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A MULTIMODAL APPROACH TO THE STUDY OF HEALTHY AND PATHOLOGICAL AGING

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General introduction

Due to falling birth rates combined with increased longevity, a remarkable increase in the proportion of aged persons in the population is evident in all highly developed nations. It has been predicted that by 2050, there will be many more older adults in wealthy, developed countries (26%) than children under 15 years old (about 16% of total population) (Cohen, 2003).

Population aging represents both an opportunity and a challenge for society. On one hand, older citizens constitute an important reserve of human capital and experience, on the other hand, the incidence of cognitive impairments connected with healthy aging and dementia will increase, with an increased burden for families and society (Hebert et al., 2013). Alzheimer's Disease (AD) is the most frequent cause of age-related dementia, accounting for about 60% of cases, and its incidence is expected to raise more and more, as around 80 million of dementia cases are foreseeable by 2040 (Ferri et al., 2005).

As medical science increases lifespan and our society becomes progressively older, there is a growing interest in understanding the cognitive and neural changes that accompany both healthy and pathological aging (Harada et al., 2013). The ultimate goal is to understand how the changes in neural structure and function map onto age-related behavior in attempts to characterize the neurobiological aspects of aging and to distinguish normal from pathological aging.

The need to distinguish normal from pathological aging and the necessity of early diagnosis, leads aging research to the field of biomarkers, biological entities that can identify the disease in a timely manner (Moretti, 2016). Electroencephalography (EEG) represents a non-invasive, easy to apply and relatively cheap tool that has gained increasing interest in the biomarkers research of dementia. Although traditional EEG biomarkers have not been considered accurate enough to be useful in clinical practice, the possibility to discover reliable and sensitive neurophysiological markers to identify the preclinical phases of the disease would enable an earlier, and potentially more effective, treatment to slow AD progression.

Another area that has gained increasing interest concerns the attempt to evaluate new strategies to delay or counteract cognitive decline that accompany both normal and pathological aging (Lautenschlager et al., 2014). Moreover, since medications used to manage cognitive and neuropsychiatric symptoms associated with AD have limited efficacy and adverse side effects, it is of great importance to develop alternative therapeutic approaches. One area which has recently garnered considerable clinical and research interest is the use of non-invasive brain stimulation techniques, such as transcranial electrical stimulation (tES), to improve physiological and pathological age-related cognitive impairments (Berryhill & Jones, 2012; Zimerman et al., 2013). It has been shown that electrical stimulation may facilitate behavioral performance in a variety of conditions (e.g. Fertonani et al., 2010; Roy et al., 2014; Harvey & Kerkhoff, 2015). A recent review (Summers et al., 2015) indicated that tES has significant positive effects on cognitive and motor functions in healthy older adults across a variety of tasks. tES has shown to be able to induce modifications of the cortical plasticity which may outlast the stimulation period itself (Sergeeva, 2011). For that reason, it could have enormous potential to slow or even reverse declines in functioning associated with aging (Ferrucci et al., 2008; Boggio et al., 2009; Boggio et al., 2012). However, the results were inconsistent and the overall efficacy of non-invasive brain stimulation as a therapeutic tool is still under debate.

The present dissertation focused on normal and pathological aging. The first aim of the current work was to (Study 1) investigate the neural/electrophysiological dynamics of multiple object processing in pathological aging, specifically in Alzheimer's Disease (AD) and in its prodromal stage, Mild Cognitive Impairment (MCI), in order to obtain possible early event-related potential (ERP) biomarkers of AD pathology. The ultimate goal of this dissertation was to obtain reliable and sensitive neurophysiological markers that distinguish normal from pathological aging.

The second aim of this thesis was to (Study 2) investigate whether transcranial electrical stimulation (tES) may be used to exogenously increase the participants' level of arousal with a subsequent improvement of behavioural performance during a task involving high mental processes. This investigation has to be framed in an attempt to find an effective approach to

use in interventions for both cognitive enhancement in normal aging and cognitive rehabilitation in pathological aging.

Healthy and pathological aging: an overview

Human aging is a multifactorial process characterized by the progressive degeneration of organ systems and tissues. It is largely determined by genetics, and influenced by a wide range of environmental factors, such as diet, exercise, exposure to microorganisms, pollutants, and ionising radiation. Aging is not a unitary process and persons age at different rates along different dimensions (Fillit et al., 2002).

There is ample evidence that human aging is accompanied by biological, physiological, psychological and neurological changes. In particular, aging affects sensory and perceptual functions. Mental, cognitive and neurophysiological age-related modifications are also well documented (Glisky, 2007). Within the physiological modifications that accompany aging, alteration in brain structure and function, intimately tied to decrements of cognitive functions across a variety of domains, are largely described (Deary et al., 2009; Drag & Bieliauskas, 2010; Salthouse, 2012).

1. Age related cognitive changes

It is well known that normal aging is accompanied by both structural and functional neurophysiological modifications, resulting in changes in cognition, that tend to noticeably and negatively affect people's everyday life (Harada et al., 2013). The magnitude of the changes can vary markedly across individuals and cognitive processes with some abilities declining, whereas others remaining relatively stable or even showing an increase with aging (Grady, 2012). Moreover, some studies pointed out that the brain of older people is not only characterized by decline in the neural efficiency, but also by increased and compensatory cerebral activations (Reuter-Lorenz & Cappell, 2008; Reuter-Lorenz & Park, 2010).

As individuals age, many aspects of information processing become less efficient, including speed of processing, working memory capacity, inhibitory function and long-term memory. At the same time, other aspects of cognitive functions such as implicit memory and knowledge storage, are protected and relatively resistant to cognitive aging. In an extensive study, Park and collaborators (Park, 2002), tested across multiple cognitive domains a sample

of individuals ranging from 20 to 90 years. The results showed widespread age-related declines in measures of working memory, long-term memory and processing speed. Only verbal ability, which is an estimate of accrued knowledge rather than a cognitive mechanism, showed to be resistant to aging (Figure 1.1).

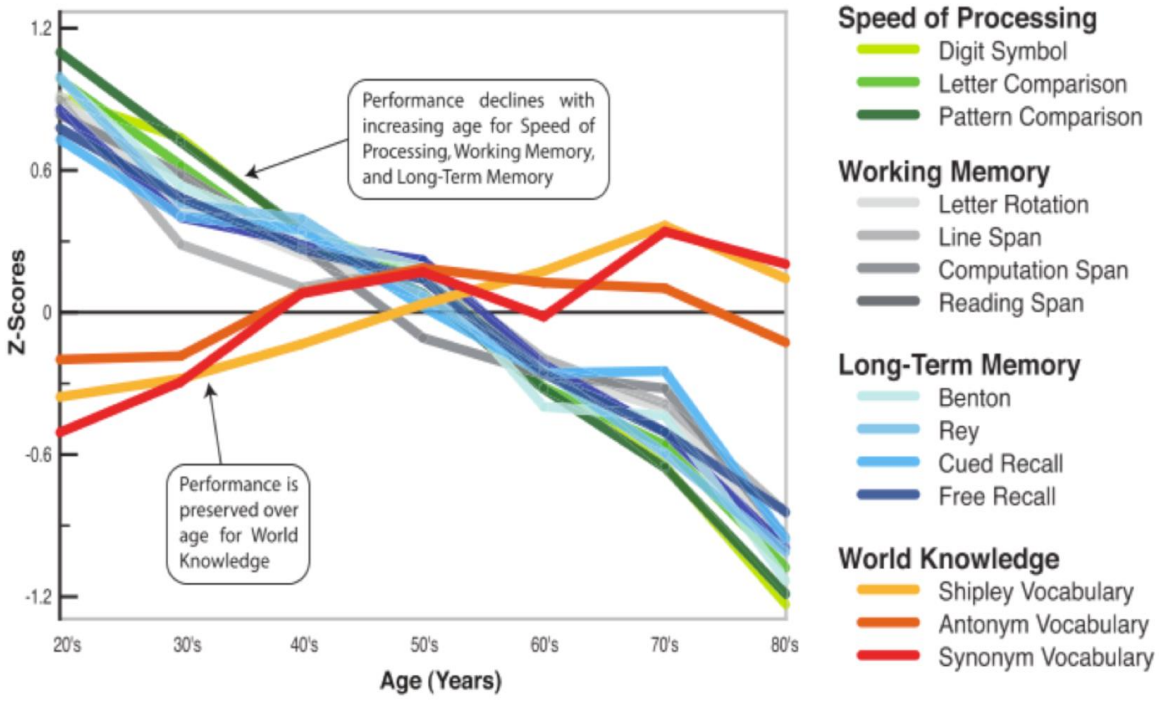

Figure 1.1 *Changes of cognitive functions across lifespan. Cross-sectional data showed age-related changes on measures of speed of processing, working memory, long-term memory, and world knowledge. Almost all measures of cognitive functions decline with age, except world knowledge, which appears even to have some improvement (adapted from Park et al., 2002).*

It is well established that the cognitive functions most affected by age are attention and memory (Albert, 1993; Madden, 2007; Glisky, 2007). In particular, some aspects of these processes like selective attention (Gazzaley et al., 2005, 2008; Zanto & Gazzaley, 2010; Geerligs et al., 2014), divided attention and attention switching (Castel & Craik, 2003), as well as episodic memory (Reuter-Lorenz & Park, 2010) and working memory (Braver & West, 2008; Saunders & Summers, 2010) show significant age-related declines.

The ability to selectively process information (attention) and to retain information in an accessible state (working memory) are critical aspects of our cognitive capacities and are domains that have a big influence on every-day life functioning.

Selective attention is one of the cognitive processes most affected by aging and has been suggested to act as a mediator of age-related decline in various complex cognitive abilities (Castel & Craik, 2003). Selective attention allows us to deal with the visual information overload we are constantly exposed to, enhancing the processing of visual information relevant to our goal (i.e., selective component), and to inhibit the processing of what is irrelevant (i.e., inhibitory component) (Desimone & Duncan, 1995; Duncan et al., 1997; Madden, 2007). Researchers have used a variety of paradigms in the investigation of age-related changes in selective attention, including visual search tasks, in which participants must locate certain items among an array of distracting items (Becker et al., 2013) and the Stroop colour-word task (Verhaeghen & Cerella, 2002), which requires participants to process one aspect of a stimulus.

Several studies suggest that older adults have particular difficulty with selective attention, and are thus particularly vulnerable to interference. For example, Gazzaley and colleagues demonstrated that older adults display deficits in focusing on a target in the presence of irrelevant stimuli. Aging decreases the ability to suppress irrelevant information, while the ability to enhance relevant information remains intact although the difficulty to ignore irrelevant stimuli decreases memory capacity for relevant ones (Gazzaley et al., 2005, 2008; Zanto & Gazzaley, 2010; Geerligs et al., 2014). Moreover, older adults show deficits in suppressing the automatic capture of attention by abrupt presentation of a stimulus and in disengaging attention once it has been captured. During a visual search paradigm, it was demonstrated that older adults are more susceptible than younger adults to bottom-up attentional capture and that benefit less from top-down mechanisms to override stimulus driven attentional captures.

It has been demonstrated that attention interacts closely to working memory (WM), a cognitive mechanism that enables humans to maintain and manipulate a limited amount of information for a brief period of time (Vogel & Machizawa, 2004). Deficits in WM have a

great impact on cognitive functions, and a large amount of cognitive decline in the elderly can be explained by decline in working memory.

Memory research in aging has been focused on both the memory storage of the information and the memory executive functions involved in coordinating storage and processing. Age related differences are present in tasks requiring mere storage (short-term memory) as well as and in tasks requiring storage and processing (working memory). However, simple storage span tasks yield smaller age effects than working memory span tasks. Age-related deficits in working memory span performance have been reported across many span tasks (Jost et al., 2011). A meta-analysis by Bopp and Verhaeghen (2005) showed that age difference on storage span tasks depends on the type of items to be remembered: letter spans is more affected by aging that digit spans. Indeed, when comparing verbal with spatial memory spans, an increase in spatial response times as a function of age has been observed (Bird et al., 2010).

While the first studies focused on the age-related decline in the maintenance of information in working memory, there is a recent interest in the study of the executive control components of working memory. The executive processes of selective attention and inhibitory control have figured prominently in several accounts of aging and working memory decline (Lustig et al., 2001). Older adults showed a particular impairment in tasks that require the ability to manipulate information and the integration of short-term storage with executive control processes (e.g., manipulation of stored content as in backward digit span, or coordination with other complex cognitive computations such as mental arithmetic in operation span).

Declines in working memory have been ascribed to an age-related change in executive attentional control and inhibition, which increases vulnerability to interference, decreasing the storage capacity of working memory during the encoding, the retention interval and the retrieval process (Saunders & Summers, 2011).

1.1. Theories of cognitive aging

Although there is a broad consensus about normal age-related cognitive decline, the specific mechanisms underlying these changes are still a topic of discussion. In the 1980s and 1990s, several unitary frameworks, which attributed age-related cognitive impairments to one

central capacity limitation in the processing system, have been proposed (Cabeza, 2012). These theories are based on the hypothesis that there is a shared ability that cross-cuts all of the tasks on which older adults are impaired. Although aging affects a range of cognitive functions, there is one central or "core" deficit underlying the myriad changes. Four main deficits have been proposed to explain the pattern of age-related declines: changes in speed of processing, changes in inhibitory ability changes in processing resources and changes in sensory mechanisms.

1.1.1. Processing-speed theory

One prominent theory suggested is the "Processing-speed theory" (for a review see Salthouse, 1996). According to Salthouse (1996), age-related cognitive deficits in attention, working memory and other cognitive tasks are ascribed to a generalized reduced speed in performing cognitive operations. The reduced speed of processing is global and has an impact on all the aspects of cognition.

The central hypothesis of this theoretical framework is that aging is associated with a decrease in the processing speed to execute some operations and this leads to impairments in cognitive functioning due to two mechanisms: the "limited time mechanism" and the "simultaneity mechanism". On one hand, too much time is required to perform the early operations and, as a consequence, the time available for higher-level operations is restricted (limited time). On the other hand, the products of early processing may no longer be available by the time that later operations are accomplished (simultaneity).

The evidence that processing speed changes may underlie much of the cognitive decline associated with aging and that controlling for speed of processing often eliminates age differences on cognitive tasks, corroborated this theory. Furthermore, longitudinal studies have shown a strong relationship between changes in speed of processing and changes in performance on a large number of cognitive tasks (Saunders & Summers, 2011).

One possible neural mechanism for explaining this slowing is the age-based decline in the structural integrity of white-matter tracts, as well as loss of brain volume (Geerligs et al, 2014). Indeed, since the myelin sheath serves to increase the speed of neural transmission

along axons, its age-related deterioration would lead to a slowing down of neural communication (Wiegand et al., 2014a, 2014b).

1.1.2. Inhibition deficit theory

Hasher, Zacks, and May proposed another theory able to account for cognitive deficits associated with aging (Hasher et al., 1988). According to the "Inhibition deficit theory", age-related declines have been attributed to deficient inhibition processes ascribed to changes in executive control, which includes a variety of processes such as updating and maintaining information in working memory, shifting mental sets, and the intentional inhibition of irrelevant information that influence multiple cognitive functions (McDowd, 1997; Lustig et al., 2001). Specifically, older adults are more likely to be influenced by irrelevant information. Failure to suppress distractors reduces working memory capacity, leaving no more space for relevant information.

Inhibitory deficits are evident when older adults are required to selectively attend to information in the environment, or to inhibit a strong association or response. On task-switch or on set-shift tasks, when subjects must first pay attention to one aspect of a stimulus (e.g. shape) and then to another (e.g. color), older adults show deficits in the ability to ignore the previously relevant dimension (e.g., the shape) (Madden, 2007). This evidence is supported by brain imaging research that documented specific age-related impairment in inhibitory control. Older and younger adults showed equivalent activations for the to-be-remembered items, but older adults displayed more distractor-related brain activity as compared to younger adults (Gazzaley et al, 2005). Moreover, inhibitory deficits may impair performance not only on tasks that directly assess inhibitory ability, but also on assessments of working memory capacity.

The inhibition deficit theory also suggests that older adults have difficulties in deleting no-longer relevant information, as evidenced in the greater proactive interference (difficulty in processing and learning new information because of already existing information) respective to younger adults (Lustig et al., 2001). Interestingly, greater proactive interference in older individuals is related to an impaired activation in a region of the left inferior frontal gyrus that has been specifically associated with interference control (Jonides et al., 1998).

1.1.3. Reduction of processing resources theory

Another theory elaborated by Craik and Byrd (Craik et al, 1982) suggested that cognitive aging is related to a reduction of the amount of "processing resources". Decline in WM capacity, conceptualized as the amount of resources needed to retain and manipulate information, could explain the plurality of age-related deficits, especially in cognitively demanding tasks. In line with this hypothesis, many studies showed that older adults are typically impaired in tasks involving high cognitive resources, whereas relatively automatic tasks requiring little or no resources are largely preserved. A reduction in age-related differences has been documented when the task provided elements that decreased the processing demands of the memory task, such as environmental supports ("environmental support hypothesis" Grady & Craik, 2000).

The reduction in the efficacy of pre-frontal cortex functioning could explain age-related reduction in processing resources theory (Castel & Craik, 2003). In line with this idea, older adults show weaker activations in the pre-frontal hemisphere usually involved in a given task as compared to younger adults. For example, older adults showed weaker activations compared to younger adults in right pre-frontal cortex (PFC) in visuospatial working memory tasks, which imply activations in the right PFC (Grady & Craik, 2000). Similarly, they show weaker activity in left pre-frontal cortex during episodic encoding tasks, which involve mainly the left PFC (Grady, 2008).

1.1.4. Sensory deficit theory

According to the Sensory deficit theory, age-related cognitive deficits could be ascribed to a general impairment in sensory processing (Lindenberger & Baltes, 1994). The evidence that in a wide range of cognitive tasks, older adults' performance correlates strongly with their sensory abilities, supports this theory. For instance, Lindenberger and Baltes (1994) collected extensive sensory, medical, cognitive and social measures from a large sample of older adults reporting strong correlations between cognitive and sensory measures. They demonstrated, indeed, that a significant proportion of the age-related variance in several cognitive tasks (e.g., reasoning, memory, speed of processing, world knowledge, verbal fluency) can be

explained by sensory functioning (visual and auditory acuity) and that once these sensory differences are statistically controlled, there are no longer age differences in cognitive functioning.

Several hypotheses attempt to explain the relation between cognitive and perceptual decline in aging. The common cause hypothesis, proposed by Baltes and Lindenberger, suggested that age-related declines in cognitive, sensory, and sensorimotor functioning can primarily be attributed to an overall neural degeneration (Lindenberger & Baltes, 1994; Lindenberger et al., 2001). The strong connection of sensory and cognitive abilities with age has been explained also by the sensory deprivation hypothesis, which states that a prolonged lack of adequate sensory input will result in cognitive deterioration due to neuronal atrophy (Valentijn et al., 2005).

Alternative explanations have also been proposed. For example, Schneider and Pichora-Fuller suggested the information degradation hypothesis, according to which degraded perceptual signal inputs, resulting either from age-related neurobiological processes (e.g., retinal degeneration) or experimental manipulations (e.g., reduced visual contrast), lead to errors in perceptual processing, which may affect non-perceptual cognitive processes (Schneider & Pichora-Fuller, 2000). Several studies experimentally manipulating older and younger adults' perceptual inputs demonstrated that both older and younger adults' cognitive performance was affected by perceptual input manipulations, but a greater effect was observed for older adults (Toner et al., 2012). This is due to the age-related slowing of perceptual processing (Salthouse, 1996; Faust & Balota, 1997; Madden, 2007) and to the insufficient cognitive top-down mechanisms (Raz & Rodrigue, 2006), which are unable to compensate for decreased bottom-up, perceptual signals in older adults.

2. Age related brain structural and functional changes

Attempts to understand age-related cognitive decline go along with the study of neurobiological aspects of aging, with the final aim to distinguish normal from pathological processes. In vivo structural neuroimaging data and post-mortem examination of brain tissue have revealed a diverse array of age-related changes in the brain (Madden & Cabeza, 2007).

Several neuroimaging studies have confirmed age-related changes in morphological characteristics of the brain, both in grey and white matter (figure 1.2). These changes are prominent in the PFC and are interpreted as mediating behavioral patterns of cognition within non-demented people (Cabeza et al., 2002). In particular, post-mortem studies showed decreased grey matter volume in the brains of older adults as compared to younger ones (Resnick et al., 2003), that seems to be related to lower synaptic density (Price et al., 2001). Synaptic changes are not uniform throughout the brain, with PFC and medial temporal lobe (MTL) structures resulted to be specially affected, while the occipital cortex remains relatively unaffected in older subjects (Raz & Rodrigue, 2006).

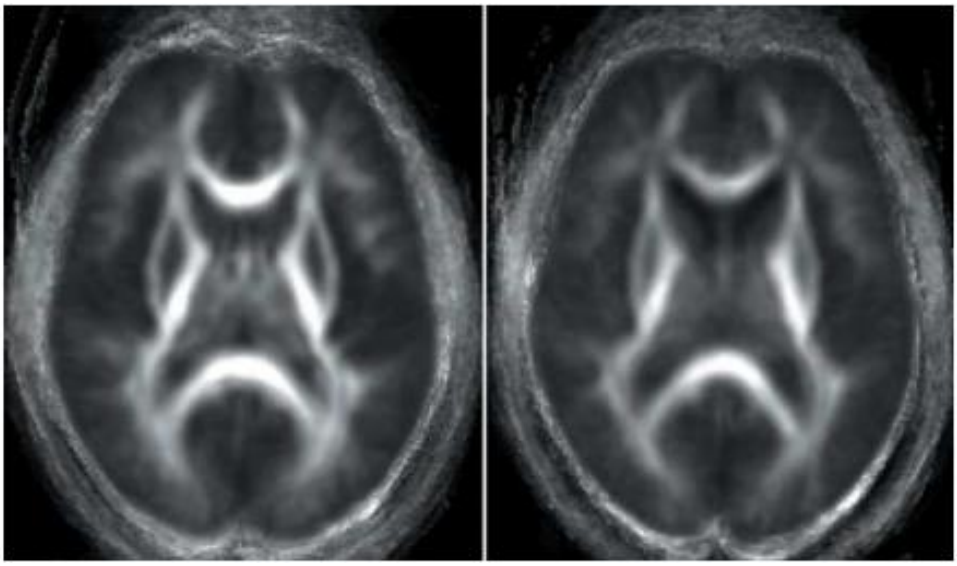

Figure 1.2 *Deterioration of white matter in aging. Differences in white matter integrity between the brains of a young (left panel) and an old (right panel) individual (adapted from Hedden & Gabrieli, 2004).*

White-matter degradation, in the form of hyperintensities, reduced white-matter integrity and volume loss, is also commonly observed among elder subjects especially in the anterior parts of the brain connecting the fronto-striatal system (Hedden & Gabrieli, 2004; Madden et al., 2009). This reduction correlates with decreases in processing speed and reasoning ability (for a review see Hedden & Gabrieli, 2004).

Besides the frontal lobes and connected regions, the hippocampal formation, a structure important to declarative memory, experiences volume loss in advanced aging that is significantly accelerated in early stages of Alzheimer's disease (for reviews see Raz &

Rodrigue, 2006). The dopaminergic system that is involved in the efficiency regulation of pre-frontal functions, is also particularly affected by aging (Mattay et al., 2002).

More recently, a number of studies have examined age-related differences in functional brain activity during cognitive task performance. These studies (Grady & Craik, 2000; Grady, 2008; Grady, 2012) confirmed a task-related age difference in brain activity most pronounced in frontally-mediated circuits supporting executively controlled processes (Cabeza, 2012), whereas brain activity underlying automatic processes (e.g., implicit memory, priming) is comparable in younger and older age (Soldan et al., 2008). The increased frontal recruitment could be interpreted to be beneficial to performance in older age, to reflect detrimental age-related changes or a dedifferentiation of cognitive functions in older age (Lindenberger et al., 2001).

2.1. Neural compensation models

In the domain of cognitive neuroscience, several models have been elaborated. These models focus on functional or structural mechanisms of cognitive aging to explain not only deficient but also preserved cognitive performance in older adults.

2.1.1. CRUNCH model

One of the most prominent models conceived is the compensation-related utilization of neural circuits hypothesis (CRUNCH, Reuter-Lorenz & Cappell, 2008). This model has attempted to account for age-related changes as a function of task difficulty, as well as to explain patterns of over-activation and under-activation in older adults (Reuter-Lorenz & Cappell, 2008; Reuter-Lorenz & Park, 2010). CRUNCH posits that the decline of neural efficiency might lead older adults to recruit more and different neural circuits than younger adults, in order to deal with task demands. This hypothesis suggests that age-related neural compensation is effective with low task demands, whereas the age-related declines in performance become apparent when task difficulty increases. Furthermore, CRUNCH is in line with the cognitive reserve model. Individuals with a bigger cognitive reserve, the whole of knowledge and competences acquired along the life, may face higher demanding tasks

(Stern, 2009) making neural over activation less likely at lower demanding tasks. CRUNCH model is also in agreement with Scaffolding Theory of Aging and Cognition (STAC; Reuter-Lorenz & Cappell, 2008), as compensatory activations may provide scaffolding to boost cognitive processes in aging.

2.1.2. PASA model

In general, two different compensatory patterns are recognized: the posterior-anterior shift in aging (PASA model) and the hemispheric asymmetry reduction in older Adults (HAROLD model).

The posterior-anterior shift in aging (PASA model) consists of an age-related decrease in occipital activity coupled with a compensatory age-related higher activation of the prefrontal cortex (Davis et al, 2008). This posterior-anterior shift in aging (PASA) has been typically attributed to functional compensation. Importantly, Davis and colleagues (2008) showed that aging results in a global shift not exclusive to the prefrontal cortex and that PASA does not appear to be contingent on task difficulty (figure 1.3).

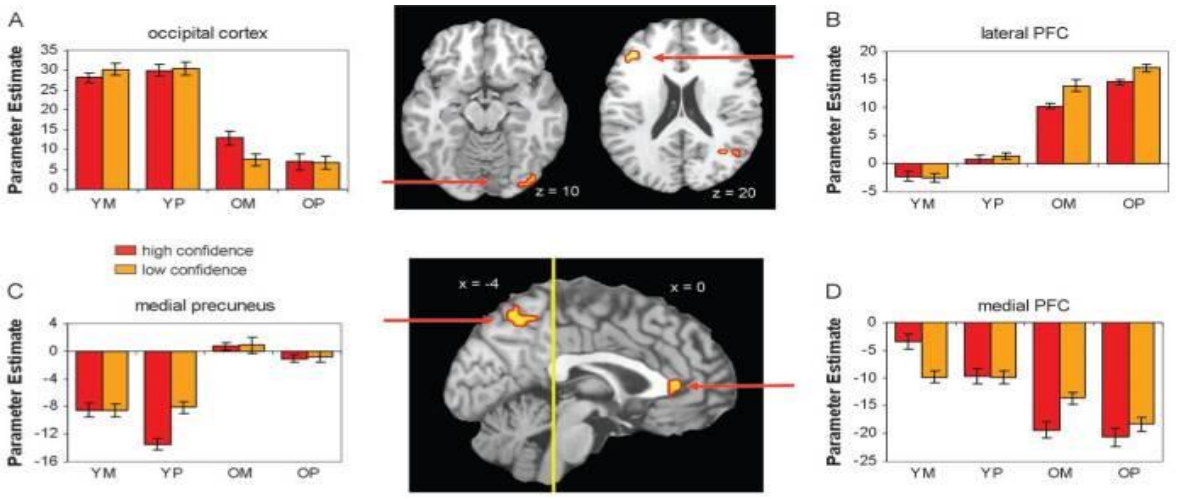

Figura 1.3 (A, B) *The PASA pattern of activations: across 2 different tasks and 2 levels of confidence, the occipital cortex showed greater activity in younger than in older adults whereas PFC showed the opposite pattern;* ***(C, D)*** *The PASA pattern for deactivations: across 2 different tasks and 2 levels of confidence, posterior midline cortex showed greater deactivations in younger than older adults, whereas the anterior midline cortex showed the opposite pattern (Davis et al, 2008).*

2.1.3. HAROLD model

Another compensatory pattern identified in the aging literature is the bilateral involvement of the PFC, which instead shows a lateralized activation in young adults (Hemispheric Asymmetry Reduction in Older Adults- HAROLD model). Older adults appear to utilize additional prefrontal cortical regions to compensate for declines in several different cognitive domains (figure 1.4). According to the compensation hypothesis, increased functional hemispheric symmetry in older adults could help counteract age-related neurocognitive deficits and may be necessary to perform adequately on the tasks (Cabeza et al., 2002).

This pattern of activation was shown by Reuter-Lorenz and colleagues (Reuter-Lorenz & Park, 2010). They investigated, by means of PET, the age-related neural changes in verbal (letters) and spatial (dot position) encoding. Younger individuals demonstrated left lateralized activation for the verbal task and right lateralized activation for the spatial one. In contrast, elderly participants had a bilateral pattern in both the two conditions. This asymmetry reduction in aging has been also observed in the domains of working memory, episodic memory, perceptual processes, and inhibitory control (Cabeza et al., 2002). Moreover, the HAROLD pattern was attributed to a compensatory mechanism, consistent with evidence that a more bilateral activity in older adults was positively correlated with successful cognitive performance (Grady & Craik, 2000; Reuter-Lorenz & Cappell, 2008) and was found only in high-performing, not in low-performing older adults (Cabeza et al., 2002).

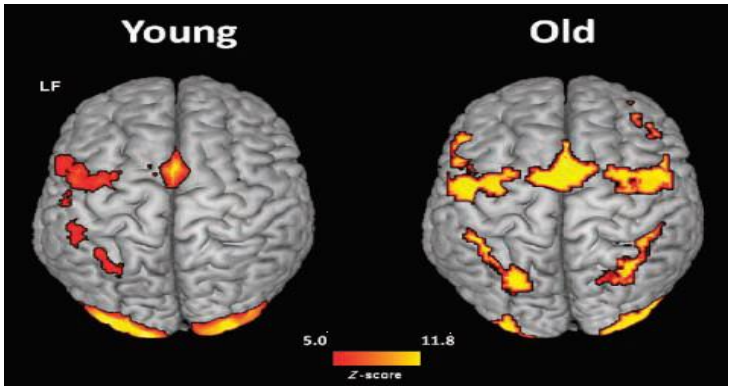

Figure 1.4 *HAROLD pattern. Older adults show activations over the homologous area recruited by young adults (adapted from Schneider-Garces et al., 2010).*

2.1.4. STAC model

A framework that takes into account both neurocognitive decline and neuroplasticity of aging brain is the STAC model (Reuter-Lorenz & Park, 2010). It claims that the aging brain is characterized by a number of neural challenges to which it must overcome and adapt. Some of these challenges are morphological, including atrophy, amyloid deposition, white matter deterioration, and dopamine receptor depletion. Others are functional and include dedifferentiation (i.e., loss of brain regional specialization), decreased activation of MTL structures, and default network dysregulation. According to STAC, brains would overcome such neural alterations by forging alternative neural circuitries, scaffolds, which allow individuals to maintain an adequate level of cognitive functioning even at advanced ages. Scaffolds encompass patterns of over-activations (e.g., PASA, HAROLD patterns), involving primarily the PFC, but also some areas within parietal, medio-temporal, and occipital regions. Compensatory scaffolding is related to experiences so that new learning, cognitive training and active cognition might all mediate new neural scaffolding in order to maintain a high level of cognitive functioning. Furthermore, mechanisms of neurogenesis, synaptogenesis, and angiogenesis, even if less efficient in older than younger individuals, remain functional and help in forging alternative neural networks. Given that individuals are always challenged by cognitive situations that the brain must face, scaffolding is a lifelong process, relied upon in an enhanced manner in the older age.

3. Age related psychophysiological modifications

Several studies have shown that aging is associated with a general attenuation of the autonomic nervous system, which leads to decreases in arousal response and results in diminished psychological activity (Boss & Seegmiller, 1981; Nigam et al., 2012).

3.1. Arousal

Arousal is a temporary state of generalized physiological and psychological activation, and it plays a crucial role in the regulation of different aspects of our lives such as sleep, anxiety

and cognition (Sara & Bouret, 2012). The modulation of arousal is regulated by several nuclei widely distributed in the brainstem. These nuclei have neuromodulatory functions throughout almost all cortical and subcortical structures. The locus coeruleus (LC), a small structure in the pons on the lateral edge of the 4th ventricle (figure 1.5), is one of these nuclei and, as demonstrated by animal model studies, is considered the preeminent noradrenergic nucleus involved in the regulation of arousal (Rajkowski et al., 1993; Aston-Jones et al.,1999).

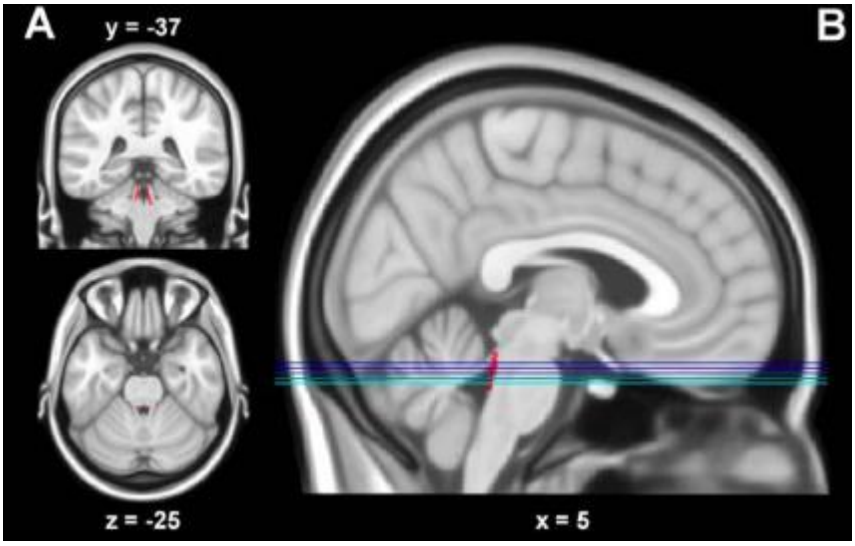

Figure 1.5 *The Locus Coeruleus (LC) is shown in red.*

The cerebral cortices of all the hemispheric lobes are extensively innervated by the LC projections, which are the only source of cortical noradrenaline (NA). The basal forebrain, amygdala and hippocampus, thalamus and hypothalamus are all structures innervated by the LC such as all the nuclei of the brainstem (premotor, motor and sensory), the cerebellum, the sympathetic and parasympathetic preganglionic neurons of the spinal cord. Considering the vast number of LC projections, its crucial role is clear in the regulation of cortical and subcortical functions. NA circuits are best known for their role in behavioral arousal and in the control of heart rate and blood pressure, but there is also a growing body of evidence from rodent, primate, and human studies that the noradrenergic system plays an important role in attention, memory and cognition (Sara & Bouret, 2012).

Primate LC neurons have shown two different functioning modes, phasic and tonic, during several tasks (Clayton, 2004). In the *phasic mode*, bursts of LC activation are observed during

the processing of motivationally relevant stimuli; as a consequence, a release of noradrenaline (NA) in different cortical structures (hippocampus, neocortex, and other projection areas) is observed. This state of activation has been related to a performance improvement during different tasks. Conversely, during the *tonic mode*, the bursts of LC activity are absent while the basic activity of the LC is increased. Therefore, subjects result in a more distractible behavior that might decrease task performance (Aston-Jones & Cohen, 2005).

Yerkes and Dodson (1908) state that the relationship between arousal and performance is represented by an inverted-U curve (figure 1.6). According to this theory, performance is optimal with moderate LC tonic activity and prominent phasic LC activation (phasic LC mode). Furthermore, although an increased level of arousal can facilitate task engaged responses, a too high level can lead to a decline of the performance caused by an excess of distractibility and anxiety.

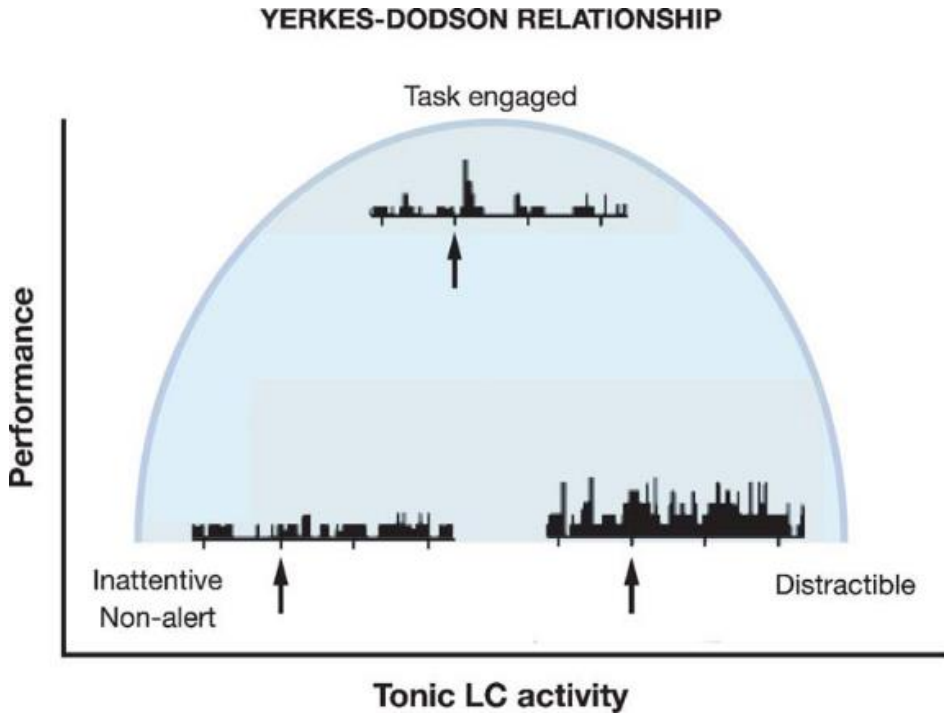

Figure 1.6 *Inverted-U relationship between LC activity and performance on tasks that require focused attention (by Aston-Jones et al., 1999).*

LC phasic mode shows a reduction of activity, as a result of a process of habituation, with the decrement of the salience of task-relevant stimuli (Sara, 2009). This pattern is not exhibited for highly salient or task-relevant stimulus events (Aston-Jones & Cohen, 2005). Different studies have shown that the LC activity is highly plastic and is not strictly related to specific attributes of the presented target, rather it responds to events in a task-related manner (Aston-Jones & Cohen, 2005). Recent evidence has demonstrated that the timing of LC phasic responses is extremely short (∼100 ms onset) and this is in contrast with traditional concepts of a slowly and nonspecific system. In the light of this evidence, Aston-Jones and Cohen (2005) proposed that the LC phasic response provides a temporal attentional filter that selectively facilitates task-relevant behaviors.

3.2. Psychophysiological indices of arousal

The main physiological indices commonly used to evaluate the level of arousal are electrodermal activity, skin conductance (SC), heart rate, electroencephalography and pupil diameter.

3.2.1. Electrodermal activity

Electrodermal activity is the umbrella term used for defining autonomic changes in the electrical properties of the skin, in response to sweat gland secretion. The most widely studied property is the SC, which can be quantified by applying an electrical potential between two points of skin contact and measuring the resulting current flow between them (Fowles et al., 1981). SC is considered a reliable measure of arousal (Dawson et al., 2000; Bagherli & Mokhtari, 2011). The time series of SC is characterized by a slowly varying tonic activity and a fast varying phasic activity. Tonic skin conductance is generally considered as the level of skin conductance in the absence of any particular discrete environmental event or external stimuli. This slow changing level is generally referred to as Skin Conductance Level (SCL). Tonic changes in the skin conductance level typically occur in a period of from tens of seconds to minutes. Phasic skin conductance measurements are typically associated with short-term events and occur in the presence of discrete environmental stimuli. Phasic changes

usually show up as abrupt increases, or "peaks" in the skin conductance that begins 1s after the stimulus with the peak of amplitude around 1s after the onset. After this rise of activation there is a slow decline to the baseline (figure 1.7). These peaks are generally referred to as Skin Conductance Responses (SCRs) (Levinson and Edelberg, 1985; Dawson et al., 2000).

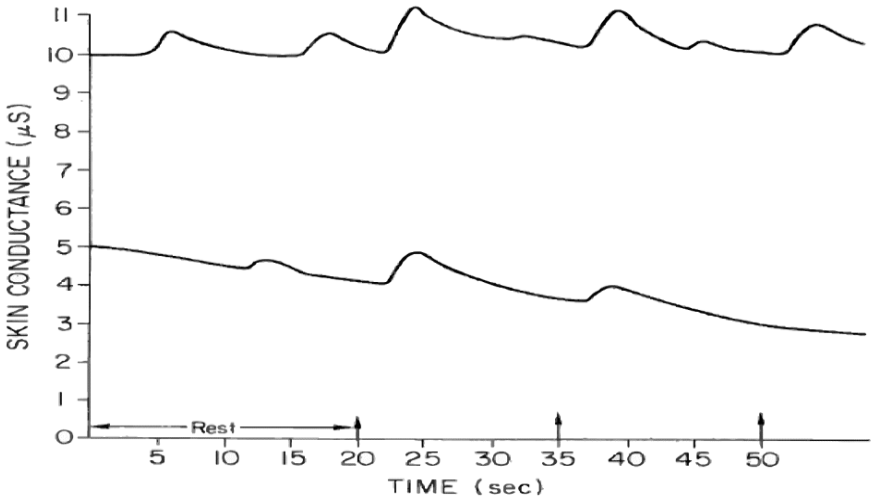

Figure 1.7 *Two skin conductance recordings during 20 seconds of rest followed by three repetition of a simple discrete stimulus. Arrows represent the presentation of the stimulus (by Dawson et al., 2000).*

3.2.2. Heart rate

Arousal is also associated with increases in heart rate, another popular measure of the autonomic response (Simons, 1988). However, the interpretation of this index is not always easy because it mainly reflects parasympathetic inhibitory influences, strongly modulated by respiratory behavior and baroreflex activation (Berntson et al., 1993).

3.2.3. Electroencephalography

Arousal is also evaluated through electroencephalography and the analyses of event-related potentials (ERPs). The P3 has been one of the most heavily investigated ERPs, peaking 300–600 ms after a task-relevant stimulus and with a maximal distribution over centro-parietal midline electrode sites. Recent evidence from animal, genetic, and pharmacological studies, suggests that the P3 may represent a cortical electrophysiological correlate of the phasic LC

response (Nieuwenhuis et al., 2005; Nieuwenhuis et al., 2011). In particular, Nieuwenhuis and colleagues (2005, 2011) claimed that the simultaneous occurrence of the P3 and the autonomic components of the orienting response reflects the co-activation of LC-NA system and of the peripheral sympathetic nervous system by their common neural substrates. In one study that recorded monkey LC neuron activity and cortical ERPs simultaneously, both phasic LC activity and fronto-parietal ERPs analogous to the human P3 were selectively evoked by target stimuli and followed closely related time courses (Aston-Jones & Cohen, 2005).

3.2.4. Pupil diameter

Whereas the P3 may index the phasic LC response, neural control over pupil size has been associated with both the sympathetic and parasympathetic nervous systems (Rajkowski et al., 1993). Rajkowski and colleagues (1993) showed that pupil diameter correlates with LC tonic activity in the monkey (figure 1.8) and it has been confirmed also in the humans (Gilzenrat et al., 2003).

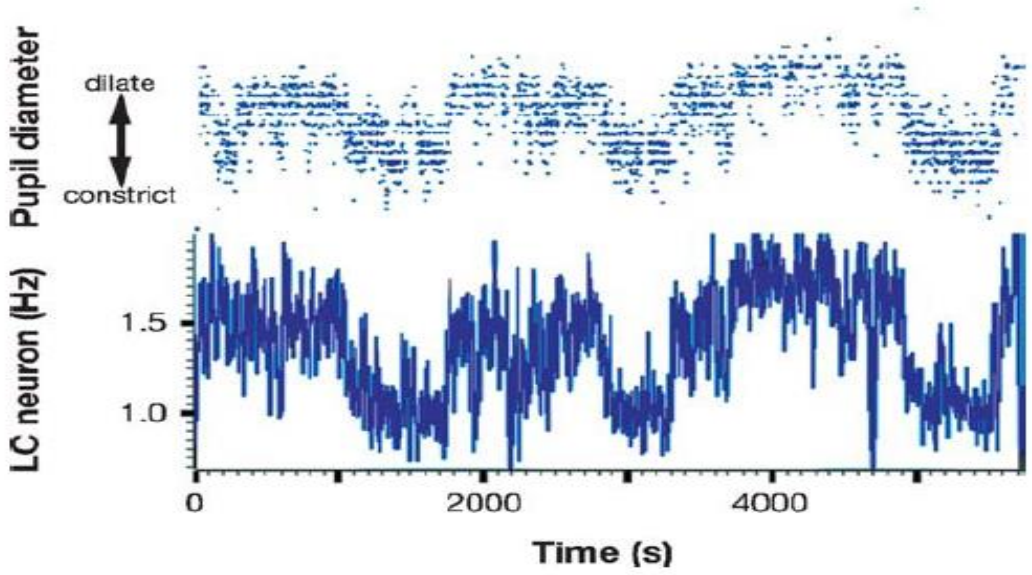

Figure 1.8 *Relationship between tonic pupil diameter and baseline firing rate of an LC neuron in monkey (by Aston-Jones & Cohen, 2005).*

Numerous studies have shown that task processing is related to wide pupil dilation, consistent with the presence of an LC phasic response to task-relevant events (Beatty, 1982; Richer & Beatty, 1987). Aston-Jones and Cohen (2005), in a study with a series of tone discrimination, showed that early in each trial series there were large phasic pupil dilations for each discrimination. Similarly, to the LC phasic responses, these dilations declined in amplitude during trials while baseline (tonic) pupil diameter increased as the task became more difficult and the expected utility of the stimuli began to decline. Baseline pupil diameter was greatest at the point at which subjects chose to abandon the current series of stimuli, consistently with the hypothesis that this choice was related to an increase in LC tonic activity. In light of this theory, high levels of tonic pupil diameter would follow a decline in task performance with a consequent reengagement in the task and an increased P3 amplitude.

Murphy and colleagues (Murphy et al., 2011) investigated the relationship between performance on an attentional task and two psychophysiological indices of LC-NA system activity: pupil diameter and P3. They used an auditory oddball task with stimuli presented through headphones, participants had to press a button for target stimuli which were presented 20% of the total stimuli. The authors proposed an inverted U-shaped relationship between the pre-stimulus pupil diameter and both P3 amplitude and task performance; according to this relationship, an optimal performance and the largest P3 amplitude occurred at the same intermediate level of prestimulus pupil diameter. Gilzenrat and colleagues (2010) presented three experiments manipulating the task utility (i.e., the costs and rewards of performance). They replicated the results present in literature confirming that task-related processing is associated with phasic dilations of the pupil in humans. The novelty contribution of their study is the conclusion that if task difficulty is further increased over a limit, the pattern of pupil dynamics reverses with a consequent phasic constriction, mirroring the dynamics of the tonic LC mode.

These finding of an inverse relationship between baseline pupil diameter and phasic pupil dilations are consistent with the observed differentiation between tonic and phasic modes of LC activity in animal literature and also correspond well to the performance dynamics predicted from the theory of Aston-Jones and Cohen (2005). Pupil size has a relatively short

temporal profile, increasing within a few seconds and returning to baseline levels relatively rapidly (Bernhardt et al., 1996).

3.3. Arousal and aging

Aging is generally associated with an attenuation of the autonomic systems (Barontini et al., 1997). As a consequence also arousal may decline with aging and therefore, some age-related cognitive declines can be attributed to this alteration. However, changes in arousal with aging have not been fully studied and there are still conflicting results.

Some evidence suggests that elderly are under aroused and task-induced responses of electrophysiological measures, such as skin conductance and pupil dilation, are smaller and deleted in the elderly than in the young (Gross & Levenson, 1993; Levenson, 1997). Barottini and colleagues, for instance, showed that whether basal heart rate was similar in all subjects and failed to correlate with age, skin conductance level decreased with aging and correlated negatively and significantly with age (Barontini et al., 1997). Another study using event-related potential (ERP) also showed age-related attenuation of physiological reactivity in older compared to younger adults, but this was not reflected in any decrements of subjective affective appraisal (Kisley et al., 2007). These results extend previous findings that sympathetic nervous system activity is altered in aging and are supported by evidence of LC-NA system changes in aging (Mather & Harley, 2016). First of all, an age related decline in LC neuron number by ~ 20-40% (German et al., 1988), was suggested, with selective cell loss in the rostral LC compartment. Clear evidence that LC tau pathology increases with age (Braak et al., 2011) was also found.

Others studies, on the contrary, did not found any age differences in sympathetic nervous system activity (e.g., Denburg et al., 2003) compared to younger adults. However older adults were less likely to show measurable skin conductance responses (e.g., non-responders) as compared to the young; when a response occurred, however, the magnitude of SCRs did not differ between young and old (Neiss et al., 2009). Also Kunzman and Grühn (2005), including several measures of autonomic activity such as heartbeat interval, finger pulse amplitude, skin conductance level and others, did not find any age difference in physiological responses to stimuli of emotional relevance. Another study of Kim and colleagues compared

pupillary responses of young and older normal participants using infrared pupillography. Results showed no differences in amplitude of pupillary response between the 2 groups. Instead, the older group showed a delayed habituation response and the interval between the maximal dilation (arousal response) and the followed constriction (habituation response) was prolonged (Kim et al., 2000).

Overall these studies reported contrasting results; there is currently no consensus in literature on age-related arousal modulation. Although, as already discussed, some research reported a general attenuation of physiological reactivity with age, others did not find any difference in autonomic responses between young and older adults. The arousal response in the elderly has been incompletely studied and are necessary more investigation to disentangle this issue.

4. Pathological aging: the Alzheimer's disease

Dementia is a clinical syndrome with a progressive course characterized by a cluster of symptoms and signs, including difficulties in memory, disturbances in language, and psychological and psychiatric changes that interfere with daily-living activities (Dubois et al., 2010).

Alzheimer Disease (AD) is the most frequent cause of age-related dementia, accounting for about 60% of cases, and its incidence is expected to rise more and more, as around 80 million of dementia cases have been foreseeable by 2040 (Ferri et al., 2005). The prevalence of AD is age-dependent, doubling every 5 years after the age of 60 years, with around 1% of those aged 65-69 years affected rising to almost 20% in those aged 85 years or over (Rossor et al., 1996). One of the most prominent clinical features of AD is the progressive memory impairment in long term-memory (episodic and semantic), short-term memory, and implicit memory functions (Nebes et al., 1992; Weintraub et al., 2012). Additionally, according to the American Psychiatric Association, one or more cognitive disturbances between aphasia, apraxia, agnosia or disturbances in executive functions is necessary to determine a diagnosis of AD.

The cognitive symptoms that appear in the first stages of the pathology are deficit in episodic memory and one of the main structure involved is the hippocampus. For that reason, the

common view of Alzheimer's disease (AD) is that of an age-related memory disorder associated with progressive brain changes in the MTLs and in the default mode network (DMN). Recent evidence, however, showed that early lesions of lateral parietal networks are responsible of selective attention deficits that accompanied memory impairment in AD patients (Finke et al., 2013). The disrupted integration within and across intrinsic brain networks, which overlap in parietal and temporal lobes, seems to be responsible of the selective competition of representations during both perception and memory (Finke et al., 2013). Over time, cerebral degeneration affects other brain regions causing a severe evolution (aphasia, apraxia, agnosia and executive dysfunction). Primary motor and sensory cortices are less damaged than other cerebral structures (Weintraub et al., 2012). In addition, with disease progression, behavioral symptoms such as delusions, agitation, changes in personality, and mood disturbances may also occur (Dubois et al., 2010).

4.1. The clinical continuum: from normal aging to AD

Current evidence considers AD as a portion of a biological and clinical continuum with amyloid plaques deposition starting even 10-30 years before the onset of the first clinical symptoms. This continuum may be divided in three main stages (Braak et al., 2011) ranging from the initial pre-symptomatic or preclinical phase (healthy elderly with no cognitive symptoms but that present AD pathological changes) to the final dementia phase with the well-known multidomain cognitive and functional impairment.

Many attempts have been made to better understand the transitional phase between normal aging and dementia. Petersen introduced the concept of mild cognitive impairment (MCI) to designate an early, but abnormal, state of cognitive impairment, interposed between the cognitive changes of normal and pathological aging (Petersen, 2004a). Different diagnosis classification systems have been proposed for the characterization of mild cognitive disorders associated with aging. They all share the common concept that a single, clinically significant impairment in cognition, for example memory, is present without addition functional or cognitive impairments necessary to diagnose dementia. Furthermore, activities of daily living are preserved and complex instrumental functions are either intact or minimally impaired in MCI patients (Petersen et al., 2014). MCI patients can be classified into two main diagnostic

entities (Petersen et al., 2014; Petersen, 2004a, 2004b): amnestic MCI, when the core neuropsychological impairment is memory, and the category of non-amnestic MCI, which represents patients who show a poor performance in neuropsychological tests assessing cognitive functions other than memory. Patients with MCI are considered at high-risk for the development of AD, with a conversion rate of about 10-15% within one year (Petersen, 2004a, 2004b).

Neuroimaging and neuropsychological attempts have been made to better understand how MCI converts to AD. Galluzzi et al. (2010) found that the medial temporal atrophy and abnormal CSF (level of beta amyloid in cerebrospinal fluid) are the most important predictors of the conversion from MCI to AD. Moreover, a recent study has demonstrated that atrophy of the hippocampus on MRI in cognitively intact elderly people predicts dementia, in particular of Alzheimer type, during a 6-year follow-up (den Heijer et al., 2006). Therefore, hippocampus atrophy could be considered a potential marker for detecting preclinical AD (figure 1.9).

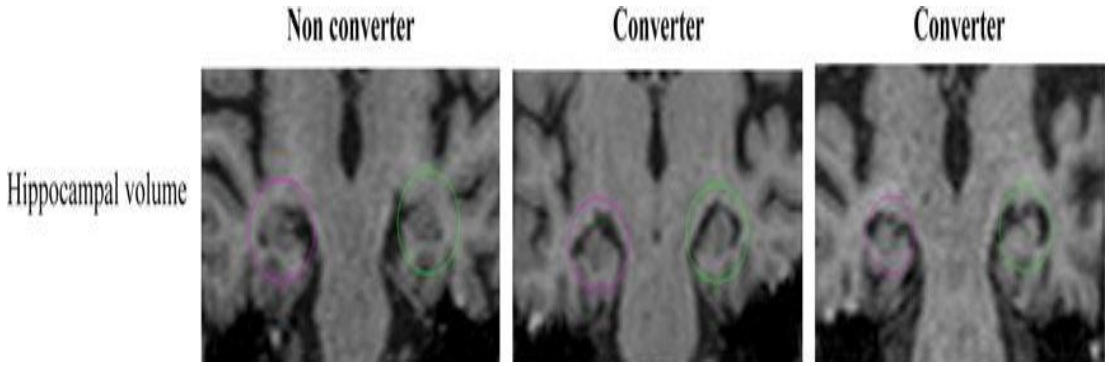

Figure 1.9 *Hippocampal volume in MCI (Galluzzi et al., 2010).*

A progressive slowing of the neocortical EEG has been reported to occur in close relation to the cognitive and behavioral changes typical of AD (Dringenberg et al, 2000). In a recent study, Moretti and colleagues (2012) investigated the association between hippocampal atrophy and increase of some EEG markers (i.e., alpha3/alpha2) both in MCI and AD patients. Results showed that the increase of alpha3/alpha2 frequency power ratio was correlated with atrophy of hippocampus in both types of patients. This study (Moretti et al., 2012) and other similar evidence (Moretti et al., 2013; Ruzzoli et al. 2016) support the

possible role of EEG markers as diagnostic and prognostic factors in both MCI and AD patients.

4.2. Neuropathology of AD

From the neuropathological point of view AD is characterized by accumulating amyloid plaques and neurofibrillary tangles typically in medial temporal structures and cerebral cortex (Albert, 1993; Weintraub et al., 2012). In particular, an accumulation of amyloid - Aβ-42, tau and phospho tau that subsequently caused neuritic plaques and tangles in MTL, seems to be responsible of the pathology (figure 1.10).

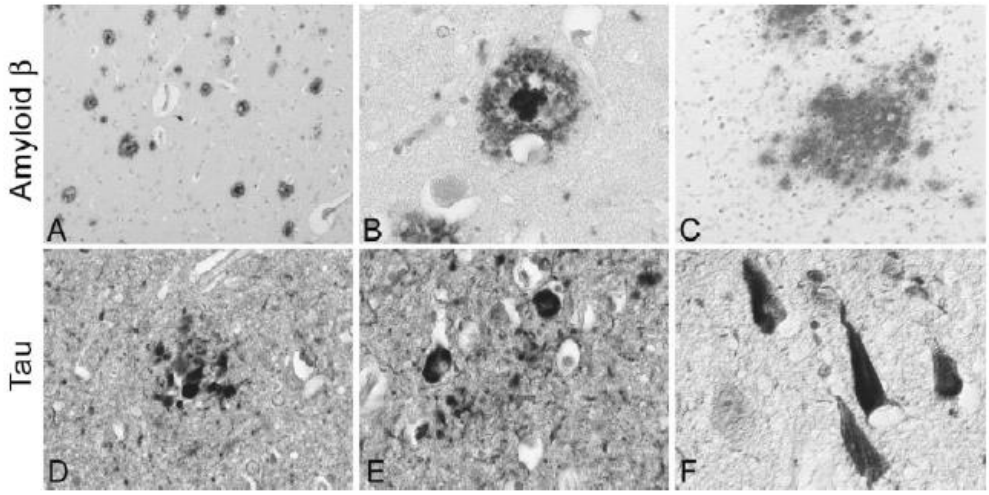

Figure 1.10 (A, B, C) *Cerebral changes in Alzheimer Disease - evolution of amyloid plaques in neocortex. D,E,F- TAU immunohistochemistry leads to neurofibrillary tangles which assume different shapes* (Serrano-Pozo et al., 2011).

The other features linked to these two core pathological hallmarks of AD are inflammation, oxidative stress, progressive synaptic and neuronal loss (Serrano-Pozo et al., 2011). Many SPECT and PET studies have also shown a reduction of glucose metabolism in tempo-parietal cortex (Resnick et al., 2003). A severe depletion of cortical cholinergic innervations is also involved in the neuropathology of AD (Supekar et al., 2008). For this reason, since the mid-1990s, cholinesterase inhibitors have been used as symptomatic treatments for AD to improve cognition and indirectly help function and behavior. However, many recent data

indicate that, by itself, cholinergic dysfunction may not be sufficient to cause the marked changes in cognition and cortical activity typical of AD. Rather, more widespread degenerative processes involving multiple neurotransmitter systems seem to be necessary to produce dementia-like cognitive and behavioral patterns (Dringenberg et al., 2000). Recent evidence suggests that the dopaminergic system could also have a prominent role in the pathophysiology of AD. Dopamine agonists may improve memory function in animal models of AD suggesting that dopaminergic agents could be a novel drug for AD (Himeno et al., 2011). However, the involvement of dopamine in the cognitive symptoms in patients with AD has been poorly explored (Martorana & Koch, 2014).

In a recent study, Koch and colleagues (2014), investigated whether the administration of dopamine agonist rotigotine (RTG) could modulate cortical plasticity in AD patients, as measured by theta burst stimulation protocols of repetitive transcranial stimulation applied over the primary motor cortex. Results showed that at baseline, AD patients were characterized by impaired cortical plasticity. Cortical plasticity increased and normalized after RTG administration revealing that dopamine agonists have the ability to restore altered mechanisms of cortical plasticity in AD patients. This provides novel implications for therapies based on dopaminergic stimulation to combat cortical dysfunction in AD (Koch et al., 2014).

4.3. New Frontiers in AD research

A first recent line of research concerns the identification and validation of biomarkers for Alzheimer's disease diagnosing. Amyloid β 42, and tau proteins are established core cerebrospinal biomarkers and to date, measurement of β-amyloid, total tau and phospho-tau-181 in cerebrospinal fluid (CSF) is the most advanced and accepted method to diagnose probable AD with high specificity and sensitivity (Scheltens et al., 2016). Novel candidate biomarkers include amyloid β oligomers, a toxic form of Aβ that causes synaptic dysfunction, and synaptic markers (dendritic protein neurogranin), which are involved in long-term potentiation and memory consolidation (Craig-Schapiro et al., 2009). CSF reflects

metabolic processes in the brain and it is a very useful fluid for AD diagnosis. However, it required an invasive collection.

Another relatively new topic of research, that holds great promise in revealing how brain network dynamics change across the lifespan and in disease, is functional connectivity investigation. AD is accompanied by loss of connections between brain systems causing the disruptions in functional networks (Supekar et al., 2008; Dennis & Thompson, 2014). Several studies examined changes in functional connectivity through fMRI (functional magnetic resonance imaging), EEG (electroencephalography), MEG (magnetoencephalography), and PET (positron emission tomography) as well as by means of multi-modal methods that combine resting state fMRI with PET imaging (Supekar et al., 2008; Friston, 2011; Damoiseaux et al., 2012; Dennis & Thompson, 2014). Overall these studies showed that connectivity and network integrity decrease in healthy aging. This decrease is accelerated in AD, with specific systems such as the default mode network hit hardest (Dennis et al., 2014). The default mode network, is the most active network in the absence of a task and is known to be involved in the retrieval of autobiographical episodic memory as well as self-referential mental processing. The different DMN structures are densely interconnected to each other. Both structural and functional aspects of DMN are affected both in normal aging and in AD (Fjell et al., 2014). Indeed, a general decrease in whole brain connectivity as well as in DMN connectivity has been reported in healthy aging (Sala-Llonch et al., 2015; Lehmann et al., 2015; Joo et al., 2016) and at a further level of severity in AD, MCI patients and in subjects at high risk for AD (Supekar et al., 2008). PET studies have found DMN hubs to be especially vulnerable to amyloid deposition, likely one factor contributing to the robust findings of decreased functional connectivity of the DMN in AD (Dennis & Thompson, 2014).

Neural and cognitive mechanisms implicated in Multiple Object Processing in Alzheimer's disease patients and in subjects with Mild Cognitive Impairment

1. Introduction

The ability to concurrently process multiple objects is fundamental in several daily activities that require detecting multiple visual inputs simultaneously (Pagano & Mazza, 2012; Pagano et al., 2014; Mazza & Caramazza, 2015).

Some cognitive theories suggested that there are at least two classes of mechanisms involved in multiple object processing (MOP): an early individuation mechanism, tightly related to attention, which enables the simultaneous identification of the items; and a later mechanism mediated by visual working memory, which enables the representation of the identified objects to be actively maintained (Trick & Pylyshyn, 1993; Pylyshyn, 2001; Xu & Chun, 2009). The early representations produced allow the visual system to individuate each object as being separate from others. When a more detailed representation of the objects is required, a later process encodes the individuated items in greater detail to produce a complete representation, ultimately leading to the full object identification (Dehaene & Cohen, 1994; Ansari et al., 2007).

The processing of multiple objects is strictly related to visual enumeration. During enumeration, our visual system needs to detect and keep several objects separate in order to count them once and only once, and to finally retrieve the numerosity of a set. For this reason, when investigating multiple object processing, many studies adopted visual enumeration tasks in which participants are required to count a variable number of target stimuli presented among distractors (Pagano & Mazza, 2012).

A peculiar feature of visual enumeration is the so-called "subitizing phenomenon" (Kaufman et al., 1949), represented by a dissociation between the performance in enumerating small (called "subitizing") and larger quantities of targets (a process usually referred to as "counting"). If the number of displayed objects is within the "subitizing' range (usually up

to 3-4 items), participants are fast and accurate, whereas if the number of displayed objects is in the counting range, they are slower and more error prone (Wutz & Melcher, 2014).

1.1. Electrophysiological correlates of multiple object processing

Given the high temporal resolution of electrophysiological measures, previous Event-Related Potential (ERP) studies (Mazza & Caramazza, 2012; Pagano & Mazza, 2012; Munneke et al., 2013) provided important hints on the temporal brain dynamics associated with the various processing stages involved in the analysis of multiple object processing. Specifically, two temporally distinct neural activations, the N2pc and the CDA, have been associated, respectively, to the object individuation mechanism and to visual working memory processes.

The N2pc (N200 posterior contralateral) is a lateralized negative component that appears over posterior electrode sites with a latency of about 200 ms after stimulus onset (Luck & Hillyard, 1994; Eimer, 1996) when the relevant information (i.e., target element) is displayed in one visual hemifield. In particular, the N2pc is defined as the difference in the activity of the two hemispheres, with the activity acquired over the electrodes ipsilateral to the target side being less negative than the contralateral ones (Eimer, 1996). The N2pc has typically been measured during visual search tasks and has been associated with attentional selection/individuation processes (Eimer, 1996; Ester et al., 2012; Pagano & Mazza, 2012). In multiple objects processing tasks, where targets are presented among distractors, the N2pc component is considered a neurophysiological marker of target selection and its amplitude increases as a function of target numerosity, reaching a limit at around three targets (Eimer, 1996; Munneke et al., 2013).

The CDA (Contralateral Delay Activity) is a lateralized and sustained posterior negative wave, which is elicited around 400 ms post-stimulus onset and indexes visual working memory abilities (Duarte et al., 2013; Pagano et al., 2014). Like N2pc, CDA represents a difference in the activity of posterior sites across hemispheres with respect to the target side, with the contralateral sites being more negative than the ipsilateral sites (Vogel & Machizawa, 2004). CDA amplitude is modulated by the number of objects that have to be

maintained in memory storage and increases as a function of target numerosity until it reaches an asymptotic limit at around three objects (Pagano et al., 2014).

1.2. Multiple Object Processing in healthy aging

It is well documented that normal aging is accompanied by alterations in brain structures and functions that are associated with cognitive changes (Glisky, 2007). Declines in perception, attention and WM performance contribute to age-related impairments in a broad range of cognitive functions, in particular when multiple rather than single elements are required to be processed.

Despite there being few studies investigating multiple objects processing in aging, there is evidence of age-related decline in the ability to simultaneously process multiple objects (Sliwinski & Buschke, 1997; Watson et al., 2002, Watson et al., 2007; Pagano et al., 2015). Nevertheless, it is still unclear whether the age-related impairment in multiple objects analysis is connected to a general decline, equally affecting all the mechanisms associated with the analysis of multiple objects, or whether it affects only a specific component.

For instance, if all the mechanisms involved in multiple object analysis were delayed and/or suppressed, a general age-related cognitive slowing (Salthouse, 1996) could be responsible for the impairments in multiple object performance. Instead, if there was a delay or suppression of one specific mechanism, that particular process could be responsible for the age-related impairment in multiple object processing (Mazza & Brignani, 2016).

The investigation of how aging modulates the distinct processes involved in enumeration is very important because it allows us to understand the basis of the cognitive decline (for a review see Mazza & Brignani, 2016). While behavioral studies do not allow successful disentanglement of this issue, recent advances in neuroimaging techniques (and EEG, in particular) have allowed researchers to shed new light on such mechanisms.

In their seminal EEG study, Pagano and colleagues (Pagano et al., 2015) clarified the nature of age-related impairment in multiple object processing reporting that a common decline of both early attentional (reflected by N2pc) and late working memory mechanisms (indexed by CDA) lays the foundation for the impaired behavioral performance. The authors reported a reduction of both N2pc and CDA responses in healthy elderly subjects when asked to

enumerate a variable number of targets (from 1 to 6) presented among distractors. Whereas the reduction of N2pc component affected all the target numerosities, CDA response was reduced only for large numerosities (more than 3 targets), thus suggesting a dissociation between a general slowing in cognitive efficiency and a selective impairment affecting one specific mechanism.

1.3. Multiple Object Processing in pathological aging

As already discussed in the previous chapter, Alzheimer's disease (AD) and Mild Cognitive Impairment (MCI) are mainly characterized by attentional and memory deficits. For this reason, the investigation of the neural correlates of multiple object processing may provide important insights to the study of pathological aging by shedding new light on the physiological mechanisms underlying these age-related diseases (Mazza & Brignani, 2016). In addition, since AD is part of a clinical and biological continuum and attentional and memory deficits are expected to appear at very early stages of dementia, evaluation of visuospatial processes is considered a promising approach in the search for predictive markers of AD (Cespòn et al., 2015). The identification and validation of biomarkers for diagnosing Alzheimer's disease is crucial in order to intervene early in the pathology (Mazza & Brignani, 2016; Moretti, 2016).

So far, only a few studies have investigated enumeration performance in samples of patients. In the late nineties, Watson and Humphreys (Watson & Humphreys, 1999) demonstrated that patients with damage to temporo-parietal regions showed a preserved enumeration function with relatively normal subitizing and counting processes when only targets were presented. However, when targets were presented among distractors, the authors reported an overall slowing down of performance and a marked increase of errors in the counting range. Also, in a previous study, Dehaene and Cohen (Dehaene & Cohen, 1994) measured enumeration performance in a sample of simultanagnosic patients who suffered from parietal, occipital or posterior temporal lesions. These patients showed preserved performance when enumerating a small number of objects (1, 2 and sometimes 3), thus showing preserved subitizing. However, their performance dramatically declined for the counting range when more than 3 items were presented. More recent brain imaging studies confirmed and further deepened

those results, reporting that multiple objects processing mainly activates temporo-parietal areas (Ansari et al., 2007; Vetter et al., 2011; Vuokko et al., 2013), among the key regions early affected by AD and which suffer from the earliest deposition of beta-amyloid, even before cognitive symptoms appear (Celone et al., 2006). Indeed, FDG-PET studies in patients with AD showed a reduced metabolism in the temporo-parietal cortex, posterior cingulate and precuneus (Jagust et al., 2007). There are few behavioral studies that have investigated enumeration abilities in samples of AD patients. Moreover, to our knowledge, there are no studies adopting this paradigm in preclinical or prodromal populations such as MCI patients. Nebes and colleagues (Nebes et al., 1992) showed that the quantity of items presented modulated the enumeration performance of AD patients. The authors reported a decline in the subitizing span of AD patients and that they were slower and more error prone when more than two targets were presented. Interestingly, the enumeration performance was found to be correlated with the severity of dementia assessed with the Clinical Dementia Rating scale (CDR; Morris, 1997). Maylor and colleagues (Maylor et al., 2005) investigated enumeration abilities in AD patients at a moderate stage of the disease (mean MMSE score of 17.3). The authors reported a reduced subitizing span in AD patients who were able to simultaneously process around 2 targets, while the span of healthy elderly controls was around 3.5 targets. Considering the overall enumeration performance, AD patients were overall slower, but not less accurate than healthy elderly controls. In line with the significant correlation with CDR found in the study by Nebes (Nebes et al., 1992), enumeration performance was related to the severity of the disease, as measured with Mini Mental State Examination (MMSE; Folstein et al., 1975). Patients with higher error rates and slower reaction times had lower MMSE scores. Furthermore, subitizing span was also associated with dementia severity, such that as dementia severity increased, subitizing span decreased. To investigate whether the enumeration impairment reported in moderate AD patients was also present at an earlier stage of the disease, Maylor and colleagues (Maylor et al., 2008) tested the ability to enumerate a variable number of targets (from 1 to 9 as in Maylor et al., 2005) in a sample of AD patients at a mild stage of the disease (mean MMSE score of 22.8). Although the results highlighted an overall impairment in enumeration abilities in mild-stage AD patients (e.g. slower

counting rate), the reduction of subitizing span was apparent only in the later stage of the disease (i.e., moderate; Maylor et al., 2008).

2. Objective of the study

The aim of the present study was to investigate the neural/electrophysiological dynamics of multiple object processing in pathological aging, specifically in AD and in its prodromal stage, MCI, in order to obtain possible early ERP biomarkers of AD pathology. The ultimate goal is to obtain reliable and sensitive neurophysiological markers for distinguishing normal from pathological aging.

In order to examine this issue, EEG activity was recorded while mild AD patients, MCI patients and healthy elderly controls performed a multiple-object enumeration task. EEG analysis focused on the aforementioned electrophysiological components N2pc and CDA, respectively associated with visual individuation/attention and working memory functions.

The rationale behind the study was the following: if pathological aging (AD or even the prodromal stage of the disease, that is MCI) impairs the ability to individuate multiple targets during enumeration, a suppression of the N2pc component in the pathological aging groups as compared to healthy elderly controls should be expected. In contrast, if the impairment in enumeration is due to a decline in active maintenance of the target elements in visual working memory, a suppression of the CDA component in the group of AD and/or MCI patients should be observed.

The investigation of EEG during multiple object processing in AD and MCI patients would greatly contribute to disclose which mechanisms involved in this process (attention selection or working memory maintenance) are the source of impairment (Mazza & Brignani, 2016). Moreover, modifications of N2pc and CDA latency or amplitude across MCI and AD patients may be an ideal marker in determining conversion from MCI to AD. However, as far as we know, this is the first attempt to investigate these mechanisms in pathological aging, specifically in AD and MCI.

3. Materials and methods

3.1. Participants

Three groups of participants were recruited in the present study: mild Alzheimer's disease patients (AD), amnestic Mild Cognitive Impairment patients (MCI) and healthy elderly controls. AD patients were diagnosed as suffering from probable AD according to National Institute of Neurological and Communicative Disorders and Alzheimer's Disease and Related Disorders Association criteria (McKhann et al., 1984). AD patients were identified as eligible for participating to the study if their age was between 60 and 85 years old, their Mini Mental State Examination (MMSE) score was greater or equal to 20 (mild AD), their Clinical Dementia Rating (CDR) scale score was less or equal to 2 and their Hachinski Ischemia score was less or equal to 4. All patients had been on a stable dose of cholinesterase inhibitors (donepezil or rivastigmine) for at least 3 months prior to the participation in the study. MCI patients were diagnosed according to the criteria proposed by Petersen (Petersen, 2004a). MCI patients were recruited if their age was between 60 and 85 years old, their MMSE score was greater or equal to 24, their CDR scale score was equal to 0.5 and their Hachinski Ischemia score was less or equal to 4. Patients with potentially confounding medical, neurological or psychiatric disorders, which could account for the onset of dementia, were not included in the study. Both AD and MCI patients were recruited through the outpatient facility of the National Institute for the Research and Care of Alzheimer's disease - IRCCS San Giovanni di Dio Fatebenefratelli in Brescia (Italy). Healthy, community-dwelling older adults aged 60 to 85 years old with no previous history of neurological or psychiatric problems were recruited from social and recreational centers located on Brescia territory. The final samples were composed of 20 AD patients, 16 amnestic MCI patients and 20 healthy elderly controls. Demographic data of the three groups of participants is reported in Table 2.1. All participants gave their written informed consent prior to the beginning of the experiment. All of the procedures were approved by the Ethic Committee of the IRCCS San Giovanni di Dio Fatebenefratelli Scientific Institute (Brescia, Italy) and were performed according to the Declaration of Helsinki for research involving human subjects.

	AD (N=20)	MCI (N=16)	Healthy elderly (N=20)
Gender (males-females)	10-10	9-7	6-14
Age (years)	76.30 (1.52)	75.00 (1.56)	69.40 (0.95)
Education (years of school)	7.80 (0.63)	8.19 (0.77)	10.65 (0.91)

Table 2.1 *Demographic characteristics relative to the three groups of participants (AD, MCI and healthy elderly) reported as mean scores (± standard error of the mean).*

3.2. Neuropsychological testing

All participants underwent a detailed neuropsychological assessment where they performed a battery of tests aimed to measure: general cognitive abilities (MMSE), memory (Rey Auditory Verbal Learning test immediate and delayed recall - RAVLT; episodic memory; digit span; spatial span; recall of Rey-Osterrieth Complex figure - ROCF) attention and visuo-spatial abilities (Attentive matrices; Trail Making test part A and B - TMT; Stroop test), language (verbal fluency), non-verbal reasoning (Raven's Colored Progressive Matrices - RCPM 47) and praxia (Copy of Rey-Osterrieth Complex figure - ROCF). Additionally, depressive symptoms were measured using the Geriatric Depression Scale (GDS). The neuropsychological assessment was administered and scored some days prior to the experimental session by a trained neuropsychologist. Healthy elderly participants who presented even just one pathological test score were not included in the sample. The tests used and the mean scores obtained by the three groups of participants are reported in Table 2.2.

	AD	MCI	Healthy elderly	AD vs healthy elderly	MCI vs healthy elderly	AD vs MCI
MMSE	22.07 (0.32)	26.16 (0.45)	27.78 (0.44)	*p < 0.001*	*p < 0.05*	*p < 0.001*
RCPM 47	26.30 (1.38)	30.84 (0.91)	32.08 (0.79)	*p ≤ 0.001*	p = 0.821	*p < 0.05*
RAVLT-immediate recall	28.00 (1.44)	37.86 (2.20)	45.81 (1.45)	*p < 0.001*	*p < 0.01*	*p < 0.001*
RAVLT-delayed recall	2.99 (0.40)	7.00 (0.81)	10.22 (0.45)	*p < 0.001*	*p < 0.001*	*p < 0.001*
Episodic memory	2.43 (0.45)	7.63 (1.21)	14.63 (0.76)	*p < 0.001*	*p < 0.001*	*p < 0.001*
ROCF-copy	27.26 (1.99)	33.41 (1.23)	35.64 (0.37)	*p < 0.001*	p = 0.568	*p < 0.01*
ROCF-recall	7.69 (0.82)	12.68 (1.87)	18.71 (1.07)	*p < 0.001*	*p < 0.01*	*p < 0.05*
Digit Span	5.50 (0.21)	5.69 (0.22)	5.63 (0.21)	p = 0.964	p = 0.996	p = 0.907
Spatial span	4.40 (0.13)	4.33 (0.23)	4.93 (0.17)	p = 0.086	p = 0.059	p = 0.988
Verbal fluency phonemic	26.85 (1.51)	29.25 (1.95)	40.15 (2.07)	*p < 0.001*	*p ≤ 0.001*	p = 0.757
Attentive matrices	35.96 (3.00)	44.55 (2.04)	46.79 (1.42)	*p < 0.01*	p = 0.869	*p < 0.05*
TMT A	76.50 (13.86)	47.40 (8.44)	27.15 (2.61)	*p ≤ 0.001*	p = 0.410	p = 0.133
TMT B	141.43 (19.05)	102.44 (20.05)	73.40 (9.38)	*p < 0.01*	p = 0.376	p = 0.319
TMT B-A	106.42 (14.84)	60.00 (12.15)	48.15 (7.43)	*p < 0.01*	p = 0.792	*p < 0.05*
Stroop-reaction times	46.84 (7.15)	26.10 (5.06)	14.91 (1.62)	*p < 0.001*	p = 0.303	*p < 0.05*
Stroop-errors	3.47 (1.54)	1.98 (0.79)	0.20 (0.18)	*p ≤ 0.05*	p = 0.485	p = 0.656
GDS	7.35 (1.25)	5.87 (0.81)	5.30 (0.94)	p = 0.404	p = 0.977	p = 0.715

Table 2.2 *Neuropsychological results relative to the three groups of participants (AD, MCI and healthy elderly) reported as the mean scores (± SEM) and post-hoc comparisons (p-values) between the groups. Italics highlight significant post-hoc comparisons.*

3.3. Stimuli and procedure

A total of 24 equiluminant red and green dots (35.5 cd/m2) were presented on a gray background (19.5 cd/m^2) and equally distributed to the left or right of a white fixation dot. The dots appeared within an invisible grid of 10 rows x 8 columns centered on the fixation dot. In each trial a variable number (ranging from 1 to 6) of targets (green dots) was presented among distractors (red dots) to either the left or right of the fixation dot. The total number of dots was held constant while the number of targets varied across trials. Targets were presented in a random order and position, but never in the two extreme columns and rows of the invisible grid or in the column adjacent to the fixation (stimuli were the same as those used in Pagano et al., 2015; Pagano et al., 2014; Pagano & Mazza, 2012). Participants were seated in a dimly lit room in front of a 17'' Dell monitor at a viewing distance of approximately 80 cm. Each trial began with a random interval (ranging from 2460 to 2540 ms) displaying the fixation dot. The stimulus array was then displayed for 400 ms[1]. After a blank frame lasting 500 ms, the response screen was displayed until the participant's response. In light of the acknowledged inability of AD patients to covertly orient attention and thus to inhibit saccades toward stimuli when they appear (see for example Crawford et al., 2013; Danckert et al., 1998), participants (AD, MCI and healthy elderly) were not explicitly asked to maintain their gaze on the central fixation dot during stimulus presentation, but instead to have their eyes on the fixation when each trial started. Otherwise, there would not have been a comparable condition among the three groups. Participants were required to count the number of targets (from 1 to 6) and verbally report their response. Given the fact that participants gave a verbal response, which was registered by the experimenter using a keyboard, it was not possible to measure reaction times but only the accuracy (i.e. error rates) of performance. A total of 600 trials (10 blocks, 60 trials each) was delivered. One practice block (18 trials) was given prior to the first block of the experimental session.

[1] As in previous studies, the duration of the visual stimuli presentation was not maintained active until the response of the subjects, but limited to a fixed interval, to adapt the paradigm at event-related potential registration (Pagano et al. 2012; Pagano et al. 2014; Pagano et al. 2015).

The stimuli were generated and responses were recorded using E-Prime 2 software (Psychology Software Tools, Pittsburgh, PA).

3.4. EEG recording and processing

Electroencephalogram was continuously recorded from an ActiCAP cap with 27 active Ag/AgCl electrodes (Brain Products, GmbH, Munich, Germany) placed according to the 10-20 International System and comprising: Fp1, Fp2, F7, F3, Fz, F4, F8, FCz, T7, C3, Cz, C4, T8, CP5, CP6, P7, P3, Pz, P4, P8, PO7, PO9, PO8, PO10, O1, Oz, O2. The signal was referenced online to the right mastoid, and then re-referenced offline to the left mastoid. The ground electrode was placed over AFz. Horizontal and vertical eye movements were detected respectively with electrodes placed at the left and right canthi and above and below the right eye. The EEG was recorded at 1000 Hz sampling rate using a time constant of 10 s as a low cut-off filter and a high cut-off of 250 Hz. The EEG signal was processed and analyzed offline using Brain Vision Analyzer 2 (Brain Products, GmbH, Munich, Germany). Continuous data was filtered off-line with a 40 Hz high cut-off filter. Brain components corresponding to ocular artifacts (blinks and saccades) were identified and discarded by applying the ICA ocular correction algorithm implemented in Brain Vision Analyzer 2 over the whole continuous signal. Epochs were created starting from 200 ms before stimulus onset and lasting 800 ms after stimulus onset. They were baseline corrected from -200 ms to 0. All epochs were then visually inspected to discard those containing remaining artifacts such as muscular activity, head movements or other sources of noise. EEG was averaged separately for each target numerosity (from 1 to 6 targets) and each target location (left or right hemifield), resulting in 12 averaged waveforms for each participant and electrode position. To obtain N2pc and CDA components, the mean differential activity at posterior electrodes (PO7 and PO8, as in Pagano et al., 2015) was computed by subtracting the ipsilateral mean amplitude from the contralateral with respect to the location of target presentation (left or right) and separately for each target numerosity. Contralateral and ipsilateral mean amplitude values were obtained by collapsing the electrode activity across target sites (PO7 was considered contralateral for right targets and ipsilateral for left targets; PO8 was considered contralateral for left targets and ipsilateral for right targets) (the same procedure used in

Pagano et al., 2015; Pagano et al., 2014; Pagano & Mazza, 2012). N2pc and CDA amplitude values were then computed by extracting the mean amplitude values in the time window ranging from 250 ms to 350 ms (N2pc) and from 450 ms to 800 ms (CDA) post-stimulus onset for each participant and each target numerosity. The mean number of averaged trials, considering all target numerosities, was 60.01 in the sample of AD patients, 51.53 in MCI patients, and 68.46 in the group of healthy elderly.

4. Results

4.1. Behavioral results

Behavioral data (mean error rates) were analyzed by means of a generalized linear mixed model (GLMM) with "numerosity" (six levels; number of targets presented from 1 to 6) as the within subject factor and "group" (three levels; healthy elderly, AD patients and MCI patients) as the between subject factor. As the three groups were not matched for age and education, we introduced the variables "age" and "education" as fixed factors. Since the assumption of normal distribution of the data was violated, we performed GLMM models on mean error rates with log link function for the gamma distributed dependent variable. Goodness of fit models were evaluated through Akaike information criterion (AIC) and Bayesian information criterion (BIC). Post-hoc comparisons were performed with Sidak correction for multiple comparisons. We performed several GLMM considering the variables "numerosity", "group", "age", "education" and their interactions and then looking for the model with the best fit of the data. For behavioral data analysis, among the several GLMM performed, the models that best fit the data did not include either the variable "age" or the variable "education", thus suggesting no effect of these variables on the results.

Mean error rates (± SEM) as a function of target numerosity and group are reported in Figure 2.1 and Table 2.3. Among the several GLMM performed, the best model (AIC = 784.27, BIC = 806.58) was the one with "numerosity" and "group" as main effects and "numerosity x group" as an interaction effect. Results showed a significant main effect of numerosity ($F_{(5,318)} = 135.497$ $p<0.001$) with an increase in error rates as a function of target numerosity. The group of AD patients demonstrated poorer performance when compared to the groups

of MCI patients and healthy elderly, and MCI patients performed worse than the group of healthy elderly, as revealed by a significant main effect of group ($F_{(10,318)} = 56.649$ $p<0.001$). Importantly, results showed a significant interaction between numerosity and group ($F_{(10,318)} = 1.978$ $p<0.05$). Post-hoc comparisons revealed that AD patients showed poorer performance than healthy elderly for all target numerosities (1-6) (all $p<0.05$), while MCI patients exhibited a decreased performance in respect to healthy elderly only for larger numerosities (2-4-5-6) (all $p<0.05$). Considering larger numerosities (4-5-6), post-hoc results revealed a significant difference between AD and MCI patients, with AD patients being less accurate in enumeration performance (all $p<0.05$).

In order to investigate the subitizing span (how many targets can be processed simultaneously) we computed the "efficiency value" for each participant considering the first target numerosity at which accuracy fell below 90% (Franconeri et al., 2007) and then multiplying it by the value of total accuracy across all target numerosities (Pagano & Mazza, 2012). We decided to compute this arbitrary value because we wanted to correlate the behavioral performance of the subjects with their performance at the neuropsychological tests. For that reasons we needed a unique index of efficiency.

In order to compare the mean efficiency values across the groups, ANOVA model for Gaussian distributed values was performed. Considering the differences between the groups in terms of age and education, we introduced these variables as covariates in the model. Covariate-adjusted results showed a significant effect of group ($F_{(2,56)} = 3.91$, $p<0.05$). In line with the general analysis on error rates, the significant main effect indicates a trend for lower capacity limits in pathological aging (AD: mean = 2.44, SEM = 0.27; MCI: mean = 3.09, SEM = 0.26) as compared to healthy aging (mean = 3.99, SEM = 0.25). However, from post-hoc comparisons with Sidak correction only the difference between healthy elderly and AD emerged as statistically significant ($p<0.05$).

N. of targets	1	2	3	4	5	6
Error rates						
AD patients	2.32 (0.63)	6.66 (1.58)	17.52 (3.11)	31.41 (5.12)	46.05 (5.80)	67.33 (6.77)
MCI patients	0.76 (0.42)	3.79 (0.93)	13.80 (2.63)	19.14 (2.53)	28.26 (3.76)	47.78 (4.48)
Healthy elderly	0.06 (0.06)	1.35 (0.28)	8.22 (1.79)	12.08 (1.63)	18.59 (1.66)	32.38 (3.27)
N2pc mean amplitude						
AD patients	-0.70 (0.21)	-1.29 (0.29)	-1.31 (0.26)	-1.61 (0.25)	-1.68 (0.29)	-1.82 (0.33)
MCI patients	-1.32 (0.20)	-1.71 (0.34)	-2.13 (0.36)	-2.05 (0.41)	-2.10 (0.42)	-1.79 (0.30)
Healthy elderly	-1.07 (0.33)	-1.61 (0.28)	-1.77 (0.35)	-1.44 (0.36)	-2.11 (0.36)	-1.68 (0.33)
CDA mean amplitude						
AD patients	-0.32 (0.16)	-0.91 (0.27)	-0.72 (0.22)	-1.07 (0.19)	-1.10 (0.30)	-1.27 (0.30)
MCI patients	-0.92 (0.16)	-1.47 (0.32)	-2.08 (0.40)	-1.94 (0.34)	-1.54 (0.24)	-1.50 (0.34)
Healthy elderly	-0.71 (0.19)	-1.03 (0.21)	-1.05 (0.24)	-1.19 (0.30)	-1.77 (0.26)	-1.32 (0.24)

Table 2.3 *Mean values (±SEM) of error rates, N2pc and CDA amplitude as a function of target numerosities in the three groups of participants (AD, MCI and healthy elderly).*

4.2. Neuropsychological results

Mean age- and education-adjusted scores obtained by the three groups of participants at each neuropsychological test are reported in table 2.2. In order to investigate in which cognitive domain the groups differed, we performed a univariate ANOVA for each neuropsychological score (age- and education-adjusted) with the variable "group" as the between-subject factor. Results indicated that the groups' performances were significantly different in the following neuropsychological tests: MMSE ($F_{(2,53)} = 57.11$, $p<0.001$), RCPM 47 ($F_{(2,53)} = 8.34$, $p<0.01$), RAVLT immediate recall ($F_{(2,53)} = 30.66$, $p<0.001$), RAVLT delayed recall ($F_{(2,53)} = 47.89$, $p<0.001$), episodic memory ($F_{(2,53)} = 61.74$, $p<0.001$), ROCF copy ($F_{(2,51)} = 10.95$, $p<0.001$), ROCF recall ($F_{(2,51)} = 19.93$, $p<0.001$), spatial span ($F_{(2,53)} = 3.67$, $p<0.05$), verbal fluency ($F_{(2,53)} = 15.35$, $p<0.001$), attentive matrices ($F_{(2,52)} = 6.69$, $p<0.01$), TMT A ($F_{(2,52)} = 7.05$, $p<0.01$), TMT B ($F_{(2,33)} = 5.29$, $p<0.05$), TMT B-A ($F_{(2,33)} = 7.14$, $p<0.01$), reaction time at Stroop test ($F_{(2,49)} = 11.49$, $p<0.001$), errors at Stroop test ($F_{(2,49)} = 3.09$, $p\leq0.05$). No significant group differences emerged in the scale assessing depressive symptoms (GDS; $F_{(2,52)} = 1.08$, $p = 0.35$). Post-hoc comparisons are reported in table 2.2. In general,

considering the tests evaluating memory functions (RAVLT immediate recall; RAVLT delayed recall; episodic memory; ROCF recall), both AD and MCI patients showed poorer performance when compared to healthy elderly (and MCI patients performed better than AD patients). Regarding the tests assessing attention (attentive matrices; TMT A; TMT B; TMT B-A; Stroop test), whereas AD patients were impaired compared to healthy elderly, MCI patients showed results similar to those of healthy elderly controls, thus demonstrating preserved attentive functions.

In order to investigate the relationship between the neuropsychological profile and the behavioral performance in the enumeration task, Pearson's correlation coefficients were calculated considering the efficiency value and the scores at each neuropsychological test. Results showed a significant correlation between the efficiency value and different neuropsychological domains: global cognitive abilities (MMSE: $r = 0.52$, $p<0.001$) (see Figure 2.1); abstract reasoning (RCPM 47: $r = 0.48$, $p<0.001$); verbal memory (RAVLT immediate recall: $r = 0.43$, $p\leq0.001$; RAVLT delayed recall: $r = 0.48$, $p<0.001$; episodic memory: $r = 0.47$, $p<0.001$); visuo-spatial memory (ROCF recall: $r = 0.40$, $p<0.01$); praxic functions (ROCF copy: $r = 0.36$, $p<0.01$) and attentive functions (attentive matrices: $r = 0.42$, $p\leq$; TMT A: $r = -0.45$, $p\leq0.001$; Stroop reaction time: $r = -0.54$, $p<0.001$; Stroop errors: $r = -0.46$, $p\leq0.001$).

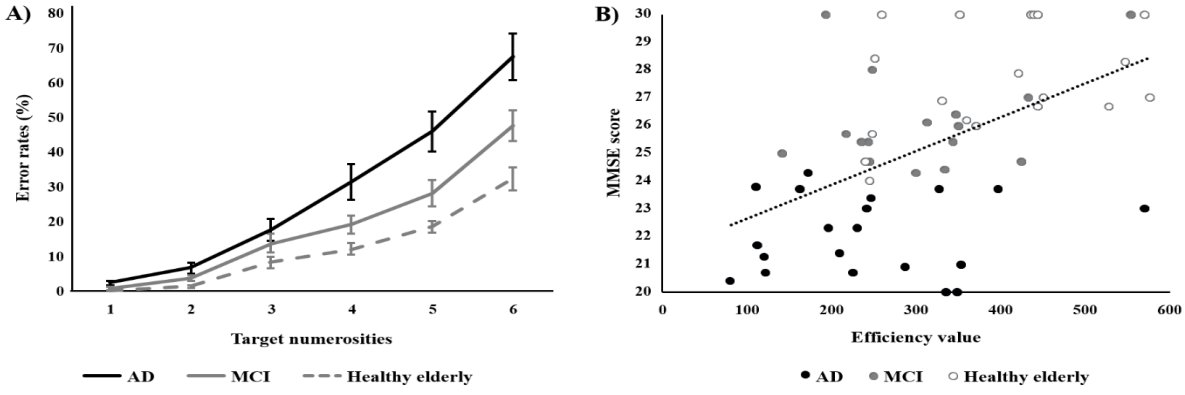

Figure 2.1. (A) *Mean error rates as a function of target numerosities for the three groups of participants (AD, MCI and healthy elderly). Error bars represent the standard error of the mean (±SEM);* ***(B)*** *Cluster plot showing the significant correlation (r=0.52, p<0.001) between the efficiency value and general cognitive abilities as assessed with MMSE for the three groups of participants (AD, MCI and healthy elderly).*

4.3. Electrophysiological results

Electrophysiological data (N2pc and CDA mean amplitude values) were analyzed by means of a generalized linear mixed model (GLMM) with "numerosity" (six levels; number of targets presented from 1 to 6) as a within subject factor and "group" (three levels; healthy elderly, AD patients and MCI patients) as a between subject factor. Furthermore, we introduced the variables "age" and "education" as fixed factors. Given that the data was normally distributed, we performed GLMM models on N2pc and CDA mean amplitude values with identity link function for the normal distributed dependent variable. Goodness of fit of models were evaluated through Akaike information criterion (AIC) and Bayesian information criterion (BIC). Post-hoc comparisons were performed with Sidak correction for multiple comparisons. We performed several GLMM considering the variables "numerosity", "group", "age" and "education" and their interactions and looking for the model with the best fit of the data. As for behavioral data analysis, among the several GLMM performed the models that best fit the data did not include either the variable "age" or the variable "education", thus suggesting no effect of this variable on electrophysiological results.

4.3.1. N2pc

Mean amplitude values (± SEM) as a function of target numerosity and group are reported in Table 2.3. Among the several GLMM performed, the best model (AIC = 1173.76, BIC = 1196.06) was the one with "numerosity" and "group" as main effects and "numerosity x group" as an interaction effect. Results showed a significant main effect of numerosity ($F_{(5,318)} = 4.181$ $p \leq 0.001$) with an increase in N2pc mean amplitude as a function of target numerosity. Post-hoc comparisons revealed higher N2pc mean amplitude values for large target numerosity (3-6) than for small target numerosity (1-2). No significant effect was found for "group" ($F_{(2,318)} = 1.87$ $p=0.156$) or for the interaction between "numerosity" and group" ($F_{(10,318)} = 0.453$ $p=0.919$). Figure 2.2 shows the grand-average N2pc component elicited by different target numerosity (from 1 to 6) in the three groups of participants.

4.3.2. Contralateral Delay Activity

Mean amplitude values (± SEM) as a function of target numerosity and group are reported in Table 2.3. Among several GLMM performed, the best model (AIC = 1055.88, BIC = 1078.18) was the one with "numerosity" and "group" as main effects and "numerosity x group" as interaction effect. The results showed a significant main effect of "numerosity" ($F(5,318) = 5.988$, $p<0.001$) with an increase in mean CDA amplitude as a function of target numerosity. In line with N2pc results, post-hoc comparisons revealed higher CDA mean amplitude values for large target numerosities (3-6) than for small target numerosities (1-2). The significant main effect of "group" ($F(2,318) = 9.18$ $p<0.000$) revealed that AD patients showed an overall reduction of CDA amplitude in comparison to MCI patients and healthy elderly, whereas MCI patients showed an increased CDA amplitude compared to both AD patients and healthy elderly. No significant effect was found for the interaction between "numerosity" and "group" ($F_{(10,318)} = 1.008$, $p=0.437$). Figure 2.2 shows the grand-average CDA component elicited by different target numerosities (from1 to 6) in the three groups of participants.

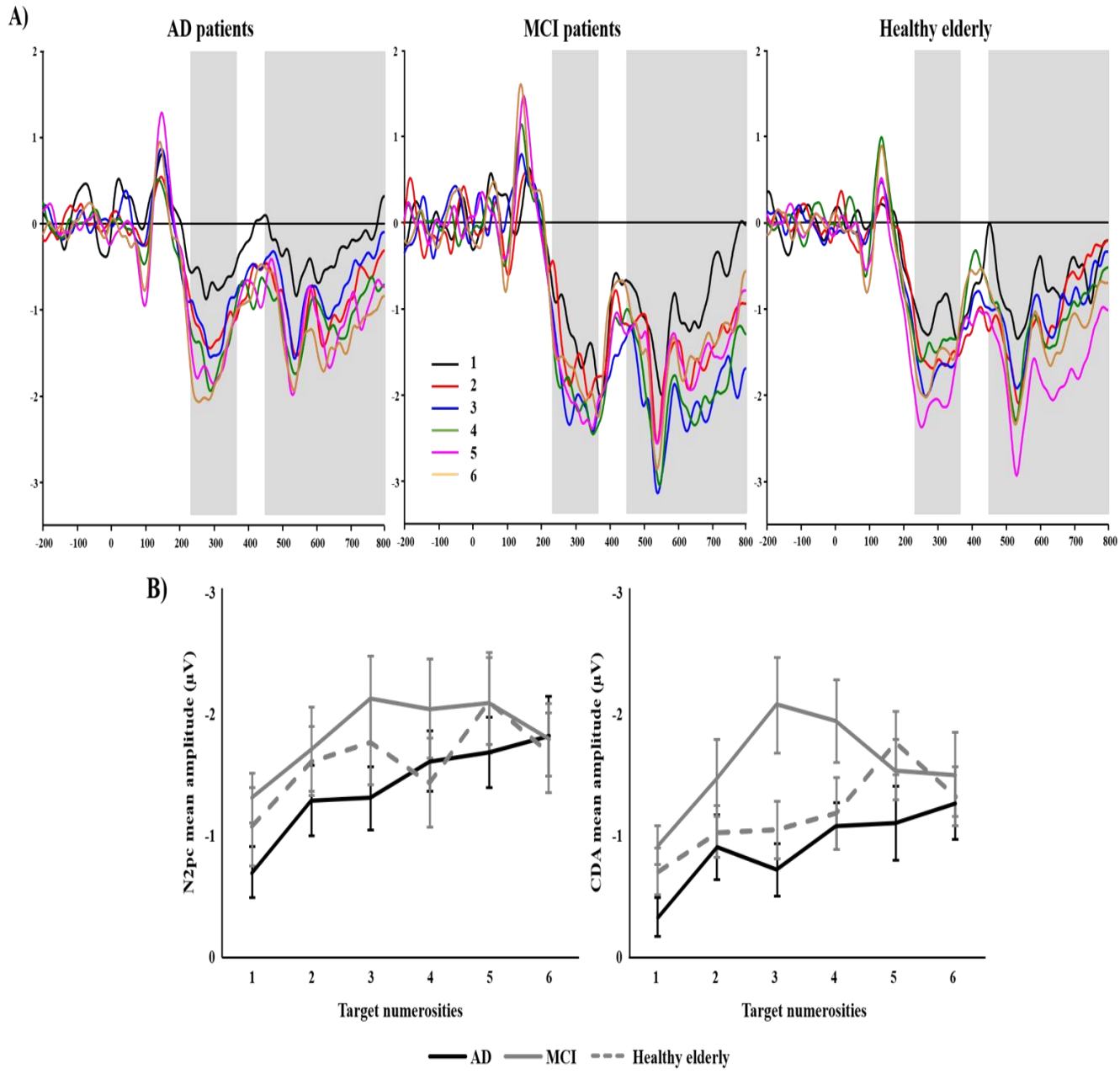

Figure 2.2 (A) *Grand-averaged ERP waveforms for N2pc (250-350 ms) and CDA (450-800 ms) represented as a function of target numerosities for the three groups of participants (AD, MCI and healthy elderly). For illustrative purposes only, data were filtered at 20 Hz;* ***(B)*** *Mean amplitude values as a function of target numerosities for the three groups of participants (AD, MCI and healthy elderly). Error bars represent the standard error of the mean (±SEM).*

5. Discussion

The results of the present study shed light on the mechanisms responsible for the impairment of multiple objects processing in AD and for the first time, revealed how MOP is affected in the prodromal stage of the disease, namely MCI. The primary aim was to provide empirical evidence for testing the possibility to adopt N2pc and CDA as indexes able to characterize the progression of attention and memory impairment from normal ageing to AD. To achieve this goal, EEG activity was recorded while mild AD patients, amnestic MCI patients and healthy elderly controls performed a multiple objects processing task where they had to enumerate a variable number of targets presented among distractors.

Considering the behavioral performance, AD patients showed an overall decline in enumeration abilities with an increase in error rates that encompasses all target numerosities. The investigation of the subitizing span showed a reduction in the number of objects that can be processed simultaneously. These results are only partly in line with previous studies. Despite Nebes and colleagues reported a decline of subitizing in AD (Nebes et al., 1992), afterwards it has been suggested that the reduction of the subitizing span was evident only for patients in a later stage of the disease (probably moderate AD) and not for mild AD patients (Maylor et al., 2008; Maylor et al., 2005). Furthermore, previous studies reported that AD patients were overall slower in the enumeration performance, but not less accurate. The present results, instead, point out a decrease in accuracy that overall encompasses all target numerosities. This discrepant finding is probably due to methodological differences between the studies. The main difference regards the length of the presentation time of visual stimuli. In the present study, the visual array containing the stimuli was briefly presented for 400 ms whereas in previous studies it was endlessly presented until the patient's response. This difference is crucial because, in the present case, participants had a very short amount of time to estimate the number of targets presented, thus making the task more difficult and more error prone.

Whereas in AD both subitizing and counting appeared to be impaired by the disease, in the prodromal stage only counting was found to decline, with preserved accuracy for small numerosities within the subitizing range. The fact that MCI patients showed a spared

performance for the low perceptual load is in line with previous studies, which reported that AD impairs enumeration abilities in the subitizing range only in a later stage of the disease (Maylor et al., 2008; Maylor et al., 2005). Considering the variability in the diagnostic criteria, it is reasonable that the disease severity of some MCI patients may overlap with that of the patients considered as mild AD patients in the study by Maylor and colleagues (Maylor et al., 2008). The evidence that subitizing is spared in the prodromal stage of the disease but not in mild AD is further upheld by the results of this study, which pointed out that the subitizing span correlated with the cognitive decline, as measured with the MMSE. Indeed, this evidence supports the vision that AD cognitive decline in subitizing span is expressed as a continuum, such that the ability to simultaneously process multiple objects decreases as a function of the cognitive impairment, with more severe patients having the most reduced subitizing span. This finding further substantiates previous evidence on the relationship between the ability to process multiple objects and the severity of the disease (Maylor et al., 2005; Nebes et al., 1992), thus envisaging the utility of multiple object processing tasks as a tool for the screening of cognitive decline. Furthermore, I found that enumeration performance correlates with various neuropsychological tests, mainly in the domain of attention (attentive matrices, TMT A, and Stroop test) and memory (verbal memory: RAVLT immediate and delayed, episodic memory; visuo-spatial memory: ROCF recall). The ability to simultaneously process multiple objects seems to rely on the efficiency of several cognitive functions, mainly involving sustained and selective attention, inhibition abilities, visuospatial long term memory and verbal short and long term memory.

The recording of EEG activity during the execution of the enumeration task allowed us to delineate the neural dynamics associated with the behavioral impairment, thus further substantiating the specific involvement of attention and working memory mechanisms in this decline. The results showed that the earlier individuation component (N2pc) was spared both in AD and in MCI patients, whereas the later component linked to visual working memory abilities (CDA) was altered in both groups of patients as compared to healthy elderly controls. Therefore, these data support that CDA might be an especially sensitive index that is useful for an early diagnosis of cognitive impairment likely due to AD.

Considering the earlier N2pc component, its amplitude was equally modulated by target numerosity, with increasing values for high perceptual load (counting) than for low perceptual load (subitizing) in all three groups of participants, thus suggesting that the mechanisms linked to attentional selection/individuation were not affected by pathological aging (neither in MCI nor in mild AD). Despite EEG studies of multiple objects processing pointing out an age-related decline of N2pc component (Pagano et al., 2015; Störmer et al., 2013), the present results indicated that the pathophysiology underlying AD did not induce a further impairment of the attentional individuation/selection mechanisms. The patients enrolled in the present study were in the early (mild AD) and prodromal phases (MCI) of the disease, so it cannot be ruled out that a decline of N2pc component may arise in the later stages of the disease (i.e. moderate AD) in comparison to normal aging. To the best of our knowledge this is the first study investigating the EEG markers of attention selection/individuation (as N2pc) in a sample of AD patients. As for N2pc, CDA amplitude increased as a function of target numerosity with higher values in the condition of high perceptual load (counting) as compared to the condition of low perceptual load (subitizing). Despite this modulation being equally observed in the three groups of participants, results showed an overall alteration of CDA amplitude in pathological aging with a specific pattern that differs according to the stage of the disease. The alteration of the CDA component in pathological aging is ascribable to the recognized impairment of working memory abilities in AD (Baddeley et al., 1991) and even in its prodromal stage (Brandt et al., 2009; Saunders & Summers, 2010, 2011). The fact that the decline in enumeration performance observed in pathological aging was underlined by the alteration of working memory processing may be considered in line with the evidence that enumeration (and specifically counting) is more efficient in individuals with greater working memory capacities due to their better ability to inhibit the processing of already counted objects (Tuholski et al., 2001). AD patients presented a global reduction of CDA amplitude, which impeded an efficient enumeration performance both for low (subitizing) and high (counting) perceptual load, leading to an overall decline in multiple object processing. Conversely, the alteration of CDA amplitude in MCI patients pointed out an hyperactivation of this component, which may be interpreted as a compensatory mechanism that enables MCI patients to perform efficiently when there

are few targets to be enumerated (low perceptual load) but it is not sufficient to supply for the more demanding performance in the counting range (high perceptual load). Concerning AD patients, the impairment of working memory mechanisms may be so severe that it impedes an efficient enumeration performance not only for the more demanding counting process, but also for the subitizing range. This is probably because AD patients are not able to inhibit previously viewed target locations, even when few items are presented. The present results need to be considered and further deepened in light of the experimental procedure adopted in this study. As mentioned before, differently from previous enumeration studies where enumeration display remained on the screen until participant's response, in our paradigm visual stimuli were only briefly presented for 400 ms. This methodological difference is crucial, as in the present case the limited amount of presentation time did not allow participants to count targets while they were displayed, thus forcing them to rely mostly on the iconic memory trace of the stimulus presented and requiring more working memory resources to elaborate that trace. Although working memory is a key mechanism underlying enumeration performance (Mazza & Caramazza, 2015; Tuholski et al., 2001), this is especially true in the case of our time-limited paradigm where the working memory component is even more crucial in determining the consequent performance.

To our knowledge, no study has examined CDA activity in a sample of AD or MCI patients. There is only one study that investigated CDA component in a sample of individuals considered at-risk to develop MCI on the base of the neuropsychological screening and reported an abnormal CDA in this sample of participants with a reduced differentiation between set sizes of the presented objects (Newsome et al., 2013). Previous EEG studies highlighted a reduction of CDA component in healthy aging during different tasks concerning multiple objects processing (Pagano et al., 2015; Sander et al., 2011; Störmer et al., 2013; Wiegand et al., 2014a, 2014b), indicating that working memory mechanisms are involved and concur in the age-related decline of multiple objects processing. This is the first study that goes beyond healthy aging, investigating the effects of pathological aging on the activation of CDA, and demonstrates that the pathophysiology underlying AD induced a further suppression of CDA amplitude during the execution of an enumeration task consistent with the severe impairment of working memory functions in AD patients. Whereas CDA

amplitude was decreased in the AD sample, the abnormal pattern of CDA was expressed as an increase in its amplitude in MCI patients. This evidence may represent a compensatory mechanism that allowed MCI patients to compensate for the cognitive decline by over-activating the areas involved in the execution of the enumeration task. The over-activation was associated with a good level of performance for the low perceptual load condition of the task (subitizing range), but with a decline in performance for high perceptual load (counting range), possibly due to the narrowness of available neural resources that were not able to sustain the behavioral performance in more demanding conditions.

These results may be interpreted in the framework of the "Compensation-related utilization of neural circuits" (CRUNCH; Reuter-Lorenz & Cappell, 2008) in the aging brain. The CRUNCH model postulates that, in conditions of low cognitive load, elderly recruit more neural resources than younger adults when their performance is equivalent, whereas for more difficult tasks (high cognitive load) the compensatory mechanisms vanish, leading to equivalent or decremented recruitment of neural resources and to a decline of elderly's performance. The aging brain can recruit additional neural resources to uphold certain cognitive functions but this compensation mechanism will run out when the limit of available resources is reached (Reuter-Lorenz & Cappell, 2008). In the framework of multiple object processing studies, a recent electrophysiological study investigating the temporal locus of neural compensation in healthy aging pointed out that an equal behavioral performance between young and old participants was associated with an over-activation of N2pc component in the elderly, thus suggesting that healthy aging is associated with an over-recruitment of the neural circuits underlying the attentional mechanisms (Pagano et al., 2016). Despite CRUNCH and other compensation models that have been studied and conceived comparing healthy elderly with young adults, several studies suggest that the mechanisms of compensation derived from healthy aging can be applied to pathological aging too (for a review see Scheller et al., 2014).

Investigating how memory networks are affected by AD pathophysiology (for a review see Chhatwal & Sperling, 2012), Celone and colleagues (Celone et al., 2006) hypothesized a nonlinear pattern of activation over the stages of AD continuum, ranging from the hyper-activation in the earlier prodromal stages of MCI to the hypo-activation as the disease

severity advances to mild AD. Accordingly, fMRI data comparing hippocampal activation across the continuum of healthy aging, MCI and mild AD, revealed that less impaired MCI patients had greater hippocampal activation as compared to healthy elderly, whereas more impaired MCI patients showed a decrease in the activation, similar to that apparent in AD patients (Celone et al., 2006).

In the context of EEG studies, parieto-occipital compensatory recruitment in MCI patients has been reported as an abnormal enhancement of P450 amplitude over posterior regions during a memory task, indicating that MCI patients need to recruit additional resources in order to carry out the cognitive task (Beuzeron-Mangina & Mangina, 2009). Crucial for the interpretation of the results of the present study, this compensatory mechanism has been observed only in the prodromal phase of AD, but not in the later stages when the diagnosis of AD has taken hold (Beuzeron-Mangina & Mangina, 2009). The supposed compensatory mechanism exhibited with the hyper-activation of CDA component in MCI patients was no longer present in AD patients, who conversely showed a suppression of the aforementioned component. A possible interpretation of this finding is that the severe neurodegeneration of the dementia phase impedes the recruitment of additional neural resources, thus not allowing compensatory mechanisms to take place as the pathological burden advances and becomes more severe (Beuzeron-Mangina & Mangina, 2009; Buckner, 2004). This evidence complies also with the findings of functional activation studies reviewed by Prvulovic and colleagues (Prvulovic et al., 2005), that demonstrated that the progression of neural degeneration can lead to phenomena of either hyper-activation (usually associated with mildly impaired performance, as in the present case of MCI patients), or hypo-activation which is linked to a greater impairment in performance, as showed here for AD patients.

6. Conclusions

Concluding, the present study showed that the impairment of multiple object processing along the continuum of AD pathology ranges from an initial decline visible only for more demanding perceptual load (counting) in the prodromal phase of the disease (MCI), to a decline that encompasses both counting and subitizing (low perceptual load) processes in the

earlier stage of the disease (mild AD). The neural dynamics underlying these enumeration deficits are associated with the decay of working memory mechanisms, as indexed by the alteration of CDA activation both in mild AD and in MCI patients. This alteration follows a non-linear pathway along the continuum of the disease: whereas in the prodromal phase there is a hyper-activation of CDA (presumably indexing a compensatory mechanism), in the mild stage of AD a reduction of its activation is evident. It remains to be investigated whether in more advanced stages of the disease (i.e. moderate AD) further decreased of CDA activation may emerge and/or the disease progression may induce also an alteration of the attentional individuation/selection mechanisms (indexed by N2pc component), which resulted spared in the present sample of MCI and mild AD patients. Together, these findings suggest that electrophysiological components may be especially sensitive markers of the very earliest stages of AD.

Arousal modulation by means of transcranial electrical stimulation in young and elderly healthy adults

1. Introduction

The fundamental role of arousal in cognition has been extensively reported by several studies on animals and humans (Berridge & Waterhouse, 2003; Jeong & Biocca, 2012; Mickley Steinmetz et al., 2012; Sharot & Phelps, 2004). In a comprehensive review, Berridge and Waterhouse (2003) cited numerous studies showing the role of arousal in attention and in the memory detection, retention, and retrieval of information. For example, Sharot and Phelps (2004) investigated the role of arousal on memory retention by examining the recognition of neutral and arousing words. Participants were required to fixate a central word while either an arousing or a neutral word briefly appeared at the periphery. Results showed that arousing stimuli are better retained than non-arousing ones. Whereas recognition of neutral words became worse over time, recognition of arousing words showed improvement and was better than neutral word recognition even after a delay of 24 hours. Authors concluded that arousal is related to selective attention during the encoding process and arousing information are more likely to be encoded than neutral stimuli. Overall these results indicated that arousal slowed forgetting, even when overt attention was not focused on the arousing stimulus. In line with this evidence, Mickley Steinmetz and colleagues (2012) showed that the selectivity of encoding arousing stimuli produced better long-term memory results than the encoding of neutral stimuli. The retention and accumulation of information was strengthened when subjects were exposed to arousing events or information. In addition, arousing information was also retrieved or remembered more vividly and accurately (Jeong & Biocca, 2012).

1.1. Arousal in rehabilitation

The importance of arousal in cognition has received empirical support from studies which applied rehabilitative interventions aiming at improving the arousal level in traumatically

brain injured patients (Levine et al., 2011; Manly et al., 2002) and in children with attention-deficit hyperactivity disorder (ADHD) (O'Connell et al., 2006).

Levine and colleagues (Levine et al., 2011) used a particular executive function intervention, called Goal Management Training (GMT), in a group of chronic traumatic brain injury patients (mostly stroke) affecting the frontal lobe. The training was based on the theory of sustained or vigilant attention and promoted a mindful approach to complex real-life tasks. The intervention trained subjects to stop ongoing behavior in order to monitor performance and adjust goals. GMT has the general purpose to promote the activation of the right frontal-thalamic-parietal sustained attention system that is required to endogenously maintain goal-directed states in working memory avoiding cue-dependent or distracted behavior that are common in patients with attentional and executive deficits (Levine et al., 2011). Outcome data reported advantages for one-session GMT over a control motor-training condition in patients' ability to solve lifelike problems. GMT reduced attentional lapses, increased behavioral consistency, and improved problem-solving performance in patients with stable brain lesions and self-reported executive deficits supporting the efficacy of this rehabilitation approach for executive functioning deficits.

Manly and colleagues (Manly et al., 2002), instead, investigated whether the provision of brief auditory stimuli would improve the performance in a complex task of patients with dysexecutive syndrome. Dysexecutive syndrome is a cluster of deficits (attention, planning, problem solving and behavioral control deficits) typically caused by prefrontal cortex damage. These patients have difficulties in planning activities and are unusually vulnerable to environmental distractors capture in everyday life activities. These auditory stimuli aimed at interrupting current activity and at cueing patients to consider their overall goal. Results showed that without the external auditory cues, patients performed worse than control volunteers. However, when exposed to the interrupting tones, their performance was significantly improved showing that alerting cues improved executive functions and, as a consequence, the performance of the patients.

O'Connell and colleagues (O'Connell et al., 2006) applied the same approach in children with ADHD, a disorder that has been linked with right frontal dysfunctions. A group of 15 ADHD children and 15 matched controls completed the Sustained Attention to Response

Task. Random, non-contingent alerting cues were introduced on two blocks of the task as a cue for participants to adopt a more supervisory stance to their performance. In order to have a measure of the arousal response produced by alerting cues, the electrodermal activity was recorded. Results showed that errors were significantly reduced in the period immediately following the presentation of the alerting cue. The authors claimed that sustained attention performance could be enhanced in children with ADHD using a simple cognitive training strategy.

1.2. Modulating arousal in human

In humans, arousal is commonly modulated by means of emotional stimuli (Dew et al., 2014; Sutherland & Mather, 2012), warning cues (Hackley, 2009) and conflict paradigms (Brown et al., 2014). Emotional stimuli, usually categorized on the bases of valence (emotionally negative, neutral and positive) and arousal (from calm to excited), have been frequently used (e.g., pictures from the International Affective Picture System (IAPS) (Lang et al., 2008); sounds from the International Affective Digital Sound (IADS) system (Bradley & Lang, 1999)). Among warning cues, both visual, acoustic and cutaneous warnings are used to modulate arousal (Zeigler et al., 2001). The Stroop task (MacLeod, 1992) has also been used to increase arousal. The task requires the participants to say the color of the letters independently of the written word. If the word "red" is written in blue, they would have to say "blue", but not "red". To do so, they need to suppress their habitual tendency to respond to the color word (red) and instead respond to the demanded ink color (blue). Since this is a highly demanding task, it is expected to increase arousal of the subject, and for this reason it has been used in different studies with this purpose (Brown et al., 2014).

1.3. Transcranial electrical stimulation (tES)

Transcranial electrical stimulation (tES) is a non-invasive brain stimulation technique that involves the delivery of a low-level intensity (~1-2 mA) current by a battery-driven stimulator between electrodes that are placed directly on the head (Nitsche & Paulus, 2000; Priori et al., 1998).

The electrodes are generally composed of conductive rubber pads inserted in saline-soaked sponges (20-35 cm^2). The current passes through the scalp and crosses the extracortical layers to reach the cortex and modulate neuronal membrane potential in a subthreshold way altering cortical excitability and activity. The currents administered with tES are too weak to elicit action potentials in cortical neurons but they can modify the response threshold of the stimulated neurons (Bindman et al., 1964; Creutzfeldt et al., 1962).

There are three main modalities of current application: direct (transcranial direct current stimulation, tDCS), alternating (transcranial alternating current stimulation, tACS), or random noise (transcranial random noise stimulation, tRNS).

1.3.1. Transcranial direct current stimulation (tDCS)

Transcranial direct current stimulation is the most prevalent form of tES (Nitsche et al., 2008). tDCS implies the application of a constant current density. At the start and end of the stimulation, the current is gradually increased/decreased until the desired level of intensity (fade-in/fade-out periods). Neurons respond to tDCS by altering their firing rates. The stimulation technique is often categorized as either anodal or cathodal, according to the polarity of the electrode placed over the area of interest. Several studies using animal models (Bindman et al., 1964; Creutzfeldt et al., 1962) suggested that cathodal tDCS reduces spontaneous neuronal firing rates, while anodal tDCS has the opposite effect. tDCS affects sodium and calcium channels (Liebetanz et al., 2002; Nitsche et al., 2003) and thus it modulates the resting potential of the neuronal membrane (Nitsche et al., 2008).

1.3.2. Transcranial alternating current stimulation (tACS)

Using tACS, it is possible to deliver an alternated sinusoidal current at a specific frequency. This gives the experimenter the advantage of stimulating at specific frequencies. During half of the cycle of a tACS oscillation, one electrode will serve as anode and the other one as cathode, and during the other half of the cycle the pattern is reversed. tASC could be used to influence brain oscillations (Herrmann et al., 2013) and could serve as an instrument for interacting with ongoing cortical oscillations (Brignani et al., 2013; Polanía et al., 2012; Thut et al., 2012), inducing entrainment (Thut et al., 2012) and thereby contributing to a better

understanding of cortical binding in cognitive processes. However, very little is known about tACS and only few studies used it. More evidence is needed in order to prove its ability to entrain cortical frequency.

1.3.3. Transcranial random noise stimulation (tRNS)

tRNS consists of the application of alternating currents in a range of frequencies, typically between 0 and 1000 Hz, but a narrower range of frequencies can be used (e.g., from 100 to 600 Hz). It has been demonstrated that a few minutes of tRNS at high frequency (101-640 Hz) on the motor cortex induces an increase in the amplitude of motor evoked potential that persists after the end of stimulation (Terney et al., 2008). A similar result was found by Fertonani and colleagues (2011) during the execution of a perceptual learning task. It has been proposed that this type of repeated random subthreshold stimulation could induce an increase in the sodium inflow and a consequent prolonged depolarization and induction of long term potentiation-like phenomena (Fertonani et al., 2011; Terney et al., 2008). Another hypothesis is based on the phenomenon of stochastic resonance (Miniussi et al., 2013), that is a signal, too weak to exceed a threshold, is amplified by adding noise (McDonnell & Abbott, 2009). For example, if a sub-threshold random noise is added to the physiological oscillation in the brain, the sum of these two signals will exceed the threshold, resulting in improved cognitive performance (Cappelletti et al., 2015).

1.4. Arousal modulation by tES

To investigate the role of arousal in animals, electrical simulation delivered by means of electrodes implanted directly on the noradrenergic nucleus locus coeruleus (LC) has been widely used. It has been shown that increasing arousal through the LC activation and promoting the consequent release of NA to the cortex during a cognitive demanding task, has a beneficial impact on the focus of attention and on stimuli processing (Clayton et al., 2004; Sara, 2009). Electrical stimulation through electrodes implanted in the region of the LC has shown facilitation of successive discrimination reversal on rats (Edelson, 1978). Others animal studies demonstrated that increasing arousal, by electrical stimulation of the LC, just

before a retention test, alleviates amnesia facilitating memory retrieval after a long retention interval in rats that had forgotten a series of left–right turns in a maze (Devauges & Sara, 1991; Sara & Devauges, 1988). More recently, Lim and colleagues (2010) showed that high-frequency stimulation of the rat hippocampus can induce long-term potentiation (LTP) in the hippocampo-prefrontal cortical (hippo-PFC) pathway, which is involved in long-term memory consolidation.

Mauri and colleagues (Mauri et al., 2015) have recently shown that it is also possible to modulate the levels of arousal in humans through transcranial electrical stimulation (tES). They used an adapted version of the continuous performance test (CPT), which is widely used to measure sustained attention (Conners et al., 2003). During the task, a series of digits, from 1 to 9, was presented in a quasi-random order in the center of the screen. Participants were instructed to respond to the target digits (8-9) using one of two response buttons. A warning digit (1) was always presented before the target digit. Bursts of tES were administered to the participants concurrently to the presentation of the warning stimuli in order to mimic the physiological phasic activation of the LC induced by the warning stimuli themselves. The hypothesis of the study was that the exogenous increase of arousal of the participants induced through the use of tES would have improved the behavioural performance. Response speed was evaluated as a measure of performance because a reduction in reaction time (RT) was already demonstrated in conditions of increased arousal, indicating a performance improvement (Bagherli & Mokhtari, 2011; VaezMousavi et al., 2009). To obtain a physiological measure of arousal, SCRs were recorded during the task. SCRs are considered a reliable measures of arousal and thought to reflect cognitive engagement during a task (Bagherli & Mokhtari, 2011; Dawson et al., 2000). Consistently with a general increase of arousal, the results of the study showed an improvement of the performance (reduction of reaction times) and a concurrent increase of SCRs during the real tES condition in comparison to placebo (sham) tES condition.

2. Objective of the study

The general objective of the present study was to investigate whether tES may be used to exogenously increase the participants' level of arousal with a subsequent improvement of the behavioural performance also during a task involving higher mental processes. This investigation has to be framed in an attempt to find an effective approach to be used in interventions for both cognitive enhancement in normal aging and cognitive rehabilitation in pathological aging.

Specifically, the first aim of the current study was to investigate whether tES was able to induce arousal modulations in healthy young individuals during a visual short-term memory task. Recent evidence has been reported showing that an increase of arousal during a short-term memory task improves the processing of high priority stimuli, enhancing processing of salient stimuli and impairing processing of relatively less-salient stimuli (Lee et al., 2015; Mather & Sutherland, 2011).

The second aim of the present study was to investigate (i) whether tEs was able to induce arousal modulations in healthy elderly individuals using the same paradigm applied to young participants and (ii) whether an increase of arousal in healthy elderly is beneficial for the cognitive performance. It is still unclear which effects an increase of arousal induces on older adults' cognitive performance. The previous research investigating this issue reveals a mixed picture. Some studies, for instance, suggests that emotional arousal has beneficial effects on associative memory in healthy older adults as in younger adults (Nashiro & Mather, 2011a); other studies, instead, revealed that arousal has a detrimental effect on aging, worsening older adults' item memory binding (Nashiro & Mather, 2011b) and information retrieval (Kukolja et al. 2008).

3. Experiment 1: Young participants

In a recent study, Sutherland and Mather (2012) reported that the increasing of arousal during a visual short-term memory task (VSTM) induced an improvement of memory for high salient stimuli and a worsening of memory for low salient stimuli. They presented an array

of 8 letters with different levels of salience (three high-salience (i.e, black) and five low-salience (i.e., grey) letters) and used emotional sounds to modulate arousal, briefly showed before the presentation of the stimuli to be remembered. Subjects were required to report vocally as many letters as they remembered regardless of their colour. The results showed that high-salience letters were overall more likely to be reported than low-salience letters. In addition, the recall of high-salience stimuli was increased after the presentation of an arousing sound, while the recall of low-salience stimuli was decreased.

Mauri and colleagues (2015) have recently shown that bursts of high frequency transcranial random noise stimulation (tRNS), administered concurrent to the presentation of relevant stimuli of a vigilance task, induced behavioural and physiological effects consistent with an increase of arousal.

In the present study, we aimed at evaluating whether arousal can be increased during a short-term memory task which required higher mental processes, using bursts of high frequency transcranial random noise stimulation (tRNS), as in the study by Mauri and colleagues (2015). We used an adapted version of the visual short-term memory task proposed by Sutherland and Mather (2012), in which bursts of tES were administered to increase arousal, instead of negative arousing sounds. To evaluate the physiological level of arousal, we recorded skin conductance response and the pupil diameter during the task execution. Several studies showed that these psychophysiological measures mirror a LC phasic response to task-relevant events (Beatty, 1982; Gilzenrat et al., 2003; Rajkowski et al., 1993; Benedek & Kaernbach, 2010; Bradley et al., 2008).

3.1. Materials and methods

3.1.1. Participants

Twenty-three healthy volunteers participated in the experiment. The data from one participant were excluded from the final analyses because of a lower accuracy (< 1.96 standard deviations) compared to the overall mean of the participants. Two participants were also discarded from the analyses because they clearly perceived the bursts of stimulation during the real tES condition. The final sample was composed by 20 participants (16 females,

mean age = 23.5 years; SD = 2.8). All of them had normal or corrected-to-normal visual acuity, evaluated with the Lighthouse visual acuity charts (Lighthouse Enterprises, New York, NY) and reported normal values (all participants = 3.90%) at the Pelli-Robson contrast sensitivity test (Pelli & Robson, 1988). All participants were right handed according to the Edinburgh handedness inventory test (Oldfield, 1971) and showed no risk factors for tES application, as assessed through safety questionnaires (Fertonani et al. 2015, 2010). Before the beginning of the experiment, participants completed the STAI-Y questionnaire (Spielberger et al., 1983) to measure the state and trait anxiety level (STAI-Y state, mean score = 30, SD = 6.2; STAI-Y trait, mean score = 38.4, SD=8.5).

The experimental methods were approved by the Ethics Committee of the IRCCS Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy. Informed consent was obtained from all participants. Participants received a financial reward at the end of the experiment.

3.1.2. Behavioral task and procedure

The task was adapted from a previous work by Sutherland and Mather (Sutherland & Mather, 2012) and is displayed in figure 3.1. Each trial started with a fixation cross displayed in the center of the screen for a variable interval of 750-3000 ms. A circular array of 8 letters appeared for 200 ms, followed by a fixed interval of 200 ms. Afterwards, an acoustic neutral signal of 250 ms, followed by the appearance of a question mark, informed subjects to vocally report as many letters as they remembered. Participants were instructed to maintain fixation on the central cross during the whole trial. After the answer, they had to press the spacebar to move to the next trial. Only accuracy, not speed of response, was emphasized.

The letters were presented in uppercase with a dimension of ~0.87° x 0.87° at a distance of 3.125° from the central fixation cross. All the Italian alphabet letters were used, except for the letter ‘I’ due to its high similarity with the lowercase letter ‘L’. Each array of letters consisted of 3 high-salience letters (black stimuli, RGB value 102 102 102) and 5 low-salience letters (grey stimuli, RGB value 250 250 250). The letters and their salience were randomly selected on a trial-by-trial basis. Subjects were instructed to vocally report as many letters as they remembered, despite their color.

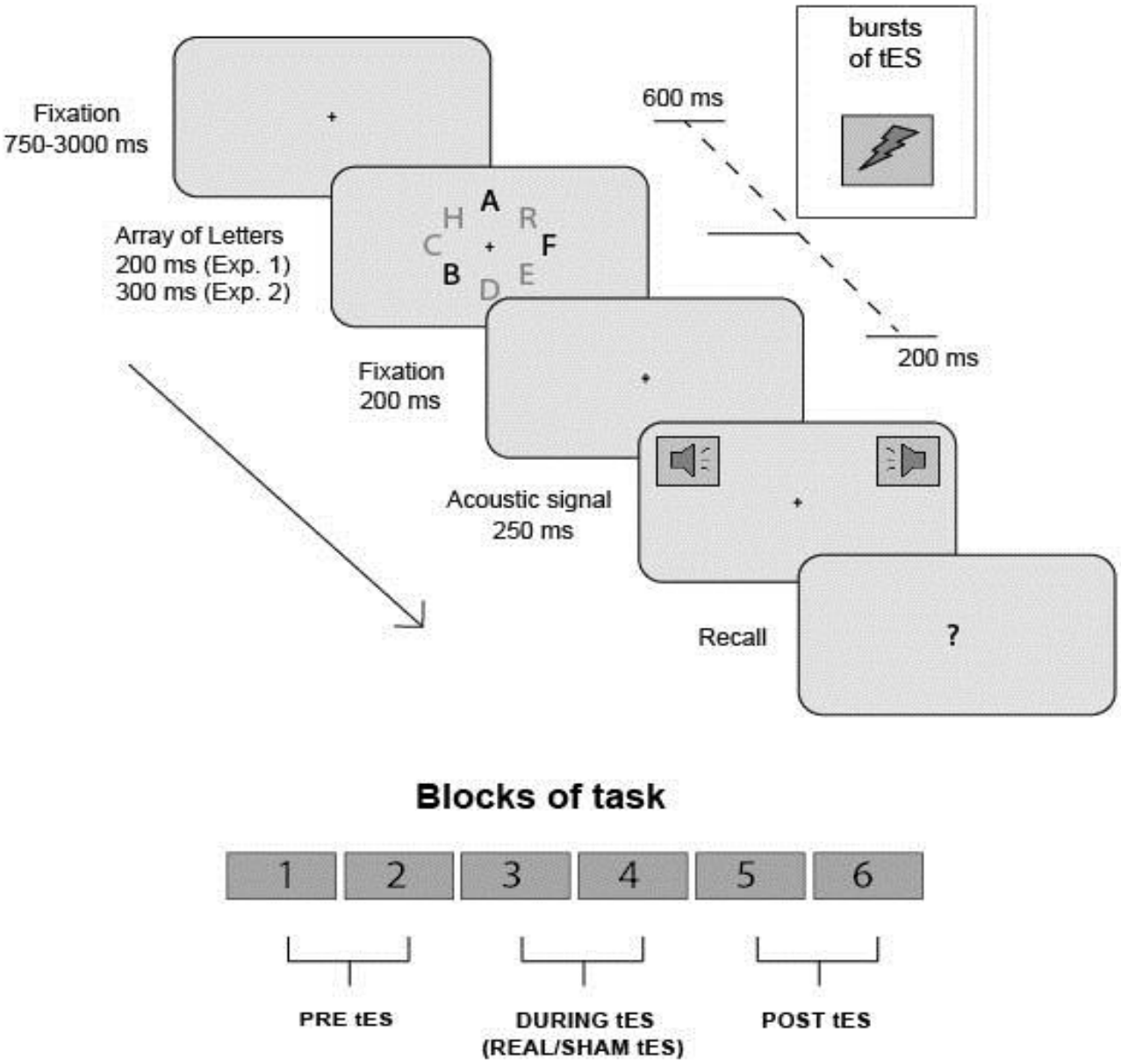

Figure 3.1 *A schematic illustration of the paradigm and the design of the experiment. Bursts of tES were applied starting 600 ms before the onset of the array of letters and lasting until 200 ms after its offset (duration = 1000 ms in Experiment 1; 1100 ms in Experiment 2). tES was applied only during the two central blocks of the task.*

Stimuli were displayed on a light gray background using a Dell LED monitor with a screen resolution of 1920x1080 pixels. The presentation was controlled by E-Prime software (Psychology Software Tools Inc., Sharpsburg, PA). The screen was located at a distance of 70 cm from the participants. The vocal responses were recorded through a microphone and digitized with GoldWave software (GoldWave, Newfoundland, CA) with a sampling rate of 11,025 Hz. The total experiment lasted approximately 40 minutes.

All participants completed two experimental sessions during which they performed the same behavioral task combined with real or sham tES (within-subject design). The temporal interval between the two experimental sessions was at least of 7 days, in order to avoid any possible after-effect of the stimulation. Each session began with a short training, to become familiar with the task (5 trials), and then continued with six blocks of task (30 trials each, 180 trials overall). tES was applied concurrently with the task execution only during blocks 3 and 4 (i.e, during-tES), whereas during blocks 1, 2, 5 and 6 participants performed the task while tES stimulator was in a stand-by state. Blocks 1 and 2 (i.e, pre-tES) served as baseline, while blocks 5 and 6 (i.e, post-tES) were thought to capture possible after-effects of tES. Half of the participants received real tES during the first experimental session and sham tES during the second one, whereas the opposite order of stimulation was followed for the other half of the participants. The order of the tES condition (real vs. sham) was balanced across participants.

3.1.3. tES

Bursts of high frequency tRNS (100-640 Hz) were delivered using a battery-driven current stimulator (BrainSTIM, EMS, Bologna, Italy) through a pair of rectangular electrodes (35 cm^2) inserted in saline-soaked sponges prepared with a conductive gel solution. The electrodes were placed over FPz and Oz as determined by the International 10-20 EEG system. The stimulation was triggered during the two central blocks of the task (during-tES) with E-Prime software, for a total of 60 bursts of stimulation. Each burst of stimulation began 600 ms before the onset of the array of letters and lasted until 200 ms after its offset (duration = 1000 ms). The stimulation parameters (current intensity = 2mA; max current density =

0.057 mA/cm^2) were below the safety limits (Woods et al., 2015). In the sham condition, the current was not delivered after the initial impedance check.

3.1.4. tES sensations

At the end of each experimental session, all participants completed a questionnaire to evaluate possible discomfort and perceived influences induced by tES on the performance (Fertonani et al., 2015, 2010). It was necessary that participants did not perceive any difference between real and sham stimulation, because the mere sensory stimulation could mimic the expected arousal effects. Only two participants, who clearly perceived the bursts of tES during the real stimulation, were able to differentiate real from sham sessions, and for this reason their data were excluded from the analyses. No participant of those included in the analyses reported any perceived sensation induced by tES application, except for one participant who reported itching at mild level during the real tES condition, supporting that bursts of stimulation are associated with the absence of perceptions in almost all the participants.

3.1.5. Pupil size recording

Pupil size was recorded from the right eye through a video infrared camera (SR Research Eyelink 1000, SR Research Ltd., Mississauga, Ontario, CA) with a sampling rate of 500 Hz. The camera was placed in front of the participants at a distance of 60 cm. Pupil dilation was measured fitting an ellipse on the pupil image and calculating the length of the major axis. At the beginning of each experimental session a nine-point calibration procedure was performed in order to calibrate the eye-tracker (calibration error was kept below 0.5° of visual angle).

3.1.6. Skin conductance recording

Skin conductance was recorded from 1.3 cm of diameter Ag/AgCl electrodes placed on the distal phalanges of the second and third finger of the participant's non-dominant hand. The electrodes were prepared with an isotonic paste (Discount Disposables, St. Albans,

Vermont), and the activity was recorded using a galvanic skin response module (BrainProducts GmbH, Munich, Germany) with a constant voltage applied across the electrodes. Electrodermal data were DC-recorded continuously with a resolution of 0.1 and digitized at a sampling rate of 5000 Hz (BrainAmp ExG MR 16 channels, BrainProducts GmbH, Munich, Germany).

3.1.7. Behavioral analyses

As measure of behavioral accuracy, we analyzed the amount of the correct reported letters as a function of salience. In order to account for the different number of the high- and low-salience letters presented, the raw number of the remembered letters was adjusted to the total number of letters presented for each salience (total number of remembered letters/total number of presented letters). We applied a repeated measures ANOVA to the proportion of the remembered letters with *salience* (2 levels, high and low), *tES condition* (2 levels, real and sham) and *block* (3 levels, pre- during- and post-tES) as within-subjects factors. Sidak's correction was applied for post-hoc comparisons where required.

3.1.8. Pupil size analyses

Pupil size data were extracted using Eyelink data-viewer (SR Research data-viewer, SR Research Ltd., Mississauga, Ontario, Canada). The measures were expressed in arbitrary units as recorded by the eye-tracker, and they were linear in true pupil diameter (Einhäuser et al., 2008). Pupil data were processed and analyzed using BrainVision Analyzer v2. As a first step, we identified blinks throughout the dataset and corrected them estimating the pupil size through a linear interpolation of adjacent samples. All the blocks of data were then segmented into epochs comprised between 500 ms before and 4000 ms after the presentation of the array of letters. Values were then baseline corrected to the 500 ms preceding the stimulus and all the segments were visually inspected to reject those with artifacts. Separate averages were obtained for pre-tES, during-tES and post-tES blocks. According to literature (Bradley et al., 2008) and to the visual inspection of the grand averages, we chose to analyze the mean pupil size in the interval between 1500 and 2500 ms after the onset of the array of letters. This temporal window allowed (i) to observe the dilation of the pupil that followed

the initial constriction due to the light reflex and (ii) to use the largest interval of clean data without the risk to overlap values between adjacent segments. The data from 5 participants were excluded from the analyses because their pupil dilation was outliers compared to the overall mean of the participants. A repeated measures ANOVA was applied to the data of the remaining 15 participants with the factors *tES condition* (2 levels, real and sham) and *block* (3 levels, pre-, during- and post-tES). Sidak's correction was applied for post-hoc comparisons where required.

3.1.9. Skin conductance analyses

The first measure of interest obtained by the skin conductance signal was the SCRs to the bursts of stimulation. In line with a previous study of Benedek and Kaernbach (2010), the integrated skin conductance response values were considered as an indicator of response magnitude in an event-related design. Data within the period of interest, that was 1-4 seconds after the onset of the bursts of stimulation, were extracted from the raw data using BrainVision Analyzer v2 and then analyzed using Ledalab software. The minimum amplitude criterion was set to 0.05 μS. In trials without bursts of stimulation (pre- and post-tES of the real condition and all blocks of the sham condition) a corresponding period of interest was analyzed. All values were standardized with the formula y=log (1+x) due to the positively skewed distributions of the SCRs (Martin & Venables, 1980). For both the real and sham conditions, the values from all blocks were normalised to the respective baseline block, as in the behavioural analyses. The data from 3 participants were excluded from the analyses because their responses were outliers compared to the overall mean of the participants. A repeated measures ANOVA was applied to the data of the 17 remaining participants with the factors *tES condition* (2 levels, real and sham) and *block* (3 levels, pre-during- and post-tES). Sidak's correction was applied for post-hoc comparisons where required.

In order to investigate in depth analogies and dissimilarities between this and our previous study (Mauri et al., 2015), we compared the SCL obtained by the skin conductance signal across the participants of the two studies. The SCL is considered as an index of general activation during a large amount of time and is not related to a specific event. Starting from

the SC recording, in both the experiments, we extracted the mean tonic value during each task block of the sham condition. We considered only sham condition because it better reflected general task-related levels of activation, irrespective of tES influence. All the values of this and our previous study (Mauri et al., 2015), were standardised with the formula y=log (1+x) as for the SCRs. Data from 17 subjects of this study (14 female, mean age = 23.4; SD = 2.8; STAI-Y state, mean score = 30.1, SD = 4.7; STAI-Y trait, mean score = 38.1, SD = 8.6) (VSTM group) and 11 subjects of Mauri and colleagues (2015) (7 female, mean age = 24.5; SD = 3.8; STAI-Y state, mean score = 31.9, SD = 7.6; STAI-Y trait, mean score = 35.8, SD = 7.4) (CPT group) were compared using a mixed-model ANOVA with the factors *group* (2 levels, group 1 and 2) and *block* (3 levels, pre- during- and post-tES). Sidak's correction was applied for post-hoc comparisons where required.

3.2. Results

3.2.1. Behavioral data

The analysis on the proportion of correct letters showed a main effect of the factor salience [$F(1,19) = 21.83$, $p = 0.01$] confirming, as expected, that high salience letters were overall more reported than low salience letters. The factor block was also significant [$F(2,38) = 12.30$ $p < 0.001$] showing that an higher number of letters was reported during- and post-tES compared to the pre-tES block. This result suggests an improvement of the performance across blocks as consequence of a learning effect (figure 3.2). No other main effect or interactions emerged from the analysis [all p's > 0.31], showing that the behavioral performance was not affected by tES application.

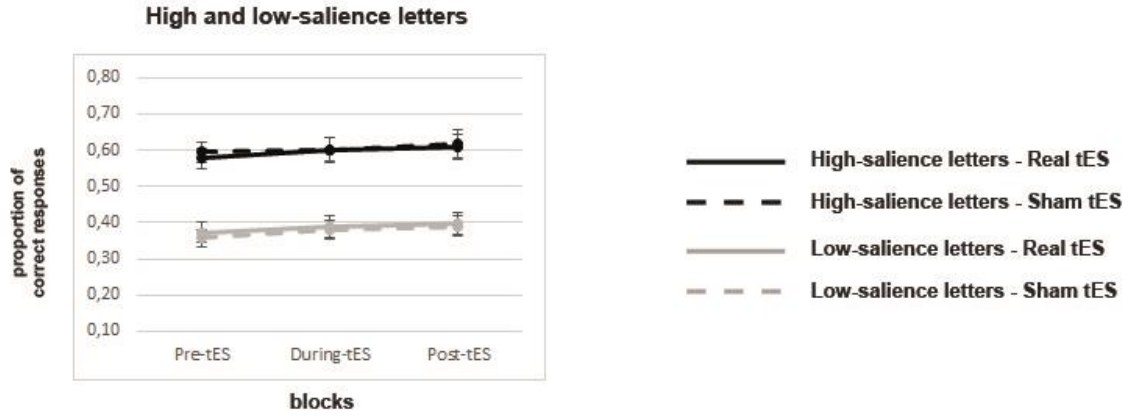

Figure 3.2 *Behavioural results of the high and low-salience letters in the real and sham tES conditions, as a function of the blocks of the tasks. The graph reports the amount of the correct reported letters for both high and low-salience letters at pre- during- and post-tES blocks. Error bars represent the standard error of the mean (±SEM).*

3.2.2. Pupil size

The main factor condition was not significant [F (1,14) = 0.28, p > 0.60], neither it was the factor block [F (2,28) = 0.33, p > 0.73]. The interaction between condition and block was significant [F (2,28) = 3.83, p = 0.03], but the post-hoc comparisons did not reveal any significant difference [all p's > 0.15]. These results suggest that pupil size did not show any variation during the whole task (figure 3.3A).

3.2.3. Skin conductance

The analysis on the SCRs revealed a significant effect of the main factor block [F (2,32) = 12.45, p < 0.001]. Post-hoc comparisons between blocks showed higher SCRs amplitude in the pre-tES block compared to during-tES and post-tES blocks, denoting a general decrease in SCRs throughout the experiment in response to a physiological habituation to the task. The main factor condition was not significant [F (1,16) = 0.49, p = > 0.49], neither it was the interaction between condition and block [F (2,32) = 2.40, p > 0.11], indicating the absence - of tES effects on SCRs (figure 3.3B).

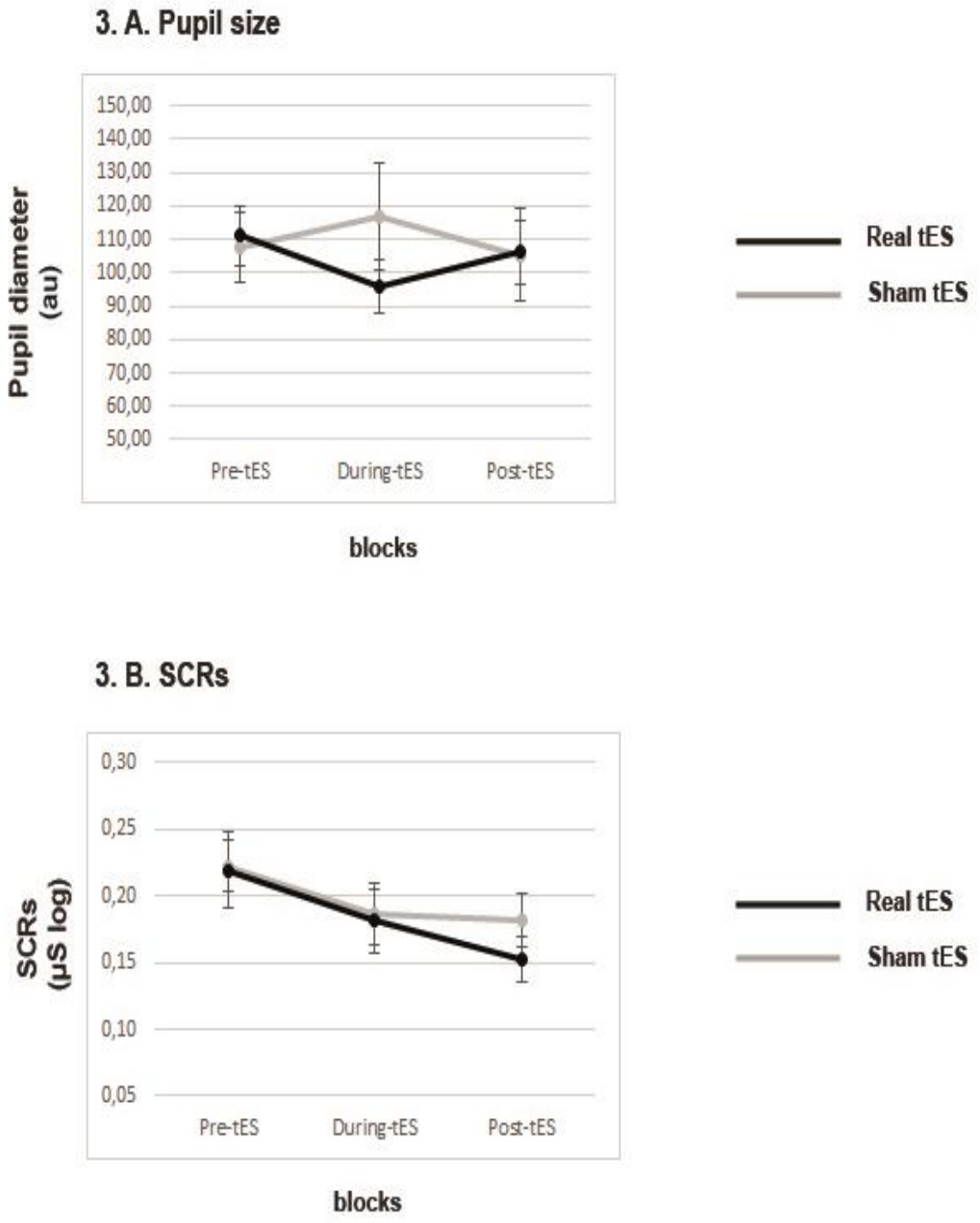

Figure 3.3. (A) *Pupil size variation in the real tES condition (in black) and in the sham tES condition (in grey), as a function of the blocks of the tasks. The horizontal axis represents the blocks of the task, while the vertical axis represents the pupil diameter expressed in arbitrary units (au);* ***(B)*** *SCRs in the real tES condition (in black) and in the sham tES condition (in grey). The horizontal axis represents the blocks of the task, while the vertical axis represents the SCRs in µS log. Error bars represent the standard error of the mean (±SEM).*

The analyses on the SCL revealed a main effect of the factor group [F (1,26) = 5.95, p = 0.02)], according to which the participants of this study (VSTM group) showed a significant higher SCL compared to participants of Mauri and colleagues' study (2015) (CPT group). The factor block was not significant [F (2,52) = 1.86, p = 0.17)], neither it was the interaction between factors [F (2,52) = 2.36, p = 0.11)] (figure 3.4).

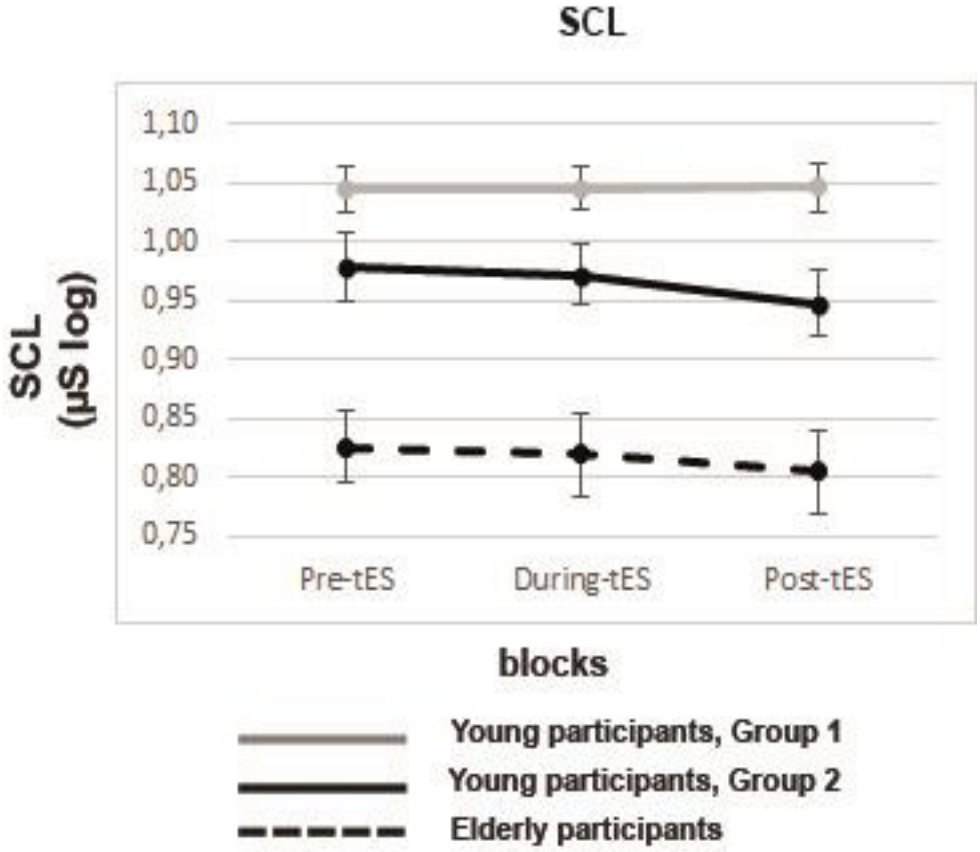

Figure 3.4 *SCL in the group 1 (current study), in the group 2 (previous study) and in elderly. The horizontal axis represents the blocks of the task, while the vertical axis represents the SCL in µS. Error bars represent the standard error of the mean (±SEM).*

The Independent Samples t-test applied at the scores of the STAI-Y of group 1 and group 2 revealed any significant difference across groups, neither for the state scale (p = 0.45), nor for the trait scale (p = 0.48). This result suggests that the subjects of the two studies showed the same levels of activation at the beginning of the experiments.

3.3. Discussion

The behavioral results on young participants showed that subjects reported a general higher number of high-salience stimuli (black letters) in comparison to low-salience stimuli (grey letters), confirming that the paradigm was correctly implemented. These data are consistent with the biased competition model of attention (Baluch & Itti, 2011), according to which stimuli compete for mental representation and high saliency stimuli are prioritized in attention, garnering more neural resources for their representation. Bottom-up effects related to high saliency stimuli, which captures attention due to their physical characteristics, are therefore fundamental in determing priority and stimuli processing (Itti & Koch, 2000). In line with this theoretical framework, various studies reported the key role of salience in visual perception, showing that high contrast stimuli are more likely to capture attention at the expense of low contrast stimuli, increasing the processing resources devoted to them (Bogler et al., 2011; Treue, 2003).

Regarding the effects induced by tES on the proportion of reported high and low salience letters, the visual trends were consistent with expectations: a higher number of high-salience letters was reported during the real stimulation compared to the sham condition, while a lower number of low-salience letters was reported during the real stimulation compared to the sham condition. However, despite these visual trends, the factor condition did not show any statistical effect, meaning that tES stimulation did not modulate the performance in a significant way. Taking into account the absence of any significant effect also in the SCRs and in the pupil diameter, we can infer that tES was not successful in modulating the level of young subjects' arousal during a VSTM task.

In the study by Mauri et al. (2015), in which tES modulated arousal related behavioral and psychophysiological responses, we used a CPT task, which is specifically designed to study sustained attention/arousal. The CPT requires a continuous monitoring of sensory information and measures the ability to detect and respond to specified targets presented infrequently and at random intervals over a prolonged time period, while simultaneously inhibiting responses to non-target stimuli (Eliason & Richman, 1987). This task has been found to be associated with neural substrates of sustained attention, including the pathways

between basal ganglia, thalamus, and frontal lobes. Right hemisphere involvement (asymmetric response) is also evident across multiple studies (Ballard, 2001; Riccio et al., 2002).

The VSTM task used here, instead, is specifically designed to assess the visual component of the working memory system. The task involves the processing, the non-permanent maintenance and the retrieval of visual information while salience effect was modulated. It requires to actively hold relatively abstract representations of a limited number of items. VSTM performance is generally connected with intraparietal sulcus activity (Xu & Chun, 2009).

The two tasks differed in regards to the cognitive effort required, which was higher in the VSTM task used in the present study in comparison to the CPT task used in Mauri et al. (2015). Considering that several studies use difficult tasks as a condition for increasing arousal of subjects who are performing it (Brown et al., 2014; Gellatly & Meyer, 1992), we can assume that the VSTM task itself increased the level of endogenous activation more than the CPT task did. Consistently with this interpretation, the STAI-Y values compiled before the beginning of the tasks did not differ between the two studies, meaning that subjects started the task from a comparable level of activation. However, the comparison of the SCL, extracted from the SC recording during the task in the sham condition, showed significantly higher levels of activation during this study compared to the levels of SCL acquired in the study of Mauri and colleagues (2015). We can suppose that the bursts of stimulation that we applied were effective to modulate a low-level state of arousal (i.e, Mauri et al. 2015), but they were not enough to increase an already high-level state of arousal, as in this study. We believe that the difference in the quantity of current stimulation induced in the two studies (60 s vs. 72 s; 60 bursts vs. 81 bursts; in the present study and in Mauri et al. 2015, respectively) did not justify the different results across the studies. Therefore, the absence of any effect related to the exogenous stimulation (tES effects) in the behavioral or physiological results, could be explained by the increased level of endogenous activation due to the task, as confirmed by the higher level of SCL compared to the previous study.

4. Experiment 2: elderly participants

So far, arousal in aging has been poorly studied. Still unclear are the age-related arousal changes and contrasting results have been reported in literature. Although some studies showed that older adults generally display physiological arousal response patterns that are similar, albeit attenuated, compared to younger adults (Denburg et al., 2003; Kunzmann & Grühn, 2005; Neiss et al., 2009), most studies demonstrated a general decline of the arousal responses in the aging population with smaller and deleted task-induced responses of electrophysiological measures, such as skin conductance and pupil dilation (Gross & Levenson, 1993, Barontini et al., 1997; Kim et al., 2000; Kisley et al., 2007). The generally age-related attenuation of the autonomic systems (Barontini et al., 1997) suggested that arousal may also decline with aging and some age-related cognitive deficits can be attributed to this alteration.

Contrasting results about the effects induced by the modulation of arousal in aging have been also reported. Some studies suggested that arousal has beneficial effects on cognitive performance in healthy older adults (Nashiro & Mather, 2011a; Sutherland & Mather 2015); others, instead, reported a detrimental effect of arousal on cognitive performance in elderly subjects (Kukolja et al., 2008; Nashiro & Mather, 2011b).

Sutherland and Mather (2015) investigated the effects of arousal on memory for stimuli salience, using arousing sounds during a short-term memory task. The authors found that presenting an emotionally negative arousing sound before briefly showing an array of letters with different levels of salience increased the memory for more salient letters in a group of older adults. Despite older age predicted an overall decline in the number of targets that were reported, arousal ratings continued to predict greater attention biases to objects with high salience. As previously found in young adults (Sutherland & Mather, 2012), results confirmed the enhancing role of emotional arousal in the effects of stimulus salience also in older adults suggesting that emotional arousal has beneficial effects on cognitive performance. In another study, Nashiro and Mather (2011a) revealed that healthy older adults remembered a greater number of arousing than non-arousing items, indicating that emotional arousal enhances item memory in normal aging.

The same authors, in another experiment (Nashiro & Mather, 2011b), reported contrasting results. They examined whether arousal would enhance younger and older adults' within-item and between-item memory binding. Data revealed that arousal improved younger adults' within-item memory binding but not that of older adults. In another study Kukolja and colleagues (2008) investigated age-related influences of the individual stress level on behavioural and neural measures during a challenging memory task such as encoding and retrieval of spatial contextual information. Results showed that increasing cortisol responses in young subjects had a beneficial effect on cognitive performance, while, on the contrary, increasing cortisol responses in older subjects had a detrimental effect on cognitive performance with a decreased efficiency to retrieve information. These data suggested that neuroendocrine responses are differentially associated with behavioural and neural measures in cognitively challenging situations in young and older subjects (Kukolja et al., 2008).

In the present study we used the same short-term memory paradigm and the same protocol of stimulation used in Experiment 1, with the aim to investigate whether tEs was able to induce arousal modulations in healthy elderly individuals. The second goal of the study was to explore the effects of an arousal enhancement on behavioural responses. As neurophysiological measures of arousal, we recorded the skin conductance and the pupil size during the task, two indexes under the control of the autonomic nervous system (Bradshaw, 1967; Kahneman & Beatty, 1966).

4.1. Materials and methods

4.1.1. Participants

Twenty-four healthy elderly volunteers (15 females, mean age = 68 years; SD = 3.5) were recruited from a larger sample of Senior University students, and participated to the study for a small monetary compensation. According to the same criteria applied in Experiment 1, data from four participants were discarded from the final analyses: three of them reported a lower accuracy or a higher number of false alarms (respect to ±1.96 standard deviations) compared

to the overall mean of the participants; another one had a clear perception of the bursts of stimulation during the experiment.

All the remaining 20 participants (11 females, mean age = 68 years; SD = 3.6) had normal or corrected-to-normal visual acuity, evaluated with the Lighthouse visual acuity charts (Lighthouse Enterprises, New York, NY) and reported normal values (all participants = 5.60%, but one = 3.90%) for the Pelli-Robson contrast sensitivity test (Pelli et al., 1988). All of them were right handed according to the Edinburgh handedness inventory test (Oldfield, 1971) and showed no risk factors for tES application, as assessed through safety questionnaires (Fertonani et al., 2015; 2010).

Subjects who had a history of seizures, implanted metal objects, heart problems or any other neurological or psychiatric disease were not included, as well as individuals who scored below 24 out of 30 on the Mini Mental State Examination (MMSE). All the participants underwent a neuropsychological evaluation, scheduled about one week before the first experimental session, in order to assess that their cognitive functioning fell within the normal range. The neuropsychological test battery included measures to assess non-verbal reasoning (Raven Colored Progressive Matrices), verbal fluency (phonemic), memory (Story Recall, Rey–Osterrieth Complex Figure recall, Rey Auditory Verbal Learning Test Immediate and delayed recall, Digit Span, Spatial Span), visuo-spatial abilities (Rey–Osterrieth Complex Figure, copy), attention and executive functions (attentional matrices, Trail-Making test A and B, Stroop test). The results of the neuropsychological tests are summarized in Table 3.1. Before the beginning of the experiment, participants completed the STAI-Y questionnaire to measure the state and trait anxiety level (STAI-Y state, mean score = 33.6, SD = 6.4; STAI-Y trait, mean score = 37.4, SD = 9.7). At the end of each experimental session participants were asked to explicitly clarify the strategies used during the execution of the task. The study was approved by the Ethics Committee of IRCCS Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy. Informed consent was obtained from all participants prior to the start of the experiment.

	Raw score	Adjusted score	*Cut-off
Mini mental state examination (MMSE)	28.54 ± 1.5	26.99 ± 2.0	≥24
Raven-colored progressive matrices	28.96 ± 3.7	31.8 ± 2.7	>18
Fluency-phonemic	34.83 ± 11.3	35.79 ± 9.9	>17
Digit span forward	5.83±1.0	5.81±1.0	>3.75
Digit span backward	3.75±0.6	-	-
Story recall	14.85 ± 3.6	15.29 ± 2.8	>8
Spatial span	4.54 ± 0.9	4.71 ± 0.9	>3.75
Rey Auditory Verbal Learning Test (Immediate recall)	41 ± 8.4	44.05 ± 6.9	>28.52
Rey Auditory Verbal Learning Test (Delayed recall)	8.96 ± 2.1	10.01 ± 1.9	>4.68
Rey-Osterrieth complex figure-recall	13.77 ± 4.8	17.11 ± 4.5	>9.47
Trail making test-A	47.63±17.9	31.42±18.3	<93
Trail making test-B	117±33.5	69.95±31.8	<282
Trail making test-B-A	72.19±24.4	40.95±20.5	<186
Stroop test (errors)	0.54 ± 0.9	0.33 ± 0.7	<4.23
Stroop test (time)	3.70 ± 0.6	21.98 ± 8.9	<36.91
Attentional Matrices	46.58 ± 6.7	45.64 ± 7.1	>30
Rey-Osterrieth complex figure-copy	33.77 ± 3.4	34.92 ± 3.0	>28.88
Geriatric Depression Scale (GDS)	4.79	3.44	24/30

Table 3.1 *Neuropsychological results relative to the healthy elderly subjects reported as the mean scores (±SD). *Cut-off scores according to Italian normative data are reported.*

4.1.2. Behavioral task, procedure and analyses

All participants completed two experimental sessions during which they received real or sham tES (within subject design). The task and the stimuli were the same as those used in Experiment 1 except for the presentation duration of the array of letters that was longer (300 ms) respect to the previous experiment, to obtain a comparable task difficulty in the two age groups. Furthermore, in order to allow healthy elderly subjects to familiarize with the task, they performed an initial training session of 10 trials, also in this case followed by six blocks of task (30 trials each) divided in pre-tES (blocks 1 and 2), during-tES (blocks 3 and 4) and post-tES (blocks 5 and 6).

As in Experiment 1, the raw number of letters reported by the subjects was transformed with a proportion based on the total number of letters presented for each salience level. A repeated

measures ANOVA was applied to the proportion of the remembered letters with *salience* (2 levels, high and low), *tES condition* (2 levels, real and sham) and *block* (3 levels, pre- during- and post-tES) as within subjects factors. Sidak's test was performed for post-hoc comparisons.

4.1.3. tES

The type of stimulation as well as the montage were the same as those used in Experiment 1. tES was delivered during the two central blocks of the task (during-tES) for a total of 60 bursts of stimulation. The only difference between the two experiments was the stimulation duration, which was slightly longer in elderly (1100 ms) because of the longer presentation of the array of letters.

4.1.4. tES sensations

As in Experiment 1, at the end of each experimental session, all participants completed a questionnaire to detect any possible physical sensation and perception induced by tES (Fertonani et al., 2015; 2010). Only one participant clearly perceived the bursts of tES during the real stimulation, differentiating real from sham session. In order to avoid any possible confounding effect, the data of this participant were not considered in the analyses. No participant of those included in the analyses reported any perceived sensation induced by tES application, neither in the real, nor in the sham condition, suggesting that also most of elderly participants did not experience any sensation during bursts of stimulation.

4.1.5. Pupil size recording and analysis

Pupil size was measured and recorded exactly as in Experiment 1. Also all the steps of processing of the signal, including the temporal interval analyzed (i.e., 1500-2500 ms after the onset of the array of letters) were exactly as in Experiment 1. The data from 13 participants were excluded from the analyses for technical problems (the increase in the frequency of eye blinks and movements in older participants resulted in system failures during the recording) or because their responses were outliers compared to the overall mean

of the participants. A repeated measures ANOVA was applied to the data of the 7 remaining subjects with the factors *tES condition* (2 levels, real and sham) and *block* (3 levels, pre- during- and post-tES).

4.1.6. Skin conductance recording and analysis

Skin conductance was measured and recorded exactly as in Experiment 1. At the same way, SCRs and SCL were extracted by the skin conductance signal and analyzed. The same parameters were used (SCRs: time window of interest: 1-4 seconds after the onset of the bursts of stimulation; minimum amplitude criterion: 0.05 μS. SCL: mean tonic value during each block of the task in the sham condition). For both the real and sham conditions, the values from all blocks were normalised to the respective baseline block, as in the previous experiment.

The data of 2 participants were excluded from the analyses on SCRs because their responses were outliers compared to the overall mean of the participants. A repeated measures ANOVA was applied to the data of the remaining 18 elderly subjects with the factors *tES condition* (2 levels, real and sham) and *block* (3 levels, pre- during- and post-tES).

4.2. Results

4.2.1. Behavioral data

The analysis performed on the proportion of correct letters revealed a main effect of the factors salience [$F(1,19) = 24.18$, $p < 0.001$] and block [$F(2,38) = 7.50$, $p = 0.002$], confirming the salience effect and the learning effect that we observed in young participants. Importantly, in elderly participants the factor tES condition interacted with block [$F(2,38) = 7.41$, $p = 0.002$] and with salience and block [$F(2,38) = 3.29$, $p = 0.048$]. In order to better understand these interactions, we analyzed the two levels of salience separately. Because the two tES conditions did not differ in the pre-tES blocks, we normalized the effects induced by tES to the pre-tES performance, by subtracting pre-tES proportion of remembered letters from that observed during-tES and post-tES blocks in real and sham condition. Two separate repeated measures ANOVA were applied testing the factors *tES condition* (2 levels, real and

sham) and *block* (2 levels, during- and post-tES). Regarding the high-salience letters, we found a main effect of tES condition [F (1,19) = 7.79, p = 0.012], revealing that the real tES reduced significantly the amount of remembered high-salience letters in comparison to the sham condition (see figure 3.5). No significant effect emerged by the analysis on low-salience letters (all p's>0.28), showing that tES did not affect the amount of the low-salience letters remembered.

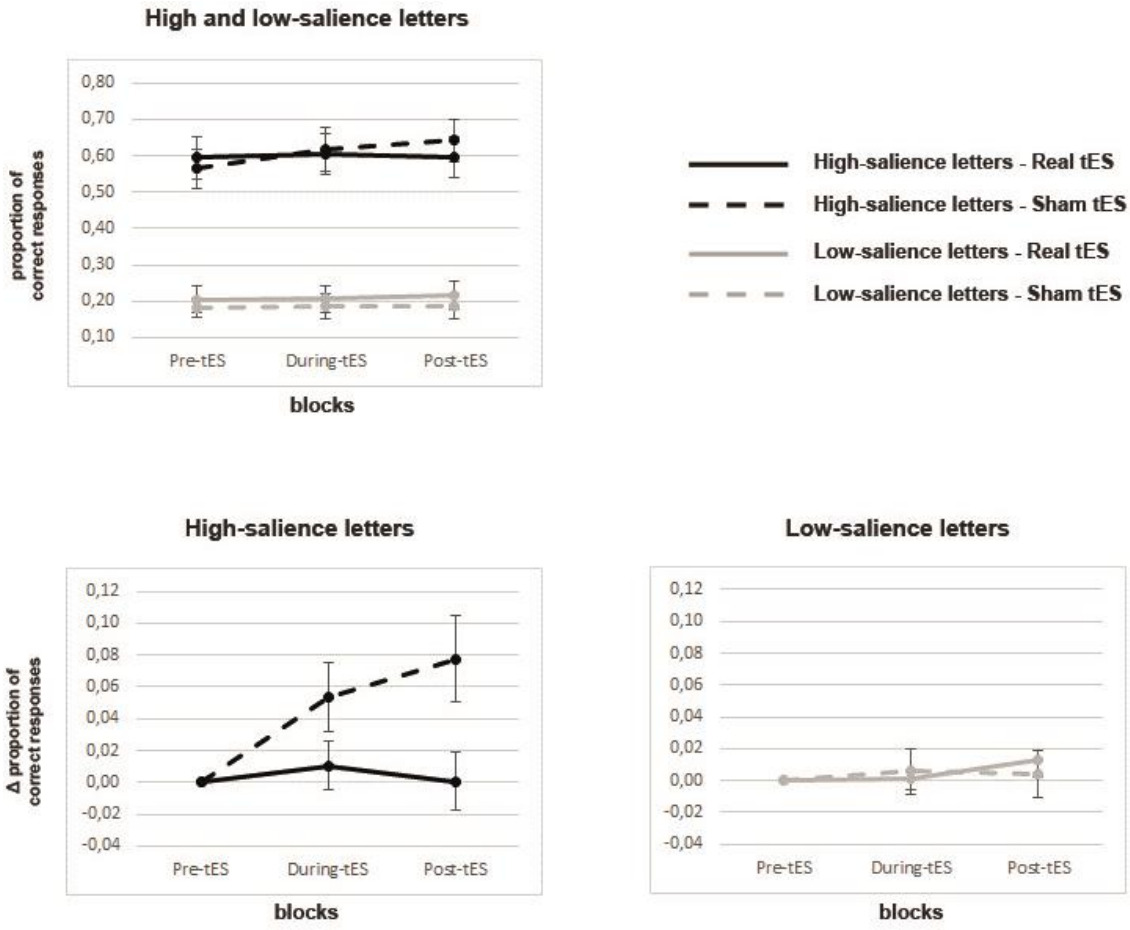

Figure 3.5 *Behavioural results of the high and low-salience letters in the real and sham tES conditions, as a function of the blocks of the tasks. On the top of the figure, the graph reports the amount of the correct reported letters for both high and low-salience letters at pre- during- and post-tES blocks. The graphs below report the normalized amount of correct reported letters, obtained by subtracting pre-tES proportion of remembered letters from that observed during-tES and post-tES blocks for high-salience letters (on the left) and low-salience letters (on the right). Error bars represent the standard error of the mean (±SEM).*

4.2.2. Pupil size

The main factor condition was not significant [F (1,6) = 0.59, p = n.s.], neither it was the factor block [F (2,12) = 0.80, p = n.s.] or their interaction [F (2,12) = 0.57, p = n.s.]. The pupil size did not show any variation during the whole task, despite for a visual trend with a non-significant decrease of the pupil size during the sham condition compared to the real stimulation condition (figure 3.6A). The lack of significant effects may be partly ascribed to the low sample size.

4.2.3. Skin conductance

The analysis on SCRs showed a main effect of tES condition [F (1,17) = 4,48, p = 0.049], according to which higher SCRs were associated to the real tES condition compared to the sham condition during the whole task (see Figure 3.6B). Another significant effect was present for the factor block [F (2,34) = 14,05, p < 0.01]. Post-hoc comparisons showed a general SCRs decrease throughout the experiment in response to a physiological habituation to the task, with a subsequent reduction of the response amplitude over time. A significant interaction between condition and block [F (2,34) = 3.92, p = 0.029] was also found, indicating that in the pre-tES block there was a significant difference (p=0.016) between the two tES conditions with higher SCRs amplitude during real tES compared to the sham condition. This difference between tES conditions decreased during the tES application (p=0.06) and disappeared in the post-tES block (p>0.2) (figure 3.6A).

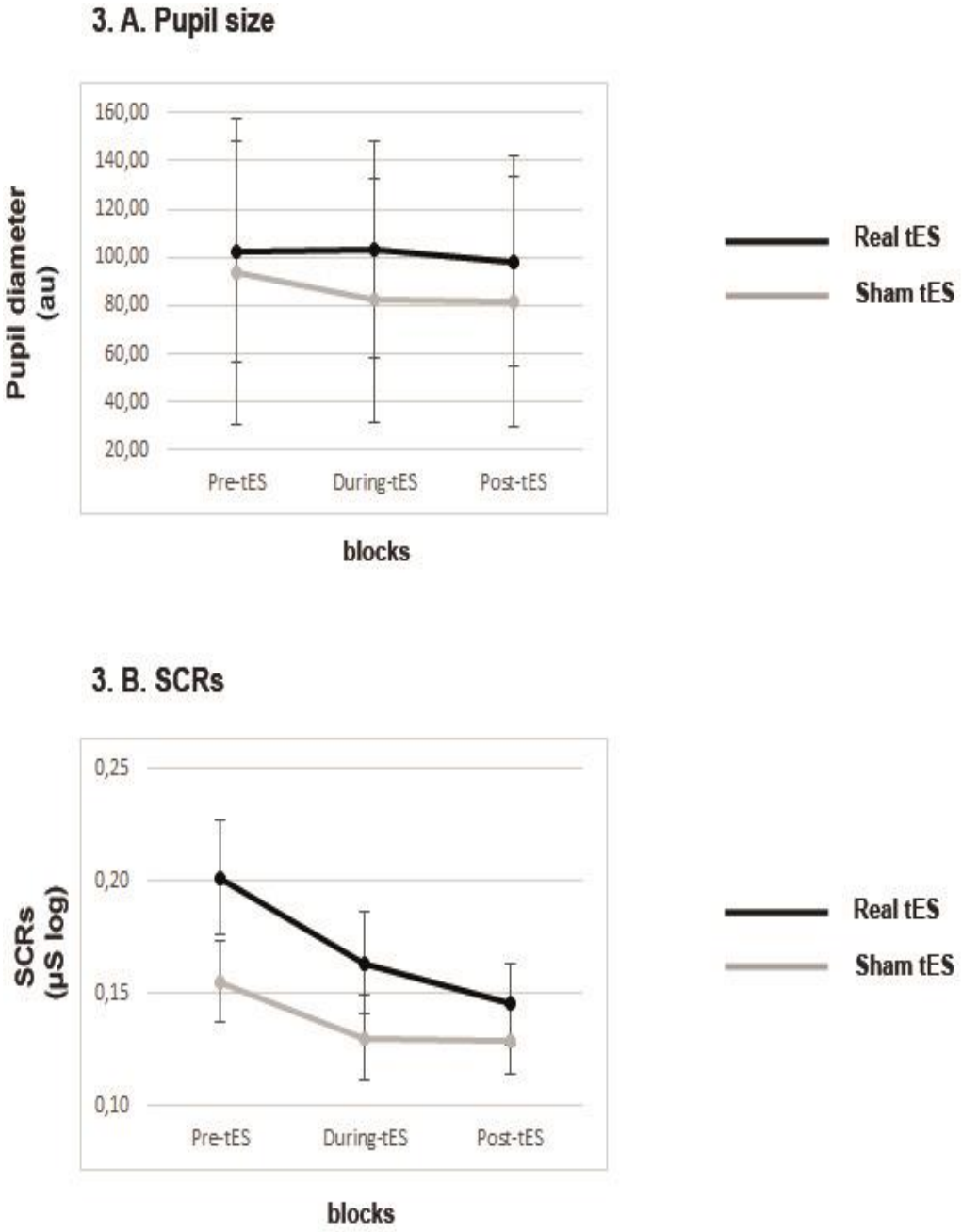

Figure 3.6. (A) *Pupil size variation in the real tES (in black) and in the sham tES condition (in grey), as a function of the blocks of the tasks. The horizontal axis represents the blocks of the task, while the vertical axis represents the pupil diameter expressed in arbitrary units (au);* ***(B)*** *SCRs in the real tES (in black) and in the sham tES condition (in grey). The horizontal axis represents the blocks of the task, while the vertical axis represents the SCRs in µS log. Error bars represent the standard error of the mean (±SEM).*

5. Comparison between young and elderly participants

We compared behavioral and psychophysiological variables between the two groups. Mixed-model ANOVA with *group* (2 levels, young and elderly), *tES condition* (2 levels, real and sham), *salience* (2 levels, high and low) and *block* (3 levels, pre- during- and post-tES) were applied at the proportion of reported letters, while mixed-model ANOVA with *group* (2 levels, young and elderly), *tES condition* (2 levels, real and sham) and *block* (3 levels, pre-during- and post-tES) were applied at skin conductance (SCRs and SCL). Sidak's tests were performed for post-hoc comparisons.

5.1. Behavioral data

In addition to the main effects of salience [$F(1,38) = 43{,}32$, $p < 0.001$] and block [$F(2,76) = 12.61$, $p < 0.001$], which have already been considered in each group singularly, the analysis revealed a significant main effect of group [$F(1,38) = 14.73$, $p = 0.001$]. A tendency to significance in the interaction between salience and group [$F(1,38) = 3.89$, $p = 0.056$] was also observed. In order to better understand this tendency, we analyzed the two levels of salience separately. Two separate mixed-model ANOVA with group (2 levels, young and elderly), tES condition (2 levels, real and sham) and block (3 levels, pre- during- and post-tES) were applied. Regarding the low-salience letters, we found a main effect of group [$F(1,38) = 19.91$, $p < 0.001$], revealing that elderly reported a lower amount of low-salience letters compared to young participants. Whereas, for high salience letters, the difference between the groups observed, vanished [$F(1,38) = 0.002$, $p = 0.96$].

Overall these results showed that, as expected, on the whole, elderly remembered fewer letters than young participants, suggesting that in normal conditions elderly people have a lower span of visual short-term memory. However, the salience factor influenced the identity of the reported letters: compared to young participants, elderly reported the same amount of high-salience letters ($p = 0.96$) but a lower amount of low-salience letters ($p < 0.001$) (see figure 3.7). Interestingly, the reduced memory span showed by elderly people is much more unbalancing toward high salience stimuli, in comparison to what happens in young subjects.

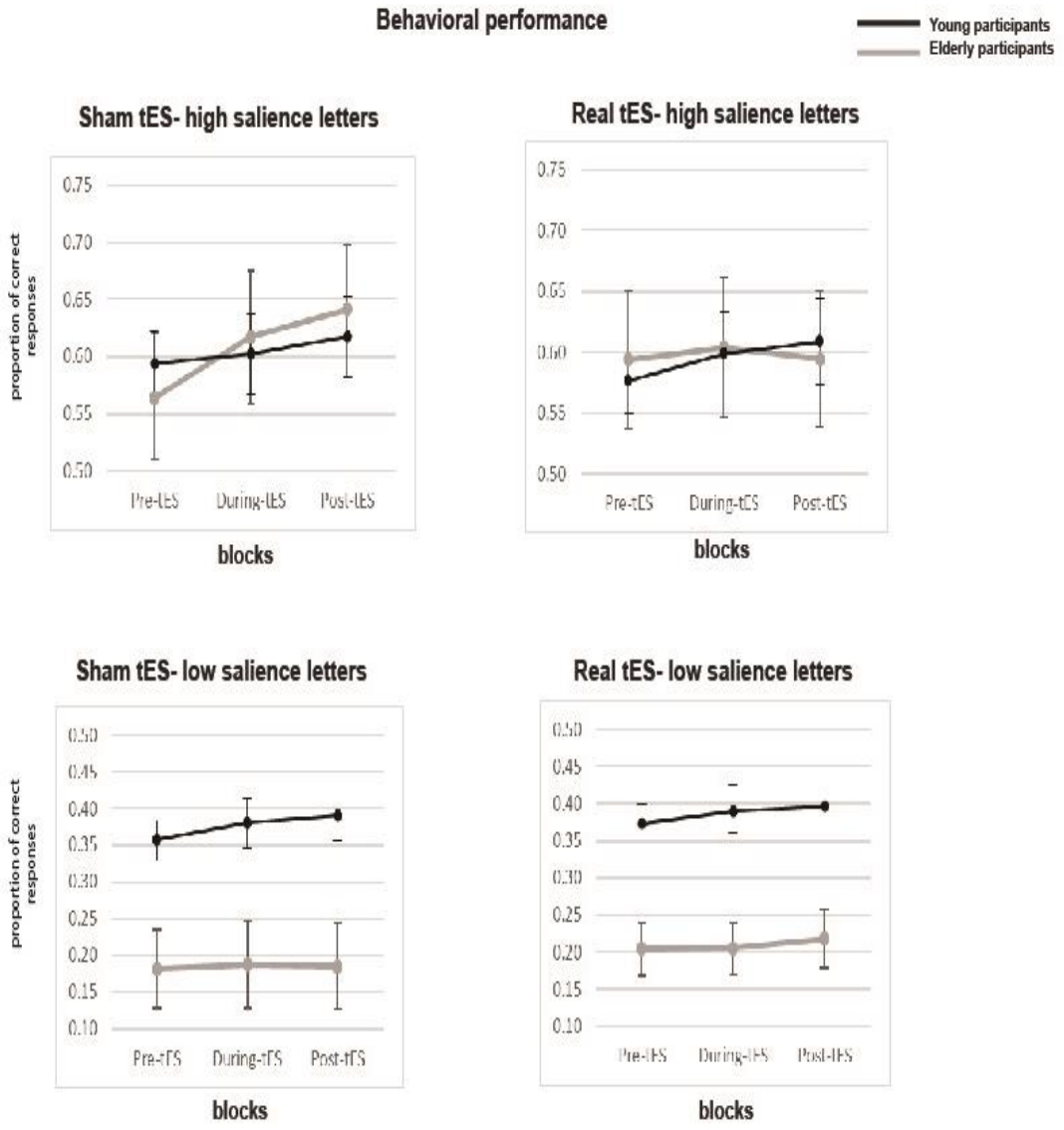

Figure 3.7 *Behavioural results of the high and low-salience letters in the two tES conditions (real and sham), both in young (in black) and in elderly subjects (in grey). The graphs on the top refer to the high-salience letters while the below ones refer to the low-salience letters. Figures on the left side refer to the sham tES condition, the right ones to the real tES condition. The horizontal axis represents the blocks of the task (the 6 blocks of task were collapsed into 3 blocks: pre-, during- and post-tES) while the vertical axis represents the proportion of correct responses reported by the subjects. Error bars represent the standard error of the mean (±SEM).*

5.2. Skin conductance

The analysis on SCRs revealed a significant main effect of block [$F(2,66) = 25.96$, $p < 0.001$], already described in each group and explained as physiological habituation to the task, and a significant interaction between tES condition and block [$F(2,66) = 5.81$, $p = 0.005$]. A general decrease in SCRs throughout the experiment emerged in the real tES condition, indicating that SCRs were affected by tES application. A higher SCRs amplitude for the pre-tES block compared to the during-tES and post-tES blocks was also observed in the sham condition, whereas the difference between during and post blocks disappeared.

A tendency toward significance emerges for the interaction between tES condition and group [$F(1,33) = 3.66$, $p = 0.056$]. Post-hoc comparisons showed lower SCRs amplitude in the sham condition for elderly subjects compared to the young group. Whereas, in the real tES condition, this difference vanished (figure 3.8).

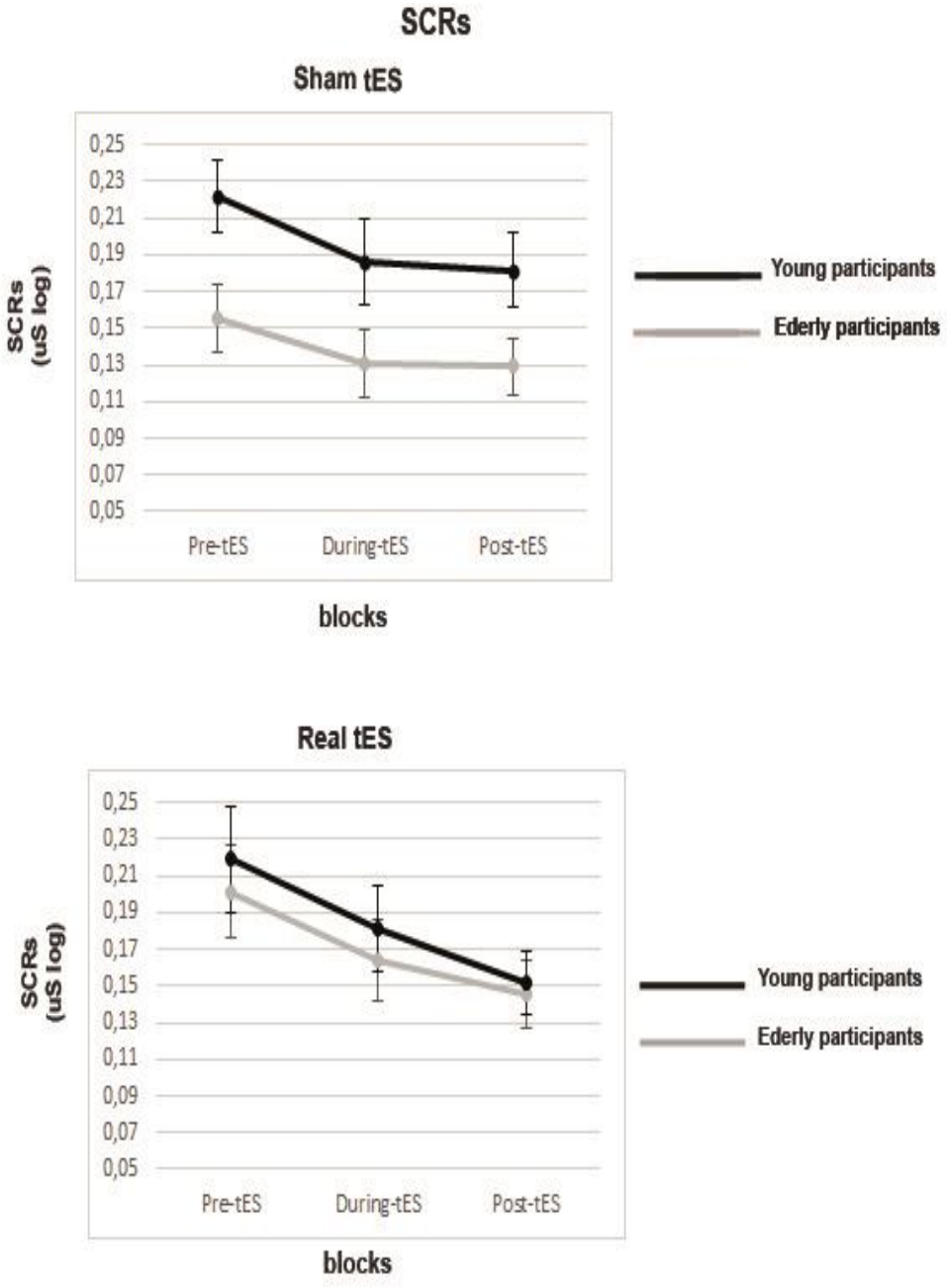

Figure 3.8 *SCRs in the sham tES (figure above) and in the real tES (figure below) condition both in young (in black) and in elderly subjects (in grey). The horizontal axis represents the blocks of the task (the 6 blocks of task were collapsed into 3 blocks: pre-, during- and post-tES), while the vertical axis represents the SCRs in μS log. Error bars represent the standard error of the mean (±SEM).*

The analyses of the SCL showed a main effect of the factor group [$F(1,33) = 34.5$, $p < 0.001$] with a significant lower level of SCL for elderly subjects compared to young subjects. Neither the factor block [$F(2,66) = 0.98$, $p = 0.37$)] nor the interaction between factors [$F(2,66) = 1.41$, $p = 0.25$)] were significant (figure 3.4).

6. Discussion

The analyses performed on the behavioral performance revealed that, on the whole, elderly remembered fewer letters than young participants. Despite the general reduction of the visual memory span, however, the elderly group showed a larger disproportion between high (black letters) and low (grey letters) salience stimuli respect to young adults. Indeed, they reported the same amount of high-salience letters, but a lower amount of low-salience letters, denoting an unbalancing toward high salience stimuli compared to young subjects.

These results could be explained taking into account that the processing of high saliency stimuli is normally prioritized in attention (biased competition model of attention- Baluch & Itti, 2011) leaving no more space for the processing of other stimuli in subjects with a reduced memory span like older adults (Bopp & Verhaeghen, 2005).

Regarding the effects induced by tES, data suggested that real stimulation induced the report of a significantly lower proportion of high salient stimuli, compared to the sham condition, suggesting a reduction of the memory span. For low salience letters, instead, the factor "tES condition" did not show any significant effect. The low baseline performance of low-salience letters may have limited the effects of tES on the performance (i.e., floor effect).

Concerning the physiological indexes, pupil diameter did not show any significant effect. Because of technical problems such as the increase in the frequency of eye blinks and movements in older participants that resulted in system failures during the recording, only a few subjects were included in the final analysis. For that reason, the lack of any significant result in the pupil diameter could be ascribed to the low sample size. SCRs, instead, showed a significant lower response in the sham condition respect to the real tES condition. However, this effect could not be ascribed to the stimulation because a difference was already present in the first block of the task revealing that elderly subjects had a different starting point in

the two conditions. Some evidence suggests that psychophysiological responses are more difficult to be measured in the elderly than in the young (Gross & Levenson, 1993; Barontini et al., 1997). Both pupil size (Winn et al., 1994) and skin conductance generally decrease with aging (Barontini et al., 1997). Older adults are also less likely to show measurable SCRs (Gavazzeni et al., 2008) (e.g., non-responders) as compared to the younger adults (Neiss et al., 2009). Furthermore, measures of skin conductance may be more difficult with the elderly since skin undergoes physiological changes; with aging, skin, is likely to become less hydrated increasing its resistance to electrical current (Barontini et al., 1997). Overall these data suggested that the physiological indexes resulted unreliable in elderly participants and thus they could not be used to draw firm conclusions on the tES modulations of the level of arousal.

We can put forward two hypotheses for explaining the cognitive worsening induced by tES in elderly participants. We can suppose that, differently from what we observed in young, the bursts of stimulation that we applied were enough to induce an increase of arousal in elderly subjects. The comparison of SCRs and SCL between the groups, indeed, showed significant lower levels in elderly respect to young subjects, suggesting a general age-related attenuation of skin conductance in physiological aging. We can, thus, infer that tES might have been strong enough to successfully modulate the level of elderly subjects' arousal during the VSTM task.

If it was the case, we have to conclude that an increase of arousal in older adults resulted in an impairment of their cognitive performance. These results are consistent with a previous study of Nashiro & Mather (2011b) that demonstrated that arousal decreased older adults' performance, reducing within-item memory binding. Also Kukolja and colleagues (2008) showed that increasing cortisol responses in older subjects had a detrimental effect on cognitive performance with a decreased efficiency to retrieve information. In another study, Wurm and collegueas (2004) examined the possible link between activation and cognitive modulation. They used a color-naming task. Words were displayed on a computer screen in a colored font and participants were required to quickly name the font color. Authors found that only for older adults the time needed to name the ink colour for high arousing words was longer (Wurm et al., 2004).

Otherwise, we can ascribe the different effects induced by tES in the two age groups to the functional brain reorganization occurring with aging. In the present study we used a fronto-occipital montage (FPz-Oz) with the aim of stimulating a large area of the brain, including the brainstem (Bikson et al., 2012; Laakso & Hirata, 2013; Sadleir et al., 2010; Wagner et al., 2014). tES may have also interacted with the activity of specific cortical areas, such as the frontal areas, which undergo relevant changes with aging. According to the PASA model the healthy aging is normally accompanied by a higher activation of PFC to compensate for a decrease in occipital activity (Davis et al, 2008). tES application could have interfered with the frontal hyper activation distinctive of elderly subjects thus resulting in an impairment of the cognitive performance.

We can also speculate that tES interfered with visual processing at the low level of the visual system pathway. As suggested by the information degradation theory, degraded perceptual signal inputs result from age-related neurobiological processes (e.g., retinal degeneration) (Schneider & Pichora-Fuller, 2000). Whether during tES application the electric current reaches the retina (Brignani et al. 2013), it could induce a detection impairment, and a subsequent reduction of the memory span, which could vary according to the participant's age to the retina state.

7. General discussion

The current study examined whether transcranial electrical stimulation (tES) can modulate arousal by improving performance during a visual short-term memory (VSTM) task in healthy young and elderly participants. Arousal plays a central role in cognition and a better behavioural performance in young subjects has been associated with an increment of arousal (Bagherli & Mokhtari, 2011; Sutherland & Mather, 2012; VaezMousavi et al., 2007). Although age-related arousal changes are still unclear, some studies showed a general attenuation of the autonomic systems (Barontini et al., 1997) and a decline of the arousal responses in the aging population (Gross & Levenson, 1993, Barontini et al., 1997; Kim et al., 2000; Kisley et al., 2007). Taking into account the importance of arousal in cognition (Berridge & Waterhouse, 2003; Brown et al., 2014; Cortoos et al., 2010; Schock et al., 2011),

the possibility to exogenously modulate it by means of a non-invasive technique such as tES, could represent enormous potential to slow cognitive decline associated with healthy and pathological aging. tES, is a non-invasive technique that has been showed to enhance cognitive abilities in a variety of task through the modulation of neuronal membrane potentials. The techniques is associated with the absence of any clear perception by the participants and based on neuroplasticity mechanisms it might also induce long-term effects (Nitsche & Paulus, 2001).

A previous study conducted by Mauri and colleagues (Mauri et al., 2015) demonstrated that it is possible to modulate arousal in healthy young subjects through bursts of transcranial electrical stimulation (tES) in a sustained attention task. The authors applied bursts of random noise high frequency tES with the aim of mimicking the physiological phasic activation of the LC related to relevant stimuli.

In Experiment 1 we investigate whether applying the same parameters of tES previously used by Mauri and colleagues (2015) it was possible to induce arousal modulations in healthy young individuals during a task involving higher mental processes, such as a VSTM task. Recently, Sutherland and Mather (2012) demonstrated that the increasing of arousal via negative emotional stimuli during a VSTM induced an improvement of memory for high salient stimuli and a worsening of memory for low salient stimuli. The behavioural results of the first study showed that tES stimulation did not modulate the performance in a significant way, probably because it was not successful in modulating the level of young subjects' arousal during the VSTM task. We can ascribe this failure to the increased level of endogenous activation that the VSTM task induced itself, rendering the tES unable to further increase it. Consistently with this interpretation, the comparison of the SCL showed significant higher levels during this study compared to the study of Mauri and colleagues (2015). Results of Experiment 1 suggest that the applied tES was effective to modulate a low-level state of arousal (i.e, Mauri et al. 2015), but it was not enough to increase an already high-level state of arousal. During the task, the absence of any variation of the SCRs and the pupil diameter, which both mirrors LC phasic response, is in line with this explanation.

In Experiment 2, we applied the same paradigm and the same type of stimulation used in Experiment 1 to investigate whether tEs was able to induce arousal modulations in healthy

elderly individuals and to explore the effects of an arousal enhancement on behavioural responses. The behavioral results showed that real stimulation induced a reduction of the memory span, while physiological indexes suggested a general age-related attenuation of the levels of arousal. For this reason, we can suppose that the stimulation was enough to increase arousal, resulting however in an impairment of the older adults' cognitive performance.

8. Conclusions

In conclusion, the results of these two studies did not allow us to draw firm conclusion about the ability of tES to increase arousal in healthy participants. On the whole, evidence reported in Experiments 1 and 2 shows that bursts of tES, applied during a VSTM task, differently modulate behavioral performance and psychophysiological indexes in young and elderly participants. These results raise the issue on the correctness to apply the knowledge on the effects of tES in young subjects to elderly subjects. Taking into account the structural and functional changes occurring in the brain of aging population, it is possibly misleading expecting the same effects of tES in both young and elderly subjects. In these studies, we used a new way to administer tES stimulation, with bursts of current instead of the continuous and longer stimulation that is commonly used (Woods et al., 2015). The possibility of using this type of stimulation protocol in normal aging and in patients, in order to modulate arousal and facilitate neurorehabilitation, is an important perspective that should be evaluated. We acknowledge that a number of questions need to be addressed yet and that further investigations exploring the effects of bursts of tES in young and older subjects are needed.

General conclusions

The present work focused on the investigation of the neurophysiological and behavioral mechanisms related to healthy and pathological aging. In order to examine this issue two studies were conducted.

The purpose of the first study was to explore the behavioral and electrophysiological dynamics of multiple object processing in mild cognitive impairment and Alzheimer's disease patients in order to identify neurophysiological markers able to reliably differentiate normal from pathological aging. This objective was achieved by measuring in AD, MCI and healthy controls both the behavioral performance and the electrophysiological components (N2pc and CDA) evoked during the execution of an enumeration task.

AD patients showed an overall decline in accuracy for all target quantities, whereas in MCI patients, only enumeration of large quantities was impaired. N2pc, an electrophysiological index of attentional selection, was spared in AD and MCI, while CDA was altered in both groups with a non-linear pattern: a reduction in AD and a hyperactivation in MCI. Particular emphasis has been given to the results obtained in MCI patients. In this group, in fact, the electrophysiological results suggested a hyperactivation process interpreted as a compensatory mechanism facilitating the retention in memory of a limited number of elements to be enumerated, which, however, failed when the memory load increased. This compensatory process vanished with the progression of cognitive decline, as evidenced by the small amplitude of the CDA component in the group of AD patients. Overall, the results suggested that multiple object processing could be considered a valid paradigm to identify behavioral and neural markers able to distinguish between the different AD stages, even in the prodromal MCI phase. In particular, CDA may be a useful neural signature to both distinguish between healthy and pathological aging and characterize the different stages along the AD continuum, possibly becoming a reliable candidate for an early diagnostic biomarker of AD pathology.

The aim of the second study, in a more applicative perspective, was the evaluation of the possibility to apply a particular non-invasive neurorehabilitation protocol, by means of

transcranial electrical stimulation (tES), in order to improve cognitive performance by increasing the level of psychophysiological arousal. This objective was achieved by investigating, in a group of healthy young subjects and in a group of healthy elderly subjects, both the behavioral performance to a short term memory task and the relative indexes of autonomic physiological activation (pupillary dilatation and skin conductance).

In young subjects, the behavioural results showed a trend consistent with a condition of increased arousal, with a higher number of high-salience stimuli and a lower number of low-salience stimuli reported during the real stimulation condition. Despite this data trend, no significant differences were present between the real and sham stimulation condition. The SCRs and the pupil diameter did not show any significant difference between the two stimulation conditions suggesting that the tES did not modulate the phasic component of arousal in healthy subjects. It has also been conducted a comparison between the SCL of the subjects who took part in this study (VSTM group) with that of a group of subjects who carried out a sustained vigilance task (CPT' group) in one tES study similar to this and published by Mauri et al. (2015). The subjects of the VSTM group showed a significant higher SCL compared to the subjects of the CPT group during the sham condition. However, the values reported at the STAI-Y state scale were not different between the groups of subjects, suggesting that both groups had a comparable level of activation before the task. The difference of the SCL during the task in the sham condition, allow us to consider the task per se as an arousing factor.

Regarding elderly subjects, the behavioral results showed that real stimulation induced a reduction of the memory span, while physiological indexes suggested a general age-related attenuation of the levels of arousal. For this reason, we can suppose that the stimulation was enough to increase arousal, resulting however in an impairment of the older adults' cognitive performance.

In conclusion, given the increase longevity in our society and the increased incidence of age-related disease (such as dementia), the issues addressed in this work are particularly relevant. The identification of possible psychophysiological markers of the disease, or even of its prodromal stages (the first research purposes), could in fact make an early diagnosis possible

for the timely implementation of curative interventions increasing the chances of an efficient treatment. The results reported lay the foundation for further investigations to establish the validity of using multiple object processing to provide early diagnostic biomarkers.

The identification of additional means of cognitive enhancement (purpose of the second study) could instead be essential to reduce the cognitive age-related deficits, and to complement/replace the treatments available in the field of pathological aging. Despite the negative results of the study, the importance of this field of research lies on the attempt to fill a void in the literature. In the first place, little is still know about the modulation of arousal in aging, secondly, the possibility of using a non-invasive brain stimulation protocol in elderly subjects and in patients, in order to modulate arousal and facilitate neurorehabilitation, is an important perspective that should be deeply evaluated. Further investigations exploring the effects of bursts of tES on other behavioural tasks and physiological indices of arousal, are needed to support this promising research line.

Because of the potential impact, high priority and a lot of attention should be given to research lines such as those developed in this thesis.

List of abbreviations

AD Alzheimer's disease

ADHD Attention-Deficit Hyperactivity Disorder

AIC Akaike information criterion

BIC Bayesian information criterion

CDA Contralateral Delay Activity

CDR Clinical Dementia Rating

CPT Continuous Performance Test

CRUNCH Compensation-Related Utilization of Neural Circuits Hypothesis

CSF Cerebrospinal Fluid

DMN Default Mode Network

EEG Electroencephalography

ERP Event-Related Potential

fMRI Functional Magnetic Resonance

GDS Geriatric Depression Scale

GLMM Generalized Linear Mixed Model

GMT Goal Management Training

HAROLD Hemispheric Asymmetry Reduction in Older Adults

LC Locus Coeruleus

MCI Mild Cognitive Impairment

MMSE Mini Mental State Examination

MOP Multiple Objects Processing

MTL Medial Temporal Lobe

NA Noradrenaline

N2pc N200 Posterior Contralateral

PASA Posterior-Anterior Shift in Aging

PFC Pre-Frontal Cortex

RAVLT Rey Auditory Verbal Learning

RCPM Raven's Colored Progressive Matrices

ROCF Rey-Osterrieth Complex figure

RT Reaction Time

RTG Rotigotine

SC Skin Conductance

SCL Skin Conductance Level; is referred to tonic activity

SCRs Skin Conductance Responses; is referred to phasic activity

STAC Scaffolding Theory of Aging and Cognition

tACS transcranial alternating current stimulation

tDCS Transcranial Direct Current Stimulation

tES Transcranial Electrical Stimulation

TMS Transcranial Magnetic Stimulation

TMT Trail Making test part A and B

tRNS transcranial random noise stimulation

VSTM Visual Short Term Memory

WM Working Memory

References

Albert, M. (1993). Neuropsychological and neurophysiological changes in healthy adult humans across the age range. *Neurobiology of Aging,* 14(6), 623-625.

Ansari, D., Lyons, I. M., van Eimeren, L., & Xu, F. (2007). Linking visual attention and number processing in the brain: the role of the temporo-parietal junction in small and large symbolic and nonsymbolic number comparison. *Journal of Cognitive Neuroscience*, 19(11), 1845–1853.

Aston-Jones, G., & Cohen, J. D. (2005). An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annual Review of Neuroscience, 28*, 403–50.

Aston-Jones, G., Rajkowski, J., & Cohen, J. (1999). Role of locus coeruleus in attention and behavioral flexibility. *Biological Psychiatry, 46*(9), 1309–1320.

Baddeley, A. D., Bressi, S., Della Sala, S., Logie, R., & Spinnler, H. (1991). The Decline of Working Memory in Alzheimer'S Disease. *Brain*, *114*(6), 2521–2542.

Bagherli, J., & Mokhtari, P. (2011). Effects of Arousal and Activation on Simple and Discriminative Reaction Time in a Stimulated Arousal State. *World Applied Sciences Journal*, *12*(10), 1877–1882.

Ballard, J. C. (2001). Assessing attention: comparison of response-inhibition and traditional continuous performance tests. *Journal of Clinical and Experimental Neuropsychology*, *23*(3), 331–350.

Baluch, F., & Itti, L. (2011). Mechanisms of top-down attention. *Trends in Neurosciences*, *34(4), 210-224.*

Barontini, M., Lazzari, J. O., Levin, G., Armando, I., & Basso, S. J. (1997). Age-related changes in sympathetic activity: Biochemical measurements and target organ responses. *Archives of Gerontology and Geriatrics*, *25*(2), 175–186.

Beatty, J. (1982). Phasic Not Tonic Pupillary Responses Vary With Auditory Vigilance Performance. *Psychophysiology*, *19*(2), 167–172.

Becker, S. I., Folk, C. L., & Remington, R. W. (2013). Attentional capture does not depend on feature similarity, but on target-nontarget relations. *Psychological Science*, *24*(5), 634–

47.

Benedek, M., & Kaernbach, C. (2010). A continuous measure of phasic electrodermal activity. *Journal of Neuroscience Methods*, *190*(1), 80–91.

Bernhardt P., Dabbs J., & Riad J. (1996). Pupillometry system for use in social psychology. *Behavior Research Methods*, *28*(1), 61–66.

Berntson, G. G., Cacioppo, J. T., & Quigley, K. S. (1993). Respiratory sinus arrhythmia: Autonomic origins, physiologicalmechanisms, and psychophysiological implications. *Psychophysiology*, *30*, 183–196.

Berridge, C. W., & Waterhouse, B. D. (2003). The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Research Reviews*, *42*(1), 33–84.

Berryhill, M. E., & Jones, K. T. (2012). tDCS selectively improves working memory in older adults with more education. *Neuroscience Letters*, *521*(2), 148–151.

Beuzeron-Mangina, H., & Mangina, C. A. (2009). Excessive compensatory recruitment as a compulsory neurophysiological mechanism in Very Early Alzheimer's Disease as compared to Mild Vascular Dementia and to age-matched normal controls. *International Journal of Psychophysiology*, *73*(2), 164–169.

Bikson, M., Rahman, A., & Datta, A. (2012). Computational models of transcranial direct current stimulation. *Clinical EEG and Neuroscience*, *43*(3), 176–83.

Bindman, L. J., Lippold, O. C. J., & Redfearn, J. W. T. (1964). The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long-lasting after-effects. *The Journal of Physiology*, *172*, 369–382.

Bird, C. M., Chan, D., Hartley, T., Pijnenburg, Y. A., Rossor, M. N., & Burgess, N. (2010). Topographical short-term memory differentiates Alzheimer's disease from frontotemporal lobar degeneration. *Hippocampus*, *20*(10), 1154–1169.

Boggio, P. S., Ferrucci, R., Mameli, F., Martins, D., Martins, O., Vergari, M., Priori, A. (2012). Prolonged visual memory enhancement after direct current stimulation in Alzheimer's disease. *Brain Stimulation*, *5*(3), 223–230.

Boggio, P. S., Khoury, L. P., Martins, D. C. S., Martins, O. E. M. S., de Macedo, E. C., & Fregni, F. (2009). Temporal cortex direct current stimulation enhances performance on a

visual recognition memory task in Alzheimer disease. *Journal of Neurology, Neurosurgery, and Psychiatry*, *80*(4), 444–7.

Bogler, C., Bode, S., & Haynes, J.-D. (2011). Decoding Successive Computational Stages of Saliency Processing. *Current Biology*, *21*(19), 1667–1671.

Bopp, K. L., & Verhaeghen, P. (2005). Aging and verbal memory span: A meta-analysis. *The Journals of Gerontology. Series B, Psychological Sciences and Social Sciences*, *60*, P223–P233.

Boss, G. R., & Seegmiller, J. E. (1981). Age-related physiological changes and their clinical significance. *The Western Journal of Medicine*, *135*(6), 434–40.

Braak, H., Thal, D. R., Ghebremedhin, E., Del Tredici, K., Nelson, P., Braak, H., Lee, V. (2011). Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years. *Journal of Neuropathology and Experimental Neurology*, *70*(11), 960–9.

Bradley, M. M., & Lang, P. J. (1999). International affective digitized sounds (IADS): Stimuli, instruction manual and affective ratings. In *Technical Report B-2*. Gainesville, FL: University of Florida.

Bradley, M. M., Miccoli, L., Escrig, M. a., & Lang, P. J. (2008). The pupil as a measure of emotional arousal and autonomic activation. *Psychophysiology*, *45*(4), 602–607.

Bradshaw, J. (1967). Pupil size as a measure of arousal during information processing. Nature, 216(5114), 515-516

Brandt, J., Aretouli, E., Neijstrom, E., Samek, J., Manning, K., Albert, M. S., & Bandeen-Roche, K. (2009). Selectivity of executive function deficits in mild cognitive impairment. *Neuropsychology*, *23*(5), 607–618.

Braver, T. S., West, R. (2008). Working memory, executive control, and aging. In: Craik, F. I., Salthouse, T., editors. *The handbook of aging and cognition*. 3rd ed. New York, Psychology Press, 311–372.

Brignani, D., Ruzzoli, M., Mauri, P., & Miniussi, C. (2013). Is Transcranial Alternating Current Stimulation Effective in Modulating Brain Oscillations? *PLoS ONE*, *8*(2), e56589.

Brown, S. B. R. E., van Steenbergen, H., Kedar, T., & Nieuwenhuis, S. (2014). Effects of arousal on cognitive control: empirical tests of the conflict-modulated Hebbian-learning

hypothesis. *Frontiers in Human Neuroscience*, *8*, 23.

Buckner, R. L. (2004). Memory and executive function in aging and ad: Multiple factors that cause decline and reserve factors that compensate. *Neuron*, *44*(1), 195–208.

Cabeza, R. (2012). Commentary: Neuroscience frontiers of cognitive aging: Approaches to cognitive neuroscience of aging. *New Frontiers in Cognitive Aging*.

Cabeza, R., Anderson, N. D., Locantore, J. K., & McIntosh, A. R. (2002). Aging Gracefully: Compensatory Brain Activity in High-Performing Older Adults. *NeuroImage*, *17*(3), 1394–1402.

Cappelletti, M., Pikkat, H., Upstill, E., Speekenbrink, M., & Walsh, V. (2015). Learning to integrate versus inhibiting information is modulated by age. *The Journal of Neuroscience*, *35*(5), 2213–25.

Castel, A. D., & Craik, F. I. M. (2003). The effects of aging and divided attention on memory for item and associative information. *Psychology and Aging*, *18*(4), 873–885.

Celone, K. A., Calhoun, V. D., Dickerson, B. C., Atri, A., Chua, E. F., Miller, S. L., Sperling, R. A. (2006). Alterations in memory networks in mild cognitive impairment and Alzheimer's disease: an independent component analysis. *The Journal of Neuroscience*, *26*(40), 10222–31.

Cespón J., Galdo-Álvarez S., Pereiro A. X., Díaz F. (2015). Differences between mild cognitive impairment subtypes as indicated by event-related potential correlates of cognitive and motor processes in a simon task. *Journal of Alzheimer's Disease*, *43*(2), 631–647.

Chhatwal, J., & Sperling, R. (2012). MRI of Mnemonic Networks across the spectrum of Normal Aging, Mild Cognitive Impairment and Alzheimer's disease. *J Alzheimers Dis*, *31*(3), S155–S167.

Clayton, E. C. (2004). Phasic Activation of Monkey Locus Ceruleus Neurons by Simple Decisions in a Forced-Choice Task. *Journal of Neuroscience*, *24*(44), 9914–9920.

Clayton, E. C., Rajkowski, J., Cohen, J. D., & Aston-Jones, G. (2004). Phasic activation of monkey locus ceruleus neurons by simple decisions in a forced-choice task. *The Journal of Neuroscience*, *24*(44), 9914–20.

Cohen, J. E. (2003). Human Population : The Next Half Century, *302*(November), 1172–

1175.

Conners, C. K., Epstein, J. N., Angold, A., & Klaric, J. (2003). Continuous performance test performance in a normative epidemiological sample. *Journal of Abnormal Child Psychology*, *31*(5), 555–62.

Cortoos, A., De Valck, E., & Cluydts, R. (2010). *Conditioned Arousal in Insomnia Patients*. Nova Science Publishers Inc.

Craig-Schapiro, R., Fagan, A. M., & Holtzman, D. M. (2009). Biomarkers of Alzheimer's disease. *Neurobiology of Disease*.

Craik, F. I. M., & Byrd, M. (1982). Aging and cognitive deficits: The role of attentional resources. In Craik, F. I. M., Trehub, S., editors. *Aging and cognitive processes*. New York, NY, Plenum, 191– 211

Crawford, T. J., Higham, S., Mayes, J., Dale, M., Shaunak, S., & Lekwuwa, G. (2013). The role of working memory and attentional disengagement on inhibitory control: Effects of aging and Alzheimer's disease. *Age*, *35*(5), 1637–1650.

Creutzfeldt, O. D., Fromm, G. H., & Hermann, K. (1962). Influence of Transcortical Neuronal d-c Currents Activity on Cortical. *Experimental Neurology*, *5*, 436–452.

Damoiseaux, J. S., Prater, K., Miller, B. L., & Greicius, M. D. (2012). Functional connectivity tracks clinical deterioration in Alzheimer's disease. *Neurobiology of Aging*, *33*(4), 828.e19–828.e30.

Danckert, J., Maruff, P., Crowe, S., & Currie, J. (1998). Inhibitory processes in covert orienting in patients with Alzheimer's disease. *Neuropsychology*, *12*(2), 225–241.

Davis, S. W., Dennis, N. A., Daselaar, S. M., Fleck, M. S., & Cabeza, R. (2008). Que PASA? The posterior-anterior shift in aging. *Cerebral Cortex*, 18, 1201–1209.

Dawson, M. E., Schell, A. M., & Filion, D. L. (2000). The Electrodermal System. In J. Cacioppo, L. Berntson, & G. Tassinary (Eds.), *Handbook of Psychophysiology* (200–223). New York: Cambridge University Press.

Deary, I. J., Corley, J., Gow, A. J., Harris, S. E., Houlihan, L. M., Marioni, R. E., Starr, J. M. (2009). Age-associated cognitive decline. *British Medical Bulletin*, *92*(1), 135–152.

Dehaene, S., & Cohen, L. (1994). Dissociable mechanisms of subitizing and counting: neuropsychological evidence from simultanagnosic patients. *Journal of Experimental*

Psychology. Human Perception and Performance, *20*(5), 958–975.

Den Heijer, T., Geerlings, M. I., Hoebeek, F. E., Hofman, A., Koudstaal, P. J., & Breteler, M. M. B. (2006). Use of hippocampal and amygdalar volumes on magnetic resonance imaging to predict dementia in cognitively intact elderly people. *Archives of General Psychiatry*, *63*(1), 57–62.

Denburg, N. L., Buchanan, T. W., Tranel, D., & Adolphs, R. (2003). Evidence for preserved emotional memory in normal older persons. *Emotion*, *3*(3), 239–253.

Dennis, E. L., & Thompson, P. M. (2014). Functional brain connectivity using fMRI in aging and Alzheimer's disease. *Neuropsychology Review*.

Desimone, R., & Duncan, J. S. (1995). Neural mechanisms of selective visual attention. *Annual Review of Neuroscience*, *18*(1), 193–222.

Devauges, V., & Sara, S. J. (1991). Memory retrieval enhancement by locus coeruleus stimulation: evidence for mediation by beta-receptors. *Behavioural Brain Research*, *43*(1), 93–97.

Dew, I. T. Z., Ritchey, M., LaBar, K. S., & Cabeza, R. (2014). Prior perceptual processing enhances the effect of emotional arousal on the neural correlates of memory retrieval. *Neurobiology of Learning and Memory*, *112*, 104–13.

Drag, L. L., & Bieliauskas, L. A. (2010). Contemporary review 2009: cognitive aging. *Journal of Geriatric Psychiatry and Neurology*, *23*(2), 75–93.

Dringenberg, H. C., Laporte, P. P., & Diavolitsis, P. (2000). Increased effectiveness of tacrine by deprenyl co-treatment in rats: EEG and behavioral evidence. *Neuroreport*, *11*(16), 3513–6.

Duarte, A., Hearons, P., Jiang, Y., Delvin, M. C., Newsome, R. N., & Verhaeghen, P. (2013). Retrospective attention enhances visual working memory in the young but not the old: An ERP study. *Psychophysiology*, *50*(5), 465–476.

Dubois, B., Feldman, H. H., Jacova, C., Cummings, J. L., DeKosky, S. T., Barberger-Gateau, P., Scheltens, P. (2010). Revising the definition of Alzheimer's disease: A new lexicon. *The Lancet Neurology*, *9*(11), 1118–1127.

Duncan, J., Humphreys, G., Ward, R. (1997). Competitive brain activity in visual attention. *Current Opinion in Neurobiology*, *7*(2), 255–261.

Segal M., Edelson, A. (1978). Effects of Priming Stimulation of Catecholamine Containing Nuclei in Rat Brain on Runway Performance. *Brain Research Bulletin*, *3*, 203–206.

Eimer, M. (1996). The N2pc component as an indicator of attentional selectivity. *Electroencephalography and Clinical Neurophysiology*, *99*(3), 225–234.

Einhäuser, W., Stout, J., Koch, C., & Carter, O. (2008). Pupil dilation reflects perceptual selection and predicts subsequent stability in perceptual rivalry. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(5), 1704–9.

Eliason, M. J., & Richman, L. C. (1987). The Continuous Performance Test in learning disabled and nondisabled children. *Journal of Learning Disabilities*, *20*(10), 614–619.

Ester, E. F., Drew, T., Klee, D., Vogel, E. K., & Awh, E. (2012). Neural Measures Reveal a Fixed Item Limit in Subitizing, *The Journal of Neuroscience*. *32*(21), 7169–7177.

Faust, M. E., & Balota, D. A. (1997). Inhibition of return and visuospatial attention in healthy older adults and individuals with dementia of the Alzheimer type. *Neuropsychology*, *11*(1), 13–29.

Ferri C.P., Prince, M.P., Brayne. C., Brodaty, H., Fratiglioni, L., Ganguli, M., Hall, K., Hasegawa, K., Hendrie, H., Huang, Y., Jorm, a., Mathers, C., Menezes, P.R., Rimmer, E., & Scazufca, M. (2005). Global prevalence of dementia: a Delphi consensus study. *The Lancet*, *366*, *2112 - 2117*, *366*(9503), 2112–2117.

Ferrucci, R., Mameli, F., Guidi, I., Mrakic-Sposta, S., Vergari, M., Marceglia, S., Priori, A. (2008). Transcranial direct current stimulation improves recognition memory in Alzheimer disease. *Neurology*, *71*(7), 493–8.

Fertonani, A., Ferrari, C., & Miniussi, C. (2015). What do you feel if I apply transcranial electric stimulation? Safety, sensations and secondary induced effects. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *126*(11), 2181–2188.

Fertonani, A., Pirulli, C., & Miniussi, C. (2011). Random noise stimulation improves neuroplasticity in perceptual learning. *The Journal of Neuroscience*, *31*(43), 15416–23.

Fertonani, A., Rosini, S., Cotelli, M., Rossini, P. M., & Miniussi, C. (2010). Naming facilitation induced by transcranial direct current stimulation. *Behav Brain Res*, *208*(2), 311–318.

Fillit, H. M., Butler, R. N., O'Connell, A. W., Albert, M. S., Birren, J. E., Cotman, C. W., Tully, T. (2002). Achieving and maintaining cognitive vitality with aging. *Mayo Clinic Proceedings. Mayo Clinic*, *77*(7), 681–696.

Finke, K., Myers, N., Bublak, P., & Sorg, C. (2013). A biased competition account of attention and memory in Alzheimer's disease. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *368*(1628), 20130062.

Fjell, A. M., McEvoy, L., Holland, D., Dale, M., & Walhovd, B. (2014). What is normal in normal aging? Effects of aging, amyloid and Alzheimer's disease on the cerebral cortex and the hippocampus. *Progress in Neurobiology*, *117*, 20–40.

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *Journal of Psychiatric Research*, *12*(3), 189–198.

Fowles, D. C., Christie, M. J., Edelberg, R., Grings, W. W., Lykken, D. T., & Venables, P. H. (1981). Publication Recommendations for Electrodermal Measurements. *Psychophysiology*, *18*(3), 232–239.

Franconeri, S. L., Alvarez, G. A., & Enns, J. T. (2007). How many locations can be selected at once? *Journal of Experimental Psychology. Human Perception and Performance*, *33*(5), 1003–1012.

Friston, K. J. (2011). Functional and effective connectivity: a review. *Brain Connect.* 1, 13–36.

Galluzzi, S., Geroldi, C., Ghidoni, R., Paghera, B., Amicucci, G., Bonetti, M., Zanetti, O., Cotelli, M., Gennarelli, M., Frisoni, G.B. (2010). The new Alzheimers criteria in a naturalistic series of patients with mild cognitive impairment. *Journal of Neurology*, 257 (12), 2004–2014.

Gavazzeni, J., Wiens, S., Fischer, H. (2008). Age effects to negative arousal differ for self-report and electrodermal activity. *Psychophysiology*, 45, 148–151.

Gazzaley, A., Clapp, W., Kelley, J., McEvoy, K., Knight, R.T., D'Esposito, M. (2008). Age related top-down suppression deficit in the early stages of cortical visual memory processing. *Proc. Natl. Acad. Sci. U. S. A.* 105, 13122-13126

Gazzaley, A., Cooney, J. W., Rissman, J., & D'Esposito, M. (2005). Top-down suppression

deficit underlies working memory impairment in normal aging. *Nature Neuroscience*, *8*(10), 1298–1300.

Geerligs, L., Saliasi, E., Maurits, N. M., Renken, R. J., & Lorist, M. M. (2014). Brain mechanisms underlying the effects of aging on different aspects of selective attention. *NeuroImage*, *91*, 52–62.

Gellatly, I. R., & Meyer, J. P. (1992). The effects of goal difficulty on physiological arousal, cognition, and task performance. *The Journal of Applied Psychology*, *77*(5), 694–704.

German, D. C., Walker, B. S., Manaye, K., Smith, W. K., Woodward, D. J., & North, A. J. (1988). The human locus coeruleus: computer reconstruction of cellular distribution. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *8*(5), 1776–1788.

Gilzenrat, M. S., Cohen, J. D., Rajkowski, J., & Aston-jones, G. (2003). Pupil dynamics predict changes in task engagement mediated by locus coeruleus. *Society of Neuroscience Abstracts*, (515.19).

Gilzenrat, M. S., Nieuwenhuis, S., Jepma, M., & Cohen, J. D. (2010). Pupil diameter tracks changes in control state predicted by the adaptive gain theory of locus coeruleus function. *Cognitive, Affective & Behavioral Neuroscience*, *10*(2), 252–69.

Glisky, E. L. (2007). Changes in Cognitive Function in Human Aging. In: Riddle, D. R., editor. *Brain Aging: Models, Methods, and Mechanisms*, Boca Raton (FL), CRC Press/Taylor & Francis. Chapter 1.

Grady, C. (2012). The cognitive neuroscience of ageing. *Nature Reviews. Neuroscience*, *13*(7), 491–505.

Grady, C. L. (2008). Cognitive neuroscience of aging. *Annals of the New York Academy of Sciences*.

Grady, C. L., & Craik, F. I. (2000). Changes in memory processing with age. *Current Opinion in Neurobiology*, *10*(2), 224–231.

Gross, J. J., & Levenson, R. W. (1993). Emotional suppression: physiology, self-report, and expressive behavior. *Journal of Personality and Social Psychology*, *64*(6), 970–986.

Hackley, S. a. (2009). The speeding of voluntary reaction by a warning signal. *Psychophysiology*, *46*(2), 225–33.

Harada, C. N., Love, M. C. N., & Triebel, K. (2013). NIH Public Access. *Clin Geriatr Med.*, *29*(4), 737–752.

Harvey, M., & Kerkhoff, G. (2015). Effects of non-invasive brain stimulation on attention: Current debates, cognitive studies and novel clinical applications. *Neuropsychologia*, *74*, 1–6.

Hasher, L., & Zacks, R. T. (1988). Working memory, comprehension, and aging: A review and a new view. In G. H. Bower (Ed.), The psychology of learning and motivation (Vol. 22, pp. 193-225). New York: Academic Press

Hebert, L. E., Weuve, J., Scherr, P. A., & Evans, D. A. (2013). Alzheimer disease in the United States (2010-2050) estimated using the 2010 census. *Neurology*, *80*(19), 1778–1783.

Hedden, T., & Gabrieli, J. D. E. (2004). Insights into the ageing mind: a view from cognitive neuroscience. *Nature Reviews. Neuroscience*, *5*(2), 87–96.

Herrmann, C. S., Rach, S., Neuling, T., & Strüber, D. (2013). Transcranial alternating current stimulation: a review of the underlying mechanisms and modulation of cognitive processes. *Frontiers in Human Neuroscience*, *7*, 279.

Himeno E., Ohyagi Y., Ma L., Nakamura N., Miyoshi K., Sakae N., et al. . (2011). Apomorphine treatment in Alzheimer mice promoting amyloid-beta degradation. Ann. Neurol. 69, 248–256.

Itti, L., & Koch, C. (2000). A saliency-based search mechanism for overt and covert shifts of visual attention. *Vision Research*, *40*(10–12), 1489–1506.

Jagust, W., Reed, B., Mungas, D., Ellis, W., & DeCarli, C. (2007). What does fluorodeoxyglucose PET imaging add to a clinical diagnosis of dementia? *Neurology*, *69*(9), 871–877.

Jeong, E. J., & Biocca, F. A. (2012). Are there optimal levels of arousal to memory? Effects of arousal, centrality, and familiarity on brand memory in video games. *Computers in Human Behavior*, *28*(2), 285–291.

Jonides, J., Marshuetz, C., Smith, E. E., Reuter-lorenz, P. A., & Koeppe, R. A. (1998). Age Differences in Behavior and PET Activation Reveal Differences in Interference Resolution in Verbal Working Memory. *Journal of Cognitive Neuroscience*, 188–196.

Joo, S. H., Lim, H. K., & Lee, C. U. (2016). Three Large-Scale Functional Brain Networks from Resting-State Functional MRI in Subjects with Different Levels of Cognitive Impairment. *Psychiatry Investigation*, *13*(1), 1.

Jost, K., Bryck, R. L., Vogel, E. K., & Mayr, U. (2011). Are old adults just like low working memory young adults? Filtering efficiency and age differences in visual working memory. *Cerebral Cortex*, *21*(5), 1147–1154.

Kahneman, D., Beatty, J. (1966). Pupil diameter and load on memory. *Science*, 154(3756):1583–1585.

Kaufman, E., Lord, M., Reese, T., & Volkmann, J. (1949). The discrimination of visual number. *The American Journal of Psychology*, *62*(4), 498–525.

Kim, M., Beversdorf, D. Q., Heilman, K. M. (2000). Arousal response with aging: pupillographic study. *Journal of International Neuropsychological Society*, 6, 348–50.

Kisley, M. A., Wood, S., & Burrows, C. L. (2007). Looking at the sunny side of life: Age-related change in an event-related potential measure of the negativity bias. *Psychological Science*, *18*(9), 838–843.

Koch, G., Di Lorenzo, F., Bonnì, S., Giacobbe, V., Bozzali, M., Caltagirone, C., & Martorana, A. (2014). Dopaminergic modulation of cortical plasticity in Alzheimer's disease patients. *Neuropsychopharmacology*, *39*(11), 2654–61.

Kukolja, J., Thiel, C. M., Wolf, O. T., & Fink, G. R. (2008). Increased cortisol levels in cognitively challenging situations are beneficial in young but not older subjects. *Psychopharmacology*, *201*(2), 293–304.

Kunzmann, U., & Gruhn, D. (2005) Age differences in emotional reactivity: the sample case of sadness. *Psychology of Aging*, 20, 47–59

Laakso, I., & Hirata, A. (2013). Computational analysis shows why transcranial alternating current stimulation induces retinal phosphenes. *Journal of Neural Engineering*, *10*(4), 46009.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (2008). *International affective picture system (IAPS): Affective ratings of pictures and instruction manual*. *Technical Report A-8*. Gainesville, FL: University of Florida.

Lautenschlager, N. T., Anstey, K. J., & Kurz, A. F. (2014). Non-pharmacological strategies

to delay cognitive decline. *Maturitas*, *79*(2), 170–173.

Lee, T.-H., Greening, S. G., & Mather, M. (2015). Encoding of goal-relevant stimuli is strengthened by emotional arousal in memory. *Frontiers in Psychology*, *6*, 1–7.

Lehmann, M., Madison, C., Ghosh, P. M., Miller, Z. A., Greicius, M. D., Kramer, J. H., Rabinovici, G. D. (2015). Loss of functional connectivity is greater outside the default mode network in nonfamilial early-onset Alzheimer's disease variants. *Neurobiology of Aging*, *36*(10), 2678–2686.

Levenson, R. W. (1997). Expressive, physiological, and subjective changes in emotion across adulthood. In Quails, S. H. & Abeles, N. editors. *Psychology and the aging revolution: how to adapt to long life.* Washington, DC, American Psychological Association, 123-140

Levinson, D. F. & Edelberg, R. (1985). Scoring criteria for response latency and habituation in electrodermal research: a critique. *Psychophysiology,* 22, 417-426

Levine, B., Schweizer, T. A., O'Connor, C., Turner, G., Gillingham, S., Stuss, D. T., Robertson, I. H. (2011). Rehabilitation of executive functioning in patients with frontal lobe brain damage with goal management training. *Frontiers in Human Neuroscience*, *5*, 9.

Liebetanz, D., Nitsche, M. A., Tergau, F., & Paulus, W. (2002). Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. *Brain*, *125*, 2238–2247.

Lim, E. P., Tan, C. H., Jay, T. M., & Dawe, G. S. (2010). Locus coeruleus stimulation and noradrenergic modulation of hippocampo-prefrontal cortex long-term potentiation. *The International Journal of Neuropsychopharmacology*, *13*(9), 1219–31.

Lindenberger, U., & Baltes, P. B. (1994). Sensory functioning and intelligence in old age: a strong connection. *Psychology and Aging*.

Lindenberger, U., Scherer, H., & Baltes, P. B. (2001). The strong connection between sensory and cognitive performance in old age: Not due to sensory acuity reductions operating during cognitive assessment. *Psychology and Aging*, *16*(2), 196–205.

Luck, S. J., & Hillyard, S. A. (1994). Electrophysiological correlates of feature analysis during visual search. *Psychophysiology*, *31*(3), 291–308.

Lustig, C., Hasher, L., & Tonev, S. T. (2001). Inhibitory control over the present and the

past. *European Journal of Cognitive Psychology*, *13*(1–2), 107–122.

MacLeod, C. M. (1992). The Stroop task: The "gold standard" of attentional measures. *Journal of Experimental Psychology: General*.

Madden, D. J. (2007). Aging and Visual Attention. *Current Direction in Psychological Science*, *16*(2), 70–74.

Madden, D. J., Bennett, I. J., & Song, A. W. (2009). Cerebral white matter integrity and cognitive aging: Contributions from diffusion tensor imaging. *Neuropsychology Review*.

Manly, T., Hawkins, K., Evans, J., Woldt, K., & Robertson, I. (2002). Rehabilitation of executive function: Facilitation of effective goal management on complex tasks using periodic auditory alerts. *Neuropsychologia*, *40*, 271–281.

Martin, I., & Venables, H. (1980). Techniques in Psychophysiology. *Psychological Medicine*, *10*.

Martorana, A., & Koch, G. (2014). Is dopamine involved in Alzheimer's disease? *Frontiers in Aging Neuroscience*, *6*.

Mather, M., & Harley, C. W. (2016). The Locus Coeruleus : Essential for Maintaining Cognitive Function and the Aging Brain. *Trends in Cognitive Sciences*, *20*(3), 214–226.

Mather, M., & Sutherland, M. R. (2011). Arousal-biased competition in perception and memory. *Perspect Psychological Science*, *6*(2), 114–133.

Mattay, V. S., Fera, F., Tessitore, A., Hariri, A. R., Das, S., Callicott, J. H., & Weinberger, D. R. (2002). Neurophysiological correlates of age-related changes in human motor function. *Neurology*, *58*(4), 630–5.

Mauri, P., Miniussi, C., Balconi, M., & Brignani, D. (2015). Bursts of transcranial electrical stimulation increase arousal in a continuous performance test. *Neuropsychologia*, *74*, 127–136.

Maylor, E. A., Sheehan, B., Watson, D. G., & Henderson, E. L. (2008). Enumeration in Alzheimer's disease and other late life psychiatric syndromes. *Neuropsychologia*, *46*(11), 2696–2708.

Maylor, E. A., Watson, D. G., & Muller, Z. (2005). Effects of Alzheimer's Disease on Visual Enumeration. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences*, *60*(3), 129–P135.

Mazza, V., & Brignani, D. (2016). Electrophysiological advances on multiple object processing in aging. *Frontiers in Aging Neuroscience*, *8*.

Mazza, V., & Caramazza, A. (2012). Perceptual Grouping and Visual Enumeration. *PLoS ONE*, *7*(11).

Mazza, V., & Caramazza, A. (2015). Multiple object individuation and subitizing in enumeration: a view from electrophysiology. *Frontiers in Human Neuroscience*, *9*, 1–7.

McDonnell, M. D., & Abbott, D. (2009). What is stochastic resonance? Definitions, misconceptions, debates, and its relevance to biology. *PLoS Computational Biology*, *5*(5), e1000348.

McDowd, J. M. (1997). Inhibition in Attention and Aging. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences*, *52B*(6), P265–P273.

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., & Stadlan, E. M. (1984). Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*, *34*(7), 939–944.

Mickley Steinmetz, K. R., Schmidt, K., Zucker, H. R., & Kensinger, E. A. (2012). The effect of emotional arousal and retention delay on subsequent-memory effects. *Cogn Neurosci*, *3*(3–4), 150–159.

Miniussi, C., Harris, J. A., & Ruzzoli, M. (2013). Modelling non-invasive brain stimulation in cognitive neuroscience. *Neuroscience and Biobehavioral Reviews*, *37*(8), 1702–1712.

Moretti, D. V. (2016). Editorial: Neurophysiology in Alzheimer's Disease and Dementia. *Frontiers in Aging Neuroscience*, *8*, 1–2.

Moretti, D. V., Paternicò, D., Binetti, G., Zanetti, O., & Frisoni, G. B. (2013). EEG upper/low alpha frequency power ratio relates to temporo-parietal brain atrophy and memory performances in mild cognitive impairment. *Frontiers in Aging Neuroscience*, *5*.

Moretti, D. V., Prestia, A., Fracassi, C., Binetti, G., Zanetti, O., & Frisoni, G. B. (2012). Specific EEG changes associated with atrophy of hippocampus in subjects with mild cognitive impairment and Alzheimer's disease. *International Journal of Alzheimer's Disease*.

Morris, J. C. (1997). Clinical dementia rating: a reliable and valid diagnostic and staging

measure for dementia of the Alzheimer type. *International Psychogeriatrics / IPA, 9 Suppl 1*, 173–178.

Munneke, J., Fait, E., & Mazza, V. (2013). Attentional processing of multiple targets and distractors. *Psychophysiology*, *50*(11), 1104–1108.

Murphy, P. R., Robertson, I. H., Balsters, J. H., & O'connell, R. G. (2011). Pupillometry and P3 index the locus coeruleus-noradrenergic arousal function in humans. *Psychophysiology*, *48*(11), 1532–43.

Nashiro, K., & Mather, M. (2011a). Effects of Emotional Arousal on Memory Binding in Normal Aging and Alzheimer's Disease. *The American Journal of Psychology*, *124*(3), 301–312.

Nashiro, K., & Mather, M. (2011b). How arousal affects younger and older adults' memory binding. *Experimental Aging Research*, *37*(1), 108–128.

Nebes, R. D., Brady, C. B., & Reynolds, C. F. 3rd. (1992). Cognitive slowing in Alzheimer's disease and geriatric depression. *Journal of Gerontology*, *47*(5), P331-6.

Neiss, M. B., Leigland, L. A., Carlson, N. E., & Janowsky, J. S. (2009). Age differences in perception and awareness of emotion. *Neurobiology of Aging*, *30*(8), 1305–1313.

Newsome, R. N., Pun, C., Smith, V. M., Ferber, S., & Barense, M. D. (2013). Neural correlates of cognitive decline in older adults at-risk for developing MCI: Evidence from the CDA and P300. *Cognitive Neuroscience*, *4*(3), 152–162.

Nieuwenhuis, S., Aston-Jones, G., & Cohen, J. D. (2005). Decision making, the P3, and the locus coeruleus-norepinephrine system. *Psychological Bulletin*, *131*(4), 510–32.

Nieuwenhuis, S., De Geus, E. J., & Aston-Jones, G. (2011). The anatomical and functional relationship between the P3 and autonomic components of the orienting response. *Psychophysiology*, *48*, 162–175.

Nigam, Y., Knight, J., Bhattacharya, S., & Bayer, A. (2012). Physiological changes associated with aging and immobility. *Journal of Aging Research*, *2012*(ii), 2012–2014.

Nitsche, M. A., Cohen, L. G., Wassermann, E. M., Priori, A., Lang, N., Antal, A., Pascual-Leone, A. (2008). Transcranial direct current stimulation: State of the art 2008. *Brain Stimulation*.

Nitsche, M. A., Fricke, K., Henschke, U., Schlitterlau, A., Liebetanz, D., Lang, N., Paulus,

W. (2003). Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. *The Journal of Physiology*, *533.1*, 293–301.

Nitsche, M. A., & Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *The Journal of Physiology*, *527 Pt 3*, 633–9.

Nitsche, M. A., & Paulus, W. (2001). Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology*, *57*(10), 1899–901.

O'Connell, R. G., Bellgrove, M. a, Dockree, P. M., & Robertson, I. H. (2006). Cognitive remediation in ADHD: effects of periodic non-contingent alerts on sustained attention to response. *Neuropsychological Rehabilitation*, *16*(6), 653–65.

Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia*.

Pagano, S., Fait, E., Brignani, D., & Mazza, V. (2016). Object individuation and compensation in healthy aging. *Neurobiology of Aging*, *40*, 145–154.

Pagano, S., Fait, E., Monti, A., Brignani, D., & Mazza, V. (2015). Electrophysiological Correlates of Subitizing in Healthy Aging. *Plos One*, *10*(6), e0131063.

Pagano, S., Lombardi, L., & Mazza, V. (2014). Brain dynamics of attention and working memory engagement in subitizing. *Brain Research*, *1543*, 244–252.

Pagano, S., & Mazza, V. (2012). Individuation of multiple targets during visual enumeration: New insights from electrophysiology. *Neuropsychologia*, *50*(5), 754–761.

Park, D. C., Lautenschlager, G., Hedden, T., Davidson, N. S., Smith, A. D., & Smith, P. K. (2002). Models of visuospatial and verbal memory across the adult life span. *Psychology and Aging*, 17(2), 299–320

Pelli, D. G., & Robson, J. G. (1988). The Design of a New Letter Chart for Measuring Contrast Sensitivity. *Clinical Vision Science*, *2*(3), 187–199.

Petersen, R. C. (2004). Mild cognitive impairment as a clinical entity and treatment target. *Archives of Neurology*, *62*(7), 1160–1163; discussion 1167.

Petersen, R. C. (2004). Mild cognitive impairment as a diagnostic entity. In *Journal of Internal Medicine* (Vol. 256, pp. 183–194).

Petersen, R. C., Caracciolo, B., Brayne, C., Gauthier, S., Jelic, V., & Fratiglioni, L. (2014). Mild cognitive impairment: A concept in evolution. *Journal of Internal Medicine*, *275*(3), 214–228.

Polanía, R., Nitsche, M. A., Korman, C., Batsikadze, G., & Paulus, W. (2012). The importance of timing in segregated theta phase-coupling for cognitive performance. *Current Biology : 22*(14), 1314–8.

Price, J. L., McKeel, D. W., & Morris, J. C. (2001). Synaptic loss and pathological change in older adults - Aging versus disease? *Neurobiology of Aging*, *22*(3), 351–354.

Priori, A., Berardelli, A., Rona, S., Accornero, N., & Manfredi, M. (1998). Polarization of the human motor cortex through the scalp. *Neuroreport*, *9*(10), 2257–60.

Prvulovic, D., Van De Ven, V., Sack, A. T., Maurer, K., & Linden, D. E. J. (2005). Functional activation imaging in aging and dementia. *Psychiatry Research - Neuroimaging*, *140*(2), 97–113.

Pylyshyn, Z. W. (2001). Visual indexes, preconceptual objects, and situated vision. *Cognition*, *80*, 127–158.

Rajkowski, J., Kubiak, P., & Aston-Jones, G. (1993). Correlations between locus coeruleus (LC) neural activity, pupil diameter and behavior in monkey support a role of LC in attention. *Society for Neuroscience Abstracts*, *19:974*.

Raz, N., & Rodrigue, K. M. (2006). Differential aging of the brain: Patterns, cognitive correlates and modifiers. *Neuroscience and Biobehavioral Reviews*, *30*(6), 730–748.

Resnick, S. M., Pham, D. L., Kraut, M. A., Zonderman, A. B., & Davatzikos, C. (2003). Longitudinal Magnetic Resonance Imaging Studies of Older Adults: A Shrinking Brain. *J. Neurosci.*, *23*(8), 3295–3301.

Reuter-Lorenz, P. A., & Cappell, K. A. (2008). Neurocognitive aging and compensation hypothesis. *Current Directions in Psychological Science*, *17*, 177–182.

Reuter-Lorenz, P. a, & Park, D. C. (2010). Human Neuroscience and the Aging Mind: at Old Problems A New Look. *Journals of Gerontology Series B-Psychological Sciences and Social Sciences*, *65*(4), 405–415.

Riccio, C. A., Reynolds, C. R., Lowe, P., & Moore, J. J. (2002). The continuous performance test: A window on the neural substrates for attention? *Archives of Clinical*

Neuropsychology, *17*(3), 235–272.

Richer, F., & Beatty, J. (1987). Contrasting Effects of Response Uncertainty on the Task-Evoked Pupillary Response and Reaction Time. *Psychophysiology*, *24*(3), 258–262.

Rossor, M. N., Fox, N. C., Freeborough, P. A., & Harvey, R. J. (1996). Clinical Features of Sporadic and Familial Alzheimer's Disease. *Neurodegeneration*, *5*(4), 393–397.

Roy, L. B., Sparing, R., Fink, G. R., & Hesse, M. D. (2014). Modulation of attention functions by anodal tDCS on right PPC. *Neuropsychologia*, *74*, 96–107.

Sadleir, R. J., Vannorsdall, T. D., Schretlen, D. J., & Gordon, B. (2010). Transcranial direct current stimulation (tDCS) in a realistic head model. *NeuroImage*, *51*(4), 1310–8.

Sala-Llonch, R., Bartrés-Faz, D., & Junqué, C. (2015). Reorganization of brain networks in aging: a review of functional connectivity studies. *Frontiers in Psychology*, *6*, 663.

Salthouse, T. A. (2012). Consequences of age-related cognitive declines. *Annual Review of Psychology*, *63*, 201–26.

Salthouse, T. A. (1996). The processing-speed theory of adult age differences in cognition. *Psychological Review*, *103*(3), 403–428.

Sander, M. C., Werkle-Bergner, M., & Lindenberger, U. (2011). Contralateral delay activity reveals life-span age differences in top-down modulation of working memory contents. *Cerebral Cortex*, *21*(12), 2809–2819.

Sara, S. J. (2009). The locus coeruleus and noradrenergic modulation of cognition. *Nature Reviews Neuroscience*, *10*(3), 211–23.

Sara, S. J., & Devauges, V. (1988). Priming stimulation of locus coeruleus facilitates memory retrieval in the rat. *Brain Research*, *438*(1–2), 299–303.

Sara, S. J. J., & Bouret, S. (2012). Orienting and Reorienting: The Locus Coeruleus Mediates Cognition through Arousal. *Neuron*, *76*(1), 130–141.

Saunders, N. L. J., & Summers, M. J. (2010). Attention and working memory deficits in mild cognitive impairment. *Journal of Clinical and Experimental Neuropsychology*, *32*(4), 350–357.

Saunders, N. L. J., & Summers, M. J. (2011). Longitudinal deficits to attention, executive, and working memory in subtypes of mild cognitive impairment. *Neuropsychology*, *25*(2), 237–248.

Scheller, E., Minkova, L., Leitner, M., & Kloppel, S. (2014). Attempted and successful compensation in preclinical and early manifest neurodegeneration - a review of task fMRI studies. *Frontiers in Psychiatry*, *5*, 1–16.

Scheltens, P., Blennow, K., Breteler, M. M. B., de Strooper, B., Frisoni, G. B., Salloway, S., & Van der Flier, W. M. (2016). Alzheimer's disease. *The Lancet*, *6736*(15), 1–13.

Schneider-Garces, N. J., Gordon, B. A., Brumback-Peltz, C. R., Shin, E., Lee, Y., Sutton, B. P., Fabiani, M. (2010). Span, CRUNCH, and beyond: working memory capacity and the aging brain. *Journal of Cognitive Neuroscience*, *22*(4), 655–69.

Schneider, B. A., & Pichora-Fuller, M. K. (2000). Implications of perceptual deterioration for cognitive aging research. In *The handbook of aging and cognition* (pp. 155–219).

Schock, L., Schwenzer, M., Sturm, W., & Mathiak, K. (2011). Alertness and visuospatial attention in clinical depression. *BMC Psychiatry*, *11*(1), 78.

Sergeeva, E. G. (2011). Brain-state-dependent non-invasive brain stimulation and functional priming: a hypothesis. *Restorative Neurology and Neuroscience*, *29*(6), 463–492.

Serrano-Pozo, A., Frosch, M. P., Masliah, E., & Hyman, B. T. (2011). Neuropathological alterations in Alzheimer disease. *Cold Spring Harbor Perspectives in Medicine*.

Sharot, T., & Phelps, E. A. (2004). How arousal modulates memory: disentangling the effects of attention and retention. *Cognitive, Affective & Behavioral Neuroscience*, *4*(3), 294–306.

Simons, R. F. (1988). Event-related slow brain potentials: A perspective from ANS psychophysiology. *Advances in Psychophysiology*, *1*, 223–267.

Sliwinski, M., & Buschke, H. (1997). Processing speed and memory in aging and dementia. *The Journals of Gerontology. Series B, Psychological Sciences and Social Sciences*, *52*(6), 308-18.

Soldan, A., Gazes, E., Hilton, H. J., & Stern, Y. (2008). Aging does not affect brain patterns of repetition effects associated with perceptual priming of novel objects associated with perceptual priming of novel objects, *20*(10), 1762–1776.

Spielberger, C. D., Gorsuch, R. L., Lushene, R., Vagg, P. R., & Jacobs, G. A. (1983). Manual for the State-Trait Anxiety Inventory. *Consulting Psychologists Press.*

Stern, Y. (2009). Cognitive reserve. *Neuropsychologia*, *47*(10), 2015–2028.

Störmer, V. S., Li, S. C., Heekeren, H. R., & Lindenberger, U. (2013). Normative shifts of cortical mechanisms of encoding contribute to adult age differences in visual-spatial working memory. *NeuroImage*, *73*, 167–175.

Summers, J. J., Kang, N., & Cauraugh, J. H. (2015). Does Transcranial Direct Current Stimulation Enhance Cognitive and Motor Functions in the Ageing Brain? A Systematic Review and Meta-Analysis. *Ageing Research Reviews*, *25*, 42–54.

Supekar, K., Menon, V., Rubin, D., Musen, M., & Greicius, M. D. (2008). Network analysis of intrinsic functional brain connectivity in Alzheimer's disease. *PLoS Computational Biology*, *4*(6).

Sutherland, M. R., & Mather, M. (2015). Negative arousal increases the effects of stimulus salience in older adults. *Experimental Aging Research*, 41(3), 259- 271.

Sutherland, M. R., & Mather, M. (2012). Negative arousal amplifies the effects of saliency in short-term memory. *Emotion*, *12*(6), 1367–72.

Terney, D., Chaieb, L., Moliadze, V., Antal, A., & Paulus, W. (2008). Increasing human brain excitability by transcranial high-frequency random noise stimulation. *The Journal of Neuroscience*, *28*(52), 14147–55.

Thut, G., Miniussi, C., & Gross, J. (2012). The functional importance of rhythmic activity in the brain. *Current Biology : 22*(16), R658-63.

Toner, C. K., Reese, B. E., Neargarder, S., Riedel, T. M., Gilmore, G. C., & Cronin-Golomb, A. (2012). Vision-fair neuropsychological assessment in normal aging, Parkinson's disease and Alzheimer's disease. *Psychology and Aging*, *27*(3), 785–90.

Treue, S. (2003). Visual attention: the where, what, how and why of saliency. *Current Opinion in Neurobiology*, *13*(4), 428–432.

Trick, L. M., & Pylyshyn, Z. W. (1993). What enumeration studies can show us about spatial attention: evidence for limited capacity preattentive processing. *Journal of Experimental Psychology. Human Perception and Performance*, *19*(2), 331–351.

Tuholski, S. W., Engle, R. W., & Baylis, G. C. (2001). Individual differences in working memory capacity and enumeration. *Memory & Cognition*, *29*(3), 484–492.

VaezMousavi, S. M., Barry, R. J., & Clarke, A. R. (2009). Individual differences in task-related activation and performance. *Physiology & Behavior*, *98*(3), 326–30.

VaezMousavi, S. M., Barry, R. J., Rushby, J. a, & Clarke, A. R. (2007). Arousal and activation effects on physiological and behavioral responding during a continuous performance task. *Acta Neurobiologiae Experimentalis*, *67*(4), 461–70.

Valentijn, S. A. M., Van Boxtel, M. P. J., Van Hooren, S. A. H., Bosma, H., Beckers, H. J. M., Ponds, R. W. H. M., & Jolles, J. (2005). Change in sensory functioning predicts change in cognitive functioning: Results from a 6-year follow-up in the Maastricht Aging Study. *Journal of the American Geriatrics Society*, *53*(3), 374–380.

Verhaeghen, P., & Cerella, J. (2002). Aging, executive control, and attention: a review of meta-analyses. *Neurosci Biobehav Rev*, *26*, 849–857.

Vetter, P., Butterworth, B., & Bahrami, B. (2011). A candidate for the attentional bottleneck: set-size specific modulation of the right TPJ during attentive enumeration. *Journal of Cognitive Neuroscience*, *23*(3), 728–736.

Vogel, E. K., & Machizawa, M. G. (2004). Neural activity predicts individual differences in visual working memory capacity. *Nature*, *428*, 748–51.

Vuokko, E., Niemivirta, M., & Helenius, P. (2013). Cortical activation patterns during subitizing and counting. *Brain Research*, *1497*, 40–52.

Wagner, S., Rampersad, S. M., Aydin, Ü., Vorwerk, J., Oostendorp, T. F., Neuling, T., Wolters, C. H. (2014). Investigation of tDCS volume conduction effects in a highly realistic head model. *Journal of Neural Engineering*, *11*(1), 16002.

Watson, D. G., & Humphreys, G. W. (1999). The magic number four and temporo-parietal damage: neurological impairments in counting targets amongst distractors. *Cognitive Neuropsychology*, *16*(7), 609–629.

Watson, D. G., Maylor, E. A., & Manson, N. (2002). Aging and enumeration: a selective deficit for the subitization of targets among distractors. *Psychological Aging*, *17*, 496–504.

Watson, D. G., Maylor, E. a, & Bruce, L. a M. (2007). The role of eye movements in subitizing and counting. *Journal of Experimental Psychology. Human Perception and Performance*, *33*(6), 1389–1399.

Weintraub, S., Wicklund, A. H., & Salmon, D. P. (2012). The neuropsychological profile of Alzheimer disease. *Cold Spring Harbor Perspectives in Medicine*, *2*(4), 1–18.

Wiegand, I., Töllner, T., Habekost, T., Dyrholm, M., Müller, H. J., & Finke, K. (2014a). Distinct neural markers of TVA-based visual processing speed and short-term storage capacity parameters. *Cerebral Cortex, 24*(8), 1967–1978.

Wiegand, I., Töllner, T., Dyrholm, M., Müller, H. J., Bundesen, C., & Finke, K. (2014b). Neural correlates of age-related decline and compensation in visual attention capacity. *Neurobiology of Aging*, *35*(9), 2161–2173.

Winn, B., Whitaker, D., Elliott, D. B., Phillips, N. J. (1994). Factors affecting light-adapted pupil size in normal human subjects. *Invest. Ophthalmol. Vis. Sci,* 35, 1132–1137

Woods, A. J., Antal, A., Bikson, M., Boggio, P. S., Brunoni, A. R., Celnik, P., Nitsche, M. A. (2015). A technical guide to tDCS, and related non-invasive brain stimulation tools. *Clinical Neurophysiology*.

Wurm L. H., Labouvie-Vief G., Aycock J., Rebucal K. A., Koch H. E. (2004). Performance in auditory and visual emotional stroop task: A comparison of older and younger adults. *Psychology and Aging,* 19, 523–535

Wutz, A., & Melcher, D. (2014). The temporal window of individuation limits visual capacity. *Frontiers in Psychology*, *5*, 1–13.

Xu, Y., & Chun, M. M. (2009). Selecting and perceiving multiple visual objects. *Trends in Cognitive Sciences*, *13*(4), 167–174.

Yerkes, R. M., Dodson, J.D. (1908). The relation of strength of stimulus to rapidity of habit-formation. *Journal of Comparative Neurology and Psychology,* 18, 459–482.

Zanto, T.P., Toy, B., Gazzaley, A., (2010). Delays in neural processing during working memory encoding in normal aging. *Neuropsychologia,* 48, 13-25.

Zeigler, B. L., Graham, F. K., & Hackley, S. A. (2001). Cross-modal warning effects on reflexive and voluntary reactions. *Psychophysiology*, *38*(6), 903–11.

Zimerman, M., Nitsch, M., Giraux, P., Gerloff, C., Cohen, L. G., & Hummel, F. C. (2013). Neuroenhancement of the aging brain: Restoring skill acquisition in old subjects. *Annals of Neurology*, *73*(1), 10–15.