NREM Sleep Spindles and Slow Wave Sleep in Younger and Elderly Women: an investigation of their influence on Declarative Memory Consolidation

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COMPULSORY DECLARATION This work has not been previously submitted in whole, or in part, for the award of any degree. It is my own work. Each significant contribution to, and quotation in this dissertation that comes from the work, or works, of other people has been attributed, and has been cited and referenced.

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Abstract

Previous research shows that slow wave sleep (SWS) and sleep spindles play an essential role in declarative memory consolidation. However, this role is not well understood in the ageing women. With advancing age, SWS and sleep spindles undergo significant decreases in duration and density, while there is a simultaneous decline in declarative memory. The primary aim of this research was to investigate the relationship between sleep architecture, sleep spindle activity, and declarative memory retention in two groups of women: 14 younger (M = 20.5±1.28 years) and 14 older females (M = 63.14±2.03 years). Participants underwent polysomnography on a baseline and experimental night and encoded a list of word-pairs of graded difficulty on the experimental night. Word-pair type included integrative, concrete and low concrete measures. Memory retention was then assessed pre- and post-sleep. Our results confirm the characteristic age-related decrease in SWS and sleep spindle activity in older adults. In the older group, SWS positively correlated with concrete word-pair retention, while spindle density and the number of spindles positively correlated with overall retention. In addition, the percentage change in spindle density, slow and fast density, and fast intensity from baseline to experimental night positively correlated with low concrete word-pairs. Finally, in the younger group, the number of spindles positively correlated with low concrete word-pairs and the percentage change in fast and slow spindle intensity correlated with concrete word-pair retention. Although younger women recalled more word-pairs than older women in both conditions, memory retention was largely preserved in both groups after sleep.

Keywords: Slow Wave Sleep, Sleep Spindles, Declarative Memory, Sleep Architecture, Ageing, Sleep-dependent memory consolidation

Introduction

An important function of sleep is to consolidate newly formed memories encoded during periods of wakefulness into the long-term memory system (Diekelmann & Born, 2010). This consolidation process is achieved during sleep via repeated reactivation of recently acquired information and occurs as a dialogue between brain systems that include the hippocampus, thalamus and neocortex (Born & Wilhelm, 2012; Walker, 2008). Through this continuous reactivation, salient memories are strengthened and assimilated into long-term memory systems.

The *Active System Consolidation* model proposes that the dialogue between brain systems that mediate memory consolidation is reliant on specific electrophysiological characteristics of Non-Rapid Eye Movement (NREM) sleep (Diekelmann & Born, 2010). Specifically, these electrophysiological processes include the interaction between sleep spindles, hippocampal ripple activity and slow brain oscillations (Ellenbogen, Payne, & Stickgold, 2006; Fogel & Smith, 2011; Stickgold & Walker, 2007). Sleep spindles in NREM stage 2 and SWS in particular, have become a focus in sleep research, as recent evidence suggests that these waveforms may play a significant role in the transfer of sleep-dependent declarative memory consolidation into long-term memory systems (Fogel & Smith, 2011).

One of the main limitations of sleep research is its over-reliance on samples made up predominantly of young adults. Due to these methodological limitations, its often difficult to generalize findings to older and elderly adult populations (Harand et al., 2012; Hornung, Danker-Hopfe, & Heuser, 2005). Age is important factor to consider because substantial changes in both sleep architecture and declarative memory accompany the ageing process. For instance, there are typical reductions in sleep spindles with advancing age (Martin et al., 2013; Wolkove, Elkholy, Baltzan, & Palayew, 2007), which is accompanied by a deterioration in declarative memory (Cherdieu, Reynaud, Ulrich, & Mazza, 2014). Research also suggests that elderly adults tend to experience more disrupted sleep compared to younger adults (Conte et al., 2012). Since healthy sleep is thought to promote memory consolidation and act as protective factor against cognitive impairment, such disrupted sleep in older adults may exert negative effects on the sleep-dependent memory consolidation process (Scullin & Bliwise, 2015). Given the changes in sleep and memory that accompany the ageing process, much of the research on younger participants may have poor generalizability to older populations. Further research is necessary to help understand the impact that ageing has on the processes that influence sleep-dependent memory consolidation.

Brief Definitions of Sleep and Memory

Sleep may be conceptualized as a natural and reversible state of reduced responsiveness to external stimuli (Rasch & Born, 2013). In mammals, it is divided into two main types: Rapid Eye Movement (REM) sleep and Non-REM (NREM) sleep (Carskadon & Dement, 2011; Walker, 2008). During a period of sleep, NREM and REM alternate in approximately 90-minute cycles (Zillmer et al., 2008). NREM sleep is further divided into four stages of decreasing cortical arousal and increasing neural synchronicity (Spriggs, 2002; Zillmer, Spiers, & Culberston, 2008). The four stages of NREM sleep consist of particular electroencephalographic (EEG) waveforms that relate to different cortical states of arousal and neural synchronicity (Diekelmann & Born, 2010; Rasch & Born, 2013). Specifically, stage 1 and 2 are light forms of sleep where arousal thresholds are low. Stage 3 and 4, on the other hand, are considered deep forms of sleep, collectively referred to as Slow Wave Sleep (SWS). Therefore, NREM sleep is characterised by stage 1, 2 and SWS.

Stage 1. Stage 1 is considered to be a transitional state between sleep and wakefulness (Carskadon & Dement, 2011). This lasts for a couple of minutes. Disrupted sleep is usually associated with an increased percentage of this stage (Carskadon & Dement, 2011). For example, increased stage 1 sleep is seen in ageing adults who tend to experience disrupted sleep (Ohayon, Carskadon, Guilleminault & Vitiello, 2004).

Stage 2. Stage 2 is a light form of sleep and contains k-complexes and sleep spindles (Carskadon & Dement, 2011). Sleep spindles are brief and recurring bursts of brain activity that last for at least 0.5 seconds and are thought to occur as a result of thalamocortical activity (Fogel & Smith, 2011; Gruber et al., 2013; Lüthi, 2014). Sleep spindles are traditionally defined as activity in the frequency range of 12-16Hz (Fogel & Smith, 2011), but can include a frequency range of 10-16Hz (Diekelmann & Born, 2010), although this may vary slightly across different studies. For example, Bódizs, Körmendi, Rigó, and Lazar (2009) used a range of 9-16Hz to identify sleep spindles. These researchers argue that inter-individual differences in sleep spindle activity exist and that traditional measure can be limiting. Sleep spindles are thought to play a role in sleep maintenance and sleep-dependent memory consolidation (Lüthi, 2014). K-complexes, on the other hand, are waveforms characterised by an acute decrease in amplitude followed by an increase in amplitude, and are believed to play a role in information processing during sleep (Fogel & Smith, 2011).

SWS. This stage predominantly contains slow brain oscillations (0.5-1Hz) and delta wave activity (1-4Hz) (Fogel & Smith, 2011; Steriade, 2006). Sleep spindles also occur in SWS (Spriggs, 2002). In a typical night of sleep, early sleep is SWS-rich (Peigneux, Laureys,

Delbeuck, & Maquet, 2001; Rasch & Born, 2013). During this stage, heart rate and breathing slows down (Fogel & Smith, 2011).

REM. REM sleep, also known as paradoxical sleep, contains heightened cortical arousal, ocular saccades and muscle atonia (Dang-Vu et al., 2006; Peigneux et al, 2001). During the night, REM sleep dominates during the second half of the night as it intensifies and lengthens (Peigneux et al, 2001). This is though to be a result of differing neuromodulator activity in REM sleep compared to NREM sleep.

During NREM sleep, cholinergic activity is suppressed while aminergic activity continues to occur (Rasch & Born, 2013). This means that acetylcholine and cortisol activity is reduced and noradrenergic and serotonergic activity is operational, although to a lesser extent than during wakefulness (Diekelmann & Born, 2010). During REM, aminergic activity is lowered whereas cholinergic activity is heightened to similar or higher levels than during wakefulness (Rasch & Born, 2013). This heightened cholinergic activity results in amplified acetylcholine and cortisol activity (Diekelmann & Born, 2010). NREM and REM, with differing neuromodulator activity and specific sleep-related field potentials, are thought to play important roles in memory consolidation (Diekelmann & Born, 2010).

*Memory***.** Memory can be divided into two main types: declarative and non-declarative memory. Non-declarative memory is comprised of memories that do not necessarily require conscious recollection and include memories that are accessible through action and behaviour (Diekelmann, Wilhelm, & Born, 2009). An example of non-declarative memory is procedural memory, which is memory for learning actions, habits, and skills (Walker, 2008). Non-declarative memory usually relies on implicit learning and a diverse network of brain systems for processing, including the cerebellum, pons and striatum (Zillmer et al., 2008).

Declarative memory, on the other hand, refers to the type of memories that are accessible through conscious recollection (Fogel & Smith, 2011). These can further be subdivided into episodic memory, which is memory of events that occur in a spatio-temporal context, and semantic memory, which relates to memory of factual information (Walker, 2008). Memories consolidation occurs over a long period of time and usually relies on brain systems that mainly include, but are not limited to, the hippocampus, thalamus and neocortex (Fogel & Smith, 2011). Over this long period of time, memories are thought to become stable once involvement of the thalamus and hippocampus reduces and the memories are permanently stored in the neocortex. Although the exact mechanism of the memory consolidation process is not entirely understood, recent evidence suggests that sleep is critical

for this process (Fogel & Smith, 2011). There is also some evidence to suggest that declarative memory difficulties associated with older age (Harand et al., 2012) and may be partly understood by alterations in the sleep-dependent memory consolidation process (Rasch & Born, 2013).

Sleep-Dependent Memory Consolidation: A Role for NREM stage 2 and SWS

Sleep-dependent memory consolidation refers to the process where new memories which were encoded during wake are subsequently strengthened during sleep (Diekelmann & Born, 2010). Research suggests that NREM sleep is necessary for, and actively plays a role in the successful consolidation of declarative memory consolidation (Born & Wilhelm, 2012; Griessenberger et al., 2012). Evidence for sleep-dependent memory consolidation comes from a number of studies that indicate that sleep may enhance the retention of and improve performance on a variety of declarative and non-declarative memory tasks (Dang-Vu et al., 2006; Diekelmann et al., 2009; Gais & Born, 2004). For example, participants who were allowed to sleep after memorizing paragraphs of text (Wagner, Gais & Born, 2001) or completing a face recognition task (Nishida, Pearsall, Buckner, & Walker, 2009), showed improved retention and better memory performance compared to participants who did not sleep. In addition, sleep interference studies highlight that participants with disrupted sleep show relatively poor performance on tasks such as learning sequences of faces and objects and recall on word-pair tasks compared to those who have uninterrupted sleep (Ellenbogen, Hulbert, Jiang, & Stickgold, 2009; Griessenberger et al., 2012; Wilhelm et al., 2011). In another example, a study by Wagner and colleagues (2004), show that sleep may enhance insight. Participant who were allowed to sleep could solve a mathematical problem that they could previously not solve. Control participants who were added to a wake group showed no improvement on solving the problem. This contributes to the view that sleep can qualitatively change previously encoded information and create stronger and more stable connections (Diekelmann & Born, 2010).

Declarative sleep-dependent memory consolidation is thought to occur through a two-stage process called *Active System Consolidation* (Born & Wilhelm, 2012) and the *Synaptic Homeostasis Hypothesis* (Tononi & Cirelli, 2014). The *Synaptic Homeostasis Hypothesis* proposes during wakeful learning synaptic potentiation occurs. Synaptic potentiation is linked to SWS regulation; such that higher amount of prior wakefulness is promotionally related to higher amounts of SWS. During SWS, synaptic downscaling occurs to restore synaptic homeostatic is thought to be linked to cognitive performance (Tononi & Cirelli, 2014). On

the other hand, in the *Active System Consolidation* model (see Figure 1.A), memories temporarily stored in the hippocampus during wakefulness are continuously reactivated during sleep to eventually become integrated into the long-term system (Dang-Vu et al., 2006). Evidence for this comes from neuroscientific studies that show neural activity observed in the hippocampus during wakeful learning also occurs during NREM sleep (Stickgold & Walker, 2007). This continual reactivation is thought to be a dialogue between brain systems that involve the hippocampus, thalamus and the neocortex in which salient memories are strengthened and redistributed (see Figure 1.A; Born & Wilhelm, 2012; Stickgold & Walker, 2007).

The dialogue between the hippocampus and the neocortex during SWS is mediated by an interaction between the slow oscillations, sleep spindles and hippocampal ripple activity as illustrated in Figure 1.B (Born & Wilhelm, 2012). Slow oscillations regulate the dialogue by grouping together neuronal activity in two ways (Andrillon et al., 2011; Steriade, 2006). Firstly, activity is grouped into hyperpolarizing down-states, which is associated with reduced spindle activity and synaptic firing in intra-cortical and thalamocortical networks (Clemens et al.,2007; Steriade, 2006). Secondly, activity is grouped into depolarizing up-states, which is associated with enhanced spindle activity (Born & Wilhelm, 2012; Clemens et al., 2007). It is during these depolarizing up-states that sleep spindles also synchronize with hippocampal ripples to cause spindle-ripple events (Clemens et al., 2011). A spindle-ripple event is where hippocampal sharp-waves attach to the trough of sleep spindle waves (see magnified circle in Figure 1.B). Once paired together, spindle-ripple events then reverberate to the surrounding neocortex. This is thought to facilitate the transfer of memory from the short-term (hippocampus) to the long-term (neocortex) memory system (Born & Wilhelm, 2012).

In addition to potentially facilitating the transfer of memories, sleep spindles are thought to play a role in synaptic plasticity. It has been proposed that once spindle-ripple events reach the surrounding neocortex, they initiate an influx of calcium ions that will set the stage for long-term potentiation (LTP) during REM sleep (Diekelmann & Born, 2010). LTP is an important process which is known to be essential to synaptic plasticity (Fogel et al., 2012). Synaptic plasticity is important for the re-organization and re-structuring of previously encoded information into the long-term memory system (Dang-Vu et al., 2006).

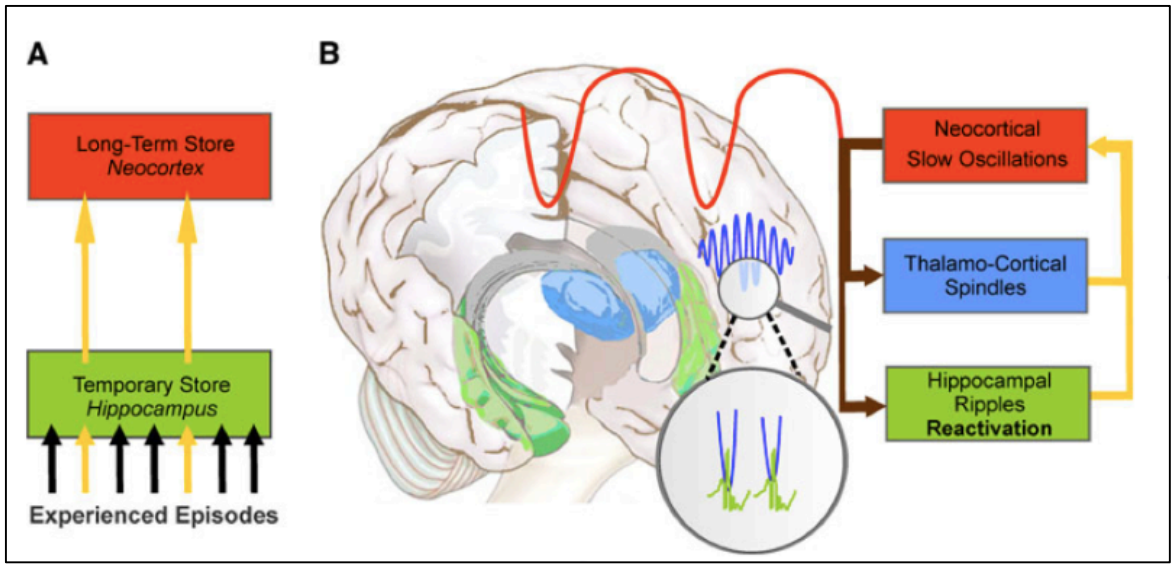

Figure 1: The hippocampal-to-neocortical dialogue. **A:** During NREM sleep, memories temporarily stored in the hippocampus are transferred to the long-term store in the neocortex. **B:** The dialogue involves the interaction between the slow oscillations, sleep spindles and hippocampal ripples to create *spindle-ripple events* (magnified circle). From: Born, J., Wilhelm, I. (2012). System consolidation of memory during sleep. Psychological Research, 76, 192-302.

However, the role of sleep spindles may not be exclusive to SWS. A large portion of studies report that spindle density in stage 2 of sleep is associated with overnight declarative memory consolidation (e.g. Genzel, Dresler, Wehrle, Grözinger, & Steiger, 2009; Gruber et al, 2015; Schabus et al., 2004; Van der Helm et al., 2011). For example, Fogel and Smith (2006) found that the duration of stage 2 and sleep spindle increased after a period of learning. In this study, no other sleep stage showed any changes. In contrast, other studies find overnight declarative memory enhancement and preservation to be associated with sleep spindles in SWS (e.g. Holz et al., 2012; Tamminen, Payne, Stickgold, Wamsley, & Gaskell, 2010). For example, one study found that overnight declarative memory enhancement and preservation to be exclusively linked to spindles in SWS (Cox, Hofman, & Talamini, 2012). The researchers argued that according to the *Active System Consolidation* model, consolidation is exclusive to SWS because of the grouping effect slow oscillations have on sleep spindles and hippocampal ripple activity (Bergmann et al., 2012; Clemens et al., 2011).

As a result of these variations, sleep spindle studies vary in how they measure sleep spindle activity, and in which stage of sleep they measure it. For example, some studies make exclusive use of stage 2 sleep spindles from night-to-night or in the first half of sleep. This is motivated by the fact that sleep spindle increases in stage 2 of sleep from a control night to a learning night (Schabus et al. 2008) and earlier sleep versus later sleep (Gruber et al., 2015)

were specifically linked to memory retention. Furthermore, other researchers make use of all NREM sleep spindles. For example, Hoedlmoser et al. (2014) included all NREM sleep spindles (that is, stage 2 and SWS) to assess changes the relationship between memory performance changes in spindle activity.

In sum, there is evidence to suggest sleep spindles may play a role in stage 2 and SWS. This may be understood through two reasons. Firstly, some researchers have argued that the k-complexes in stage 2 may be akin to a slow oscillation seen in SWS (Steriade, 2006). This may imply that sleep-dependent declarative memory consolidation could rely on both stage 2 and SWS. Secondly, Genzel and colleagues (2014) propose that the replay described in *Active System Consolidation* occurs throughout NREM sleep. Specifically, they suggest that reactivation and replay is dominant in stage 2 while synaptic downscaling of weaker memories occurs in SWS (Genzel, Kroes, Dresler, & Battaglia, 2014). Although reactivation continues in SWS, salient memories would not undergo erasure and continue to consolidate. Thus, sleep spindles in stage 2 and SWS may have a role to play in these processes, but further research is required to investigate the effects of NREM sleep spindles, slow oscillations and hippocampal ripple activity given the sometimes different findings in previous research (Fogel & Smith, 2011; Genzel et al., 2014).

Finally, sleep spindle studies can also vary in the agreement about what aspect of sleep spindle activity is responsible for memory consolidation. For example, Schabus et al. (2008) note that the absolute value of spindle activity measured in a subject on any given night may reflect an individual's learning ability, while the relative increase from night to night may be linked to memory consolidation following elaborate encoding. Other researchers measure sleep spindle intensity instead of the traditional spindle density (e.g. Gruber et al., 2015, Hoedlmoser et al., 2014). Spindle intensity is measured as the product of the mean amplitude and the mean duration of sleep spindles, while sleep spindle density is measured as the number of spindles per minute (Hoedlmoser et al., 2014). Thus further research is required to investigate what aspects of sleep spindle activity are important for memory consolidation (Fogel & Smith, 2011).

The Significance of the Sleep Spindle in Memory Consolidation

Sleep spindle studies consistently find that improved retention on declarative memory tasks is positively correlated with sleep spindle in stage 2 and SWS across the night (Lüthi, 2014). For example, Gais, Mölle, Helms and Born, (2002) found that spindle density in stage 2 was positively correlated with recall on a word-pair associated task of 336 unrelated words.

Similarly, Schabus et al. (2004) found only those participants who showed an increase in spindle intensity during a period of stage 2 sleep displayed an enhancement in memory performance on a word-pair associate recall task. In another example, Tamminen et al. (2010), found that NREM sleep spindles were positively associated with the lexical integration of newly-learned words. These results are consistent among other declarative tasks, such as face-scene associations or item-context associations (e.g. Bergmann, Mölle, Diedrichs, Born, & Siebner, 2012; Marshall, Helgadottir, Mölle, & Born, 2006; Van der Helm, Gujar, Nishida, & Walker, 2011).

There is some evidence to suggest that sleep spindles may play an active role in sleep-dependent memory consolidation. For instance, a study by Mednick et al. (2013) where stage 2 sleep spindle density was selectively enhanced or suppressed, found that experimentally manipulating these waveforms had a significant effect on declarative memory. Sleep spindles were manipulated by using two different hypnotics, zolpidem and sodium oxybate, to respectively increase or decrease sleep spindles density (Mednick et al., 2013). Selectively increased stage 2 sleep spindle density was associated with significantly superior performance on verbal recall compared to the performance of the group with selectively decreased spindle density. In a related study, Kaestner, Wixted, and Mednick (2013), using the same experimental hypnotics, found a similar trend but using a different declarative task.

Sleep spindle activity may also be related to an individual's learning abilities. For instance, high spindle densities have been linked to cognitive abilities such as working memory and planning (Chatburn et al., 2013; Hoedlmoser et al., 2014; Schabus et al., 2006; Schabus et al., 2008). In contrast, abnormalities in sleep spindle amplitude have been associated with brain atrophy and mental retardation (Spinoza & Garzon, 2007). However, the relationship between cognitive ability, traditionally measured by IQ, and sleep spindles is inconsistent and remains uncertain (Fogel, Smith, & Beninger, 2010; Gruber et al., 2013; Tucker & Fishbein, 2008).

Research indicates that two types of sleep spindles exist: slow spindles, with a frequency range of 11-13Hz, occur in the frontal regions; and fast spindles, with a frequency range of 13-15Hz, occur in central EEG regions (Barakat et al., 2011). These two types of sleep spindles are believed to originate from separate origins in the brain and may play different roles in the memory consolidation process (Fogel & Smith, 2011). Although the different roles of these spindle types are not yet clear, some research suggests that fast spindles are associated with overnight performance gain on motor sequence-learning (MSL) task (Barakat et al., 2011), are enhanced after learning a visuomotor skill (Tamaki, Matsuoka,

Nittono, & Hori, 2009), and have been found to synchronize to the depolarising slow oscillations after learning a word paired associate task (Mölle, Bergmann, Marshall, & Born, 2011). In the study by Mölle et al. (2011), slow spindles were found to follow fast spindles and did not synchronise with the slow oscillations. From this study, the role of slow spindles is not clear. It is also not clear whether spindle features, such the amplitude and duration, play a role in sleep-dependent memory consolidation (Fogel & Smith, 2011).

In general, sleep disruption has shown to impair cognition (Dang-Vu et al., 2006; Diekelmann & Born, 2010; Guzman-Marin & McGinty, 2006; Vassalli & Dijk, 2009). For example, sleep disruptions are often comorbid with psychiatric and neurodegenerative diseases (Wulff, Gatti, Wettstein, & Foster, 2010). Recent research has begun to focus on the specific aspect of sleep that might be involved in impaired cognition from disrupted sleep. There is some evidence to suggest that there is a role for sleep spindle activity in cognitive functioning. For example, sleep spindle deficits have been implicated in impaired cognitive processes. In a study by Latreille et al., 2014, they reported that sleep spindle abnormalities were associated with the development of dementia in a sample of patients with Parkinson's Disease. In another example, patients with schizophrenia tend to exhibit reduced sleep spindle density (Ferrarelli, et al., 2010; Ferrarelli, et al., 2007) and also show poor performance on memory tasks after a night of sleep compared to healthy individuals (Manoach & Stickgold, 2009; Wamsley et al., 2012).

There is also some evidence to suggest that sleep spindles may reflect, to some degree, the integrity of the thalamocortical network and may eventually be used a biomarker for healthy brain functioning (Leresche, Lambert, Errington, & Crunelli, 2012; Limoges, Mottron, Bolduc, Berthiaume, & Godbout, 2005). As a result, sleep spindles may have potential clinical significance for patients and individuals who experience sleep spindle deficits and poor memory (De Gennaro & Ferrara, 2003; Urakami, Loannides, & Kostopoulos, 2012). This has led researchers to include investigations into the impact of sleep spindle deficits in vulnerable populations, such as the elderly. This is important as healthy sleep is thought to promote sleep-dependent memory consolidation and may act as a potential protective factor against cognitive impairment often associated with the ageing population (Scullin & Bliwise, 2015).

Sleep-Dependent Declarative Memory Consolidation in the Elderly

Increasing age is associated with changes in sleep spindle activity (Fogel & Smith, 2011). Specifically, in older adults sleep spindle density, amplitude and duration show a

marked decrease, while there is a slight increase in spindle frequency (Crowley, Trinder, Kim, Carrington, & Colrain, 2002; Crowley, 2011; Fogel et al., 2012; Knoblauch et al., 2005; Martin et al., 2013; Nicolas, Petit, Rompré, & Montplaisir, 2001). These reductions become noticeable from the age of thirty (Backhaus et al, 2007; Martin et al., 2013).

The elderly experience relatively more difficulty falling asleep (Carskadon & Dement, 2011; Conte, Carobbi, Errico, & Ficca, 2012) more disrupted sleep with multiple awakenings during the night, and experience less SWS (Cajochen, Münch, Knoblauch, Blatter, & Wirz-Justice, 2006; Conte et al., 2012; Cirelli, 2012).

Studies on sleep architecture and memory retention in older adults. In general, studies of sleep-dependent memory consolidation in the elderly are less common and report mixed results especially with regards to declarative memory consolidation (Carotenuto & Esposito, 2014; Harand et al., 2012; Hornung et al., 2005). For example, Aly and Moscovitch (2010) showed that for a sample of adults aged 69-80, the amount of time spent asleep positively correlated with retention on a declarative task involving the memorization of short stories from the Logical Memory portion of the Wechsler Memory Scale III (WMS-III) and from a series of questions relating to personal events. In another study, Wilson, Baran, Pace-Schott, Ivry and Spencer (2012), also found that older adults performed better on a word-pair task after a period of sleep compared to a period of wake. In contrast, some studies have reported that sleep does not enhance memory in older adults. For example, Cherdieu et al (2014) found that older adults (mean age: 68,9 years) did not improve on a visuospatial object-location task compared to younger adults after a period of sleep. Specifically, memory retention was preserved in the younger adults but not older adults, who experienced a deterioration in performance. This finding was also evident in older adults on a declarative word-pair retention task (e.g. Mary, Schreiner, & Peignuex, 2013; Scullin, 2012; Westerberg et al., 2012).

Researchers using pharmacological interventions to manipulate sleep parameters in older adults have yielded some intriguing results. For example, a study in which an acetylcholinesterase inhibitor (donepezil) was used to selectively enhance the percentage of REM density in older adults found an enhanced performance on word list recall (Schredel, Weber, Leins, & Heuser, 2001). In a related study, Hornung et al. (2005) also used donepezil to manipulate REM sleep and found this manipulation significantly improved performance on a mirror-tracing task. Sleep and its effect on sleep-dependent memory consolidation in the elderly is thus somewhat unclear; however, the literature suggests that age-related changes in sleep may have an influence subsequent memory consolidation. There is limited research on

the pharmacological manipulation of sleep spindle activity in older adults. This may be an important area of research as age-related decreases in sleep spindle activity could be related to poor sleep-dependent memory consolidation in older adults (Harand et al., 2012). In sum, this pharmacological manipulation of sleep spindles suggests that sleep spindle may play an active role in consolidating declarative memory.

The role of sleep spindles for memory retention in older adults. Only a handful of studies have directly assessed the relationship between sleep spindles and both declarative and procedural memory performance in the elderly. A study by Seeck-Hirschner et al. (2012), found that an elderly group of women performed significantly better on a declarative task after a period of sleep and was associated with stage 2 spindle density. Lafortune et al. (2014), also found that sleep stage 2 spindle density and REM sleep were good predictors of next-morning cognitive performance. Specifically, higher spindle density predicted better performance on verbal learning, visual attention, and verbal fluency. Overnight memory retention has also been associated with slow spindle activity in stage 3 in younger adults, but with overall NREM (stage 2 and 3) oscillations in older adults (Wislowska, Heib, Hoedlmoser, Griessenberger, & Schabus, 2013). Whereas the literature consistently suggests that sleep spindle activity is associated with overnight memory consolidation in younger adults, the literature for older adults is inconsistent (Harand et al., 2012).

Peters et al. (2008) assessed the relationship between procedural memory retention and spindle characteristics after a night of sleep across young and older adults. They found that spindle activity increased during sleep after task acquisition in both groups and participants also displayed improved task performance following periods of sleep. In another study by Rauchs et al. (2008), researchers compared memory performance on an episodic memory task among patients with Alzheimer's disease (AD), healthy elderly adults and young adults. Episodic memory post-sleep was preserved in the younger participants and the healthy elderly participants compared to AD patients. However, there was also an age-related decrease in spindle intensity in the healthy older adults and the AD patients (Rauchs et al., 2008). Because both the younger and healthy older adults exhibited ceiling effects for the learning task, the researchers examined the relationship between spindles and memory for the AD group only. They found that performance on immediate recall positively correlated with spindle intensity (Rauchs et al., 2008). Research involved in uncovering the potential link between impaired declarative memory and decreases in sleep spindle activity is necessary to understand sleep-dependent memory consolidation mechanisms in the ageing population.

In sum, there is need for further research to establish how spindle activity differs across ages, and also to investigate the cognitive correlates of such differences. It is unclear whether older adults experience cognitive-related benefits from sleep and it is not clear how sleep-dependent memory consolidation may change throughout the ageing process (Scullin & Bliwise, 2015). Some studies suggest that older adults do not experience cognitive-related benefits from sleep because of a weakening in the hippocampal-to-neocortical dialogue seen in *Active System Consolidation* (McCrae et al., 2012; Wislowska et al., 2013). Specifically, this weakening may be due to decreases in SWS and sleep spindle activity. Age-related decreases in SWS and spindle activity may be linked to structural shrinkage in the hippocampus and prefrontal cortex that is sometimes seen in older adults (Cavanaugh & Blanchard-Fields, 2011; Guzman-Marin & McGinty, 2006). Consequently, structural shrinkage has been associated with disrupted sleep and poor performance on memory retention tasks (Mander et al., 2013).

Factors to Consider in Sleep-dependent Memory Consolidation Research

There are several factors that can affect sleep-dependent memory consolidation (Conte & Ficca, 2013). These can include an awareness of confounding factors, such as task difficulty, learning ability, and intrinsic motivation, that can influence the process of memory acquisition, consolidation and recall and the process of sleep.

Psychological factors, such a level of awareness during learning, intentionality, individual learning strategies and capabilities have been shown to affect sleep-dependent memory consolidation processes (Conte & Ficca, 2013; Diekelmann et al., 2009). For example, there is some evidence to suggest that sleep selectively enhances memories that are behaviourally relevant, and thus a motivational component is attached to sleep-dependent consolidation (Diekelmann & Born, 2010). In a study by Wilhelm et al. (2011), post-learning sleep was associated with better recall on a word-pair task and increases in spindle and slow oscillation activity when participants were told that their recall would be tested again in the morning after sleep. Those participants who were not told this showed no significant changes in their sleep or in their recall (Wilhelm et al. 2011).

The level of engagement during task acquisition, as well as the level of task difficulty, can impact on subsequent sleep quality, sleep spindle activity and memory performance (Conte et al, 2012; Schmidt et al., 2006). Popular memory tasks, such as the verbal paired associates (VPA-15) test, that entail a small sample of items to be remembered tend to display ceiling effects on memory performance (Utll, 2005; Utll, Graff, &Richter, 2002). On

the other hand, using word-pairs that require participants to actively elaborate on associations during encoding is reliably associated with subsequent changes in sleep (Payne et al, 2012; Schmidt et al., 2006). This may imply that sleep preferentially consolidates word-pairs that require novel and elaborate associations. Sleep studies that rely solely on semantically related word-pairs may not be modulated by sleep compared to unrelated word-pairs (Payne et al., 2012). This is because related word-pars entail pre-existing connections (for example, '*river-ship')*. Pre-existing connections could be thought of as already strongly associated memories that would not necessarily need further strengthening during sleep (Diekelmann & Born, 2010). Thus sleep studies could include tasks with a sufficient level of engagement and difficulty. This is particularly relevant to the elderly because they have been shown to experience an 'association deficit' (Badham, Estes, & Maylor, 2012; Naveh-Benjamin, Hussain, Gueza & Bar-On, 2003). Specifically, older adults tend not to make use of mnemonic strategies to aid the encoding process. Using tasks adapted for the elderly, like for example, integrative word-pairs (which make up a coherent phrase like 'lemon-cake'), has been shown to alleviate this association deficit (Jones & Golonka, 2012). However, the effect of using integrative word-pairs on subsequent changes in sleep and in sleep-dependent memory consolidation in the elderly is unclear.

Previously, a large portion of sleep studies did not take into account sex-related differences in sleep and memory performance on different types of tasks (Dzaja et al., 2005). This is an important consideration because some studies suggest that there are significant differences when it comes to sleep architecture and spindles in men and women (Dzaja et al., 2005; Grander et al., 2012). For example, women report higher incidences of sleep disturbance and tiredness, regardless of age. The prevalence of insomnia is higher in women, whereas sleep apnea and REM sleep behaviour disorder is higher in men. In their review on sleep in women, Dzaja et al. (2005) report that it is not clear whether women are at a higher risk for sleep loss (which may be due to factors such as hormonal fluctuations or infant care-taking responsibilities), and it's also not clear how age may mediate these processes. There are some studies that report sex-related memory performance differences. For example, Genzel et al. (2012) suggest that tasks may be classified in terms of the degree of 'maleness' or 'femaleness'. This comes from research that appears to indicate that women tend to outperform men for verbal, episodic, and emotional tasks, while men seem to have an advantage in visuospatial tasks (Genzel et al., 2012; Kuriyama, Mishma, Soshi, Honma, & Kim, 2011; McDevitt, Rokem, Silver, & Mednick, 2014). However, sex-related differences in sleep-dependent memory performance need to be replicated.

In terms of sex-related sleep spindle differences, some studies have reported that women with major depressive disorder (MDD) show increases in spindle amplitude, density and duration, whereas men with MDD show no such increases (Plante et al., 2013). There is also a rapid decline in spindle density from middle-aged to elderly men, while such reductions are only seen much later in women (Martin et al., 2013). Martin et al. (2013), did however find that older women tended to exhibit more pronounced spindle frequency reductions than men. In relation to cognitive ability in younger women (for children and adolescents) there is some evidence to suggest that slow spindle duration is associated with higher fluid intelligence in females, compared with male counterparts (Ujma et al., 2015; Ujma et al., 2014). Similarly, positive associations of intelligence with spindle characteristics in females have been found in other studuies (Ujma, Sandor Szakadat, Gombos, & Bodizs, 2016). For example, Bodizs Gombos, Ujma, & Kovács (2014) found that fast spindle density correlated postively with fluid intellengce in adolescent females while fast spindle frequency positively correlated for males. Thus the relationship between congition and sleep spindle parameters in males and females is complex and requires further study.

There is also some evidence to suggest that sleep spindle activity, such as spindle frequency, may vary during the ovulatory menstrual cycle (e.g. Baker & Driver, 2004, 2007). The menstrual cycle occurs over a duration of approximately 28 days and entails changes in four reproductive hormones: luteinizing hormone, follicle-stimulating hormone, estrogen and progesterone (Baker and Driver, 2007). In other studies, authors showed that women who were taking medroxyprogesterone acetate (a synthetic form of human progesterone) in comparison to matched controls, displayed higher spindle densities (Plante & Goldstein, 2013). This comes from circumstantial evidence that progesterone can affect the production of sleep spindles in women. However, some polysomnographic studies show that sleep appears to remains relatively stable across menstrual cycle, although higher oestrogen levels have generally been associated with improved quality of sleep (Moline, Broch, Zak, & Gross, 2003).

Few studies on how the menstrual cycle may affect sleep-dependent memory consolidation exist. One study found women in their mid-luteal phase showed a significant increase in spindle activity after learning and improved performance compared to women in other points in their cycle (Genzel et al, 2012). In addition, this study further suggested that estrogen correlated with offline declarative learning, whereas progesterone correlated with motor learning. A study by Soni et al. (2013), showed that women might be more susceptible to consolidating negative memories during the early luteal phase, where estradiol is low and

progesterone is high. In another example, Genzel et al. (2012) showed that menstruating women failed to show a benefit from sleep on a declarative task compared to non-menstruating women and a group of men. These researchers found that the lack of sleep-dependent memory consolidation was associated with decreased sleep spindle activity, which they suggested may be due to low levels of the estrogen and progesterone.

During menopause, which is the cessation of menstrual periods, older women experience changes in hormonal fluctuations, such as a decrease in estrogen (Moline et al., 2003). The prevalence of insomnia and other sleep disruptions is particularly high in menopausal women (Moline et al., 2003). In addition, there is mixed data on the effects of hormone replacement therapy (HRT) on subsequent sleep, a common medication taken by women in menopause (Moline et al., 2003). However, little is known about how menopause and its associated changes may affect sleep spindles and memory consolidation.

In conclusion, there are many factors that need to be taken into account when conducting studies on sleep-dependent memory consolidation. Being aware of such factors will produce more robust results and help form a clearer focus on mechanisms responsible for memory consolidation, especially in the ageing population.

Rationale and Aims

Research indicates that sleep plays a significant role in declarative memory consolidation (Born & Wilhelm, 2012). Recent studies further suggest that spindle activity, which includes spindle density and intensity, that occurs during NREM stage 2 and SWS is associated with improvements on declarative memory tasks (Diekelmann & Born, 2010; Fogel & Smith, 2011). The relative contribution of stage 2 versus SWS sleep spindles to sleep-dependent memory consolidation is unclear (Fogel & Smith, 2011). However, research consistently finds that both NREM stage 2 sleep and NREM SWS spindles are associated with memory retention (e.g. Cox et al., 2012; Van der Helm et al., 2011). Thus sleep spindle activity in NREM stage 2 and SWS has become an important focus in sleep research because of its link to learning and memory, synaptic plasticity, and its potential significance in clinical populations (Astori et al., 2013; Mednick et al., 2013). This study, therefore, included an analysis of NREM stage 2 and SWS spindle activity to explore sleep-dependent memory consolidation in younger and older adults.

In summary, little is known about how the process of sleep-dependent memory consolidation may be affected in older adults. Previous research shows that older adults tend to experience memory performance and retention difficulties (Badham, Etes & Maylor,

2012). In addition, research on declarative memory is particularly important because episodic memory, which is a component of declarative memory, is most often affected in older adults (Harand et al., 2012). These difficulties may be partly understood through age-related structural changes due to the ageing process (Cavanaugh & Blanchard-Fields, 2011). Research that has examined the declarative memory consolidation process during sleep in the elderly yield mixed results. In some cases, memory is enhanced after a period of sleep (Wilson et al., 2011), and in others it is not (Scullin, 2012). Research suggests that the hippocampal-to-neocortical dialogue may be compromised in ageing adults as a result of changes in sleep spindle characteristics, such as reductions in density, amplitude and duration (Fogel & Smith, 2011; Hornung et al., 2005; Scullin & Bliwise, 2015). There is also evidence to suggest that there may be sex-related differences in spindle activity (e.g. Martin et al., 2013). In addition, menstrual cycle phase may affect sleep spindle activity and subsequent memory consolidation (Genzel et al., 2012). Therefore, further research is needed to investigate the role that NREM spindle activity plays in sleep-dependent memory consolidation in women and particularly, the elderly.

Aims

This study comprised of three general aims.

1. To investigate memory performance pre-sleep and post-sleep in our two samples of younger (18-30 years) and older women (60-65 years). This was carried out in order to confirm the common finding that younger adults significantly outperform older adults on declarative memory word-pair tasks (e.g. Rauchs et al., 2008). We aimed to see this trend in both conditions of pre and post-sleep.
2. To characterise sleep architecture and sleep spindle activity between the two groups during a baseline and an experimental night of polysomnography. Sleep spindle activity included measurement of the average spindle frequency, amplitude and duration. In addition, it entailed the average number of detected spindles, spindle density and lastly, spindle intensity.
    a. A related aim was to investigate the changes within each group for sleep architecture and spindle activity from the baseline to the experimental night.
3. To investigate the relationship between sleep architecture and spindle activity with post-sleep memory retention.

Hypotheses

There are three major hypotheses for this study:

1. Younger women will outperform older women on all declarative memory tasks, both pre- and post sleep.
2. Older women will show age-related changes in sleep architecture and spindle activity compared to younger women.
    a. Specifically, younger women will have a higher percentage of SWS and REM compared to older women (Ohayon et al., 2004).
    b. Younger women will have higher sleep spindle density (Martin et al., 2013) and intensity compared to older women.
    c. Regarding sleep spindle characteristics, younger women will display sleep spindles with relatively longer durations, higher amplitudes, slower frequencies and higher numbers of detected sleep spindles (Martin et al., 2013)
3. SWS, sleep spindle density and sleep spindle intensity will positively correlate with retention scores on the declarative memory task for both groups. However, a stronger correlational will be found in younger women compared to older women.

METHODS

Design and Setting

This was a cross-sectional study, using a mixed-participant design. There were two groups of participants: Younger females (aged 18-30) and older females (aged 60-65). Each participant was measured on consecutive nights of baseline and experimental polysomnography.

The study took place at the University of Cape Town's Sleep Laboratory (UCTSL). The sleep laboratory consists of a control room and two video-monitored, sound-attenuated, and electrically shielded test rooms. It is equipped with two sets of Nihon-Kohden Polysomnographs.

Participants

A total of twenty-nine participants took part in this study. Participants were recruited from Cape Town and the surrounding areas through advertisement placed around the university of Cape Town (UCT) campus, in local newspapers and social clubs. The younger group of female participants (n =15) were recruited from the university of Cape Town. The older group of female participants (n =14) were recruited through newspaper and newsletter

advertisements, as well as various senior citizen associations, such as The University of the Third Age (U3A) (https://sites.google.com/site/u3asafrica/). The recruitment process, including participant eligibility, and inclusion and exclusion criteria is shown in figure 2.

Eligibility. Participants were assigned to one of two groups: a 'younger' group (18-30yrs) or an 'older' group (60-65yrs). Eligible participants were those who were female, of the correct age, reported healthy sleep habits measured by the Pittsburgh Sleep Quality Index, had no significant depressive symptoms, and were not currently using medications that have known effects on sleep architecture.

A maximum age of 30 years for the younger group was chosen because sleep spindles reportedly show a decline after the age of 30 (Backhaus et al., 2007). In addition, research has shown that from the age of 60, there is a significant reduction in sleep spindles compared to middle aged adults (Crowley, 2011; Martin et al., 2013). The narrow age range for the older women accommodates concerns regarding a higher probability for rapid cognitive decline often seen after 65 years of age (Nyberg, Lövden, Riklund, Lindenberger, & Bäckman, 2012).

Exclusion Criteria. Participants were excluded if they were taking psychiatric medication, hormonal medications (such as the contraception pill), sleep medication (such as sleeping pills) and finally respiratory-related medication that may contribute to disorders such as sleep-specific breathing disorders. In addition, full-time smokers were excluded because nicotine use has shown to affect sleep architecture (Crowley, 2011).

Potential participants were excluded if they did not have a grade-12 certificate because the screening involved a measurement of intelligence and mental state. These assessments can be susceptible to biases at an education level, favouring individuals who have higher education (Strauss, Sherman, & Spreen, 2006). Thus, all potential participants without leaving-school diplomas were excluded in an attempt to avoid any of these biases. This was also done because the declarative memory tasks involved verbal ability.

The second phase of the recruitment process entailed screening for depressive symptoms (BDI-II), sleeping difficulties (PSQI), mental impairment (MMSE) and cognitive ability (WASI; verbal strength). Participants were excluded if they scored above 14 (indicating more than mild depression) on the BDI-II, above 5 (indicating sleep disturbances) on the PSQI and below 90 (indicating below average intelligence) on the WASI. Younger women were excluded if they scored below 29 on the MMSE, while older women were excluded if they scored below 27.

Younger female participants were excluded if they reported any irregularities in their menstrual cycles or took contraceptive medication. On the other hand, older participants were excluded when they reported use of Hormone Replacement Therapy (HRT) or if they were pre-menopausal.

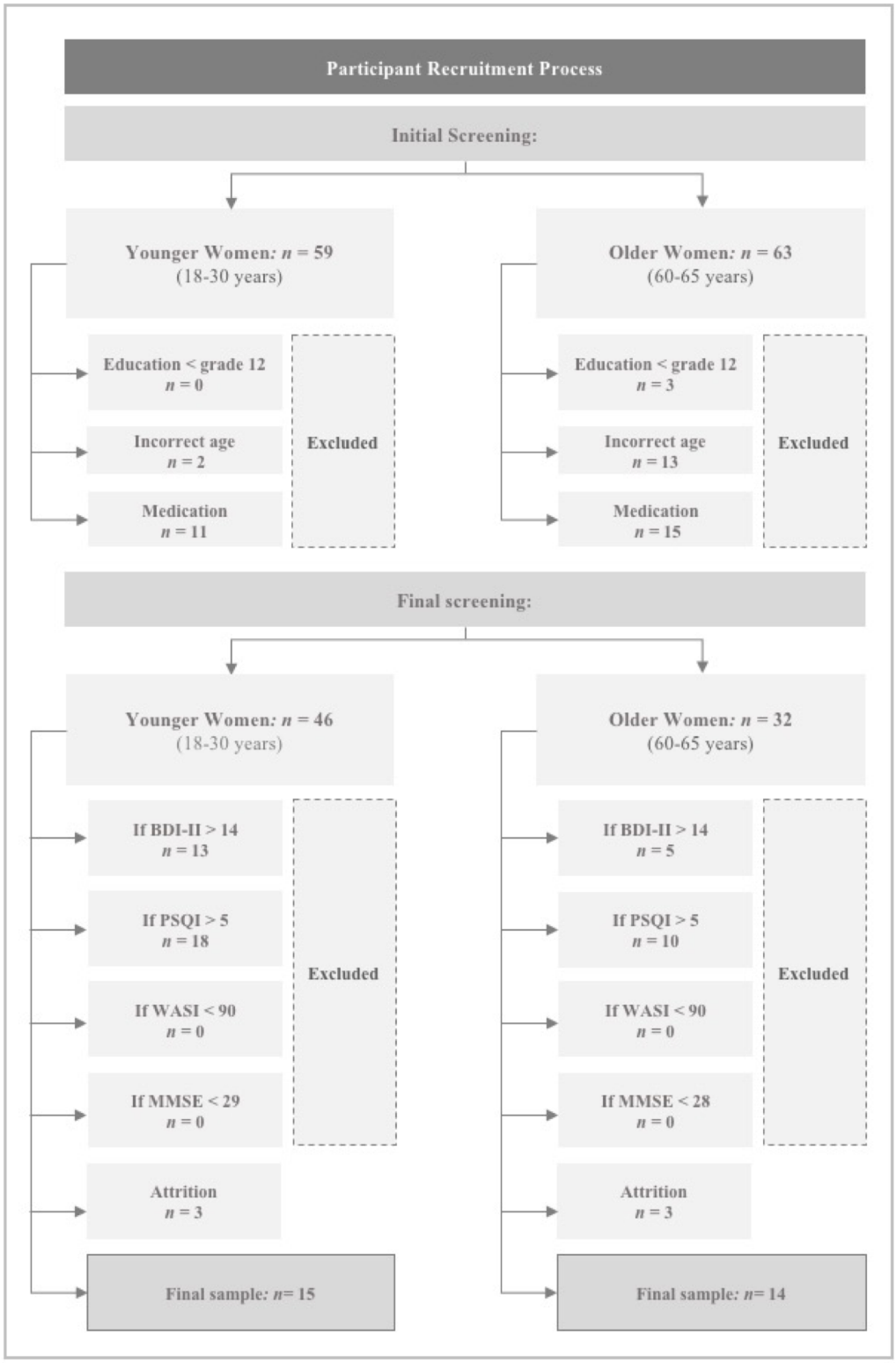

Figure 2: A flow diagram illustrating the screening and exclusion criteria leading to the final sample.

Materials and Apparatuses

Screening measures.

Demographic and Personal Information form (Appendix B). Participants were asked to complete a demographic and personal information form created for the purposes of this study. This served as an initial screening form, and attained information on age, level of education, and medication use. In addition, information regarding their menses was obtained for two reasons. Firstly, we wanted to exclude participants with potential irregularities in their menses. This is because irregular menses can be associated with altered circadian rhythms (Baker & Driver, 2007; Moline et al., 2003). And secondly, there is some evidence to suggest that sleep spindle activity and subsequent memory consolidation may be affected by the menstrual cycle (Genzel et al., 2012). Thus we aimed to avoid testing the younger women during their menses. Information regarding the menstrual cycle was also asked of the older women. Specifically, we wanted to confirm that they were in the menopausal phase and not in the perimenopausal phase as this transitional state can affect sleep differently to women during menopause (Moline et al., 2003).

Mini-mental State Examination (MMSE; Folstein et al., 1975 – Appendix D*).* The purpose of the MMSE is to screen for cognitive impairments in individuals aged 18-85, and is specifically suitable to use in elderly populations. This brief assessment takes 5-10 minutes to administer The MMSE assesses orientation in time and place, language, attention and calculation, and immediate as well as delayed recall. The examiner asks the questions and records the participant's responses. Participants can score a maximum of 30 points, with higher scores indicating the absence of mental impairment.

A decline in MMSE score is associated with advancing age, and is seen to begin at ages 55-60 and accelerates from the age of 75 years onwards (Strauss et al., 2006). Individuals with higher levels of education and IQ tend to score higher on the MMSE (Strauss et al., 2006). This is because the language used in assessments like the MMSE can contain biases towards test-takers with higher levels of education (Strauss et al., 2006).

The MMSE has demonstrated moderate internal consistency (r = .59), test-retest reliability (.80-.95), and shows moderate to high levels of construct validity (Strauss et al., 2006). Normative data shows that individuals between the age of 18-30 tend to score 29, while individuals between the age of 60-65 tend to score in 28 at the education level of high school diploma and university experience.

Beck Depression Inventory – Second Edition (BDI-II; Beck, Steer, & Brown, 1996 - Appendix E). The BDI-II is a self-report measure of depressive symptomatology which takes approximately 5-10 minutes to administer. The inventory consists of 21 items that are rated on a 4-point scale (0 – 3, 3 is the most intense) to measure intensity of feelings during the preceeding two weeks. Scores can range from 0 – 63, with higher scores indicating more depressive symptoms. Total scores classify people as either minimally depressed (0-13), mildly depressed (14-19), moderately depressed (20 – 28), or severely depressed (29-63). For the purposes of this study, the cut-off score was 14 because depression can alter typical sleep patterns (Plante et al., 2013). Thus participants were screened for mild, moderate and severe depression.

The BDI-II is suitable to use on adolescents and adults (13-86 years of age; Strauss et al., 2006). This inventory has also been shown to overlap with other measurements of depression, such as the Geriatric Depression Scale (GDS), Depression Anxiety Stress Scales – Depression (DASS-D) and the Self-Rating Depression Scale (SDS) (Wang & Gorenstein, 2013). In addition, there is overlap with other measurements such as the Scale for Suicide Ideation (SSI), the Short Psychological Well-Being Scale (SPWB) and the Drug Abuse Screening Test (DAST). Thus there is strong evidence for construct and discriminant validity for the BDI-II. The inventory has demonstrated high internal consistency (0.9) and test-retest reliability (0.73 – 0.96; Wang & Gorenstein, 2013).

Pittsburgh Sleep Quality Index (PSQI; Buysse, Reynolds, Monk, Berman, & Kupfer, 1989 – Appendix F). The PSQI assesses sleep quality and detects potential sleep-related problems (Aloba, Adewuya, Ola, & Mapayi, 2007). It is a self-rated questionnaire with 24 items that address seven areas; namely: sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction (Aloba et al., 2007). It is scored on a likert-scale of 0-3 (with higher scores indicating more sleep problems) and overall score is generated by the addition of all the scores. A score of above five suggests the presence of sleep disturbance (Buysse et al., (1989), thus participants who scored six and above were excluded from the study. The test is administered and completed within 5 -10 minutes and has been validated among several populations including university students, psychiatric patients, older adults and non-western cultures (Aloba et al., 2007; Beaudreau et al., 2012).

Wechsler Abbreviated Scale of Intelligence (WASI; The Psychological Corporation, 1999). The WASI is a brief screening assessment for intellectual functioning and may be administered to individuals ranging from 6 to 89 years of age (Strauss et al., 2006). It assesses verbal and non-verbal ability. It consists of four subtests based on vocabulary, block design, similarities and matrix reasoning. The WASI takes approximately 30 minutes to administer and scoring follows age-based norms provided in the test manual. Scores range in the following categories: *extremely low* IQ (0-70), *borderline* IQ (70-80), *low average* (80-90), *average* IQ (90-110), *high average* (110-120), *superior* (120-130) and *very superior* (130+). The cut-off range for the current studies sample was 90.

The WASI has good internal consistency (on average the reliability is .93) and test-retest reliability (for adults .88). The construct validity for this measure ranges from .84 for performance IQ and .88 for verbal IQ, and is linked to the Wechsler Adult Intelligence Scale –III (WAIS-III). WASI scores were used in order to control for between-group differences in verbal ability, which has been shown to influence performance on verbal memory tasks (Strauss et al., 2006). Full-scale IQ and verbal IQ are reported in the study.

Experimental Measures.

Polysomnography. A polysomnogram is the standard measurement for quantifying sleep and its associated physical phenomena (Spriggs, 2002). There are several parameters that are commonly measured in a basic Polysomnograph (Spriggs, 2002). Firstly, electroencephalography (EEG) is used to record brain activity as the participant enters and passes through the different stages of cortical arousal. Secondly, electrooculography (EOG) is used to record the participant's eye movements during sleep. This is used to identify slow rolling eye movements in stage one and ocular saccades in REM sleep. Thirdly, electromyography (EMG) is used to detect the participant's muscle activity and may be used to identify the onset of REM sleep, where the body enters a temporary state of paralysis (Zillmer & Spiers, 2008). And lastly, the electrocardiography (ECG) which records the heart rate of the participant. Thus, the four channels (EEG, EOG, EMG and ECG) were used to capture sleep stages and sleep features, such as sleep spindles.

Sleep was recorded using a Nihon Kohden EEG equipment and Polysmith analysis software. Each recording was in accordance with the International 10-20 system, which is a guide to the montage of electrode set-up. Reference electrodes were placed on the left and right ear mastoids and the sampling rate for the EEG was set at 500Hz. The upper impedance limit was 5 ohms. The guidelines from the American Academy of Sleep Medicine (AASM)

Manual for the scoring of sleep and Associated Events (2007) were used to identify and score sleep architecture. Sleep architecture is scored for sleep stages and various sleep variables. The following were used in this study: NREM stage 1, NREM stage 2, NREM SWS and REM sleep. In addition, wake after sleep onset (WASO), total sleep time (TST), sleep efficiency and number of detected arousals were scored. WASO relates to the amount of time spent awake after initial sleep onset. Sleep efficiency refers to the total amount of time spent asleep out of the amount of time in bed (Spriggs, 2002). Thus for this study, participants are allotted an 8-hour period time in which to sleep. The 8-hour period of potential sleep time represents a typical night of sleep for adults (Carskadon & Dement, 2011; Spriggs, 2002).

Sleep data was recorded for each participant over the adaptation, baseline and experimental night. For this study, data was taken only from the baseline and experimental night, since the adaptation night was used to allow participants the opportunity to acclimatize to the sleep laboratory. Sleep latency could not be scored because of a technical error during the scoring. An independent qualified sleep scorer analysed a portion of the data. An agreement of 80% per scored epoch was reached between two scorers, indicating sufficient inter-rater reliability.

Spindle Detection. An automated algorithm was chosen to detect sleep spindles because it provided the most time-efficient and systematic source of analysis. Traditionally, human sleep scorer experts manually count sleep spindles based on visual criteria (O'Reilly, Godbout, Carrier, & Lina, 2015; Ray et al., 2015). Counted sleep spindles can vary between 200-1000 during a night of sleep (Huupponen et al., 2007). Acceptable inter-rater reliability for human scorers usually relies on the aggregate score of four or more experts because it can be a difficult and subjective task when, for example, sleep spindles are partially obscured by artefact or different sleep spindle scoring criteria are employed (Devuyst et al., 2006; Wendt et al., 2015). This is particularly relevant regarding data for psychiatric patients or older adults where sleep spindles are less visually obvious (Devyust, Dutoit, Stenuit, & kerkhofs, 2011). Thus manual sleep spindle detection methods based on visual identification are criticized for being a tedious, difficult, lengthy and sometimes inaccurate process (Bódizs et al., 2009; O'Reilly et al., 2015; Warby et al., 2014).

Sleep spindles were detected using an automated algorithm using the Somnolyzer 24 x 7 Siesta Spindle Detector (The Siesta Group, Vienna, Austria), which is based on the established sleep analysis criteria of Schimicek et al. (1994). The Somnolyzer was validated

as a reliable automated classification system as revealed by an epoch-by-epoch agreement of 80% (Cohen's Kappa: 0.72) with human expert scorers (Anderer et al., 2005).

Spindle detection via the automated algorithm was achieved in two main steps. The first step involved identifying potential sleep spindles on artifact-free EEG data. This meant that the data was band-pass filtered at a frequency range of 11-16Hz. The following additional thresholds for sleep spindle parameters were: minimum durations of 0.3 seconds (s) and a maximum of 2s, minimum amplitudes of 12 microvolts (μV). This results in a high sensitivity for detection of sleep spindles. However, this also meant that a large amount of false positive detections may occur. Thus to account for this, a second step entailed (from the possible range of detected sleep spindles) a linear discriminate analysis (LDA) to reduce the number of false positives and to gain a higher specificity of truly detected spindles. However, this method does not take into account individual variability in sleep spindle frequency and/or amplitude (Bódizs et al., 2009).

Sleep spindle activity was analysed in stage 2 and SWS, collectively referred to as NREM sleep spindle activity. NREM sleep spindle activity was chosen because of the main hypothesis that sleep spindle activity increases follow learning in stage 2 (Mednick et al., 2013; Wamsley et al., 2013) and in SWS (Cox et al., 2012; Genzel et al., 2014). For this study, the following sleep spindle variables were assessed: spindle density, spindle intensity, and spindle type (slow (11-13Hz) or fast (13-16Hz)). In addition, the average amplitude, frequency, duration and number of each detected spindle was also assessed.

Epworth Sleepiness Scale (ESS; Johns, 1991 – Appendix G). This self-administered questionnaire is used to assess average daytime sleepiness and differentiates between average sleepiness and excessive sleepiness (Johns, 1991). There are eight questions in which an individual is asked to rate how likely they are to fall asleep on a scale of 0-3, where 0 indicates an unlikely chance of dozing or falling asleep, and 3 indicates a high chance of dozing or falling asleep. A total score of 10 or more points reflects abnormal daytime sleepiness. This tool may be used to assess average sleepiness in older adults and should take no longer than five minutes to complete (Smyth, 2012). Good internal consistency has been reported for the ESS, with a Chronbach's alpha between 0.74 and 0.88 (Smyth, 2012; Johns, 1992). The original instructions for the ESS require subjective evaluations of sleepiness over a recent period of time, but for the purposes of this research (which requires a present-moment evaluation of sleepiness) a minor change to the instructions were made. Instead of asking participants to rate their sleepiness over a period of recent time (one week),

participants were asked to rate their sleepiness in the moment of completing the questionnaire. This was used to assess relative vigilance in participants before they attempted to fall asleep and before commencing with the memory recall task in the morning after waking.

*Pittsburgh Sleep Diary (*PSD; Monk, 1994 – Appendix H). The self-administered PSD is used to assess subjective sleep and waking behaviours and is divided into two sections which are completed before sleep (bedtime questionnaire) and after sleep (waketime questionnaire). For this study, the waketime questionnaire is was used to assess subjective sleep quality. The waketime questionnaire comprises of 11 questions and takes approximately five minutes to complete (Monk et al., 1994). Scores on the questionnaire give an indication of the subjective experiences of the following sleep variables: (1) sleep latency (SL), (2) frequency of nightly awakenings (FNA), (3) the amount of time spent awake after initial sleep onset (WASO), (4) sleep efficiency (SE), (5) sleep quality, (6) final mood upon awakening and (7) alertness upon awakening. Sleep latency refers to the amount of time it takes to for initial sleep onset, while sleep efficiency refers to the amount of time spent asleep relative to the amount of time spent lying in bed. The PSD has shown good reliability and validity (e.g. Smith & Wegener, 2003). In addition, it is noted that the PSD is sensitive to age-related differences of sleep continuity and is thus suitable to use in older adults. For this study, subjective sleep quality, final mood on awakening and level of alertness was used to assess potential confounding influences on memory task performance since perceived sleep experiences and next-morning physical states (e.g., alertness) can influence the sleep-dependent memory consolidation process during encoding, consolidation and recall (Conte & Ficca, 2013).

Declarative Memory: word-pair associates task (Appendix I). A word-pair task consisting of 90 word-pairs was created to assess neutral declarative memory. The task consisted of three subtasks as a function of word-pair type: (1) semantically unrelated integrative word-pairs, (2) high concrete word-pairs and (3) semantically unrelated low concrete word-pairs. These word-pair subtypes were used to introduce varying difficulty to the overall task, and ultimately assess the probable effects learning difficulty may have on subsequent sleep and memory retention as suggested by Smith et al. (2006) and Conte (2012).

Introducing difficulty was developed in several ways. Firstly, a list of integrative word-pairs was chosen. This addressed the association-deficit hypothesis, which states that older adults tend to neglect mnemonic strategies when encoding, which can result in poor encoding

and subsequent consolidation and recall (Badham, Estes, & Maylor, 2012). Badham et al. (2012) propose that integrative word-pairs ameliorate this deficit. In their study, they used integrative word-pair based on Estes and Jones (2009) integrative word-pair list, which consisted of coherent phrases that do not require elaborated encoding (e.g. *travel-book).* A random sample of 30 integrative word-pairs were drawn from the 45 word-pairs used in Badham et al.'s study (2012), which was found to alleviate age-related memory differences. This list served to provide a baseline level of difficulty.

Secondly, semantic word-pairs (e.g. *concert-harp)* are easier to remember than unrelated word-pairs (e.g. *concert-square)* (Badham et al. 2012). In addition, research suggests that sleep may preferentially consolidate semantically unrelated word-pairs (Payne et al., 2012). The semantically unrelated word-pairs were further divided into levels of high concrete word-pairs (e.g. *bird-comb*) and low concrete word-pairs (e.g. *something-atom)*. Low concrete word-pairs may increase the level of difficulty as proposed by Smith et al. (2006).

In order to create lists for low and high concrete word-pairs, a random sample of 30 word-pairs was taken from an online database http://www.wordnorms.com/ (Buchanan, Holmes, Teasley & Hutchison, 2013). Here, an excel sheet of 12 498 words and word combinations were downloaded. Each word and word-pair combination is supplemented with feature information, such as the part of speech (e.g. noun versus adjective), word length, forward and backward target word probability, and semantic relatedness. The online database aimed to expand on previous word databases and norms.

For this study two important word-pair features were utilized: level of concreteness and word-pair semantic relatedness. The subsequent word-pair lists were generated through the following steps. Firstly, only word-pairs that consisted of noun-to-noun words were considered. Secondly, emotionally negative word-pairs such as *'trouble-punishment'* and *'inferior-hate'* were eliminated since this task focused on neutral declarative memory. Thirdly, the level of concreteness was pre-defined on the online database, which was measured on an increasing scale from 1-7, where 1 was high concrete and 7 was low concrete (abstract) (Buchanan, Holmes, Teasley & Hutchison, 2013). Two lists, separated by high concreteness (where word had values were > 4.94) and low concreteness (where word values ≤ 4.95) were created.

The final step involved transforming the semantically related word-pairs into semantically unrelated word-pairs. Each word-pair consists of a cue word and a target word; for example, bird (cue) and nest (target) or hair (cue) and comb (target). In excel, each cue

word and each target word was divided into its own column and randomised to create unrelated word-pairs; for example, '*bird-comb'* and '*hair-nest'*. Once this was achieved, a random sample of 30 high concrete word-pairs and a random sample of 30 low concrete word-pairs was drawn.

Procedure

The Research Ethics committee in the Psychology Department at the University of Cape Town approved the protocols for this study.

Ethical considerations: Informed consent, potential risks, and confidentiality. Informed consent was obtained from each participant. The informed consent document entailed information about the purpose of the study and what was required from the potential participants. In addition, they were briefed about the potential risks involved in participating in the study. Specifically, they were told that they would spend three nights at the Universality of Cape Town's Sleep Laboratory. They were told that this involved wearing an EEG while sleeping and that this took place in the laboratory, which is an unfamiliar place to them. Participants were assured that wearing an EEG was not dangerous and that security measures were put in place to ensure their safety while participating in the study. This included telling them that the sleep laboratory is a lockable facility and that Campus Protection Services (CPS) guards were available at all times. CPS staff are also capable of attending to medical emergencies as they are trained in first aid.

Participants were made aware of their right to refuse to take part in the study and their right to withdraw from it at any stage during the experiment, without incurring any penalty. Participants were informed that all their information obtained from the study would remain confidential. During the data collection phase, study pseudonyms were used. Only other researchers directly involved in the study had access to their information.

Special attention was paid to the elderly because this age group can undergo some age-related physical and cognitive changes that may put them at risk of having health-related concerns (Cavanaugh & Blanchard-Fields, 2011; McCrae, 2009). The participants were asked about their current medication use and general health. Thus during the recruitment process, both age groups were screened for any chronic health issues that may put them at risk outside of normal day-to-day living. This may include aspects of health like cardiovascular diseases, which are more prevalent among the elderly (Kociuba et al., 2010).

Study Procedure. Once informed consent was obtained, candidates were screened at UCT in a private room in the Psychology Department or at the homes of the elderly

participants. Eligible participants were then invited to take part in the study and participation times were arranged between the researcher and the participant. During data collection, participants were asked to refrain from daytime napping and consuming alcoholic or caffeinated beverages. Bed times were scheduled according to individual night and bedtime routines obtained from their PSQI results. Participants were instructed to arrive 2-3 hours in advance to allow for the Polysomnogram set-up.

The three nights spent at the sleep laboratory comprised of an adaptation night, a baseline night and an experimental night (Figure 3**A**), with the first night serving as the adaption night. The adaptation night enabled the participants to habituate to the laboratory environment and control for first-night effects. These effects include more-than-usual awakenings during the night and difficulty in maintaining sleep (Newell, Mairesse, Verbanck, & Neu, 2012). It has been shown that individuals who experience poor sleep from sleeping in unfamiliar settings can subsequently experience recovery sleep the following night (Bonnet, Berry, & Arand, 1991; Wu, et al., 2006). For example, they may experience a REM rebound and have atypical sleep architecture. Thus, a minimum of one day and a maximum of one week (depending on the participant's personal work schedule) elapsed between the adaptation night and the baseline night. The baseline night was consecutively followed by the experimental night, where the participants were asked to participate in the word-pair memory task.

The three night were set up identically to each other (see figure 3**B**). The participants were setup for Polysomnographic recording and before bed they are asked to fill out the ESS. In the morning, they were again asked to fill out the ESS and also the PSD after the EEG was removed. However, on the third evening (the experimental night), participants were exposed to a learning task, which took approximately 60 minutes to complete (see figure 3**C**). Participants were seated in front of a computer screen and shown three lists of 30 word-pairs. The word-pairs were displayed using E-prime software (Psychology Software Tools, Pittsburgh). During the task, participants had two rounds to learn the word-pairs, each followed by a cued-recall. Participants were instructed that a word-pair would be seen on the screen, and their task was to remember the word pair because later on the researcher would ask them to recall the second word of the word-pair. Three practice examples were then executed and the task commenced. During each encoding and recall, the word-pairs were automatically randomised in each list.

Each word-pair was displayed on a computer monitor for 3 seconds, which was followed by a white fixation cross where participants were instructed to imagine a

relationship between the word-pairs in order to help them form associations. This was done to control for inter-individual mnemonic strategies. Following this, the white cross then changed to a red colour, where participants anticipated a new word-pair would to learn. A randomised cued recall task followed both encoding sessions in the evening. Once participants had awoken participants completed another recall (figure 3**C**).

In the morning after the completion of each night, participants were compensated R100 (a total of R300 for three nights) for their time. Participants were then debriefed.

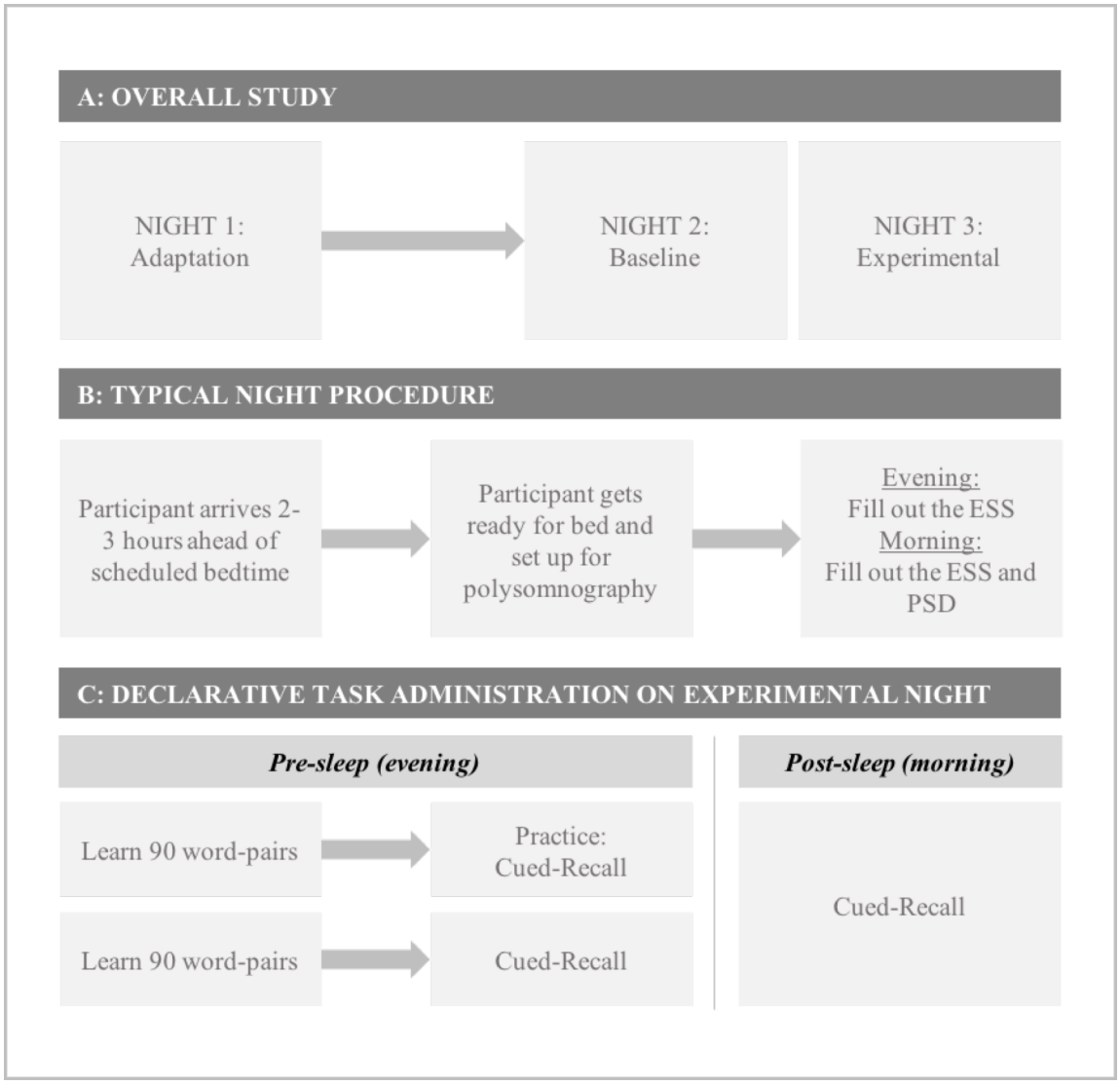

Figure 3: Study Design Protocol. Participants were tested over three nights. The first night is separated from the second and third to account for recover sleep (min one day – max 7 days). The first night served as an adaption night. Baseline and learning nights were consecutive. All nights followed the same procedure. On the learning night, the declarative task was added.

STATISTICAL ANALYSES

All analyses were completed using R and SPSS (Version 22). Unless noted otherwise, we set the threshold for statistical significance (α) at .05.

Deriving Outcome Variables. The following section covers the procedure that was used to derive the memory variables of the word-pair task. Recall was assessed three times during the study resulting in three memory variables: (1) Pre-sleep memory performance, which refers to the immediate cued-recall of the word-pairs on the experimental night, (2) post-sleep memory performance, which refers to the cued-recall of the word-pairs after a period of sleep, and finally (3) overall memory retention, which was was derived as the retention from the evening performance to morning performance. This was calculated as post-sleep memory performance divided by pre-sleep memory performance x 100. All three memory variables were calculated for each word-pair measure.

Overall memory retention was calculated for four word-pair measures: (i) overall word-pair retention, which referred to the overall percentage retention of the 90 word-pairs, (ii) overall integrative word-pair retention, which referred to the overall percentage retention for the 30 integrative word-pairs, (iii) overall concrete word-pair retention, which referred to the overall percentage retention of the 30 concrete word-pairs, and (iv) overall low concrete word-pair retention, which referred to the overall percentage retention of the 30 low concrete word-pairs.

In addition to the memory variables, sleep spindle variables were categorized in the following way: sleep spindle activity comprised of (1) spindle density, which is the average number of spindles per minute [number of spindles/stage minutes], and (2) spindle intensity, refers to the summation of the average sleep spindle amplitude and duration [average amplitude X average duration] (Schabus et al., 2006). In addition to the overall density and intensity, these two measures were also subdivided into spindle type. Firstly into slow (11-13Hz) spindles and secondly into fast (13-15Hz) spindles.

Inferential statistics. Independent-sample *t*-tests were performed on the demographic screening scores for this study to assess between-group differences. To analyze sleep architecture and spindle activity, the statistical analyses were carried out in four stages.

Stage 1: A series of 2 x 2 (Condition [pre-sleep vs post-sleep] x Group [Young vs Old]) mixed Analysis of Variance (ANOVA) were performed to assess between- and within-group differences on memory performance. Memory performance was calculated for the four

word-pair measures, which are overall word-pairs, integrative word-pairs, concrete word-pairs and low concrete word-pairs before and after a period of sleep.

Stage 2: A series of mixed ANOVAs assessed changes in sleep variables for the baseline and experimental night between the 2 groups. Specifically, data for two categories of sleep variables were analysed. The first category related to sleep architecture (stage 1, stage 2, SWS, REM, WASO, sleep efficiency and arousal index). The second sleep variable related to spindle activity. Spindle activity consists of spindle density, spindle intensity, spindle type (fast and slow), amplitude, frequency, duration and number of detected spindles. For these comparisons, a series of 2x2 ANOVAs (Night [Baseline vs Experimental] x Group [Young vs Old]) were performed.

In addition, independent *t*-tests assessed between-group differences for the relative percentage change in sleep spindle activity on the experimental night of the study.

Stage 3: In the third stage of analysis, one-tailed Pearson Correlations were performed to assess the relationships between sleep variables (sleep architecture and sleep spindle activity) and memory retention scores. In addition, the relationship between the overall percentage change in sleep spindle activity and memory retention was assessed.

Stage 4. In addition to analyzing the sleep and declarative memory data, self-reported measures were examined. Separate dependent sample *t*-tests were performed to assess differences in subjective sleep-related experiences from the baseline to experimental night. These included sleepiness from the ESS, and sleep quality, final mood upon awakening and alertness upon awakening from the PSD. Finally, one-tailed Pearson Correlations were performed to assess the relationships between self-reported measures of perceived sleep quality, alertness, sleepiness and mood upon awakening and memory retention scores.

RESULTS

Demographic Characteristics

Twenty-eight women participants were included in the final data analysis. Of the 15 younger women tested, one of the participant's data was lost. The final sample included 14 younger women (M = 20.5±1.28 years of age) and 14 older women (M = 63.14± 2.03 years of age). The ethnicity of younger sample of women consisted of 26% Caucasian, 40% Black, 26% Coloured and 6.6% Other. On the other hand, the ethnicity of older sample of women consisted of 86% Caucasian and 17% Coloured. The screening measures (BDI, MMSE, PSQI, WASI) for this study served two purposes. Firstly, it was used to ensure that eligibility

criteria were met, and secondly to ensure that eligible participants within each group were matched as closely as possible in terms these variables. As shown in table 1, there were no significant between-group differences for the screening measures (all *p*s > .081).

Table 1

Between-group comparisons on the Screening Measures

Variable	Young (n = 14)	Old (n = 14)	p	ESE
PSQI	3.71 (1.06)	2.79 (1.57)	.081	0.69
BDI	5.15 (3.95)	6.55 (3.72)	.387	0.36
MMSE	29.64 (0.63)	29.42 (0.76)	.424	0.32
WASI	108.82 (8.06)	114.75 (12.20)	.141	0.57
Verbal IQ	116.36 (8.00)	121.07 (8.51)	.144	0.57

Note: Means are presented with standard deviations in parentheses. ESE = effect size estimate, in this case Cohen's *d*.

Six out of the fourteen older women recruited for this study indicated that they were on medication. As previously stated, medication use is more prevalent in the ageing population (Wolkove et al., 2007). Each medication that was reported was independently researched for known sleep-related effects on respiratory events during sleep and evaluated in light of the screening measures for sleep quality (PSQI) and depressive symptoms (BDI). The six women on medication indicated no sleeping difficulties, depressive symptoms, psychiatric illness history and/or psychiatric medication or mental impairment (MMSE). To ensure there was no effect of medication use on sleep spindle activity, an independent *t*-test was performed to explore sleep spindle activity between older women who were and those who weren't on medication. This is important as some medication can selectively increase sleep spindle activity (Mednick et al., 2013). The analysis revealed no significant differences in spindle activity for women on medication versus women free of medication (all *p*s > .408).

1. Pre-Sleep versus Post-Sleep Declarative Memory Performance in Younger and Older Women.

Difficulty of word-pairs. Before the analysis was conducted for memory performance, a one-way ANOVA was conducted to confirm that the three word-pair categories differed in terms of difficulty. The analysis detected a main effect of word-pair type $F(2,81) = 30.68$; $p \leq$

.001, indicating that memory retention for the three different word-pair categories differed significantly. Post-hoc comparisons revealed a statistically significant difference between retention of integrative and low concrete word-pairs ($p \leq .001$) and between concrete and low concrete word-pairs ($p \leq .001$). However, retention of integrative and concrete word-pairs retention was not statistically different ($p = .107$).

The order of means shown in Table 2 show that women scored the best in the integrative word-pair measure and worst in the low concrete word-pair measure. This suggests that there was an element of difficulty in the word-pair type.

Table 2
Memory Performance of word-pairs for younger and older women Pre-Sleep and Post-Sleep on the Experimental Night

	Younger Group (n = 14)		Older Group (n = 14)		ANOVA					
					Main Effect of Condition		Main Effect of Age		Interaction Effect	
Retention (%)	Pre-sleep	Post-sleep	Pre-sleep	Post-sleep	p	ESE	p	ESE	p	ESE
Overall	84.07 (14.77)	83.86 (14.97)	64.92 (15.42)	61.43 (15.46)	.001**	0.51	.001**	0.34	.001**	0.45
Integrative	95.71 (5.29)	96.19 (4.69)	90 (14.91)	88.81 (15.11)	.582	0.01	*.129*	0.09	*.205*	0.06
Concrete	87.62 (12.57)	88.57 (13.25)	78.33 (17.63)	73.81 (17.92)	.039*	0.15	.049*	0.14	.003**	0.30
Low-Concrete	68.81 (29.46)	66.67 (29.76)	26.67 (24.32)	21.43 (22.75)	.001**	0.33	.001**	0.42	.143	0.08

Note. Means are presented with standard deviations in parentheses. ESE = Effect size estimate, in this case partial eta^2.
* p<.05 and ** p< .01

Table 2 also shows the results for the pre- and post-sleep memory performance from the experimental night between the younger and older women pre-sleep and post-sleep performance. The following results are discussed per word-pair measure.

Overall integrative word-pair performance. There was no significant main effect of condition ($F(1, 26) = 0.31$, $p = .582$, ESE = 0.01), age ($F(1, 26) = 2.45$, $p = .129$, ESE = 0.09). This means that there was no main effect pre- or post-sleep. There was also no interaction effect ($F(1, 26) = 1.69$, $p = .205$, ESE = 0.06). However, an important note is that younger women's memory performance increased from pre-to-post sleep, whereas the older women's performance decreased (see Table 2). To investigate if any trends were evident, post-hoc pairwise comparisons were performed. A pairwise comparison revealed a small trend toward significance in post-sleep memory performance between the younger and older women $t(52) = -3.34$, $p = .087$. Perhaps the lack of between-or-within group differences are due to ceiling effects since both younger and older women retained over 90% of the word-pairs before the delayed recall.

Overall concrete word-pair retention. The analysis detected a main effect of age $F(1, 26) = 4.27$, $p = .049$, ESE = 0.14. Specifically, younger women remembered more word-pairs ($M = 88.1 \pm 4.11$) than older women ($M = 76.07 \pm 4.11$). Again, this is in line with our hypothesis and current literature.

There was also a main effect of condition $F(1, 26) = 4.74$, $p = .039$, ESE = 0.15 detected, with the order of means suggesting that more word-pairs were recalled pre-sleep ($M = 82.98 \pm 15.75$) compared to post-sleep ($M = 81.19 \pm 17.19$). However, a significant interaction effect was detected $F(1, 26) = 11.15$, $p = .003$, ESE = .30). Post-hoc pairwise comparisons revealed that there was a statistically significant difference in post-sleep memory performance between younger and older women $t(52) = -3.51$, $p = .015$. This is in contrast to a non-significant difference in pre-sleep memory performance between younger and older women $t(52) = -1.58$, $p = .120$. This suggests that sleep may have played a role in better memory recall in the morning for the younger women. Specifically, younger women showed a slight, albeit non-significant, increase in retention from pre- to post-sleep (pre-sleep: $M = 87.62 \pm 12.57$; post-sleep: $M = 88.57 \pm 13.25$) (see figure's 4 & 5). In contrast, older women showed a slight, but again non-significant, decrease in retention post-sleep ($M = 73.81 \pm 17.92$) compared to the pre-sleep condition ($M = 78.33 \pm 17.63$) (see figure 5).

Overall low concrete word-pair performance. Analyses detected a significant main effect of age for this word-pair measure $F(1, 26) = 18.87$, $p = .001$, ESE = 0.42. The younger women recalled more word-pairs than the older women ($M = 67.74 \pm 7.11$ and $M = 24.05 \pm 7.11$, respectively). In addition, the analyses detected a main effect of condition ($F(1, 26) = 13.00$, $p = .001$, ESE = .33), with memory performance better pre-sleep compared to post sleep (see figure's 4 and 5). No significant interaction effect was detected ($F(1, 26) = 4.28$, $p = .143$, ESE = 0.08). However, it is noted that younger younger women showed a slight decrease in memory retention pre- to post-sleep, whereas older women showed a larger decrease (see Table 2). To investigate differences between younger and older women in each condition (that is, pre-sleep versus post-sleep), post-hoc pairwise comparisons were performed. The results showed that younger women recalled significantly more word-pairs pre-sleep compared to older women $t(52) = -4.17$, $p < .001$. Similarly, younger women recalled significantly more word-pairs post-sleep than older women $t(52) = -4.51$, $p < .001$. This suggests that there was no significant change within each group, but rather age-related differences between the two groups in both conditions.

Overall word-pair performance. A significant main effect of age for overall word-pair memory performance was detected ($F(1, 26) = 13.22$, $p < .001$, ESE = 0.34). The results indicate that younger women retained more word-pairs ($M = 83.96 \pm 4.04$) compared to older women ($M = 63.18 \pm 4.04$). This is in line with current literature which suggests that older adults, and in our case older women, experience declarative memory difficulties compared to their younger counterparts (Harand et al., 2012).

In addition, a main effect of condition was detected ($F(1, 26) = 26.75$, $p < .001$, ESE = 0.50) with the order of means suggesting that pre-sleep performance ($M = 74.5 \pm 17.73$) was higher than post-sleep performance ($M = 72.64 \pm 18.79$). However, a significant interaction effect was also detected between condition and age ($F(1, 26) = 20.94$, $p < .001$, ESE = 0.45). Post-hoc pairwise comparisons explored this effect further. Analyses detected a significant difference for memory performance between younger and older women pre-sleep $t(52) = -3.34$, $p = .002$, and post-sleep $t(52) = -3.92$, $p < .001$. This suggests that age-related differences in memory performance was evident in both conditions. However, there were no significant changes within in group over the two conditions (all ps > .544). This may suggest that memory performance was preserved in both groups.

In sum, there were clear age-related differences between younger and older women with regards to memory performance. This was the case for all word-pair measures, except integrative word-pairs. In general, younger women tended to remember significantly more than older women. While younger women showed an increase in concrete word-pair memory performance post-sleep, older women showed a slight, but non-significant, decrease. Despite slight decreases in post-sleep memory performances, there were no significant decreases in all four word-pair measures for both groups. This may suggest that memory performance was largely preserved in younger and older women from pre-sleep to post-sleep (see figure's 4 and 5).

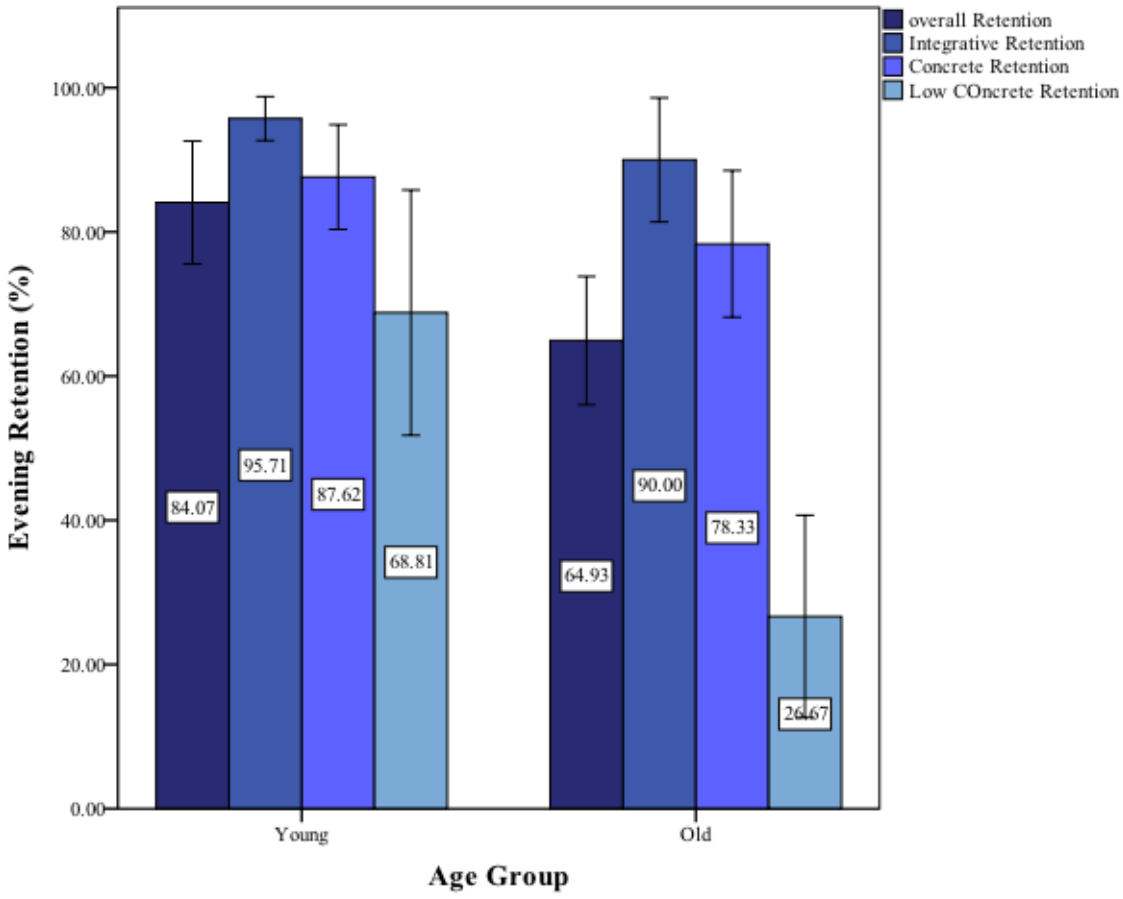

Figure 4: Evening Retention (%): a comparison of word-pair categories between Young and Older Women

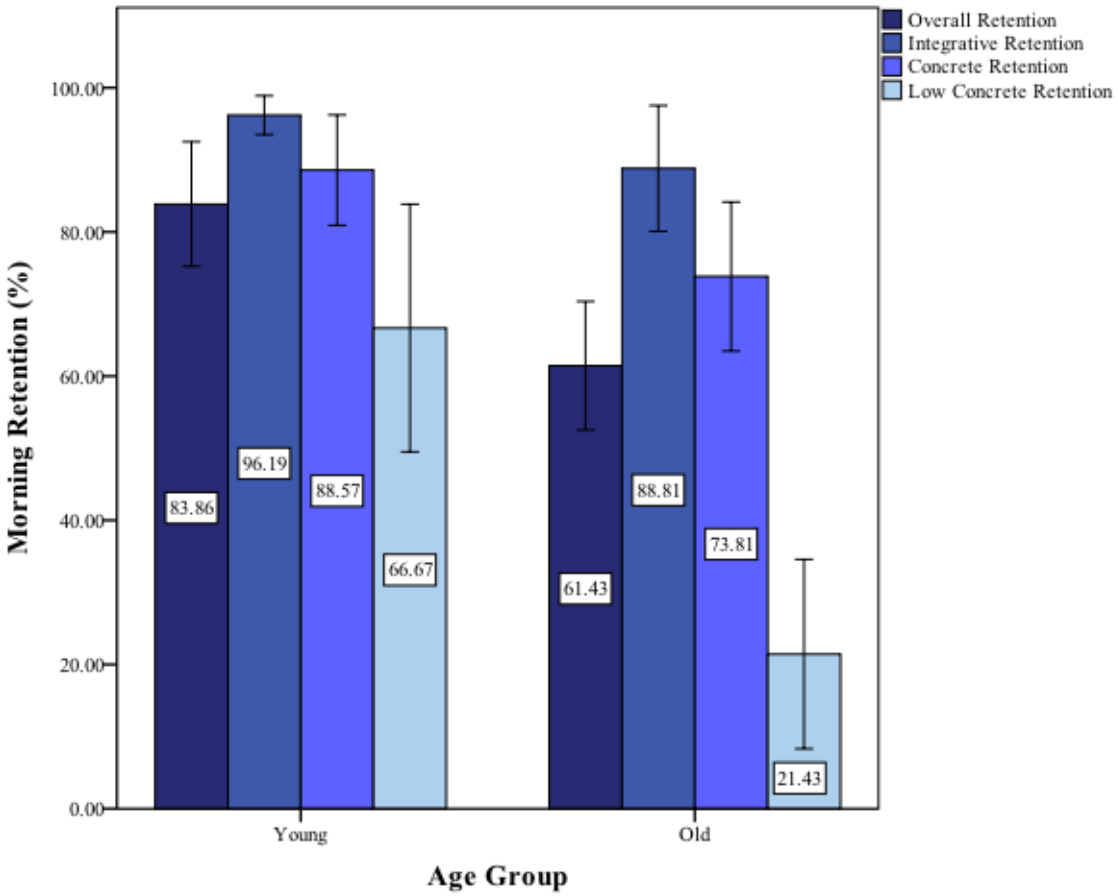

Figure 5: Morning Retention (%): a comparison of word-pair categories between Young and Older Women

2. Sleep Architecture and Sleep Spindle Activity in Younger and Older Women

2.1 Sleep Architecture

Table 3 shows the results for sleep stages, WASO, TST, Sleep Efficiency, Arousal index and on the baseline and experimental night.

Sleep Stages: Stage 1. There was a significant main effect of age ($F(1, 26) = 6.30$, $p = .019$,ESE = 0.20), with older women experiencing more stage 1 sleep compared to younger women. The analyses detected no main effect of night ($p = .580$). However, while younger women showed a slight decrease in stage 1 sleep from baseline to experimental night, older women showed a slight increase.

Table 3
Sleep Architecture on Baseline and Experimental Nights for Younger and Older Women

	Younger Group (n = 14)		Older Group (n = 14)		ANOVA results			
					Main effect of Night		Main effect of Age	
Sleep Variable	Baseline	Experimental	Baseline	Experimental	p	ESE	p	ESE
Stage (%)								
N1	7.98 (2.78)	7.79 (2.98)	10.69 (3.65)	11.45 (4.65)	.580	0.01	.019*	0.20
N2	49.47 (5.13)	47.16 (6.25)	55.34 (6.42)	49.03 (8.64)	.001**	0.34	.178	0.07
SWS	24.65 (3.73)	24.7 (4.78)	16.35 (3.35)	19.12 (3.82)	.061	0.12	.001**	0.53
REM	17.79 (4.43)	20.38 (5.07)	17.58 (5.91)	20.43 (7.52)	.006**	0.3	.918	0.00
WASO (Min)	18.39 (21.2)	15.21 (15.73)	50.79 (38.4)	60.43 (33.68)	.620	0.01	.001**	0.43
TST (Hrs.)	7.58 (0.52)	7.56 (0.36)	6.99 (0.67)	6.79 (0.63)	.387	0.03	.001**	0.38
Efficiency (%)	94.36 (6.28)	94.19 (4.52)	86.38 (8.66)	84.69 (7.75)	.547	0.01	.001**	0.39
Arousals	9.8 (4.35)	8.67 (3.15)	19.67 (10.06)	20.49 (12.16)	.848	0.00	.001**	0.33

Note. Means are presented with standard deviations in parentheses. ESE = effect size estimate, in this case, partial eta^2. N1 = stage 1; N2 = stage 2; SWS = Slow wave sleep; REM = Rapid Eye Movement; WASO = wake after sleep onset; TST = total sleep time in hours; Efficiency = Sleep efficiency.
* p<.05 and ** p<.01

Stage 2. There was a main effect of night, $F(1, 26) = 12.87$, $p = .001$, ESE = 0.34. Of note is that both younger and older women experienced less stage 2 on the experimental night where learning and recall occurred compared to the baseline night. Although older women tended to experience a higher percentage of stage 2 compared to younger women, there was no significant main effect of age, ($F(1, 26) = 1.92$, $p = .178$, ESE = 0.07) nor a significant interaction effect ($F(1, 26) = 2.39$, $p = .134$, ESE = 0.08). We had expected to find a significant age-related difference as the literature suggests that older adults tend to experience more stage 2 than their younger counterparts (e.g. Harand et al., 2012; Ohayon et al., 2004). Nevertheless, older women did experience more stage 2.

SWS. There was a significant main effect of age for SWS ($F(1, 26) = 29.87$, $p = .001$, ESE = 0.53). The results indicate that the younger women experienced more SWS than older women. This was expected (Ohayon et al., 2004) and in line with our hypothesis. It can be seen from the means in Table 3, that younger women experienced a higher percentage of SWS on both the baseline and experimental night compared to the older women. Specifically, on both the baseline and experimental night, younger women has significantly more SWS than older women ($t(3, 52) = -5.67$, $p < .001$ and $t(3, 52) = -3.76$, $p < .001$, respectively).

Analyses detected no main effect of night ($F(1, 26) = 29.87$, $p = .061$, ESE = 0.53), nor a significant interaction effect ($F(1, 26) = 29.87$, $p = .070$, ESE = 0.53). However, both these values represent a trend towards significance. From Table 3, the means suggest that older women experienced a considerable increase in SWS on the experimental night. On the other hand, younger women's SWS remained relatively stable across the baseline and experimental night. This was confirmed with a post-hoc pairwise analyses: there was no significant change in SWS from the baseline to experimental night for younger women $t(3, 52) = -.036$, $p < .971$, but a trend towards a significant increase for the older women, $t(3, 52) = -1.95$, $p = .057$.

REM. The analyses detected no main effect of age ($F(1, 26) = 0.01$, $p = .918$, ESE = 0.00). Some literature indicates that there are age-related decreases in REM sleep (e.g. Harand et al. 2012, Ohayon et al., 2004) while other studies indicate that REM sleep remains relatively unchanged in older adults (Rasch & Born, 2013). Both younger and older women, in this case, showed similar results for REM sleep percentage (see Table 3).

However, there was a significant main effect of night ($F(1, 26) = 8.58$, $p = .006$, ESE = 0.03). This indicates that there was a significant increase in REM sleep on the experimental night. There was, however, no detected interaction effect ($F(1, 26) = 0.01$, $p = .914$, ESE = 0.00). The means in Table 3 show that both the younger and older women experienced an increase in REM sleep on the experimental night when they were required to complete the declarative retention task ($M = 17.65 \pm 5.00$ and $M = 20.33 \pm 6.14$, respectively).

In sum, sleep stage percentage within the younger and older women (see figure 6) generally followed age-related norms. Older women experienced the characteristic decrease in SWS and increase in stage 1 and 2. However, we did not see any significant age-related differences in REM between the two groups. Although both groups did experience a significant increase in REM sleep on the experimental night.

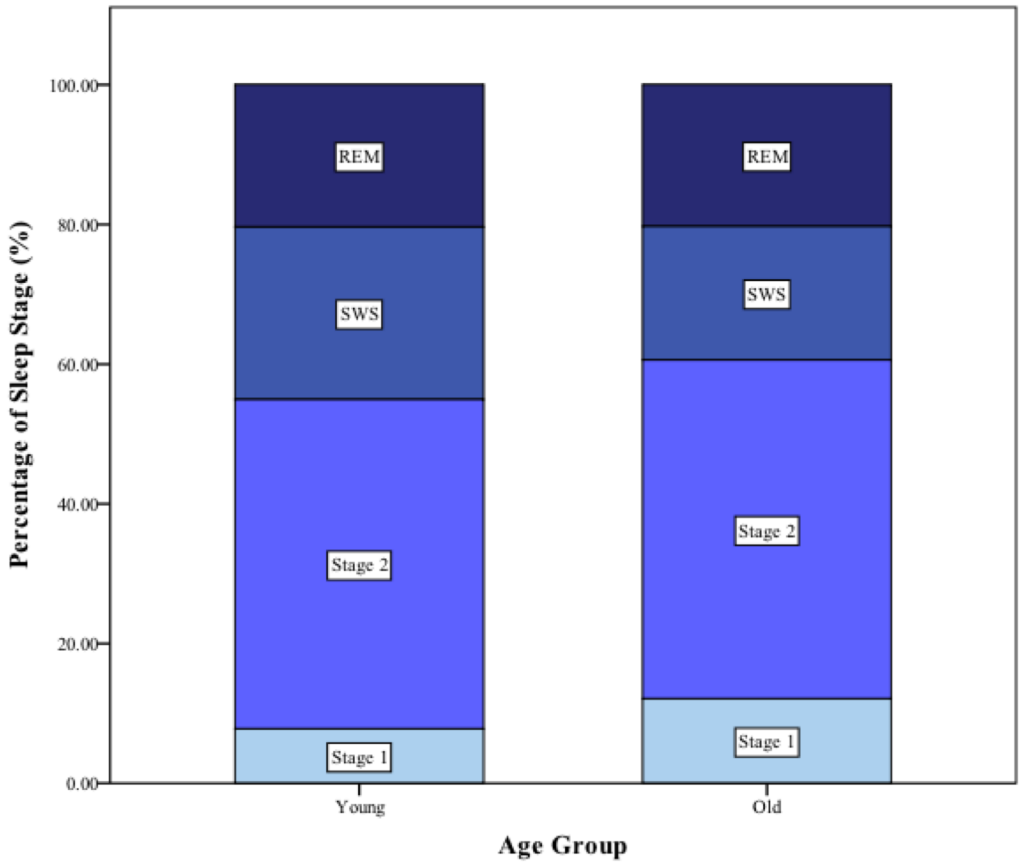

Figure 6: The Experimental Night: A comparison of sleep stages between younger and older women.

Sleep architecture Variables: WASO, TST, Sleep Efficiency and Arousal Index. There were no main effects of night (all $Fs < 0.77$, $ps > .387$) nor any significant interaction effects (all $Fs < 1.41$, $ps > .246$) for these variables. However, there were several main effects of age (refer to figure's 7 and 8).

WASO. Overall, younger women experienced less WASO compared to older women ($F(1, 26) = 19.66$, $p < .001$, ESE = 0.43). When looking at the means in Table 3, the results indicate that younger women experienced less WASO (minutes) on both the baseline and the experimental night compared to older women. Although there were no statistically significant changes from baseline to experimental night, younger women experienced less WASO on the experimental night (see figure 8). This is in contrast to the older women who experienced more WASO on the experimental night (see figure 8). This was slightly unexpected as pre-sleep learning has shown to reduce aspects of sleep architecture like time spent awake in older adults (Conte et al., 2012).

TST. There was a significant difference between time spent asleep in the younger and older women TST, ($F(1, 26) = 15.84$, $p < .001$, ESE = 0.38). Younger women experienced a higher TST (hours) compared to older women. When looking at the means in Table 3, younger women experienced a higher TST on both the baseline and experimental night compared to older women (see figure's 7 and 8). However, unlike results for WASO, both groups showed slightly less TST on the experimental night that involved learning the word-pairs.

Sleep Efficiency. There was an age-related difference found for Sleep Efficiency ($F(1, 26) = 16.32$, $p < .001$, ESE = 39). Younger women had better sleep efficiency compared to older women. In figure 7 and 8, the results show that this is the case for both the baseline and experimental night. Although there was a non-significant main effect for night, the means indicated in Table 3 show that both groups experienced a slight increase in sleep efficiency on the experimental night.

Arousal Index. Younger and older women significantly differed on their Arousal Index ($F(1, 26) = 15.84$,, $p < .001$, ESE = 0.33). Younger women experienced few arousals than the older women. The means in table 3 indicate that this was the case for both the baseline and

the experimental night. However, the younger women experienced a slight decrease in arousals on the experimental night whereas older women experienced a slight increase.

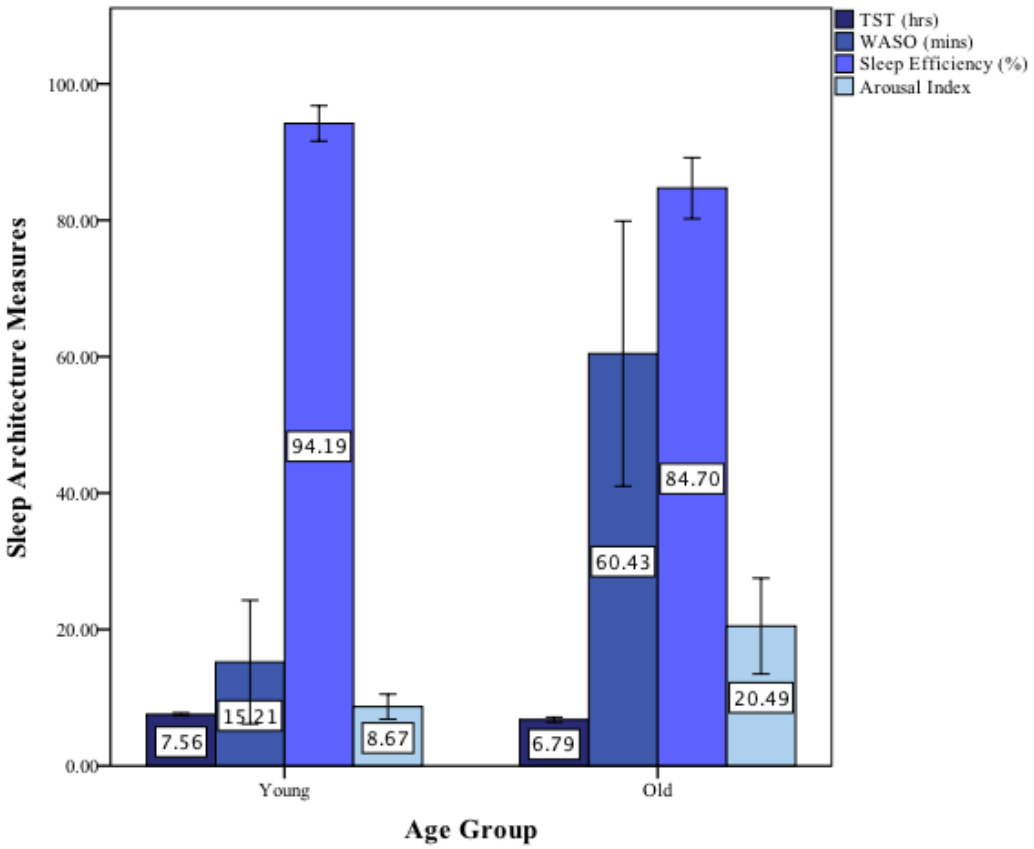

Figure 7: Baseline Night: Comparison of sleep architecture measures Between Younger and Older Women.

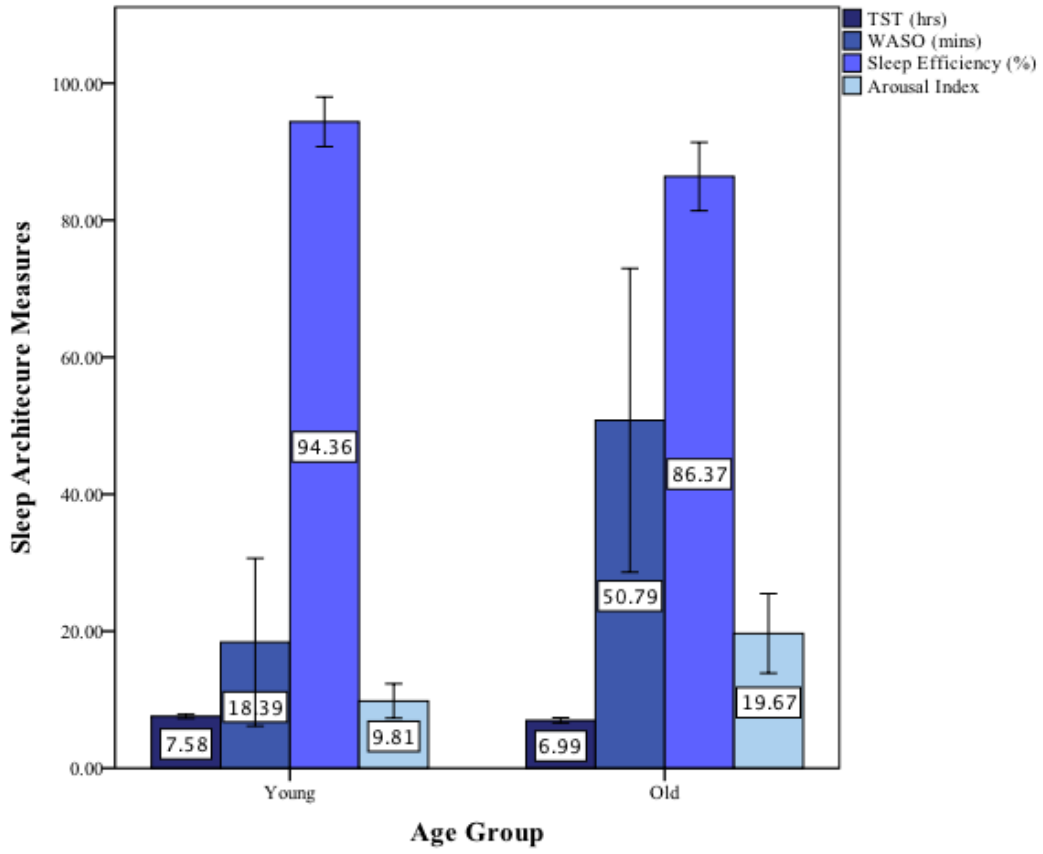

Figure 8: Experimental Night: Comparison of sleep architecture measures Between Younger and Older Women.

2.2 Sleep Spindle Activity

For all sleep spindle activity variables, no main effects of night (all *F*s < 0.72, *p*s > .258) and no interaction effects (all *F*s < 0.16, *p*s > .286) were detected. However, there were significant main effects for age (see Table 4). These are presented below.

Sleep Spindle Density. Figure 9 illustrates spindle densities between younger and older women on the experimental night. A significant main effect of age for general spindle density ($F(1, 26) = 6.65$, $p = .016$, ESE = 0.20) and slow spindle density ($F(1, 26) = 6.65$, $p = .034$, ESE = 0.16) was found. However, fast spindle density failed to reach significance ($F(1, 26) = 2.40$, $p = .133$, ESE = 0.08). This indicated that younger women had a significantly higher sleep spindle density and slow sleep spindle density compared to older women. Although fast sleep spindle density failed to reach significance, the means in Table 4 indicate that younger younger women still had a higher density compared to older women.

Table 4

NREM Sleep Spindle Activity on Baseline and Experimental Nights for Younger and Older Women.

	Younger Group (n = 14)		Older Group (n = 14)		ANOVA Main Effect of Age	
NREM Spindle Activity	Baseline	Experimental	Baseline	Experimental	p	ESE
SD	3.89 (1.92)	3.92 (1.72)	2.31 (1.39)	2.35 (1.46)	.016*	0.20
Slow SD	1.82 (1.56)	1.87 (1.46)	0.83 (0.86)	0.82 (0.77)	.034*	0.16
Fast SD	2.06 (1.16)	2.05 (0.98)	1.49 (0.79)	1.53 (0.86)	.133	0.08
Intensity	47.31 (10.68)	47.89 (5.06)	39.98 (3.25)	40.68 (3.17)	.003**	0.29
Slow Intensity	48.37 (7.04)	48 (6.61)	41.23 (3.94)	41.99 (3.31)	.002**	0.32
Fast Intensity	45.56 (4.77)	45.93 (4.04)	39.21 (3.41)	39.8 (3.31)	.001**	0.42
Amplitude	48.51 (3.35)	48.75 (2.74)	49.99 (3.59)	50.51 (3.00)	.179	0.25
Duration	0.98 (0.13)	1.03 (0.27)	0.8 (0.08)	0.81 (0.07)	.001**	0.07
Frequency	13.08 (0.29)	13.1 (0.34)	13.28 (0.26)	13.27 (0.22)	.092	0.35
Number	1437.5 (688.61)	1416.93 (624.62)	816.07 (507.76)	769.14 (458.38)	.007**	0.11

Note. Means are presented with standard deviations in parentheses. SD = spindle density. Intensity refers to the measurement of amplitude and duration. ESE = effect size estimate, in this case, partial eta^2.
* p<.05 and ** p<.01

Sleep Spindle Intensity. A significant main effect of age was found for all sleep spindle intensity measures, including slow and fast sleep spindle intensity (see figure 10). Firstly, younger women displayed a higher sleep spindle intensity compared to older women *(F*(1, 26) = 10.55, *p* = .003, ESE = 0.29). Secondly, younger women displayed a significantly higher slow spindle intensity compared to older women (*F*(1, 26) = 12.19, *p* = .002, ESE = 0.32). Lastly, younger women displayed a significantly higher fast sleep spindle intensity than the older women (*F*(1, 26) = 19.02, *p* < .001, ESE = 0.42).

In all instances, the means presented in Table 4 show that older women experienced a slight increase in spindle intensity from baseline to experimental night, whereas younger women showed a slight increase for overall intensity and fast spindle intensity only.

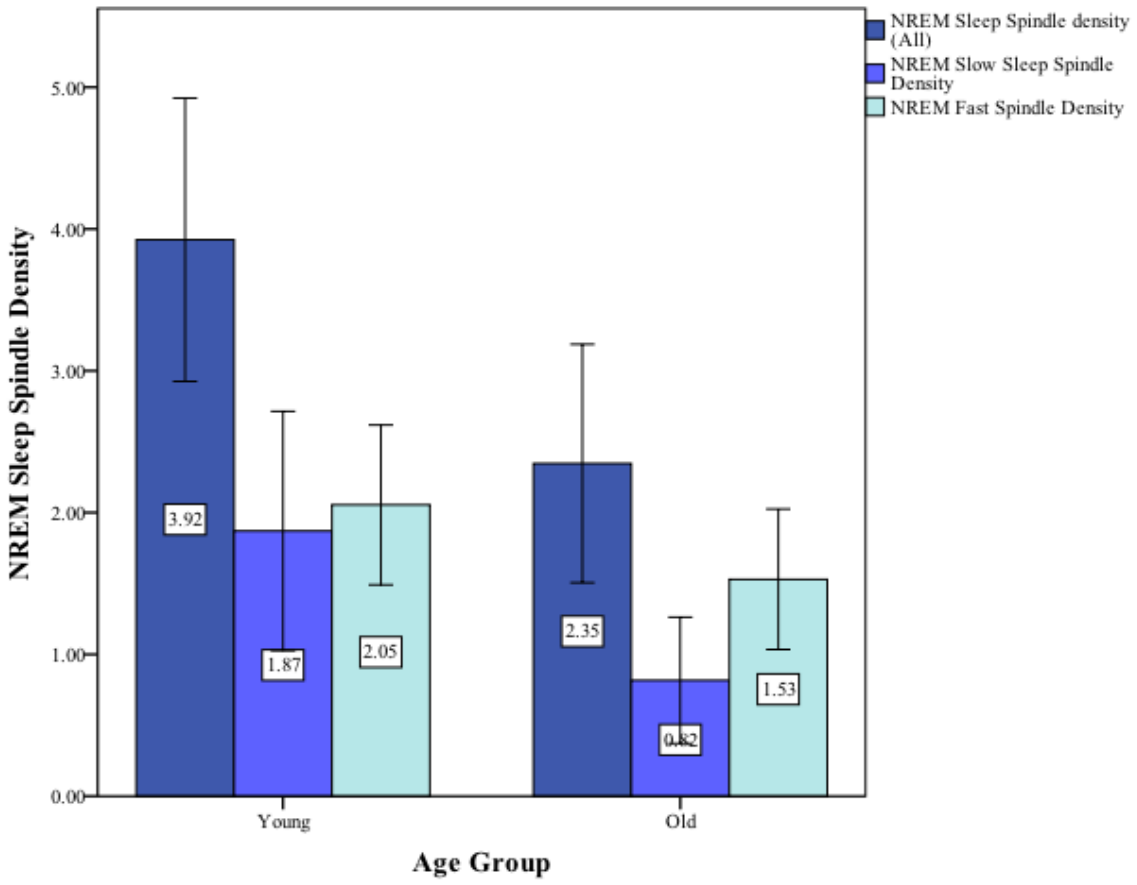

Figure 9: Sleep Spindle Density Comparison Between Younger and Older Women on the Experimental Night. Error bars represent 95% confidence intervals.

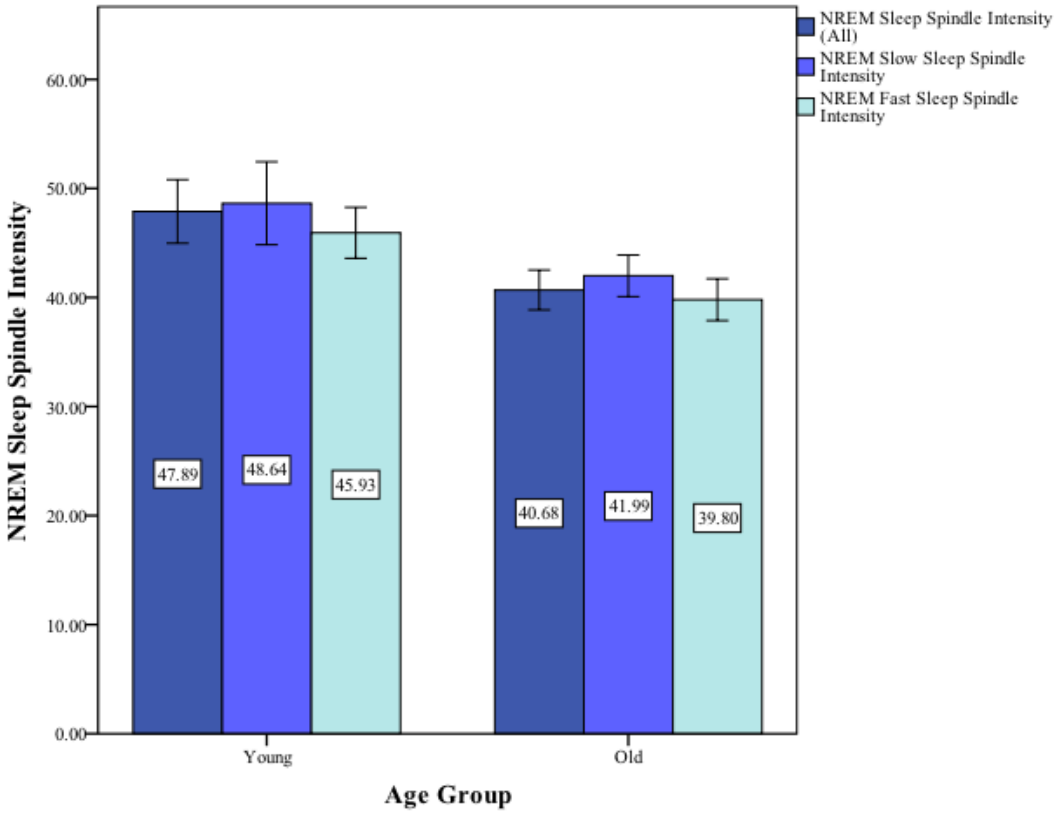

Figure 10: Sleep Spindle Intensity Comparison Between Younger and Older Women on the Experimental Night. Error bars represent 95% confidence intervals.

Sleep Spindles Features: average duration, average frequency, average amplitude and average number of detected spindles. Analyses detected significant main effects of age for the average duration and average number of detected sleep spindles. Specifically, younger women had longer sleep spindle durations compared to older women ($F(1, 26) = 13.81$, $p = .001$, ESE = 0.07), and younger women displayed a significantly higher number of spindles compared to older women, ($F(1, 26) = 8.62$, $p = .007$, ESE = 0.11). Figure 11 shows the mean differences between younger women on the baseline ($M = 1437.5 \pm 688.61$) and experimental night ($M = 1416.93 \pm 624.62$) compared to older women on the baseline ($M = 816.07 \pm 507.76$) and experimental night ($M = 769.14 \pm 458.38$) for number of spindles. Interestingly, both younger and older women showed a decrease in the number of spindles on the experimental night. However, this result failed to reach significance ($p = .656$).

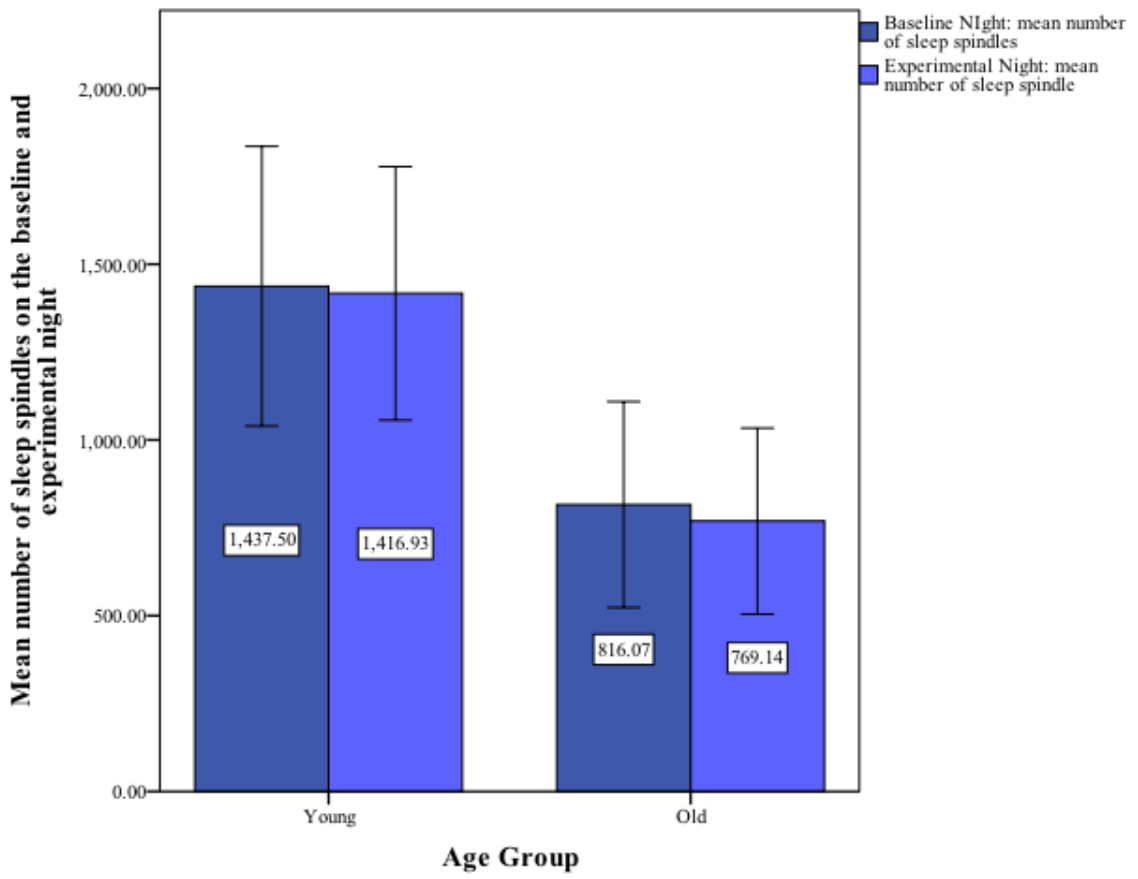

Figure 11: Number of Detected Sleep Spindles on the Baseline and Experimental Night in Younger and Older Women. Error bars have 95% confidence intervals.

The percentage change in spindle activity scores in younger and older women. Some research suggests that instead of using the absolute value of detected sleep spindles, it is rather the relative percentage change in activity from one night of sleep to the next that reflects the memory consolidating effects of sleep spindles (Schabus et al., 2008). In order to assess whether there was a significant change in sleep spindle activity from baseline to experimental night between groups, separate independent sample *t*-tests were performed.

Independent *t*-test comparisons detected no significant difference in percentage change for any of the spindle activity variables for younger and older women from the baseline to experimental night (all *p*s > .296). Although changes failed to reach significance, younger and older women showed small increases in sleep spindle activity from the baseline to experimental night. However, in the older group, there was a decrease in the average frequency of sleep spindle activity and in the number of detected sleep spindles.

Table 5
The Relative Percentage Change in NREM Sleep Spindle Activity between Younger and Older Women

	Younger Group (n = 14)		Older Group (n = 14)				
NREM Spindles	M	SD	M	SD	*t*-statistic	*p*	ESE
SD change (%)	4.2	15.43	2.10	17.63	-0.34	.738	0.13
Slow SD change (%)	9.5	25.60	5.8	23.37	-0.40	.695	0.15
Fast SD change (%)	4.9	28.42	3.0	20.42	-0.20	.847	0.08
Intensity change (%)	4.2	14.85	1.9	5.60	-0.54	.596	0.20
Slow Intensity change (%)	0.9	6.63	2.4	9.53	0.49	.630	0.18
Fast Intensity change (%)	1.1	4.86	1.7	6.44	0.31	.757	0.11
Amplitude change (%)	0.6	2.76	1.1	2.97	0.48	.638	0.17
Duration change (%)	5.1	15.15	0.6	4.48	-1.07	.296	0.40
Frequency change (%)	0.2	1.09	-0.1	0.88	-0.66	.514	0.30
Number change (%)	0.7	11.12	-3.2	22.63	-0.58	.567	0.22

Note: Degrees of freedom = 26 for all variables. SD change = Spindle density change. ESE = effect size estimate, in this case, Cohen's *d*. M = means and SD = standard deviation.

3. The Relationship between Sleep Architecture and Sleep Spindle Activity with Declarative Memory Consolidation

3.1 Sleep Architecture and Declarative Memory Consolidation

Separate one-tailed Pearson correlations were performed to assess the relationship between sleep architecture on the experimental night and memory consolidation, measured as retention in the different word-pair categories (overall, integrative, concrete and low concrete). The results are presented in Table 4.

For the older group, there were no significant associations between WASO, TST, and sleep efficiency with memory retention in any of the categories (all *ps* > .093). However, number of arousals positively correlated with the retention of low concrete word-pairs (r = .620, p = .009). This was not anticipated. Using the absolute deviation around the median did not reveal any outliers. Regarding sleep stages and the outlined hypotheses for the older group, a positive correlation was found between SWS and the overall retention of concrete word-pairs *only* (r = .479, p = .042; see figure 12). This indicated that the more SWS the older women experienced on the experimental night, the better their overall retention for concrete word-pairs. There were no other significant correlations between sleep stages and memory retention for the older women (all *p*s > .083).

For the younger group, the sleep architecture variables WASO, TST, sleep efficiency and number of arousals showed several signification correlations with memory retention. Specifically, TST positively correlated with the overall percentage of word-pair retention (r = .517, p = .029), the overall retention of concrete word-pairs (r = .509, p = .031) and low concrete word-pairs (r = .854, $p \leq .01$). Thus, the more sleep that the young women had during their 8-hour allotted sleep-time, the better their memory.

Similarly, sleep efficiency positively correlated with the overall percentage of word-pair retention (r = .493, p = .037), the overall retention of concrete word-pairs (r = .490, p = .038) and low concrete word-pairs (r = .839, $p \leq .01$). Thus, those who experienced higher sleep efficiency had better memory retention. WASO negatively correlated with the overall retention of low concrete word-pairs (r = .722, p = .002), while positively correlated with the overall retention of word-pairs (r = .574, p = .016). This means that the more time spent awake after first sleep onset, the worse the memory retention for low concrete word-pairs, but better overall word-pair retention. This positive correlation was not anticipated and does not fit with the expected outcomes from sleep and memory literature. Further analyses were performed to investigate this result. One outlier was detected using the absolute deviation around the median which further revealed that WASO did not significantly correlate with overall retention of word-pairs (r = -.249, p = .206).

Table 6
The Relationship between Sleep Architecture and Memory Retention in Younger and Older Women.

Variable	N1 (%)	N2 (%)	SWS (%)	REM (%)	TST (hrs.)	Eff. (%)	Overall Retention (%)	Integrative (%)	Concrete (%)	Low Concrete (%)
N1 %	**1.000**	**-.232**	**.089**	**-.474**	**-.455**	**-.467**	**-.354**	**-.281**	**.109**	**.136**
N2 %	.079	**1.000**	**-.691**	**-.670**	**.552**	**.555**	**.286**	**.201**	**-.391**	**.305**
SWS %	.018	-.821	**1.000**	**.249**	**-.501**	**-.507**	**-.119**	**-.071**	**.479 ***	**-.249**
REM %	-.702	-.512	.058	**1.000**	**-.084**	**-.076**	**-.027**	**-.004**	**.147**	**-.340**
TST (hrs.)	-.468	.489	-.431	.082	**1.000**	**.999**	**.296**	**.376**	**-.369**	**.198**
Eff. %	-.459	.471	-.424	.092	.998	**1.000**	**.301**	**.368**	**-.365**	**.203**
Overall Retention (%)	-.659 **	.391	-.343	.239	.517 *	.493 *	**1.000**	**.483**	**.177**	**.196**
Integrative (%)	.024	-.582 *	.438	.319	-.298	-.285	.067	**1.000**	**-.360**	**-.338**
Concrete (%)	-.720 **	.173	-.247	.439	.509 *	.490 *	.759	-.118	**1.000**	**-.152**
Low Concrete (%)	-.423	.731 **	-.683 **	-.002	.854 **	.839 **	.705	-.384	.499	**1.000**

Note: Results presented are Pearson's *r* correlation coefficients. The bold scores in the greyed-out triangle relate to results for the older women. The score diagonal to it relates to the younger women. N1 = stage 1, N2 = stage 2, SWS = slow wave sleep, REM = Rapid eye movement sleep, TST = total sleep time, EFF = sleep efficiency.
* $p<.05$ and ** $p<.01$.

In the younger group, the percentage of SWS negatively correlated with low concrete word-pairs ($r = 683$, $p = .004$) and did not reach significance for concrete word-pairs ($p = .197$), as it did in the older group. However, unlike for older women, younger women did show a moderately strong positive correlation between the percentage of stage 2 sleep and low concrete word-pairs ($r = .731$, $p = .001$; see figure 13). This may be of significance as sleep spindle activity predominantly occur in this stage (Spriggs, 2002). In addition, the amount of stage 1 sleep negatively correlated with the overall percentage of concrete word-pairs ($r = .720$, $p = .002$), which meant those who experienced a higher amount of stage 1 sleep had worse concrete word-pair memory retention. The data showed no significant correlations between REM and memory retention in the younger women.

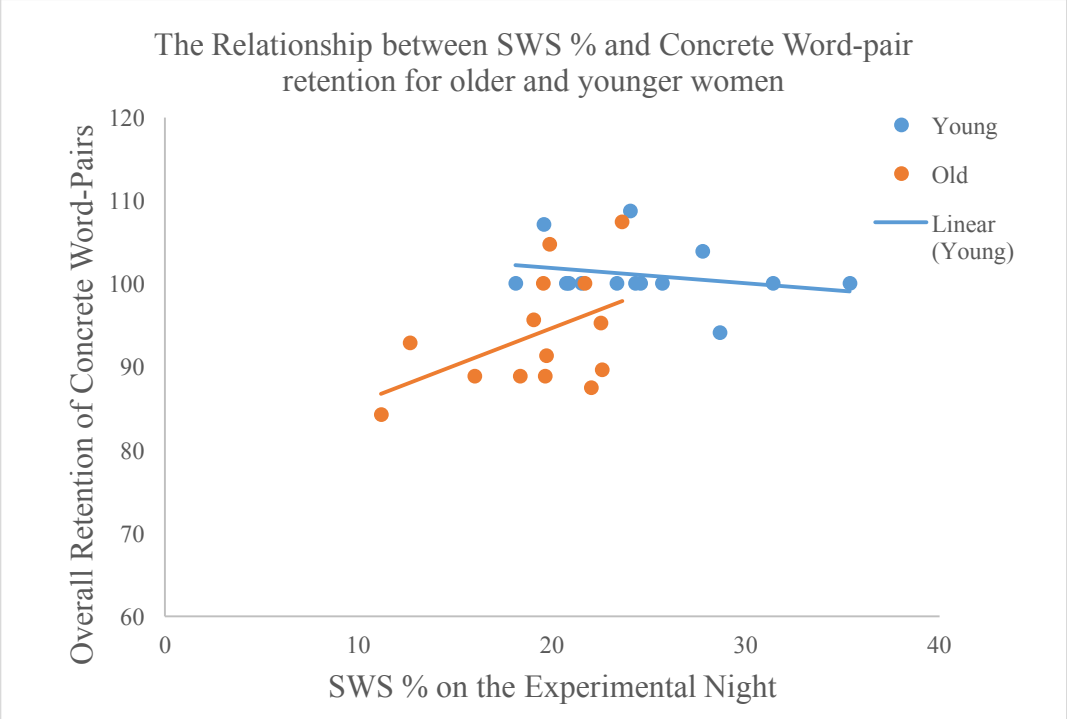

Figure 12: SWS Percentage and Overall Concrete word-pair retention in younger and older women.

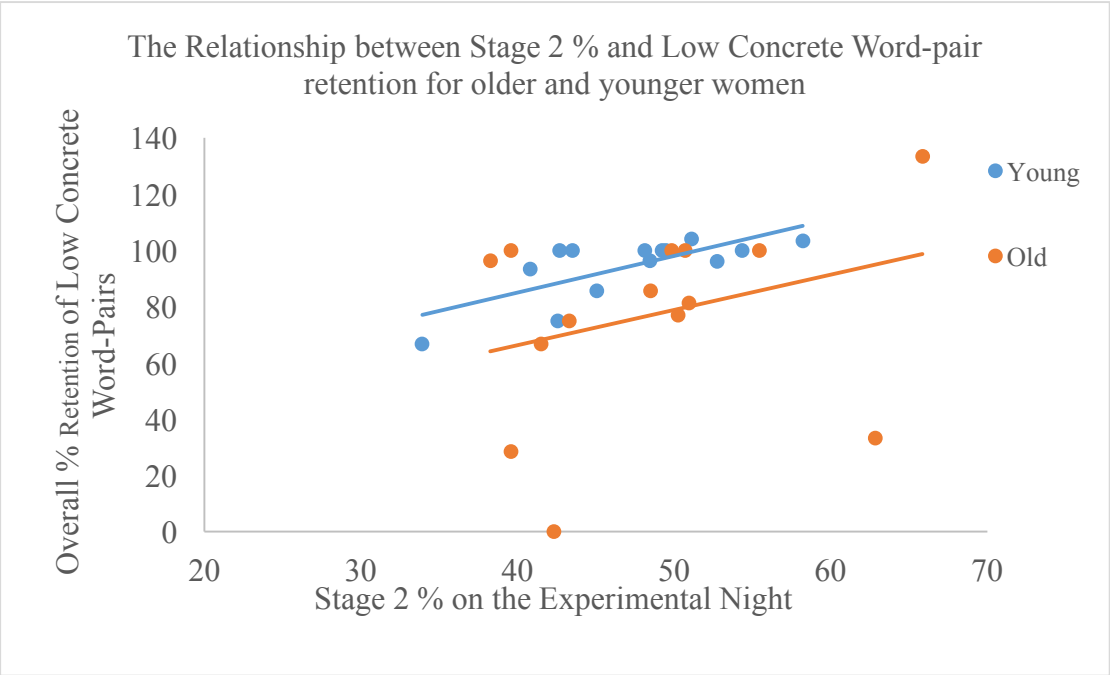

Figure 13: Stage 2 Percentage and Overall Low Concrete word-pair retention in younger and older women.

3.2 The Relationship between Sleep Spindle Activity and Declarative Memory Consolidation in Older and Younger Women

Two sets of one-tailed Pearson correlations were performed. The first analysis assessed whether the absolute values of spindle activity detected on the experimental night correlated with overnight memory retention. The second analysis assessed the relationship between the relative percentage change in spindle activity (from baseline to experimental night) and overnight memory retention.

Firstly, when analysing the relationship between spindle activity on the experimental night with memory retention there were significant correlations for both groups, however, these correlations differed within each age group. The only similarity between older and younger women was that the number of detected sleep spindles positively correlated with overall retention (see figure 15).

In the older group, spindle density positively correlated with overall retention (r = .468, p = .046; see figure 14). This was in line with our major hypothesis, which states that sleep spindle density would positively correlate with memory retention. In addition, fast spindle density (r = .475, p = .043), and number of spindles (r = .506, p = .032), positively correlated with overall memory retention. These results indicated that a higher spindle density and number of sleep spindles are associated with better memory retention. Slow spindle density, intensity (including slow and fast), amplitude, frequency and duration did not correlate with memory retention in any word-pair measure (all ps > .059).

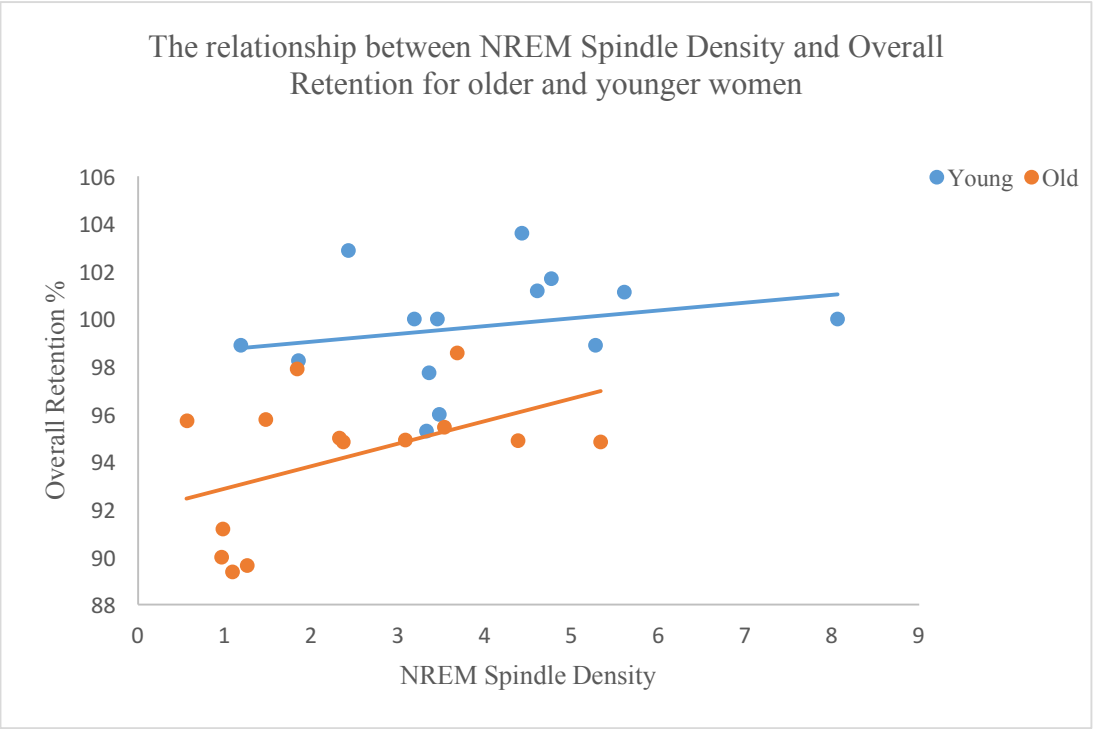

Figure 14: NREM Spindle Density and Overall Retention in younger and older women.

Table 7
Correlation Matrix for NREM Sleep Spindle Activity on the Experimental Night with Memory Retention for Younger and Older.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. SD	**1.000**	**.881**	**.905**	**.753**	**.613**	**.695**	**.300**	**-.339**	**.460**	**.959**	**.468***	**.151**	**-.126**	**.390**
2. Slow SD	.826	**1.000**	**.595**	**.525**	**.529**	**.384**	**.243**	**-.665**	**.288**	**.795**	**.355**	**-.054**	**.091**	**.254**
3. Fast SD	.534	-.036	**1.000**	**.806**	**.564**	**.834**	**.291**	**.023**	**.522**	**.913**	**.475***	**.305**	**-.296**	**.434**
4. Intensity	.670	.666	.188	**1.000**	**.850**	**.962**	**.118**	**-.241**	**.797**	**.686**	**.170**	**-.022**	**-.168**	**.358**
5. Slow Intensity	.615	.708	.029	.973	**1.000**	**.697**	**.051**	**-.432**	**.691**	**.478**	**.062**	**-.084**	**-.059**	**.050**
6. Fast Intensity	.607	.419	.448	.883	.768	**1.000**	**.121**	**-.029**	**.771**	**.672**	**.188**	**.033**	**-.226**	**.438**
7. Amplitude	.230	.153	.178	-.101	-.163	-.050	**1.000**	**.236**	**-.499**	**.230**	**.146**	**.301**	**-.064**	**.164**
8. Frequency	-.333	-.730	.505	-.448	-.575	-.121	.289	**1.000**	**-.325**	**-.222**	**.064**	**.321**	**-.346**	**.124**
9. Duration	.400	.384	.133	.773	.807	.619	-.271	-.286	**1.000**	**.447**	**.064**	**-.189**	**-.136**	**.242**
10. Number	.989	.823	.518	.708	.655	.648	.269	-.307	.469	**1.000**	**.506***	**.204**	**-.191**	**.411**
11. Overall (%)	.236	.245	.051	.059	.075	-.043	-.189	-.065	.185	.256	**1.000**	**.483**	**.177**	**.196**
12. Integrative (%)	-.130	-.293	.208	-.262	-.250	-.364	-.012	.137	-.174	-.188	.067	**1.000**	**-.360**	**-.338**
13. Concrete (%)	-.054	.120	-.275	-.136	-.119	-.192	-.362	-.183	-.041	-.061	.759	-.118	**1.000**	**-.152**
14. Low Concrete (%)	.427	.368	.204	.197	.130	.257	.185	.005	.206	.485*	.705	-.384	.499	**1.000**

Note: Results presented are Pearson's *r* correlation coefficients. The bold scores in the greyed-out triangle relate to results for the older women. The score diagonal to it relate to the younger women. SD = spindle density.
* $p<.05$ and ** $p<.01$.

In the younger group, the number of spindles positively correlated with overall retention of low concrete word-pairs (r = .485, p = .039; see figure 15). Thus, the higher the number of spindles present, the better the retention for the most difficult word-pair measure, low concrete word-pairs. As Table 7 shows, there were no other significant correlations between spindle activity and retention in the younger group. This was contrary to our hypothesis as we expected spindle density to positively correlate with overall memory retention, concrete word-pair retention and low concrete word-pair retention. There was, however, a borderline positive correlation of NREM spindle density positively correlating with low concrete word-pair retention (p = .064).

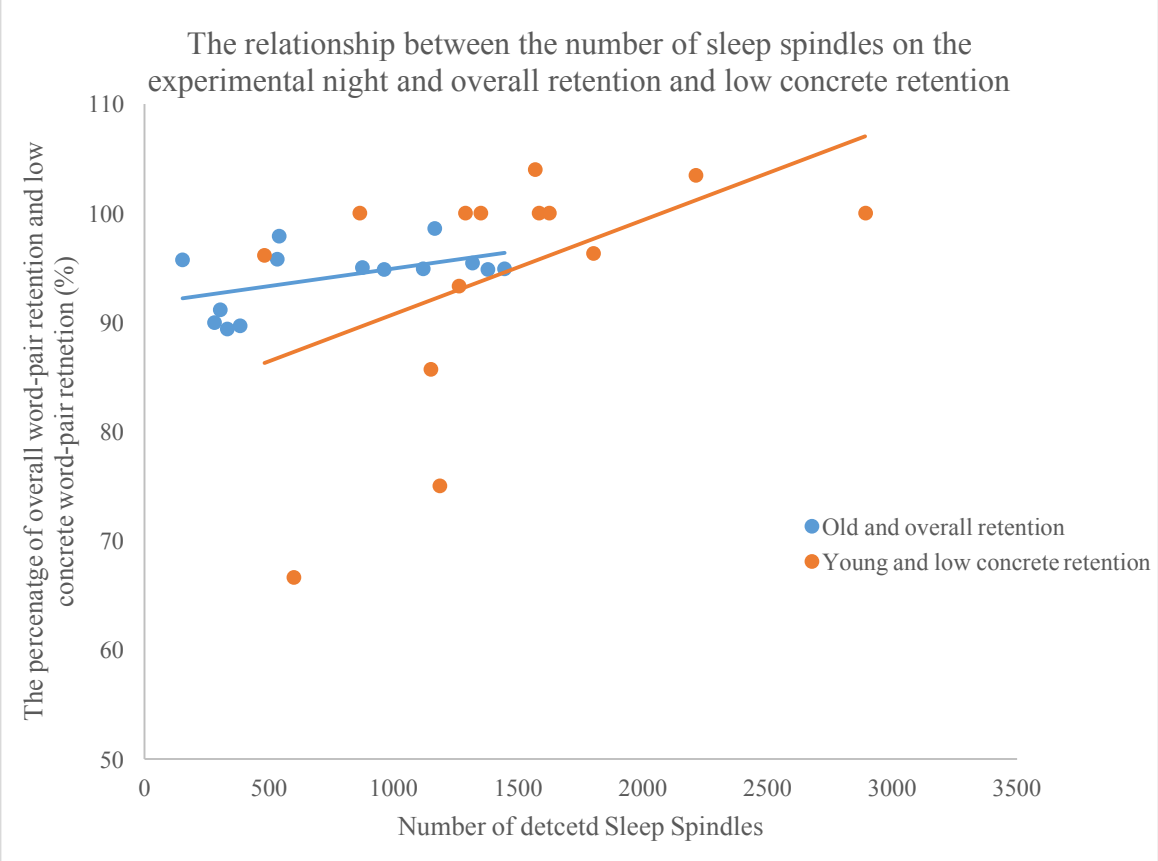

Figure 15: NREM Spindle Density and Overall Retention in younger and older women

One-tailed Pearson correlation coefficients were also calculated to assess whether there was a relationship between percentage change in sleep spindle activity and memory retention. The results of these correlations are presented in Table 8. In the younger group, the change in slow and fast spindle intensity positively correlated with overall percentage retention of concrete word-pair (r = .593, p = .013 and r = .542, p = .023, respectively).

Table 8
Correlation Matrix for the percentage change in NREM Sleep Spindle Activity on the Experimental Night with Overall Memory Retention for Younger and Older

NREM Spindles Activity	Change in SD (%)	Change in Slow SD (%)	Change in Fast SD (%)	Change in Intensity (%)	Change in slow intensity (%)	Change in fast intensity (%)	Overall Retention (%)	Integrative (%)	Concrete (%)	Low Concrete (%)
Change in SD (%)	**1.000**	**.590**	**.764**	**.449**	**.511**	**.379**	**-.006**	**-.496***	**-.034**	**.700****
Change in Slow SD (%)	.648	**1.000**	**.100**	**.647**	**.433**	**.459**	**-.415**	**-.615****	**.150**	**.530***
Change in Fast SD (%)	.616	-.045	**1.000**	**.102**	**.242**	**.330**	**.203**	**-.434**	**-.034**	**.572***
Change in Intensity (%)	-.225	.205	-.284	**1.000**	**.558**	**.803**	**-.372**	**-.291**	**-.136**	**.439**
Change in slow intensity (%)	.557	.513	.225	.024	**1.000**	**.108**	**-.417**	**-.592***	**.187**	**.075**
Change in fast intensity (%)	.269	.232	.199	.355	.815	**1.000**	**-.211**	**-.200**	**-.292**	**.584***
Overall Retention (%)	-.057	.162	-.136	-.014	.341	.298	**1.000**	**.483**	**.177**	**.196**
Integrative (%)	-.182	.174	-.385	.156	-.260	-.319	.067	**1.000**	**-.360**	**-.338**
Concrete (%)	.205	.389	-.031	.087	.593*	.542*	.759	-.118	**1.000**	**-.152**
Low Concrete (%)	-.321	-.304	.049	.056	.183	.336	.705	-.384	.499	**1.000**

Note: Results presented are Pearson's *r* correlation coefficients. The bold scores in the greyed-out triangle relates to results for the older women. The score diagonal to it relate to the younger women. SD = spindle density.
* $p<.05$ and ** $p<.01$.

This suggests that the larger the increase in fast and slow spindle intensity from baseline to experimental night, the higher the retention for concrete word-pairs. There were no other significant correlations (all *p*s > .085).

In the older group, the change in overall spindle density (r = .700, p = .003), in fast spindle density (r = .572, p = .016), and in slow spindle density (r = .647, p = .006), positively correlated with the overall retention of low concrete word-pairs. Thus larger increases in spindle density were associated with better the memory retention for the low concrete word-pair measure. In addition, the increase in fast spindle intensity positively correlated with overall retention of low concrete word-pairs (r = .584, p = .014). Thus, the larger the increase in fast spindle intensity, the higher the retention for the low concrete word-pairs. Interestingly, the percentage change in overall sleep spindle density (r = -.496, p = .036), in slow sleep spindle density (r = -.615p = .010), and the percentage of change in overall sleep spindle slow intensity (r = -.592, p = .013) showed a negative correlation with overall retention percentage of integrative word-pairs. Thus, the larger the increase in spindle density the poorer the memory retention for this word-pair measure.

4. Self-Reported Measures of Sleep Quality and Sleepiness in Younger and Older Women

As a secondary analysis, self-reported measures of sleep were investigated to see whether perceived experience influenced subsequent sleep and memory retention (see Table 9). Participants were asked to score, along a likert scale (where 1 was the least/worst and 10 was the most/best) for self-reported sleep quality, mood and alertness upon awakening. This was filled in each morning after a period of 8 hours of sleep. Participants also filled out the ESS, which assessed their level of sleepiness.

A series of paired sample *t*-tests were performed within each group to assess whether there any significant changes from baseline to experimental night. The analysis revealed younger and older women did not show any significant differences from baseline to experimental night for perceived level of sleepiness, sleep quality, mood and alertness upon awakening (Younger: all *p*s> .234, Older: all *p*s > .331). This showed that being exposed to a declarative task did not significantly change their perceived level of sleep quality. However, on average, younger women did show high scores for the ESS pre-sleep as they scored over 10 on both nights, which indicates they was abnormally sleepy. However, this was before bedtime. The morning ESS showed normalized sleepiness ratings. The ESS was performed to

see whether completing a learning task just before sleep impacted each individual's rating of sleepiness compared to their baseline recording.

One-tailed Pearson correlations assessed whether perceived tiredness or sleep quality was associated with memory retention. This is important to consider as motivation and other intrinsic factors may contribute to successful encoding and subsequent consolidation (Conte & Ficca, 2013). In the older group, the self-reported SQ positively correlated with overall retention (r = .527, p = .032) and overall retention of integrative word-pairs (r = 575, p = .020). This meant that those who reported better SQ had better memory for those word-pair categories. There were no other significant correlations (all ps > .080). For the younger group, perceived level of alertness upon waking from the experimental night showed a positive correlation with overall retention (r = .473, p = .044). There were no other significant correlations (all ps > .190). This means that those who were more alert upon awakening had better memory retention.

Table 9
Paired t-test comparing Self-Reported Measure in Younger and Older Women

	Young (n = 14)		Old (n = 14)		Young		Old	
Variable	Baseline	Experimental	Baseline	Experimental	p	ESE	p	ESE
PSD Morning								
SQ	7.71 (1.54)	7.71 (1.89)	7.23 (2.39)	7.53 (2.18)	1.00	0.00	.711	0.17
Mood	7.78 (1.58)	8.21 (1.72)	8.46 (1.33)	8.69 (1.38)	.234	0.25	.489	0.13
Alertness	6.42 (2.88)	6.18 (2.52)	6.69 (2.25)	6.38 (1.85)	.588	0.10	.651	0.17
ESS Evening	10.71 (4.76)	11.64 (4.23)	8.14 (3.42)	8.92 (3.77)	.507	0.20	.331	0.28
ESS Morning	7.07 (4.96)	8.00 (5.00)	7.35 (5.05)	7.00 (4.69)	.304	0.19	.646	0.08

Note: Means are presented with standard deviations in parentheses. PSD = Pittsburgh Sleep Diary. Self-reported SQ = self-reported sleep quality. ESS = Epworth Sleepiness Scale. ESE = size effect estimate, in this case Cohen's d.

DISCUSSION

This study aimed to investigate sleep-dependent memory consolidation in younger and older women. Specifically, this pertained to SWS and sleep spindle activity and its relationship to declarative memory performance and retention. Previous research suggests that SWS and sleep spindle activity is actively involved in the consolidation of declarative memories (Born & Wilhelm, 2012; Diekelmann & Born, 2010; Stickgold & Walker, 2007). Previous sleep research also reports that older adults tend to show age-related declines in

SWS and sleep spindle activity that can coincide with a weakening in declarative memory (Harand et al., 2012; Hornung et al., 2005; Martin et al., 2013; Ohayon et al., 2004). This research aimed to investigate the question of whether sleep-dependent declarative memory consolidation is altered in older age, and what role the various changes in sleep architecture and spindle activity play (Scullin, 2015).

There were three main hypotheses for this study. Firstly, it was hypothesized that younger women would perform better and retain more word-pairs than older women. Secondly, it was hypothesized that sleep architecture and sleep spindle activity would show age-related declines, as suggested by previous studies (e.g. Martin et al., 2013; Crowley, 2011). And finally, it was hypothesized that a positive relationship exists between SWS, sleep spindle activity and memory retention for both younger and older women. In our case, this relationship is hypothesized to be evident in older women who report having no sleeping difficulties or disorders. However, older women should show a weaker relationship than younger women, since advancing age is associated with a decline in SWS and sleep spindle activity and marked by memory impairments (Harand et al., 2012; Hornung et al., 2005).

Memory Performance Pre-sleep and Post-sleep

The results of this study support the hypothesis that older women show a decline in declarative memory ability compared to younger women (Carotenuto & Espositio, 2014; Harand et al., 2012). Specifically, younger women performed significantly better on both the pre-sleep and post-sleep memory performance of the word-pair task compared to the older women. This trend extended to the word-pair measures of overall retention, concrete retention and low concrete retention.

Integrative word-pair performance. No age-related differences were found between the younger and older women for this word-pair type. These result support the hypothesis put forward by Estes and Jones (2009) that integrative word-pairs may alleviate typical age-related differences in memory recall. This was evident for both the pre-sleep and post-sleep condition. There was also no significant change in performance from the pre- to post-sleep conditions. However, younger women experienced a slight increase in word-pair recall post-sleep, whereas older women did not. This means that even though older women performed similarly to their younger counterparts, they still recalled less word-pairs. A potential reason for the lack significant increases in post-sleep retention may be that integrative word-pairs are not modulated by sleep and potentially they are too easy. This is evidenced by the high scores

in both groups pre-sleep. According to some researchers, sleep-dependent memory may process memories that need further strengthening (Conte & Ficca, 2013; Diekelmann & Born, 2010). If the integrative word-pairs performance and retention did not require further strengthening, then sleep may not have played a role in sleep-dependent memory consolidation for this cohort.

Concrete word-pair performance. Unrelated concrete word-pairs are thought to entail an element of difficulty that requires explicit encoding and elaboration (Payne et al., 2012). A significant age-related difference was evident between the two groups. In the post-sleep performance condition, the younger experienced an increase in word-pair recall whereas older women did not. Although these relative increases and decreases were not significant, this may suggest that sleep may have played a role in the enhanced recall. The potentially modulating effect of sleep is in line with previous studies (Nishida, Pearsall, Buckner, & Walker, 2009; Wagner, Gais & Born, 2001). However, since we did not contain a wake-condition to compare our delayed recall after sleep to, it is not certain that the period of sleep was responsible for improved post-sleep retention. Nonetheless, these results are in line with numerous studies, which suggests that sleep plays a beneficial role in promoting memory consolidation (Born & Wilhelm, 2012; Diekelmann, Wilhelm, & Born, 2009).

Low concrete word-pair performance. This word-pair was the most challenging for the younger and older women. This supports the view that low concrete word-pairs involves difficult encoding (Schmidt et al., 2006). Here again, a significant age-related difference was found, with younger women outperforming older women both pre- and post-sleep. The younger and older women showed a slight decrease in post-sleep performance. But again, these decreases were not significant. Thus word-pair performance was preserved to some extent in both groups.

Using this word-pair measure may not be suitable for older adult populations. Underperforming on such a task may result in poor motivation and disengaged encoding, which can play a role in subsequent sleep-dependent memory consolidation (Conte & Ficca, 2013).

Overall word-pair performance. There was a significant age-related difference in the overall word-pair performance between younger and older women. However, the older women did not experience a significant decrease in post-sleep recall. Thus the performance in

the younger and older women was again largely preserved from pre-sleep to post-sleep. This is contrary to other sleep studies on older adults that find recall in not preserved post-sleep (e.g. Scullin, 2012).

Although memory was seemingly preserved in both groups, there was a lack of recall enhancement that other studies have found following a period of sleep (e.g. Gais & Born, 2004). A lack of enhancement in the younger women after a period of sleep was somewhat unexpected. However, this lack of a significant increase in post-sleep recall may be due to the fact that overall retention is the combined performance of all three word-pair measures. Specifically, the very low performance in the low-concrete word-pair measure may be skewing a sleep benefit seen on the recall of the integrative and concrete word-pairs. This reasoning, however, does not apply to the older women. Where the younger women showed a slight increase, older women recalled less word-pairs in all three word-pair types. However, as Diekelmann & Born (2010) suggest, a lack of enhancement does not mean that sleep was not actively involved. Here, sleep may have played a role in preserving memory retention.

Sleep Architecture in Younger and Older Women and its relationship with Declarative Memory Consolidation

Sleep architecture changes significantly over the lifespan (Spriggs, 2002). Older adults experience an age-related decline in various sleep architecture variables. A prominent change associated with ageing adults is the decrease in SWS and sleep spindle activity (Fogel & Smith, 2011; Harand et al., 2012; Hornung et al., 2005; Martin et al., 2013). Sleep stage 1 and 2 begin to increase in length while REM is decreased in duration (Ohayon et al., Spriggs, 2002). Other age-related changes include frequent arousals, increased sleep latency, shorter sleep duration, and decreased sleep efficiency (Hornung et al., 2005; Ohayon et al., 2004).

Sleep stages and their relationship with declarative memory retention. The older women showed a higher percentage of stage 1 sleep compared to younger women and is in line with previous research (Ohayon et al., 2004). The results indicate that the older women spent more time transitioning between wake and sleep than younger women. Older women also displayed a higher percentage of stage 2 sleep and experienced less SWS than their younger counterparts. Again, this in line with previous studies (Carskadon & Dement, 2011; Conte et al., 2012; Harand et al., 2012). SWS declines may be linked to cortical tissue loss and thalamocortical regulation changes (Hornung et al., 2005). Since the *active system consolidation* model proposes that declarative memory consolidation occurs in SWS, less

time in this stage may result in reduced memory consolidation. This may also be due to the fact that delta activity and slow oscillations undergo major reduction in the ageing population (Fogel et al., 2012).

Despite the significantly lower percentage of SWS in older women, a positive correlation in SWS with the overall retention of concrete word-pairs was found in older women. This was the only significant correlation in the elderly women regarding sleep stages. Higher amounts SWS in older women was associated with better retention of concrete word-pairs. This result supports the view that older adults can experience a sleep-related benefit for declarative memory recall (Lafortune et al., 2014; Wilson et al., 2012). The same relationship was not seen in the younger women, however. This relationship may be due several factors. Firstly, there may be ceiling effects as pre-sleep recall was already high on the integrative (recall was above 90%) and concrete word-pairs (recall was above 85%). If this lack of a significant correlation in younger women was due to ceiling effects, it supports the view that sleep may preferentially consolidate encoded memories that are weakly encoded which require strengthening (Diekelmann & Born, 2010).

Interestingly, stage 2 sleep was positively associated with better recall on low concrete word-pairs in the younger women. This meant that more stage 2 sleep was associated with better retention on the recall of low concrete word-pairs, where memories were weakly encoded because of the nature of the task. In addition, there was an uncharacteristic negative association between the amount of SWS and recall for the concrete word-pairs in the younger women. This indicated that better recall was associated with less SWS. The differences experienced by each group may suggest that sleep stages by themselves do not necessarily explain sleep-dependent memory consolidation as some research has suggested (Diekelmann & Born, 2010). Instead research is rather focused on identifying the neurophysiological mechanisms within the stages that may be responsible for consolidation. These neurophysiological mechanisms include slow oscillations, spindle activity and hippocampal ripple activity (Stickgold & Walker, 2007). This is important because sleep spindle activity occurs in stage 2 and SWS (Fogel& Smith, 2011). It has been suggested that stage 2 and SWS spindle activity may share similar roles (Diekelmann & Born, 2010). In contrast, it has been postulated that spindle activity in stage 2 and SWS may play different roles. Specifically, stage 2 has been associated with neuronal replay while SWS is responsible for synaptic downscaling (Genzel et al., 2014). Here, low concrete word-pairs may be associated with stage 2 in the younger women because of the neuronal replay that may have been occurred. The implications for the older women, however, is not clear. Our

results suggest that age may be a mediating factor in the role that sleep stages plays for the retention of different word-pair tasks.

The percentage of REM sleep was not significantly different between younger and older women. Some studies report that older adults experience a characteristic decrease in REM sleep (e.g. Ohayon et al., 2004) while others suggest that it may not be significantly affected in late life (Rasch & Born, 2013). Lafortune et al. (2013) hypothesize that normal percentages of REM sleep in healthy older adults may reflect the integrity of cholinergic activity. Cholinergic activity is also linked to sleep-dependent memory consolidation ((Diekelmann & Born, 2010; Rasch & Born, 2013). A lack of significantly decreased REM sleep in this sample of older women may suggest that REM sleep deterioration may not always be associated with the ageing process.

Typical proportions of REM sleep fall within 20-25% of total sleep (Ohayon et al., 2004; Spriggs, 2002; Zillmer et al., 2008). Both the younger and older women experienced slightly less than 20-25% on the baseline night which may have been due to continuing acclimatization to the laboratory setting. On the experimental night both groups of women experienced a significant increase in REM sleep. This may be due to a small REM rebound from the previous night or it may be associated with an enhancement of memory retention. As previously stated, REM can play a role in memory consolidation (Rasch & Born, 2013). However, there were no significant correlations between REM sleep and retention for any of the word-pair categories.

Sleep efficiency, Arousals, TST, WASO and their relationship with declarative memory retention. Age-related differences were present for these sleep variables. Again, this is in line with previous research that report major decreases in sleep time and efficiency alongside an increase in awakenings and arousals in older adults (Conte et al., 2012; Fogel et al., 2012; Harand et al., 2021).

The relationship between these variables and memory retention however, is less clear. In the younger group, TST and sleep efficiency positively correlated with overall retention, concrete word-pair retention and low-concrete word-pair retention. This meant that more time spent asleep and higher sleep efficiency correlated with better recall. This result contributes to the broad hypothesis that sleep is vitally involved in memory consolidation (Walker, 2008). However, it is argued that TST and longer periods of sleep are not necessarily determinant factors for better recall (Diekelmann & Born, 2010). Sleep-dependent memory consolidation has been shown to occur in naps (Schmidt et al., 2006) and

during the first half of the sleep cycle (Gruber et al., 2015). WASO negatively correlated with low concrete word-pairs, implying that more time spent awake after sleep onset correlated with worse recall. This makes sense as disrupted sleep can negatively influence memory consolidation (Dang-Vu et al., 2006; Diekelmann & Born, 2010; Guzman-Marin & McGinty, 2006; Vassalli & Dijk, 2009). On the other hand, a positive correlation with the retention of low concrete word-pairs and number of arousals was found for the older women. This result is conflicting and is potentially spurious as it cannot be explained on physiological basis nor by any outliers.

In sum, the results indicate that sleep architecture seemed to play a different role in memory retention for the two age groups. SWS showed a beneficial effect for concrete retention for the older women, but not for the younger women. Younger women, in contrast, showed the opposite relationship, where stage 2 showed a significant relationship for retention of low-concrete word-pairs. This may be because sleep architecture changes over the lifespan, which can have different implications for subsequent sleep-dependent memory consolidation (Scullin & Bliwise, 2015). This may also be due to the type of word-pairs used or ceiling effects. Exclusive use of concrete word-pairs may have elicited a different result. This may also be the case for exclusive use of integrative word-pairs and low concrete word-pairs. However, an alternate view is that memory consolidation may be mediated by NREM spindle activity in both these stages.

Sleep Spindle Activity in Younger and Older Women and its relationship with Declarative Memory Consolidation

NREM spindle activity in younger and older women showed age-related differences, which is in line with current literature (Crowley, 2011 Knoblauch et al., 2005, Martin et al., 2013). However, there were no significant increases in sleep spindle activity from baseline to experimental night, which is contrary to the hypotheses for this study and to various sleep spindle studies (e.g. Schabus et al., 2008). Nevertheless, there were significant correlations found in each group.

NREM Spindle Density. Younger women had significantly higher spindle density compared to older women, which corroborates previous findings (e.g. Martin et al., 2013). This included significantly higher slow spindle density, which seemed the most affected in the older group. Slow spindles are thought to be generated from the frontal region (Bakarat et al., 2011), which may explain this marked difference. Older adults experience major

reductions in frontal activity, which is sometimes associated with age-related shrinkage of hippocampal and prefrontal cortex regions (Cavanaugh & Blanchard-Fields, 2011; Fogel et al., 2012). In contrast, there were no age-related differences for fast sleep spindles, which are thought to be generated from central locations (Bakarat et al., 2011).

The change in spindle density and slow density negatively correlated integrative word-pairs in the older women. This may be further evidence that this type of word-pair is not necessarily modulated by sleep. Although the correlation was not significant, younger women also showed a negative correlation between spindle density and fast spindle density.

Fast spindle density has previously been found to be related to word-pair retention (Molle et al., 2011), although the role for slow sleep spindles in declarative memory is unclear (Fogel & smith, 2011). There was no significant correlation for slow or fast spindle density with declarative memory retention in the older women. However, there was a positive correlation in general sleep spindle density and the overall retention. This meant that better recall was associated with higher spindle density in the older women. Furthermore, the change in spindle density (including fast and slow density) from baseline to experimental night was positively correlated with better recall for the low concrete word-pair task. These results support the hypothesis that sleep spindle activity is important for declarative memory consolidation (Born & Wilhelm, 2012; Stickgold & Walker, 2007). Moreover, this echoes previous research findings evident in younger adults (Schabus et al., 2008). These results also confirm that sleep spindle activity may be related to declarative memory retention in a cohort of older women, which is similar to the finding in Seeck-Hirschner et al. (2012).

However, the same relationship was not found for the younger women. Again, this may be due to ceiling effects. A lack of significant association for the difficult word-pair measure may also be related to the idea that the younger and older women potentially found this measure too difficult.

NREM spindle Intensity. There were age-related differences in sleep intensity for the overall intensity, slow intensity and fast intensity of sleep spindles. This is in line with other studies that show older adults have significantly lower sleep spindle intensity (e.g. Rauchs et al., 2008). There were no correlations for between memory retention and spindle intensity in either group. However, the relative change in fast intensity from the baseline to experimental night was positively associated with better retention of low concrete word-pairs in older women. This meant that those who experienced a bigger increase in fast spindle intensity had better recall for the most difficult word-pair measure. In contrast, the change in fast and slow

spindle intensity was positively associated with concrete retention in the younger group. This indicates that the larger the increase in slow and fast spindle intensity during NREM stage 2 and SWS, the better their recall for the concrete word-pair measure. Although we postulated that sleep spindle activity would be positively associated with memory retention in younger and older women, we did not anticipate this varying outcome. Even though we did not find a similar and coherent trend between the two groups, there is still an evident relationship between spindle activity and memory retention.

Lastly, the percentage change of slow intensity negatively correlated with integrative word-pairs in the older women. The same was found with the younger women although the result was not significant. Again, integrative word-pairs may be too easy. Previous studies suggest that using tasks that are engaging and entail a level of difficulty elicit a sleep-dependent memory effect compared to non-engaging and easier tasks (Conte & Ficca, 2013; Payne et al., 2012; Schmidt et al., 2006)

NREM spindle features: duration, amplitude, frequency, number. In terms of NREM spindle features, there were two significant age-related differences. These included number and duration, which has been found in previous literature (e.g. Crowley, 2011; Knoblauch et al., 2005). However, previous literature has also found a significant age-related difference for a decrease in amplitude and an increase in frequency for older adults (Crowley et al., 2002, Fogel et al., 2012), which was not found in the present study. The reason for this result is uncertain, but it is recognized that this cohort of older women showed an amplitude similar to younger adults. For example, in a study by Martin al. (2013), elderly participants (60-73 years of age) had a maximum spindle amplitude of approximately 30 μV. The older women in this study showed an average amplitude of $M = 50.25 \pm 3.29$. The differences between spindle frequency is less clear. Potentially, a significant increase in spindle frequency may be associated with impaired consolidation. The discrepancy between our results and other studies may be that the age criteria for the older women entailed a much narrower age range than studies like Martin et al. (2013).

Younger women had significantly higher numbers of detected sleep spindles and longer durations compared to older women. The developmental changes are well-documented (e.g. Martin et al., 2013; Nicolas et al., 2001). However, little is known about how these features relate declarative memory consolidation. Although the number of sleep spindles are not usually associated with better retention, this relationship was found in this study. Specifically, both the younger and older women showed positive correlations for the number

of sleep spindles and memory retention. However, older women showed this significant correlation with overall retention, while younger women showed the correlation with low concrete word-pair retention. The implication here is that more spindles present in NREM sleep can result in better recall on a word-pair task. But again, the results vary by age group and task difficulty.

These results contribute to the research which reports that older adults do not necessarily experience a weakening in sleep-dependent memory consolidation (e.g. Aly & Moscovitch, 2010). Younger and older women experienced a sleep spindle-related benefit in the retention of declarative word-pairs, as we hypothesized. However, the exact relationship is complicated by the fact that different aspects of sleep spindles were associated with retention on different types of word-pair categories. In order to understand exactly how these mechanisms function and change in different age groups, further research is required.

Limitations

The prevalence of illness and sleep disorders is high in the ageing population (Cavanaugh & Blanchard-Fields, 2011; Wolkove et al., 2007). Similarly, medication use increases with older age and many drugs may interact with sleep to possibly exacerbate sleep problems (Crowley, 2011; Wolkove et al., 2007, Molony, 2009). For example, Theophylline, which is used for aiding in respiratory problems, may contribute to the development and maintenance of insomnia in older adults (Wolkove et al., 2007). Furthermore, Sleep Disordered Breathing (which contains a range of respiratory events) is more common among older adults and affects sleep quality, sometimes leading to excessive daytime sleepiness (Crowley, 2011). This means that attaining a sample of medication-free older women was unlikely. This poses a weakness for sleep research in the older participants.

For this study, NREM sleep spindle activity was assessed to investigate age-related differences in younger and older women. It was also used to investigate the relationship with declarative memory retention. NREM spindle activity was chosen because previous literature indicates that sleep spindle activity in both stage 2 and SWS is related to the consolidation of memories. The relative contribution of stage 2 versus SWS sleep spindle activity was not assessed. Thus it was not possible to investigate how this may have played a role in the consolidation of different word-pair categories, especially since SWS correlated with concrete word-pairs in older women and stage 2 correlated with low concrete word-pairs in younger women. Moreover, the automated spindle detection method did not take into account individual spindle variability and may limit the extent of the results.

In addition, the inclusion of other declarative and procedural memory tasks may have resulted in different outcomes. Moreover, using all three word-pair types may have potentially confounded the results. However, this research shows that word-pair type may be associated with different stages and and that this could be influenced by age.

Lastly, multiple statistical test was performed on a small sample. A Bonferroni correction for multiple tests was not used which is major limitation of this study.

Conclusions and Future Directions

Younger women remembered more pre- and post-sleep compared to older women. Although younger women remembered more word-pairs, memory was preserved in both groups as there were no significant differences from pre to post-sleep in either groups. This may suggest that older adults can experience a sleep-related benefit for declarative retention. This is contrary to the idea that older adults experience a weakening in the sleep-dependent memory consolidation mechanism as some researchers suggest (e.g. Scullin, 2012). Older women had significantly lower SWS and sleep spindle activity than younger women, which is a developmental characteristic of older adults (Martin et al., 2013). We did not find significantly lower amplitude and fast spindle density, nor significantly increased frequency, however. To our knowledge, there are no studies which propose the ideal amount of sleep spindle activity with respect to developmental ages. Knowledge regarding this may help to identify where and when sleep spindle activity begins to falter in older adults.

Stage 2 and SWS correlated with different word-pair types in the younger and older women. This highlights the fact that there may different roles for stage 2 spindles versus SWS spindles (Genzel et al., 2012). In addition, it is unknown whether the memory consolidating effect of sleep spindles act in isolation or in the presence of other activity, like slow oscillations (Fogel & Smith, 2011). This question arises because active system consolidation proposes that sleep spindles couple with hippocampal ripple activity through the grouping effect of slow oscillations (Diekelmann and Born, 2010). Researchers propose that the time-locked sequencing of sleep spindles with hippocampal ripple activity in the presence of slow oscillations is responsible for successful consolidation (Clemens, 2011; Mölle, Bergmann, Marshall, & Born, 2011). But during stage 2, we do not see this grouping. However, the coupling could be achieved through k-complexes in stage 2. For example, Steriade (2006) argues that the k-complex is akin to slow oscillation found in SWS. Future studies should include the investigation of which aspects of sleep spindles are important for declarative consolidation (Fogel & Smith, 2011).

Future studies should also carefully consider methodology when planning a sleep study. Specifically, the type of task can have a profound effect on the expected results. From this study, it appears that suing unrelated concrete word-pairs may elicit a sleep-effect on memory retention. However, level of difficulty introduced by low concrete word-pairs may not be a reasonable decision to use with older adults. Instead, increasing the amount of word-pairs may be a better solution to task difficulty (Conte et al., 2012). However, if the sample consists of entirely younger adults, low concrete word-pairs may potentially be useful. This may be especially relevant as spindle activity has been specifically associated with low concrete word-pairs in our study and others (e.g. Schmidt et al., 2006).

In most sleep studies, absolute sleep spindle density is commonly used. However, sleep spindle intensity, spindle type and the relative changes in spindle activity are equally important measures to consider for sleep-dependent memory consolidation (Schabus et al., 2008). Future studies should make use of a range of sleep spindle activity measures to attain an overall picture as sleep spindle density can be limiting.

Clinical Significance. The changes in SWS and sleep spindle activity in the ageing population are exacerbated in clinical populations such as those affected by Alzheimer's Disease (e.g. Rauchs et al., 2008). Although much research is still needed to clarify the role SWS and sleep spindle activity plays in declarative memory consolidation, it is evident that sleep-specific field potentials such as slow oscillations and sleep spindles may actively contribute to the consolidation process (Diekelmann & Born, 2010). In addition, the age-related changes seen in older adults are associated with deterioration of grey matter and may serve as biological marker for structural changes related to the brain (Fogel et al., 2016). Studies such as Mednick et al. (2013), which investigate the effect of selectively increased sleep spindle density on declarative retention are important. This is because the potential manipulation of sleep spindle activity to selectively enhance memory for vulnerable populations like the elderly may help to protect against further decline. Thus a goal of sleep spindle research should include studies on enhancing memory retention through the manipulation of sleep spindle activity.

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Appendix A:
Informed Consent Document

This document is for the permission to use the participant's information collected during the research and the release of sleeping patterns, performance results on memory tests, and other personal data.

You are invited to participate in a research study at the University of Cape Town. This document is for your information and for your explicit permission to use data that is collected throughout the study. Information about your sleep architecture, memory abilities and other sleep-related details would be collected and analysed. Your participation in this research is voluntary. If you have any questions, please direct them to the Principle Investigator (the leader of the research study), who will provide more information and answer the queries.

Please take time to go through this document before you decide whether to participate in this research.

1. **Title of the Research Study**

   Older age as a mediating factor in the role that sleep spindles play for memory consolidation.

2. **Principle Investigator**
   Siobhan McCreesh
   Contact number: 0734247296

3. **What is the purpose of this research?**
   This study explores how changes in sleep architecture may be related to memory changes in older adults.

4. **What will happen if you participate in this study?**
   You will be asked to come to the sleep laboratory at the University of Cape Town (UCT) for two nights.

   Before you come in for the three nights of sleep and testing, the Principle Investigator will assess you on a few short questionnaires and tests This will help the researcher to identify particular patterns that are important to know prior to the study.

5. **How many people are expected to participate in this study?**
   40

6. **What are the possible risks for you?**
   You will be asked to sleep in the sleep laboratory at the university, which may feel uncomfortable for you. Precautions have been put in place to ensure your safety and as much comfort as possible. The sleep laboratory rooms have beds, clean linen and clean, functioning toilets.

   Electrodes will be placed onto your head before sleep and then removed in the morning. The electrodes are padded and lubricated so that any discomfort you may feel is minimised. The electrodes are not harmful.

7. **What are the possible benefits for you?**
   There is no guarantee of any benefits. However, you may gain a better understanding of sleep and how it relates to memory and age.

8. What are the benefits of this study for others?

This research will help inform sleep research society on the importance of sleep and it relates to other aspects of our life. If there are relationships that exist between a decline in memory processing and sleeping difficulties in older adults then this research will guide further research to help address these issues. It is important to know how our sleep changes as we age, and how our memory abilities change with it.

9. Will it cost you any money to partake in this study?

It will not cost you any money to partake in this study.

10. Can you withdraw from this study?

You are free to withdraw from this study at any point in time. There will be no penalty if you choose to do so.

11. If you decide to withdraw, can your information be used?

If you decide to withdraw from this research, the information that has already been collected may be used.

12. How will your information be kept secret (confidential) after the data has been collected?

Your information will be recorded and stored in computers with security passwords or kept in secure filing cabinets. The researchers and University of Cape Town representatives will only view your information. Your information will not be released without your explicit permission unless law requires it.

13. What information will be collected from you?

You will be asked about your demographic information and knowledge of menstrual cycle timing. Data will be collected regarding your sleep architecture and performance on memory tasks. If your data is used in a compiled data set, the information used will include records of age, sleep architecture and performance, and *not* information that can lead back to you, so your name and address (and such like) will not be used.

14. How does the researcher benefit from this study?

The researcher is undertaking this study for the aim to attain a Masters degree. This will help the researcher advance in their career and do further research.

15. Signatures

I (as a study representative) acknowledge that I have explained the purpose, the procedures, the possible benefits and risks. It has also been explained how the research participant's performance, sleep architecture and other information will be collected and used.

Signature of Person Obtaining Consent and Authorization Date

_____ _____

By signing this document, you acknowledge that you have been informed of the purpose, the procedures, the possible benefits and risks in this study. You are also informed about how your performance, sleep architecture and other information will be collected and used. You have been given the opportunity to ask any questions and know that you can ask other questions if you need.

You voluntarily agree to participate in the study and authorize the collection, recording, sharing and use of information collected by the researchers involved in this study.

Signature of Person Consenting and Authorizing Date

_____ _____

Appendix B – Demographic and personal information form

1. Name:

2. Age:

3. Gender/sex: Please tick

Male	Female

4. What is your primary language? Please specify below.

5. Are you currently on any medication? If yes, please list ALL medication.

6. Do you have any pre-existing medical conditions? If yes, please state ALL.

7. Please list any other health-related issues.

8. Menstrual cycle: Please tick where appropriate

(i) Is your menstrual cycle interval regular?

Yes	No

(ii) What is the interval (in days) for your menstrual cycle?
(Interval means from day 1 of menstruation until the onset of the next menstrual period. Usually it is about 28 days.)

(iii) Are you on any medication, like the contraceptive pill or Hormone Replacement Therapy (HRT), for your menstrual cycle?

Yes	No

(iv) Please indicate if you are in the menopausal phase.
(Menopause is when your period has not occurred for a year or longer.)

Yes	No

Appendix C: Receipt of Participation

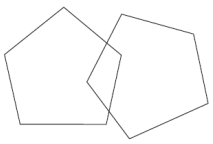
NREM Sleep Spindles and Slow Wave Sleep in Younger and Elderly Women: an investigation of their influence on Declarative Memory Consolidation

I, ……., hereby state that I have received compensation of R100.00 for the participation in this sleep study. This money was handed to me by Siobhan McCreesh.

Signature: …….. Date:……

Appendix D: Mini-Mental State Examination (MMSE)

STANDARDIZED MINI-MENTAL STATE EXAMINATION (SMMSE)

	QUESTION	TIME ALLOWED	SCORE
1	a. ***What year is this?***	10 seconds	/1
	b. ***Which season is this?***	10 seconds	/1
	c. ***What month is this?***	10 seconds	/1
	d. ***What is today's date?***	10 seconds	/1
	e. ***What day of the week is this?***	10 seconds	/1
2	a. ***What country are we in?***	10 seconds	/1
	b. ***What province are we in?***	10 seconds	/1
	c. ***What city/town are we in?***	10 seconds	/1
	d. ***IN HOME – What is the street address of this house?*** ***IN FACILITY – What is the name of this building?***	10 seconds	/1
	e. ***IN HOME – What room are we in? IN FACILITY – What floor are we on?***	10 seconds	/1
3	**SAY:** ***I am going to name three objects. When I am finished, I want you to repeat them. Remember what they are because I am going to ask you to name them again in a few minutes.*** Say the following words slowly at 1-second intervals - **ball/ car/ man**	20 seconds	/3
4	***Spell the word WORLD. Now spell it backwards.***	30 seconds	/5
5	***Now what were the three objects I asked you to remember?***	10 seconds	/3
6	**SHOW** wristwatch. **ASK:** ***What is this called?***	10 seconds	/1
7	**SHOW** pencil. **ASK**: ***What is this called?***	10 seconds	/1
8	**SAY:** ***I would like you to repeat this phrase after me: No ifs, ands or buts.***	10 seconds	/1
9	**SAY:** ***Read the words on the page and then do what it says.*** Then hand the person the sheet with CLOSE YOUR EYES on it. If the subject reads and does not close their eyes, repeat up to three times. Score only if subject closes eyes	10 seconds	/1
10	**HAND** the person a pencil and paper. **SAY:** ***Write any complete sentence on that piece of paper.*** (Note: The sentence must make sense. Ignore spelling errors)	30 seconds	/1
11	**PLACE** design, eraser and pencil in front of the person. **SAY**: ***Copy this design please.***  Allow multiple tries. Wait until person is finished and hands it back. Score only for correctly copied diagram with a 4-sided figure between two 5-sided figures.	1 minute	/1
12	**ASK** the person if he is right or left-handed. Take a piece of paper and hold it up in front of the person. **SAY**: ***Take this paper in your right/left hand*** (whichever is non-dominant), ***fold the paper in half once with both hands and put the paper down on the floor*** . Score 1 point for each instruction executed correctly.	30 seconds	
	Takes paper correctly in hand		/1
	Folds it in half		/1
	Puts it on the floor		/1
	TOTAL TEST SCORE		**/30**

Note: This tool is provided for use in British Columbia with permission by Dr. William Molloy. This questionnaire should not be further modified or reproduced without the written consent of Dr. D. William Molloy.

Provided by the Alzheimer's Drug Therapy Initiative for physician use.

Appendix E: Beck Depression Inventory II (BDI-II)

Roche

V 0477

Beck Depression Inventory

CRTN: ____ CRF number: ____ Page 14 patient inits: ____

Baseline

BDI-II®

Date:

Name: _____ Marital Status: _____ Age: _____ Sex: _____

Occupation: _____ Education: _____

Instructions: This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the **one statement** in each group that best describes the way you have been feeling during the **past two weeks, including today**. Circle the number beside the statement you have picked. If several statements in the group seem to apply equally well, circle the highest number for that group. Be sure that you do not choose more than one statement for any group, including Item 16 (Changes in Sleeping Pattern) or Item 18 (Changes in Appetite).

1. Sadness
- 0 I do not feel sad.
- 1 I feel sad much of the time.
- 2 I am sad all the time.
- 3 I am so sad or unhappy that I can't stand it.

2. Pessimism
- 0 I am not discouraged about my future.
- 1 I feel more discouraged about my future than I used to be.
- 2 I do not expect things to work out for me.
- 3 I feel my future is hopeless and will only get worse.

3. Past Failure
- 0 I do not feel like a failure.
- 1 I have failed more than I should have.
- 2 As I look back, I see a lot of failures.
- 3 I feel I am a total failure as a person.

4. Loss of Pleasure
- 0 I get as much pleasure as I ever did from the things I enjoy.
- 1 I don't enjoy things as much as I used to.
- 2 I get very little pleasure from the things I used to enjoy.
- 3 I can't get any pleasure from the things I used to enjoy.

5. Guilty Feelings
- 0 I don't feel particularly guilty.
- 1 I feel guilty over many things I have done or should have done.
- 2 I feel quite guilty most of the time.
- 3 I feel guilty all of the time.

6. Punishment Feelings
- 0 I don't feel I am being punished.
- 1 I feel I may be punished.
- 2 I expect to be punished.
- 3 I feel I am being punished.

7. Self-Dislike
- 0 I feel the same about myself as ever.
- 1 I have lost confidence in myself.
- 2 I am disappointed in myself.
- 3 I dislike myself.

8. Self-Criticalness
- 0 I don't criticize or blame myself more than usual.
- 1 I am more critical of myself than I used to be.
- 2 I criticize myself for all of my faults.
- 3 I blame myself for everything bad that happens.

9. Suicidal Thoughts or Wishes
- 0 I don't have any thoughts of killing myself.
- 1 I have thoughts of killing myself, but I would not carry them out.
- 2 I would like to kill myself.
- 3 I would kill myself if I had the chance.

10. Crying
- 0 I don't cry anymore than I used to.
- 1 I cry more than I used to.
- 2 I cry over every little thing.
- 3 I feel like crying, but I can't.

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Subtotal Page 1

Continued on Back

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NR15645

Roche

Beck Depression Inventory — **Baseline**

V 0477 CRTN: _____ CRF number: _____ Page 15 patient inits: _____

11. Agitation

0 I am no more restless or wound up than usual.
1 I feel more restless or wound up than usual.
2 I am so restless or agitated that it's hard to stay still.
3 I am so restless or agitated that I have to keep moving or doing something.

12. Loss of Interest

0 I have not lost interest in other people or activities.
1 I am less interested in other people or things than before.
2 I have lost most of my interest in other people or things.
3 It's hard to get interested in anything.

13. Indecisiveness

0 I make decisions about as well as ever.
1 I find it more difficult to make decisions than usual.
2 I have much greater difficulty in making decisions than I used to.
3 I have trouble making any decisions.

14. Worthlessness

0 I do not feel I am worthless.
1 I don't consider myself as worthwhile and useful as I used to.
2 I feel more worthless as compared to other people.
3 I feel utterly worthless.

15. Loss of Energy

0 I have as much energy as ever.
1 I have less energy than I used to have.
2 I don't have enough energy to do very much.
3 I don't have enough energy to do anything.

16. Changes in Sleeping Pattern

0 I have not experienced any change in my sleeping pattern.
1a I sleep somewhat more than usual.
1b I sleep somewhat less than usual.
2a I sleep a lot more than usual.
2b I sleep a lot less than usual.
3a I sleep most of the day.
3b I wake up 1–2 hours early and can't get back to sleep.

17. Irritability

0 I am no more irritable than usual.
1 I am more irritable than usual.
2 I am much more irritable than usual.
3 I am irritable all the time.

18. Changes in Appetite

0 I have not experienced any change in my appetite.
1a My appetite is somewhat less than usual.
1b My appetite is somewhat greater than usual.
2a My appetite is much less than before.
2b My appetite is much greater than usual.
3a I have no appetite at all.
3b I crave food all the time.

19. Concentration Difficulty

0 I can concentrate as well as ever.
1 I can't concentrate as well as usual.
2 It's hard to keep my mind on anything for very long.
3 I find I can't concentrate on anything.

20. Tiredness or Fatigue

0 I am no more tired or fatigued than usual.
1 I get more tired or fatigued more easily than usual.
2 I am too tired or fatigued to do a lot of the things I used to do.
3 I am too tired or fatigued to do most of the things I used to do.

21. Loss of Interest in Sex

0 I have not noticed any recent change in my interest in sex.
1 I am less interested in sex than I used to be.
2 I am much less interested in sex now.
3 I have lost interest in sex completely.

3 4 5 6 7 8 9 10 11 12 A B C D E

_____ Subtotal Page 2
_____ Subtotal Page 1
_____ Total Score

NR15645

Appendix F: Pittsburgh Sleep Quality Index (PSQI)

Name_____ Date_____

Sleep Quality Assessment (PSQI)

What is PSQI, and what is it measuring?

The Pittsburgh Sleep Quality Index (PSQI) is an effective instrument used to measure the quality and patterns of sleep in adults. It differentiates "poor" from "good" sleep quality by measuring seven areas (components): subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, and daytime dysfunction over the last month.

INSTRUCTIONS:

The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

During the past month,

1. When have you usually gone to bed? _____
2. How long (in minutes) has it taken you to fall asleep each night? _____
3. What time have you usually gotten up in the morning? _____
4. A. How many hours of actual sleep did you get at night? _____
   B. How many hours were you in bed? _____

5. During the past month, how often have you had trouble sleeping because you	Not during the past month (0)	Less than once a week (1)	Once or twice a week (2)	Three or more times a week (3)
A. Cannot get to sleep within 30 minutes				
B. Wake up in the middle of the night or early morning				
C. Have to get up to use the bathroom				
D. Cannot breathe comfortably				
E. Cough or snore loudly				
F. Feel too cold				
G. Feel too hot				
H. Have bad dreams				
I. Have pain				
J. Other reason (s), please describe, including how often you have had trouble sleeping because of this reason (s):				
6. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?				
7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?				
8. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?				
9. During the past month, how would you rate your sleep quality overall?	Very good (0)	Fairly good (1)	Fairly bad (2)	Very bad (3)

Scoring

Component 1	#9 Score	C1 _____
Component 2	#2 Score (<15min (0), 16-30min (1), 31-60 min (2), >60min (3)) + #5a Score (if sum is equal 0=0; 1-2=1; 3-4=2; 5-6=3)	C2 _____
Component 3	#4 Score (>7(0), 6-7 (1), 5-6 (2), <5 (3)	C3 _____
Component 4	(total # of hours asleep) / (total # of hours in bed) x 100 >85%=0, 75%-84%=!, 65%-74%=2, <65%=3	C4 _____
Component 5	# sum of scores 5b to 5j (0=0; 1-9=1; 10-18=2; 19-27=3)	C5 _____
Component 6	#6 Score	C6 _____
Component 7	#7 Score + #8 score (0=0; 1-2=1; 3-4=2; 5-6=3)	C7 _____

Add the seven component scores together _____ ***Global PSQI*** _____

A total score of "5" or greater is indicative of poor sleep quality.
If you scored "5" or more it is suggested that you discuss your sleep habits with a healthcare provider

Appendix G: The Epworth Sleepiness scale (ESS)

The Epworth Sleepiness Scale (ESS)
Adapted version

How likely are you to doze off or fall asleep in the following situations, in contrast to feeling just tired? This refers to your usual way of life in recent times. Even if you have not done some of these things recently try to work out how they would have affected you in the moment. Use the following scale to choose the most appropriate number for each situation:

0 = would **never** doze
1 = **slight chance** of dozing
2 = **moderate chance** of dozing
3 = **high chance** of dozing

SITUATION	CHANCE OF DOZING (0-3)
Sitting and reading	
Watching television	
Sitting inactive in a public place (e.g. a theater or meeting)	
As a passenger in a car for an hour without a break	
Lying down to rest in the afternoon when circumstance permit	
Sitting and talking to someone	
Sitting quietly after a lunch without alcohol	
In a car, while stopped for a few minutes in the traffic	
TOTAL SCORE	

Johns, M.W. (1991). A new method for measuring daytime sleepiness: The Epworth sleepiness scale. Sleep, 14 , 540-545.

Appendix H: Pittsburgh Sleep Diary (PSD)

SLEEP DIARY **WAKETIME** **KEEP BY BED**

Please fill out this part of the diary first thing in the morning.

day ..... date .....

went to bed last night at .....

attempted to fall asleep at .....

minutes until fell asleep .....

finally woke at .....

Awakened by *(check one)*: alarm clock/radio ☐
someone whom I asked to wake me ☐
noises ☐
just woke ☐

After falling asleep, woke up this many times during the night (circle)
0 1 2 3 4 5 or more

total number of minutes awake .....

- woke to use bathroom (circle # times)
  0 1 2 3 4 5 or more
- awakened by noises/child/bedpartner (circle # times)
  0 1 2 3 4 5 or more
- awakened due to discomfort or physical complaint (circle # times)
  0 1 2 3 4 5 or more
- just woke (circle # times)
  0 1 2 3 4 5 or more

Ratings (place a mark somewhere along the line):

Sleep Quality:
very bad _____ very good

Mood on Final Wakening:
very tense _____ very calm

Alertness on Final Wakening:
very sleepy _____ very alert

Appendix I: Declarative Word-Pair Task

Recall is recorded twice in the evening and once in the morning.

Word Type	Cue	Target	Correct			Intrusions
Integrative	Apartment	Dog				
Integrative	Law	School				
Integrative	Ocean	Fish				
Integrative	Travel	Book				
Integrative	Velvet	Dress				
Integrative	Linen	Pants				
Integrative	Race	Car				
Integrative	Fireplace	Wood				
Integrative	Town	Church				
Integrative	Steel	Pipe				
Integrative	Donor	Heart				
Integrative	Maple	Leaf				
Integrative	Corporate	Plane				
Integrative	Plastic	Toy				
Integrative	Border	Land				
Integrative	Mountain	Snow				
Integrative	Jungle	Snake				
Integrative	Brass	Horn				
Integrative	Summer	Rain				
Integrative	Jelly	Grape				
Integrative	Bathroom	Soap				
Integrative	Monkey	Foot				
Integrative	Rice	Paper				
Integrative	Strawberry	Milk				
Integrative	Trick	Rabbit				
Integrative	Box	Wine				
Integrative	Soup	Can				
Integrative	Halloween	Ghost				
Integrative	Parade	Horse				
Integrative	Necklace	Diamond				

Word Type	Cue	Target	Correct			Intrusions
Unrelated & concrete	Star	Oyster				
Unrelated & concrete	Cliff	Bee				
Unrelated & concrete	Spinach	Tape				
Unrelated & concrete	Seal	Movie				
Unrelated & concrete	Pillow	Knee				
Unrelated & concrete	Hat	Uncle				
Unrelated & concrete	People	Clarinet				
Unrelated & concrete	Towel	Cup				
Unrelated & concrete	Earth	Mouse				
Unrelated & concrete	Frog	Stair				
Unrelated & concrete	Nose	Owner				
Unrelated & concrete	Bean	Flag				
Unrelated & concrete	Chair	River				
Unrelated & concrete	Tile	Purse				
Unrelated & concrete	Banana	Head				
Unrelated & concrete	Stamp	Wrist				
Unrelated & concrete	Boss	Eye				
Unrelated & concrete	Garbage	Sweater				
Unrelated & concrete	Coral	King				
Unrelated & concrete	Olive	Mink				
Unrelated & concrete	Magazine	Tiger				
Unrelated & concrete	Film	Spring				
Unrelated & concrete	Muscle	Stage				
Unrelated & concrete	Africa	Winter				
Unrelated & concrete	Sink	clam				
Unrelated & concrete	Nurse	Stereo				
Unrelated & concrete	Curtain	Egg				
Unrelated & concrete	Cotton	Band				
Unrelated & concrete	Bird	Comb				
Unrelated & concrete	Tornado	Log				

Unrelated & abstract	Direction	Graduation				
Unrelated & abstract	Personality	Descent				
Unrelated & abstract	Situation	Atom				
Unrelated & abstract	Legend	Capability				
Unrelated & abstract	Language	Fortune				
Unrelated & abstract	Style	Truth				
Unrelated & abstract	Zone	Goal				
Unrelated & abstract	Duty	Fantasy				
Unrelated & abstract	Patience	Evidence				
Unrelated & abstract	Definition	Emotion				
Unrelated & abstract	Fiction	Lot				
Unrelated & abstract	Area	Agreement				
Unrelated & abstract	Business	Freedom				
Unrelated & abstract	Purpose	Hours				
Unrelated & abstract	Climate	Speech				
Unrelated & abstract	Excuse	Temperature				
Unrelated & abstract	Length	Electricity				
Unrelated & abstract	Addition	Vision				
Unrelated & abstract	Advice	Mile				
Unrelated & abstract	Decision	Self				
Unrelated & abstract	Chemistry	Ledge				
Unrelated & abstract	Foresight	Geometry				
Unrelated & abstract	Society	Crescent				
Unrelated & abstract	Construction	Energy				
Unrelated & abstract	Neuron	Something				
Unrelated & abstract	Nothing	Honesty				
Unrelated & abstract	Thing	Mind				
Unrelated & abstract	Habit	Security				
Unrelated & abstract	Virtue	Helper				
Unrelated & abstract	Bravery	Knowledge				