[151] Y.-K. Chang, C.-h. Chu, C.-C. Wang, Y.-C. Wang, T.-F. Song, C.-L. Tsai, and J. Etnier, "Dose-response relation between exercise duration and cognition," *Medicine and science in sports and exercise*, vol. 47, 05 2014.

[152] J. Northey, N. Cherbuin, K. Pumpa, D. Smee, and B. Rattray, "Exercise interventions for cognitive function in adults older than 50: a systematic review with meta-analysis," *British Journal of Sports Medicine*, vol. 52, pp. 154–160, 04 2017.

[153] D. Turner, M. Hu, E. Generaal, D. Bos, M. Ikram, A. Heshmatollah, L. Fani, M. Ikram, B. Penninx, and P. Cuijpers, "Physical exercise interventions targeting cognitive functioning and the cognitive domains in nondementia samples: A systematic review of meta-analyses," *Journal of Geriatric Psychiatry and Neurology*, vol. 34, 04 2020.

[154] B. Klimova and R. Dostalova, "The impact of physical activities on cognitive performance among healthy older individuals," *Brain Sciences*, vol. 10, p. 377, 06 2020.

[155] L. F. Biazus-Sehn, F. B. Schuch, J. Firth, and F. de Souza Stigger, "Effects of physical exercise on cognitive function of older adults with mild cognitive impairment: A systematic review and meta-analysis," *Archives of Gerontology and Geriatrics*, vol. 89, p. 104048, 2020.

[156] J. Carvalho, F. Machado, D. Barros, A. Sampaio, I. Marques-Aleixo, L. Bohn,

A. Pizarro, L. Teixeira, J. Magalhães, and O. Ribeiro, ""body & brain": effects of a multicomponent exercise intervention on physical and cognitive function of adults with dementia -study protocol for a quasi- experimental controlled trial," *BMC Geriatrics*, vol. 21, p. 156, 03 2021.

[157] X. Huang, X. Zhao, B. Li, Y. Cai, S. Zhang, Q. Wan, and F. Yu, "Comparative efficacy of various exercise interventions on cognitive function in patients with mild cognitive impairment or dementia: A systematic review and network meta-analysis," *Journal of Sport and Health Science*, vol. 11, no. 2, pp. 212–223, 2022.

[158] M. Sellami, M. Gasmi, J. Denham, L. D. Hayes, D. Stratton, J. Padulo, and N. L. Bragazzi, "Effects of acute and chronic exercise on immunological parameters in the elderly aged: Can physical activity counteract the effects of aging?," *Frontiers in Immunology*, vol. 9, 2018.

[159] M. Endres, K. Gertz, U. Lindauer, J. Katchanov, J. Schultze, H. Schröck, G. Nickenig, W. Kuschinsky, U. Dirnagl, and U. Laufs, "Mechanisms of stroke protection by physical activity," *Annals of neurology*, vol. 54, pp. 582–590, 12 2003.

[160] S. Vaynman, Z. Ying, and F. Gomez-Pinilla, "Vaynman s, ying z, gomez-pinilla f. hippocampal bdnf mediates the efficacy of exercise on synaptic plasticity and cognition. eur j neurosci 20: 2580-2590," *The European journal of neuroscience*, vol. 20, pp. 2580–2590, 12 2004.

[161] J. Marmeleira, "An examination of the mechanisms underlying the effects of physical activity on brain and cognition," *European Review of Aging and Physical Activity*, vol. 10, pp. 83–94, 2013.

[162] E. S. Bliss, R. H. X. Wong, P. R. C. Howe, and D. E. Mills, "The effects of aerobic exercise training on cerebrovascular and cognitive function in sedentary, obese, older adults," *Frontiers in Aging Neuroscience*, vol. 14, 2022.

[163] S. Chapman, S. Aslan, J. Spence, L. Defina, M. Keebler, N. Didehbani, and H. Lu, "Shorter term aerobic exercise improves brain, cognition, and cardiovascular fitness in aging," *Frontiers in aging neuroscience*, vol. 5, p. 75, 11 2013.

[164] S. Heijnen, B. Hommel, A. Kibele, and L. Colzato, "Neuromodulation of aerobic exercise—a review," *Frontiers in Psychology*, vol. 6, p. 1890, 01 2016.

[165] R. Leckie, L. Oberlin, M. Voss, R. Prakash, A. Szabo-Reed, L. Chaddock-Heyman, S. Phillips, N. Gothe, E. Mailey, V. Vieira-Potter, S. Martin, B. Pence, M. Lin, R. Parasuraman, P. Greenwood, K. Fryxell, J. Woods, E. Mcauley, A. Kramer, and K. Erickson, "Bdnf mediates improvements in

executive function following a 1-year exercise intervention," *Frontiers in human neuroscience*, vol. 8, p. 985, 12 2014.

[166] T. Huang, K. Larsen, M. Ried-Larsen, N. Møller, and L. Andersen, "The effects of physical activity and exercise on brain-derived neurotrophic factor in healthy humans: A review," *Scandinavian journal of medicine & science in sports*, vol. 24, pp. 1–10, 04 2013.

[167] R. Peters, "Ageing and the brain," *Postgraduate medical journal*, vol. 82, pp. 84–88, 03 2006.

[168] T. McMorris, *Exercise and Cognitive Function: a neuroendocrinological explanation*, pp. 41 – 68. Willey & Blackwell, Chichester, West Sussex, UK, 05 2009.

[169] T. McMorris and B. J. Hale, "Is there an acute exercise-induced physiological/biochemical threshold which triggers increased speed of cognitive functioning? a meta-analytic investigation," *Journal of Sport and Health Science*, vol. 4, no. 1, pp. 4–13, 2015.

[170] R. Meeusen, I. Smolders, S. Sarre, K. L. D. Meirleir, H. A. Keizer, M. Serneels, G. Ebinger, and Y. Michotte, "Endurance training effects on neurotransmitter release in rat striatum: an in vivo microdialysis study," *Acta physiologica Scandinavica*, vol. 159, no. 4, pp. 335–341, 1997.

[171] M. K. Dalsgaard, P. Ott, F. Dela, A. Juul, B. K. Pedersen, J. Warberg, J. Fahrenkrug, and N. H. Secher, "The csf and arterial to internal jugular venous hormonal differences during exercise in humans," *Experimental physiology*, vol. 89, no. 3, pp. 271–277, 2004.

[172] G. Wang, N. Volkow, J. Fowler, D. Franceschi, J. Logan, N. Pappas, C. Wong, and N. Netusil, "Pet studies of the effects of aerobic exercise on human striatal dopamine release," *Journal of nuclear medicine: official publication, Society of Nuclear Medicine*, vol. 41, pp. 1352–1356, 08 2000.

[173] N. Ayala and M. Heath, "Pupillometry reveals the role of arousal in a postexercise benefit to executive function," *Brain Sciences*, vol. 11, p. 1048, 08 2021.

[174] M. Mather, R. Huang, D. Clewett, S. E. Nielsen, R. Velasco, K. Tu, S. Han, and B. L. Kennedy, "Isometric exercise facilitates attention to salient events in women via the noradrenergic system," *NeuroImage*, vol. 210, p. 116560, 2020.

[175] A. L. McGowan, M. C. Chandler, J. W. Brascamp, and M. B. Pontifex, "Pupillometric indices of locus-coeruleus activation are not modulated following single bouts of exercise," *International Journal of Psychophysiology: official jour-*

nal of the International Organization of Psychophysiology, vol. 140, pp. 41–52, 2019.

[176] T. Lohman, A. Roche, and R. Martorell, *Anthropometric Standardization Reference Manual*. Human Kinetics Books, 1988.

[177] G. Holland, K. Tanaka, R. Shigematsu, and M. Nakagaichi, "Flexibility and physical functions of older adults: A review," *Journal of Aging and Physical Activity*, vol. 10, pp. 169–206, 04 2002.

[178] P. Seidenberg and A. Beutler, *The Sports Medicine Resource Manual*. 01 2008.

[179] M. Voss, S. Heo, R. Prakash, K. Erickson, H. Alves, L. Chaddock, A. Szabo-Reed, E. Mailey, T. Wójcicki, S. Phillips, N. Gothe, E. Mcauley, B. Sutton, and A. Kramer, "The influence of aerobic fitness on cerebral white matter integrity and cognitive function in older adults: Results of a one-year exercise intervention," *Human brain mapping*, vol. 34, p. 2972–2985, 11 2013.

[180] G. Raghuveer, J. Hartz, D. Lubans, T. Takken, J. Wiltz, M. Mietus-Snyder, A. Perak, C. Baker-Smith, N. Pietris, and N. Edwards, "Cardiorespiratory fitness in youth: An important marker of health: A scientific statement from the american heart association," *Circulation*, vol. 142, p. e101–e118, 07 2020.

[181] S. Kodama, K. Saito, S. Tanaka, M. Maki, Y. Yachi, M. Asumi, A. Sugawara, K. Totsuka, H. Shimano, Y. Ohashi, N. Yamada, and H. Sone, "Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: A meta-analysis," *JAMA: the journal of the American Medical Association*, vol. 301, pp. 2024–2035, 06 2009.

[182] H. Tanaka, K. D. Monahan, and D. R. Seals, "Age-predicted maximal heart rate revisited," *Journal of the American College of Cardiology*, vol. 37, no. 1, pp. 153–156, 2001.

[183] G. A. V. Borg, "Psychophysical bases of perceived exertion.," *Medicine and science in sports and exercise*, vol. 14, no. 5, pp. 377–81, 1982.

[184] D. H. Brainard, "The psychophysics toolbox.," *Spatial vision*, vol. 10, no. 4, pp. 433–436, 1997.

[185] L. Thaler, A. Schütz, M. Goodale, and K. Gegenfurtner, "What is the best fixation target? the effect of target shape on stability of fixational eye movements," *Vision Research*, vol. 76, pp. 31–42, 2013.

[186] P. K. Pilly and A. R. Seitz, "What a difference a parameter makes: A psychophysical comparison of random dot motion algorithms," *Vision Research*, vol. 49, no. 13, pp. 1599–1612, 2009.

[187] S. Research, "Eyelink 1000 plus - the most flexible eye tracker." Accessed: 2023-02-23.

[188] A. Delorme and S. Makeig, "Eeglab: an open-source toolbox for analysis of single-trial eeg dynamics," *Journal of Neuroscience Methods*, vol. 134, pp. 9–21, 2004.

[189] R. Petersen, G. Smith, S. Waring, R. Ivnik, E. Tangalos, and E. Kokmen, "Mild cognitive impairment: Clinical characterization and outcome," *Archives of neurology*, vol. 56, pp. 303–308, 04 1999.

[190] Z. Nasreddine, N. Phillips, V. Bédirian, S. Charbonneau, V. Whitehead, I. Collin, J. Cummings, and H. Chertkow, "The montreal cognitive assessment, moca: A brief screening tool for mild cognitive impairment," *Journal of the American Geriatrics Society*, vol. 53, pp. 695–699, 05 2005.

[191] S. Freitas, M. R. Simões, L. Alves, D. Duro, and I. Santana, "Montreal cognitive assessment (moca): Validation study for frontotemporal dementia," *Journal of Geriatric Psychiatry and Neurology*, vol. 25, no. 3, pp. 146–154, 2012.

[192] S. Hoops, S. Nazem, A. Siderowf, J. Duda, S. Xie, M. Stern, and D. Weintraub, "Validity of the moca and mmse in the detection of mci and dementia in parkinson disease," *Neurology*, vol. 73, pp. 1738–45, 11 2009.

[193] S. Freitas, M. R. Simões, L. Alves, M. Vicente, and I. Santana, "Montreal cognitive assessment (moca): Validation study for vascular dementia," *Journal of the International Neuropsychological Society*, vol. 18, no. 6, p. 1031–1040, 2012.

[194] L. Koski, H. Xie, and S. Konsztowicz, "Improving precision in the quantification of cognition using the montreal cognitive assessment and the mini-mental state examination," *International psychogeriatrics*, vol. 23, pp. 1107–1115, 02 2011.

[195] J. Cardoso, B. Apagueno, P. Lysne, L. Hoyos, E. Porges, J. Riley, R. Fillingim, A. Woods, R. Cohen, and Y. Cruz-Almeida, "Pain and the montreal cognitive assessment (moca) in aging," *Pain Medicine*, vol. 22, 03 2021.

[196] J. H. Wymer, K. Rayls, and M. T. Wagner, "Utility of a clinically derived abbreviated form of the wais-iii," *Archives of Clinical Neuropsychology*, vol. 18, no. 8, pp. 917–927, 2003.

[197] H. Jung, D. Yi, M. Byun, J. Lee, Y. Lee, H. Ahn, and D. Lee, "Functional neural correlates of the wais-iv block design test in older adult with mild cognitive impairment and alzheimer's disease," *Neuroscience*, vol. 463, pp. 197–203, 05 2021.

[198] H. Joung, D. Yi, H. Ahn, Y. Lee, M. S. Byun, K. Sung, D. Han, and D. Y. Lee, "Normative study of the block design test for adults aged 55 years and older

in korean aging population," *Psychiatry Investigation*, vol. 18, pp. 539–544, 06 2021.

[199] H. Hassan, Z. Said, and N. Ibrahim, "Validity and reliability of beck depression inventory (bdi) bahasa melayu version: A pilot study on public servants having symptoms of depression," *Linguistics and Culture Review*, vol. 6, pp. 1–12, 11 2021.

[200] A. Beck, R. Steer, and G. Brown, *Manual for the Beck Depression Inventory-II*, 1996.

[201] R. Campos and B. Goncalves, "The portuguese version of the beck depression inventory-ii (bdi-ii) preliminary psychometric data with two nonclinical samples," *European Journal of Psychological Assessment*, vol. 27, pp. 258–264, 01 2011.

[202] E. Ponciano and A. Cardoso, I.and Pereira, "Adaptação de uma versão experimental em língua portuguesa do beck depression inventory-second edition (bdi-ii) em estudantes do ensino superior," in *Acão social e aconselhamento psicológico no ensino superior e intervenção*, 2004.

[203] B. J. Baum, "Principles of saliva secretion.," *Annals of the New York Academy of Sciences*, vol. 694, p. 17–23, 1993.

[204] U. Nater and N. Rohleder, "Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research," *Psychoneuroendocrinology*, vol. 34, no. 4, pp. 486–496, 2009.

[205] R. Chatterton, K. Vogelsong, Y. Lu, A. Ellman, and G. Hudgens, "Salivary alpha-amylase as a measure of endogenous adrenergic activity," *Clinical Physiology*, vol. 16, pp. 433–448, 07 1996.

[206] M. Navazesh, "Methods for collecting saliva," *Annals of the New York Academy of Sciences*, vol. 694, no. 1, pp. 72–77, 1993.

[207] L. Salimetrics, *Salivary α-Amylase kinetic enzyme assay kit*, 04 2019.

[208] J. W. de Gee, C. M. C. Correa, M. Weaver, T. H. Donner, and S. van Gaal, "Pupil dilation and the slow wave erp reflect surprise about choice outcome resulting from intrinsic variability in decision confidence," *Cerebral cortex (New York, N.Y.: 1991)*, vol. 31, no. 7, p. 3565–3578, 2021.

[209] S. L. Bachman, S. Attanti, and M. Mather, "Isometric handgrip exercise speeds working memory responses in younger and older adults," *Psychology and aging*, 2023.