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Age-Related Changes in Sleep-Dependent Consolidation of Visuo-Spatial Memory

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AGE-RELATED CHANGES IN SLEEP-DEPENDENT CONSOLIDATION OF VISUO-SPATIAL MEMORY

A Thesis Presented

by

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ABSTRACT

AGE-RELATED CHANGES IN SLEEP-DEPENDENT CONSOLIDATION OF VISUO-SPATIAL MEMORY

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Healthy aging is associated with a reduction in slow-wave sleep (SWS), crucial for declarative memory consolidation in young adults; consequently, previously observed benefits of sleep on declarative learning in older adults could reflect a passive role of sleep in protecting memories from waking interference, rather than an active, stabilizing effect. To dissociate the passive and active roles of sleep, a visuo-spatial task was administered; memory was probed after a 12 hr interval consisting of either daytime wake or overnight sleep and post-wake/post-sleep stability of the memories was tested following task-related interference. Ninety five older adults (mean=65.43 yrs; SD=7.6 yrs) and 137 young adults (mean= 21.22yrs; SD=2.62 yrs) were tested across either an "Interference" or a "No Interference" condition (without exposure to the interference). In both young and older adults, sleep significantly benefitted performance compared to wake, such that the memories were more resistant to subsequent interference. For young adults, post-sleep performance was correlated with time spent in SWS and delta power density during SWS early in the night. Additionally, the interaction between NREM and REM early in the night played an important role in stabilizing the memories. There were no significant correlations between sleep parameters and over-sleep performance changes in older adults;

however, high performing older adults benefitted from greater amounts of REM sleep early in the night, and from the interaction between NREM and REM during this time period. These results suggest that the active role of sleep in declarative memory consolidation persists in an aging population.

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CHAPTER I

INTRODUCTION

Sleep is a period of physical inactivity and a state of mental quiescence, serving as a physiological state that is crucial for rest, healing and growth. Over the years however, animal and human researchers have gathered evidence to show that the function of sleep is more complex, and is extended to learning and memory (Spencer, 2013). For instance, studies in rodents have shown that sleep deprivation is detrimental to performance on tasks such as the Morris Water Maze task and in contextual fear conditioning (Dorokhov et al., 2011; Yang et al., 2011; Hagewoud et al., 2011). Similarly in humans, sleep deprivation has been linked to diminished motor skills and learning abilities across a range of cognitive tasks (Holland, 1968; Kilgore, 2010). The observation that sleep is beneficial to *memory* in humans came from the seminal work by Jenkins and Dallenbach in 1924, which demonstrated less forgetting over a period of sleep compared to wake. However, it was not until decades later that researchers began systematically studying the role of sleep in memory consolidation across the various memory domains by means of behavioral, molecular, electrophysiological and neuroimaging techniques.

Memory consolidation refers to the process by which newly acquired memories are strengthened, stabilized and committed to long-term neocortical stores (Walker and Stickgold, 2006). Although these steps in memory processing occur while awake, it has increasingly been demonstrated that memory consolidation occurs maximally over sleep. Most of the work related to sleep-dependent memory consolidation in humans has been conducted in young adult samples, where performance on a memory task is tested following an interval of sleep compared an equivalent interval spent awake (Walker and Stickgold, 2006; Diekelmann and Born, 2010). These studies have provided strong support for the notion that memories are actively

consolidated over sleep. However, sleep, much like other physiological processes, undergoes radical changes with age; concurrent with these changes in sleep, is a decline in cognitive abilities, specifically with relation to episodic memory (Ronnlund et al., 2005). An emerging question, therefore, is whether sleep-dependent memory consolidation is preserved in older adults. To answer this, a few studies have compared post-sleep and post-wake performance in older adults; collectively they have demonstrated a role of sleep in memory consolidation in older adults that is task-dependent. Specifically, although non-declarative memories do not appear to be benefited by a period of sleep any more so than a period of wake, the benefit of sleep on declarative memory seems to persist with age (Wilson et al., 2012). The current study's goal is to examine whether the mechanism of this benefit of sleep in older adults is similar to that observed in young adults, and if not, whether it is attributable to specific changes in sleep architecture.

Sleep States and Physiology

Sleep is a heterogenous process that is characterized by distinct neurobiological states known as sleep stages. Aserinsky and Kleitman (1955) were the first to make the distinction between non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep, one that marked a very important development in the study of sleep's role in memory processing; it has since become evident that each stage of sleep is associated with the activation of different brain structures with varying functional connectivity (Wagner et al., 2010), changing metabolic activity (Madsen et al., 1991; Cirelli and Tononi, 2011), and consequently, differential involvement in memory consolidation (Walker and Stickgold, 2006, Spencer, 2013).

Sleep onset is typically characterized by the transition from waking state to NREM Stage 1 (N1), defined by the appearance of theta waves (4-7 Hz; Iber et al., 2007). N1 is then followed

by NREM Stage 2 (N2) that is marked by K-complexes and sleep spindles that occur intermittently across the background theta activity, and both of which play an important role in sleep maintenance (Urakami, Ioannides and Kostopoulos, 2012 ;Nicholas, Trinder and Colrain, 2002). K-complexes consist of a sharp negative peak followed by a positive peak, creating a waveform that lasts 0.5 - 1 s. Sleep spindles are short bursts (approximately 0.5 s) of high frequency thalamocortical waves (11-16 Hz). The next stage in the progression into deeper sleep states is NREM Stage 3 (N3), also known as slow-wave sleep (SWS) as it is characterized by low frequency delta waves (0.5 – 4 Hz). Finally, REM sleep, named so due to the rapid ocular saccades associated with it, is also referred to as paradoxical sleep; this is owing to the fact that REM is characterized by EEG activity between 30 and 80 Hz, much like that of wake, but is also associated with muscle atonia (Iber et al., 2007).

The adult brain typically alternates through these stages of sleep in a cyclic fashion; cycles in healthy adults are approximately 90 minutes in duration and consist of a progression from lighter to deeper NREM stages, and ending in a REM bout (Carskadon and Dement, 2011; Schulz, 1980). The relative distribution of each of the stages varies across the sleep cycles: Specifically, there is a greater percentage of SWS during the sleep cycles early in the night, while the percentage of REM sleep is greater in the sleep cycles later in the night (Carskadon and Dement, 2011). Figure 1A shows a typical night in a healthy young adult. There are several underlying driving forces that are responsible for the observed distribution of SWS and REM across the night: SWS quantity and physiology is strongly driven by the homeostatic sleep drive (Borbély, 1981; Borbély, 1982). The greater the "sleep pressure" when entering into sleep state, the greater the amount of time spent in SWS, and greater the EEG spectral power of the delta waves associated with it. As sleep pressure declines over the course of the night, the length of the

SWS bouts and delta power of the slow-wave activity (SWA) reduce as well. REM sleep, on the other hand, is particularly sensitive to body temperature, such that REM sleep propensity increases with increasing body temperature (Czeisler et al., 1980). Consequently, REM bouts get longer as body temperature rises closer to sleep offset. In addition to the relative distribution of the different stages of sleep, cycle length also varies across the night (Carskadon and Dement, 2011). The first sleep cycle length is typically between 70 and 100 minutes, while the second cycle tends to be longer with an average length of 90-120 minutes.

The quantity and distribution of sleep stages, the length of sleep cycles as well as spectral characteristics of each sleep stage vary not only as a consequence of factors such as immune function, physical and mental fatigue (Spiegel, Lepoult and Cauter, 1999; Bryant, Trinder and Curtis, 2004), but also as a function of age (Pace-Schott and Spencer, 2011). While examining age-related changes in the role of sleep in memory consolidation, it is crucial to consider the arrangement of the different stages of sleep across the night in addition to the quantity and quality of these stages, since each of these characteristics of sleep architecture have a distinct role in memory consolidation in young adults.

Cognitive Benefits of Sleep in Young Adults

Sleep benefits learning and memory in young adults on a range of memory tasks, spanning non-declarative and declarative memory domains. The category of non-declarative memory encompasses skill learning, priming and conditioning (Squire, 2004); however, research in sleep-dependent memory consolidation has predominantly focused on skill learning. Researchers have used tasks that require swift reactions, with the assumption that successful learning is reflected by a reduction in reaction times as well as increases in accuracy. Using a motor sequence learning task, where participants are required to type a sequence of numbers as

quickly as they can, reaction times were found to be faster following an interval of sleep compared to wake, with a concurrent increase in accuracy, thus reflecting greater improvements in performance over sleep (Walker et al., 2002; Spencer et al., 2006). Similarly, visuo-motor skill was observed to be superior following overnight sleep relative to pre-sleep performance on a mirror tracing task (Tamaki et al., 2009), as well as on a pursuit rotor task (Fogel and Smith, 2006).

Declarative memories include semantic memories that are related to factual information and classified as "general knowledge," as well as episodic memories that are associated with specific events in an individual's past (Squire, 2004). The majority of studies that probe declarative memory employ episodic memory tasks that, in their simplest form, require recall of previously presented information. For instance in one such study, participants learned a list of word pairs and were asked to recall the correct associations following overnight sleep or after a day spent awake (Wilson et al., 2012). Memory for the word pairs was found to be superior following an interval of sleep compared to wake. Similarly, in another study, recall of stories and personal events was greater following an interval of sleep compared to wake (Aly and Moskovitch, 2010). Yet another declarative task, a spatial memory task, also yielded similar results across a number of studies (Rasch et al., 2007; Talamini et al., 2008; Rudoy et al., 2009).

Therefore, there is sufficient evidence to support the notion that sleep in young adults plays a crucial role in the processing of memories, in all of their diverse forms. Owing to the diverse nature of sleep itself, it is consequently not surprising that the different stages of sleep contribute to the consolidation of memories across these memory domains to varying degrees.

The Mechanism of Sleep-Dependent Memory Consolidation: Animal Models and Young Adults

Stemming from Jenkins and Dallenbach's (1924) observation that memory is benefited by a period of sleep, and the distinction between NREM and REM sleep made by Aserinsky and Kleitman (1953), numerous studies have been conducted in order to understand the mechanism underlying the cognitive benefits of sleep, particularly the relative contributions of each sleep stage. Early research in this field had a specific eye toward the role of REM in learning and memory (Smith and Butler, 1982; Smith and Weeden, 1990; Smith and Wong, 1991). Accumulating evidence in animal models using selective sleep deprivation techniques suggested that REM is critical for non-declarative, but not for declarative learning (Smith, 1996; Smith and Rose, 1996; Smith and Rose, 1997). Similarly, in humans, time spent in REM sleep was found to be positively correlated with learning gains, specifically with relation to non-declarative tasks (Grieser, Greenberg and Harrison, 1972; Dotto, 1996). However, since this effect was not consistently demonstrated (Vertes and Siegel, 2000; Siegel, 2001) and the effect appeared to be dependent on the type of memory task used (Stickgold et al., 2001), researchers were prompted to consider the role of NREM sleep in memory consolidation as well.

As mentioned previously, the percentage of SWS is greater early in the night and steadily declines as the night progresses (Borbély, 1981; Borbély, 1982); on the other hand, the percentage of REM increases in the second half of the night (Czeisler et al., 1980). Therefore, in order to dissociate the role of NREM, specifically SWS, and REM sleep on memory consolidation, Plihal and Born (1997) studied the effects of early and late night sleep on a non-declarative mirror tracing task and a declarative word-pair learning task using a split-night paradigm in young adults. They demonstrated that declarative memory was benefited by sleep

early in the night, while late night sleep benefited non-declarative memory; since sleep early in the night is rich in SWS and late night sleep in REM, the authors suggested that declarative memory consolidation was driven by SWS, whereas non-declarative memory consolidation was REM-dependent.

It is now known that the neurobiology of SWS indeed presents an ideal mechanism for declarative memory consolidation. The process of memory consolidation involves the transfer of newly acquired memories to more stable cortical representations (Walker and Stickgold, 2006). Consequently, in the case of episodic memories that engage the hippocampus during initial acquisition (Tulving and Markowitsch, 1998), episodic memory consolidation involves a transfer of these hippocampal-dependent memory traces to neocortical locations, a process that is neurophysiologically supported by SWS (Buzsáki, 1996). During SWS, high-frequency oscillations (~200 Hz) known as "sharp-wave ripples" are observed within the hippocampus, and are temporally correlated with neocortical spindles (Siapas and Wilson, 1998). Furthermore, slow oscillations occurring across the cortex during SWS seem to orchestrate the synchrony between hippocampal ripples and neocortical spindles, thereby facilitating the hippocampal-neocortical dialogue necessary for declarative memory consolidation (Spencer, 2013). In fact, hippocampal ripples seem to selectively bias the occurrence of sleep spindles in specific neocortical areas associated with recently encoded information (Siapas and Wilson, 1998). Even more convincingly however, hippocampal "replay" of newly acquired memories has been observed in both animal models and in humans. Wilson and McNaughton (1994) recorded activity in the hippocampal place cells in rats during encoding as well as during post-training SWS, following exposure to a spatial navigation task. They found that the sequential firing of the hippocampal place cells during encoding was selectively replicated during post-training SWS.

Likewise in humans, using cerebral blood flow measurements, Peigneux and colleagues (2004) found that hippocampal areas that were activated during encoding of a spatial task were reactivated during post-training SWS, and importantly, greater reactivation was associated with greater performance gains.

In addition to the contribution of SWS, a growing body of literature suggests that sleep spindle activity during N2 also plays a role in declarative memory consolidation (Fogel et al., 2002; Walker, 2002). Using a word-pair learning task, Schabus and colleagues (2004) demonstrated a relationship between N2 sleep spindle activity and performance gains post-sleep. Furthermore, Gais and colleagues (2002) reported learning-dependent *increases* in N2 spindle activity following training on a declarative task compared to a non-learning condition, and this increase in spindle activity correlated with performance benefits on the task. Therefore, it is apparent that sleep-dependent consolidation of declarative memories is linked to physiological mechanisms occurring *throughout* NREM sleep.

A notion that is gaining popularity is that NREM sleep does not act independently in the consolidation of declarative memories; the sequential contributions of NREM and REM stages are now being considered, with each stage serving different, but perhaps not independent, functions in the consolidation process (Giuditta, 1995; Diekelmann and Born, 2010; Spencer, 2013). The synaptic homeostasis model, first described by Tononi and Cirelli (2003), states that during NREM sleep, global downscaling of synaptic strengths occurs. Owing to the increase in synaptic potentiation across wake, the process of synaptic depotentiation is crucial for creating an ideal neural environment for additional learning post-sleep. However, since memory consolidation is driven by long-term potentiation (LTP) at the cellular level (Chauvette, Seigneur and Timofeev, 2012), it is unclear how the depotentiating nature of SWS, as suggested by Tonini

and Cirelli (2003), could contribute to it. Two recent studies have taken a closer look at the synaptic changes occurring across NREM and REM sleep: First, on the premise that downscaling of synaptic strength should result in reduced somatosensory cortical-evoked local field potentials (LFP) as a result of electrical stimulation, Chauvette and colleagues (2012) surprisingly demonstrated an *increase* in somatosensory cortical-evoked LFP following SWS, thus concluding that SWS is associated with synaptic upscaling rather than downscaling as previously believed. Second, Grosmark and colleagues (2012) recorded LFP in hippocampal CA1 neurons during NREM and intervening REM episodes, and found that it is in fact, REM sleep that is responsible for the reduction in neuronal excitability over sleep.

These findings do not, by any means, preclude the role of either SWS or REM in declarative memory consolidation. Rather, they provide support for the sequential role of NREM and REM episodes on memory consolidation. In line with this, Born and Feld (2012) provided the hypothesis that although REM is responsible for global downscaling of synaptic strength, NREM is crucial for the local upscaling of neuronal circuits involved in recently acquired memories. Interestingly, an earlier study by Ficca and colleagues (2000) demonstrated that disturbed NREM-REM sleep cycles resulted in impaired recall of verbal material after a night of sleep in young adults. Together these studies indicate that although NREM and REM sleep contribute to memory consolidation in different ways, they do not do so independently; in fact, the contributions of NREM and REM to the process of memory consolidation are perhaps contingent on their occurrence in succession, or in other words, on the arrangement and integrity of sleep cycles.

Age-Related Changes in Sleep Physiology and its Relation to Cognition

Aging is associated with diminished sleep quality, attributable in part by higher incidences of neuropsychiatric and sleep disorders in older populations (Neikrug and Ancoli-Israel, 2010). However, sleep quality is also diminished in healthy, normal aging (Pace-Schott and Spencer, 2011). Figure 1B shows a typical night in a healthy older adult. Older adults experience increased sleep onset latency and frequent awakenings through the course of the night, resulting in a marked increase in wake after sleep onset (WASO) compared to young adults. Consequently, sleep efficiency, or the amount of time spent asleep relative to time spent in bed, is significantly reduced in older adults compared to young adults (Buysse et al., 2005). Considering the role of sleep spindles and K-complexes in sleep maintenance (Urakami, Ioannides and Kostopoulos, 2012 ;Nicholas, Trinder and Colrain, 2002), the progressive increase in WASO with age could be a result of an age-related decrease in sleep spindle number, density and duration (Nicolas et al., 2001), as well as in a reduction in K-complex number and density (Crowley et al., 2002). Additionally, aging is associated with a reduction in EEG spectral power in the theta, sigma and delta frequency ranges (Carrier et al., 2001). The most drastic age-related change in sleep architecture however, is the reduction in SWS (Colrain et al., 2010), reflected by both the reduction in the total amount of SWS as well as shorter durations of the SWS bouts (Lombardo et al., 1998). Finally, there is some evidence to show that older adults also have reduced REM density compared to young adults (Darchia et al., 2003), and have decreased propensity to awaken from REM sleep (Dijk et al., 2001).

Only recently has sleep-dependent memory consolidation been probed in healthy aging. In the non-declarative domain, Spencer and colleagues (2007) compared performance on a motor sequence learning task, and found that while young adults showed a sleep benefit, performance

changes over sleep did not differ from changes over wake for the older adult group. This result was replicated by Siengsukon and Boyd (2008) and by Wilson and colleagues (2012), giving rise to the idea that sleep-dependent memory consolidation is reduced in older adults. Owing to the changes in SWS with age, one would expect a similar reduction in the processing of declarative memories over sleep. Consistent with this notion, Mander and colleagues (2013) demonstrated that medial prefrontal cortex gray matter atrophy associated with aging is paralleled by reduction in NREM SWA, and the extent of the reduction in NREM SWA correlated with post-sleep episodic memory impairment. Furthermore, following sleep-dependent declarative memory consolidation in young adults, there is a reduction in task-related hippocampal activation with a concurrent increase in hippocampal-neocortical connectivity (Takashima et al., 2006); however, this was not the case in older adults (Mander et al., 2013). Evidence of reduced hippocampal-prefrontal functional connectivity (Salat et al., 2005) as well as reduced connectivity within the hippocampus during SWS (Terry, Anderson and Horne, 2004) has been observed in aged rats as well.

In contrast with these findings, Aly and Moscovitch (2010) probed autobiographical memory in older adults and reported that recall of stories and personal events was superior following a period of sleep compared with a period of wake. Likewise, using a word-pair associates task, Wilson and colleagues (2012) demonstrated that older adults recalled significantly more novel word pairs following sleep compared to wake. Collectively these studies suggest that sleep-dependent declarative memory consolidation may indeed be preserved in aging.

The inconsistencies in the declarative memory literature facilitate the need to dissociate the passive and active roles of sleep in memory consolidation: Specifically, since the benefit of

sleep for older adults, where observed, is reflected by a maintenance in performance, or in other words less forgetting, sleep may merely protect memories from ongoing waking interference as opposed to actively strengthening, stabilizing and relocating memories as they do in young adults. Through the use of behavioral manipulations and electrophysiology, the current study is an attempt at filling this gap in the literature by exploring whether *active* consolidation of visuo-spatial memories occurs in older adults.

Current Study: Motivation and Hypotheses

Proponents of the hypothesis that the role of sleep in memory consolidation is one that is passive and short-lived state that sleep is beneficial to memory insofar as it provides a period of time, undisturbed by interference from waking activities, where newly encoded information is preserved without much decay (Vertes, 2004; Vertes and Siegel, 2005). Since such a theory does not advocate that sleep *strengthens* memory traces, memories would once again become labile and susceptible to interference when the individual awakens and engages in daytime activities.

Although the passive role of sleep is one mechanism through which memory is benefited, sufficient evidence has been accumulated against this being the *sole* mechanism, specifically in young adults. Firstly, the benefit of sleep has indeed been found to be long-lasting. If learning is immediately followed by an interval of sleep, the benefits of sleep on memory are maintained 24 hours later despite an additional interval consisting of daytime activities (Wilson et al., 2012). Furthermore, impaired memory for learned information following 12 hours of wake is found to recover 24 hours later once participants have had the opportunity to sleep; however, the delayed benefits of sleep to memory are diminished compared to the effects of sleep immediately following learning. Secondly, region-specific reactivation of hippocampal and neocortical ensembles during SWS resulting in more efficient memory recall post-sleep, provides

mechanistic support for the active role of sleep in memory consolidation (Peigneux et al., 2004). Thirdly, sleep fragmentation and disruption of NREM-REM sleep cycles, without a concurrent increase in awakenings, has been shown to be detrimental to memory consolidation (Ficca et al., 2000). Such an observation contradicts the hypothesis that merely time spent asleep is sufficient for protecting memories, but rather supports the existence of specific sleep-dependent processes that are crucial for consolidation to occur.

Ellenbogen and colleagues (2006) used an interference paradigm to demonstrate that memories that are consolidated over sleep are actively strengthened such that they are resistant to subsequent interference. They trained participants on a word-pair learning task using A-B pairs; after a period of 12 hours consisting of either daytime wake or overnight sleep, they trained the same participants on interfering A-C word pairs. When memory for the original A-B word pairs was tested following interference, they found that the performance of the group that slept in between sessions was significantly superior to the group that stayed awake. Diekelmann and colleagues (2012) extended this finding to spatial learning using task-related odor cues during post-training wake or SWS to test the effects of memory reactivation on memory stability. They found that reactivation of memories during wake rendered them labile, while the opposite occurred during SWS, wherein memories were strengthened and thus more resistant to interference. Furthermore, reactivation during SWS, not wake, resulted in activation of hippocampal and posterior cortical regions, consistent with the mechanism of declarative memory consolidation via a hippocampal-neocortical dialogue (Peigneux et al., 2004).

The active role of sleep in memory consolidation is less clear in older adults, particularly in light of the inconsistent behavioral outcomes of the sleep-dependent declarative memory studies. In order to dissociate the passive and active roles that sleep plays in cognitive processes,

a visuo-spatial learning paradigm was used in this study, given that, 1) it engages the hippocampus (Shrager et al., 2007), 2) such a paradigm has been used before to adequately demonstrate the relationship between declarative learning, reactivation of memory traces in the hippocampus during SWS and post-sleep benefits on performance in young adults (Diekelmann et al., 2012), 3) such a task has never previously been used to probe sleep-dependent declarative memory consolidation in older adults, and 4) Cherdieu and colleagues (2014), using a visuo-spatial learning paradigm in young and older adults demonstrated that while young adults show a distinct benefit of sleep on this task, older adults, in fact, do not. Although not to a level of significance, sleep did indeed provide a modest level of protection against decay compared to wake in older adults, leading Cherdieu and colleagues (2014) to conclude that sleep-dependent memory consolidation is *reduced* in older adults. Such a benefit of sleep could be a result of the passive role of sleep in protecting memories from decay alone. However, both Cherdieu and colleagues (2014) and Mazzoni and colleagues (1999) have demonstrated a strong positive correlation between proportion of time spent in sleep cycles relative to total sleep time, and performance on a declarative task in older adults, suggesting a reliance on sleep physiology and not merely time spent asleep.

The current study aimed to provide some insight into whether older adults lack the active role of sleep in the consolidation of visuo-spatial memories, or if it is simply reduced. This is done through 1) the use of an interference paradigm as per Ellenbogen and colleagues [2006] and Diekelmann and colleagues (2011), 2) looking at the contribution of early night sleep in particular, on memory performance, with respect to quantity, quality and arrangement of the sleep stages, and 3) examining age-related changes in the EEG spectral characteristics of NREM sleep, and its effect on memory consolidation.

Our a-priori predictions for young adults were: 1) Young adults would benefit from a 12-hour interval of sleep in both, the No Interference and the Interference conditions, such that memory decay would be much reduced following sleep compared to an intervening interval of wake, 2) the benefit of sleep in young adults would be closely related to the amount of time spent in SWS early in the night, such that greater amounts of SWS early in the night would result in superior performance post-sleep, 3) greater delta power and sigma power density of the SWA early in the night would correlate with performance benefits post-sleep, 4) greater sigma power density of N2 across the night would correlate with better memory, 5) performance post-sleep would be related to the amount of time spent in sleep cycles relative to time spent asleep, such that greater the percentage of time spent in sleep cycles, better the performance post-sleep, and finally, 6) the interaction between NREM and REM episodes early in the night would play an important role in the memory consolidation process.

With regard to older adults, we predicted: 1) Sleep physiology in older adults would be markedly different from young adults in that they would have reduced SWS, reduced delta and sigma power density, as well as disrupted sleep cycles and, 2) owing to these changes in sleep physiology, we would see reduced sleep-dependent memory consolidation, such that the benefits of an interval of sleep would only be evident in the Interference condition, where the active role of sleep in episodic memory consolidation would be unmasked.

CHAPTER II

METHODS

Participants

One hundred and thirty seven healthy young adults between the ages of 18 and 30 yrs and 95 healthy older adults between the ages of 50 and 79 yrs participated in this study. In order to ensure that the sample consisted of healthy individuals, participants were excluded if they had been diagnosed with a neurological disease, congestive heart failure, had a previous myocardial infarction or heart surgery, or a history of stroke or head trauma. Additionally, participants were excluded if they used sleep-affecting medications, or if they habitually slept less than 5 hours or more than 11 hours per day. In order to accurately perform the behavioral task, we also ensured that participants had unimpaired, or corrected vision (20/30 or less) as assessed with a standard vision chart.

Participants were assigned to either the Wake group or the Sleep group, and to either the "No Interference" or the "Interference" condition. A subset of the Sleep group was administered polysomnography (PSG) recording procedures. There were therefore, a total of 8 experimental groups in this study: Young Adult No Interference Wake (N=33), Young Adults No Interference Sleep (without PSG, N=23; with PSG, N = 17), Young Adult Interference Wake (N=32), Young Adult Interference Sleep (without PSG, N=17; with PSG, N = 15), Older Adult No Interference Wake (N=20), Older Adults No Interference Sleep (without PSG, N=13; with PSG, N = 14), Older Adult Interference Wake (N=23), Older Interference Sleep (without PSG, N=15; with PSG, N = 10).

For older adult participants alone, we administered the Mini-Mental State Exam (MMSE, Rovner and Folstein, 1987) and the National Adult Reading Test (NART: Nelson, 1991), as measures of cognitive function and intelligence levels respectively.

Sleep Assessments

In addition to prescreening, participants were queried for average sleep time using the Pittsburgh Sleep Quality Index (PSQI), a questionnaire used to determine an individual's sleep quality over the previous 30 days (PSQI; Buysse et al., 1989). The PSQI includes questions probing subjective sleep quality, sleep latency, sleep duration, sleep efficiency, disturbances during sleep, the use of sleep-affecting medications, and daytime somnolence; it has been determined to be a reliable (Cronbach's α=0.87) and valid instrument for the measurement of sleep disturbances (Bakhaus et al., 2002). An abbreviated Wake-Time Diary was given in the morning session to assess subjective sleep quantity and quality during the preceding night (Smith et al., 2003). To assess daytime activities including napping and caffeine intake, an abbreviated Sleep-Time Diary was given in the evening session.

In addition, during each session of the study, participants were given the Stanford Sleepiness Scale (SSS). Responses range from 1 (feeling active, wide-awake) to 7 (almost in reverie, struggling to remain awake), providing a measure of self-reported sleepiness (Hoddes et. al., 1973). This information was taken in order to assess differences in subjective sleepiness across groups and conditions.

Procedures

Figure 2 describes the experimental procedures in this two-session study. Following informed consent procedures, session one began with completion of the PSQI, Wake-Time Diary (for the Wake groups) or the Sleep-Time Diary (for the Sleep group), and the SSS. Subsequently,

participants completed the *preview*, the *encoding phase*, and the *immediate recall phase* of the visuo-spatial learning task. The exact time of testing during session one was recorded for each participant, and by means of the Sleep-Time and Wake-Time diaries, we determined: 1) "Sleep inertia," or the amount of time between sleep offset and commencement of the experimental procedures in the morning (for the Wake group), and 2) "sleep delay," or the amount time between conclusion of experimental procedures and sleep onset in the evening session (for the Sleep group).

Session one and two were separated by a 12-hr interval. Session one took place between 8-10 AM for those assigned to the Wake group, with session two occurring 12 hrs later between 8-10 PM, following and interval spent fully awake. Participants were instructed not to nap or consume alcohol during this time. Alternatively, the Sleep group performed session one between 8-10 PM and session two 12-hrs later between 8-10 AM the following morning, after an interval consisting of overnight sleep (during which PSG recordings were conducted for a subset of this group). The SSS and Sleep-Time Diary (for the Wake group) or the Wake-Time Diary (for the Sleep group) were completed at the start of session two. Subsequently, participants were introduced to either the No Interference or the Interference conditions, followed by the *delayed recall phase*.

The Visuo-Spatial Task

The task used to probe declarative memory was a visuo-spatial learning task similar to the game Memory (also known as Concentration), and was adapted from the paradigm used by Diekelmann and colleagues (2011). Twenty images were presented on a computer screen, arranged in a 5x4 matrix (Fig. 3A). The images represented pictures of common nouns (for example, "nurse," "dog," or "cherries"). Participants were informed that a preview of the image

matrix would be presented to them and were instructed to memorize the locations of the images as they would be tested on their memory for those locations later in the session. Based on pilot data, in order to equate learning across age groups, the image preview was presented for 30 s for young adults and 60 s for older adults. Subsequently, the images were virtually "flipped over," and participants began the *encoding phase*. In this phase, a single image matching an item in the matrix was displayed on the right of the screen (Fig. 3A) and participants were asked to click on the location within the matrix where the matching image was located. The correct image for the selected location was then revealed to the participant, providing them with feedback as to whether they were correct, and giving them additional opportunities to learn the image locations. After all items in the matrix had been tested, if accuracy was < 15%, the preview of the image matrix was presented again (30 s for young adults and 60 s for older adults). The encoding phase continued until the participant reached a criterion of 63% correct or until the full set of images had been probed 10 times.

After a 20 min period, during which they completed a set of questionnaires, participants were informed that they were to be tested on their memory for the image locations in the *immediate recall phase* (Fig. 3B). They were also informed that unlike the encoding phase, each image location would be tested just once, and no feedback would be provided (in other words, the correct image for the selected location would not be displayed following a response). This was done in order to prevent further learning, and to provide an unbiased baseline measurement of their knowledge of the spatial locations of each image, no feedback was provided.

Session two procedures differed for the Interference and the No Interference conditions. For the Interference condition, participants were introduced to a new matrix, composed of the same images used during encoding, but the items were in new locations. The preview and

encoding phases were identical to those in session one with the exception that encoding continued until accuracy was 63% or when all items had been probed 4 times (this was lower than session one in order to avoid substantial levels of interference, resulting in a floor effect with respect to memory for the original image locations). After a 30 min wait period, the participants were retested for delayed recall on the original locations in the *delayed recall phase* (Fig. 3B). During the 30 mins between the Interference round and delayed recall, participants were shown a movie ("Planet Earth") in order to prevent active rehearsal of the image locations and to equate activities across participants.

In the No Interference condition, to equate the time in the lab prior to the delayed recall phase in the Interference condition, the participants first watched "Planet Earth" for 30 mins, followed by the delayed recall phase. There was no additional encoding in the No Interference condition.

Polysomnography

For a subset of the participants that were assigned to the Sleep groups, we followed session one with the addition of PSG. One hour or more prior to the participant's estimated bedtime, the standard PSG montage was applied in the participant's residence. The montage included six EEG leads (O_1, O_2, C_3, C_4, F_1, F_2, Cz,), two EOG leads (one on the side of each eye), two chin EMG leads, two mastoid electrodes and one ground electrode on the forehead, using the Aura PSG wireless/ambulatory system (Grass Technologies, Astro-Med Inc., West Warwick, RI).

PSG data was analyzed according to the specifications provided in the revised American Academy of Sleep Medicine manual (Iber et al., 2007). All records were scored for NREM-REM sleep cycles as per the criteria provided by Griessenberger and colleagues (2012). A sleep cycle

was defined as a period of NREM followed by REM, lasting a minimum of 30 mins, and not interrupted by a period of continuous wake greater than 2 mins. NREM or REM bouts that were <2 mins in duration were included in the previous sleep stage, and REM bouts that were < 15 mins apart were combined and considered as a single REM bout. Finally, each individual's record was divided in half, such that the contribution of NREM, and specifically SWS, could be examined during the first half of the night.

EEG spectral power analyses were conducted using the BrainVision Analyzer 2.0 software (Brain Products, Munich, Germany). Raw data was first subjected to segmentation: delta power density (0.5-4 Hz) and sigma power density (12-16 Hz) was calculated during SWS during the first half of the night, while sigma power density was calculated during N2 across the night (Fogel, Smith and Cote 2007). Segmented data was then filtered for frequencies between 0.3 and 35 Hz, followed by semi-automatic raw data inspection for large artifacts, such as arousals, motion artifacts and transient electrical interference that render epochs unscorable, in the EEG channels only: Artifacts were automatically detected by the software, but were subsequently confirmed or rejected by visual inspection. Inspected data was then segmented into 4 s bins and subjected to semi-automatic artifact rejection for the detection of more minute frequency and amplitude fluctuations that may have been missed during raw data inspection. Finally, in order to measure spectral power density (μV^2/Hz), central and frontal electrodes were subjected to Fast-Fourier transform analysis using a 10% Hanning window with no overlap; spectral EEG power density for delta and sigma frequency ranges was calculated and averaged across the central and frontal electrodes (Marshall et al., 2006).

Statistical Analyses

In order to compare group differences in the questionnaire measures (PSQI, SSS1 and SSS2), independent samples t-tests were used. Likewise, baseline performance was compared between age groups, and between Wake and Sleep groups within each age group, using two measures: Number of loops required to reach criterion during encoding and accuracy during the immediate recall phase (proportion of images correctly recalled). For all t-tests, if the Levene's test for homogeneity was found to be significant, the adjusted t-statistics and *p*-values are reported.

Performance changes from session one to two were calculated by subtracting accuracy during the delayed recall phase from accuracy during the immediate recall phase. However, in order to control for differences in baseline performance on the task, specifically accuracy at immediate recall, an Adjusted Score was used as the dependent variable in our analyses. This was done by using the following formula: Adjusted Score = (Delayed Recall – Immediate Recall) / Immediate Recall

To test the effects of independent variables, Group (Wake vs. Sleep) and Condition (No Interference vs. Interference) on performance on the visuo-spatial task (Adjusted Score), a two-way between subjects ANOVA within each age group was conducted. In order to examine whether there was an age-related reduction in active sleep-dependent memory consolidation, a two-way Group by Age Group (Young vs. Old) was conducted within the interference condition alone; this was done on the premise that a significant Group x Age Group interaction would suggest that sleep differentially affects the stability of the visuo-spatial memories across the two age groups. If the independent samples t-tests conducted previously to compare scores on the

PSQI, SSS1 and SSS2 revealed any significant difference between the Wake and Sleep groups for either of the age groups, those measures were used as covariates in ANOVAs.

Aging is associated with an increase in WASO and consequently a reduction in sleep efficiency (Pace-Schott and Spencer, 2011); therefore, we compared these two measures between young and older adults using independent samples t-tests. Owing to the role of NREM sleep, particularly SWS, in declarative memory consolidation in the early part of the night (Plihal and Born, 1997), the effects of the amount of time spent in NREM and SWS during the first half of the night on performance changes on the visuo-spatial task was assessed in young and older adults. Furthermore, to test whether the first bout of SWS following sleep onset is associated with declarative memory consolidation independently, the amount of NREM and SWS in the first sleep cycle of the night specifically, was measured. Independent samples t-tests were performed to compare the amount of time spent in SWS and NREM during the first half of the night, and during the first sleep cycle of the night. In addition, we examined EEG spectral power density in young and older adults in the delta and sigma frequency ranges during SWS in the first half of the night. Owing to the contribution of N2 in declarative memory consolidation (Fogel et al., 2002), we compared the amount of time spent in N2, as well as sigma power density during N2, across the night between the two age groups. Given the suggestion of the involvement of REM sleep in memory consolidation by means of global synaptic depotentiation (Grosmark et al., 2013), we also examined the age-related differences in the distribution of REM sleep during the first and second halves of the night.

To explore the relationship between each of the sleep parameters and performance changes over sleep (Adjusted Score), Pearson's correlations were conducted. For these analyses, if the behavioral results revealed a main effect of Group with no Group x Condition interaction,

the two experimental conditions (NI and I) were combined in an effort to increase the power to detect the relationship between behavioral changes and the various sleep parameters. Since delta power density during SWS is known to be particularly sensitive to sleep pressure (Borbély, 1981; Borbély, 1982), Pearson's correlation between sleepiness scores during session one and amount of SWS and delta power density across the night for each age group was conducted. If this correlation was found to be significant, SSS1 scores were used as a covariate in a partial correlation between delta power density and the Adjusted Score. All correlational analyses involving older adults were controlled for age as well, owing to the progressive changes in sleep structure and physiology across our selected age group (50-80 years).

It has been suggested previously that uninterrupted, organized and continuous NREM/REM sleep cycles are more efficient in the process of memory consolidation, than the individual sleep stages considered independently (Ficca et al., 2000). Thus, the relationship between time spent in uninterrupted sleep cycles and the Adjusted Score was examined. Finally, in order to test the importance of the first NREM-REM-NREM triplet on the Adjusted Score, a multiple regression analysis was performed, specifically to explore the interaction between the three stages. This was done such that the independent effects of each of those stages, *as well as* their combined effects on over-sleep consolidation of visuo-spatial memories could be explored. The interaction terms in multiple regression analyses, if found to be significant, suggest that the impact of one variable depends on the level of the other. Furthermore, if the interaction is significant, then the interpretation of the main effects of each variable cannot be considered alone, as such an interpretation is incomplete and does not represent the true relationship between the variables and the outcome. Therefore, in order to look at the relationship between NREM and REM sleep in the first NREM-REM-NREM triplet of the night, the following

variables were centered around their average values and entered into the multiple regression: Total amount of NREM in the first sleep cycle (SC1 NREM), total amount of REM in the first sleep cycle (SC1 REM), total amount of NREM in the second sleep cycle (SC2 NREM), interaction terms SC1 NREM x SC1 REM, SC1 REM x SC2 NREM, SC1 NREM x SC2 NREM and SC1 NREM x SC1 REM, SC2 NREM.

CHAPTER III

RESULTS

Sample Descriptives

The average age of the young adult sample was 21.22 yrs (SD = 2.62), and consisted of 56 males and 81 females. The Wake-Time and Sleep-Time diaries were used to exclude individuals if they had consumed alcohol or excessive amounts of caffeine either prior to the experiment or in between sessions, if they had < 4 hrs of sleep on the experimental night or if they had napped during the day in between sessions. None of the young adults consumed alcohol or excessive caffeine either prior to the experiment or in between sessions. However, 9 young adults were excluded for taking a daytime-nap in between experimental sessions and 9 additional individuals were excluded for having a PSQI score >7 indicating significant sleep disturbances. Therefore, analyses are based on 119 young adult participants. Table 1 provides descriptive statistics for the final young adult sample.

The average age of the older adult sample was 65.43 yrs (SD = 7.60), consisting of 24 males and 71 females. All older adult participants scored >27 out of a possible 30 on the MMSE, and none of the participants scored <70% on the NART. There were no older adults that were excluded for alcohol or excessive caffeine consumption prior to the experiment, or in between sessions. However, 4 older adults were excluded for taking a daytime-nap in between sessions, and an additional 12 older adults for having a PSQI score >7. Thus analyses are based on 84 older adult participants. Table 1 provides descriptive statistics for the final older adult sample.

Group Differences

Table 2 provides mean scores for young and older adults for each of the questionnaire measures. Independent samples t-tests revealed that there were no significant differences

between the two age groups for subjective sleep quality measured through the PSQI ($t(193) = 1.69$, $p = 0.093$). However, young adults reported being significantly more sleepy compared to older adults during session one (SSS1; $t(93) = 3.60$, $p < 0.001$) and during session two, at trend-level (SSS2; $t(189.54) = 1.87$, $p = 0.064$).

For young adults, there were no significant differences between the Wake and Sleep groups for scores on the SSS1 ($t(117) = -0.302$, $p = 0.763$), SSS2 ($t(117) = -0.568$, $p = 0.571$), or the PSQI ($t(117) = -0.993$, $p = 0.323$). For older adults however, although there were no significant differences between the Wake and Sleep groups for the PSQI ($t(74) = 1.665$, $p = 0.1$), the older adult Sleep groups reported feeling significantly sleepier during session one compared to the Wake groups (SSS1; $t(74) = -2.572$, $p = 0.012$), while the Wake groups reported being sleepier than the Sleep groups during session two (SSS2; $t(74) = 2.079$, $p = 0.041$). Owing to these differences in self-reported sleepiness between sessions in older adults, sleepiness during both session one and two were controlled for when making behavioral comparisons between the Wake and Sleep groups.

Performance on the Visuo-Spatial Task

Baseline Differences

We compared young and older adults with respect to the two measures of baseline performance in the first session (Table 2): the number of loops required to reach criterion during the encoding phase, and accuracy in the immediate recall phase (proportion of image locations correctly recalled). Older adults required significantly more loops during the encoding phase to reach criterion compared to young adults ($t(77) = -2.863$, $p = 0.04$). Furthermore, accuracy during the immediate recall phase was greater for young adults compared to older adults ($t(195) = 2.971$, $p = 0.003$).

Young adults in the Wake group had a shorter delay between sleep offset in the morning and behavioral testing, and thus had greater "sleep inertia" than did the older adults (YA, Mean = 1.29 hrs, SD = 0.64; OA, Mean = 2.68 hrs, SD = 1.30; $t(26.595) = -4.726$, $p < 0.001$). However, sleep inertia did not affect encoding performance for either young or older adults with respect to number of loops required to reach criterion (YA, Pearson r = -.261, $p = 0.329$; OA, Pearson r = 0.553, $p = 0.255$) or accuracy at immediate recall (YA, Pearson r = 0.125, $p = 0.435$; OA, Pearson r = 0.326, $p = 0.529$).

There were no significant differences between the young adult Wake and Sleep groups with respect to number of loops required to reach criterion ($t(55) = 0.473$, $p = 0.638$), or accuracy during immediate recall ($t(117) = 0.345$, $p = 0.731$). Likewise, the older adult Wake and Sleep groups were comparable with respect to number of loops required to reach criterion ($t(20) = 0.913$, $p = 0.372$), and accuracy during immediate recall ($t(76) = -0.499$, $p = 0.619$).

Change in Performance over Wake and Sleep Intervals

A two-way Group (Wake vs. Sleep) by Condition (NI vs. I) ANOVA in young adults revealed a main effect of Group ($F(1,114) = 7.899$, $p = 0.006$) and a main effect of Condition ($F(1,114) = 100.10$, $p < 0.001$). The Group x Condition interaction was not significant ($F(1,114) = 2.323$, $p = 0.13$). Likewise, a two-way Group (Wake vs. Sleep) by Condition (NI vs. I) ANCOVA with SSS1 and SSS2 scores as covariates in older adults revealed a trend-level main effect of Group ($F(1,69) = 3.595$, $p = 0.062$; Fig. 4A) and a main effect of Condition ($F(1,69) = 93.736$, $p < 0.001$). The Group x Condition interaction was not significant ($F(1,69) = 0.266$, $p = 0.608$; Fig. 4A).

Given a suggestion of a sleep benefit in older adults, to answer whether the role of sleep in strengthening and stabilizing declarative memories is reduced in older adults relative to young

adults, we performed a two-way Group (Wake vs. Sleep) by Age Group (Young vs. Old) ANCOVA with SSS1 and SSS2 scores as covariates, in the interference condition alone. The two-way ANCOVA revealed a significant main effect of Group ($F(1,187) = 7.726$, $p = 0.006$; Fig. 4B), but no main effect of Age Group ($F(1,187) = 1.188$, $p = 0.277$; Fig. 4B), or Group x Age Group interaction ($F(1,187) = 0.099$, $p = 0.753$). Additionally, in order to answer whether the extent of encoding of the interference image locations affected recall of the original image locations, we examined the relationship between the number of loops required to reach criterion during the interference round and the Adjusted Score: We found no relationship between the two measures for either the Wake or Sleep groups for young (Wake, Pearson r = -0.141, $p = 0.541$; Sleep, Pearson r = 0.166, $p = 0.485$) and older adults (Wake, Pearson r = 0.167, $p = 0.623$; Sleep, Pearson r = -0.411, $p = 0.491$).

To explore the relationship between baseline performance on the visuo-spatial task and performance changes over wake and sleep intervals, we performed Pearson correlations between each of the measures of baseline performance (number of loops required to reach criterion and accuracy at immediate recall) and the "Forgetting Rate" for the original image locations. The Forgetting Rate was calculated using the following formula: Forgetting Rate = Accuracy at Delayed Recall – Accuracy at Immediate Recall. All analyses were controlled for sleepiness during session one and two. For young adults, there was no significant relationship between the number of loops required to reach criterion during the encoding phase and the Forgetting Rate in either the Wake (Pearson r = 0.260, $p = 0.313$) or the Sleep groups (Pearson r = 0.038, $p = 0.824$). Likewise, no significant correlation was found between accuracy at immediate recall and the Forgetting Rate in the Wake (Pearson r = -0.138, $p = 0.350$) or the Sleep group (Pearson r = -0.185, $p = 0.146$) for young adults. For older adults however, accuracy at immediate recall was

negatively correlated with the Forgetting Rate in the Wake group (Pearson r = -0.716, $p < 0.001$), as well as the Sleep group at trend-level (Pearson r = -0.272, $p = 0.074$). There was no relationship between the Forgetting Rate and the number of loops required to reach criterion during the encoding phase in either the Wake (Pearson r = 0.718, $p = 0.282$) or the Sleep groups (Pearson r = 0.120, $p = 0.697$).

Given the indication that older adults forget learned visuo-spatial material at a more rapid rate than young adults, we compared the Forgetting Rate for the two age groups (combining Wake and Sleep groups) using an independent samples t-test: although there was no significant difference between young and older adults with respect to their Forgetting Rate ($t(77) = 0.900$, $p = 0.369$), the data was in the direction of an age-related increase in the proportion of images forgotten over time as (Young adults, M = 0.16, SD = 0.17; Older adults, M = 0.18, SD = 0.19).

Finally, we examined the effects of the amount of time spent awake following task encoding and prior to sleep onset on performance changes over sleep. Young adults had greater "sleep delay" than did the older adults (YA, Mean = 3.57 hrs, SD = 1.10; OA, Mean = 2.94, SD = 0.84; $t(96) = 2.942$, $p = 0.004$), and greater sleep delay was associated with poorer performance on the task post-sleep (a lower Adjusted Score) in young adults (trend-level, Pearson r = 0.230, $p = 0.070$), but not in older adults (Pearson r = 0.001, $p = 0.999$).

Group Differences in Sleep Architecture

Sleep Integrity and Sleep Stage Organization

Independent samples t-tests revealed no significant differences between the two age groups for sleep efficiency ($t(51) = 0.281$, $p = 0.780$); however, older adults had significantly greater WASO than young adults ($t(33.23) = -3.616$, $p = 0.001$; Table 3). With respect to the organization of the sleep stages, no significant differences were observed between young and

older adults for average number of cycles (t(42) = 0.437, p = 0.664; Fig. 5A). However, the average cycle length was significantly greater for young adults than older adults (t(42) = 2.155, p = 0.037; Fig. 5B). Additionally, young adults spent a greater amount of time in complete, uninterrupted sleep cycles than did older adults (TCT; t(42) = 2.107, p = 0.041; Fig. 5B) and this remained so when looking at the percent time spent in sleep cycles relative to total sleep time (TCT/TST ; t(42) = 2.694, p = 0.01; Fig. 5B).

NREM Sleep

Table 3 provides the mean values for each sleep measure in young and older adults. There were no significant differences between young and older adults for time spent in SWS in the first half of the night (t(46) = 1.236, p = 0.233; Fig. 6A) or the second half of the night (t(46) = -0.770, p = 0.445; Fig. 6B), and this was true in terms of percent SWS during the first and second halves of the night as well (First half, t(45.983) = 1.392, p = 0.171; Second half, t(46) = -1.024, p = 0.311). However, although there was no significant difference between young and older adults with respect to percent SWS during the first sleep cycle of the night (t(40) = 1.260, p = 0.215), young adults spent a significantly greater amount of time in SWS in the first sleep cycle than did the older adults (t(33.39) = 2.088, p = 0.044; Fig. 7). Furthermore, During SWS in first half of the night, young adults had greater delta power density (t(40) = 4.441, $p < 0.001$; Fig. 8A), as well as greater sigma power density (t(35) = 2.409, p = 0.021; Fig. 8B) compared to older adults.

Young adults spent marginally more time in NREM sleep during the first half of the night compared to older adults (t(46) = 1.469, p = 0.149; Fig. 6A); however, this difference was significant when looking at percent time spent in NREM sleep during the first half of the night (t(441) = 2.371, p = 0.020). There was no difference between young and older adults in terms of

time spent in NREM sleep (t(46) = 1.446, $p = 0.155$; Fig. 6B), or percent time spent in NREM sleep during the second half of the night (t(41) = 0.394, $p = 0.696$). However, young adults spent significantly more time than older adults in NREM sleep during the first sleep cycle (t(32.22) = 3.106, $p = 0.043$; Fig. 7), and this was true with respect to percent NREM sleep during the first sleep cycle as well (t(40) = 2.704, $p = 0.010$). Finally, although there was no difference between young and older adults with respect to the amount of time spent in N2 across the night (t(51) = 0.838, $p = 0.406$; Table 3) or percent time spent in N2 across the night (t(51) = 0.219, $p = 0.828$), sigma power density during N2 was significantly greater in young adults compared to older adults (t(38) = 2.231, $p = 0.032$; Fig. 9).

REM Sleep

Older adults showed a more diffuse distribution of REM sleep across the night compared to young adults: Specifically, compared to young adults, older adults spent a greater amount of time in REM sleep during the first half of the night (t(46) = -3.849, $p < 0.001$; Fig. 6A), as well as during the first sleep cycle (trend-level; t(40) = -1.795, $p = 0.08$; Fig. 7), and this was true in terms of percent time spent in REM sleep as well (First half, t(46) = -4.148, $p < 0.001$; First sleep cycle, t(40) = -3.182, $p = 0.003$). However, compared to young adults, older adults spent significantly less time in REM sleep (t(46) = 2.111, $p = 0.04$; Fig. 6B), and percent time in REM sleep during the second half of the night (t(46) = 1.993, $p = 0.052$).

Relationship between Sleep Measures and Performance on the Visuo-Spatial Task

Since the behavioral results revealed no significant Group x Condition interaction in either age group, and as such, sleep appears to benefit visuo-spatial memory consolidation in both conditions equivalently; therefore, No Interference and Interference conditions were

combined for the purpose of all correlations between sleep measures and post-sleep performance changes on the visuo-spatial task.

The Role of Sleep Integrity and Sleep Stage Organization

WASO and sleep efficiency are indicators of poor sleep quality; hence, the effects of the two measures on over-sleep performance changes on the visuo-spatial task (the Adjusted Score) were measured. No significant relationship was found in young or older adults between the Adjusted score and either WASO (YA Pearson r = 0.215, I = 0.253; OA Pearson r = -0.207, p = 0.355) or sleep efficiency (YA Pearson r = 0.091, p = 0.632; OA Pearson r = 0.108, p = 0.631). Additionally, there was no significant correlation for either age group between the Adjusted Score and either TCT (YA Pearson r = 0.223, p= 0.305; OA Pearson r = -0.023, p= 0.930) or TCT/TST (YA Pearson r = -0.001, p= 0.994; OA Pearson r = 0.047, p= 0.857).

The Role of NREM Sleep

For young adults, there was a positive correlation between the Adjusted Score and time spent in SWS in the first half of the night (trend-level, Pearson r = 0.346, p= 0.072; Fig. 10A), but no relationship with time spent in SWS in the first sleep cycle (Pearson r = 0.172, p = 0.455). There was a trend-level relationship between the Adjusted score and time spent in N2 across the night (Pearson r = 0.342, p = 0.065). In addition to this, there was a positive correlation between the Adjusted Score and time spent in the combined NREM sleep measure during the first half of the night (Pearson r = 0.467, p= 0.012; Fig. 10B). For older adults, no relationship was found between over-sleep performance changes and time spent in N2 across the night, SWS or NREM sleep in the first half of the night or during the first sleep cycle of the night.

For young adults, there was a significant positive correlation between SSS1 scores and delta power density across the night (Pearson r = 0.465, $p = 0.025$). Consequently, we controlled for SSS1 scores when examining the relationship between post-sleep performance changes on the visuo-spatial task and delta power density in young adults: Delta power density during the first half of the night was significantly correlated with the Adjusted Score (Pearson r = 0.477, $p = 0.045$; Fig. 11). No significant relationship between SSS1 scores and delta power density was observed in older adults; however, despite this, we controlled for sleepiness in the correlations between the Adjusted Score and delta power density in older adults as well since older adults were significantly sleepier during session one compared to session two: There was no significant relationship between the Adjusted Score and delta power density during the first half of the night for older adults (Pearson r = 0.096, $p = 0.722$). In addition to this, sigma power density in neither SWS during the first half of the night (Pearson r = 0.083, $p = 0.808$) nor N2 across the night (Pearson r = -0.003, $p = 0.994$) was correlated with performance changes over sleep for young or older adults.

The Role of REM Sleep

For young adults, greater amount of REM sleep during the first sleep cycle of the night was correlated with poorer post-sleep performance on the task (trend-level, Pearson r = -0.399, $p = 0.073$). However, as demonstrated by Ficca and colleagues (2000), the role of REM sleep in declarative memory consolidation is one that is contingent on prior NREM sleep, and is dependent on the integrity of the NREM/REM sleep cycles. In order to explore this notion, and to test whether the interaction between NREM in the first sleep cycle (SC1 NREM), REM in the first sleep cycle (SC1 REM) and NREM in the second sleep cycle (SC2 NREM), or in other words, the first NREM-REM-NREM triplet, a multiple regression analysis using centered

variables was performed. In young adults, the multiple regression analysis revealed a significant 2-way interaction between SC1 REM and SC2 NREM (β = 2.894, t(20) = 2.106, p = 0.055), as well as a significant 3-way interaction between SC1 NREM, SC1 REM and SC2 NREM (β = 2.858, t(20) = 2.347, p = 0.035). A visual representation of the regression lines for each variable is displayed in Figure 12A. The multiple linear regression analysis did not result in any significant interactions in older adults.

In addition to exploring the sequential effects of NREM and REM sleep during the first triplet of the night, we also calculated the "triplet onset latency," or the amount of time following sleep onset before the onset of the first NERM-REM-NREM triplet that was uninterrupted by a period of wake > 2 mins. We found no difference between young and older adults with respect to triplet onset latency (t(40) = -0.594, p = 0.556). Furthermore, over-sleep performance changes were not related to the triplet onset latency for either the young adults (Pearson r = -0.138, p = 0.539), or the older adults (Pearson r = 0.274, p = 0.272).

A median split analysis based on accuracy at immediate recall within the older adult group was conducted, resulting in two "performance" groups: Low Performers (N=31) and High Performers (N=44). Although the low performing older adults did not differ from the high performing older adults with respect to age (t(73) = -0.083, p = 0.934), the Low Performers displayed diminished learning capacities compared to the High Performers, reflected by greater number of loops required to reach criterion at encoding (trend-level, t(20) = 1.934, p = 0.067), lower accuracy at encoding (t(72) = -2.765, p = 0.007), during immediate recall (t(73) = -11.019, p < 0.001), and during the interference round (t(36) = -2.837, p = 0.007).

Unlike the Low Performers, the high performing older adults did not differ from the young adults with respect to the accuracy at immediate recall (t(132.565) = -1.673, p = 0.10).

However, in order to test whether the High Performers behaved more like the young adult group in terms of the underlying sleep-dependent mechanism of visuo-spatial memory consolidation, Pearson correlations between time spent in SWS, NREM and REM in the first half of the night and the Adjusted Score were conducted. Although the Low Performers and the High Performers did not differ with respect to total time or percent time spent in either of the sleep stages, the proportion of time spent in sleep cycles, or the triplet onset latency, for the High Performers alone, greater amounts of REM sleep in the first half of the night alone was associated with performance changes post-sleep (N=10, Pearson r = 0.717, p = 0.020). Additionally, a multiple regression analysis was performed to determine whether the interaction between SC1 NREM, SC1 REM and SC2 NREM significantly predicted the Adjusted Score. For High Performers, there was a significant 3-way interaction between SC1 NREM, SC1 REM and SC2 NREM (β = 5.178, t(9) = 3.890, p = 0.03; Fig. 12B), suggesting that for High Performers, these stages interact in a way that is beneficial to declarative memory consolidation, much like it does in young adults.

CHAPTER IV

DISCUSSION

Cherdieu and colleagues (2013) demonstrated an age-related reduction in sleep-dependent visuo-spatial memory consolidation; they attributed this decline in over-sleep consolidation to changes in sleep parameters occurring with age, namely a reduction in sleep efficiency and percent time spent in sleep cycles. Specifically, they found those individuals (young and older adults alike) that spent a greater amount of time in sleep cycles tended to have a lower forgetting rate. Considering the importance of SWS in declarative memory consolidation, it was surprising however, that Cherdieu and colleagues (2013) did not report any differences between young and older adults with reference to time spent in SWS, nor a significant relationship between SWS and performance changes on the task for either age group. However, their comparisons were made for time spent in SWS *across* the night, while much of the literature suggests that the benefits of SWS on declarative memory is driven by SWS early in the night. Therefore, based on their results, it remained unclear what the underlying causes for the observed age-related reduction in declarative memory consolidation are, particularly whether the active, SWS-mediated consolidation mechanisms persist in older adults.

In the current study, we introduced task-related interference to the visuo-spatial paradigm, allowing us to investigate the stability of the memories following sleep. Furthermore, we focused on the architecture and spectral characteristics of early night sleep in order to better understand how age-related changes in sleep may contribute to changes in declarative memory consolidation. We report a benefit of sleep on visuo-spatial memory consolidation in young and older adults; specifically, sleep actively strengthened memories, rendering them more resistant to subsequent interference compared to an equivalent interval of wake. Importantly, this effect did not appear to

be reduced in older adults relative to young adults. In young adults, the benefit of sleep was driven by time spent in SWS during the first half of the night, and was closely related to delta power density of the SWA during this time. Additionally, although REM sleep early in the night did not confer any benefits to visuo-spatial memory consolidation on its own, bouts of REM sleep nested within, and interacting with, NREM sleep, positively benefited post-sleep performance on the task. We did not find any of these associations between sleep parameters and performance changes over sleep on the visuo-spatial task in older adults. However, high performing older adults (as per their baseline performance on the task), positively benefited from REM sleep during the first half of the night; furthermore, the interaction between NREM and REM bouts early in the night appeared to benefit memory consolidation in high performing older adults much like in the young adults. Thus, the current study demonstrates that sleep continues to play an active role in declarative memory consolidation with age; however, the physiological mechanisms of such a benefit appear to be closely related to cognitive abilities.

Young Adults: Mechanism of Sleep-Dependent Consolidation

In the young adult group, not only was performance post-sleep superior to post-wake, but greater amount of time spent in NREM sleep, specifically SWS, during the first half of the night, was associated with better performance on the task the following morning. These results are in line with previous literature that states that declarative memory consolidation is SWS-dependent, occurring maximally during the early part of the night that is dominated by SWS (Plihal and Born, 1997; Peigneux et al., 2004). Furthermore, Siapas and Wilson demonstrated that SWA across the neocortex is responsible for synchronizing the occurrence of hippocampal sharp-wave ripples and neocortical spindles, thereby facilitating the transfer of hippocampal-dependent memories to long-term neocortical stores. Likewise, we report a strong correlation between performance benefits on the visuo-spatial task and SWA (delta power density) during SWS.

A number of recent studies suggest that SWS does not act independently in declarative memory consolidation; rather, alternating NREM *and* REM bouts contribute to the consolidation of declarative memories in a sequential fashion (Giuditta, 1995; Diekelmann and Born, 2010; Spencer, 2013). To this effect, Grosmark and colleagues (2013) demonstrated that interleaving REM episodes are responsible for a reduction in neuronal discharge rates, as opposed to NREM that is responsible for an *increase* in the same owing to the high-frequency sharp-wave ripples. The current study did not directly measure changes in synaptic strengths occurring over NREM and REM sleep bouts; however, the results of the regression analyses revealed an important role of the sequential effects of NREM and REM bouts during the first NREM-REM-NREM triplet on visuo-spatial memory consolidation. Using variables centered around the mean for the purpose of the analysis, REM sleep in the first sleep cycle was found to benefit memory consolidation if preceded by a normative amount of NREM in the first sleep cycle, and if followed by the same.

Therefore, the benefit of sleep on performance on the visuo-spatial task in young adults is sensitive to the organization of sleep stages, perhaps reflecting three crucial steps in the process: 1) Wilhelm and colleagues (2011) demonstrated that retrieval expectancy in an experimental setup is sufficient to "tag" memories as important, thus prioritizing their consolidation over sleep. Therefore, in the context of the current study, the knowledge that memory for the image locations would be tested during session two may have resulted in prioritization of those memories, and consequently, local upscaling during SWA occurring soon after sleep onset (the first NREM bout) of the neuronal circuits that were activated while encoding those image locations, (Chauvette et al., 2012), 2) Subsequent REM sleep resulted in global downscaling of synaptic strengths in the hippocampus, serving as a "filter," wherein memories that were not

previously tagged as important were depotentiated (Grosmark et al., 2012), and 3) SWA and thalamocortical spindle activity following REM-dependent global downscaling of synaptic strengths, further strengthened and stabilized the memory traces associated with the image locations, ultimately resulting in the reorganization of the memories into neocortical stores from where they could be retrieved more efficiently the following morning.

Age-Related Changes in Performance and Sleep Architecture

Despite training until criterion during the encoding phase, we observed age-related differences in baseline performance. Specifically, older adults required more loops at encoding to reach criterion, a result that is consistent with the aging literature; it has previously been demonstrated that young adults have steeper learning curves compared to older adults with respect to declarative learning tasks (Vakil and Agmon-Ashkenazi, 1997; Davis et al., 2003). Furthermore, older adults in the current study were less accurate at immediate recall than young adults, in line with cross-sectional as well as longitudinal studies that have shown a decline in episodic memory with age, particularly after the age of 60 (Ronnlund et al., 2005). Structural changes in the prefrontal cortex that occur with age have a large impact on higher level executive functions, including those used to successfully perform a task requiring context-dependent memory, as in the case of the visuo-spatial task used here (Cabeza et al., 2000). However, much like the present study, previous sleep-dependent memory consolidation studies have reported similar baseline differences in performance on episodic memory tasks, while demonstrating equivalent levels of over-sleep consolidation between age groups (Aly and Moskovitch, 2010; Wilson et al., 2012).

The young and older adult samples in this study were comparable with respect to subjective sleep quality as measured through the PSQI; however, young adults reported being

significantly sleepier during both morning and evening sessions. This could perhaps be due to a recruitment bias; college students, a population typically known to have inconsistent sleep habits, often complain of sleep disturbances, fatigue and daytime sleepiness (Buboltz, Brown and Soper, 2001; Lund et al., 2010). On the other hand, since older adults have reduced daytime sleepiness despite a concurrent increase in night-time awakenings, it has been previously suggested that aging is associated with reduced sleep pressure (Pace-Schott and Spencer, 2011). Consistent with this notion, we found no correlation between subjective sleepiness prior to bed-time and subsequent time spent in SWS or delta power density of the slow waves for older adults. Considering that SWS was particularly sensitive to sleep pressure in young adults, this result supports previous reports of age-related changes in homeostatic sleep pressure.

Although self-reported sleepiness was lower in the older adults relative to young adults, older participants were significantly sleepier in the evening compared to the morning session. Aging has been linked to a shift towards "morningness", such that older adults are more likely to be morning rather than evening chronotypes (Mecacci et al., 1986). Despite this shift in chonotype however, we do not report any circadian differences in learning between the older adult Wake and Sleep groups with respect to number of loops required to reach criterion, or accuracy at immediate recall.

In order to explore whether the extent of initial learning affects post-wake or post-sleep performance on the visuo-spatial task, we explored the relationship between baseline performance and the Forgetting Rate of the image locations. For young adults, neither the number of loops required to reach criterion during the encoding phase, nor accuracy at immediate recall, was correlated with the Forgetting Rate. However for older adults, accuracy at immediate recall significantly predicted performance changes, such that superior performance at

immediate recall resulted in *increased* forgetting following a period of wake, and following a period of sleep, albeit to a lesser degree. Thus for older adults, initial level of acquisition was associated with an increase in memory decay over time, a finding that is consistent with Davis and colleagues (2003) who demonstrated a greater decline in memory for declarative information over a 24-hr period in older adults compared to young adults.

Sleep physiology was observed to be markedly different in older adults compared to young adults: Firstly, older adults appeared to have a broader distribution of SWS and REM across the night, an observation that has previously been reported in aging populations (Lombardo, 1998) and has been attributed in part by changes in the circadian regulation of sleep (Pace-Schott and Spencer, 2011). A phase advance and reduction in amplitude of the circadian rhythms associated with hormones such as melatonin and cortisol, as well as with core body temperature, have been observed in older compared to young adults (Duffy et al., 2002; Monk, 2005). Sleep stages are particularly sensitive to body temperature, and to hormonal and neurochemical modulations (Porkka-Heiskanen, Zitting and Wigren, 2013); thus, alterations to circadian rhythms could result in changes in sleep physiology and in the disruption of sleep cycles. Indeed, compared to young adults, older adults in the present study and those in the study conducted by Cherdieu and colleagues (2013) spent less time in uninterrupted sleep cycles relative to total sleep time, and had shorter sleep cycle lengths on average. Additionally, we also report age-related changes in the electrophysiological properties of SWS and N2: Older adults had reduced delta and sigma power densities during SWS, as well as reduced sigma power density in N2, corroborating previous reports in aging populations (Cajochen et al., 2006; Carrier et al., 2001; Ohayon et al., 2004).

Owing to the importance of SWA early in the night and of sleep cycle integrity on declarative memory consolidation (Peigneux et al., 2004; Westerberg et al., 2012; Ficca et al., 2000) one would expect to see reduced over-sleep consolidation of visuo-spatial memories for older adults. On the contrary, and in line with previous reports (Aly and Moscovitch, 2010; Wilson et al., 2012), we observed that older adults benefited equally from a period of overnight sleep compared to young adults with respect to performance on the visuo-spatial task. Furthermore, this benefit rendered the memories more resistant to subsequent interference compared to a period of wake, suggesting that the memories were not merely passively protected from daytime interference, but rather that they were actively strengthened.

Older Adults: Mechanism of Sleep-Dependent Consolidation of Visuo-Spatial Memories

In young adults, hippocampal activity varies as declarative information goes through the process of encoding, consolidation and retrieval. Encoding of declarative memories engages the hippocampus (Buzsáki, 1989); however, by means of the hippocampal-neocortical dialogue during SWS, declarative memories increasingly become hippocampal-independent, such that at retrieval, *less* hippocampal activation is associated with superior memory (Takashima et al., 2006). Aging is associated with changes in each of these stages of declarative memory consolidation, resulting in a reduction of the efficacy of the process: 1) Owing to the decline in hippocampal function and integrity with age (Buckley and Schatzberg, 2005), older adults engage the prefrontal and rhinal cortical regions in addition to the hippocampus during encoding of declarative information, as a way of compensating for the hippocampal deficits (Daselaar et al., 2006; Murty et al., 2009), 2) Mander and colleagues (2013) demonstrated that aging is associated with reduced task-dependent hippocampal-neocortical connectivity over time, and 3) Unlike young adults, older adults increasingly use “familiarity-based” neural networks, rather

than “recollection-based” networks at retrieval, resulting in greater engagement of the rhinal cortex, perhaps due to increased top-down frontal modulations (Daselaar et al., 2006). Thus, as a result of age-related changes in the processing of declarative information and in sleep physiology, older adults may simply require multiple bouts of sleep in order to strengthen, stabilize and relocate memories to the same extent as young adults do during a single sleep bout. Specifically, owing to a reduction in the integrity of sleep architecture with age, sleep-dependent declarative memory consolidation may not occur predominantly during the early part of the night as is the case in young adults, but rather across the night, and perhaps across subsequent sleep bouts as well.

Davis and colleagues (2003) demonstrated greater inter-individual differences in episodic recall with increasing age; thus, we explored whether the sleep-dependent mechanisms of visuo-spatial memory consolidation was dependent on the level of initial acquisition. High performing older adults showed the same effects as those reported in the complete older adult sample with the exception of one singular effect: Greater amounts of REM sleep in the first half of the night was associated with *less* forgetting post-sleep. As mentioned previously, REM is indeed beneficial to declarative memory consolidation owing to its synaptic depotentiating effects (Grosmark et al., 2012); older adults may perhaps be more reliant on this property of REM and therefore benefit from having larger bouts of REM early in the night. Additionally, it has been observed that during REM, theta waves facilitate LTP in the hippocampus (Stickgold et al., 2001). SWS and REM-mediated LTP both contribute to episodic memory consolidation in young adults; however, the decrease in SWS with a concurrent increase in REM early in the night in older adults perhaps facilitates a greater dependence on REM-mediated memory consolidation processes of declarative memory consolidation. However, the interaction between NREM and

REM bouts in the first NREM-REM-NREM triplet was one that resulted in a positive effect on visuo-spatial memory in high performing older adults, suggesting that the sequential roles of these stages early in the night continue to provide cognitive benefits in aging for those individuals that have a greater degree of initial learning.

In summary, this study provides evidence for the role of sleep in active memory consolidation of episodic memories, specifically with relation to a visuo-spatial task in young and older adults. The underlying mechanism of memory stabilization appears to depend on initial performance on the task; specifically, the sequential effects of NREM and REM bouts on memory consolidation early in the night are preserved in high performing older adults. However, unlike young adults, they also show a greater dependence on REM sleep early in the night, perhaps reflecting a reduction in the efficacy of declarative memory consolidation owing to the decline in the quantity and physiological properties of SWS.

Limitations and Future Directions

The older adult sample in this study represented a wide age range (50-80 years); since cognition and sleep undergo drastic changes after middle-age, this age range may represent individuals along varying time points on the aging spectrum. To overcome this issue, future studies should concentrate on a smaller and more specific age range. Additionally, a number of young and older adults were found to have high scores on the PSQI, which is indicative of poor habitual sleep quality. In order to avoid this, more stringent enrollment criteria might be used. For instance, by means of sleep diaries, participants' sleep habits could be monitored for a number of days prior to the experiment, and those individuals with inconsistent sleep habits may be excluded from the study.

Finally, no correlation was observed between performance benefits on the task and sigma activity either during SWS in the first half of the night, or during N2 across the night. Perhaps sigma power density alone is not a good measure of sleep spindle activity alone, and spindle frequency and density would be more informative in the context of memory consolidation. In fact, studies have shown a close relationship between spindle frequency/density and declarative memory consolidation (Ruch et al., 2012; Cox, Hofman and Talamini, 2012; van der Helm et al.,
2011), and thus future studies should include these measures in addition to sigma power density.

Conclusions

We compared post-wake and post-sleep performance on a visuo-spatial learning task in healthy young and older adults, specifically with the goal of dissociating the passive and active roles of sleep in declarative memory consolidation. Sleep was found to protect declarative memories from subsequent interference in both young and older adults, providing support for the active, strengthening effects of sleep on declarative memories. For young adults, the data also supported the SWS-dependent mechanisms for memory stabilization and additionally, provided evidence for the sequential role of NREM and REM sleep in this process. Owing to age-related changes in sleep architecture, older adults did not show similar effects. However, older adults
that demonstrated superior learning performance at baseline, appeared to rely on some of the same neural mechanisms for sleep-dependent declarative memory consolidation as young adults. Consequently, future research should focus on understanding how age-related cognitive decline impacts the traditional hippocampal-dependent declarative memory system, and how this might affect sleep-dependent memory consolidation.

APPENDIX A

TABLES

Table 1. Sample descriptive statistics. Handedness, Left-handed = L, Right-handed = R, Ambidextrous = Ambi; Sex, Male = M, Female = F; Unknown = U. *p*-values are provided for Wake vs. Sleep comparisons within each age group.

Descriptive	Young Adults		*p*-value	Older Adults		*p*-value
	Wake	Sleep		Wake	Sleep	
No Interference						
N	24	38		13	24	
Mean Age (SD)	21.52 (2.50)	20.66 (2.37)	0.182	65.54 (7.56)	63.17 (7.44)	0.363
Sex	11M, 13F	18M, 20F	0.973	4M, 9F	9M, 15F	0.878
Handedness	1L,22R,1U	4L,34R	0.402	2L,11R	4L,20R	0.922
Interference						
N	27	30		18	23	
Mean Age (SD)	21.81(3.22)	21.29(2.61)	0.506	67.29(7.27)	66.26(6.93)	0.671
Sex	10M, 17F	12M, 18F	0.667	4M, 14F	6M, 17F	0.54
Handedness	3L,24R	3L,25R,2U	0.963	3L.12R,3U	2L,20R,1Ambi	0.229

Table 2. Mean scores across experimental groups on the questionnaire measures: Pittsburgh Sleep Quality Index (PSQI), Stanford Sleepiness Scale during session one (SSS1) and session two (SSS2). Higher scores indicate poorer outcomes. Also shown here are differences in baseline performance on the visuo-spatial task across experimental groups a per two measures: Number of loops required to reach criterion during the encoding phase, and accuracy during the immediate recall phase (proportion of correct responses). Values in parentheses represent standard deviations.

Experimental Group	PSQI	SSS1	SSS2	Number of Loops at Encoding	Accuracy at Immediate Recall
Young Adults	4.12 (1.87)	3 (1.22)	2.6 (1.44)	2.3 (1.38)	0.7 (0.13)
Wake	3.92 (1.81)	2.96 (1.15)	2.51 (1.42)	2.42 (1.12)	0.7 (0.13)
Sleep	4.26 (1.91)	3.03 (1.28)	2.66 (1.46)	2.24 (1.5)	0.7 (0.13)
Older Adults	3.67 (1.70)	2.38 (1.08)	2.26 (1.05)	3.64 (2.77)	0.64 (0.19)
Wake	4.07 (1.66)	2 (0.95)	2.57 (1.04)	4.43 (3.1)	0.64 (0.12)
Sleep	3.41 (1.68)	2.63 (1.1)	2.07 (1.02)	3.27 (2.63)	0.65 (0.12)

Table 3. Overnight sleep-related statistics and relative distribution of sleep stages across the night and for SWS, NREM and REM during the first two sleep cycles of the night, for young (N=30) and older adults (N=23). Values in parentheses represent standard deviations.

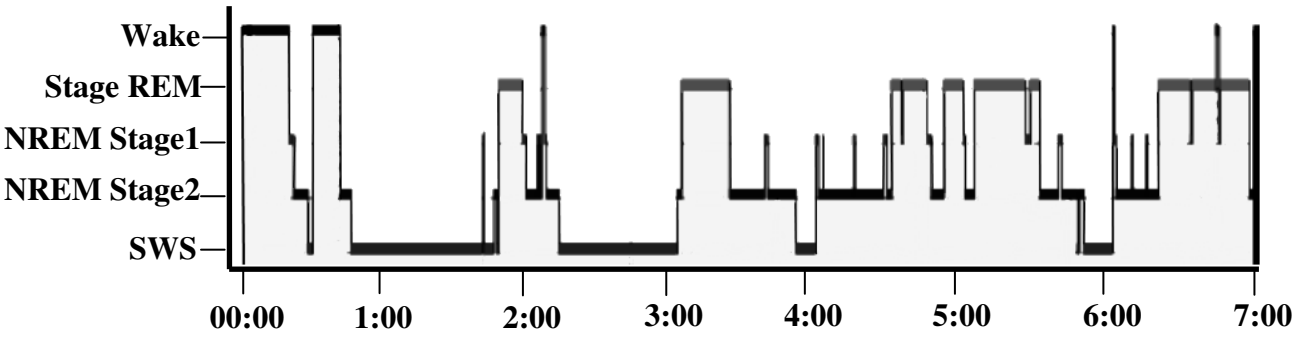
Sleep Measure (mins)	Young Adults	Older Adults	*p*-value
Across the Night			
TST	407.60 (68.02)	396.33 (55.43)	0.521
Sleep Latency	13.33 (16)	11.3 (10.58)	0.601
WASO	17.59 (19.35)	45.7 (33.21)	0.001
Sleep Efficieny (%)	88 (16)	87 (9)	0.764
N1	45.35 (18.46)	53.7 (24.27)	0.161
N2	166.44 (54.89)	155.17 (38.59)	0.406
SWS	124.73 (45.53)	115.48 (33.33)	0.416
NREM	291.17 (58.50)	270.65 (38.98)	0.153
REM	71.08 (25.86)	69.7 (24.89)	0.845
First Sleep Cycle	118.2 (55.76)	96.53 (33.23)	0.131
Total SWS	62.39 (31.09)	46.38 (17.24)	0.044
Total NREM	96.36 (48.36)	71.65 (25.17)	0.043
Total REM	12.18 (7.91)	16.83 (8.85)	0.080
Second Sleep Cycle	116.25 (44.21)	91.33 (27.58)	0.042
Total SWS	33.46 (19.01)	33.08 (16.97)	0.948
Total NREM	111.35 (148.23)	61.44 (21.14)	0.124
Total REM	26.93 (13.09)	21.28 (13.73)	0.182

APPENDIX B

FIGURES

Figure 1. Overnight sleep hypnogram for a typical a) young adult and b) an older adult.

a)



b)

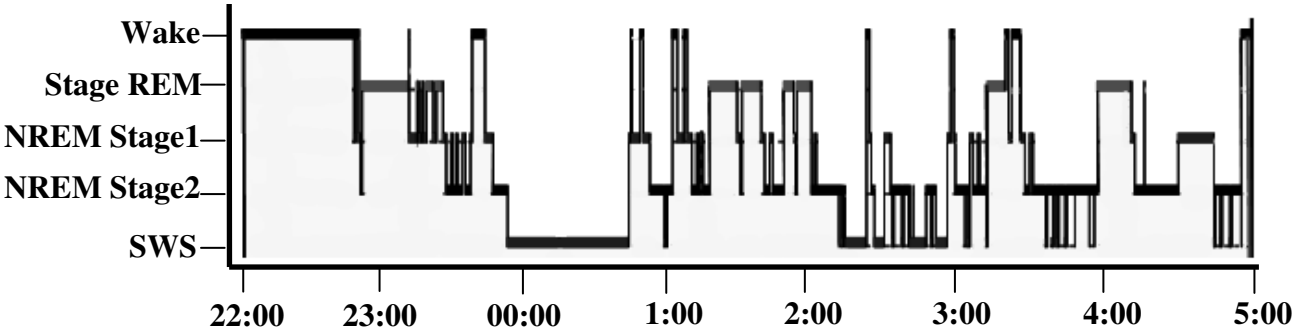


Figure 2. Experimental procedures for the Wake and Sleep groups.

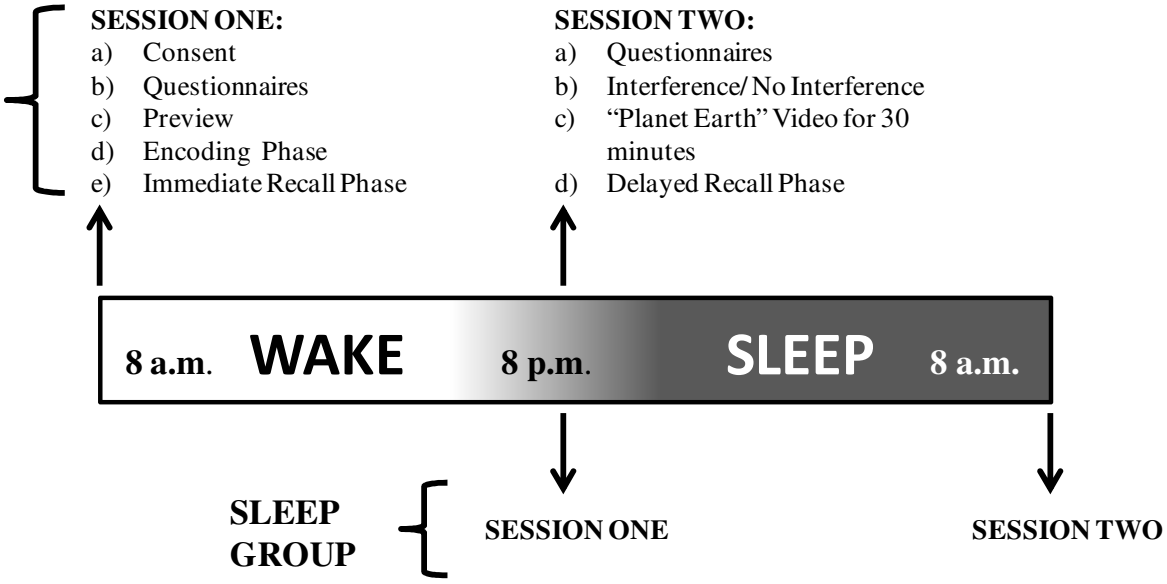
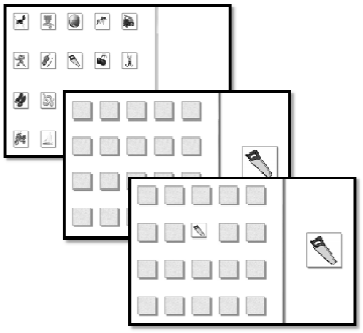


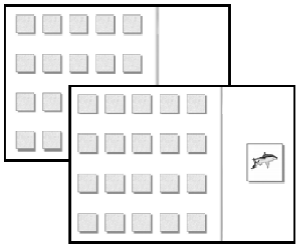
Figure 3. The visuo-spatial task consisted of a) a preview of the images followed by the encoding phase where feedback was provided, b) an immediate recall phase where feedback is not provided and c) an interference round where images are presented in different locations. The delayed recall phase was identical to the immediate recall phase.

a)



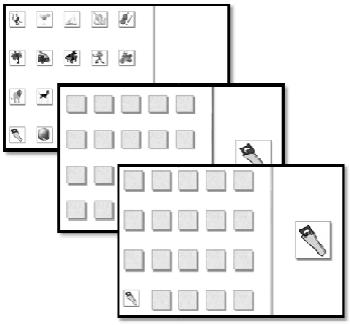
Preview and Encoding Phase

b)



Immediate Recall (and Delayed Recall) Phase

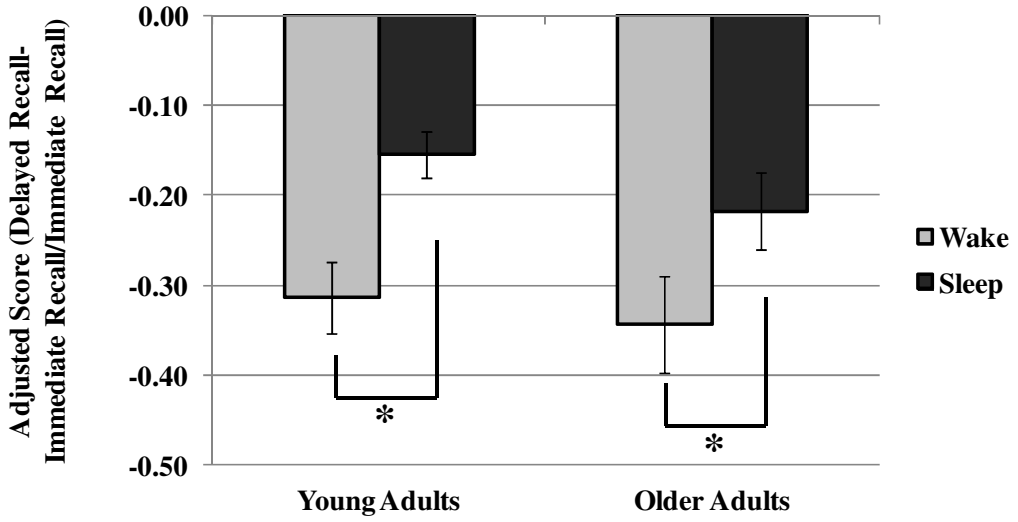
c)



Interference Condition: Preview and Encoding

Figure 4. Behavioral effects of a 12-hour interval of daytime wake or overnight sleep on visuo-spatial memory consolidation in young and older adults for a) No Interference and Interference conditions combined, and b) for the Interference condition alone. Comparisons were made in terms of the Adjusted Score ((Delayed Recall-Immediate Recall)/Immediate Recall). * p-value < 0.05

a)



b)

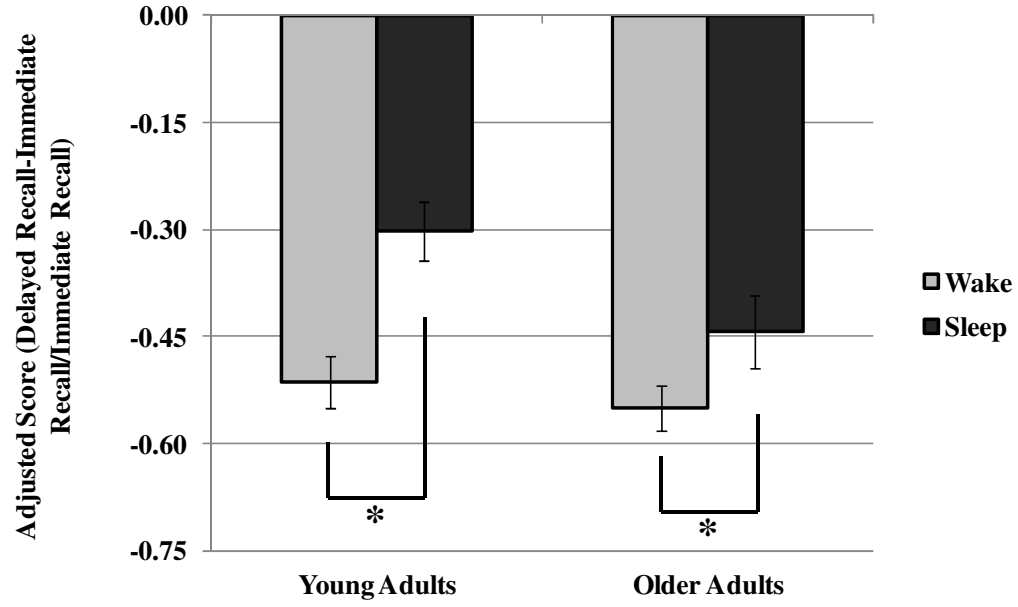
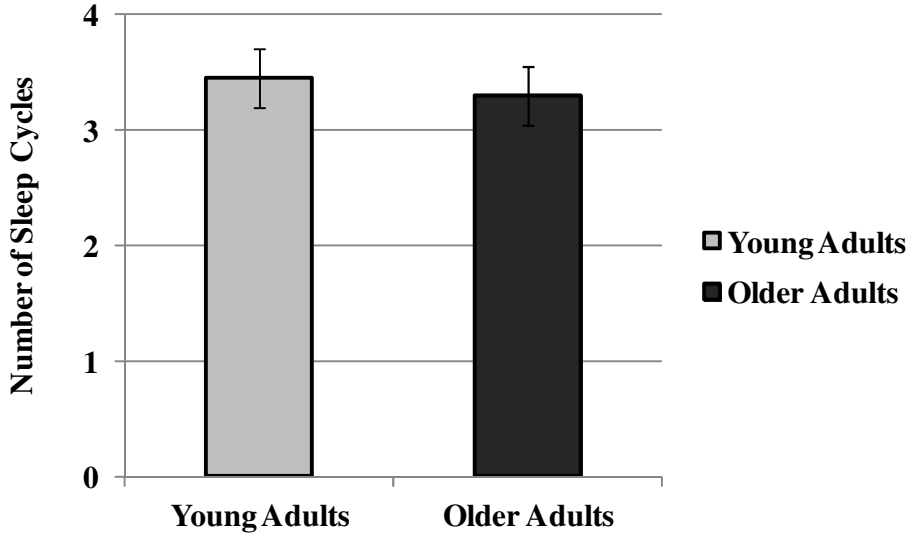


Figure 5. Differences between young and older adults with respect to sleep cycle characteristics: a) Number of sleep cycles and b) Average Cycle Time, Total Cycle Time (TCT) and percent time spent in sleep cycles relative to total sleep time (TCT/TST). * p-value <0.05.

a)



b)

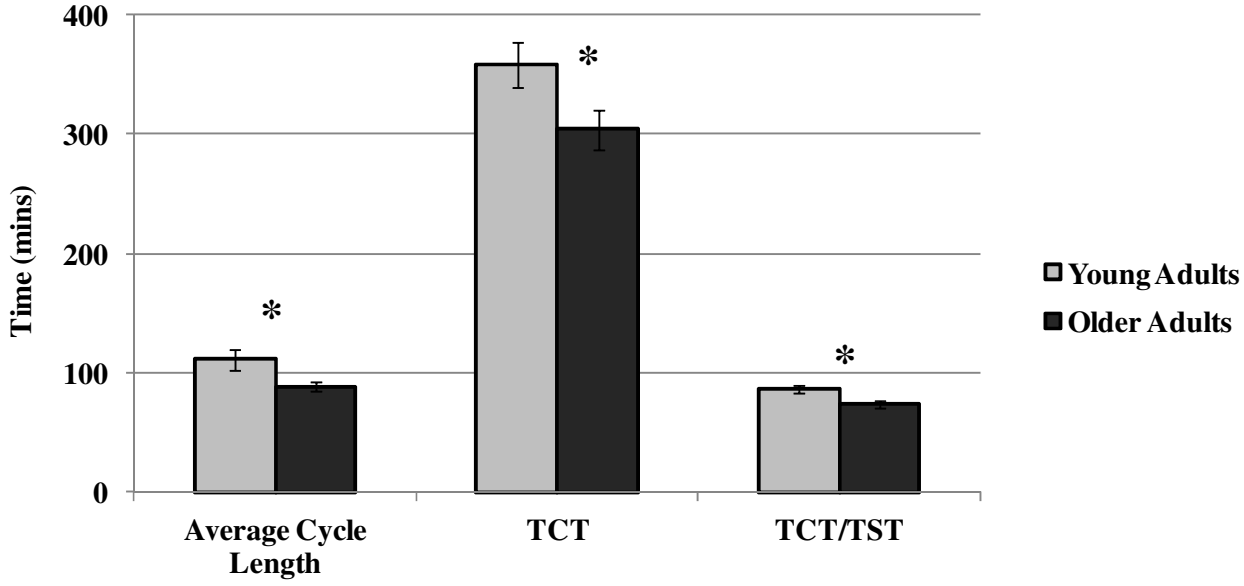
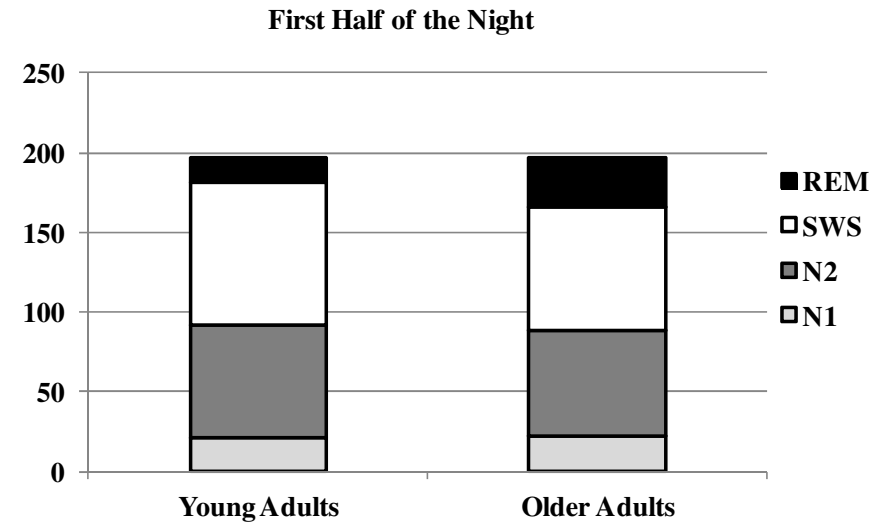


Figure 6. Differences between young and older adults with respect to early vs. late night sleep architecture, namely the amount of time spent in SWS, REM and NREM (N2 and SWS combined) sleep during a) the first half of the night, and b) during the second half of the night. $^{*}p$-value <0.05, # trend-level p-value.

a)



b)

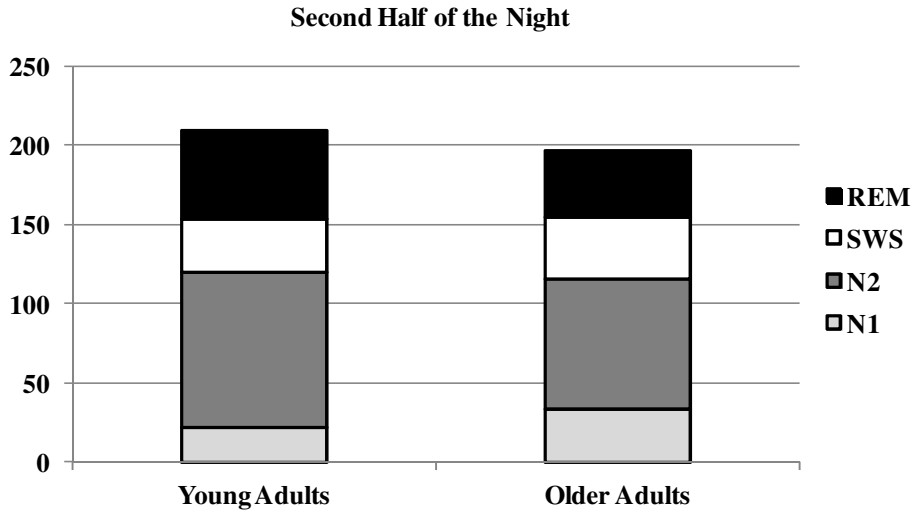


Figure 7. Distribution of sleep stages during the first sleep cycle in young (N=24) and older adults (N=22). $^{*}p$-value <0.05, # trend-level p-value.

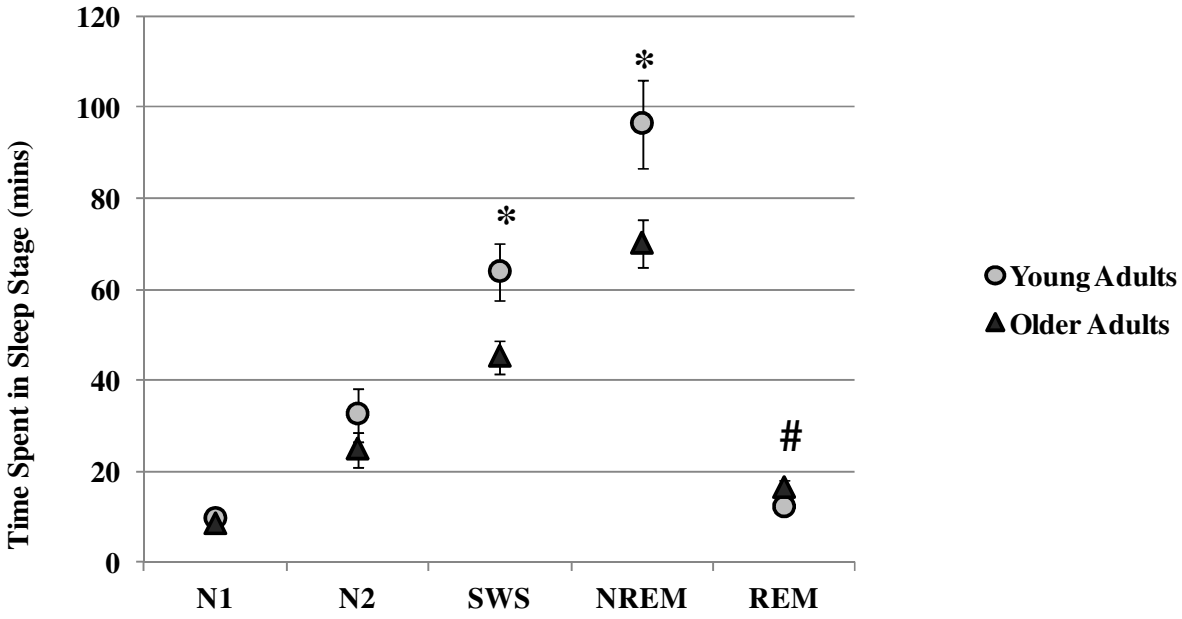
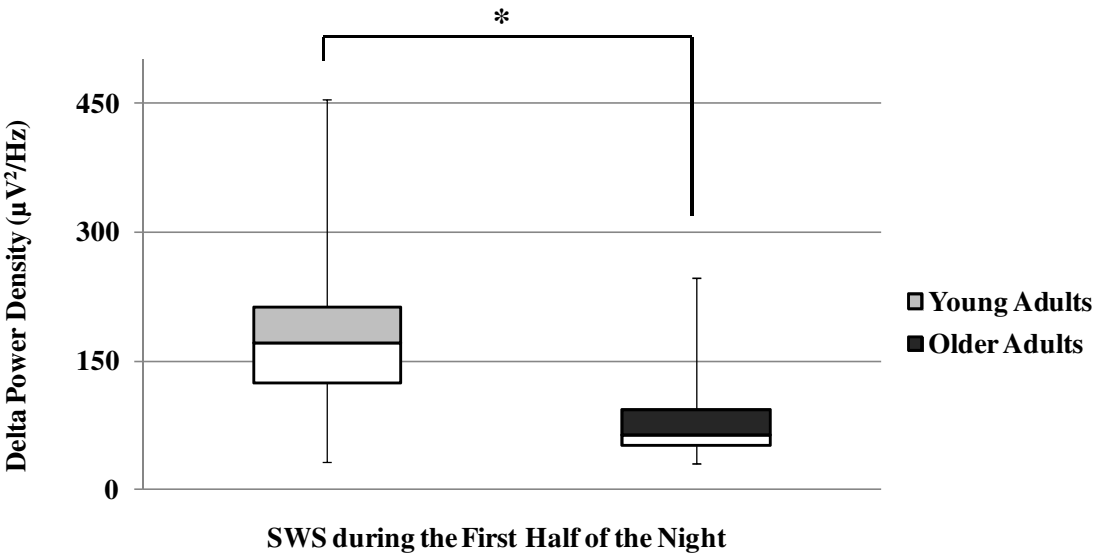


Figure 8. Differences between young and older adults with respect to EEG spectral power during SWS averaged across the central and frontal derivations for a) delta power density, and b) sigma power density. Whiskers represent minimum and maximum values, while the band indicates the median. $^{*}p$-value <0.05.

a)



b)

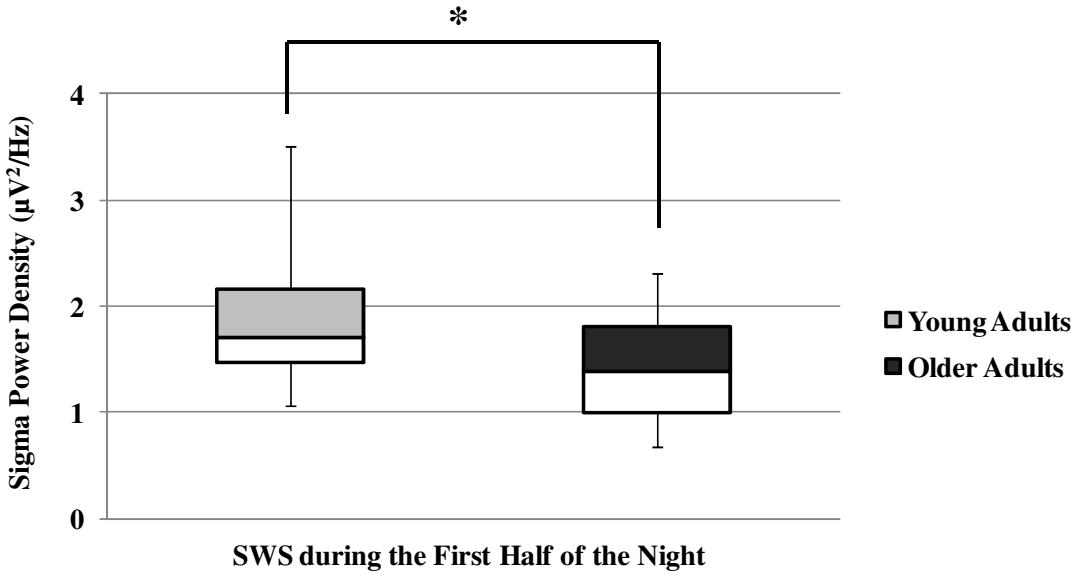


Figure 9. Differences between young and older adults with respect to sigma power density during N2 across the night averaged across the central and frontal derivations. Whiskers represent minimum and maximum values, while the band indicates the median. $^{*}p$-value <0.05.

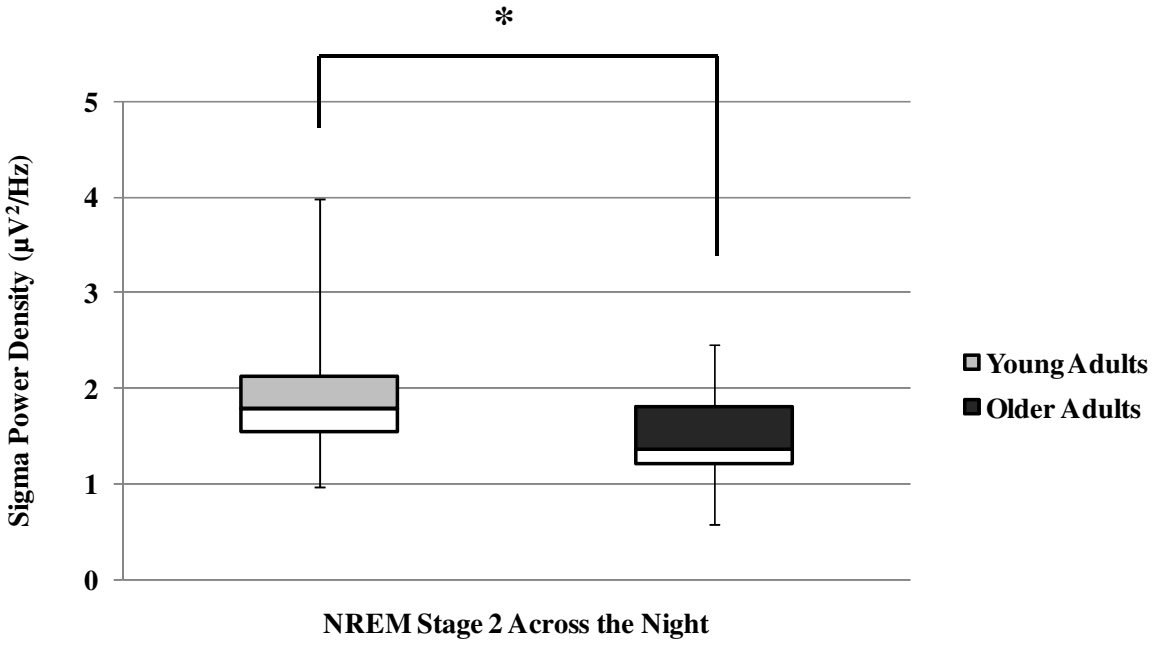
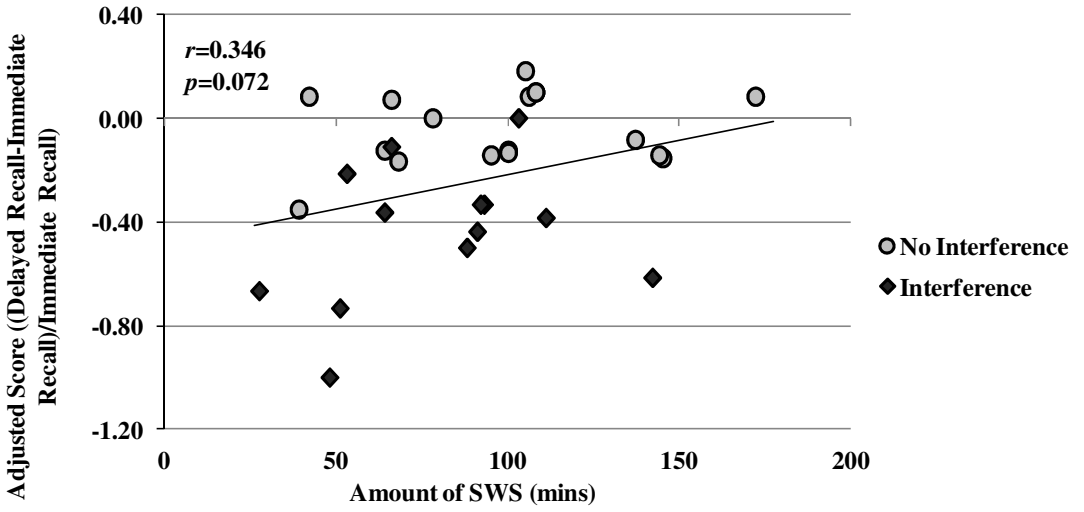


Figure 10. Relationship between post-sleep changes in performance on the visuo-spatial task (Adjusted Score) and time spent in a) SWS during the first half of the night, and b) NREM during the first half of the night, for young adults (N=30).

a)



b)

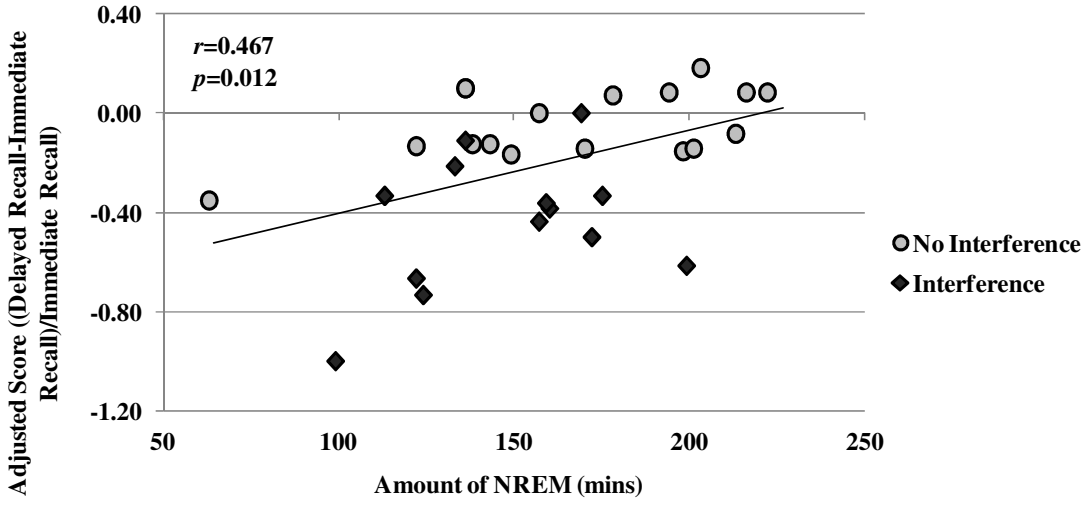


Figure 11. Relationship between delta power density during SWS for the first half of the night and post-sleep performance changes on the visuo-spatial task in young adults.

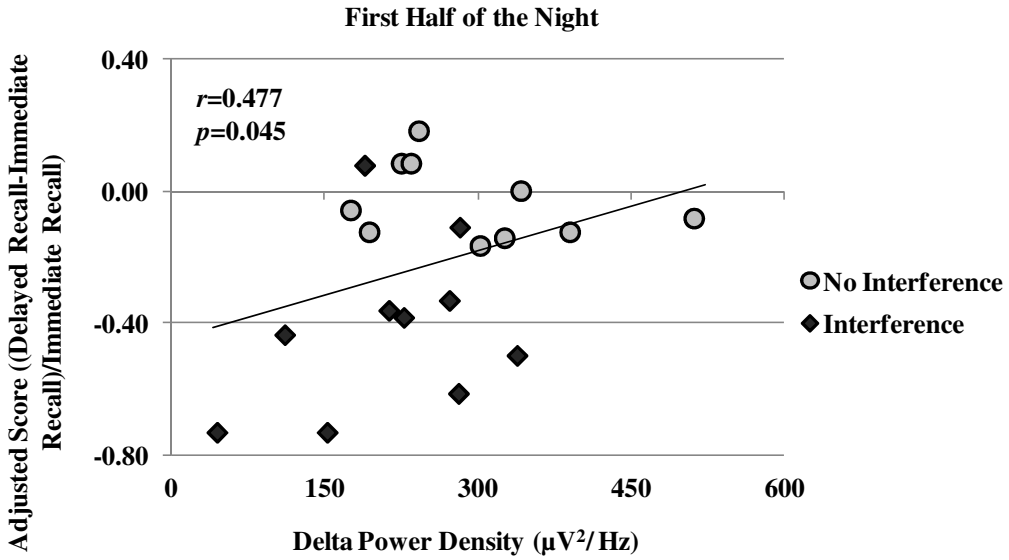
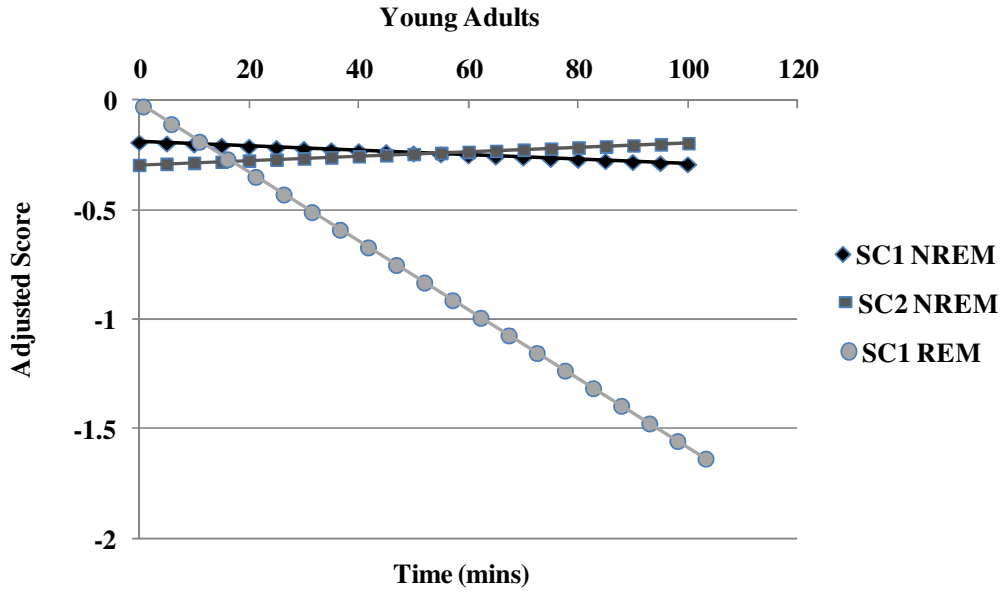
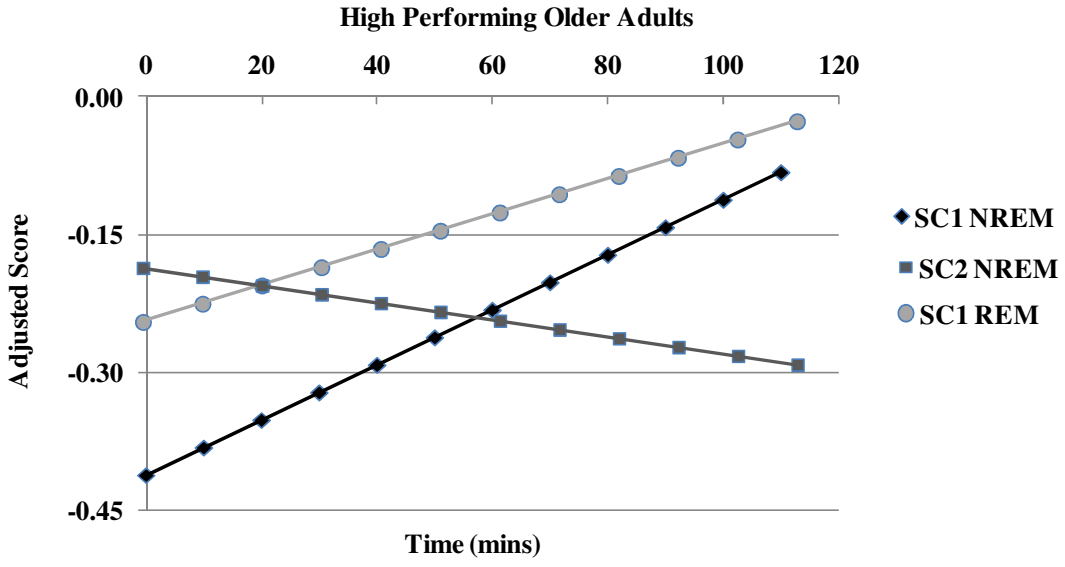


Figure 12. Graphical representation of the results of the multiple regression analysis showing the interaction between NREM sleep and REM sleep during the first sleep cycle (SC1 NREM and SC1 REM respectively), and NREM sleep during the second sleep cycle (SC2 NREM) in a) young adults, and in b) high performing older adults. The intercept and slope for each regression line were used to create a simulation of the relationship between Y and X. Thus, data points do not represent actual data from the young and older adult samples.

a)



b)



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