Effects of age on sleep and consolidation of motor learning

Christel Alessandra Gudberg

A thesis submitted in partial fulfilment of the requirements for the degree of
Doctor of Philosophy in the University of Oxford

University College Michaelmas Term 2013

"I have never taken any exercise, except sleeping and resting."

Mark Twain (1835-1910)

Effects of age on sleep and consolidation of motor learning

Christel Alessandra Gudberg

University College **Michaelmas Term 2013**

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the University of Oxford

General Abstract

Background: Our ability to consolidate what we learn changes with age. However, little is known about the neurophysiological underpinnings of consolidation of motor learning in ageing. This is largely because studies have repeatedly demonstrated a deficit in sleep-dependent consolidation of motor learning in older adults. This thesis aims to reassess commonly held assumptions about consolidation in ageing, as well as to examine the neurophysiological and neurochemical mechanisms that support the learning and consolidation processes.

Methods: Most of the studies in this thesis are based on the design of a novel whole-hand task for use in older adults, which reduces dependency on fine motor skill. This thesis adopts a number of converging measures to examine learning and memory including electroencephalograhy (EEG), magnetic resonance spectroscopy (MRS), actigraphy recordings, as well as behavioural and self-reported measures of sleep.

Results: Findings show significant improvements in learning with the adapted motor task in older adults. Importantly, this task reveals significant sleep-dependent enhancements in older adults, which are comparable to those seen in younger controls. Functional changes in sleep architecture with ageing show overall decline in slow wave sleep. Sleep-dependent improvements were specifically associated with activity in stage 3 slow wave sleep and increased hemispheric differences regardless of age. Changes in GABA concentrations with learning on a visuomotor tracking task showed marked variability across participants, and no clear associations were found between GABA and consolidation.

Conclusion: The evidence presented in this thesis highlight the complex dynamics underlying sleep consolidation, and challenges a commonly held assumption about consolidation in older adults. Specifically, the studies presented here show that observed declines in motor consolidation with ageing may be contaminated by age-related deficits fine motor skill. By removing such kinematic constraints, it was possible to detect marked improvements in motor performance also in older adults despite age-related changes in sleep architecture.

This thesis contains approximately 39,000 words.

Table of contents

i. Abbreviations

AASM	American Academy of Sleep Medicine
ANOVA	Analysis of Variance
Cr	Creatine
DLPFC	Dorsolateral Prefrontal Cortex
DMN	Default Mode Network
EEG	Electroencephalograhy
EMG	Electromyography
EOG	Electrooculography
FA	Fractional Anisotropy
fMRI	Functional Magnetic Resonance Imaging
GABA	γ-Aminobutyric Acid
Glx	Composite measure of glutamate and glutamine
jMRUI	Java-based graphical user interface
LORETA	Low-Resolution (brain) Electromagnetic Tomography
LS	Light Sleep
LTP	Long-Term Potentiation
M1	Primary Motor Cortex
mPFC	Medial Prefrontal Cortex
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NAA	*N*-Acetyl-Aspartate
NMDA	*N*-Methyl-D-Aspartate
NREM	Non-Rapid Eye Movement
PPM	Parts Per Million
PRESS	Point Resolved Spectroscopy
PSQI	Pittsburgh Sleep Quality Index
REM	Rapid Eye Movement
SD	Standard Deviation
SEM	Standard Error of the Mean
SICI	Short-Interval Intracortical Inhibition
SNR	Signal-to-Noise Ratio
SRTT	Serial Reaction Time Task
STFT	Short-Time Fourier Transform
SWA	Slow Wave Activity
SWS	Slow Wave Sleep
t0	Training Session
t1	Retest Session 1
t2	Retest Session 2
tDCS	Transcranial Direct Current Stimulation
TE	Echo Time
TMS	Transcranial Magnetic Stimulation
TR	Repetition Time

ii. List of figures

iii. List of tables

iv. Acknowledgements

I want to thank a number of people who have been instrumental in helping with, supporting, or otherwise facilitating the work that has gone into this DPhil thesis.

Firstly, the many participants, young and old, who have taken part in the studies that make up this thesis. Without their willingness and commitment this work would not have been possible.

Heidi Johansen-Berg, for her unfailingly sound advice and guidance over the past three years. She is cemented as a role model in work and life.

Katharina Wulff for crucially supporting the sleep side of things and endless EEG knowledge.

The Medical Research Council for providing my funding.

Charlotte Stagg for teaching me how to use and analyse MR spectroscopy.

Jamie Near for providing the MATLAB scripts and troubleshooting assistance for preprocessing MRS data.

Kate Porcheret for hours of EEG tutorial, and for double scoring a sample of the EEG data for the inter-rater reliability analysis (internal scorer).

Alpar Lazar, who acted as the external scorer for a sample of the EEG data.

The MSc and FHS students who helped with data collection and scoring on a subset of the EEG.

Jill O'Reilly for programming help for the sequence learning task.

Claire Sexton for help with recruitment of older adults for the behavioural studies.

The FMRIB computing guys, Duncan, Dave, Matt, and Chris, for (almost always) graciously entertaining the assorted queries and troubleshooting I bring their way.

The scanner operators who have provided radiographer support.

Anne Taylor for administrative and general support in years one and two.

My lab group for being an inspiring group of plasticians, and for constructive feedback at lab meetings.

Jacinta O'Shea for her two hats as constructive lab member and college advisor, and 'checking in' when needed.

Friends and staff at University College for creating a home away from home. For support in everything from housing to travel grants.

My housemates over the past three years for being a source of distraction when needed, Sunday brunches, and chats in hallways.

Aji for useful discussions on early drafts of this project, for support over the years, and for a different perspective.

Daniel for his continued support in all matters, but particularly for indispensable help with the delta finding procedure for Chapter 3.

And last, but not least, Helen for being a constant source of support and proofreading, and for always believing in me.

Chapter 1

Chapter 1 | General Introduction

The overarching aim of this thesis is to investigate the impact of age on motor learning and off-line consolidation, as well as to examine the neurophysiological mechanisms that support these processes. A parallel focus of this thesis is how neurochemical alterations in specific brain regions interact with behaviour and off-line processes, such as sleep-wake consolidation. In this introductory chapter I will consider research from various systems-level and neural network approaches aimed at characterising, for instance, the neuroanatomical underpinnings of motor learning and fine movement kinematics, and their functional changes with ageing.

1.1 Human motor learning and consolidation

The first section of this chapter aims to clarify specific definitions of memory and consolidation that will be adopted in this thesis. The present work will primarily focus on *sleep-specific* consolidation within *explicit motor* memory, however, it will be relevant for a detailed account to consider how these specific mechanisms and components fit into the broader dynamics of learning and memory.

1.1.1 What is memory consolidation?

Following the acquisition of new memories, initially labile traces undergo post-encoding (off-line) processing including consolidation. Consolidation is defined as a process or series of processes, which aid in stabilising and making more robust learned material (Brawn, Fenn, Nusbaum, & Margoliash, 2008; Diekelmann, Wilhelm, & Born, 2009; Rasch & Born, 2013; Stickgold, 2009). Depending on the type of material being

learnt, these processes may offer additional off-line gains that are not a direct function of further practice (Doyon et al., 2009; Robertson, Pascual-Leone, & Press, 2004). In the case of some types of memories, off-line gains may be associated with consolidation processes specifically during sleep. Such sleep-dependent effects have been consistently shown by a large number of studies (Fischer, Hallschmid, Elsner, & Born, 2002; Robertson et al., 2004; Walker, Brakefield, Hobson, & Stickgold, 2003; Walker, Brakefield, Morgan, Hobson, & Stickgold, 2002). However, the off-line gains associated with mechanisms of consolidation do not always require a period of sleep to develop, and the degree of sleep-dependence relates largely to the memory trace being processed. Distinctions between different types of memory are discussed in the following section.

1.1.2 Definitions of memory: dichotomies & distinct properties

Since the earliest logged accounts (e.g., James, 1890; Maine de Biran, 1929), memory has been conceptualised as a dichotomy – rather than a unitary faculty. However, experimental discourse examining these different memory systems began only a few decades ago. Milner (1962) showed, for instance, that a patient (H.M.) was able to learn a mirror drawing task over several sessions spaced across days despite severe amnesia and an inability to recollect having previously attempted the task. Since then, broad characterisations of these memory dichotomies have taken various (related) forms (e.g., declarative and procedural, Cohen & Squire, 1980; or explicit and implicit, Graf & Schater, 1985), often leading to the misconception that declarative equals explicit memory, while procedural is synonymous with implicit learning. While this may sometimes be the case, many procedural tasks can also be conceived of as involving explicit awareness. This thesis will focus on motor sequence learning tasks. In

a number of such tasks, including an explicit version of the serial reaction time task (SRTT), participants are overtly made aware of the underlying sequence being repeated. Therefore, it would be erroneous to classify such tasks as purely implicit. Moreover, how we might classify tasks also closely relates to, and can be further enlightened by, the processes of off-line consolidation.

1.1.2.1 Importance of sleep for memory consolidation

The importance of sleep in memory consolidation was evident from early studies demonstrating the adverse effects of sleep deprivation on learning (Fishbein 1971; Karni et al. 1994; Linden et al. 1975; Smith and Kelly 1988).

More recently, behavioural studies have compared performance before and after consolidation in healthy younger adults (see sections 1.3 and 2.1 for age-related changes). Gains in performance (Figure 1.1) following a single session of training on an explicit motor sequence are typically found to require specifically an off-line period of sleep (Robertson et al., 2004; Fischer et al., 2002; Walker et al., 2002; 2003), whereas off-line performance improvements on an implicit version of the same task may be observable after the simple passage of time (Robertson et al., 2004). These findings suggest that inherent qualities in the learning material, specifically in terms of awareness, may be represented and subsequently processed differently in the memory system.

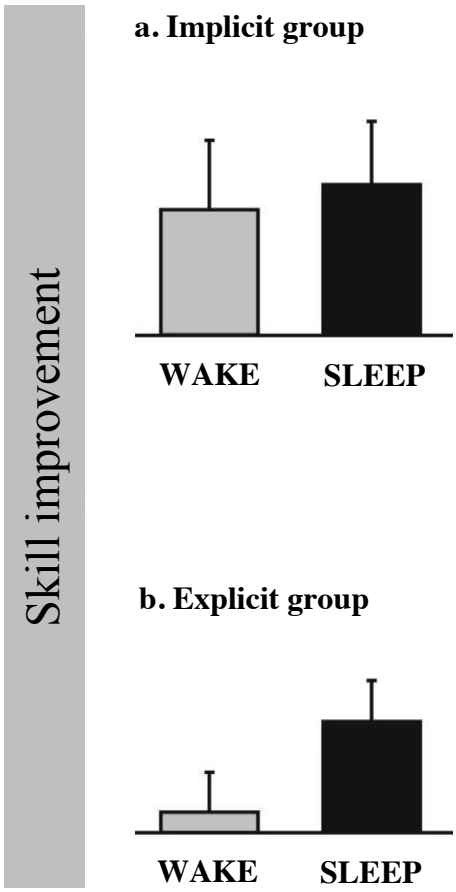


Figure 1.1. Consolidation with sleep and wakefulness during implicit and explicit motor learning. Explicit learning on a motor sequence task (b) facilitates sleep-dependent consolidation gains, whereas the implicit equivalent (a) shows consolidation gains during both wakefulness and sleep. Graphs adapted from Robertson et al. (2004).

However, recent evidence has also highlighted that such differential processing may be even more intricate than previously thought. Specifically, consolidation studies have investigated whether distinct properties of a single (motor) task may rely on different consolidation states. For example, Cohen, Pascual-Leone, Press and Robertson (2005) found that while the spatial or goal-based component of a task required a post-training consolidation period of sleep for observable gains in performance, the movement property of the same task-behaviour relied specifically on wakefulness (and not just the simple passage of time) to elicit improvements in performance. Similar

results were also reported in a recent paper (Albouy et al., 2013) showing that (daytime) sleep preferentially enhanced the allocentric (spatial) representation of motor sequence learning. However, unlike Cohen et al. (2005), this study found that the egocentric (motor) component of the task was only maintained, without further gains, regardless of whether the consolidation period involved sleep or wakefulness. Meanwhile, both task-design and -duration varied between the two studies, and the study by Albouy and colleagues specifically investigated sleep during a 90-minute daytime nap (see section 1.4.8.2 for a discussion of nap paradigms for investigating sleep). Therefore the exact nature of 'preferential' consolidation, and what dissociates different memories, still remains to be clarified. However, it may be appropriate to think of a given task as generally involving a variety of components, rather than being purely implicit or explicit in content. For the purposes of the present thesis, I will adopt the definition of memory proposed by Robertson and Cohen (2006), namely that a memory may be thought of as the collective information that has been encoded during a single task – thereby comprising a variety of components within a single memory that may require processing in discrete brain networks.

1.1.2.2 Neurophysiology and neurochemistry of motor learning and consolidation

Evidence from neuroimaging studies provides further support for the idea that distinct brain circuits may be recruited for specific behavioural components of a motor learning task (see Figure 1.2). For instance, Grafton, Hazeltine, and Ivry (1998), using functional magnetic resonance imaging (fMRI), showed that during training, while goal-based learning was associated with activations in the fronto-parietal areas of the brain, movement-based learning preferentially recruited key motor regions, such as primary motor cortex (M1) and the supplementary motor area. In terms of subsequent

consolidation, Peigneux et al. (2006) found that following motor skill learning a broad network of these areas were also recruited, including M1 and parietal regions.

A dissociation in activation patterns has also been demonstrated that is thought to depend largely on the *phase* of learning. For instance, recent studies have shown activations of the premotor cortex, dorsolateral prefrontal cortex (DLPFC), as well as the cerebellum and basal ganglia during the beginning of explicit motor learning (Doyon et al., 2009; Floyer-Lea & Matthews, 2005), whereas later stages of practice have been associated with activations of the cerebellum and basal ganglia (Doyon et al., 2002; Lehéricy et al., 2005). This would be consistent with a reduction in distributed activation patterns associated with improved motor skill across practice (Steele & Penhune, 2010; Steele, Scholz, Douaud, Johansen-Berg, & Penhune, 2012).

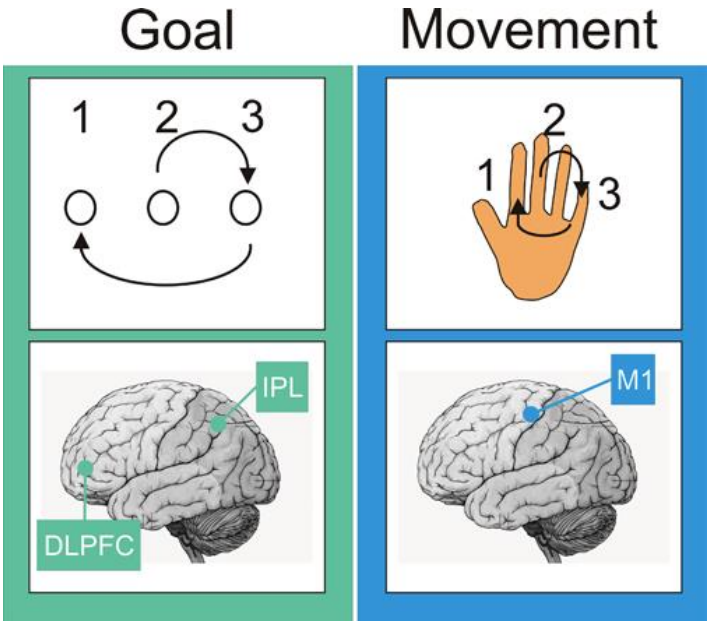


Figure 1.2. Differential encoding of task-based components. Sketch of potential brain areas involved during the acquisition of goal- and movement-based learning, respectively. Source: Robertson (2009).

Evidence is also available on the neurochemical underpinnings of motor learning. Interactions between key neurotransmitters are thought to support learning-related changes in cortical synaptic strength and plasticity. Relevant to the scope of this thesis these primarily include glutamate and γ-aminobutyric acid (GABA). GABA is the primary inhibitory neurotransmitter found across the adult mammalian brain, and is synthesised predominantly from glutamate, which is the major excitatory neurotransmitter. Studies using magnetic resonance spectroscopy (MRS) have proposed a link between specific motor behaviour and such underlying neurochemical processes in specific regions in the brain (Sumner, Edden, Bompas, Evans, & Singh, 2010; Floyer-Lea et al., 2006). One study showed gradual decreases in GABA neurotransmitter in M1 during 30 minutes of learning on a visuomotor tracking task (Floyer-Lea et al., 2006). This finding is particularly interesting because it is one of the few human studies looking at MRS-observed GABA and learning across time. However, the study did not report any correlation between behavioural performance and the neurochemical measures of GABA.

In addition, GABA has a hypothesised role to play in sleep and potentially in the consolidation of memories. For instance, specific GABA receptor agonists have been implicated in various key sleep processes including increasing sleep continuity, reducing latency to fall asleep, as well as enhancing non-rapid eye movement (NREM) sleep stages (including sleep phenomena such as spindles; Lancel, 1999), and the majority of pharmacological treatments of insomnia are aimed at regulating GABA receptors (Winsky-Sommerer, 2009). However, while GABAergic processes may be involved in sleep onset and maintenance, it is not clear whether or how such processes relate directly to functions of consolidation. Chapter 4 of this thesis will use MRS to test

for changes in GABA during motor learning and relate this to subsequent off-line consolidation.

In sections 1.2.2 and 1.3.2 I will further consider the neurophysiological correlates of memory consolidation. However, firstly, it may be relevant to examine some of the underlying mechanisms that support sleep function itself.

1.2 The architecture & functions of sleep

The above sections have included evidence that suggests an important role for sleep in the consolidation of memories, or specific components of memory. Human sleep is maintained and influenced by a number of factors including the circadian rhythm, and psychological and arousal processes. However, what exactly is sleep? How does it vary across different species? And what specific sleep processes are involved in consolidating memories in humans?

Briefly, sleep can be thought of as a state of consciousness, or alternations in consciousness, which oscillate between states of reduced awareness of external real-world stimuli to a complete loss of consciousness (Stickgold & Walker, 2009). While many characteristics of sleep have been elucidated by a rapidly growing body of research, the precise functions of sleep have yet to be clearly defined. However, sleep is thought to be involved in a number of important processes including those supporting the immune and memory systems.

Sleep can be characterised by two distinct states known as non-rapid eye movement (NREM) and REM sleep. Both types are present in all mammalian species. NREM sleep in humans is further subdivided into four stages (see Figure 1.3). REM sleep is also subdivided into further stages, namely phasic and tonic stages of sleep. See Box 1.1 for a characterisation of the sleep stages. Methods for assessing sleep stages (e.g., Rechtschaffen & Kales, 1968) and quantifying sleep characteristics are discussed in section 1.4.

Sleep progression cycles through these various stages in a repetitive, relatively consistent pattern across the night. The duration of a full sleep cycle is usually between 90-120 minutes, with typically four to five cycles per night, each consisting of a

combination of NREM and REM sleep. While similar sleep stages and phenomena are repeated throughout nocturnal sleep, their frequency and duration vary in a relatively consistent pattern. For example, NREM slow wave sleep (SWS) is particularly represented in the first half of the night, whereas REM sleep is especially prevalent in the latter half, and such differences may relate, for instance, to homeostatic functions, as well as sleep need (discussed in section 1.2.2.1). However, the characterisation of sleep stages relies on the careful assessment of specific underlying sleep phenomena closely associated with the different functions of sleep.

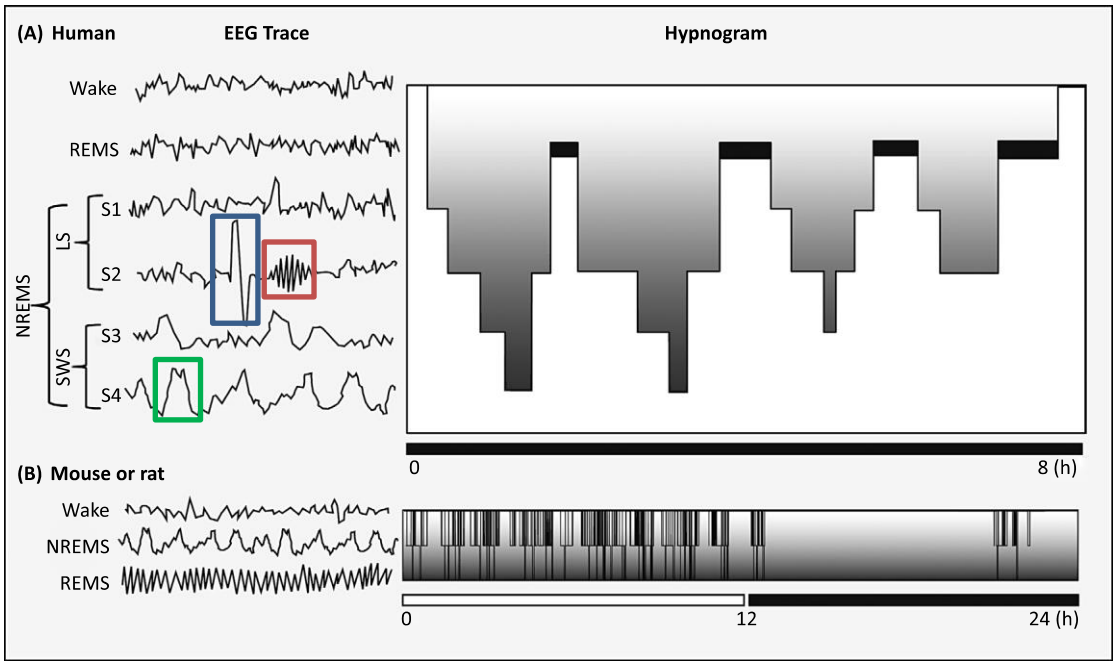


Figure 1.3. Depiction of sleep stages across human and animal sleep. Sleep repetitively progresses and regresses through lighter (LS) and deeper (SWS) NREM stages, as well as REM sleep (A). These fluctuations are depicted in the 'staircase' output, or hypnogram, on the right. SWS is especially prevalent during early sleep, and is represented by the deeper 'troughs' in the hypnogram, while stage 2 and REM sleep (black 'peaks') are particularly characteristic of later cycles. Key sleep signals are highlighted by the three boxes on the EEG trace (k-complex: blue; spindle: red; delta wave: green). In nocturnal animals such as mice (B) sleep cycles through shorter bursts of NREM and REM stages, as well as periods of wakefulness. The stages of NREM sleep are typically not subdivided into light and deep sleep in the animal literature. Figure adapted from: Genzel, Kroes, Dresler and Battaglia (2013).

Box 1.1. Stages of sleep and related EEG activity. As sleep progresses, a number of recurring sleep phenomena are visible in the EEG signal and are outlined below. The gold-standard for sleep staging is the approach proposed by Rechtschaffen and Kales (1968).

Stage 1

The earliest detection of light sleep is characterised by a decrease in alpha waves, and eye blinks dissipate into slow eye rolls until the EOG channels are relatively quiet. Towards the next phase of sleep, slow cortical oscillations begin to synchronise local neuronal networks, and initiate and coordinate other EEG signals.

Stage 2

As sleep deepens, the first sleep spindles become visible, and initially 'fast' k-complexes begin to slow and widen in shape. This is thought to reflect the inactivation of *N*-methyl-d-aspartate (NMDA) components, primarily due to membrane hyperpolarisation, as sleep deepens (Amzica & Steriade, 1998). The initially local synchronisations of the slow oscillation extend to wider networks increasing in connectivity, in turn becoming visible in the surface EEG as slow wave activity along with an increase in the number of spindles.

Stage 3

During the initial phase of slow wave sleep, the incidence of spindles begins to diminish, and slow and fast delta rhythms dominate the sleep epochs – with the occasional presence of slow k-complexes increasingly indistinguishable from other slow wave activity.

Stage 4

In the deepest form of sleep, the epoch is dominated largely by high-amplitude, slow delta waves in the healthy younger adult, but greatly reduced with older age.

REM

The progression to rapid eye-movement sleep is particularly dominant during later cycles of nocturnal sleep, and is characterised by rapid activity in the eye channels resembling wakeful eye inflections/movements. Submental muscle tone is very low during this stage, reflecting generalised muscle atonia during REM sleep (Hishikawa & Shimizu, 1995). This stage is typically associated with dream mentation.

NREM sleep occupies the predominant part of each sleep cycle and elicits EEG activity including spindles, k-complexes and delta waves that are thought to be important for memory processing during sleep. However, how are such sleep phenomena initiated and grouped?

1.2.1 Delta activity (0-4Hz)

Early invasive studies in animals (Steriade, Nunez, & Amzica, 1993) demonstrated the subdivision of specific oscillations within the delta frequency band recorded from single cells: the slow oscillation or rhythm (< 1Hz) and the slightly faster delta rhythm (1-4Hz). *In vivo* studies in (anaesthetised) cats have shown that the slow rhythm consists of distinct biphasic UP and DOWN states in cortico-thalamic neurons (Steriade et al., 1993), each reflecting neuronal depolarisation and hyperpolarisation, respectively (Destexhe, Hughes, Rudolph, & Crunelli, 2007). This contrasts with REM and wake states, which are characterised predominantly by depolarisation and asynchronous firing (Chauvette et al., 2010). The slow oscillation is thought to be the primary candidate mechanism for triggering and orchestrating other sleep signals including spindles and faster delta waves (Amzica & Steriade, 1998). However, while the slow oscillation likely transmits across thalamo-cortical pathways (e.g., in the synchronisation of thalamic-generated spindles; Contreras & Steriade, 1995), studies have shown that its expression is preserved even if thalamic projections are disrupted, whereas if cortico-cortical synaptic linkages are reversibly inactivated, the synchronisation of the slow oscillation is severely affected (Amzica & Steriade, 1995). Therefore it is thought that the slow oscillation is primarily generated within cortical neurons. However, it remains unclear how the cellular slow oscillation manifests in the signal recorded with scalp electrodes in humans. It has been proposed that it is likely

represented in the polymorphic slow delta band oscillations, predominantly k-complexes, visible in the signal of surface EEG (Amzica and Steriade, 1998), and is thought to account for more than 30 percent of slow wave sleep (Genzel et al., 2013).

It may here be relevant to briefly clarify the terminology adopted in this thesis when referring to activity in the delta frequency band, as use of terminology can vary between studies, which has led to misunderstandings particularly when comparing between animal and human studies (for discussion, see Genzel et al., 2013). A distinction is here made between the specific mechanism *slow oscillation* (< 1Hz) and its faster correlates *delta rhythms* (1-4Hz) to remain consistent with previous work (e.g., Amzica & Steriade, 1998). However, the terms *slow waves* and *slow activity* will be used interchangeably in this thesis to refer generally to activity in the delta frequency band (0-4Hz). As such, the broader term *slow wave activity* (SWA) is a more general term that encompasses slow waves indiscriminate of sleep stages, whereas *slow wave sleep* (SWS) is usually used to describe slow waves specifically in stages 3 and 4 NREM sleep in humans. Wherever possible I will try to describe existing literature according to these explicit definitions.

While it is thought that the slow rhythm (< 1Hz) vitally supports NREM activity (Steriade et al., 1993; Massimini, Huber, Ferrarelli, Hill, & Tononi, 2004), it was until recently still unclear how slow wave activity transmits across cortical networks in humans (i.e., global or local synchronicity), where it originates within the cerebral cortex, and the extent to which this is represented in the surface EEG.

1.2.1.1 Local or global phenomenon?

Work over the last decade has demonstrated that, in humans, slowly oscillating scalp recorded activity (~ 1Hz) repetitively propagates, much like a travelling wave, across the cerebral cortex (Massimini et al., 2004), suggesting more localised synchronisation and sequential propagation across networks. The origins of the waves are also thought to be consistent across volunteers, and associated primarily with central and prefrontal sites (Figure 1.4). Massimini and colleagues also demonstrated a preferential directionality of waves, more frequently travelling largely antero-posteriorly, and at a rate of approximately 2.7m/sec. Studies since then have reported further evidence to support more localised (synchronous) activity during deeper stages of sleep. For instance, functional imaging studies (e.g., Spoormaker et al., 2010) have shown that while lighter sleep is associated with increases in extensive cross-cortical connectivity (including activation of the default mode network; Horovitz et al., 2008), slow wave sleep reduces cortico-cortical connectivity, and is instead associated with more localised activations. This further suggests a link between distinct sleep activity and levels of regional synchronisation.

The above paragraphs outline some of the basic mechanisms of sleep, and their inherent properties (including origination and directionality). However, for a comprehensive assessment of sleep it will be relevant to examine how specific sleep phenomena might support behavioural function via processes such as consolidation. The subsequent section will consider converging evidence and emerging hypotheses that may shed light on this relationship.

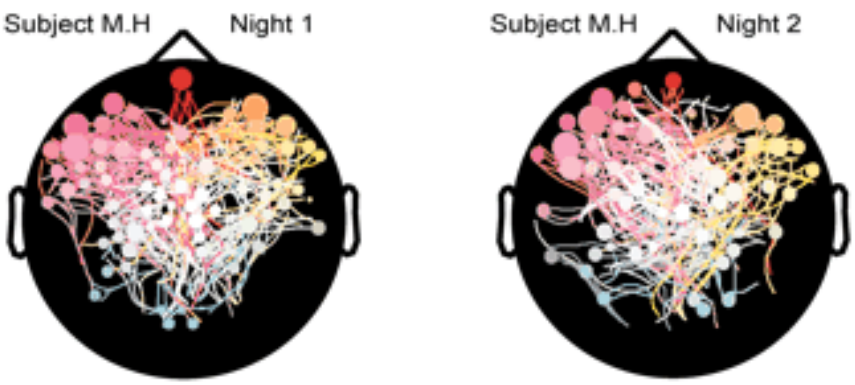


Figure 1.4. The origins and propagation of slow wave activity in the human cortex. Larger circles denote more frequent origination at specific sites in a single participant over two recording nights. It is noteworthy that sites of origin and travelling patterns remain largely similar across nights. Source: Massimini et al. (2004).

1.2.2 EEG correlates of memory consolidation

As described in section 1.1.2.1, there is behavioural evidence that certain types of memory benefit from off-line consolidation during sleep. What processes occur during sleep to support this consolidation? Ribeiro & Nicolelis (2004, p. 686) eloquently described the role of sleep as likely "[harbouring] both mechanisms postulated by Donald Hebb to be necessary and sufficient to explain memory consolidation: postacquisition neuronal reverberation, and structural synaptic plasticity". Current hypotheses of memory consolidation broadly reflect these distinct mechanisms proposed by Hebb (1949).

Two competing perspectives of specific sleep function in memory consolidation have been proposed that can be broadly categorised into 'active' (systems) consolidation thought to operate via reprocessing mechanisms such as *replay*, and homeostatic (synaptic) processes aimed at regulating neuronal networks for increased functional efficiency. While these models or hypotheses allude to the on-going debate about the functional involvement of key sleep processes in consolidation, they are not

necessarily contradictory perspectives, and may collectively offer insights into distinct (but possibly parallel) mechanisms of the consolidation process. It is possible to consider the two hypotheses as distinct parts of a continuum of regional activity during sleep, with the 'synaptic' model assuming more localised synchronisation of cortical networks, whereas the 'systems' model suggests a more widespread or global level of functional and synchronised connectivity (Genzel et al., 2013).

1.2.2.1 Synaptic homeostatic hypothesis of consolidation during sleep

The synaptic homeostasis hypothesis posits that a primary role for the slow oscillation is to promote homeostasis and 'downscaling' of synaptic potentiation within cortical circuits that were induced during active wakefulness and learning (Tononi & Cirelli, 2003, 2006). Specifically, the hypothesis (Figure 1.5) makes four claims, that (i) active wakefulness is associated with synaptic potentiation in neural networks; (ii) synaptic potentiation in turn is related to homeostatic regulation of slow wave activity; (iii) slow wave activity facilitates synaptic downscaling; and (iv) downscaling during sleep promotes neural functional efficiency. The model dissociates this type of downscaling (which is hypothesised to be a more generalised process encompassing most neurons) from long-term depression (thought to be more selective), or depotentiation (affecting only recently potentiated synapses).

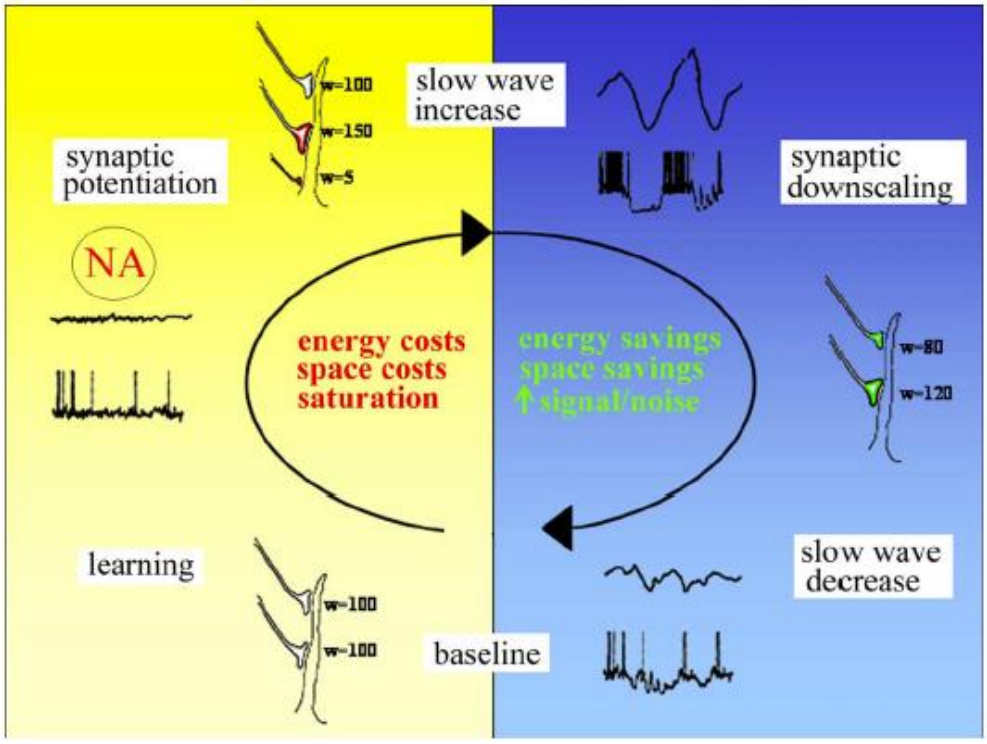


Figure 1.5. Simplified diagram of the processes of the synaptic homeostasis hypothesis. Increases in synaptic weights during wakefulness (yellow) are modulated during sleep (blue) by slow wave activity 'downscaling' of synaptic potentiation. This in turn recalibrates synaptic weights to a sustainable baseline thereby allowing new learning to take place. NA = noradrenergic system, which is more active during wakefulness and is thought to be an important driver of synaptic potentiation (Cirelli & Tononi, 2000). Source: Tononi and Cirelli (2006).

This model is supported by a number of neurophysiological findings. For instance, one study (Knott, Quairiaux, Genoud, & Welker, 2002) found increases in total synaptic density of topographically corresponding cortical neurons after 24 hours of single whisker stimulation in mice. Interestingly, the density of excitatory synapses returned to (pre-stimulation) baseline levels within four days of stimulation. Inhibitory synaptic density remained at the elevated levels. This suggests the local induction of increased synaptic potentiation in response to a sensorimotor stimulus applied during wakefulness. The study did not examine density levels specifically following a period of sleep, therefore it is not possible to consider sleep-specific changes in synaptic potentiation. However, the results highlight the transitory state of, at least, excitatory

synaptic gains, as well as the potential differential off-line processing of excitatory and inhibitory synaptic potentiation.

It is well established that slow wave activity is greatest in the early cycles of the night and decreases as sleep progresses, and this activity is thought to be a marker of sleep need (Hanlon, Faraguna, Vyazovskiy, Tononi, & Cirelli, 2009; Landsness et al., 2009). According to the homeostatic hypothesis, greater synaptic potentiation during wakefulness (for instance, due to more time spent awake) would be associated with increased slow wave activity during the following period of sleep (Tononi & Cirelli, 2003). Moreover, rather than being an epiphenomenon, this model proposes a functional role of SWA in down-regulating synaptic weights. These hypotheses are supported by studies showing that markers of synaptic potentiation are associated with a subsequent increase in slow wave activity in both animals (Meerlo, de Bruin, Strijkstra, & Daan, 2001) and humans (Huber, Ghilardi, Massimini, & Tononi, 2004). Research into more localised effects of learning (Krueger & Obál, 1993; Huber et al., 2004) further propose that local changes in synaptic density may also lead to local differences in SWA. Repetitive low-frequency stimulation at 1Hz has previously been shown to facilitate depotentiation in the rat hippocampus (Kemp & Bashir, 1997; Wagner & Alger, 1995). This is at the frequency of the slow oscillation. Moreover, it may be that the distinct biphasic UP (depolarising) and DOWN (hyperpolarising) states of the slow oscillation (Steriade et al., 1993) underlie this downscaling by producing insufficient pre-synaptic input to facilitate the post-synaptic neuron, which is thought to lead to synaptic depression (Tononi & Cirelli, 2003). While the hypothesis does not clearly describe how such regulatory mechanisms at the synaptic level directly relate to off-line processes of memory function, such as consolidation during sleep, it is suggested in the model that downscaling during sleep promotes neural functional efficiency, thereby

improving the resulting signal-to-noise ratio, which could in turn facilitate more efficient memory processing.

1.2.2.2 Active consolidation during sleep

An alternative to the synaptic homeostasis hypothesis underlying consolidation during sleep is the idea of 'active' consolidation. According to the standard model of systems consolidation, sleep supports an 'active' process, whereby memory traces are reprocessed and transferred across distributed networks via cortico-hippocampal projections (Girardeau, Benchenane, Wiener, Buzsáki, & Zugaro, 2009; Genzel et al., 2013). There is evidence to support the involvement of sleep processes in more active consolidation. For instance, a number of converging studies have shown the reactivation of firing patterns during sleep that were activated during wakeful learning (Ji & Wilson, 2007; Lee & Wilson, 2002; O'Neill et al., 2008). Moreover, activity in the theta frequency band (4-8Hz), which is associated with increased arousal states such as learning (Kahana et al., 1999), is highly prevalent across, for instance, REM sleep (Sederberg et al., 2003; Cantero et al., 2003). Recent studies have also shown that it may be possible to experimentally induce memory reactivation during sleep in both rats (Bendor & Wilson, 2012) and humans (Antony, Gobel, O'Hare, Reber, & Paller, 2012; Rasch, Buchel, Gais, & Born, 2007) by presenting an auditory or odour cue that was introduced during wakeful learning. The potential role of sleep in more 'active' consolidation processes is further supported by research suggesting that activity during sleep may likely facilitate mechanisms of synaptic potentiation. For instance, seminal work by Ribeiro, Goyal, Mello and Pavlides (1999) demonstrated plasticity-related gene expression during active wakefulness in rats (here, exposure to a rich sensorimotor environment; Figure 1.6b), consistent with previous reports (e.g., Cirelli & Tononi,

2000; Silva, 2003). Importantly, the study found that during subsequent sleep this gene was down-regulated specifically during NREM sleep (Figure 1.6b'), but then re-expressed during REM sleep (Figure 1.6b'') in cortical and hippocampal areas. In contrast to previous reports suggesting that expression of plasticity-related genes are limited or entirely switched off during sleep (e.g., Cirelli & Tononi, 2000; Silva, 2003), these findings suggest that synaptic potentiation may not only be possible during sleep, but also be a regular feature of specific stages of sleep associated, for instance, with REM activity. A recent experiment applying transcranial direct current stimulation (tDCS) to the premotor cortex during REM sleep, using a paradigm thought to enhance excitability, found a post-sleep improvement in the recall of the motor sequence learned (Nitsche et al., 2010).

Collectively, there is convincing evidence supporting the importance of sleep in both down-regulating (e.g., during slow wave activity) and up-regulating (e.g., through REM activity) the expression of plasticity-related genes in the consolidation of memories. Such plasticity-dependent up- and down-regulations could map onto, and underlie, key sleep functions outlined above including 'active' consolidation (through processes of synaptic potentiation, for instance, during REM sleep) and homeostatic 'downscaling' regulatory processes of slow wave activity (aimed at maintaining an efficient functional environment). It is possible therefore that the two hypotheses reflect different, but complementary, processes supporting sleep function that operate during distinct sleep phases/activity and crucially depend on specific attributes of regional activity (e.g., global versus local; Genzel et al., 2013). However, several mechanisms remain unclear, including whether and/or how different synaptic processes (e.g., excitatory versus inhibitory) are regulated during sleep. Further work is needed to elucidate the functional role of these more subtle mechanisms of memory processing.

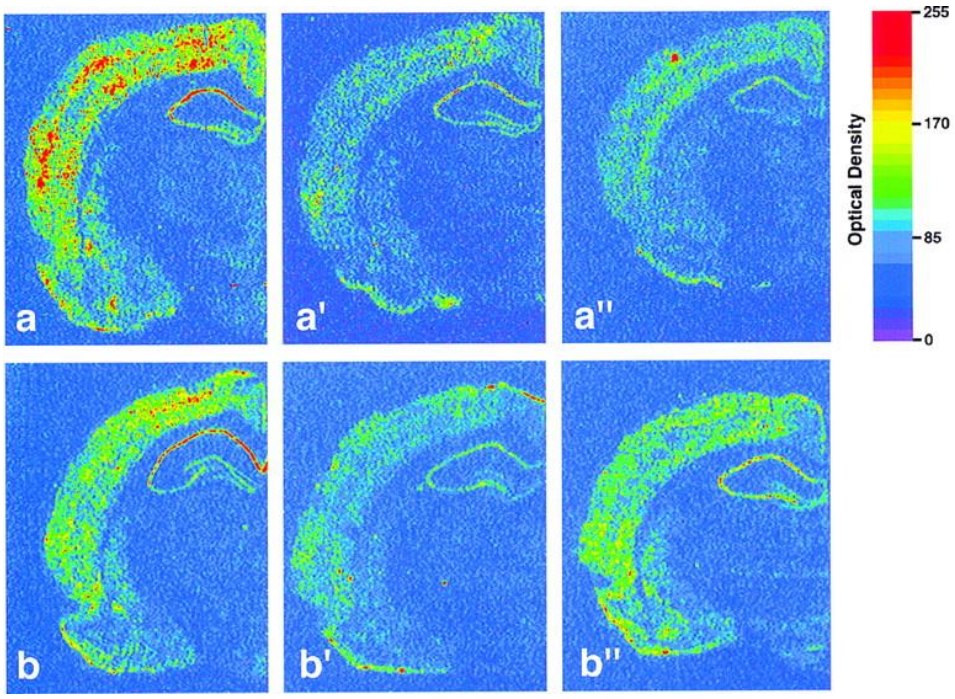


Figure 1.6. Plasticity-related gene expression during NREM and REM stages in rats. Autoradiograms of frontal sections showing differences in levels of expression in the rat cortex and hippocampus after active wakefulness (b-b'') compared with restricted controls (a-a''). Wakefulness (a and b) was associated with higher levels of gene expression in both groups, followed by a deactivation during NREM sleep (a' and b'). However, in rats that experienced an enriched environment levels increased during REM sleep (b''). Source: Ribeiro et al. (1999).

1.3 Effects of age

Studies investigating the effects of sleep on learning in humans have largely involved samples of healthy younger adults. Only relatively few studies have looked at sleep-dependent consolidation of motor learning in ageing, although these studies largely report consistent age-related deficits. However, potential mechanisms for observed deficits in older groups are poorly understood. For instance, it is unclear during which processes age-related divergences begin to manifest.

Temporally, it may be helpful to differentiate between two distinct phases during which deficits may affect memory processing: (i) during the online or in-session phase of encoding and motor skill acquisition, and/or (ii) during the off-line consolidation phase post-training. The following sections outline some of the current evidence for the effects of age on motor consolidation, and draw on the literature on age-related changes in sleep architecture and skill acquisition to examine how these factors might relate to the consolidation process.

1.3.1 Effects of age on off-line consolidation of motor memories

Behavioural studies that have attempted to reproduce sleep-dependent effects on motor consolidation in older adults are largely consistent in the finding that older age is associated with a marked decline in motor consolidation (Brown, Robertson, & Press, 2009; Fogel et al., 2012; Spencer, Gouw, & Ivry, 2007; Tucker, McKinley, & Stickgold, 2011; Wilson, Baran, Pace-Schott, Ivry, & Spencer, 2012). For example, Wilson and colleagues (2012) investigated sleep-dependent consolidation of motor sequence learning in younger and older adults, and found that sleep benefitted motor consolidation in younger, but not older, participants. Moreover, to date no study has

reported sleep-dependent effects in older volunteers, and only one study has been able to show age-equivalence in learning with retention (Fraser et al., 2009). That is, Fraser et al. found that a modified version of the SRTT, adapted to contain a high degree of variable content, showed comparable learning in younger and older adults, and that older (but not younger) adults demonstrated gains in accuracy at retest after a night of sleep. It is important to note, however, that this does not conclude any sleep-dependent effects. Given that the study design did not include a comparable period of wakefulness as a control condition, to adequately address the sleep-dependency question, this makes it equally possible that consolidation of the adapted SRT task took place over the simple passage of time rather than being specifically sleep-dependent, and by extension makes it difficult to compare results to existing sleep literature. Moreover, the reported finding that retention effects were seen *only* for the older sample and were not observed in the younger volunteers is inconsistent with most previous studies that have documented sleep-effects (only) in younger controls. It is possible that this finding reflects a previously unreported reversed effect of learning and retention between the two samples (i.e., a task that produces retention only in older adults) that might be attributable to subtle differences in the task used. However, such a hypothesis is not robustly clarified by Fraser et al., and would require extensive further tests. Therefore, it is not possible to conclude any role of sleep in off-line consolidation of motor memories in older adults on the basis of this study alone.

A second study (Tucker et al., 2011) showed that with additional practice after the consolidation period, older adults could reach a similar level to younger controls, however this required an extended consolidation period of 24 hours (see section 2.1). Together, this evidence suggests marked deficits in off-line consolidation of motor learning in older adults. However, given an extended period of consolidation (at least 24

hours) combined with extensive additional practice, older participants may reach similar performance levels as younger controls.

1.3.2 Effects of age on sleep architecture

It is well established that ageing is associated with changes in sleep architecture. For instance, studies have shown changes in factors including total sleep time and time spent in specific sleep stages (Figure 1.7). Specifically, converging evidence has shown an increase in the time spent in stage 1 and stage 2 NREM sleep, as well as overall reductions in slow wave sleep (for review, see Ohayon, Carskadon, Guilleminault, & Vitiello, 2004). Studies have also reported decreases in sleep duration and efficiency (including greater fragmentation; Huang et al., 2002; Phillips & Ancoli-Israel, 2001) and in the number of sleep spindles (Crowley, Trinder, Kim, Carrington, & Colrain, 2002; Nicolas, Petit, Rompré, & Montplaisir, 2001). Indirect changes to sleep processes may also take the form of changes in the circadian cycle, brain morphometry and functionality. Recent evidence suggests that structural changes with age, such as atrophy in the medial prefrontal cortex (mPFC) could influence key sleep architecture including NREM slow waves (Mander et al., 2013).

Changes to specific activity associated with sleep stages have also been reported. For example, a recent study (Colrain et al., 2010) showed morphological changes in k-complexes across the lifespan (Figure 1.8). This is supported by earlier work suggesting a decrease in the incidence and amplitude of slow wave activity with older age (Crowley et al., 2002; Crowley et al., 2004).

Together the available evidence suggests changes in key sleep patterns and activity with ageing including reductions in slow wave activity such as k-complexes. In

light of evidence supporting a role for slow wave activity in processes of consolidation (see section 1.2.2), it is possible that changes to such activity underlies observed age-related deficits in motor consolidation. However, there is inconsistent evidence regarding the involvement of slow wave activity in the processing of different memories. For example, research comparing the effects of age on motor sequence and word-pair associative learning showed deficits isolated to motor consolidation, while sleep-dependent consolidation on the word pair task remained intact (Wilson et al., 2012). In contrast, another study showed that age-related reductions in slow wave activity was associated with decreases in episodic memory (Mander et al., 2013). Therefore it is not clear how specific processes of sleep support different types of memories, or how these relationships change with age.

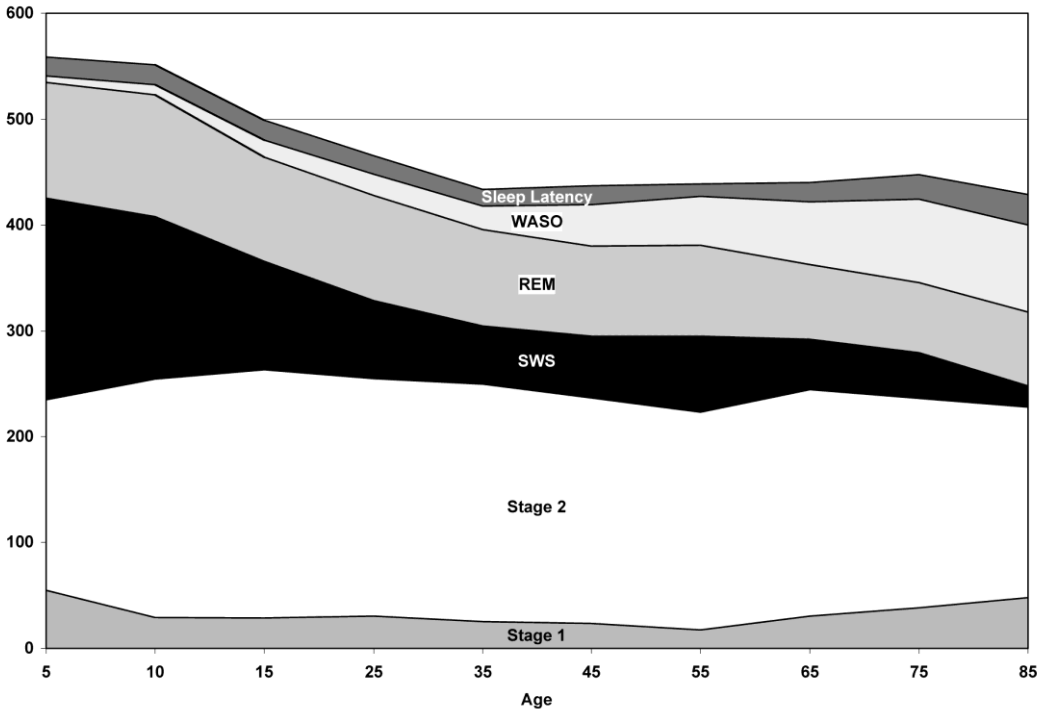


Figure 1.7. Changes in sleep architecture as a function of age. Shows the relative increases and decreases (in minutes) in specific sleep stages and measures (e.g., latency: time to fall asleep; WASO: wake after sleep onset) across a range of ages (5-85 years). Source: Ohayon et al. (2004).

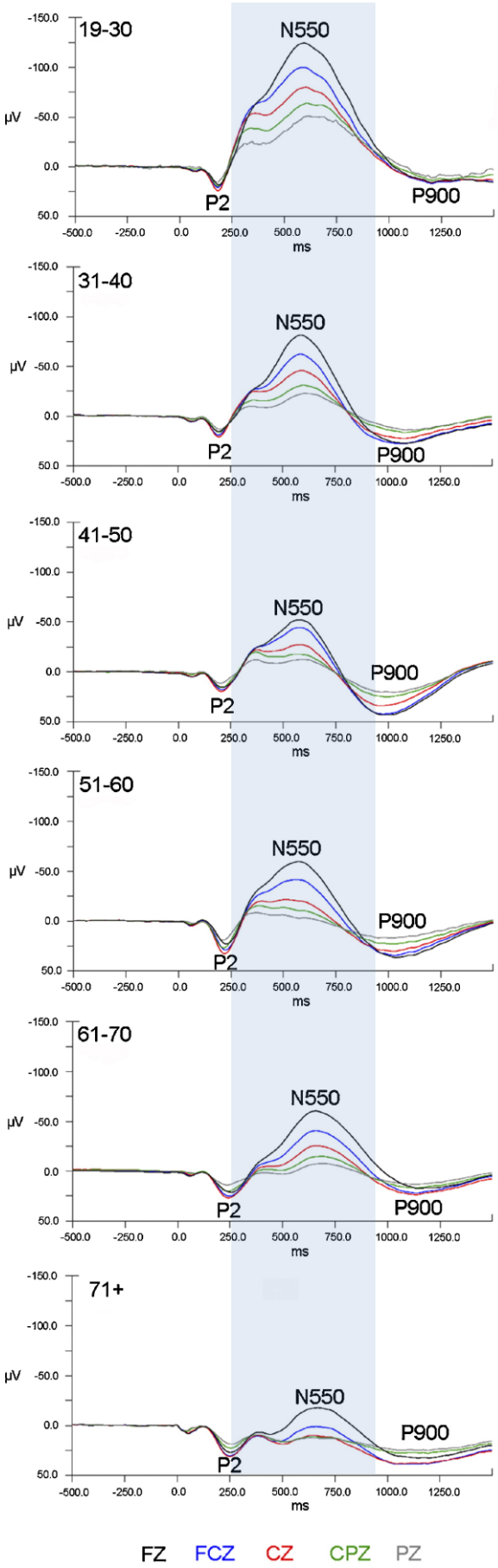


Figure 1.8. Changes in k-complex morphology across different age groups. Older age has been associated with marked decreases in amplitude of the N550 component of the k-complex in a sample of 19-71^{+} years, which is thought to be a reliable marker of ageing. Source: Colrain et al. (2010).

1.3.3 Age-related changes in the acquisition of motor learning

While changes in sleep processes have frequently been attributed a likely causal factor in age-related deficits in motor consolidation, it may also be that changes in the brain with ageing affect processes during the acquisition phase of learning. The next section will examine the available evidence supporting such acquisition-related changes, and evaluate them in relation to observed deficits in motor consolidation.

As described previously (section 1.1.2.2), neuroimaging studies have established that specific brain circuits are recruited for different aspects and phases of motor learning in humans. Research investigating age-related changes in activation during motor performance suggests that there may be changes in cortico-basal ganglia functional circuitry that occur with ageing (Marchand et al., 2011). For instance, one study (Mattay et al., 2002) found that during simple motor learning older adults showed greater activation in areas including the premotor cortex and cerebellum, and also recruited a wider network (e.g., putamen). This is thought to reflect compensatory activity including mechanisms of reorganisation in the ageing brain (Mattay et al., 2002). This is further supported by evidence (Naccarato et al., 2006) showing bilateral recruitment of M1 was necessary to support the same performance on a paced motor task in older adults compared to younger controls. Interestingly, in a separate study, age-associated cortical over-activations were not influenced by task-related variables such as movement rate, suggesting a generalised up-regulation with ageing that may be independent from functional motor demand (Riecker et al., 2006). However, changes with ageing are not necessarily linked with compensatory processes. For example, Marchand et al. (2011) found an association between older age and increased cortico-basal ganglia connectivity during motor performance, which in turn correlated with poorer outcome on a separate behavioural task when controlling for age. The

researchers concluded that this likely reflected a decline in functional circuitry rather than the involvement of compensatory processes. However, it is likely that both age-related compensatory mechanisms such as reorganisation and increased cortical-subcortical connectivity are indicative of underlying neuro-degeneration.

While there is growing evidence to indicate changes in functional architecture with older age that may affect general motor performance, it remains unclear how specific mechanisms of motor function are affected by such changes. Sarcopenia, the loss of muscle mass, is stipulated to be primarily involved in age-related changes in mechanisms such a muscle force (Klass, Baudry, & Duchateau, 2007). However, emerging evidence suggests that structural and functional cortical correlates of ageing may also influence key motor abilities including fine motor dexterity. Recent studies have shown significant reductions in fine motor speed and dexterity (Ashendorf, Vanderslice-Barr, & McCaffrey, 2009; Marmon, Pascoe, Schwartz, & Enoka, 2011; Ranganathan, Siemionow, Sahgal, & Yue, 2001), as well as finger strength and hand coordination (Soer et al., 2012) with older age. One potential mechanism underlying fine motor kinematics is cortical inhibition. Inhibitory processes are important for fine motor performance in order to suppress other irrelevant (antagonistic) muscle activations that may cause interference. Research into surround inhibition, a neural mechanism of inhibition, showed that movement, for instance, of the index finger suppressed motor excitability acting on neighbouring muscles (Sohn & Hallett, 2004). In addition to muscular deterioration, it is in part these cortical inhibitory mechanisms that are thought to change with ageing (Marneweck, Loftus, & Hammond, 2011). For instance, studies have shown age-related increases in the coactivation of agonist and antagonist muscles (Klass et al., 2007). However, these studies typically investigated age effects on proximal muscle groups, which are thought to be more severely affected

with age. Recent work by Marneweck, Loftus and Hammond (2011) examined specific muscles in the hand (i.e., first dorsal interosseus) and found a reduction in short-interval intracortical inhibition (SICI) in older adults compared to younger controls, suggesting an age-related decrease in cortical inhibitory processing.

Such processes of fine motor control may be particularly relevant for the execution of sequential finger movement important for motor learning tasks frequently used to investigate sleep consolidation. A study by Orban et al. (2011) investigated potential neural correlates of sequential motor learning in healthy younger adults, and they found associated activations in subcortical structures comprising the cerebellum, thalamus and putamen, as well as primary, supplementary and pre-supplementary motor cortices suggesting that extensive networks may be functionally recruited during sequential movements. A number of studies have shown age-related degeneration of several of these areas, including the cerebellum, thalamus and putamen (Good et al., 2001; Raz et al., 2005; Salat et al., 2004). Therefore, it seems likely that some overlap may exist between functionally relevant regions for sequential motor kinematics and key areas associated with morphological degeneration with ageing.

In summary, while it is well known that ageing can lead to changes in sleep patterns and activity, there is also convincing evidence of age-related effects on motor performance, specifically requiring fine motor kinematics. These effects are thought to relate to muscular changes in fibre mass, but also to cortical changes in inhibitory processes with ageing. However, regardless of the underlying causal mechanisms, it is increasingly apparent from converging evidence that ageing may be associated with severe deterioration in fine motor function including sequential dexterity, speed and force. Therefore, it may be that fine motor deficits during learning could mask potential

sleep effects in older adults. For this reason, one aim of this thesis was to develop a task to assess motor consolidation that was less heavily dependent on fine motor dexterity.

1.4 Methods for studying learning & consolidation

The studies in this thesis adopt a combination of techniques ranging from neurophysiological approaches, including electroencephalography (EEG) and human spectroscopy, to more qualitative and behavioural paradigms in order to assess changes with sleep and ageing. EEG is particularly suited for addressing highly specific temporal properties of brain activity, for which most neuroscientific methods in humans are still too coarse. This section outlines some of the basic principles underlying the techniques adopted in this thesis, as well as relevant methodological considerations.

1.4.1 Electroencephalography of sleep

Demonstrated in its crudest form in 1875 by Richard Caton, EEG allows for the real-time recording of electrical signals in the brain from aggregate field potentials. This method is particularly relevant for measuring (submillisecond) temporal properties of group-level neuronal activity in humans (Michel et al., 2004), including event-related phenomena (e.g., during task performance) and continuous time-series data (e.g., in order to characterise distinct EEG components associated with specific stages of sleep). The temporal strength of EEG also lies in its capacity to dissociate between sequential and parallel electrical activity in the brain.

Hans Berger was the first to propose that electrical patterns altered with sleep (1929). From this period until the late 1960s, various characterisations of sleep stages (e.g., stages A-E; Loomis et al., 1937) and analysis approaches (e.g., averaging five minute epochs; Blake et al., 1939) were adopted, until the discovery of rapid eye movements during sleep (Aserinsky, 1953), and the repetitive cycling between REM and NREM stages of sleep (Dement & Kleitman, 1957). This subsequently led to the

first consensus based scoring guidelines developed and published by Rechtschaffen and Kales in 1968. Despite efforts to update these scoring guidelines (i.e., American Academy of Sleep Medicine; AASM, 2007), the manual proposed by Rechtschaffen and Kales remains the gold-standard for biophysical sleep staging and scoring. One of the major distinguishing features separating the two scoring guidelines relates to the characterisation of slow wave sleep. According to the original scoring guidelines proposed by Rechtschaffen and Kales (1968) a distinction is made between stages 3 and 4 NREM sleep. However, this division is collapsed in the more recent update (AASM, 2007), wherein activity in stages 3 and 4 are collectively referred to as SWS. For the purposes of this thesis, it was important to keep the two stages separate due, for instance, to age-related differences in sleep architecture, particularly relating to a decline in (stage 4) slow wave sleep with older age, as well as evidence suggesting that mechanisms underlying slow wave activity may differ between these stages (Amzica & Steriade, 1998).

1.4.2 EEG acquisition

Several montages of electrodes can be adopted for recording brain potentials with EEG depending on requirements, although these are usually based on some configuration of the 10-20 system outlined by Jasper (1999). A number of studies support the use of bilateral electrode placements for the measurement of sleep, as specific sleep phenomena may be more easily observed at bilateral locations (e.g., C3 and C4 for spindles, which are important for scoring stage 2 NREM sleep; DeGennaro et al., 2000; Silber et al., 2007; McCormick et al., 1997; Whitney et al., 1998). Moreover, previous studies have demonstrated hemispheric differences in sleep activity (Nishida & Walker, 2007), as well as highly localised processing associated with motor

learning (Huber et al., 2004). For the purposes of this thesis no assumptions were made regarding the specific underlying spatial location of recorded signals (see methodological considerations in section 1.4.4), although broad inter-hemispheric differences were considered. In addition, there is a trade-off between high-density EEG and setup time (given that these were all manually placed electrodes), particularly relevant in relation to the older participant group. As such, a selective montage of (bilateral) electrodes (Figure 1.9) was suitable for the scope of this thesis. Additional channels were included for recording electrooculography (EOG) and submental electromyography (EMG), which are particularly important for scoring REM sleep (see Figure 3.2 in Chapter 3 for a characterisation of each sleep stage).

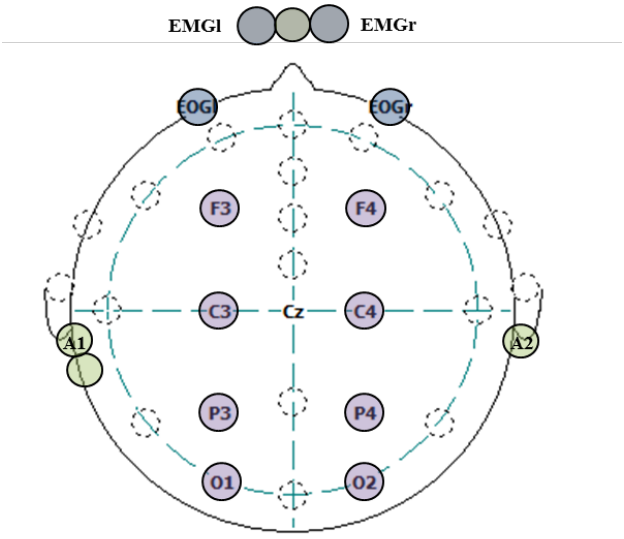


Figure 1.9. Sketch of scalp electrode placement during sleep recording. Recording electrodes for EEG (purple) were positioned bilaterally (F3, F4, C3, C4, P3, P4, O1, O2) with reference electrodes (green) at A1 (for right hemisphere and EOG electrodes) and A2 (for left hemisphere electrodes). EOG recording electrodes were placed above and below the eyes, while submental EMG was recorded from the muscles below the chin (grey). All measurements were obtained using Ag-AgCl electrodes for maximum signal conductance.

1.4.3 EEG analysis

The following paragraphs provide a brief overview of the steps involved in deriving meaningful output from the raw EEG signal. Sleep comprises dynamic phenomena that repeat with varying frequency throughout the period of sleep, and can vary considerably between participants and age groups. However, specific standardised approaches are applied to accurately characterise signal phenomena and allow for the subsequent segmentation into well-known sleep stages.

1.4.3.1 Qualitative (staging) analysis of EEG data

For sleep analysis, we typically aim to determine characteristics of, and changes in, the measured EEG signal, which relate in turn to (i) the rhythmic fluctuations, or frequency, of the signal (measured in Hz or cycles per second), (ii) the magnitude, or amplitude, of the signal (measured in mV), and (iii) the overall shape, or morphology, of specific patterns in the signal (e.g., spindles and k-complexes; Pace-Schott, 2009). Assessment of these properties (see section 1.2 above) allows us to make specific qualitative judgements regarding the characterisation of particular sleep stages. Analysis of a night of sleep is done through careful inspection epoch-by-epoch (an epoch is the restricted time window used for analysis that is subsequently scored as one of seven physiological states: namely, awake, stages 1, 2, 3, or 4 NREM sleep, REM sleep, or movement time). In this thesis stages were scored based on 30-second epochs (see methods section 3.2), which is in line with adopted scoring rules (Rechtschaffen & Kales, 1968), as well as the recommended epoch length for sleep scoring by a recent review (Silber et al., 2007).

In addition to the more qualitative scoring approach, it is also possible to quantitatively assess aspects of the EEG signal, including the use of spectral power analysis. However, these are complementary analysis approaches that offer additional levels of insight, and will both be used for the purposes of EEG analysis in Chapter 3 of this thesis.

1.4.3.2 Quantitative (spectral) analysis of EEG data

A permutation of spectral analysis (specifically of the fast Fourier transform, FFT) is the short-time FT (STFT), which is more appropriate for continuous (non-stationary) time-series data. This allows us to preserve a measure of the temporal resolution in signal frequency analysis by segmenting the data into specific slices (e.g., using a Hamming window that moves with time; Hamming, 1989). Fourier transform is then applied to (now-stationary) signal frames. This in turn allows us to compare quantitative changes in the data (e.g., in frequency and amplitude) against specific criteria. For the purposes of this thesis this included the quantification of measures (e.g., power density) of slow wave activity (SWA) during specific stages of sleep (see section 3.2 for full details of specific criteria used). Briefly, a characteristic property of SWA (and SWS) is the presence of slow waves (i.e., 0-4Hz), which are here defined as only activity that meets the scoring criteria (i.e., $\geq$ 75μV and of $\geq$ 0.5sec duration) of Rechtschaffen and Kales (1968). This increased specificity in defining delta band activity allows us to consider quantitative changes in slow wave bandwidth (e.g., incidence and power density) within particular visually scored stages of sleep (e.g., NREM sleep). For some types of analyses it may also be appropriate to include lower amplitude delta band activity (for discussion, see Colrain et al., 2010), however these are more difficult to relate meaningfully to the sleep staging used in this thesis.

1.4.4 Methodological considerations

The following paragraphs outline a number of methodological issues that influence the hypotheses that can be generated on the basis of, and the level of detail that can be obtained with, surface-level EEG measurements.

While temporal resolution is a key strength of EEG, one of the fundamental issues with using surface (scalp) electrodes to measure electrical potentials is its spatial resolution. Recorded signals represent aggregate neuronal output beyond the circumference of the (~8mm) recording electrode, and frequently from more distant networks. Specifically, the electromagnetic inverse problem (Helmholtz, 1853) dictates that a level of ambiguity exists in the measured surface potentials due to contributions from numerous configurations of underlying (intracranial) sources (Michel et al., 2004). Potential reasons for this spatial uncertainty relate to the attenuation of signal due to intervening layers (e.g., the scalp and skull) between the electrodes and the signals they are attempting to record (Figure 1.10). Therefore, it is difficult to determine the exact underlying spatial origin of measured potentials.

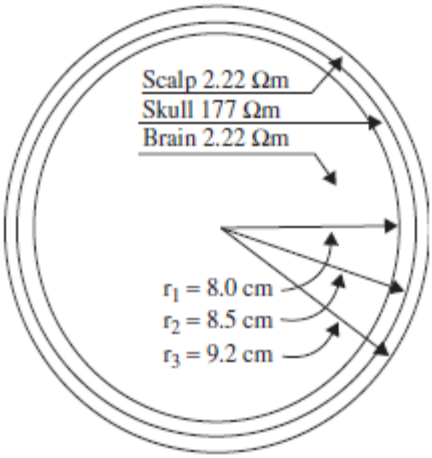


Figure 1.10. Intervening layers of the brain. Depiction of relative layer thicknesses and resistance levels. Surface electrodes are placed on the outer layer of the scalp. In this thesis, in order to reduce resistance and increase the signal-to-noise ratio all impedance levels were kept below 5kΩ. Source: Sanei and Chambers (2007).

A number of solutions to the inverse problem have been proposed (Michel et al., 2004), with source analysis models, such as the Low-Resolution (brain) Electromagnetic Tomography (LORETA) model (Pascual-Marqui et al., 1999), applied specifically to sleep phenomena more recently (e.g., Anderer et al., 2001; Ventouras et al., 2010). Source-based models like LORETA attempt to solve the spatial issue based on a priori mathematical assumptions relating to the correlational nature of more proximal neuronal signals (Ventouras et al., 2010), and has for instance been applied for source localisation of spindle activity during NREM sleep (e.g., Anderer et al., 2001). However, the assumptions adopted by these models are by definition based on a priori constraints, and the true physiological generators remain unknown (Michel et al., 2004).

An important consideration when comparing across animal and human studies is the often contradictory use of terminology. This is partly due to the complexity of the electrophysiology of sleep and the different techniques used in invasive compared to non-invasive studies. For instance, while the term SWS in humans is used to denote specifically stages 3 and 4 NREM sleep, SWS in animal studies usually refers to slow wave activity across all stages of NREM sleep. Moreover, although slow wave activity observed with surface electrodes may potentially reflect underlying slow oscillations at the cellular level, it likely represents aggregate activity across a wide range of networks as discussed previously. Therefore it is difficult to directly relate findings from single-cell recordings to those obtained with surface EEG.

However, it may be relevant to note that according to the scoring criteria (e.g., Rechtschaffen & Kales, 1968) used to characterise delta waves in human EEG, only slowly oscillating, high-amplitude events of longer duration than half of a second are considered ‘genuine’ delta waves. This is equivalent to a ≤2Hz delta frequency oscillation, and faster delta rhythms (2-4Hz) were not included in the present

characterisation of slow wave activity (see section 1.2 above). Therefore, although the neurophysiological scale of measurement may differ between animal and human studies, there may be some overlap in terminology at least in terms of the frequency range that is considered.

1.4.5 Actigraphy recorded sleep quality

While nocturnal recordings with EEG are appropriate for shorter periods (e.g., two or three nights), participants usually do not tolerate longer-term overnight EEG measurements due to the demanding and intrusive aspects of this type of measurement, including setup time for manually placing the electrodes on the scalp, which typically averages 1.5 to 2 hours per session. By contrast actigraphy is well tolerated over longer timescales, and is good measure to characterise sleep and circadian patterns over longer recording periods due to its relatively unobtrusive nature. Actigraphy requires participants to wear a simple wrist-worn accelerometer that measures amount of activity across the recording period. Participants complete a daily activity diary for the recording period (see appendix), which is used for subsequent analysis. It is important to note that actigraphy provides only an indirect measure of sleep, and contains an accelerometer that measures activity and rest cycles (Figure 1.11).

Analysis of the data involves a semi-automated procedure to derive various indirect measures of sleep (including sleep efficiency and latency, which are the most commonly reported measures).

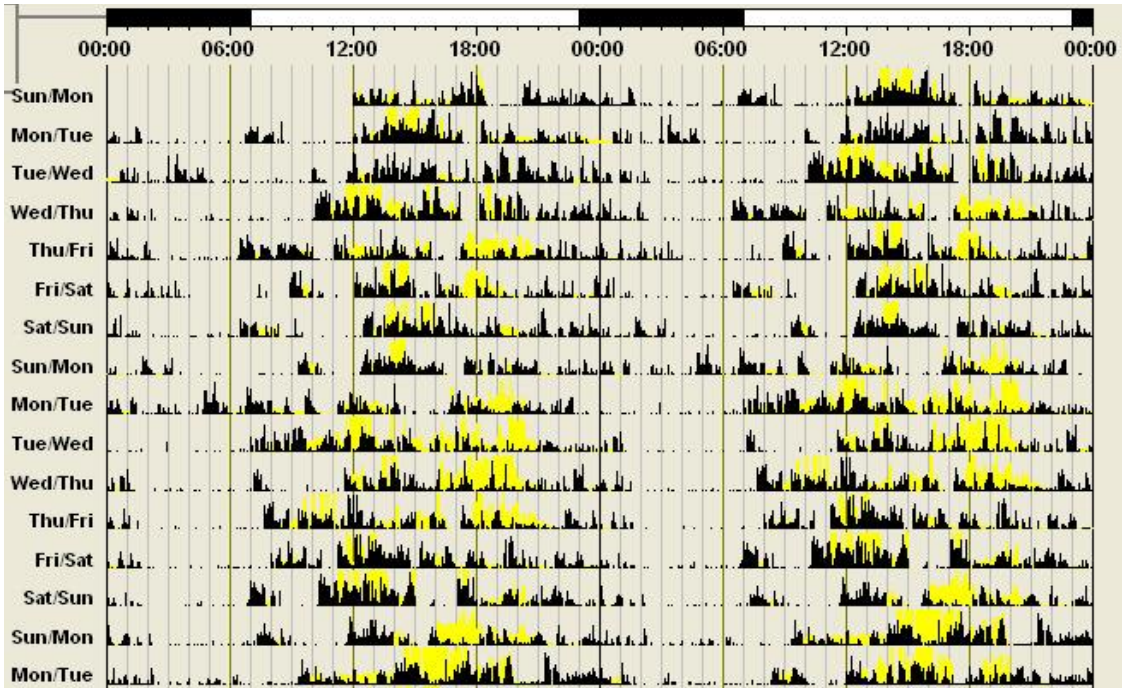


Figure 1.11. Example dataset recorded with actigraphy. Data shows activity spikes (black) interspersed with rest/no movement. Yellow colouring denotes recorded light source input.

1.4.6 Self-reported sleep quality

The Pittsburgh Sleep Quality Index (PSQI) was adopted to measure self-reported sleep quality of participants (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989; see appendix). This measure assesses several sleep parameters over the previous month (including typical bedtime and sleep duration) to provide a global score denoting the level of sleep quality. As this is a highly subjective measure of self-reported sleep the next section outlines potential methodological considerations of this questionnaire in relation to the more objective measure of sleep quality (i.e., actigraphy).

1.4.7 Methodological considerations

Actigraphy recording produces various measures of activity and rest. For the purposes of this thesis this section will focus on the three main outputs (sleep latency,

efficiency and actual sleep time). While actual sleep time is more widely adopted as a measure of sleep quality, it is unclear how the sleep parameters relate to each other. Therefore, the next section will consider the internal consistency of actigraphy, as well as the consistency between the two indirect measures of sleep (actigraphy and PSQI).

1.4.7.1 Internal consistency of actigraphy

The measure of sleep latency has previously been questioned because it is difficult to accurately determine the time between bedtime and first sleep onset based on movement data. This also affects the measure of sleep efficiency, as it is derived as a function of sleep latency. Therefore, actual sleep time has often been used as a more accepted measure of sleep quality. However, this measure also has certain inherent restrictions. For instance, actual sleep time is derived by subtracting periods of wakefulness, however characterisations of arousal are equally affected by the indirect nature of the approach because it is unclear how measures of movement recorded with actigraphy relates to actual wakefulness during the night. However, to assess at least internal consistency between these measures, correlational analyses were adopted to assess the relationships between these measures and showed strong and significant associations between actual sleep time and measures of sleep efficiency ($r = .77$) and latency ($r = -.71$), respectively. While it is true that the exact point of sleep onset cannot be determined using this indirect technique, this does suggest a level of consistency across parameters.

It should be noted that for some of the studies in this thesis (i.e., Chapter 2) recordings only took place over 24 hours, and so give a very restricted indication of potential dynamics of sleep quality that may themselves vary considerably over time.

Therefore, in those studies the measures obtained served only to shed light on the sleep quality relating to the night after a specific learning intervention.

1.4.7.2 External consistency

No correlations (all below r = .1) were observed between self-reports and the more objective sleep quality parameters derived from actigraphy recordings, suggesting poor consistency between the indirect sleep quality measures.

1.4.8 Behavioural assessment of sleep-dependent motor learning – confounds and considerations

The motor tasks adopted in this thesis are based on (or adapted from) classic tasks and paradigms used in the learning and consolidation literature (e.g., Walker et al., 2002; 2003). While tasks will be described in detail in section 2.2, the following paragraphs will consider the importance of specific key design features of the motor sequence learning paradigms, namely (i) whether considerations relating to the inherent differences between sleep and wakefulness states might contribute to observed sleep-effects, and (ii) the extent to which the quantity of the measured sleep period (e.g., diurnal nap versus nocturnal full night of sleep) have differential effects on behavioural outcome measures.

1.4.8.1 Interference of motor activity

One intuitive concern frequently raised concerning sleep studies relates to the inherent differences that exist between wakeful and sleep states. One such concern

posits that sleep offers a period of less interference (both in terms of attentional factors, but also in terms of, for instance, use-dependent motoric interference) associated with wakefulness day-to-day – after all, participants do not necessarily refrain from moving their task-related hand during the daytime period between test sessions. However, a study by Walker et al. (2002) tested this hypothesis by enforcing restricted hand movement during the intervening period of wakefulness. Their findings showed significant sleep-specific improvements over and above the wakeful period regardless of whether participants were in the hand rest condition or allowed to move their hand freely during the day, suggesting that the lack of improvement seen during wakefulness is unlikely due to motoric interference.

1.4.8.2 Mechanisms related to quantity & quality of sleep

The previous sections have briefly considered instances in which sleep has often been shown to be a key factor in consolidation-related gains following training. However, studies have occasionally adopted shorter sleep paradigms than a full night of sleep (e.g., nap studies), and it is not clear how this might affect outcomes.

Although the consensus seems to be that both shorter (e.g., nap or half-night), as well as longer (full nocturnal) sleep periods benefit memory consolidation, the size of the gain may differ depending on the length of the period, and may also relate to qualitative features of the sleep process. A three-hour period of (early nocturnal) sleep also showed benefits in performance, however the magnitude of the effect was three times inferior to that of a full night of sleep (Gais, Plihal, Wagner, & Born, 2000). Moreover, total sleep time has been found to correlate significantly with (overnight) performance improvement (Stickgold, James, & Hobson, 2000), suggesting that more

sleep leads to increased gains. However, too much sleep has also been associated with detrimental effects. For instance, research investigating mortality rates as a function of sleep duration suggests that both short and long sleep is associated with increased risk of mortality including cardiovascular-related (Gallicchio & Kalesan, 2009).

In the case of naps, sleep-dependent gains have also been demonstrated, although they may depend on a sufficient representation of both REM and SWS (Mednick, Nakayama, & Stickgold, 2003). However, how well daytime naps compare with the magnitude of observed improvements with (full) nocturnal sleep is variable. While Stickgold and colleagues (2000) reported a comparable effect size to the nap study (Mednick et al., 2003), compared to Gais et al. (2000) the benefit with shorter sleep was again inferior in magnitude (about half) to a full night of sleep. In the case of motor sequence learning, sleep gains with a daytime nap were also found (approximately 10-15 percent improvement; Korman et al., 2007; Nishida & Walker, 2007).

Collectively, there is evidence to suggest sleep-related improvements across different amounts of sleep. However, the conclusions drawn from these studies should be weighed against limitations inherent in comparing different study designs that may vary in important attributes, such as differences in the architecture of sleep stages, and variances in homeostatic and circadian processes between nocturnal and diurnal sleep, as well as between early and late sleep (Diekelmann et al., 2009).

1.4.9 Magnetic resonance spectroscopy

Magnetic resonance spectroscopy (MRS) is a powerful non-invasive tool to study various metabolic processes in health and disease, and allows for the in vivo

quantification of neurochemicals in human participants (Stagg, Bachtiar, & Johansen-Berg, 2011). This section outlines some of the basic principles underlying the acquisition and analysis of spectroscopy, as well as methodological considerations of the technique.

1.4.9.1 Spectral principles & acquisition

Unlike other imaging techniques that allow us to obtain information from multiple sites simultaneously and then define our region of interest, in MRS we acquire an average spectrum from a single predefined volume chosen a priori (Sumner et al., 2010). Moreover, while in MRI an a priori (though inaccurate) assumption is that the magnetic field affects all atoms in tissue equally, contrast in MRS is achieved as a result of the minute differences experienced by atoms, which allows us to distinguish between chemicals along the spectrum (Figure 1.12). How the magnetic field strength is experienced by the atom relates partly to the electrical environment (e.g., number of electrons) surrounding the nucleus (Figure 1.13), which produces different shielding effects from the main magnetic field (B_0). This in turn allows us to observe different chemical signals at relative points along the frequency spectrum (the 'chemical shift', and here measured in parts per million (ppm) – a dimensionless term). While the chemical shift and positioning of each signal along the spectrum remains constant under conditions where the main magnetic strength is also kept constant, they will vary with changes in B_0 (e.g., 3T-7T).

One of the applications of MRS for studying learning and consolidation is its capacity to measure GABA concentrations in humans. Despite sparse evidence, previous work (e.g., Floyer-Lea et al., 2006) has demonstrated concomitant changes in

GABA in-session with a (short) behavioural intervention, suggesting that the temporal resolution of this technique is sufficient to detect even short-term fluctuations with learning.

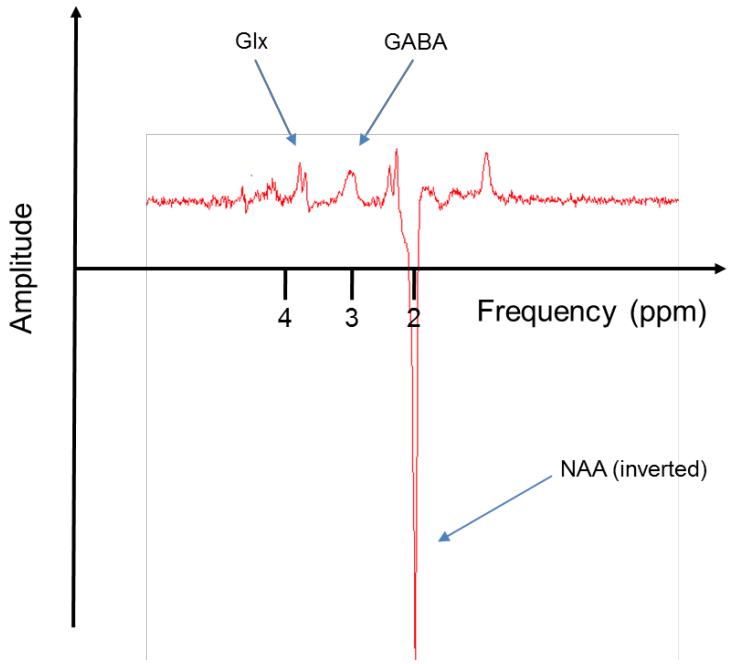


Figure 1.12. Depiction of the chemical shift of relevant molecules. Chemicals are located at characteristic frequencies along the spectrum. *N*-acetyl-aspartate (NAA) is inverted due to the GABA-editing procedure.

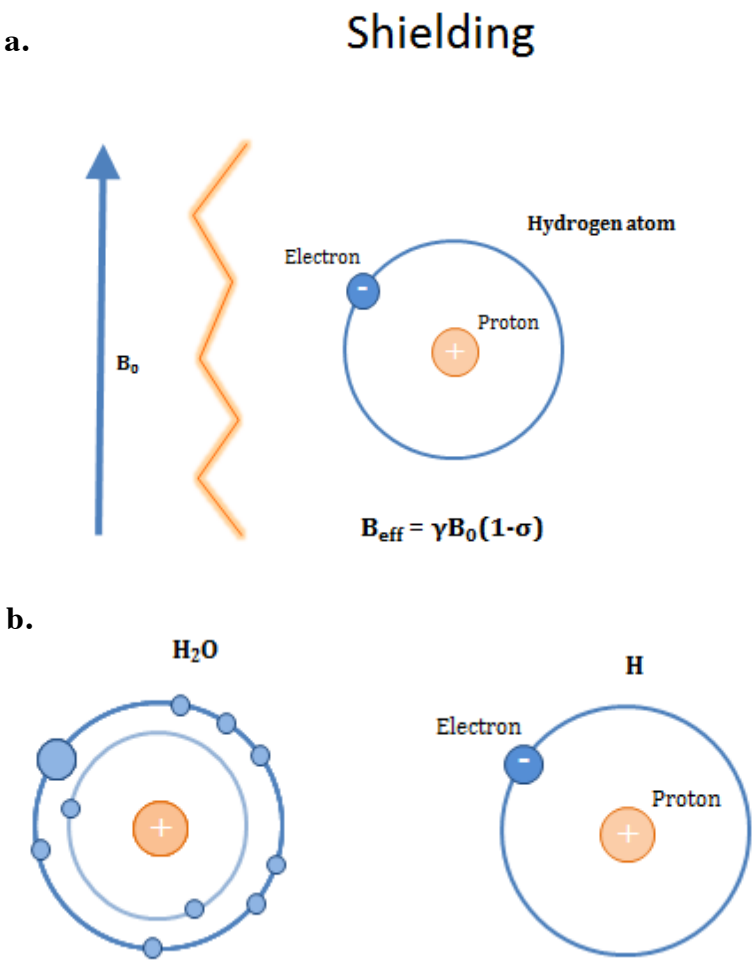


Figure 1.13. Simplified diagram of the basic principles of MR spectroscopy. Each nucleus is surrounded by a number of moving electrons, which produce their own small magnetic field (a). This acts as a form of shield against the main magnetic field (B_0), which in turn changes the efficiency of the magnetic field (B_{eff}) experienced by the nucleus. This shield is a dimensionless constant, and varies depending on the electrical environment of the nucleus or nuclei (b). It is this difference in proton-sensitivity to B_0 that provides the contrast, or chemical shift, on which spectroscopy relies.

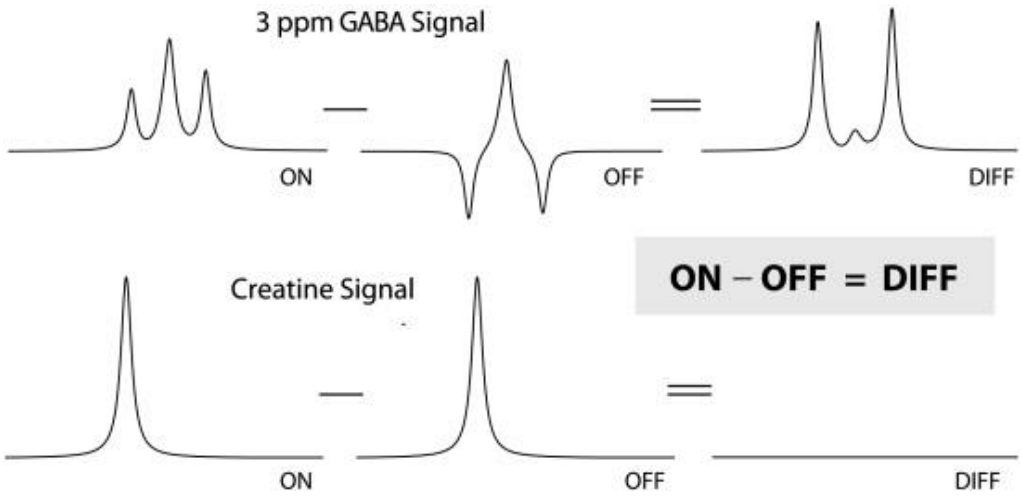


Figure 1.14. Sketch of the editing sequence for GABA. Scans are acquired with (ON) and without (OFF) editing pulses. Subtraction of these scans allows GABA to become visible in the difference (DIFF) spectrum. Source: Mullins et al. (2012).

In this thesis the acquisition protocol used was the Point Resolved Spectroscopy (PRESS) sequence, which is optimised for scanning at 3T and can be combined with the frequency selective sequence MEGA (named after its authors; Menscher et al., 1996) to allow subsequent GABA-editing. This is necessary because of stronger overlying neurotransmitters (mainly creatine, Cr) that occur at the same frequency as GABA (~3.0ppm). MEGA-PRESS applies editing pulses to separate GABA from overlapping frequencies (Figure 1.14) and allows for simultaneous suppression of the large water peak (for review, see Mullins et al., 2012).

1.4.9.2 Spectral analysis

After initial preprocessing of the acquired spectra in both MATLAB and jMRUI (e.g., to inspect spectral phasing and remove any residual water peak), model fitting is performed by manual selection of spectral peaks including the two main GABA peaks and the inverted *N*-acetyl-aspartate (NAA) peak. This subsequently allows for the

quantification of relative concentrations within a given spectrum and the assessment of changes in concentrations over time. However, this is a subjective analysis technique that depends on specific a priori assumptions (e.g., linewidth of waveforms). Therefore, all spectral preprocessing and analyses were replicated at least three times by the current author for all participants, and several samples from all analysis steps were independently assessed by two experienced spectroscopy researchers.

1.4.9.3 Methodological considerations

This section outlines two main technical considerations of the approach relating in turn to the spatial resolution of the obtained MRS measurements, as well as the reference adopted for quantifying GABA concentrations.

Firstly, as this technique measures the total GABA concentration from the acquired voxel volume, it is not possible using this technique alone to assess underlying local changes in synaptic activity (Stagg et al., 2011). Due to this spatial limitation the specific location of the concentrations measured is unclear, including whether measured chemicals originate from intra- or extra-cellular pools of GABA concentrations. This in turn limits the hypotheses that can be posed using this technique in relation to localising changes in GABA concentrations. Recent work has combined MRS with transcranial magnetic stimulation (TMS) in the human motor cortex to shed light on the relationship between MRS-measured GABA and synaptic GABA activity, and concluded that MRS-detected GABA may particularly reflect extra-synaptic GABA pools at rest (e.g., Stagg et al., 2011). However, it is possible that other GABA pools contribute to measures of dynamic change (Stagg et al., 2011), so the relationship between MRS-assessed GABA and the underlying cellular substrates remains unclear.

Secondly, one of the main questions related to quantifying biochemical concentrations with this technique is the choice of reference peak. The jMRUI analysis steps output absolute quantities of GABA concentrations, but differences across time (due, for instance, to head motion during a longer scan) could result in the false appearance of changes in concentrations, which can be problematic for paradigms investigating changes over longer acquisitions such as during behavioural learning on a task. Therefore it is important to reference concentrations to a more stable peak. NAA and Cr are often used as reference peaks because concentrations are not thought to change with a simple behavioural intervention (change in NAA is for example associated with neurological diseases such as multiple sclerosis) and are thought to remain relatively stable across time. This thesis will adopt NAA as the reference peak for the study in Chapter 4, which aims in part to replicate a previous motor learning study (Floyer-Lea et al., 2006), and Cr was not acquired separately in the original study.

Chapter 2

Chapter 2 | Motor learning & sleep with ageing

Our ability to consolidate what we learn changes with age. Current perspectives suggest a deficit in the processes of motor consolidation in older healthy adults. The first series of behavioural studies in this thesis set out to challenge this commonly held assumption about ageing, and show that this deficit is not specific to off-line processes of consolidation, but rather is related to kinematic constraints during the acquisition phase of fine motor learning. By removing such constraints, the current work reveals significant improvements in performance after sleep in older adults that are also comparable to those seen in younger controls.

2.1 Introduction

The formation of memories in humans is underpinned by highly specialised processes of encoding, consolidation and retention. Initially labile new memory traces undergo post-encoding processing, which aids in stabilising and integrating learned material over time (Brawn et al., 2008; Diekelmann et al., 2009; Rasch & Born, 2013; Stickgold, 2009) and frequently enables further post-learning improvements associated with off-line consolidation (Doyon et al., 2009; Robertson et al., 2004; Trempe & Proteau, 2010). Depending on the type of material being learnt these off-line gains may occur during wakefulness and/or during sleep.

After a single session of learning a novel motor sequence, healthy young adults consistently show off-line improvements in performance, and in the case of explicit sequence learning particularly following a 12-hour period that includes sleep (Robertson et al., 2004; Fischer et al., 2002; Walker et al., 2002; 2003). By contrast, a growing number of studies have found that such off-line improvements are lacking in healthy

older adults (Spencer et al., 2007; Wilson et al 2012; Tucker et al., 2011; Brown et al., 2009; Fogel et al., 2012). To date no studies have demonstrated sleep-dependent consolidation of motor sequence learning in older adults. Multiple factors may contribute to this age-related discrepancy. For example, there is evidence to suggest that a longer period of off-line consolidation coupled with an extended training window after consolidation, may be necessary to facilitate post-learning improvements in older age groups. A recent study by Tucker et al. (2011) comparing consolidation of motor learning in younger and older healthy volunteers found that, after a 24-hour consolidation period including both wake and sleep, younger adults showed improvements in performance while older adults showed only maintenance of performance. With further blocks of practise after the 24-hour consolidation period, the older adults displayed similar increases in performance to those seen in younger adults immediately after 24 hours. Although this finding suggests that performance improvements *can* be achieved in older adults, it also suggests that an extended period of consolidation combined with additional practice on the task may be required, and does not resolve the issue of sleep-dependency in older adults. One aim of the current study is to disentangle the effects of sleep and the passage of time on motor consolidation in older adults.

Another issue that has been overlooked by previous studies of motor consolidation in older adults is the degree to which age-related variation in movement dexterity may contribute to observed differences. There is evidence to suggest significant reductions in fine motor speed and dexterity (Ashendorf et al., 2009; Marmon et al., 2011; Ranganathan et al., 2001), as well as finger strength (Soer et al., 2012) with older age. These reductions are thought to partly reflect changes in cortical inhibition with ageing (see section 1.3.2 for a discussion of the importance of cortical

inhibition for fine motor control). Volumetric changes in gray and white matter structures with age are well-established (Lemaitre et al., 2005; Lemaitre et al., 2012; Raz et al., 1997; Smith, Chebrolu, Wekstein, Schmitt, & Markesbery, 2007), and are known to affect primary motor and somatosensory cortices (Good et al., 2001; Salat et al., 2004), as well as subcortical structures important for the coordination of movement such as the cerebellum (Raz et al., 2005). Age-related effects on brain structure and function may in turn be associated with differences in performance on tasks requiring fine motor skill (for review, see Seidler et al., 2010). For example, a recent diffusion MRI study suggested that slower motor performance in older adults was in part explained by reduced fractional anisotropy (FA) in several intrahemispheric tracts (Voineskos et al., 2012). Moreover, Mattay et al. (2002) found that relative to younger participants, older adults performing a simple motor pressing task displayed more widespread and stronger levels of activation across a network that included the cerebellum, premotor and supplementary motor areas. Specific to sequence learning, age-related decline in sub-cortical structures, such as cerebellum, thalamus and putamen (Good et al., 2001; Raz et al., 2005; Salat et al., 2004), may additionally impact on sequential 'switching' between finger presses or transitions (e.g., characterised by 'chunking' or temporal grouping of elements) (Orban et al., 2011), a key component of sequence learning.

In summary, there is little evidence for consolidation of motor sequence learning in older adults. Furthermore, older adults exhibit poorer fine motor dexterity compared to younger adults, which may stem from age-related volumetric changes in brain morphometry and reduced cortical inhibition supporting fine motor control. Therefore, previously observed deficits in consolidation may not result solely from the off-line consolidation process itself, but may also arise from deficits during 'online' encoding of

the memory trace. In order to more accurately assess consolidation in older age, we aimed to reduce task-dependency on fine motor skill. To this end, we tested off-line consolidation of motor learning in both younger and older adults using either a classic version of the motor sequence task, requiring individual finger movements, or an adapted version of the same task, using whole hand movements.

2.2 Methods

PARTICIPANTS: A total of 91 healthy right-handed participants gave written informed consent to participate in accordance with local ethics committee guidelines, and were pseudo-randomly assigned to groups based on their age (range: 18-35, younger; 50-85, older; 51 females; Table 2.1). Participants had no prior history of neurological, psychiatric, or sleep disorders, drug or alcohol abuse, and were instructed to remain caffeine, alcohol and drug free (apart from prescribed medication not expected to have an influence on sleep quality) for the duration of the study, as well as 12 hours prior to taking part. Participants were also instructed to refrain from napping during the day, confirmed verbally at the relevant post-training retest session. One participant reported having a nap after initial training, and one participant consistently reproduced only the first 4 digits of the number sequence at retest. Consolidation data from these two participants were therefore excluded from further analysis. Nine participants took part in more than one condition (a maximum of two conditions). In these cases, different sequences with completely unique grammars were used for each condition, consistent with previous work comparing sleep effects after performance on two unique sequences in the same participants (Walker et al., 2003). However, additionally, in the current study testing for different conditions took place at least one month apart. It has also previously been shown that sequence learning (unlike declarative tasks) does not offer any savings effect (Keisler & Willingham, 2007), even with the same sequence (i.e., initial implicit training of a sequence did not facilitate any savings/advantage when participants relearnt the same sequence explicitly after a temporal interval).

Table 2.1. Participant details across tasks and age groups. SEM = standard error of the mean.

Age group	Task	Group	n	Mean age (± SEM)
Younger adults	Classic	AM	13	24.50 (± .89)
Younger adults	Classic	PM	10	24.40 (± .82)
Younger adults	Adapted	AM	13	24.31 (± .94)
Younger adults	Adapted	PM	13	25.46 (± .95)
Older adults	Classic	AM	10	67.22 (± 3.19)
Older adults	Classic	PM	11	67.9 (± 2.99)
Older adults	Adapted	AM	10	66.30 (± 2.77)
Older adults	Adapted	PM	11	65.18 (± 3.22)

SEQUENCE LEARNING TASKS: Depending on the group to which participants were assigned they performed either a standard finger sequence task (classic; Figure 2.1a; Walker et al., 2002, 2003) or an adapted whole-hand sequence task (adapted; Figure 2.1b). Tasks were matched on all physical attributes apart from requiring either fine-finger or whole-hand movements to complete the task. For the adapted task, configuration and spacing (radius) of buttons are based on the average forearm length between a standard man and woman. Values are derived from (Plagenhoef, Evans, & Abdelnour, 1983), and were also tested against data from participants in the current study. A 5-digit numeric sequence was presented on the screen during the entire period participants performed the sequence to prevent any working memory component. To avoid providing accuracy feedback, responses elicited only a white dot, which moved from left to right in accordance with the number pressed to indicate the response had been recorded. Participants were instructed to repeat the sequence as fast and as accurately as possible for 30 seconds followed by a 30-second rest period. Each participant performed 12 blocks (sequence + rest) during training (t0) lasting 12 minutes in total. At the retest sessions (t1, t2) participants performed only two consecutive

blocks of 30 seconds each to reduce any influence of additional practice or training between retest sessions.

TESTING PROCEDURE: Training (t0) took place between 8-10:30am (for the AM-group) or 8-10:30pm (for the PM-group). The two retest sessions took place 12 (t1) and 24 (t2) hours following training (Figure 2.2). For morning sessions, testing took place at least one hour after participants woke up. Before the start of each session, participants were given the Stanford Sleep Scale to indicate their level of subjective alertness (Hoddes, Zarcone, Smythe, Phillips, & Dement, 1973; see appendix). Participants wore an activity monitor (digital accelerometer; CamNtech Actiwatch-Light) and were asked to keep an activity log for the duration of the study (24 hours), which were used as a measure of sleep efficiency and indirect sleep quality. Actigraphy and short-term sleep diaries have been shown to be effective for documenting sleep-wake patterns (Rogers, Caruso, & Aldrich, 1993; Sadeh & Acebo, 2002).

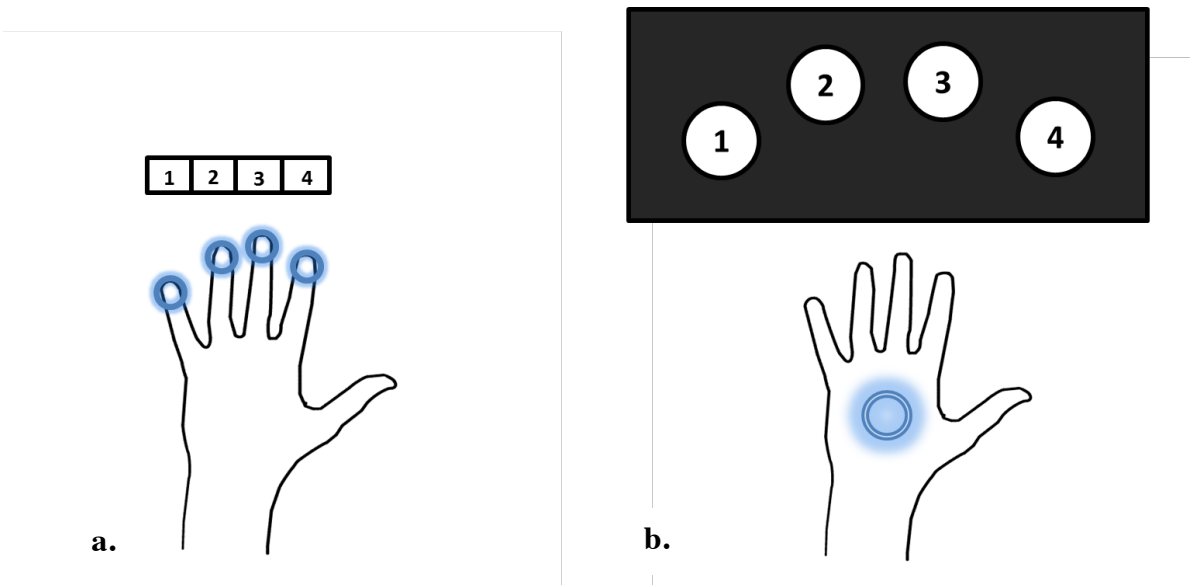


Figure 2.1. Sketch of task design and setup for the classic (a) and adapted (b) versions of the sequence learning task. Participants performed the 5-digit sequence with their non-dominant (left) hand.

Participants also completed the Pittsburgh Sleep Quality Index (PSQI), which is a measure of self-reported sleep quality over the previous month (Buysse et al., 1989).

BEHAVIOURAL MEASURES: Performance rate (number of correct sequences per block) was used as a measure of learning. Number of correct sequences per block is an amalgam performance measure that comprises rate of sequences executed per unit time (block). However, because we are here interested in the number of sequences completed in a given block (where block duration remains constant at 30 seconds), for the purposes of this work, and in accordance with previous studies (e.g., Karni et al., 1995), this measure will be referred to as performance rate. Consolidation scores were calculated as the difference in performance rate from the end of training (average of final two blocks of training at t0) to the first retest session (average of the 2 blocks performed at t1) and from the first retest to the second retest (average of the first 2 blocks performed at t2), consistent with previous work (e.g., Nishida and Walker, 2007; Walker et al., 2003, 2002). For the AM-group, the t0-t1 interval consisted of wakefulness and the t1-t2 interval consisted of sleep; the order of the intervals was reversed for the PM-group (Figure 1c). Actigraphy recordings were used to derive sleep-wake patterns, including indirect measures of sleep quality and quantity, and were scored using manual editing based on the activity logs and an automated scoring algorithm using Sleep Analysis v7.23 software (CamNtech). PSQI questionnaire data were scored manually based on scoring criteria outlined by Buysse and colleagues (1989). A higher global PSQI score is associated with poorer self-reported sleep (range 0-21) with a score greater than 5 suggesting poor sleep, as validated against clinical and laboratory measures (Buysse et al., 1989).

STATISTICAL ANALYSIS: Primary statistical analyses are based on repeated measures analysis of variance (ANOVA) with within-group factors of performance rate

per block for the analyses on in-session training, and sleep vs wake for the analyses on off-line consolidation, and between-subject factors of age group (younger, older), task (classic, adapted) and training time (AM, PM), and were followed up with ANOVAs split by age group and/or task to test for task- and age-specific effects. For ANOVAs split by age group, participant age was also included as a covariate to control for age variation within the group. One-sample *t*-tests were used to compare performance change after consolidation periods (t1-t0, t2-t1) against zero. Independent samples *t*-tests and one-way ANOVAs were used to compare sleep quality measures between age and task groups. Pearson's coefficient was adopted for all correlational analyses, and linear regression was used for predictive regression analyses.

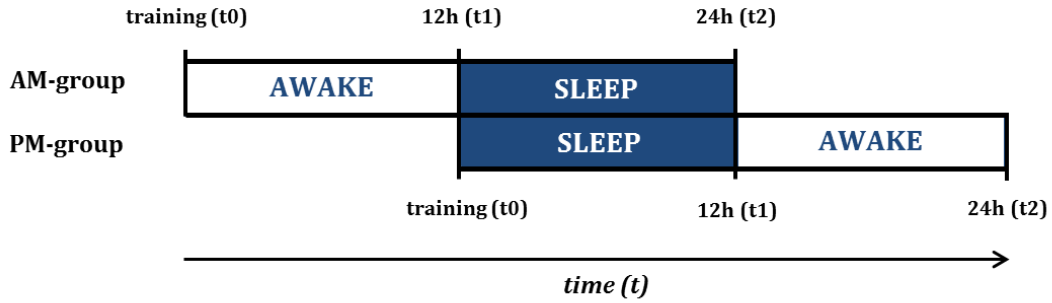


Figure 2.2. Sketch of sleep-wake design. Participants were pseudo-randomly assigned to receive their initial training (t0) either in the morning (AM-group) or in the evening (PM-group), with retests 12 (t1) and 24 (t2) hours after training.

2.3 Results

Within-session behavioural performance

To investigate changes in performance across blocks within the initial training session we ran a repeated measures ANOVA on within-session values for average performance rate per block with between-subject factors of age group (younger, older), training time (AM, PM), and task (classic, adapted), and the within-subject factor of block (blocks 1-12). Results reveal significant main effects of block ($F(7.65,634.60) = 117.61$, $p < .001$), age group ($F(1,83) = 47.62$, $p < .001$) and task ($F(1,83) = 8.66$, $p < .005$). These main effects reflect improving performance over blocks, and better performance on average in the younger group, and with the adapted task. In addition, we found significant interactions of block by age group ($F(7.65,634.60) = 2.24$, $p < .05$) and task by age group ($F(1,83) = 6.79$, $p < .05$), and a significant 3-way interaction of task x age group x time of day ($F(1,83) = 4.55$, $p < .05$).

Given these interactions with age group, we followed up with repeated measures ANOVAs split by age group. Despite the interaction between age group and block in the initial ANOVA, we found trends for significant main effects of block in both younger ($F(7.49,322.03) = 1.87$, $p = .07$) and older participants ($F(5.49,192.08) = 2.00$, $p = .07$), suggesting that performance improved across training in both age groups and across both tasks. However, consistent with the significant interaction between task and age group in the initial ANOVA, we found a significant main effect of task for older ($F(1,35) = 12.87$, $p < .005$), but not for younger participants ($F(1,43) = .22$, $p = .64$). This reflects markedly reduced performance rates on the classic task, compared to the adapted task, in the older group only (Figure 2.3). Older participants also showed a trending interaction between block and task ($F(5.49,192.08) = 2.03$, $p = .07$), whereas the younger groups did not ($F(7.53,338.77) = .51$, $p = .84$), suggesting different learning

profiles for the two tasks in the older group (despite a comparable level of overall improvement in performance across tasks (mean change in # correct sequences from b1 to b12: 7.45 ± 1.16 SEM, adapted; 7.32 ± .98 SEM, classic). On the whole, older age was associated with poorer baseline performances across tasks ($r = -.49$, $p < .001$).

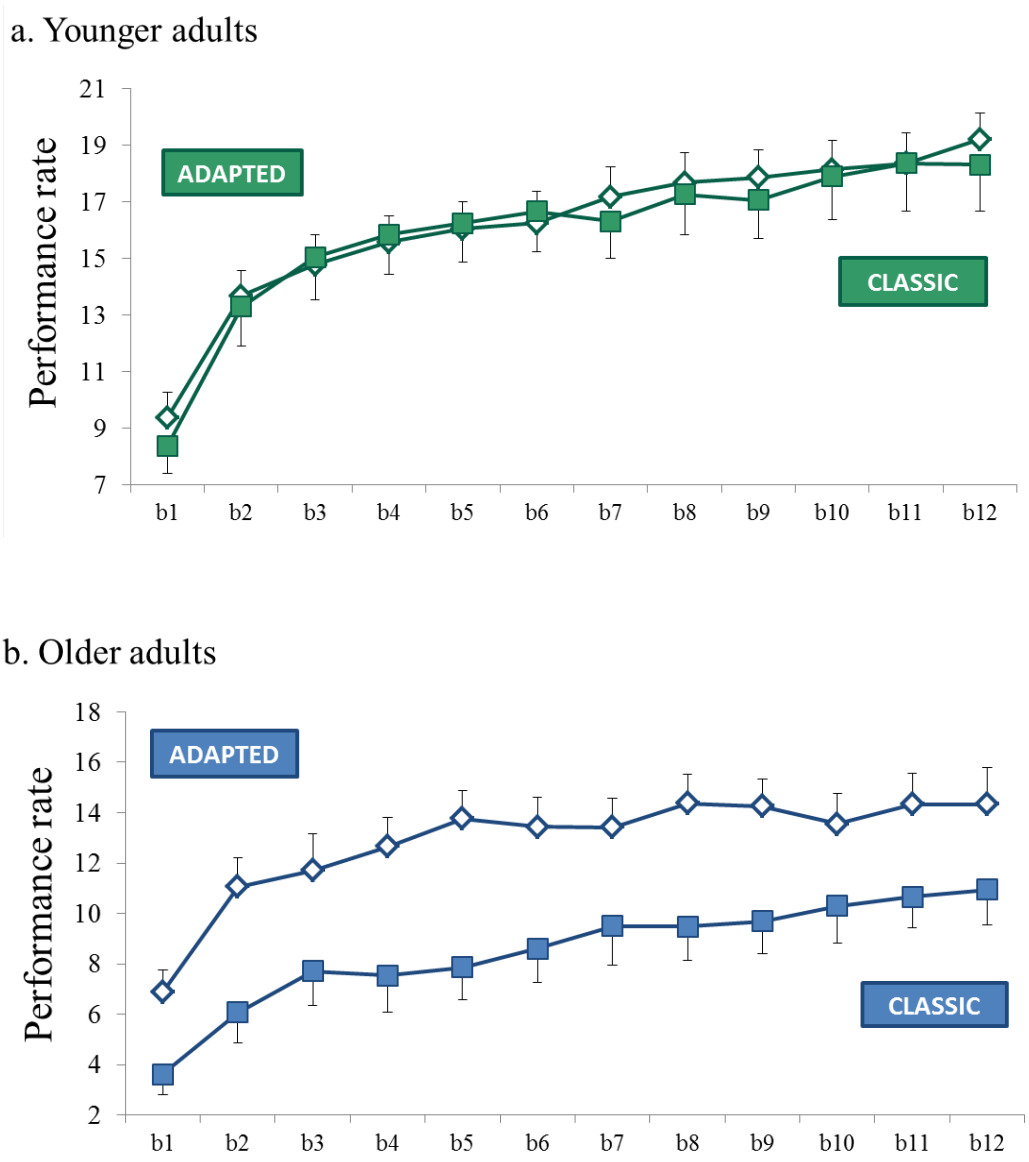


Figure 2.3. Learning curves in younger (a) and older (b) participants. Mean number of correct sequences performed per block ('b') was our measure of performance rate during the training session (classic task: closed squares and adapted task: open diamonds). Performances across tasks were close to identical in the younger groups. Equivalent learning curves in older adults show markedly poorer levels of performance on the classic compared to the adapted task.

To test for the possibility of time of day effects in initial training profiles, we compared performance on the baseline and final blocks of training respectively, taking into account task and age-group, but found no evidence for differences between AM and PM-groups ($F(1,87) = .80$, $p = .37$; mean baseline: 6.87 ± 3.67 SD, AM-group; 7.51 ± 3.92 SD, PM-group; mean final performance: 15.61 ± 5.06 SD, AM-group; 15.87 ± 5.49 SD, PM-group).

Off-line effects across groups

To test whether sleep significantly benefits task performance over and above the simple passage of time, and whether this effect is modulated by age, we ran a repeated measures ANOVA on the change in performance between sessions, including a within-subject factor indicating whether the period included sleep or wakefulness (sleep, wake), as well as between-subject factors of age group (younger, older), task (classic, adapted), and training time (AM, PM). Results show significant main effects of sleep vs wake ($F(1,81) = 6.87$, $p < .05$), age group ($F(1,81) = 16.61$, $p < .001$) and task ($F(1,81) = 6.59$, $p < .05$). These reflected overall greater off-line improvements in performance with sleep, with the classic task, and in the younger group. We also found significant 3- and 4-way interactions (sleep vs wake x age group x training time ($F(1,81) = 4.52$, $p < .05$); sleep vs wake x age group x task x training time ($F(1,81) = 4.86$, $p < .05$)).

To further follow up on these interactions we ran additional ANOVAs split by task and age group.

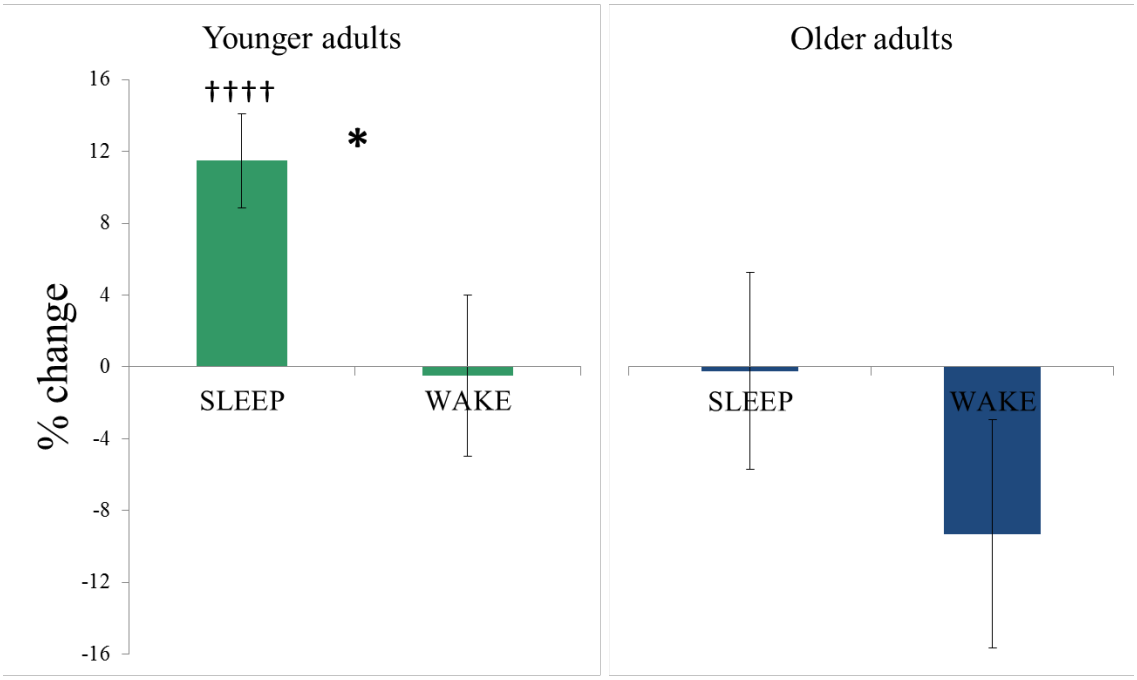
Off-line effects – Classic task

In line with previous research in younger groups we find significant sleep-dependent consolidation in younger adults (sleep vs wake: $F(1,19) = 4.65$, $p < .05$), with significant improvements found during sleep (one-sample t-test: $t(21) = 4.38$, $p < .001$), but not wakefulness ($t(21) = -.11$, $p = .91$) (Figure 2.4a). By contrast, we find no evidence for sleep-dependent consolidation in older adults (sleep vs wake $F(1,15) = 1.83$, $p = .20$) (Figure 2.4b), and find no significant improvements after either sleep ($t(19) = -.04$, $p = .97$) or an equivalent period of wakefulness ($t(19) = -1.47$, $p = .16$). There were no significant training time of day effects for either group (younger, $F(1,19) = .002$, $p = .96$; older, $F(1,15) = .09$, $p = .77$), but we did find a significant interaction between training time of day and sleep vs wake in older adults ($F(1,15) = 8.59$, $p < .05$), that was not present in the younger adults ($F(1,19) = 1.02$, $p = .33$). This reflected greater improvements during sleep compared to wake for those who trained in the morning (AM-group), but the opposite pattern for those trained in the evening (PM-group; Figure 2.5).

Off-line effects – Adapted task

In contrast to the findings for the classic task, we did find evidence for sleep-dependent consolidation in the older adults with the adapted task (Figure 2.5b), reflected in a significant main effect of sleep vs wake ($F(1,18) = 5.87$, $p < .05$), with significant improvements found during sleep ($t(20) = 2.91$, $p < .01$) but not wakefulness ($t(20) = -.36$, $p = .72$), as well as a significant interaction with age ($F(1,18) = 4.64$, $p < .05$) in the older adults. No other main effects or interactions were found.

a. Classic sequence learning



b. Adapted sequence learning

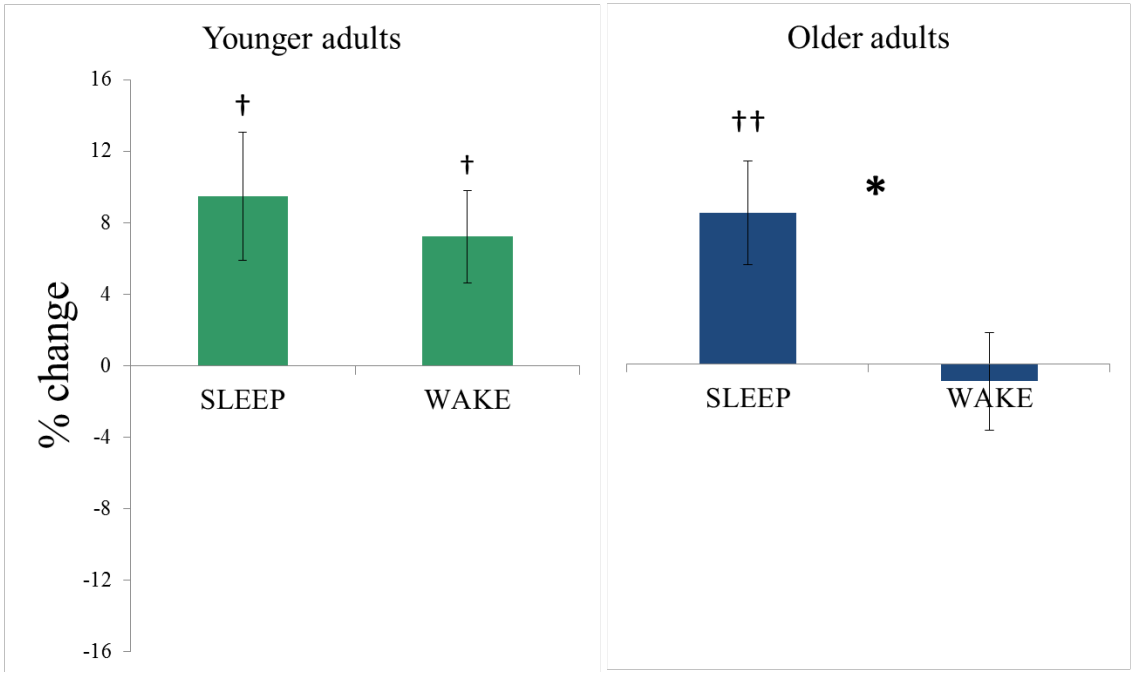


Figure 2.4. Off-line consolidation for classic and adapted tasks. Bar chart (a) showing significant improvements in performance after sleep, but not after wake, for younger but not older adults on the classic task; in contrast, after a night of sleep post-training on the adapted task (b) older adults show significant sleep-dependent improvements (* = $p < .05$, main effect of sleep vs wake; † = $p < .05$, †† = $p < .01$, †††† = $p < .001$, one-sample t-test versus zero).

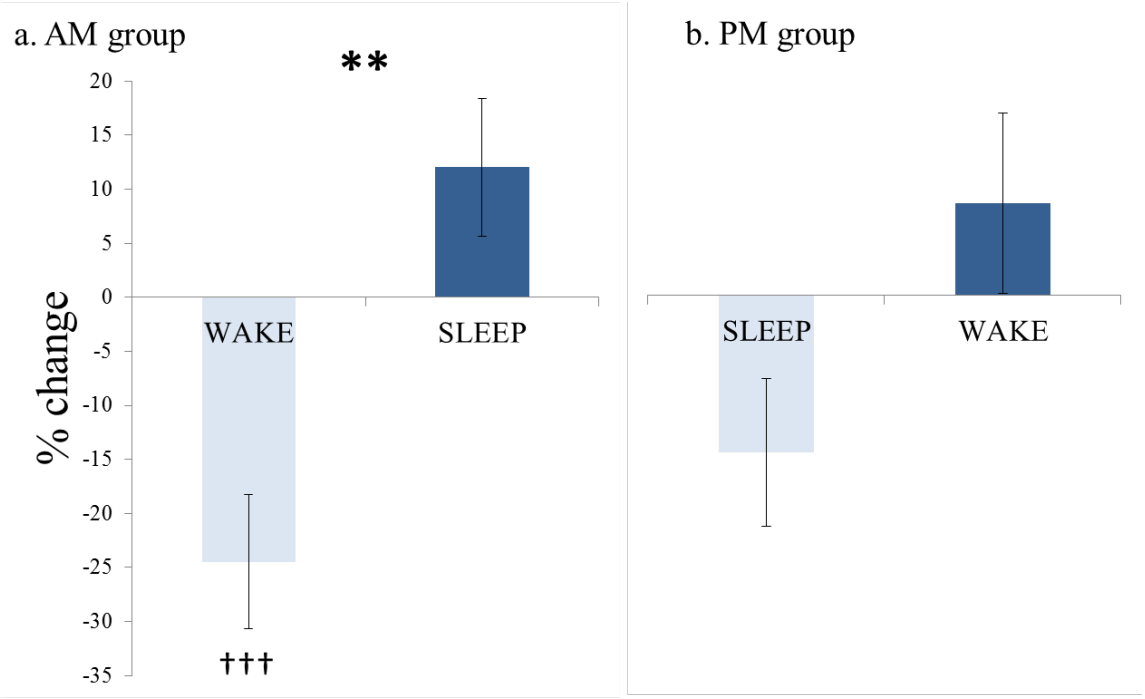


Figure 2.5. Time of day effect for classic sequence learning in older adults. Graph shows that performance worsened after the first period of consolidation (light bars) but improved after the second consolidation period (dark bars) regardless of whether these periods contained sleep or wakefulness. However, the deterioration in performance was significant only if the initial consolidation period contained wake (a; $t(9) = -3.99$, $p < .005$†††), while only trending if it contained sleep (b; $t(9) = -2.13$, $p = .06$; one-sample t-test), suggesting less forgetting with sleep consolidation. Sleep and wakefulness differed significantly only in the AM-group: $t(9) = 3.42$, $p < .01$**; but not PM-group: $t(9) = -1.62$, $p = .14$, paired-samples t-test). Larger SEMs in the PM-group, particularly after wakefulness, suggest a greater degree of variability in performances within this group.

In younger adults, there was no evidence for sleep-dependent effects with the adapted task (sleep vs wake, $F(1,22) = 1.57$, $p = .22$), with significant improvements found during both sleep ($t(25) = 2.65$, $p < .05$) and wakefulness ($t(25) = 2.78$, $p < .05$; Figure 2.5b). No other main effects or interactions were found.

Older adults: Summary

The results described above suggest distinct processes of consolidation for the adapted and classic tasks in older adults. These are summarised graphically in Figure 2.6. Specifically, whereas the adapted task shows sleep-dependent consolidation (Figure

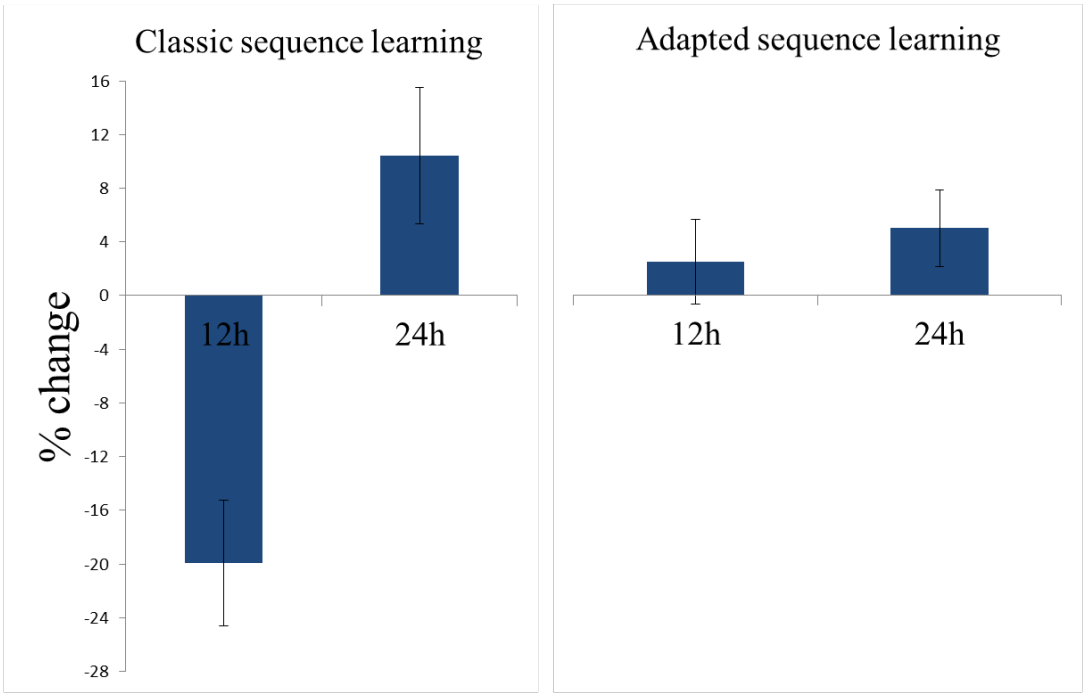
2.6b), the classic task shows greater performance gains with prolonged periods of consolidation (Figure 2.6a), regardless of whether they contain sleep or wakefulness.

Correlations between age, online & off-line gains in performance

Across groups and tasks we find significant negative correlations between improvement in performance rate with overnight sleep and age ($r = -.29$, $p < .01$) such that the older participants were, the less they consolidated during sleep. A similar, though weaker, relationship was found between age and off-line performance improvements during wakefulness, though this failed to reach statistical significance ($r = -.19$; $p = .08$).

We further considered the relationship between in-session learning and off-line improvements experienced post-sleep: Across groups we find significant correlations between change in performance rate after sleep and overall learning in-session ($r = -.41$, $p < .001$) such that the more online learning that takes places during training, the less off-line consolidation is shown to occur with sleep (Figure 2.7). No correlation was found between improvement in-session and following (off-line) wakefulness ($r = .07$, $p = .53$). Direct comparison between these two correlations reveals that the coefficients differ significantly (Fisher's r-to-z, $p < .05$).

a. 12 v 24 hours



b. Sleep v wakefulness

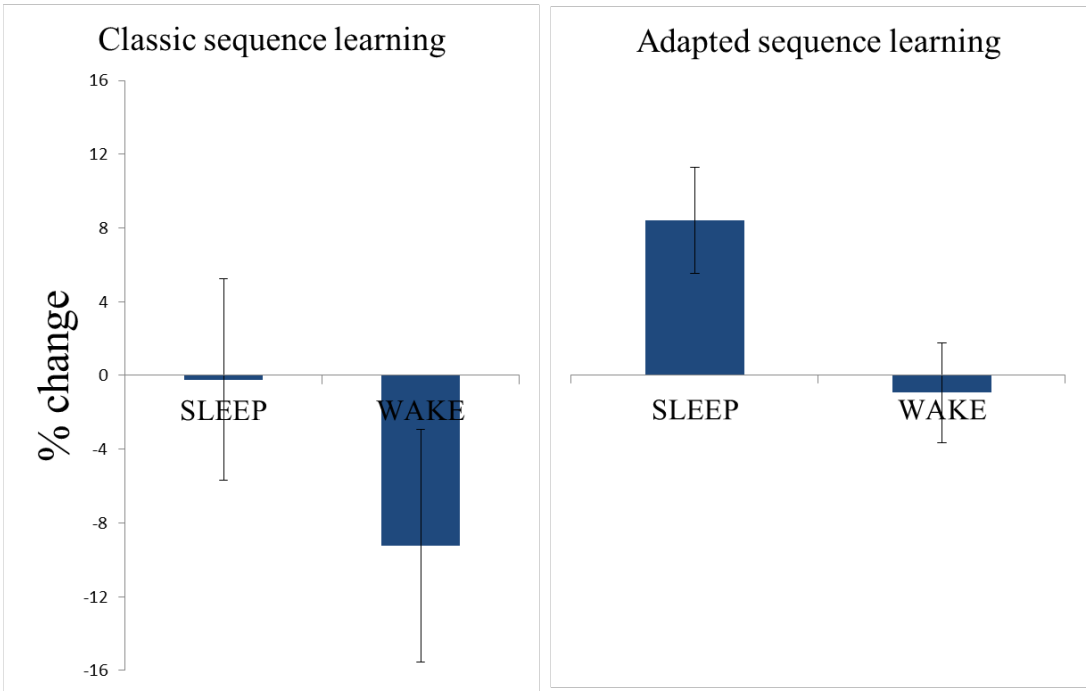


Figure 2.6. Summary of consolidation effects in older adults. Bar chart (a) showing that 24 hours offer markedly greater gains than a shorter 12-hour period on the classic but not on the adapted task (reflected by the interaction between training time of day and sleep vs wake reported above); whereas (b) shows that greater gains are found for sleep compared to wake for the adapted but not the classic task (reflected by the significant effect of sleep compared with wake for the adapted task reported above).

Next, we wanted to see if there was a relationship between poorer starting performances in older adults and off-line consolidation: when controlling for training time, we find a significant correlation between starting performance and sleep consolidation ($r = .38$, $p < .05$) for the older participants, but not for younger controls ($r = .03$, $p = .86$), but comparison between these two correlations only reveals a trend for them to differ (Fisher's r-to-z, $p = .11$). A linear regression model containing starting performance and time of day as predictor variables also significantly predicts sleep consolidation ($R^2 = .23$, $p < .01$) in older adults.

Above we find a significant role for sleep in benefiting learning on the adapted task, whereas an extended period of consolidation was needed in older adults performing the classic task.

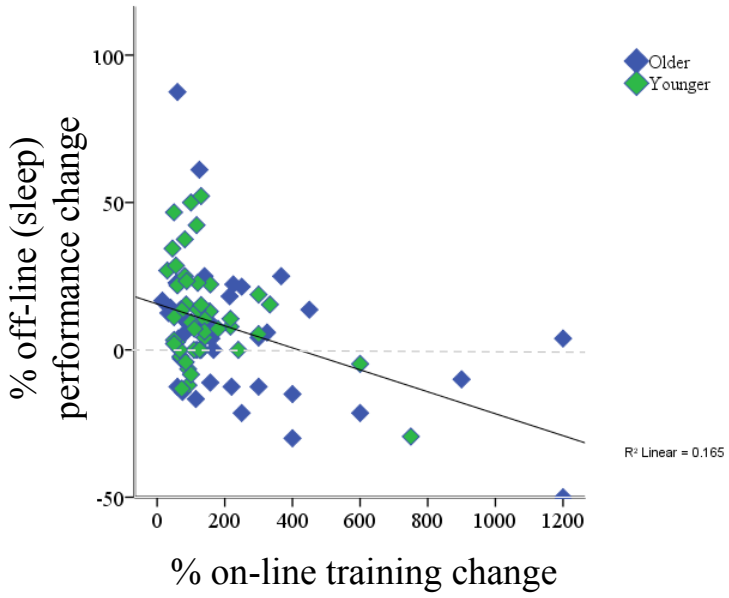


Figure 2.7. Correlation between change in performance in-session and with sleep. Online improvement is relative to baseline performance (block 1), while sleep change is relative to post-training (final two blocks) performance. Dotted line signifies threshold for consolidation (improvement) with sleep below which participants' performances worsened overnight.

However, we wanted to determine if it was possible to predict, regardless of task, the *type* of consolidation period (sleep-specific/extended) older participants would most benefit from. In order to consider this, only participants who slept in the first 12-hour period after training on either of the two tasks (i.e., both PM-groups) were included and divided into categories depending on whether they showed greater improvements in performance after consolidation with sleep (12 hours, t1) or after an extended period of time (24-hours, t2). A second regression model showed that age was the single strongest predictor of the type of consolidation profile in older adults (sleep-specific vs extended; $R^2 = .46$, $F(1,18) = 14.71$, $p = .001$). In other words, age predicted 46% of the variance in consolidation profile in older adults, such that within the older groups, increased age was associated with needing a longer consolidation period, regardless of which task was performed (mean age: 72.67 ± 2.65, *extended consolidation*; 59.30 ± 2.30, *sleep-specific consolidation*).

Sleep quality measures

We also asked if indirect measures of sleep quality (i.e., questionnaire data, actigraphy measurements, activity logs) change with age and correlate with behavioural measures.

PSQI

An independent-samples t-test on the global sleep quality score of the PSQI questionnaire with age group as a factor found a significant effect of age group ($t(78) = 2.27$, $p < .05$), suggesting that self-reported sleep quality is significantly worse in older adults (Figure 2.8).

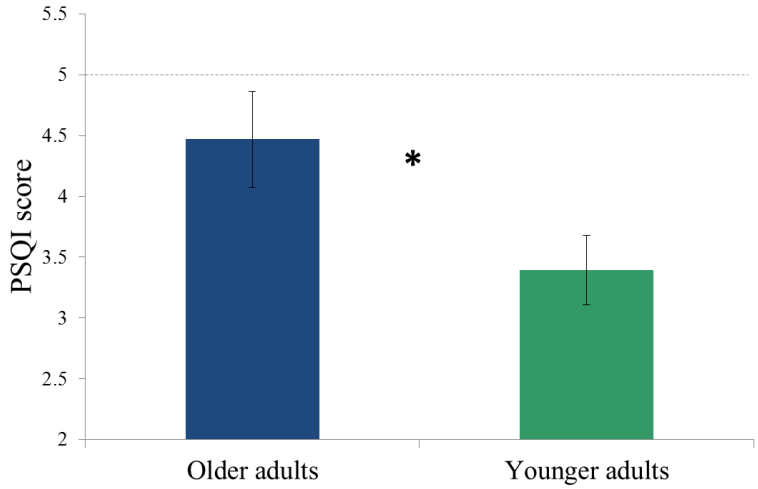


Figure 2.8. Bar graph shows mean score on the Pittsburgh Sleep Quality Index for the two age groups. A higher global score is associated with poorer self-reported sleep (range 0-21, with a score greater than 5 suggesting poor sleep as validated against clinical and laboratory measures, Buysse et al., 1989). While none of the participants here fell under the category of poor sleepers, the older age groups showed increased global scores relative to the younger participants.

Actigraphy

A one-way ANOVA on the actigraphy recordings with age group as a factor also suggested age-related differences in recorded sleep-wake patterns. We found significant differences between age groups in the time they woke up ($F(1,53) = 22.24$, $p < .001$) and got up ($F(1,53) = 18.24$, $p < .001$), and trends with sleep start ($F1,53) = 3.75$, $p = .06$) and actual sleep time ($F(1,53) = 2.23$, $p = .14$), suggesting that older adults on average woke up and got up earlier, and generally slept marginally less than the younger participant group (Table 2.2).

Correlations between sleep measures and behaviour

No correlations were found between PQSI or actigraphy measures and behavioural measures of consolidation during sleep, either when pooling across participants/tasks or when considering each age group and/or task separately.

Table 2.2. Actigraphy measures for younger and older adults. Shows the values (mean ± SEM) for specific actigraphy measures for both age groups. Values are reported in hours/percentages as indicated.

Actigraphy (h:min)	Younger (μ ± SEM)	Older (μ ± SEM)
Bed time	23:44 ± 00:09	23:23 ± 00:10
Sleep start	00:01 ± 00:09	23:35 ± 00:10
Sleep end	07:30 ± 00:07	06:33 ± 00:09
Get up time	07:36 ± 00:06	06:44 ± 00:08
Actual sleep time	06:24 ± 00:09	06:11 ± 00:11
Sleep latency	00:17 ± 00:04	00:11 ± 00:03
Sleep efficiency (%)	81.40 ± 1.03	84.23 ± 1.69

2.4 Discussion

These findings provide, to our knowledge, the first evidence of sleep-dependent consolidation of motor learning in older adults. We find a clear dissociation of consolidation effects between two versions of a motor sequence task, and suggest that previously reported age-related deficits in consolidation are potentially rooted primarily in age-related discrepancies in performance during the acquisition phase of fine motor learning. Our findings reveal strong sleep-dependent consolidation effects in older adults when kinematic constraints related to fine finger movement are removed. Specifically, we show that while older adults performing the classic sequence learning task (requiring fine motor control of individual fingers) do not show sleep-dependent consolidation effects, older adults performing the kinematically adapted task (using whole hand rather than individual finger movements) show sleep-dependent consolidation.

The finding of sleep-dependent consolidation of motor learning in older adults contrasts with previous studies reporting absent or reduced consolidation during sleep in older adults (Fogel et al., 2013; Spencer et al., 2007; Tucker et al., 2011; Wilson et al., 2012). However, there is growing evidence to suggest that fine motor ability significantly diminishes with age (Ashendorf et al., 2009; Marmon et al., 2011; Ranganathan et al., 2001; Remko et al., 2010), a skill upon which most previous studies of motor learning rely. Such decline may arise in part as a result of age-related changes in brain morphometry (Lemaitre et al., 2005; Lemaitre et al., 2012; Raz et al., 1997; Smith et al., 2007; Good et al., 2001; Salat et al., 2004) within regions that have also been shown to play a key role in fine motor function and, particularly, in sequence learning (Mattay et al., 2002; Orban et al., 2011; Voineskos et al., 2012). We propose that such morphological changes may consequently relate to how well older adults are

able to execute tasks that place a high demand on fine movement dexterity. We find that, by removing this kinematic constraint, distinct task-dependent learning profiles emerge within the older participant groups, with markedly improved performances on the adapted sequence task relative to the classic task. Strikingly, these observed differences in learning profiles strongly predicted variances in performance-improvement following consolidation with sleep.

Although, overall, older adults seemed to improve equally across the training sessions on both tasks, older age was significantly associated with poorer performance at baseline. Also, while better starting performances subsequently predicted a greater sleep gain specifically in older adults, *less* improvement within the initial training session was associated with greater benefits following sleep, regardless of age.

The finding that distinct learning profiles during motor acquisition are relevant for consolidation is also consistent with previous literature in younger age groups. For instance, studies have suggested a role for both depth of encoding and task complexity on post-learning consolidation (Kuriyama, Stickgold, & Walker, 2004). One study found that, relative to a simpler 5-element bimanual task, performance on a 9-element version of the SRTT showed greater off-line (sleep) gains in healthy younger adults (Kuriyama et al., 2004). Importantly, greater task complexity was associated with poorer performance during training as evidenced by a lower performance score at the end of training for the more difficult task. These results are in line with the current findings, as well as the hypothesis that a weaker memory trace benefits more from sleep than more deeply encoded learning (Diekelman et al., 2009). However, at least in the case of older adults, too weak a memory trace during training may also hinder sleep-dependent consolidation.

While poor performance during initial training may inhibit performance gains related specifically to sleep, improvements with consolidation in general may not necessarily be entirely obstructed. We provide compelling evidence to suggest that performance improvements, on tasks requiring fine motor skill, may still be elicited in older adults when given an extended consolidation period of 24 hours, particularly (significantly) if sleep was included in the latter half. This is partially consistent with research showing that older adults were able to maintain their performance after a 24-hour period of consolidation (Tucker et al., 2011).

Recent studies have begun to look more closely at distinct attributes of motor learning to dissociate between off-line processes that preferentially consolidate particular features of task performance. For instance, Cohen et al. (2005) found that while the goal-based, or spatial, component of motor sequence training was associated with sleep-specific gains, the movement component of the memory trace was associated with improvements only following wakefulness. A recent study (Albouy et al., 2013) similarly found that only the spatial representation of motor sequence learning was enhanced following (daytime) sleep, while the motoric component was not. Differential processing of specific components has also been found to apply to underlying neural mechanisms of encoding (Hikosaka et al., 1999; Verwey & Wright, 2004). Specifically, distinct learning components within the same task were shown to rely on different neural circuits including the dorsolateral prefrontal cortex (spatial component), and M1 and striatal areas (movement component; Grafton et al., 1998; Hikosaka, Nakamura, Sakai, & Nakahara, 2002). This is consistent with navigational maze-studies in rodents that showed differential recruitment of neural networks depending on the learning (spatial/response) component of the task (Packard & McGaugh, 1996).

These findings are particularly relevant given that the majority of (explicit) motor sequence tasks include both spatial components and movement component, which renders them susceptible to several distinct consolidation mechanisms post-training. The current data support a role for complementary and inclusive consolidation processes. While we find a statistically significant role for sleep in enhancing learning on the classic sequence task in younger adults, it is apparent that a large proportion of participants in both age groups still consolidate, or go on to consolidate further, after a period of wakefulness. We include these values in the model below (Figure 2.9) as they highlight the subtle, and often complementary, nuances of consolidation across sleep and wakefulness. Meanwhile, this effect has also been recorded by a number of earlier studies that showed modest (non-significant) improvements after a consolidation period of wakefulness in healthy younger adults (e.g., Walker et al., 2002; 2003; Nishida & Walker, 2007). Given the 'mixed' nature of explicit sequence tasks, it is therefore plausible that distinct components may require differential consolidation mechanisms (Cohen et al., 2005; Albouy et al., 2013).

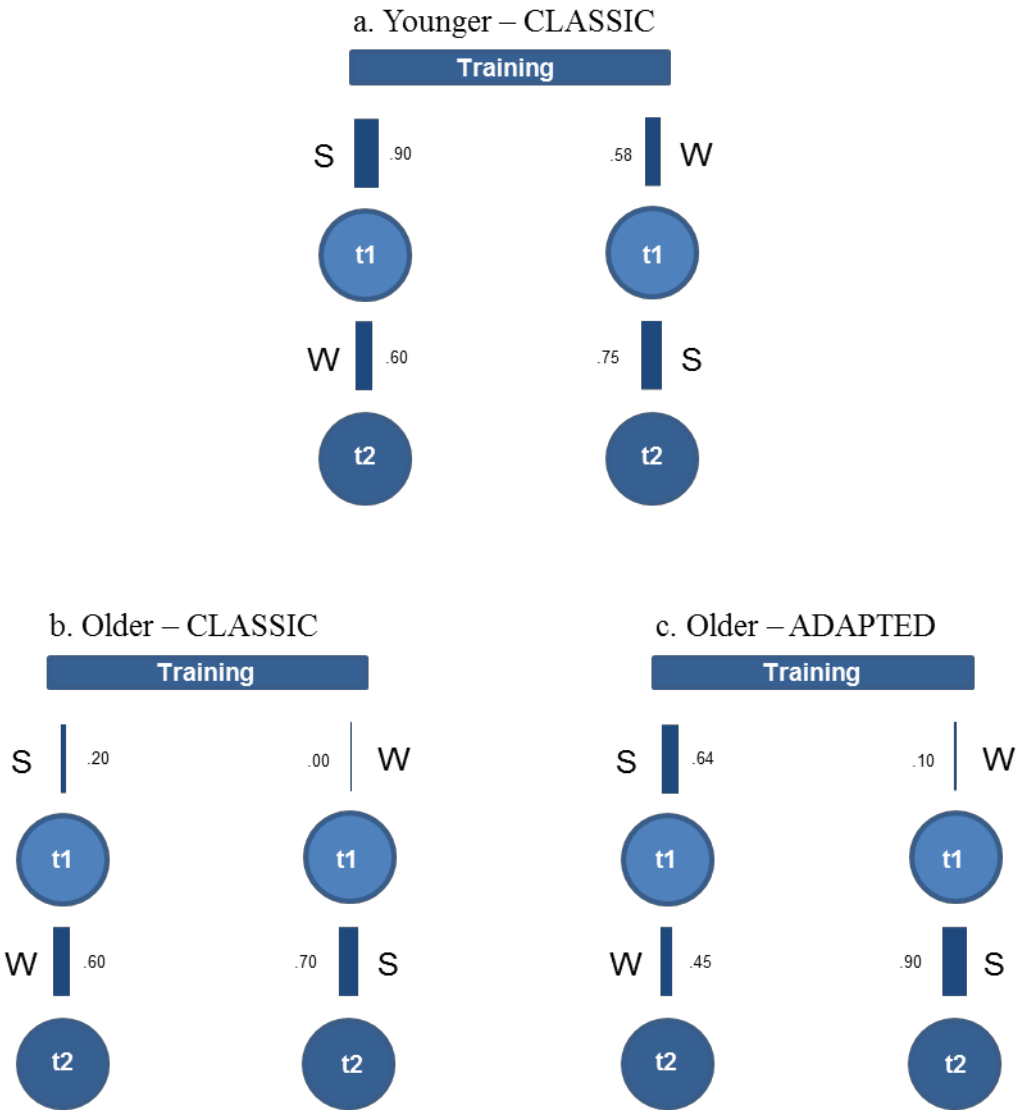


Figure 2.9. Probability weightings based on task-related improvement in younger and older adults. Decimal value weightings represent proportion of participants with performance gains at retest 1 (t1) or retest 2 (t2) after 12 and 24 hours, respectively, of a given consolidation process (sleep, S or wake, W). The younger group performing the classic task (a) show the expected pattern of sleep-dependent consolidation, with greater gains seen for consolidation periods that include sleep. The older participants performing the classic task (b) do not show the sleep-dependent patterns seen in the younger group but instead show reliance on 24-hour processes for consolidation gains. The older group performing the adapted task (c) show a pattern more in line with the weighting seen in younger controls, favouring sleep-dependent consolidation rather than a reliance on a 24-hour consolidation period. Note that proportions do not denote size of the consolidation effect, and values are not mutually exclusive (i.e., participants may have consolidated across both sleep and wakefulness, one or the other, or neither).

2.4.1 Limitations

As with previous sleep studies, it is possible that the changes observed in performance across sessions were due to circadian fluctuations associated with time of day that affected performance levels, rather than a genuine effect of consolidation. However, across groups there was no significant difference in performance between participants who trained in the morning (AM-group) or those trained in the evening (PM-group).

Fatigue at the end of training is another criticism of previous sleep studies, i.e., that the consistent sleep-dependent gain in performance seen in younger adults may result from an underestimation of actual performance levels at the end of training masked by fatigue or reactive inhibition (Brawn, Fenn, Nusbaum, & Margoliash, 2010). According to this hypothesis, if the post-training measurement is delayed for a short period (e.g., five minutes), performance measures are improved and no subsequent consolidation-specific gains are observed (Brawn et al., 2010). However, Robertson et al. (2004) found that a 15-minute interval between sessions did not produce any improvement in skill (and in fact performance levels decreased following the break). Moreover, Brawn et al.'s (2010) variant of the sequence task included both the addition of warm-up blocks and a greater number of practice blocks than previously adopted with this task, which introduces a practice-related confound, and makes it difficult to compare their results against existing findings. We did not include any interim rest period before our post-training measurement of performance, although the intermittent 30-s rest periods throughout training should in part prevent excessive fatigue.

A potential further consideration concerns the indirect measures of sleep used in this chapter, namely derived from the actigraphy recordings. Specifically, the younger participants in this study exhibit relatively lower sleep efficiency (81%) than the older

adults (84%); although these are comparable to previously reported values in healthy adults without sleep disturbances (approx. 79%; Bagai et al., 2013). However, the difference between age groups observed here could be related to the way the measure of sleep efficiency is calculated. Although the overall concordance rates between actigraphy and the gold-standard sleep recording technique (polysomnography) are reported to be over 85% in different healthy age groups (Acebo & LeBourgeois, 2006), it has been suggested that the capacity for actigraphy to detect wake states may be relatively low (Paquet, Kawinska, & Carrier, 2007), averaging at below chance level (de Souza et al., 2003). This likely contributes to an inaccurate estimation of sleep, which may particularly be affected when participants are awake but lie immobile in bed. It should also be noted that recordings in this chapter only took place for the 24 hours participants were involved in the study, and was included only as a relatively crude measure of the sleep-wake cycle to augment the subjective self-reported measures of sleep.

2.4.2 Conclusion

It has been suggested that, while the ability of consolidation to improve motor performance after training is lacking in older adults, the training or skill acquisition itself remains largely intact (Brown et al, 2009; King, Fogel, Albouy, & Doyon, 2013). We provide evidence that, while intact, there are significant decreases in skill acquisition in older adults on the classic sequence task.

In summary, we provide for the first time evidence to suggest the presence of sleep effects in older adults. Adopting a kinematically adapted version of a classic sequence learning task, we show that, by removing the demand on fine motor skill

placed by most traditional sequence tasks, sleep-specific consolidation can be revealed also in older adults. Moreover, sleep gains are significantly associated with learning profiles during training in both younger and older adults.

We further show that performance on the classic sequence task can improve across time in older adults, although this requires a longer consolidation period that includes both sleep and wakefulness. We hypothesise that age-related changes in brain morphometry, which underpin key processes for fine motor function, may contribute to deficits during the encoding stage of learning and subsequent consolidation. These findings have implications for future investigations of consolidation in ageing. However further studies are needed to tease out specific mechanisms that affect sleep-dependent consolidation in the ageing brain.

Chapter 3

Chapter 3 | Sleep architecture & function in ageing

The second experimental chapter of this thesis will build on the design and work described in Chapter 2. Specifically, it is my aim to investigate the neural correlates of sleep-dependent consolidation with the novel hand-tapping task I devised for this project. Importantly, with the finding that older adults are able to consolidate overnight on this adapted sequence learning task, the experiments in this chapter aim to address electrophysiological activity underlying sleep during consolidation in ageing. The following paragraphs detail the outcome of data collected from healthy younger (control) participants, as well as from healthy older volunteers.

3.1 Introduction

As outlined in section 1.3 on ageing, converging studies have shown several consistent changes in sleep architecture that emerge with older age. These changes are typically probed with EEG analysis techniques that may be broadly categorised into biophysical/qualitative methods of visually differentiating sleep stages, and computerised signal analysis/quantitative approaches including spectral analysis (Achermann, 2009). Studies adopting the biophysical approaches have reliably observed changes to both light and deep sleep with ageing (see Figure 1.7 in section 1.3.2). For instance, increases in both stage 1 and stage 2 sleep have been consistently related to older age, as well as decreases in slow wave sleep (for meta-analysis, see for instance Ohayon et al., 2004), with completely missing stages 3 and 4 NREM sleep reported in some instances (e.g., Sarajishvili et al., 1974). The more quantitative techniques (encompassing spectral and period-amplitude approaches) show consistent age-related changes in the large-amplitude, low-frequency delta activity (< 4Hz) that is

characteristic of slow wave activity (SWA) prevalent in stages 3 and 4 slow wave sleep (Colrain et al., 2010; Colrain, Webster, & Hirst, 1999). Looking at particular components within the delta frequency range, research suggests that the N550 component, a marker of k-complex delta activity, changes significantly with ageing, with 50 percent of amplitude variance explained by age (Colrain et al., 2010). Such age-related reductions in k-complex amplitude are also thought to reflect a more general deficit in the generation of slow wave delta activity and underlying cortical neural degeneration in older adults (K. Crowley, 2011). Specifically, older age has been reliably associated with a reduction in the amplitude of delta activity, more so than a global decline in the number of slow waves (Colrain et al., 2010; for review, see Crowley, 2011) – although age-related decreases in delta wave incidence have also been noted previously (e.g., Feinberg & Campbell, 2003). Meanwhile, the latter study also reported a greater impact of age on delta amplitude compared to incidence, suggesting a particular sensitivity of delta band signal amplitude to the effects of ageing. Studies have also shown that the power density of delta activity, a measure of energy of the power spectrum usually normalised to the bandwidth or time, is likewise significantly decreased with older age (Ehlers & Kupfer, 1997; Feinberg & Campbell, 2003). Early studies posited, however, that observed age differences in delta activity are predominant early in the lifespan (Feinberg, 1974), and that little further decrease is observed from middle age onwards (Ehlers & Kupfer, 1989). However, more recent evidence supports a more continuous (linear) decrease in delta activity across the lifespan, which has been replicated by different research groups (e.g., Bonnet & Arand, 2007; Colrain et al., 2010).

Collectively, these findings suggest a global change in the quantitative and morphological attributes of delta activity as we age. However, no studies to date have

examined these age-related changes in sleep architecture relative to post-sleep enhancements in motor performance, and the importance of diminishing delta activity in motor consolidation for older age groups remains largely unknown.

In addition to replicating the sleep-dependent consolidation seen in older adults in Chapter 2, the main aim of the second experimental chapter is to elucidate the effect of ageing on sleep architecture and function, looking specifically at the implications for motor memory consolidation. It may be relevant at this point to briefly reiterate the terminology used in this chapter, as definitions can vary confusingly across studies and investigations of human and animal sleep. As this study uses surface EEG solely in humans, the term slow wave sleep (SWS) will be used only to describe slow waves in stages 3 and 4 NREM sleep, whereas the term SWA encompasses slow waves indiscriminate of stages (see section 1.2.1, for further details of the terminology adopted in this thesis).

Summarily, the present chapter aims to i. replicate sleep-dependent motor consolidation in older adults, ii. clarify the effect of ageing on sleep architecture and other sleep parameters (e.g., actigraphy, self-reports), iii. determine the stage(s) of sleep involved in motor sequence consolidation, and iv. quantitatively assess specific parameters of slow wave sleep (e.g., delta activity) relative to ageing, consolidation, and inter-hemispheric differences.

3.2 Methods

PARTICIPANTS: A total of 22 healthy right-handed participants (n = 12 younger, 7 female, 25.25 ± .35 years; n = 10 older, 5 female, 62.20 ± 3.17 years) gave written informed consent to participate in accordance with local ethics committee guidelines. The inclusion of the two (younger and older) age groups is supported by previous work (see for example Ohayon et al., 2004), which showed an increased effect size for studies that compared younger versus older adults (compared with other groupings). Participants had no prior history of neurological, psychiatric, or sleep disorders, drug or alcohol abuse, and were instructed to remain caffeine, alcohol and drug-free (apart from prescribed medication previously determined not to have an influence on sleep quality) for the duration of the electroencephalographic recording of the study, as well as 12 hours prior to the start of these sessions. Participants were also instructed to refrain from napping during the daytime after their behavioural training session, which was confirmed verbally at the post-training retest (t1) session.

SEQUENCE LEARNING TASK: Participants in this experiment performed the adapted sequence learning task described in Chapter 2 (see section 2.2 for task design and details of the behavioural measures used).

STUDY PROCEDURE: Participants were enrolled in a three-week study period comprising three main parts (Figure 3.1). This period involved i. wearing an activity monitor and keeping a sleep diary, ii. a brief brain imaging scan (although brain imaging data are not included in this thesis), and iii. three nights of EEG recording during sleep, including the nights before and after learning the novel motor task. As sleep-dependent consolidation has already been demonstrated for the task used here (see Chapter 2), and our aim in this chapter was simply to test for correlations between

consolidation and sleep measures, we did not attempt to control for time of day effects in this experiment and trained all participants at the same time of day. As for the AM-group in Chapter 2, training (t0) took place between 8-10:30am. The two retest sessions took place 12 (t1) and 24 (t2) hours following training (see Figure 2.2, AM-Group, in previous methods section for behavioural design). Testing for morning sessions took place at least one hour after participants woke up. To assess levels of subjective alertness, the Stanford Sleep Scale was administered at the beginning of each behavioural session (Hoddes et al., 1973).

EEG DATA ACQUISITION & ANALYSIS: EEG, EOG and submental EMG were recorded by portable biomedical waveform recorders (24Mbytes, Actiwave, CamNtech). Sixteen electrodes were placed bilaterally on the scalp, above/below the eyes, and around the chin according to a restricted version of the 10-20 system (Jasper, 1999; see section 1.4.2 for in depth details and rationale for electrode montage) and were referenced to the mastoid (right side-A1, left side-A2): F3, F4, C3, C4, P3, P4, O3, O4, EOG1, EOG2. The two EMG channels were referenced to the chin. Sample rates were 128Hz to allow for sufficient recording duration (a higher sampling rate for the portable EEG would have reduced recording time to approximately 6 hours, which would be insufficient for longer sleepers). Sleep stages were subsequently visually scored for 30-second epochs according to the scoring criteria of Rechtschaffen and Kales (1968; see Figure 3.2. for examples of the various sleep stages).

SPECTRAL DENSITY & DELTA FINDING PROCEDURE: The incidence of delta waves in a given epoch and their corresponding power density values ($\mu V^2/Hz$) were determined using a semi-automated MATLAB procedure, the output of which was visually inspected to ensure accuracy. The EEG signal was first low-pass filtered to reduce levels of high-frequency noise in the data, and then the power spectral density

was calculated using a short-time fourier transform (STFT) with a Hamming window size of 64 (to maximise temporal resolution whilst minimising frequency uncertainty). The analysis calculated the power spectra for frequencies 0 to 4Hz, in 0.5Hz bins, with a resulting temporal resolution of 0.25sec. A delta-finding filter routine was then applied in which only data with 0.5sec or longer duration *and* with a peak-to-peak amplitude of 75μV or higher were determined to be genuine delta waves in accordance with the Rechtschaffen and Kales (1968) scoring rules. The number of delta waves and their power density were recorded for each sleep stage separately (stages 1-4) and also for both the electrode location over the motor cortex in the hemisphere contralateral to the hand that carried out the motor learning task (C4) and the corresponding location in the opposite hemisphere (C3) to allow for subsequent interhemispheric comparisons.

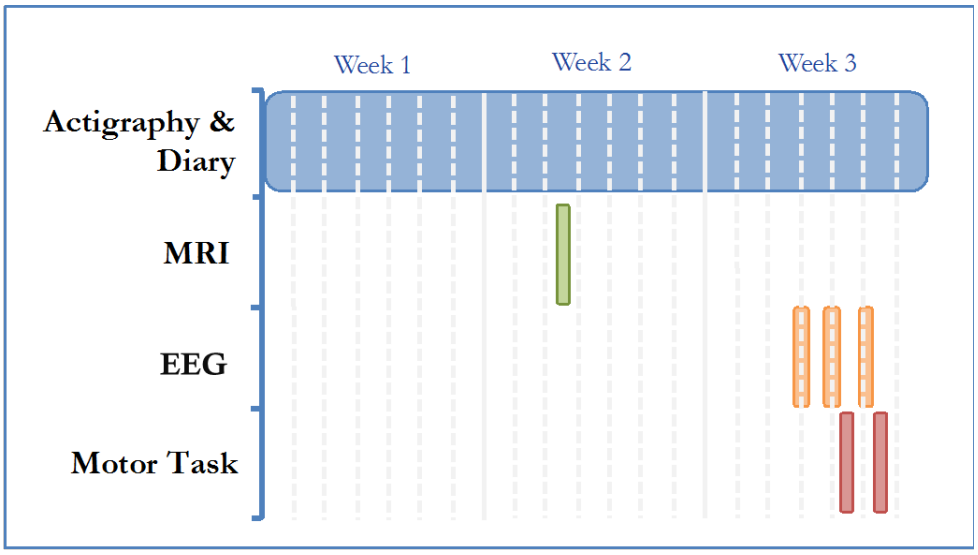


Figure 3.1. Sketch of participation involvement across a three-week period. Participants were enrolled for three weeks during which different measurements were recorded (including EEG, actigraphy and MRI). Participants also came in for an introductory meeting to discuss the study further, take consent and begin activity monitoring.

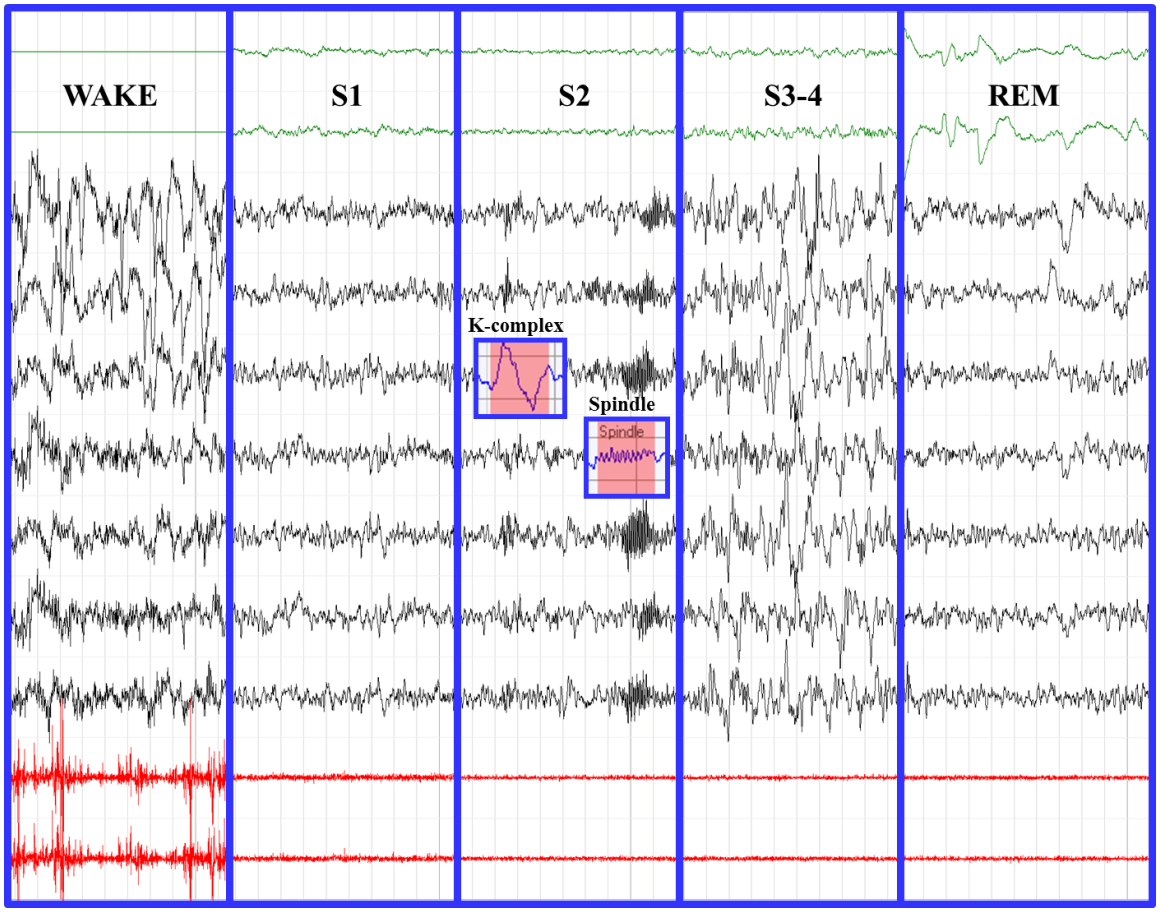


Figure 3.2. Representative waveform activity during healthy sleep. The different REM and NREM (S1-4) sleep stages are represented in addition to wakefulness. Similar to wakefulness (and REM), stage 1 is defined by mixed frequency, low amplitude oscillations, but with alpha activity taking up less than 50 percent of the epoch. REM sleep is distinguished by its rapid-eye movements in the EOG channels (green lines), as well as the presence of saw-tooth waves and frequently ample alpha band activity. Stage 2 is characterised particularly by the presence of spindle activity in the sigma band, as well as k-complexes (magnified in the EEG trace). Stage 3 contains between 20-50 percent of delta waves, whereas stage 4 is dominated (> 50 percent) by delta oscillations.

ACTIGRAPHY & QUESTIONNAIRE MEASURES: Participants wore an activity monitor (digital accelerometer; CamNtech Actiwatch-Light) on their left wrist and were asked to keep an activity log for the full three week duration of the study. While nocturnal recordings with EEG are appropriate for shorter periods (e.g., two or three nights), participants usually do not tolerate longer-term overnight EEG measurements due to the demanding and intrusive aspects of this type of measurement, including setup

time for manually placing the electrodes on the scalp, which typically averages 1.5 to 2 hours per session. By contrast actigraphy is well tolerated over longer timescales, and three weeks of actigraphic recording has been shown to be a good measure to characterise sleep and circadian patterns over longer recording periods. Participants also completed the Pittsburgh Sleep Quality Index (PSQI), which is a measure of self-reported sleep quality over the previous month (Buysse et al., 1989).

STATISTICAL ANALYSIS: Primary statistical analyses are based on repeated measures analysis of variance (ANOVA) with a between-subject factor of age group (younger, older). For ANOVAs split by age group, participant age was also included as a covariate of no interest to co-vary out age-related variability within age groups. One-sample *t*-tests were used to compare performance change after consolidation periods (t1-t0, t2-t1) against zero. Independent samples *t*-tests and one-way ANOVAs were used to compare sleep quality and sleepiness measures between age groups. Pearson's coefficient was adopted for all correlational analyses.

Inter-rater reliability was assessed between the current author and two expert scorers (one internal scorer within the research department and one external scorer from a different University). Inter-rater reliability was based on epoch-by-epoch comparison and generated a Kappa range of κ = 0.75-0.77 (82-84 percent) for a subset of younger and older participants (previously recorded levels of agreement are at approximately κ = 0.74/81 percent for Rechtschaffen & Kales scoring; see Danker-Hopfe et al., 2004 for comparison).

3.3 Results

Sleep architecture across ageing

One of the aims of this chapter was to consider age-related changes in sleep architecture across the two age groups. Table 3.1 shows the key sleep measures and summarises the ways in which sleep differed between the two groups (for representative hypnograms, see Figure 3.3). Interestingly, overall sleep time was relatively comparable between age groups, with the older group sleeping slightly longer on average. However, in line with previous research (Ohayon et al., 2004), we found that younger and older adults differed in the proportion of SWS, highlighted in this dataset by a reduction in stage 3 NREM sleep, as well as a complete absence of stage 4 sleep.

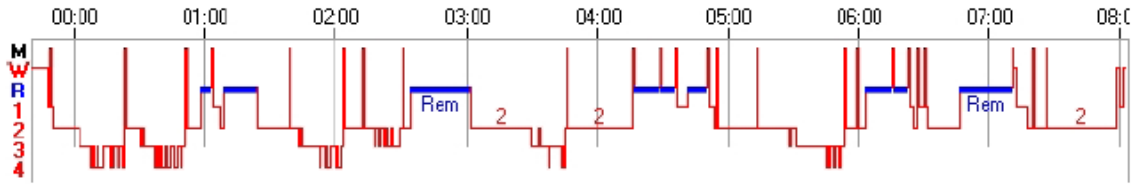
Within-session behavioural performance

To replicate the behavioural findings from Chapter 2, we ran a repeated measures ANOVA with a between-subject factor of age group (younger, older) and a within-subject factor of block (blocks 1-12). In line with the Chapter 2 findings (section 2.3), results again reveal significant main effects of block ($F(11,220) = 23.88$, $p < .001$) and age group ($F(1,20) = 8.40$, $p < .01$; Figure 3.4) for the adapted hand-tapping task. These main effects reflect improving performance across blocks, and better performance on average in the younger group. However, unlike in Chapter 2, we did not find a significant interaction between block and age group ($F(11,220) = 1.00$, $p = .44$), suggesting that, in this smaller sample, change in performance with learning did not vary significantly between age groups.

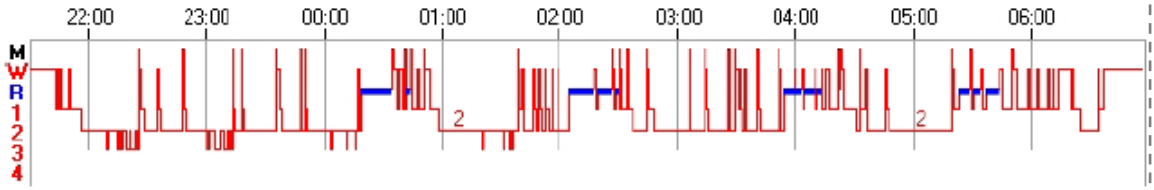
Table 3.1. Sleep measures across age groups. All sleep parameters are included below. Note that older adults did not show any stage 4 NREM sleep. μ = mean; SEM = standard error of the mean. Significant *p*-values: * < .05, independent-samples t-test; ††† < .001, one-sample t-test versus zero.

	Younger (μ ± SEM)	Older (μ ± SEM)	*p*-value
Age	25.25 ± .35	62.20 ± 3.17	
n (f)	12 (7)	10 (5)	
EEG measures			
Total sleep time (h:min)	06:43 ± 00:13	07:10 ± 00:15	.19
Stage 1 (%)	8.30 ± 1.38	12.11 ± 1.69	.09
Stage 2 (%)	52.65 ± 1.89	59.46 ± 2.08	.03*
Stage 3 (%)	8.59 ± 1.11	4.34 ± 1.60	.04*
Stage 4 (%)	8.25 ± 1.53	–	.000†††
REM (%)	22.21 ± 1.79	24.10 ± 1.30	.42
Sleep efficiency (%)	91.33 ± .53	85.51 ± .75	.06
Sleep latency (min)	12:33 ± 01:30	05:05 ± 00:25	.14
PSQI Score	3.44 ± .73	3.63 ± 1.19	.64
Stanford Sleepiness Score (1-7)			
AM training (t0)	2.43 ± .48	2.38 ± .18	.91
PM retest (t1)	2.57 ± .20	2.25 ± .37	.47
AM retest (t2)	2.93 ± .58	2.38 ± .18	.35
Actigraphy			
Sleep efficiency (%)	77.96 ± 4.70	88.84 ± 1.43	.06
Sleep latency (min)	25:00 ± 07:13	09:60 ± 02:56	.04*

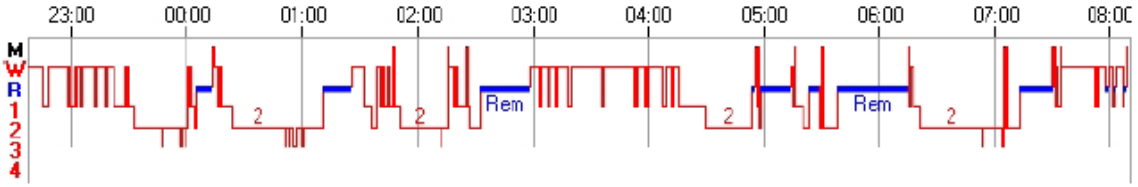
a. Younger participant – representative hypnogram in healthy control (24 years)



b. Older participant I – youngest of the age group (52 years)



c. Older participant II – oldest of the age group (79 years)



d. Older participant III – example hypnogram with absence of stage 3 (69 years)

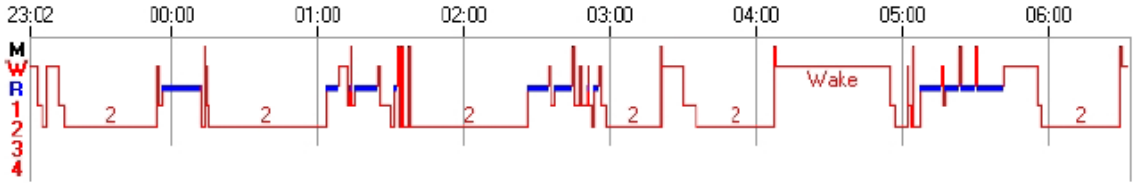


Figure 3.3. Representative sleep hypnograms across age groups. Hypnograms from younger (a) and older (b-d) participants. Hypnograms in the older adults are characterised by the absence of stage 4 NREM sleep, a regular feature in the sleep patterns of healthy younger adults. Note also the presence of stage 3 NREM sleep in the oldest participant (79 years), suggesting that a straightforward/linear relationship between increased age and reduction in slow wave activity, as seen in the group averages, may not be a universal phenomenon at the individual level.

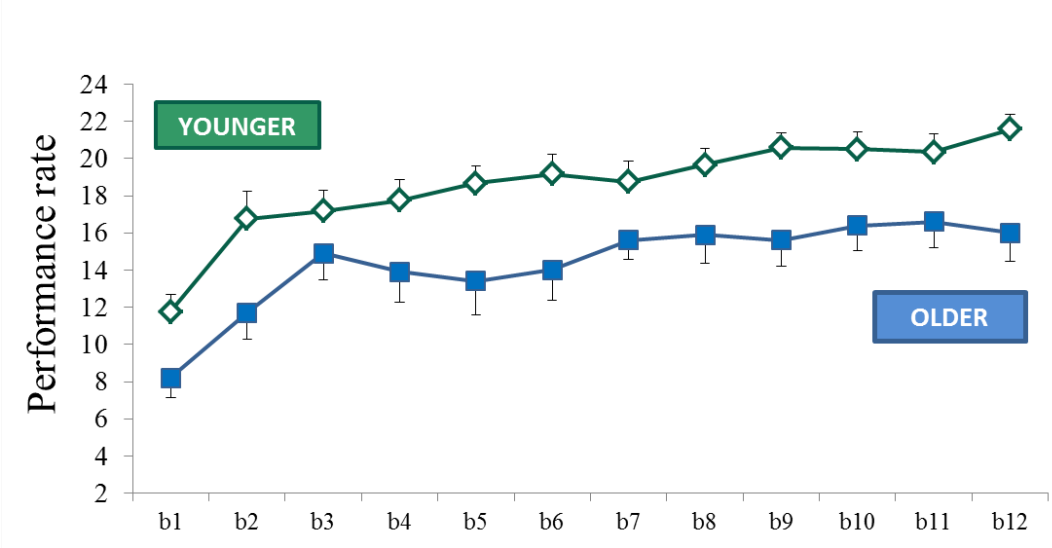


Figure 3.4. Learning curves across age groups: on the adapted task for younger (open diamonds, green) and older (closed squares, blue) adults. Mean number of correct sequences performed per block ('b') during the training session. Error bars represent standard error of the mean (SEM).

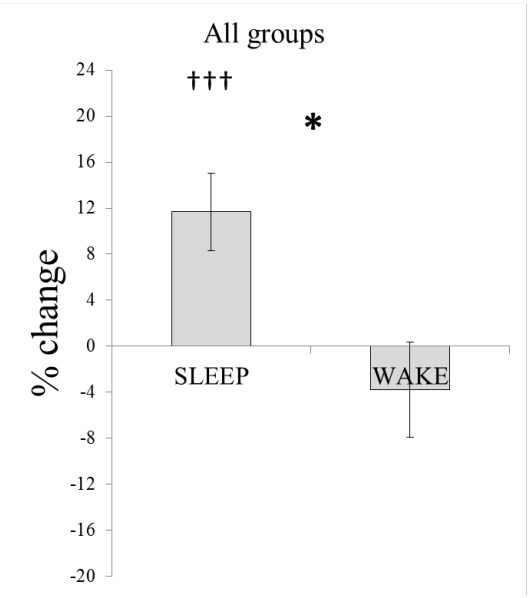
Off-line effects across groups

To also test in this sample whether sleep significantly benefits task performance over and above the simple passage of time, and whether this effect is modulated by age, we ran a repeated measures ANOVA on the change in performance between sessions, including a within-subject factor indicating whether the period included sleep or wakefulness (sleep, wake), as well as between-subject factors of age group (younger, older). It should be noted, however, that in this experiment we did not control for time of day effects as all subjects were initially trained in the morning so that the wake consolidation period always precedes the sleep consolidation period. Replicating the sleep effect in Chapter 2 (section 2.3), results show significant main effects of sleep vs wake ($F(1,20) = 6.60$, $p < .05$; Figure 3.5), with significant improvements found after a

night of sleep ($t(21) = 3.26$, $p < .005$), but not wakefulness ($t(21) = -.90$, $p = .38$). We find no significant main effect of age group ($F(1,20) = 5.20$, $p = .41$). This reflected overall greater off-line improvements in performance with sleep, which did not vary significantly across age groups on the adapted hand-tapping task.

However, as shown in Chapter 2 (section 2.3), younger adults alone did not show sleep-dependence on the adapted version of the task, but instead improved across both a period of wakefulness and sleep. Therefore it is possible that the sleep effect noted here is driven predominantly by the older age group. To examine if this absence of sleep-specificity occurred in the present sample of younger adults we ran further repeated measures ANOVAs split by age group.

a. Adapted sequence learning



b. Adapted sequence learning

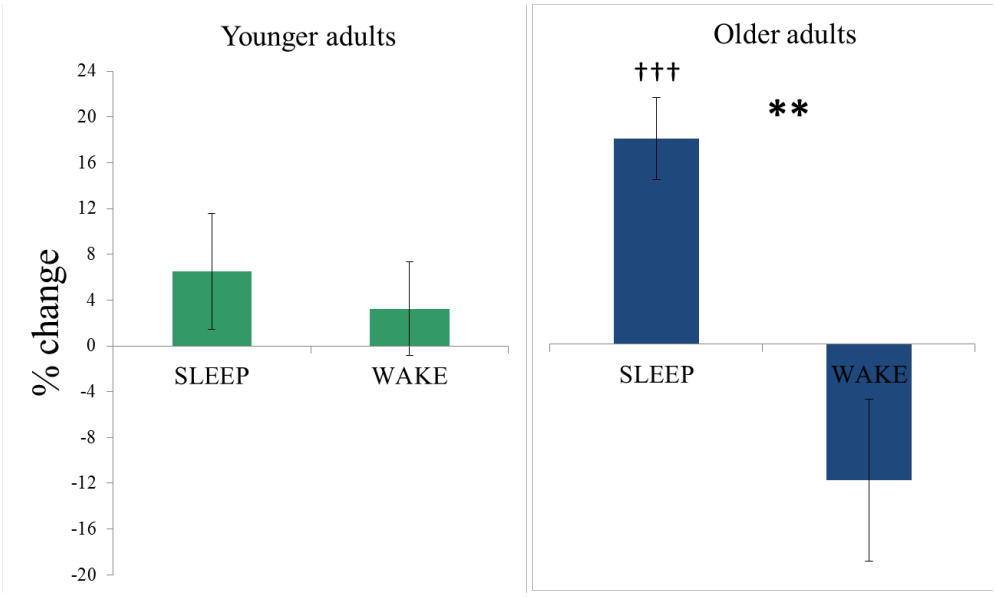


Figure 3.5. Off-line consolidation for adapted task across age groups. Bar chart (a) showing significant improvements in performance after sleep, but not after wake, across age groups, (b) after a night of sleep post-training on the adapted task only older adults show significant sleep-dependent improvements (* = $p < .05$, ** = $p < .01$ main effect of sleep vs wake; ††† = $p < .005$, one-sample t-test versus zero). Error bars reflect SEM.

Off-line effects split by age group

In line with previous findings in this thesis we find further evidence for sleep-dependent consolidation in the older adults, reflected in a significant main effect of sleep vs wake within this age-group ($F(1,9) = 12.16$, $p < .01$), with significant improvements found with sleep ($t(9) = 4.97$, $p < .005$), but not wakefulness ($t(9) = -1.73$, $p = .12$).

However, consistent with Chapter 2, there is no evidence for sleep-dependent effects in the younger group with the adapted task (sleep vs wake, $F(1,11) = .36$, $p = .56$), and we did not find any significant improvements with either sleep ($t(11) = 1.40$, $p = .19$) or wakefulness ($t(11) = .58$, $p = .58$).

Correlations between sleep stages & off-line gains in performance

We tested for correlations between off-line gains in performance with sleep and duration of different sleep stages. Across age groups, we find a significant positive correlation between behavioural improvement with sleep and duration of stage 3 SWS when controlling for age ($r = .45$, $p < .05$; Figure 3.6). That is, the more time spent in stage 3 NREM SWS, the greater the off-line improvement in performance after a period of sleep. No other stages correlated significantly with the behavioural consolidation measures. Based on this result, and to limit the issue of multiple comparisons, the focus of the subsequent analyses in this chapter will be largely concentrated on electrophysiology and age-related changes in stage 3 NREM sleep.

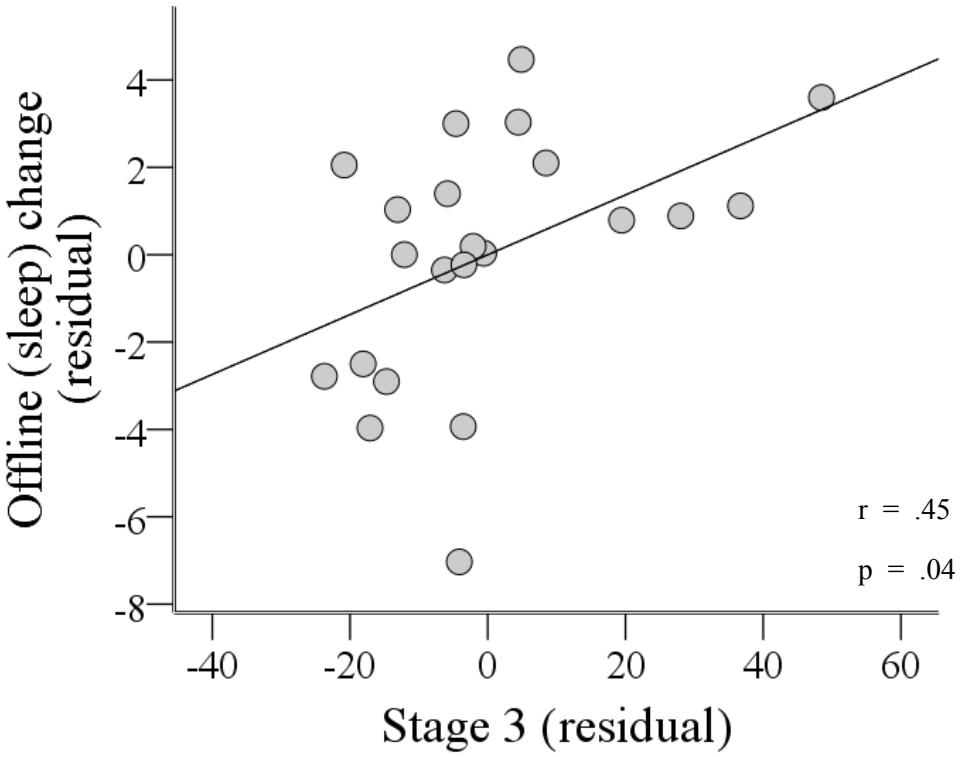


Figure 3.6. Sleep change and stage 3 NREM sleep duration across age groups. Scatter plot showing partial correlation between sleep change and absolute time spent in stage 3 NREM sleep. Axes/values are residuals (i.e., after regressing out age) to allow visualisation of the partial correlation (n=22).

To determine if this correlation also holds within each age group we ran further partial correlations split by age group (also controlling for age), and find positive correlations of a similar strength within the individual age groups, but which only show trends towards significance due to the reduced sample sizes for younger ($r = .47$, $p = .15$) and older ($r = .46$, $p = .21$) participant groups ($n = 12$ and 10, respectively). We also found a negative correlation between age and time spent in stage 3 SWS ($r = -.45$, $p < .05$), suggesting that older age was associated with less SWS.

Measures of delta activity: age and interhemispheric differences

To further investigate the role of stage 3 SWS in motor sequence consolidation, I tested the hypothesis that specific attributes of SWS (e.g., delta band activity) are involved in the consolidation process. To do this, I ran further partial correlations between behavioural improvement with sleep and both the number and power density of delta waves in stage 3 NREM sleep for both the learning (C4, contralateral to the trained hand) and non-learning (C3, ipsilateral to the trained hand) hemispheres as described in the following sections.

Incidence of delta events

Controlling for age of participants, we find that performance enhancements following sleep correlated significantly with the incidence of recorded stage 3 delta waves in each hemisphere separately (Figure 3.7).

While the mean number of delta waves was slightly higher in the learning hemisphere (C4: 645.10 ± 135.85 SEM) relative to the non-learning hemisphere (C3: 622.15 ± 135.54 SEM), a follow up repeated measures ANOVA did not show a main effect of hemisphere (C4, C3: $F(1,18) = 1.12$, $p = .30$), and only a non-significant trend of age group (younger, older: $F(1,18) = 2.99$, $p = .10$), suggesting that the number of delta waves during stage 3 NREM sleep was comparable across both learning and non-learning hemispheres, as well as across age groups, during the behavioural intervention night. Although a significant negative correlation between delta incidence and age (C4: $r = -.45$, $p < .05$) suggested that older age was associated with less delta activity. Hemispheres correlated highly on the delta incidence measure for each NREM sleep stage (stage 1: $r = .97$, $p < .001$; stage 2: $r = .85$, $p < .001$; stage 3: $r = .99$, $p < .001$).

Since the current older sample did not display any stage 4 sleep, this stage is not included above, but a separate analysis of the younger group confirms the bilateral relationship also for this stage ($r = .99$, $p = .000$).

Average power density of delta events

Another useful measure of delta activity is average power density per delta event, which tells us about the amplitude of a typical delta event in a given stage. This in turn allows us to further consider whether there is a relationship between average delta power density and behavioural improvement after sleep, as well as any interhemispheric differences.

While hemispheres did not differ significantly on the delta incidence measure reported in the previous section, a repeated measures ANOVA on stage 3 delta power density, with between-subject factor of age group and within-subject factor of hemisphere, reveals significant main effects of both hemisphere (C4, C3: $F(1,18) = 9.40$, $p < .01$) and age group (younger, older: $F(1,18) = 16.17$, $p < .005$), suggesting that stage 3 power density differed significantly between hemispheres, with greater average delta power density in the learning versus the non-learning hemisphere during the behavioural intervention night (Figure 3.8). Moreover, older adults displayed significantly diminished mean power density across hemispheres compared to the younger controls, and a linear regression analysis indicated that just over 42 percent of the variance in mean power density could be explained by age ($p < .005$), such that older age predicted reduced delta power density. Strong correlations between hemispheres were also found for the power density measure for stage 2 and 3 NREM stages (stage 2: $r = .86$, $p < .001$; stage 3: $r = .95$, $p < .001$), while only low correspondence was found between hemispheres in stage 1 ($r = .26$, $p = .26$).

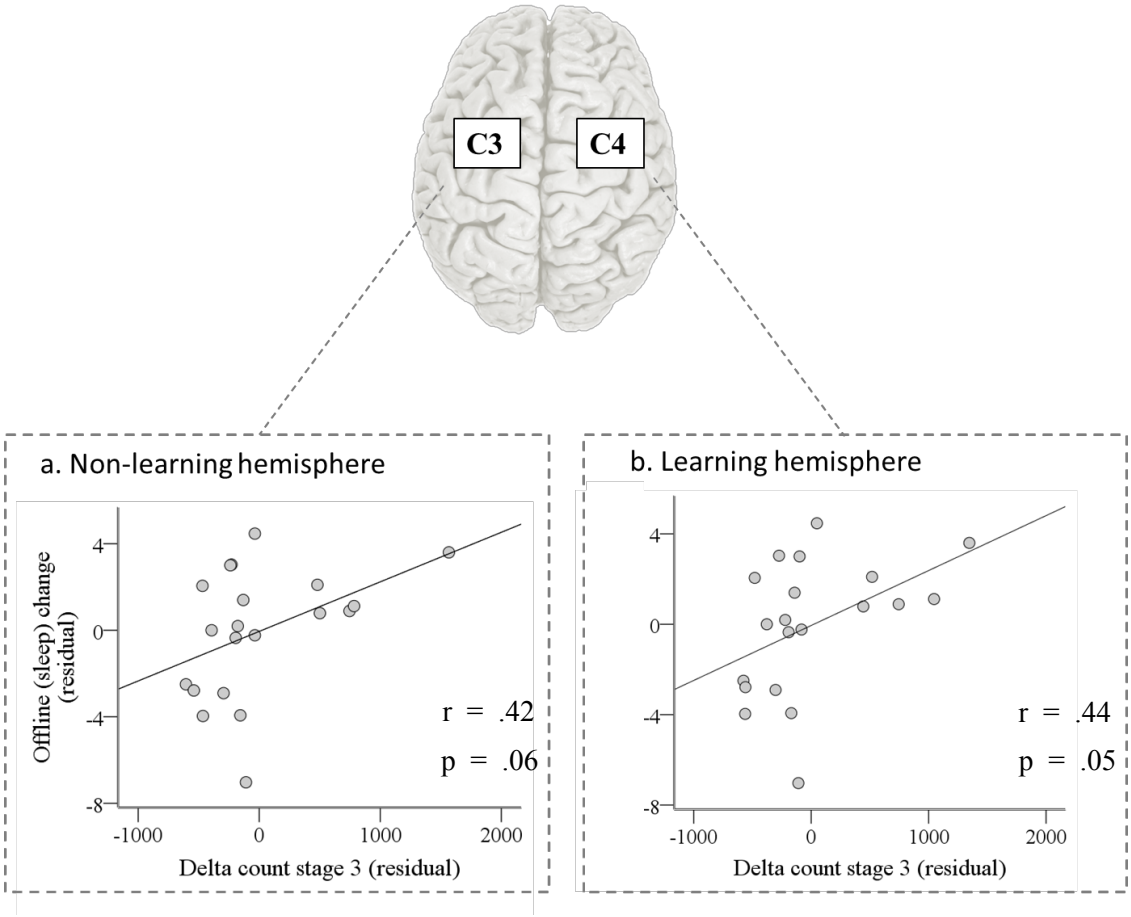


Figure 3.7. Delta count and off-line (sleep) change across age groups. Partial correlation plots of delta activity from (a) non-learning (C3) and (b) learning (C4) hemispheres during stage 3 NREM sleep relative to change in performance following nocturnal sleep. Values are residuals (again regressed on age; n=20).

To investigate the relation between delta power density and off-line consolidation, a bivariate analysis shows strong correlations between change in motor performance after sleep in the older group and average stage 3 power density across hemispheres ($r = .82$, $p < .005$; Figure 3.9), as well as in each hemisphere separately, with a slightly stronger (though not significantly different) relationship occurring with the learning hemisphere (C4: $r = .86$, $p < .005$; C3: $r = .75$, $p < .05$). Correlations between delta power density and behavioural improvement with sleep were not present in the younger age group (for younger group, bilateral: $r = .09$, $p = .81$; C4: $r = .20$, $p = .58$; C3: $r = -.06$, $p = .87$).

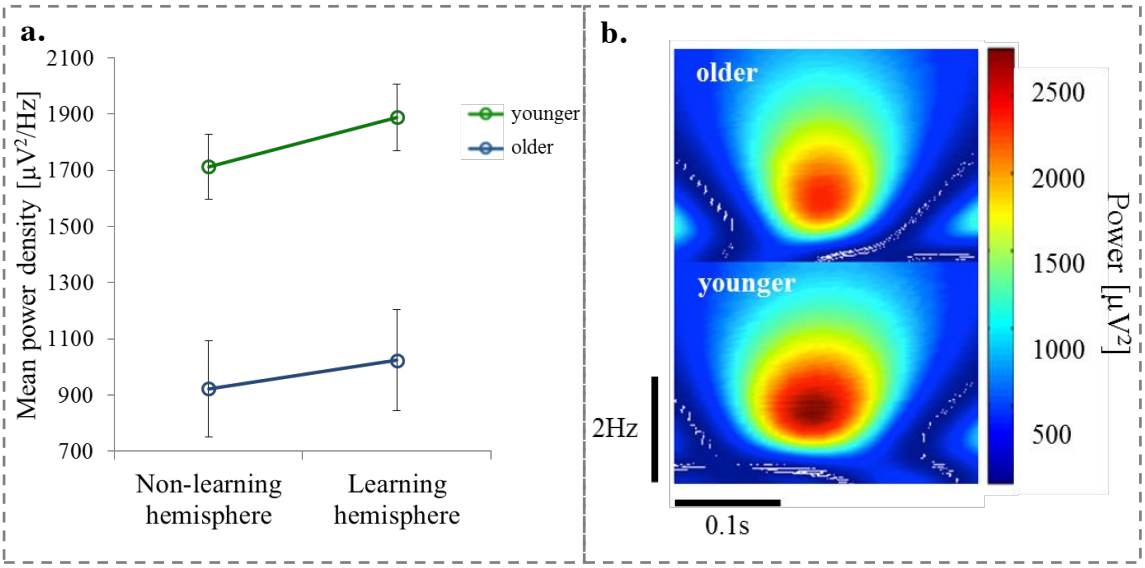


Figure 3.8. Age and interhemispheric differences in delta power during stage 3 sleep. Shows (a) the average power density of delta events in stage 3 NREM sleep in younger and older adults, and within learning and non-learning hemispheres. Error bars depict SEM; (b) surface plots of a stage 3 delta wave at the central derivation C4 (learning hemisphere) for an older (top) and younger (bottom) participant. Plots highlight the observed reductions in power density in older adults.

Exploratory analyses of other sleep stages did not reveal any significant relationships with sleep improvement, although a trend for a correlation was found between delta power density during stage 2 NREM sleep and sleep change with age as a covariate of no interest (C4: r = .44, p = .06; C3: r = .40, p = .09). No trend was found for delta incidence during this stage (C4: r = .07, p = .78; C3: r = -.05, p = .84).

Inspection of the scatter plots in the older group (Figure 3.9a-c) indicates a potential split between two groups that may be defined by the presence or absence of SWS (and the presence or absence of sleep-dependent improvement). However, due to the small sample size, and subsequent uneven split between hypothesised groups, a non-parametric related-samples Wilcoxon signed rank test was used to compare between (SWS and non-SWS) groups. Results show a non-significant difference between group (p = 1.00).

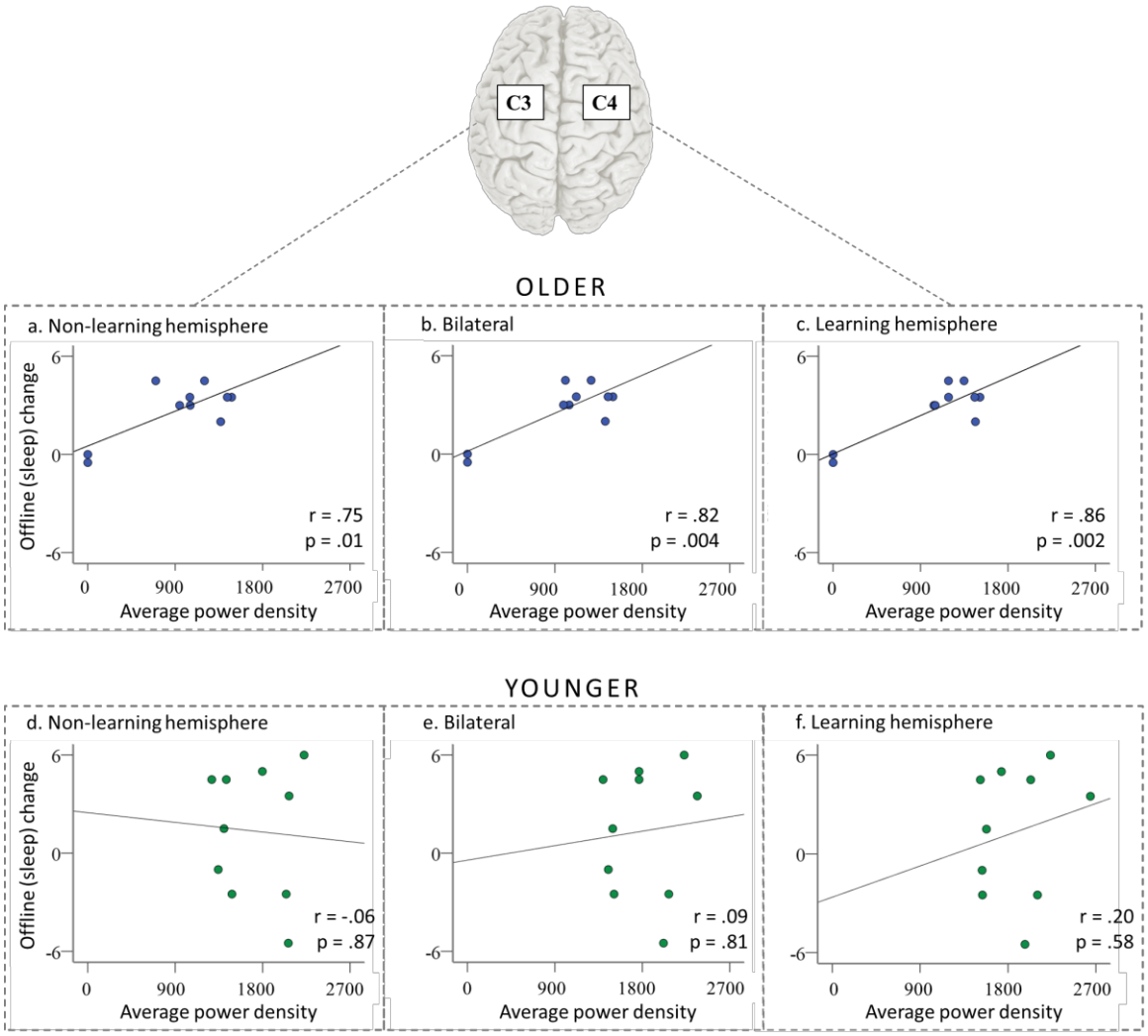


Figure 3.9. Average delta power density (stage 3) and sleep change. Correlations between consolidation with sleep (correct sequences/block) and the average power density (μV^2/Hz) of a delta event during stage 3 NREM sleep in the non-learning (C3) and learning (C4) hemispheres, as well as bilaterally, for older (a-c; n=10) and younger (d-f; n=10) participants.

It may also be that hemispheric differences in sleep architecture after learning predict the degree of sleep improvement across age groups. Hemispheric differences following learning have previously been observed in both humans (Walker, Stickgold, Alsop, Gaab, & Schlaug, 2005) and rodents (Hanlon et al., 2009), and is also supported by the significant main effect of hemisphere (learning vs non-learning) reported for the power density measure above. However, we wanted to examine whether this inter-hemispheric difference also related to behavioural improvement observed with sleep. To

do this, a difference score (subtracting the non-learning from the learning hemisphere) was calculated (similar to previous work looking at hemispheric differences in EEG sleep signal; Nishida & Walker, 2007).

Correlations with hemispheric differences in delta power density

Across age groups the difference between hemispheres in average delta power density showed a trend for correlation with behavioural improvement after sleep ($r = .43$, $p = .06$), again after controlling for age (Figure 3.10).

Together, these results suggest that while learning and non-learning hemispheres remain comparable in terms of the actual number of delta waves after learning, the power density of the delta activity is significantly greater in the learning hemisphere after a behavioural (motor skill) intervention.

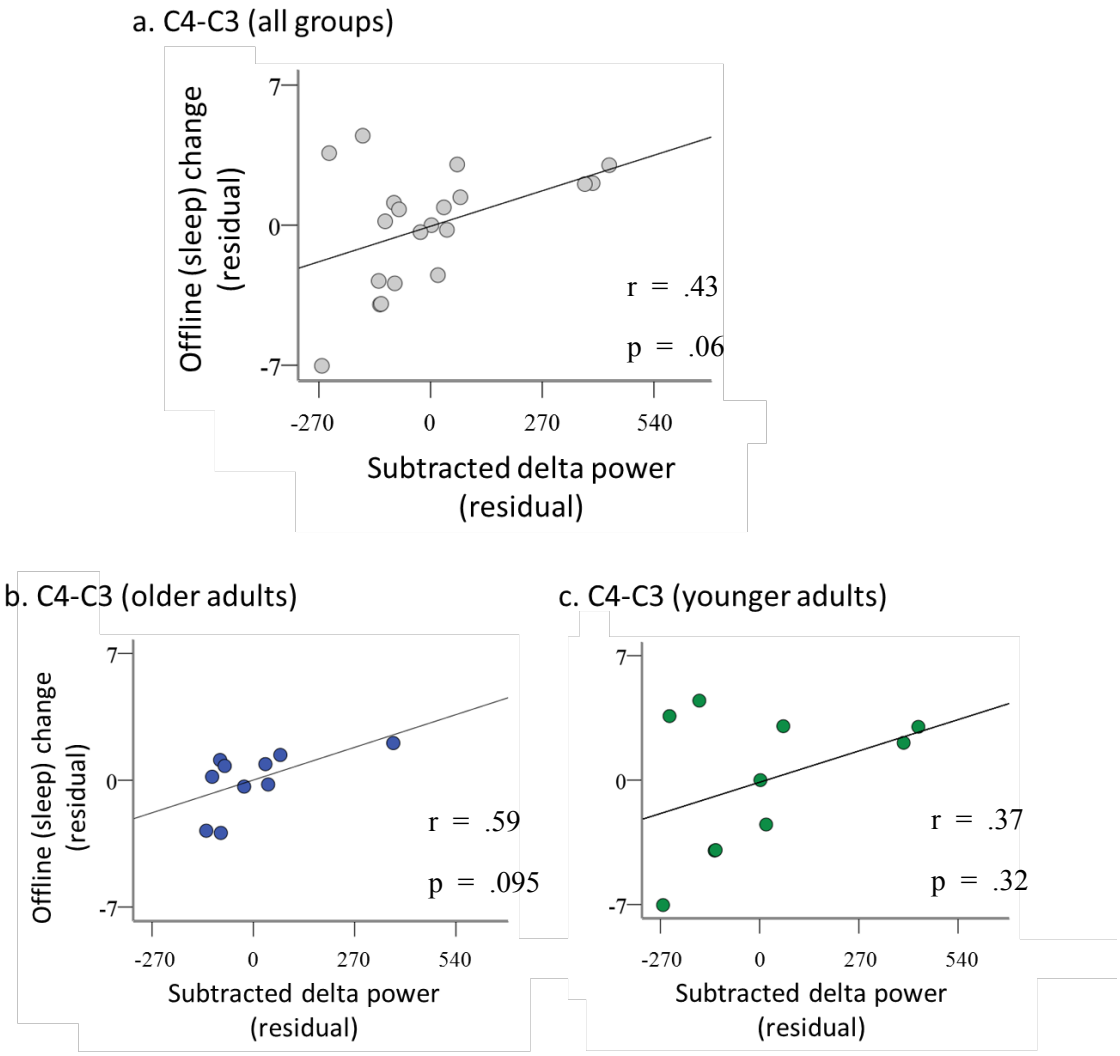


Figure 3.10. Subtracted stage 3 power density and change with sleep. Partial correlation plots with both age groups (a; n=20), and separately for older (b) and younger (c) participants. Values are residuals as regressed on age.

3.4 Discussion

The results of this chapter show for the first time that slow wave sleep (SWS) is implicated in motor sequence consolidation in both younger and older adults. Despite significant changes in sleep architecture in older adults, we found behavioural evidence for consolidation of motor learning, which related to overnight EEG measures. Specifically, greater post-sleep performance improvement was associated with more time spent in stage 3 NREM sleep. We also show that having greater spectral power density of delta activity associated with this stage may play a significant role in enhancing motor memory consolidation regardless of age.

The role of interhemispheric differences was also explored in this study. While learning and non-learning hemispheres were largely identical in the incidence of delta waves, delta power density in the learning hemisphere was significantly greater compared to the non-learning hemisphere.

3.4.1 Slow wave activity, sleep stages, and motor consolidation

The implication of SWS and, specifically, delta band spectral power in motor sequence consolidation is consistent with previous literature in younger adults. For instance, Huber et al. (2004) found a significant correlation between slow wave activity in the 0-4Hz range and post-sleep performance on a visuomotor adaptation task, such that greater improvement was associated with increased SWA. However, their measure of SWA did not include a specific characterisation of SWS, but instead was assessed indiscriminately across NREM sleep stages. Research on rodents has also shown a significant increase in SWA (specifically, spectral power) following learning on a reaching task, whereas the study did not find any differences in the spindle frequency

range (Hanlon et al., 2009). The importance of SWA for consolidation and subsequent encoding is also supported by evidence showing a link between SWA and wakeful synaptic potentiation. For instance, research has shown that the suppression of SWA negatively affected subsequent wakeful encoding (Van Der Werf, Altena, Vis, Koene, & Van Someren, 2011). Conversely, wakeful memory acquisition (associated with synaptic potentiation) elicited enhanced slow wave activity during subsequent sleep in both humans (Huber et al., 2004) and rats (Hanlon et al., 2009), whereas daytime arm immobilisation (thought to lead to synaptic depression) was associated with a decrease in SWA in the contralateral hemisphere during the following night of sleep (Huber et al., 2006). These findings are in line with the synaptic 'downscaling' hypothesis and evidence outlined in section 1.2.2.1.

Together with the present findings, there is evidence to support a role for delta band activity in motor memory. This is consistent with studies showing an association between NREM sleep and explicit motor learning (Robertson et al., 2004), as well as its proposed role in consolidating goal-based (spatial) components of motor learning (Robertson, 2009). Further, the role of SWA in declarative memory consolidation is well established (Marr, 1971; Squire 1992; McClelland et al., 1995; Rasch et al., 2007), suggesting that NREM sleep may be particularly involved in the consolidation of explicit memory traces. For instance, a number of studies have associated the involvement of NREM sleep in motor consolidation if the memory trace involved explicit awareness of the motor skill and underlying sequence, however, if acquired without explicit knowledge, consolidation of the task was associated instead with REM sleep (Robertson, 2009; Cohen et al., 2005). This is consistent with the present results, as the task here involved explicit knowledge of the motor sequence being learnt (and the sequence was displayed on the screen during all training and retest blocks). However,

while a number of studies have found consolidation-related correlates across NREM sleep, there are also reports suggesting a role specifically for stage 2 (NREM) sleep (Fischer et al., 2002; Walker et al., 2003; Fogel & Smith, 2006). While this seems immediately at odds with studies observing a role for SWS in motor consolidation (Fogel, Smith, & Cote, 2007; Huber et al., 2004), as well as the findings within this chapter, the following paragraphs outline two explanations that may at least in part account for this apparent conflict.

The first relates to the issue of human variability in qualitatively assessing, and visually scoring, EEG signal. It is well known that variability exists between sleep scorers, which highlights the need to establish reasonable inter-rater reliability between scorers. However studies assessing the inter-rater variability across research centres suggest that agreement may vary considerably across sleep stages, and some of the greatest decreases in reliability were found during the scoring of stage 2 and SWS (Danker-Hopfe et al., 2004). Such divergences may in part reflect differential interpretations of the scoring guidelines, as well as in the technique adopted to dissociate these two stages. According to the Rechtshaffen and Kales (1968) criteria, stage 2 and 3 NREM sleep are frequently only differentiated by the presence of 'sufficient' delta activity (i.e., $\geq$ 20 percent, which, in a 30-second epoch, translates to only 'true' delta events comprising at least 6 seconds, but no more than 14 seconds of the epoch). Therefore it is possible that divergences in the visual characterisation of delta events and their incidence may contribute to observed variability in the coding of stages 2 and 3 NREM sleep.

Secondly, sleep phenomena closely associated with memory consolidation (here, for instance, k-complexes) are frequent in both stage 2 and SWS. K-complexes are thought, in part, to be a manifestation of the slow oscillation at the surface-EEG level

(Amzica & Steriade, 1998), and have been implicated in the replay function of memory consolidation (Peyrache, Khamassi, Benchenane, Wiener, & Battaglia, 2009), as well as in potentially facilitating synaptic potentiation during sleep (Chauvette et al., 2012). Therefore, EEG oscillations present in both light and deep sleep may be involved in the processes of memory consolidation, and may help explain seemingly conflicting reports that associate motor consolidation with only stage 2 or only SWS. The present data further support this interpretation. While we did not find any significant association between stage 2 NREM sleep and overnight consolidation, there was a trend for a correlation between delta power density during stage 2 sleep and sleep change. Moreover, as studies unearth increasingly detailed processes and correlates of sleep phenomena in relation to memory consolidation, it will arguably become clearer that no solitary signal event, or even sleep stage, may singularly account for the processes of consolidation even within a specific memory system, and that these processes are likely all complimentary and essential mechanisms that are either directly or indirectly involved in aiding memory processing.

3.4.2 Hemispheric differences

Finally, it is interesting to note the differences observed in the present study between the learning and non-learning hemispheres. Although spontaneous delta slow waves in theory may arise from any cortical site in either hemisphere, studies have suggested the presence of preferential generators at central and frontal sites (Massimini et al., 2004), with slow waves shown to frequently (> 46 percent of slow waves) originate in the left insula and medial cingulate gyrus (Murphy et al., 2009). It is important to note that while the seed location for slow wave onset may be relatively consistent across participants, slow wave activity is not an isolated local phenomenon,

and instead sweeps cortical areas much like a travelling wave, usually from anterior to posterior regions (Massimini et al., 2004; see section 1.2.1.1). However, that slow wave activity has a widespread and wave-like cortical influence, or even its preferential source of onset, does not tell us anything about the functional properties of the underlying neuronal networks. For instance, it remains unclear whether the power or intensity of the slow oscillation is greatest at its location of origin, if it diminishes gradually as it induces neighbouring cortical networks, and how these properties relate to memory consolidation.

It has been proposed that a primary role for the slow oscillation is to promote homeostasis within cortical circuits that were induced during active wakefulness and learning (see section 1.2.2.1 for a discussion of the synaptic homeostasis hypothesis; Tononi & Cirelli, 2003; Tononi & Cirelli, 2006). That is, greater synaptic potentiation during wakefulness would be associated with increased slow wave activity during the following period of sleep (Tononi & Cirelli, 2003). This in turn would be associated with an increased signal-to-noise ratio (SNR), allowing for improved consolidation. In line with the homeostasis hypothesis, one prediction would be that uni-manual motor training would elicit higher synaptic potentiation during wakeful learning in the contralateral hemisphere. This prediction is supported by recent findings. For instance, the classic motor sequence task, on which the present learning paradigm is based, has been shown to elicit activations in the contralateral hemisphere to the learning hand during subsequent sleep (Walker et al., 2005). Moreover, motor learning in rats has been shown to elicit local increases in the contralateral hemisphere to the limb used for reaching (Hanlon et al., 2009). These reports are consistent with the present findings of enhanced spectral power in slow waves in the contralateral (learning) hemisphere. In other words, the interhemispheric differences in slow wave activity seen in the present

study may here reflect an increase in homeostatic regulation in the hemisphere that underwent significant learning, which may in turn benefit such learning. However, due to the spatial resolution of this work we do not make any specific assumptions regarding more localised activity within the learning hemisphere.

Further evidence of (learning-dependent) hemispheric differences comes from the spindle literature. For instance, Nishida and Walker (2007) showed that spindle power was related to enhanced consolidation with a daytime nap. Importantly, it was only the *difference* in hemispheres (learning versus non-learning) that significantly correlated with the sleep effect, such that greater power in the learning hemisphere correlated with better post-nap performance. Moreover, there is also emerging evidence supporting a potential link between spindle and slow wave activity. For example, studies have demonstrated a concurrent increase in spindle power density when slow wave activity is elicited with electric or magnetic stimulation (Marshall, Helgadóttir, Mölle, & Born, 2006; Massimini et al., 2007). This suggests there may be an underlying functional relationship between slow wave and spindle activity (Hoffman et al., 2007), and is consistent with work proposing a more indirect (sleep maintenance) role for spindle activity in enabling memory consolidation (e.g., by suppressing noise processing during specific sleep periods; Kim et al., 2012).

3.4.3 Age-related differences in sleep architecture

There were significant differences between older and younger participants on a number of sleep- and learning-related parameters. The age-related differences in sleep architecture discussed in this chapter are consistent with previous reports (Ohayon et al., 2004; Sarajishvili et al., 1974: Colrain et al., 1999; (Smith, Karacan, & Yang, 1977);

Colrain et al., 2010; Ehlers & Kupfer, 1997; Feinberg & Campbell, 2003). For instance, we find an overall increase in both stages 1 and 2 NREM sleep, as well as decreases in slow wave sleep with ageing (see Figure 1.7 in section 1.3.2 for comparison). Moreover, we show that older adults on average had less spectral power density in slow wave activity than younger controls, with power density decreasing significantly with age. The present data further suggest that 42 percent of the variance in signal power density could be explained by age, which is comparable to values reported previously (e.g., 50 percent; Colrain et al., 2010). Together these findings suggest that the power density of cortical slow waves is reduced with ageing, and allude to underlying age-related changes in neuronal function that in turn affect the processes supporting sleep function. For instance, it is thought that the reduction in slow wave power seen with ageing may be a consequence of general cortical degeneration resulting in a reduced quantity of cortical neurons. In light of converging evidence suggesting decreases in gray matter volume with ageing (e.g., Lemaitre et al., 2012, Smith et al., 2007), and given that slow wave activity is a predominantly cortical phenomenon (Amzica & Steriade, 1995; see section 1.2.1), it seems plausible that age-related cortical decline could be causally linked to the reductions seen in the power of slow wave activity (Crowley, 2011). Moreover, the complete lack of stage 4 SWS, as well as a marked reduction also in stage 3 with older age may in turn have an effect on homeostatic functions that rely crucially on slow wave activity. As discussed in section 1.2.2.1, slow wave activity has been implicated in the regulation and downscaling of synaptic potentiation in local networks. The repetitive nature of slow frequencies common especially in SWS is thought to facilitate this synaptic depression in turn improving the SNR and renormalizing local networks for new learning to occur (Tononi & Cirelli, 2003). However, age-related changes in slow wave activity could lead to diminished efficacy

of these homeostatic functions, thereby potentially compromising processes of consolidation and the capacity for new learning.

3.4.4 Limitations

A potential limitation of the present study – and studies in general that use the Rechtschaffen and Kales (1968) scoring criteria – is the requirement for delta waves to be at least 75μV. Given the growing evidence showing a decline in the amplitude and power of delta activity with ageing, it may be that scoring to such strict (amplitude) criteria would lead to the underrepresentation of SWS in the older sample (Colrain et al., 2010). Such a hypothesis would, however, also presuppose the omission of delta waves that fall below the stated amplitude due to age-related changes, which in turn would be apparent by a reduced incidence of recorded delta waves in the current analyses. However, the present study did not find any significant difference in the incidence of (≥ 75μV) delta waves between younger and older adults. In addition, a recent review found the 75μV criterion to be a sufficiently sensitive measure of SWS across different age groups (Silber et al., 2007). Nonetheless, it is possible that such a threshold still omits a proportion of age-related lower-amplitude events. However, to fully address this concern would require a re-assessment of the terminology used to define sleep phenomena (e.g., delta waves), and highlights an inherent limitation of the biophysical, qualitative approach to sleep staging. This limitation was also recognized by Rechtschaffen and Kales, who state theirs are arbitrary scoring criteria to facilitate a standardised approach. Moreover, applying standardised constraints offers the increased probability to compare across sleep studies and allows studies investigating more quantitative attributes of the EEG signal (e.g., changes in incidence, amplitude, power

density) to relate results to sleep stages in a meaningful way (e.g., delta events occurring specifically during stage 3 NREM sleep).

A further confound relates to the inclusion of k-complexes scored as delta waves. As outlined by Rechtschaffen and Kales, k-complexes can be difficult to dissociate from other delta events due to the similarity in frequency and amplitude. Moreover, as mentioned in the introductory paragraphs, k-complexes belong to the delta frequency band and, similar to other delta waves, diminish in amplitude across the lifespan, and this is thought to be a reliable marker of ageing (Colrain et al., 2010). Therefore the inclusion of such delta waveforms in the overall delta measure would be unlikely to have negatively affected results.

The present findings also highlighted the emergence of distinct groupings (e.g., based on the presence or absence of SWS), suggesting potential implications for consolidation and behavioural outcomes. However, analysis of these preliminary groupings is restricted by the limited sample size that results from dividing the groups. Further work is needed that considers a larger sample size to assess the specific dynamics and implications of such groupings.

As briefly discussed in Chapter 2, adopting actigraphy for dissociating sleep-wake cycles carries some degree of inaccuracy due to the way outcome measures (e.g., sleep efficiency) are derived. This issue is further evidenced by the observed discrepancy between actigraphy- and EEG-derived measures of sleep efficiency and latency in this chapter. While, in the younger sample, the actigraphy approach seemed to underestimate the 'true' sleep efficiency as compared with EEG, conversely actigraphy may have overestimated this measure in the older group (perhaps due to more immobility in bed during wake states). Although both techniques derive the sleep

efficiency measure in a similar way, as a function of sleep time relative to time in bed, EEG provides additional (direct) information and high temporal resolution to allow the dissociation between movement epochs and actual periods of wakefulness, thereby ensuring a greater degree of accuracy in identifying sleep-wake transitions.

Finally, this study included only one time point for training for both age groups, which did not allow us to test for any time of day effect within this chapter. However, these extended groupings were included in Chapter 2, which consistent with previous research did not find any time of day effect.

3.4.5 Conclusion

Despite substantial age-related changes in sleep architecture, we show significant consolidation of motor learning in older adults that in turn is associated with slow wave sleep and increased spectral power density of the underlying slow wave signal. The present findings also support a role for slow wave activity that is localised to the 'learning' hemisphere, with increased power density associated with enhanced post-sleep improvement in both age groups.

As we move towards a more integrative framework of sleep, such processes and their co-dependent functions in memory consolidation will become clearer. However, there is increasing evidence suggesting the involvement of delta rhythms in synchronous regulatory processes including synaptic homeostasis in local networks. Further studies are needed that begin to examine these phenomena, not in isolation, but as complementary mechanisms that are collectively involved in facilitating memory processing and general sleep function.

Chapter 4

Chapter 4 | Neurochemical changes with motor learning

The previous two experimental chapters have focused on the processes of motor learning and consolidation at the systems level, however such plastic effects are crucially underpinned by changes at the cellular and neurochemical level. Motor learning has, for instance, been associated with increases in excitability of the sensorimotor cortex, as well as cortical functional reorganisation. It has been suggested that this plasticity may be facilitated by decreases in local concentrations of specific neurotransmitters and the subsequent reduction in cortical inhibition (Floyer-Lea et al., 2006). The final experimental chapter investigates the time-course of neurochemical modulation over a period of learning on a visuomotor tracking task, and moreover considers the role that such neurochemical changes might play in post-learning processes of consolidation.

4.1 Introduction

As summarised in Chapter 1, γ-amino-butyric acid (GABA) is the primary inhibitory neurotransmitter found across the adult mammalian brain, and is synthesised predominantly from glutamate, which is the major excitatory neurotransmitter. Interactions between these neurotransmitters are thought to support learning-related changes in cortical synaptic strength and plasticity, including long-term potentiation (LTP). For instance, local field potential studies in rats have shown that LTP, induced by tetanic stimulation, is markedly reduced following administration of a GABA-receptor agonist, which leads to increased local GABA concentration (Trepel & Racine, 2000). In humans, it is also possible to observe gross changes in GABA concentration with MRS.

Studies have suggested a potential link between human motor learning and these underlying physiological processes in specific regions in the brain. In particular, a study by Floyer-Lea et al. (2006) found that gradual decreases in GABA neurotransmitter in M1 were observed during 30 minutes of learning on a visuomotor tracking task. The change in GABA concentration was specific to sequence tracking, and was not observed for an equivalent random tracking task, or during rest. This finding is particularly interesting because it is one of the few human studies looking at spectroscopy-observed changes in GABA with learning. However, it has not, to my knowledge, been replicated subsequently. Moreover, in the original study by Floyer-Lea et al. (2006), the researchers were unable to determine any statistical relationship between the physiological changes they observed in GABA and the improvement in behavioural performance with learning. Therefore, the first aim of the current study was to adopt the exact learning paradigm that was used in the original paper to see if it would be possible to replicate the observed changes in GABA during learning. Moreover, we also aimed to investigate individual differences to clarify if these changes statistically co-vary.

GABA also has a hypothesised involvement in processes of memory consolidation, although this relationship is not clear. For instance, studies probing the effect of drug-interventions on off-line consolidation have shown that a GABA-receptor agonist (baclofen) administered following training enhanced memory consolidation in rats (Swartzwelder et al, 1987). However, human volunteers treated with baclofen as a pharmacological intervention showed subsequent impairments in memory (Sandyk & Gillman, 1985). In line with the focus of this thesis, I also wanted to investigate the potential role between hypothesized learning-induced changes in GABA and off-line consolidation.

As outlined in Chapter 1, consolidation is defined as an off-line process whereby a memory trace becomes strengthened and more robust, and certain types of motor skills have been shown to benefit significantly from post-learning consolidation that is specific to sleep. To be consistent with the previous studies in this thesis, we included a measure of sleep-dependency for the visuomotor tracking task that had been used in the previous MRS study (Floyer-Lea et al., 2006). However, unlike the other tasks in this thesis that included a clear explicit sequence, it is debatable how to characterise this task in terms of explicit and implicit content (see section 1.1.2 for a discussion of the relationship between implicit/explicit components and consolidation). That is, the visuomotor tracking task was explicit in the sense that participants were informed that their task was to apply force to a device in order to track the movements of a visually-displayed target bar. The target bar moved according to a repeating sinusoidal sequence, however they remained naïve as to what this sequence was. It should also be noted that, unlike implicit/explicit variants of the SRTT, which rely predominantly on explicit (and distinct) digit presses on a keyboard, the responses on the visuomotor tracking task, here, involved a more fluid transition across a sinusoidal pattern, which further relied upon variations in the frequency/time domain (Figure 4.1 depicts the amplitude and frequency aspects of the tracked wavelength). As such, participants may be less likely to deduce, and hold explicitly in their working memory, the underlying sequence they were tracking. Given that implicit motor learning tasks do not show clear evidence for sleep-dependent consolidation (Doyon et al., 2009; Robertson et al., 2004), we would therefore hypothesise that any consolidation-related improvement in performance would not necessarily be sleep-specific for this task.

In summary, the current chapter aims to clarify the time-course of the change in GABA during learning, as well as the relationship between GABA and off-line consolidation. Inter-individual differences are also reported.

4.2 Methods

PARTICIPANTS: Twenty-two healthy right-handed participants (14 women, mean age: 25 ± 3 years) were recruited in accordance with local ethics guidelines. All participants performed a visuomotor tracking task for 30 minutes during spectroscopy. A subset (n = 16) were retested at two additional time points (after 12 hours and 24 hours) to investigate off-line consolidation effects. One participant was excluded on grounds that the spectrum acquired was too noisy (e.g., due to head motion) to allow accurate model fitting, therefore only their behavioural data were included for the learning curve and for comparison with off-line behavioural changes.

TASK: The learning paradigm was modelled on the previous study (Floyer-Lea et al., 2006) showing GABA changes with learning. Participants performed a visuomotor tracking task for 30 minutes during which MRS-measurements of GABA were obtained. The MR compatible pressure sensor ('squeezy') device was the same as had been used for the original study. At the beginning of each session, the device was calibrated relative to individual participant's maximum voluntary force to normalise task difficulty. Participants viewed two vertical bars on the screen during the experiment: a target bar (left), which was accompanied by an analogous response bar (right). Applying force to the pressure sensor caused the response bar to increase in height. Participants were instructed to adjust their force on the pressure sensor in order to match the height of the response bar to that of the target bar, which moved vertically in a repeating eight-second sinusoidal pattern (Figure 4.1). Blocks lasted 30 seconds and were interleaved with 30-second rest blocks. A subset of participants was retested at two additional time points – after a 12- and 24-hour consolidation period. Participants were either trained on the task (during spectroscopy) in the evening (PM-group), with retests the following morning and evening, or were trained (and scanned) in the morning (AM-

group), and retested in the evening and following morning (Figure 4.2). Groups were counterbalanced as to which period included sleep or wakefulness.

ANALYSIS OF TASK DATA: A measure of error was calculated for each five-minute block corresponding to the block duration for spectral acquisitions. This was the (absolute) difference in tracking position (target position minus participant position) on the screen, averaged across five-minute blocks, and was used as a measure of learning in the main analyses.

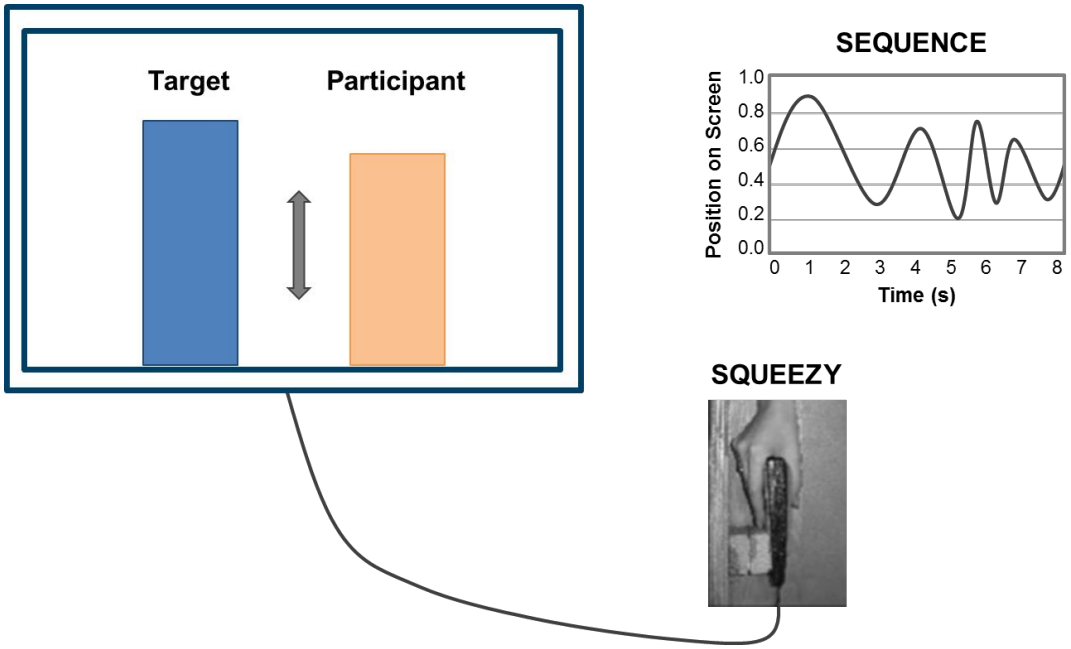


Figure 4.1. Task paradigm for motor learning during MR spectroscopy. Participants followed a target bar (blue) with their own response bar (orange). The repeating sequence in which the target bar moved is shown in the wavelength graph.

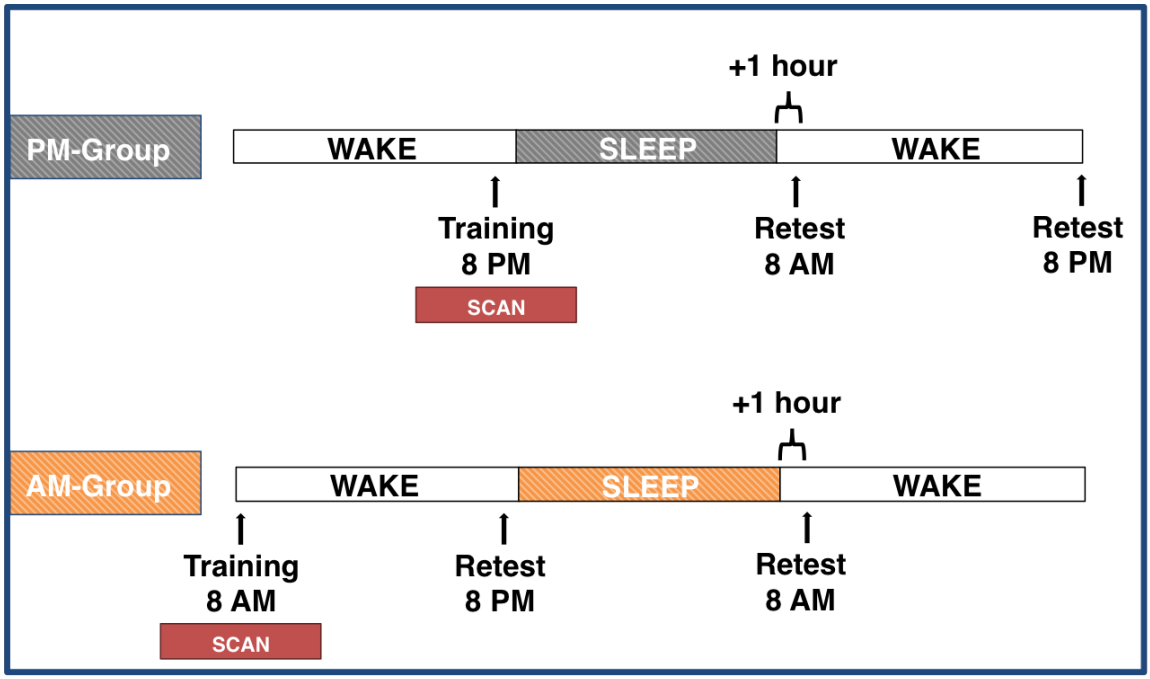


Figure 4.2. Group design for the two consolidation groups. Participants were either tested and scanned in the evening, with retests the following morning and evening, or were tested and scanned in the morning, with retests in the evening and following morning (PM and AM, respectively).

SPECTRAL ACQUISITIONS: GABA-edited spectra were acquired at 3T from a $2cm^3$ voxel centred over the hand region of the left primary motor cortex (Yousry et al., 1997), contralateral to the hand used for the motor learning task. We did not take measurements from control voxels in the present experiment, however Floyer-Lea et al. (2006) acquired from both contra- and ipsilateral sensorimotor cortices to test for the regional specificity of GABA changes with motor learning and did not find any decrease in the ipsilateral sensorimotor cortex.

Blocks of approximately five-minute measurements were obtained using a MEGA-PRESS sequence for simultaneous three-dimensional voxel localization, water suppression, and editing (TE = 68ms, TR = 2000ms). To remove the creatine resonance overlapping with GABA around 3.0 ppm and suppress the water resonance (which would otherwise dwarf the residual resonances), a double-banded 180 degree Gaussian

pulse was applied, firstly, at the frequency of water (4.7 ppm), and secondly at the frequency of the GABA resonance and again at the water frequency. The difference spectrum between the two edited spectra resulted in the GABA triplet resonance being visible without the overlapping creatine resonance peak or the sizeable water peak (Figure 4.3). NAA was inverted in the edited spectra. The acquired creatine and choline measurements were saved in a separate file for later analysis.

ANALYSIS OF MRS DATA: Data processing of the acquired spectra was performed using a MATLAB processing script to de-noise and combine the spectral blocks for subsequent analysis and model fitting using the jMRUI software package (Stefan et al., 2009). Linewidths from the unedited spectra were derived with the jMRUI package, and all Glx and GABA peaks in the edited spectra were fixed to the linewidth of the creatine peak from the corresponding acquisition block. The linewidth of NAA in the edited spectra was fixed to the corresponding unedited NAA linewidth. This was to ensure optimal model fit in the edited spectra. Concentrations are expressed relative to NAA amplitude in order to normalise estimated concentrations.

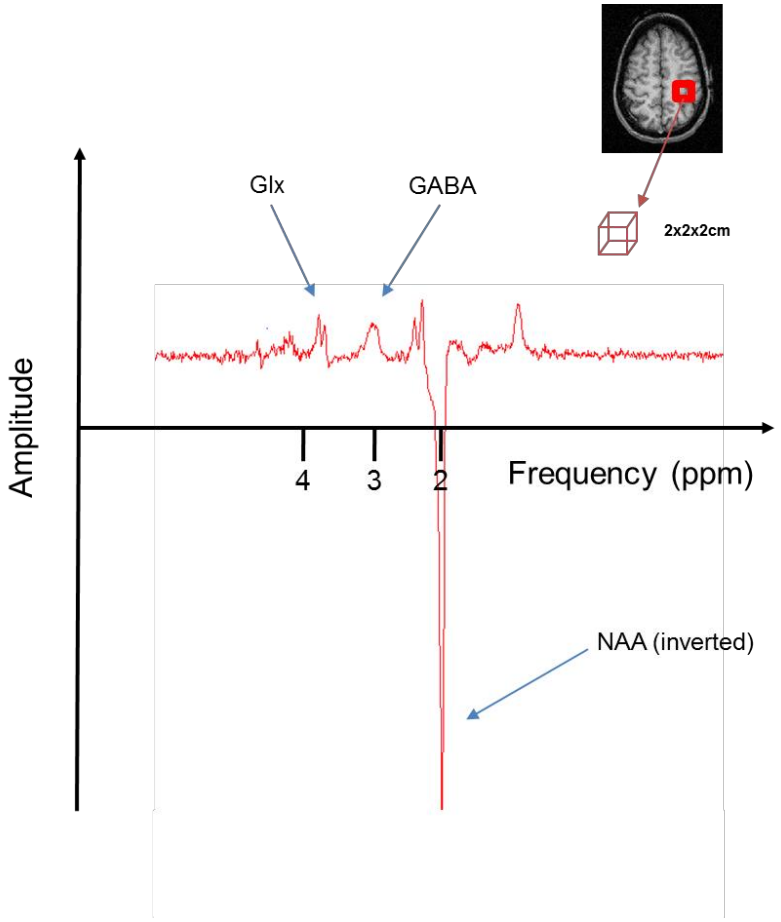


Figure 4.3. Typical GABA-edited spectrum at baseline. MR spectroscopy is a sensitive technique to assess the relative quantification of neurochemicals *in vivo*. Figure depicts the relevant neurochemicals located at characteristic frequencies along the spectrum. Glx: composite measure of glutamate and glutamine; NAA: *N*-acetylasparatic acid.

STATISTICS: To compare the effects of sleep relative to wakefulness, mean error was computed for the final two blocks of training (t0) as a measure of post-training performance. Mean error was also derived for the two blocks at each of the retest sessions (t1 and t2, respectively) to estimate performance levels after consolidation (sleep, wake). Primary statistical analyses are based on repeated measures analysis of variance (ANOVA) with a between-subject factor of training time (AM, AM). Paired-samples *t*-tests were used to compare performances after consolidation periods. Pearson's coefficient was adopted for all correlational analyses.

4.3 Results

Within-session behavioural performance

Thirty minutes of practice on the motor task led to significant reductions in tracking error over time (Figure 4.4; $F(1.33,25.33) = 46.87$, $p < .001$). On average, tracking error plateaued after around 10-20 minutes of practice.

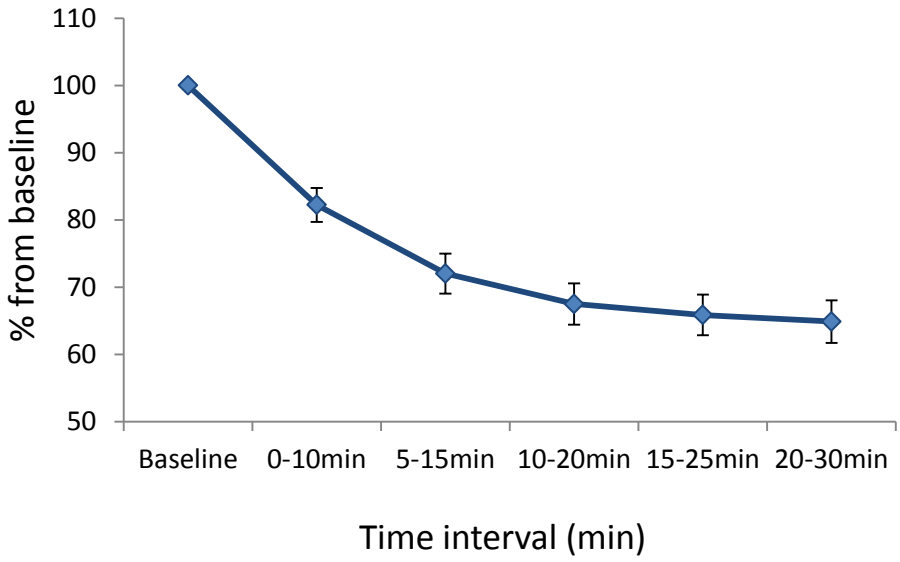


Figure 4.4. Learning curves during visuomotor tracking task. Depicts mean performance curve (+/- SEM) across the period of learning (30 minutes). Blocks are rolling averages to correspond with the acquired GABA measures.

Off-line consolidation effects

Participants showed significantly improved performance following a consolidation period of 12 hours (t1; $t(15) = 5.27$, $p < .001$), but did not demonstrate further gains after an additional 12 hours (t2; $t(15) = 1.31$, $p = .21$; Figure 4.5). However, this gain was not sleep-dependent (sleep vs wake; $t(15) = .64$, $p = .53$; Figure 4.6), and occurred during the first 12-hour period of consolidation regardless of whether this period involved sleep or wakefulness.

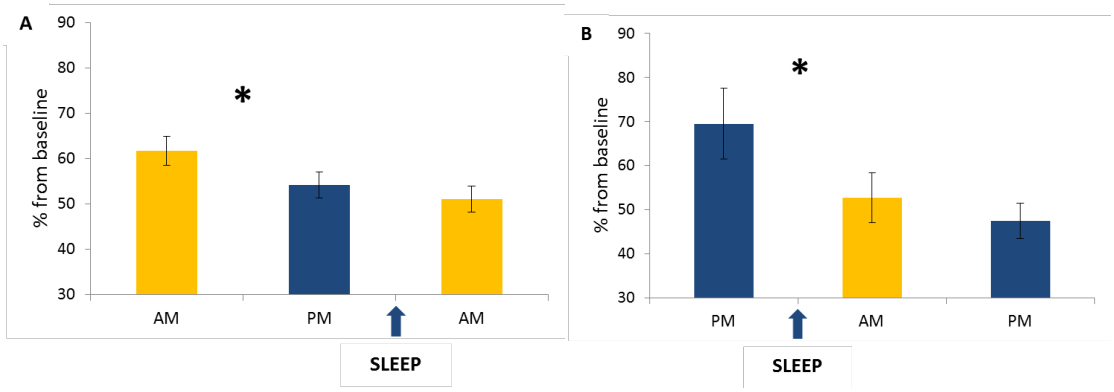


Figure 4.5. Performance changes following consolidation. A. AM-group that trained first in the morning. B. PM-group that had their initial training session in the evening. The consolidation effect was not sleep-specific and occurred after the first 12-hour consolidation period (t1).

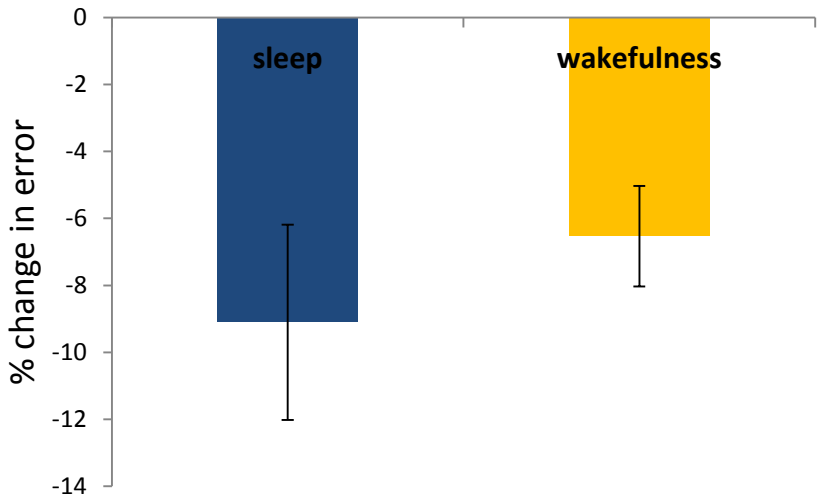


Figure 4.6. Percentage change in performance after sleep and wakefulness. While sleep evoked a greater decrease in error than the simple passage of time, there is a n.s. difference in performance between sleep and wakefulness.

Correlations between online and off-line gains in performance

Here we find significant negative correlations between within-session improvement in (absolute) error during training and off-line consolidation gains. Specifically, performance during each of the individual learning blocks significantly predicts the level of off-line behavioural gains observed (Table 4.1). Moreover, the

power and significance of learning blocks to predict off-line improvements increases temporally, such that performance after 15 minutes of learning moderately predicts off-line gains ($r = -.55$, $p < .05$), with final training performance as the strongest predictor of off-line gains ($r = -.74$, $p < .005$; Figure 4.7). In other words, the worse the final training performance, the greater an improvement is seen in performance following a 12-hour period of consolidation. What is compelling about these results is that they are consistent with findings from the previous studies in this thesis (Chapter 2) that show a similar trade-off between online (learning) and off-line (consolidation) mechanisms indicating that such a dynamic may be comparable across different motor tasks (and not specific to explicit sequence learning) and performance measures (rate versus error). We also find a significant correlation between baseline performance and the level of overall improvement in-session ($r = -.66$, $p < .005$). That is, a higher baseline performance score predicted greater decreases in error with training (Figure 4.8).

Within-session changes in GABA concentration

Inconsistent with the results of Floyer-Lea et al. (2006) we do not find a clear GABA decrease across a period of learning (Figure 4.9).

Table 4.1. Correlations between online performance level and off-line gains (visuomotor). Correlations for absolute values increase both in power and significance with progressing blocks, suggesting that performance levels during later learning blocks were particularly indicative of off-line performance gains. Asterisks denote significant correlations (*, $p < .05$; **, $p < .01$; ***, $p < .005$).

	Performance during training				
	0-10 min	5-15 min	10-20 min	15-25 min	20-30 min
Drop in error (after 12h)	-.410	-.550*	-.554**	-.646***	-.735***
p-value	.115	.027	.007	.001	.001

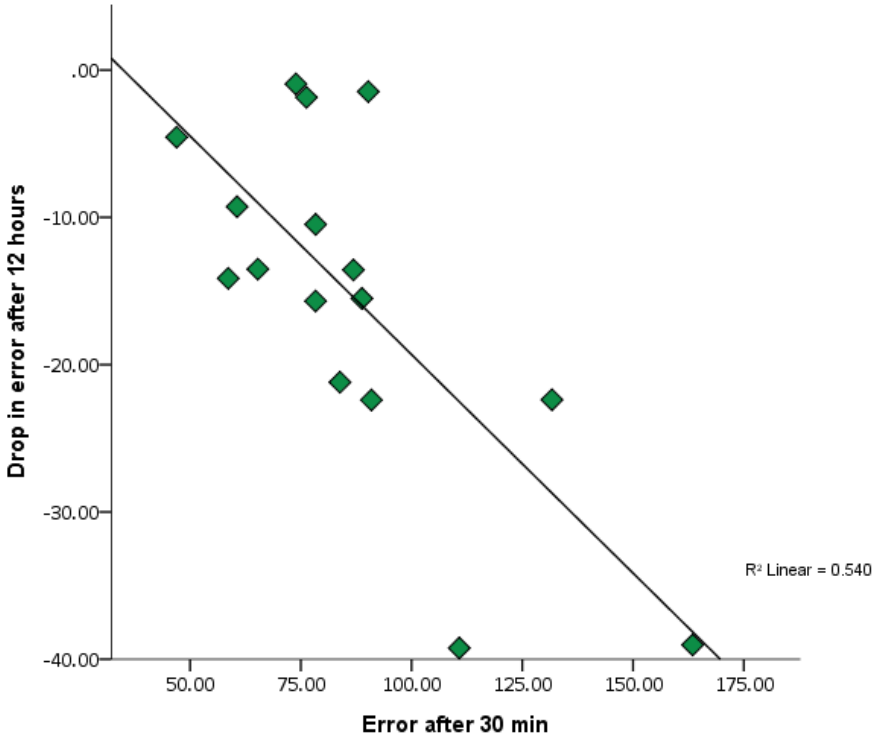


Figure 4.7. Correlation between error after 30 minutes and change in error after 12 hours. A lower tracking error denotes better performance at the end of training, while a greater drop in error signifies improved performance off-line (n=16).

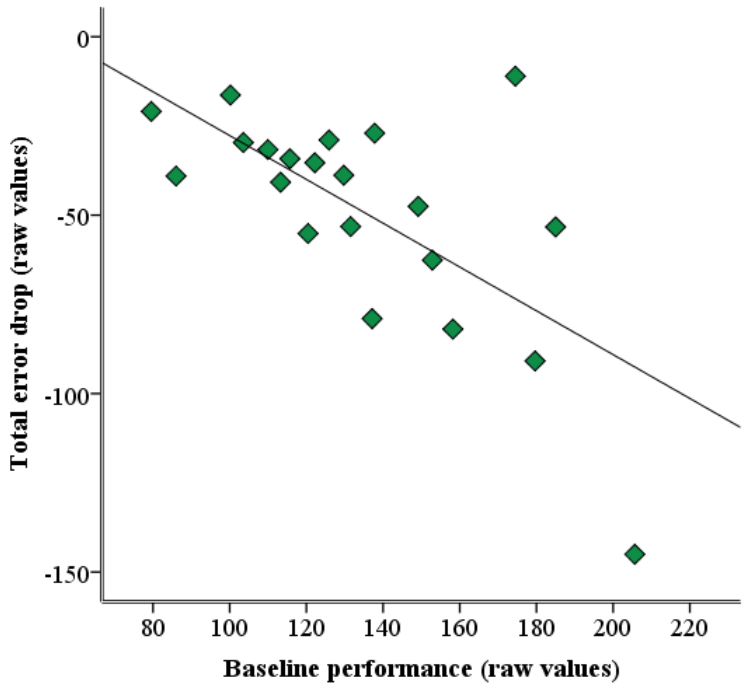
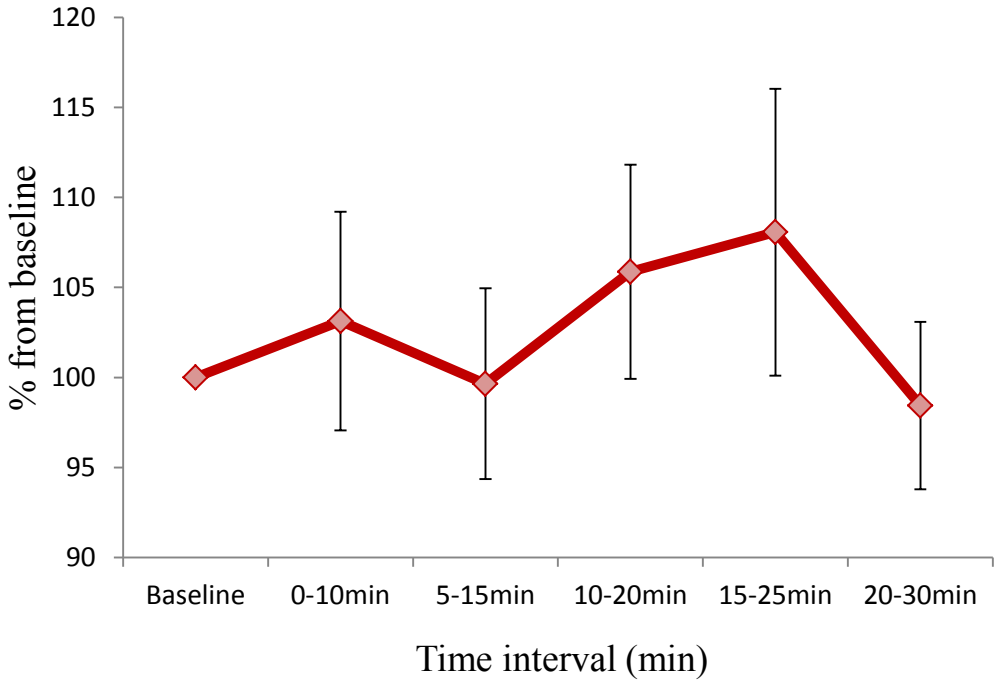


Figure 4.8. Baseline error predicts overall performance change in-session. Absolute tracking error at the beginning of training predicts the overall amount of in-session improvement in performance (n=21).

a.



b.

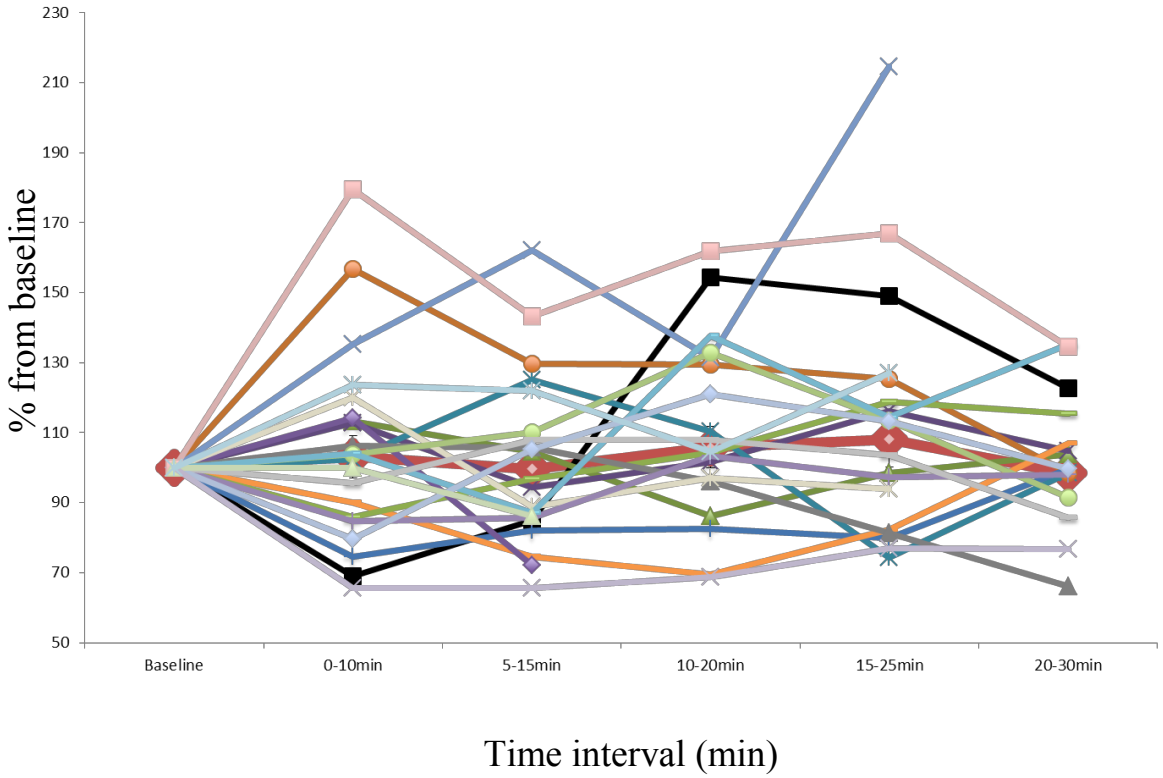


Figure 4.9. Changes in GABA concentration during visuomotor learning. While there are peaks and troughs in the averaged concentrations of GABA across the 30-minute learning period, these are not significant ($F(3.20,48.02) = .93$, $p = .44$) and do not correspond to the decreases proposed by Floyer-Lea et al. (2006). Large error bars (SEM) in the group average (a) hint at the degree of variance seen across participants (b).

Correlations with in-session GABA concentration

We tested for correlations between GABA change and change in performance during learning. No correlations were significant for multiple comparisons (see Table 4.2). A trend ($p < .05$ uncorrected) for a negative correlation between GABA change at 10-20 minutes and error change at 5-15 minutes was found, such that more learning (greater error change) was associated with a smaller GABA drop. However, given the number of relationships tested this result should be interpreted with caution. No other correlations were found (including between GABA at baseline and training performance/off-line gains).

Table 4.2. Correlations between online performance and GABA. No significant correlations were found after correction for multiple comparisons. A trend (*, $p < .05$ uncorrected) for a negative correlation was found between change in performance after 15 minutes and change in GABA after 20 minutes.

%	GABA change 0-10 min	GABA change 5-15 min	GABA change 10-20 min	GABA change 15-25 min	GABA change 20-30 min
Error change 0-10 min	.064	.327	-.090	.024	-.010
Error change 5-15 min	.084	.062	-.487*	.258	.228
Error change 10-20 min	-.042	-.040	-.268	.260	-.338
Error change 15-25 min	-.024	-.082	.304	.071	.198
Error change 20-30 min	-.017	-.080	.202	.014	.450

4.4 Discussion

Inconsistent with the original study (Floyer-Lea et al., 2006), we do not find any evidence for GABA decreases during motor learning. The trajectory of GABA change varies across participants, with some participants showing marked decreases in GABA with learning, while others display distinct increases during training. This indicates a greater degree of variability in the modulation of GABA during learning than has previously been suggested. However, in line with Floyer-Lea et al. we also do not find any significant, or consistent, relationships between changes in GABA concentration and behavioural performance (i.e., both in-session training and off-line performance improvements).

We do however find strong behavioural associations between training performance and consolidation-specific gains. Interestingly, the relationship between online and off-line measures suggest the presence of a trade-off dynamic similar to earlier work in this thesis. That is, the greater improvement that was seen during the training session, the less improvement followed with consolidation. In other words, this finding suggests that poorer performers benefit more from off-line processes of consolidation on visuomotor learning, and is consistent with results from previous studies in this thesis (Chapter 2) that showed a similar dynamic between online and off-line changes in performance.

In addition, we were able to characterise performance gains after visuomotor learning, which are particularly noticeable after the first 12-hour consolidation period, regardless of whether this period included sleep or wakefulness. The observed lack of sleep-dependency is consistent with previous research on implicit motor learning that suggests that the simple passage of time may be sufficient for consolidation, reflecting

different processes needed depending on the nature of task demands (Doyon et al., 2009; Robertson et al., 2004).

Taken together, these findings support a more general trade-off theory between learning acquisition and consolidation that spans across different motor learning tasks (both implicit and explicit) and as well different performance measures (including error and performance rate). Moreover, consolidation gains were apparent after a short 12-hour period off-line, and were not sleep specific.

While we do not see any significant changes in GABA at the group-level, such changes (both decreases and increases) were present in individual trajectories across learning. One possibility is that individual differences in GABA response may obscure any changes at the group level. Indeed, we found a trend for a negative correlation between the degree of GABA change and the degree of error change at specific time periods, though this result needs to be interpreted with caution given that it did not survive correction for multiple comparisons. Given the size of the voxel used to measure GABA levels ($2cm^3$), one explanation for the lack of a group effect drop in GABA with learning may lie in the relationship between concentrations of GABA in different parts of the voxel measured (Figure 4.10). For instance, it may be that GABA decreases very focally within the voxel measured, in only that part of the hand region that represents the task-relevant group of muscles (red circle). Meanwhile, GABA may likely start to increase in the surrounding hand region (blue circle), as a means of balancing the levels of GABA within the area. Depending on individual differences, people with superior motor skills will typically have a comparatively larger hand representation region. This area may therefore take up more of the measured voxel. This has previously been shown in the case of professional musicians. For instance, Bangert & Schlaug (2006) found visually detectable differences in the anatomy of the precentral

gyrus between musicians and non-musicians, with professional musicians having a significantly more pronounced hand-knob. Therefore, the changes in GABA we observe may be proportional and driven by individual differences in motor skill, such that the net effect of GABA-change within the voxel will be minimal, or in fact positive. It could also be that the size of the overall hand region is the same, but that instead the sub-region is more focal in some learners, or indeed that the voxel contains both a larger surround and a more focal centre. In all cases, the net effect would be minimal change, or an increase, in overall GABA concentration. However, this interpretation is speculative as we did not find any significant correlations between individual differences in motor performance and variation in GABA change that survived correction for multiple comparisons.

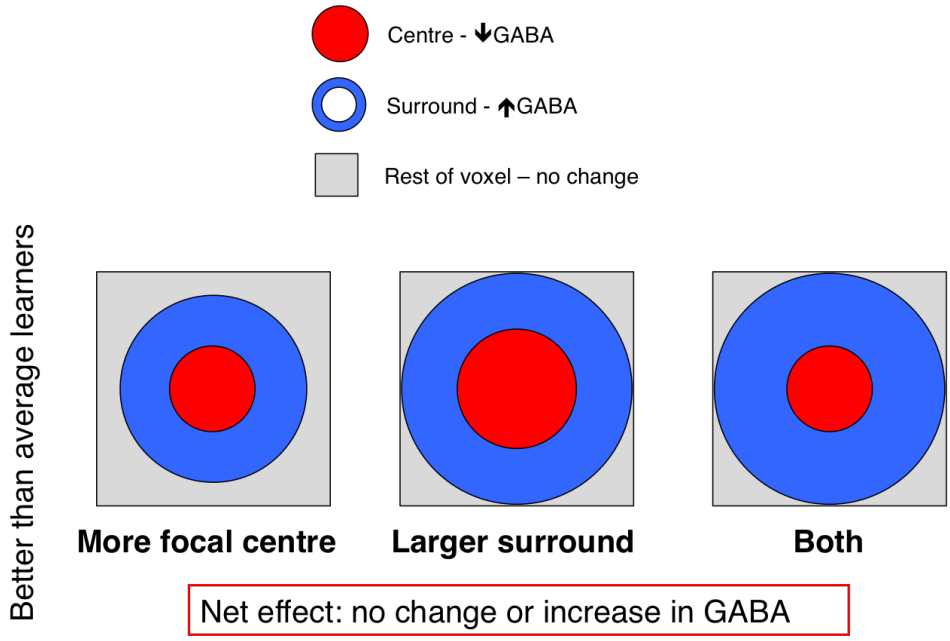


Figure 4.10. Sketch of surround inhibition explanation for the observed negative GABA result at the group level. Possible permutations of GABA concentration measured in the acquired voxel relative to inter-individual factors such as motor skill level.

4.4.1 Limitations

With a negative result, it is always possible that limited power, or experimental noise or error, has contributed to the finding. The original paper by Floyer-Lea and colleagues included 13 subjects in the motor learning condition, in contrast to the 22 subjects used here. The current study should therefore be appropriately powered to detect the effect. The individual subject GABA traces (Figure 4.9) reveal high variability across individuals, as well as some very large individual values (e.g., 50% change in GABA) that appear to be biologically implausible. MRS is a noisy technique and obtaining reliable and consistent measurements is challenging. We applied strict quality control assessments in data analysis to ensure that only spectra of reasonable quality would be included in the analysis. Nevertheless, it is possible that measurement noise, biological noise, subject motion or other artefacts contributed to reduce the quality of the observed measurements.

It is important to note that MRS is limited in its spatial resolution and specificity for GABA characterization. This is because there exists a number of separate "pools" of GABA (both within the neuron, but also pre- and post-synaptically). The MR spectroscopic measurement cannot distinguish between these different functional pools. Whereas typically it is assumed that, for baseline measurements of GABA, MRS is mainly sensitive to extra-synaptic GABA around the neuron, it remains unclear how, or whether, this changes after a behavioural intervention. It may also be that some GABA pools are invisible to MR spectroscopy and so are excluded from the estimated levels of concentration (Stagg et al., 2011).

It may be possible that the observed trade-off between behavioural measures of online and off-line changes in performance could be attributed to participants reaching their individual maximum level of improvement possible by the time of the first retest

session (i.e., signifying a floor effect). However, given that we see further (non-significant) decreases in error even after the first 12-hour consolidation period (e.g., at the second retest session), it is unlikely that this trade-off is due predominantly to such an effect.

4.4.2 Conclusion

In contrast to a previous study (Floyer-Lea et al., 2006), we find no evidence for reduction in GABA concentration during motor learning, despite evidence for clear improvements in performance at the group-level. Moreover, despite significant improvements in performance following a period of consolidation post-learning, we also do not find any consistent associations between neurochemical changes and these off-line performance gains. Meanwhile, consistent with previous work in this thesis, we find further support for a trade-off dynamic between skill acquisition and off-line gains following processes of consolidation.

Chapter 5

Chapter 5 | General discussion

This thesis has examined the impact of age on motor learning and consolidation, and the neurophysiological and neurochemical correlates of these processes. While age has been consistently demonstrated to show deficits in sleep-dependent consolidation, the findings in this thesis provide evidence for fine motor deficits during learning that likely mask the sleep effect in older adults. Findings in Chapter 2 and 3 show that addressing such learning-related deficits reveal significant sleep-dependency of motor learning in older groups. GABAergic changes in key motor areas of the brain have a hypothesised role in supporting motor learning and possibly subsequent consolidation. However, results in Chapter 4 highlight the complex dynamics of this process and suggests large variability across individual participants. Chapter 3 explored some of the underlying neurophysiology supporting sleep and memory consolidation, and demonstrated the key involvement of slow wave sleep, and specifically power density of delta activity (≤2Hz). This is in line with previous research suggesting a prominent role for slow wave activity in off-line processes of motor memory. Older adults demonstrate similar reliance on slow wave sleep for consolidating memories, although deficits were observed across participants in the older group. The following paragraphs will discuss key findings in detail, outlining future directions and conclusions that can be drawn from this work.

5.1 Age-related changes in fine motor skill

One main aim of this thesis was to develop a task to assess motor consolidation in older adults that did not depend on fine motor control. This aim was motivated by converging evidence that has linked ageing with various changes in brain structure and connectivity (Good et al., 2001; Salat et al., 2004; Lemaitre et al., 2012; Raz et al., 2005; Smith et al., 2007) thought to support important motor function including fine motor control (Orban et al., 2011), suggesting that tasks requiring fine motor control may be more demanding for older adults. While relatively few studies have investigated the effects of ageing on the consolidation of motor learning, the majority of these studies have indiscriminately applied motor learning paradigms that work well in younger participants without taking into account the implications of changes to fundamental motor function in the ageing population. This in turn means that, based on these studies, it is not possible to dissociate actual deficits in consolidation in older adults from the potential masking effects of underlying kinematic deficits. The behavioural studies in this thesis set out to examine whether changes to the kinematic properties of motor learning could eliminate confounding effects of fine motor deficits, and allow for the subsequent detection of sleep-dependent consolidation also in older groups.

5.2 Detecting sleep-dependent consolidation in older adults

The studies in this thesis (Chapters 2 and 3) showed for the first time that if we take into account the changes in fine motor skill associated with ageing, such as by modifying a task that previously heavily relied on fine finger control, it is possible to show consolidation effects in older adults that are comparable to healthy younger

controls in terms of both (i) duration of the consolidation period (12 hours, 24 hours), and (ii) the consolidation process needed (sleep, wake). Importantly, these results showed that consolidation with an adapted motor task was achieved without further practice or the need for an extended period of consolidation that have previously been suggested (e.g., Tucker et al., 2011).

While younger adults demonstrated near identical performance levels across the adapted and classic sequence tasks, older groups showed markedly greater performance levels during learning on the adapted task. However, it is not possible based on the above data to make inferences about underlying brain processes that dissociate the two tasks. One approach for assessing potential task-related dynamics is to look for differences in functional activations and connectivity during task performance. It would be of particular interest to compare such hypothesised differences in older and younger adults, and investigate how different brain processes during learning go on to either facilitate or suppress motor consolidation in older age groups.

5.3 Compensatory processes with ageing

As has been described comprehensively in earlier chapters, the brain changes with older age, and some of these changes may in turn lead to compensatory processes and connectivity (Mattay et al., 2002; Naccarato et al., 2006) in order to support function in the face of grey and white matter decline. The findings in this thesis support a potential consolidation-related compensatory mechanism with ageing. Specifically, the behavioural studies in Chapter 2 showed that while older adults were able to demonstrate sleep-dependent consolidation on the kinematically adapted sequence task, they may also still be able to consolidate on the original version of the task. However,

this was shown to require a longer period of consolidation (e.g., 24 hours), which was not specific to sleep, in order to reach the same levels of improvement as offered by the adapted task after a shorter (12 hours) period of consolidation, which was sleep-specific.

This suggests that in cases where the sleep state alone is insufficient, such as with more kinematically demanding tasks in older age, memory consolidation may be able to operate via distinct, and possibly compensatory, mechanisms relating to the *duration* of the consolidation period, in addition to the state of consciousness experienced during that period (sleep, wake). This leads to intriguing questions regarding the nature of sleep mechanisms in (motor) consolidation and how they relate to wakefulness states. While memory components may inherently favour, or demand, the processing of specifically sleep or specifically wakefulness (e.g., Cohen et al., 2005), or (non-specifically) just the simple passage of time (e.g., Doyon et al., 2009) – it seems that there may be cases for which additional processing time may be needed regardless of the consolidation state.

In theory, this leads to the question of whether the wakeful state, given enough time, is sufficient to emulate the consolidation effects of sleep. In practice, however, there are valid methodological issues in empirically testing such a hypothesis given the relatively inflexible nature of the human sleep-wake cycle and circadian rhythm, which is approximately 24 hours. Therefore, while the notion that time awake could potentially offer similar benefits as sleep (though requiring an extended period), it would be difficult to assess without considering sleep deprivation paradigms, which bring along their own range of methodological issues for studying memory and consolidation. However, brain activity associated with wakefulness could provide preliminary support for such a hypothesis. Recent studies have shown that many areas of the default mode network (DMN) likely overlap with areas activated during slow wave activity (Murphy

et al., 2009), and these areas have for instance been shown to be recruited during specific stages of sleep (Spoormaker et al., 2010; Genzel et al., 2013). Moreover, it is well known that electrophysiology during sleep and wakefulness share overlapping properties (such as activity in the alpha and theta frequency band; Kahana et al., 1999; Sederberg et al., 2003; Cantero et al., 2003). Together these findings provide compelling evidence suggesting a possible overlap in the recruitment of brain areas and specific neurophysiology between sleep and wakefulness states.

5.4 Nuances of consolidation

Figure 2.9 in Chapter 2 outlined some of the nuances of the consolidation process across sleep and wakefulness, and showed that even in healthy younger participants performing the classic task, processes during both sleep and wakefulness may benefit performance. However, in the case of this task, there was a (significant) preference for consolidation with sleep for the younger participants.

Despite hypothesised similarities between sleep and wakefulness, it may also be that the sleep state offers an expedited or more efficient segue to achieving consolidation that would otherwise take longer or would be less efficient during wakeful states – even for memories that do not necessarily show sleep-dependent effects. This is supported by the behavioural studies in this thesis when considering the two time-of-day groups (AM, PM; see for instance Figures 2.4, 4.5). Importantly, initial sleep offered greater enhancement than an equivalent initial period of wakefulness also for a task that showed a lack of sleep-dependency (Figure 4.5). This supports the hypothesis that sleep may provide a more efficient processing environment than wakefulness for the consolidation of (motor) memories. Considering some of the evidence outlined in

Chapter 1, there may be several reasons for the potential superiority of sleep in memory consolidation.

5.5 Processes supporting the role of sleep in consolidation

Analysis of the underlying neurophysiology during sleep in Chapter 3 demonstrated preliminary evidence for the role of sleep in consolidation in older adults. While older adults showed changes in sleep architecture they still consolidated to a similar extent as younger adults. Consolidation-related improvement was in turn shown to rely heavily on processes occurring during SWS, including measures relating to power density of slow wave activity. That is, both younger and older adults demonstrated recruitment of the same stage of NREM sleep (stage 3), suggesting that, in terms of the adapted task, processes of consolidation in older adults may be comparable to younger controls. However, the present findings also highlighted the emergence of distinct groupings (e.g., based on the presence or absence of SWS), suggesting potential implications for consolidation and behavioural outcomes. Although further work is needed that considers a larger sample size to assess the specific dynamics and implications of such groupings. It may here be relevant to note an apparent inconsistency in the older age group: overall the older participants in Chapter 3 showed a reduction in delta activity in the form of a lower (average) power density measure than the younger controls. However, apart from two participants who did not display any SWS at all, older adults still exhibited subsequent sleep-dependent improvements in motor performance on the adapted task that were comparable to improvements with sleep in the younger controls. Moreover, while only older participants who completely lacked SWS also showed a deficit in sleep-dependent gain

(i.e., no gain/poorer performance), a number of the younger controls showed a worsening in performance after sleep, yet had still experienced SWS and also displayed average power density of slow wave activity. However, it is possible that variability in the *amount* of SWS between participants played a role, and was shown (in section 3.3) to correlate with the size of the sleep-dependent gain. But this does not explain why reduced SWS should lead to an absence in consolidation only in the younger controls, especially since the older group generally had less SWS compared to controls. It may here be that older adults are somehow making more efficient use of their remaining SWS. However, it is important to note that for the adapted task there is a general shift in consolidation process in the younger adults. That is, unlike the sleep-dependent (for young) classic task adopted in Chapter 2, the adapted sequence task is no longer sleep-dependent in the younger controls, and some of the variance in sleep effects seen in the younger group may be accounted for by how much they consolidated during the preceding equivalent period of wakefulness. Therefore a direct comparison in effect sizes between the older (sleep-dependent) and younger (sleep-independent) groups is less meaningful for the adapted task, and differences in consolidation process could account for the discrepancy in the 'sleep architecture-sleep gain' relationship observed between age groups.

A core mechanism of (NREM) sleep is the slow oscillation, which is thought to initiate and orchestrate many sleep phenomena including cortically generated slow waves. A recent model of synaptic downscaling (Tononi & Cirelli, 2003; see section 1.2.2.1) provides compelling evidence implicating slow wave activity (SWA) in homeostatic processes during sleep. Specifically, SWA has been associated with the downscaling of synaptic potentiation. This is thought to result in an increased signal-to-

noise ratio of neuronal networks to facilitate maintenance of an efficient functional environment for further processes, as well as future learning.

A parallel process of sleep is thought to relate to a more 'active' role in memory consolidation (see section 1.2.2.2). Activity during parts of sleep has been linked to the reactivation of 'wake-like' firing patterns (Ji & Wilson, 2007; Lee & Wilson, 2002; O'Neill et al., 2008) and EEG activity, for instance, associated with REM sleep (Sederberg et al., 2003; Cantero et al., 2003), and is thought to be a more global process involving information transfer across distributed networks and areas (Genzel et al., 2013). There is also convincing evidence to support the role of specific sleep activity in up-regulating LTP-related genes and facilitating synaptic potentiation (e.g., Ribeiro et al., 1999). Synaptic potentiation was previously thought to be primarily associated with wakefulness due to the involvement of the noradrenergic system (Silva, 2003; Cirelli & Tononi, 2000).

Together, a number of functional mechanisms associated with sleep are likely involved in supporting the processes of memory consolidation, some of the most important of which include slow wave activity. The present thesis has primarily considered slow wave activity during NREM stages of slow wave sleep (particularly, stage 3), which was shown to be significantly correlated with improved behavioural outcome following sleep consolidation. The work in this thesis also supports the potential role of localised increases in SWA after learning. For instance, findings from Chapter 3 showed a significant effect of power density of slow wave activity in particular within the learning hemisphere. Hemispheric differences were not found for the incidence of slow waves, suggesting that morphological changes in slow activity may be more important for consolidation. Chapter 3 also showed age-related changes in

slow wave activity highlighted by the reduction in slow wave power density with ageing. This finding is consistent with previous reports (e.g., Colrain et al., 2010).

Future research is needed that adopts converging measures, such as high-density EEG and source-based modelling together with functional imaging techniques to assess both local and global dynamics of synchronicity and connectivity within the same participant samples. This may also elucidate the temporal sequence of key sleep processes and how they relate to memory consolidation. In addition to correlational approaches, sleep studies are gradually adopting more experimental techniques to facilitate particular sleep processes such as reactivation. For instance, recent studies have shown that it may be possible to experimentally reactivate memories during sleep by presenting an auditory or odour cue that was presented during learning (Antony et al., 2012; Bendor & Wilson, 2012; Rasch et al., 2007). However, it is unclear how underlying sleep mechanisms including spindles and slow wave activity support consolidation processes such as memory reactivation. Studies that adopt similar experimental paradigms to facilitate sleep processes are needed to further clarify the relative contribution of such mechanisms to consolidation and, importantly, characterise specific interactions between them.

5.6 Variability in the neurochemical underpinnings of motor learning

Chapter 4 highlighted the importance of inter-individual differences in neurochemistry and motor learning shown, for instance, by the large degree of variability in GABA trajectories across the learning period (including the presence of GABA increases during learning for a number of participants). Such differences could, for instance, relate to individual differences in motor skill (see section 4.4). However,

despite this variability findings showed a trend for a correlation between the change in GABA and learning after 15-20 minutes. That is, better performance during initial practice was associated with less of a GABA decrease. However, caution must be taken in interpreting such group-level averages in light of the large individual differences, and further research is needed to consider underlying reasons for observed variability in the trajectory of GABA change.

Further research would also benefit from adopting brain imaging techniques like MRS to look at neurochemical changes across a range of motor learning tasks. For example, while the paradigm adopted in Chapter 4 allowed me to draw direct comparisons with a previous study (Floyer-Lea et al., 2006), it would be interesting to take both the classic sequence task and the adapted task developed for Chapters 2 and 3 into the scanner to look at GABA changes during a shorter explicit motor learning task shown to facilitate sleep-dependency. An extension of the work in this thesis would also be to consider changes in neurochemistry with ageing, adopting a similar sleep design. However, here it would be particularly interesting to consider longer-term changes in GABAergic processes, for instance, by measuring GABA concentrations before and after a period of consolidation (e.g., measuring GABA during the training session, with shorter follow up scans during retest sessions) to assess changes over time. Although GABA levels returned to baseline levels for most participants by the end of the learning session in the present study, GABAergic processes, at least at the synaptic level, have been shown to be less transitory. For instance, animal studies (e.g., Knott et al., 2002) have previously shown that following whisker stimulation, synaptic density rapidly returned to baseline for excitatory synapses, whereas inhibitory GABAergic synaptic density remained several days following motoric stimulation. This suggests that while excitatory synaptic gains may be a more transitory state (for instance, down-regulated

by homeostatic processes during sleep), changes in GABAergic synapses may linger over longer periods following synaptic potentiation. However, one limitation of the approach is spatial resolution (see section 1.4.9.3) for measuring local synaptic changes, though it is still possible to assess broader changes over time and consolidation. It is thought that MRS may largely reflect extra-synaptic pools of GABA at rest (Stagg et al., 2011), although future work is needed to elucidate the relationship between the MRS technique and synaptic changes with a behavioural intervention.

5.7 General conclusions

In summary, there is evidence suggesting age-related changes in functional sleep architecture, as well as fine motor skill. That is, with older age is associated with increased difficulty in executing movements that require fine kinematic control. The work in this thesis suggests that this in turn reduces the amount of learning older adults demonstrate on a simple sequence learning task, and subsequently reduces the benefits associated with sleep consolidation. This may be a fundamental reason for the lack of observed sleep effects in older adults. The studies in this thesis show that if confounding fine motor constraints are reduced, older adults demonstrate significant sleep-dependent consolidation of motor learning.

That is not to say that there are no differences, overall, in sleep architecture between younger and older adults. The results in Chapter 3 show that on average older adults display a number of changes in sleep activity including reduced slow wave sleep. However, it may be relevant to note that while the majority of older participants demonstrated significant reductions compared to younger controls, it was only those participants that displayed a complete absence of both stage 3 and stage 4 that failed to

consolidate overnight. Therefore, even a relatively small amount of SWS was sufficient to offer a degree of consolidation, although this varied linearly (and positively) with duration.

However, as outlined above, there are a number of sleep processes involved in the consolidation of memories, including local regulatory mechanisms at the synaptic level, as well as potential systems-level information transfer requiring more global synchronous activity. Therefore, it is unlikely that a specific sleep stage or sleep phenomenon, such as slow wave activity, is singularly responsible for the entire consolidation process.

The next challenging steps in sleep research will be to merge these separate strands of research that largely focus on distinct sleep mechanisms in isolation, in order to create testable hypotheses that aim to integrate these processes into a single framework of sleep, how specific processes interact, and how they support consolidation.

6.1 References

Acebo, C., & LeBourgeois, M. K. (2006). Actigraphy. *Respiratory Care Clinics of North America*, *12*(1), 23–30.

Achermann, P. (2009). EEG analysis applied to sleep. *Epileptologie*, *26*, 28–33.

Albouy, G., Fogel, S., Pottiez, H., Nguyen, V. A., Ray, L., Lungu, O., Doyon, J. (2013). Daytime sleep enhances consolidation of the spatial but not motoric representation of motor sequence memory. *PLoS ONE*, *8*(1), e52805.

American Academy of Sleep Medicine (2007). *The AASM manual for the scoring of sleep and associated events: rules, terminology and technical specifications*. Westchester: American Academy of Sleep Medicine.

Amzica, F., & Steriade, M. (1995). Disconnection of intracortical synaptic linkages disrupts synchronization of a slow oscillation. *The Journal of Neuroscience*, *15*(6), 4658–4677.

Amzica, F., & Steriade, M. (1998). Electrophysiological correlates of sleep delta waves. *Electroencephalography and Clinical Neurophysiology*, *107*(2), 69–83.

Anderer, P., Klösch, G., Gruber, G., Trenker, E., Pascual-Marqui, R. D., Zeitlhofer, J., Saletu, B. (2001). Low-resolution brain electromagnetic tomography revealed simultaneously active frontal and parietal sleep spindle sources in the human cortex. *Neuroscience*, *103*(3), 581–592.

Antony, J. W., Gobel, E. W., O'Hare, J. K., Reber, P. J., & Paller, K. A. (2012). Cued memory reactivation during sleep influences skill learning. *Nature Neuroscience*, *15*(8), 1114–1116.

Aserinsky, E., & Kleitman, N. (1953) Regularly occurring periods of eye motility and concomitant phenomena during sleep. *Science*, *118*, 273-4.

Ashendorf, L., Vanderslice-Barr, J. L., & McCaffrey, R. J. (2009). Motor Tests and Cognition in Healthy Older Adults. *Applied Neuropsychology*, *16*(3), 171–176.

Bagai, K., Wakwe, C. I., Malow, B., Black, B. K., Biaggioni, I., Paranjape, S. Y., & Raj, S. R. (2013). Estimation of sleep disturbances using wrist actigraphy in patients with postural tachycardia syndrome. *Autonomic Neuroscience, 177(2)*, 260–265.

Bangert, M., & Schlaug, G. (2006). Specialization of the specialized in features of external human brain morphology. *European Journal of Neuroscience*, *24*(6), 1832–1834.

Bendor, D., & Wilson, M. A. (2012). Biasing the content of hippocampal replay during sleep. *Nature Neuroscience*, *15*(10), 1439–1444.

Berger, H. (1929). Über das Elektroenkelphalogramm des Menschen. *Arch Pyschiatr Nervenkr*, *87*, 527-70.

Blake, H., Gerard, R., Kleitman, N. (1939). Factors including brain potentials during sleep. *J Neurophysiol*, *2*, 48-60.

Bonnet, M. H., & Arand, D. L., 2007. EEG arousal norms by age. *J. Clin. Sleep Med*, *3*(3), 271–274.

Brawn, T. P., Fenn, K. M., Nusbaum, H. C., & Margoliash, D. (2008). Consolidation of sensorimotor learning during sleep. *Learning & Memory*, *15*(11), 815–819.

Brawn, T. P., Fenn, K. M., Nusbaum, H. C., & Margoliash, D. (2010). Consolidating the Effects of Waking and Sleep on Motor-Sequence Learning. *Journal of Neuroscience*, *30*(42), 13977–13982.

Brown, R. M., Robertson, E. M., & Press, D. Z. (2009). Sequence skill acquisition and off-line learning in normal aging. *PloS One*, *4*(8), e6683.

Buysse, D. J., Reynolds, C. F., 3rd, Monk, T. H., Berman, S. R., & Kupfer, D. J. (1989). The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Research*, *28*(2), 193–213.

Cantero, J. L., Atienza, M., Stickgold, R., Kahana, M. J., Madsen, J. R., and Kocsis, B. (2003). Sleep-dependent theta oscillations in the human hippocampus and neocortex. *J. Neurosci., 23*, 10897–10903.

Chauvette, S., Volgushev, M., Timofeev, I. (2010) Origin of active states in local neocortical networks during slow sleep oscillation. *Cereb Cortex, 20*, 2660–2674.

Cirelli, C., & Tononi, G. (2000). Differential Expression of Plasticity-Related Genes in Waking and Sleep and Their Regulation by the Noradrenergic System. *The Journal of Neuroscience*, *20*(24), 9187–9194.

Cohen, N. J., & Squire, L. R. (1980). Preserved learning and retention of pattern analyzing skill in amnesia: Dissociation of knowing how and knowing that. *Science*, *210*, 207–209.

Contreras, D., & Steriade, M. (1995). Cellular basis of EEG slow rhythms: a study of dynamic corticothalamic relationships. *J. Neurosci., 15*, 604–622.

Cohen, D. A., Pascual-Leone, A., Press, D. Z., & Robertson, E. M. (2005). Off-line learning of motor skill memory: a double dissociation of goal and movement. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(50), 18237–18241.

Colrain, I. M., Crowley, K. E., Nicholas, C. L., Afifi, L., Baker, F. C., Padilla, M., … Trinder, J. (2010). Sleep evoked delta frequency responses show a linear decline in amplitude across the adult lifespan. *Neurobiology of Aging*, *31*(5), 874–883.

Colrain, I. M., Webster, K. E., & Hirst, G. (1999). The N550 component of the evoked K-complex: A modality non-specific response? *Journal of Sleep Research*, *8*(4), 273–280.

Crowley, K. (2011). Sleep and sleep disorders in older adults. *Neuropsychology Review*, *21*(1), 41–53.

Crowley, K. E., Colrain, I. M. (2004). A review of the evidence for P2 being an independent component process: age, sleep and modality. *Clin*. *Neurophysiol*., *115*(4), 732–744.

Crowley, K., Trinder, J., Kim, Y., Carrington, M., & Colrain, I. M. (2002). The effects of normal aging on sleep spindle and K-complex production. *Clinical Neurophysiology*, *113*(10), 1615–1622.

Danker-Hopfe, H., Kunz, D., Gruber, G., Klösch, G., Lorenzo, J. L., Himanen, S. L., … Dorffner, G. (2004). Interrater reliability between scorers from eight European sleep laboratories in subjects with different sleep disorders. *Journal of Sleep Research*, *13*(1), 63–69.

De Gennaro, L., Ferrara, M., Bertini, M. (2000). Topographical distribution of spindles: variations between and within NREM sleep cycles. *Sleep Res, 3*, 155-60.

Dement, W., Kleitman, N. (1957). Cyclic variations in EEG during sleep and their relation to eye movements, body motility, and dreaming. *Clin Neurophysiol, 9*, 673-90.

De Souza, L., Benedito-Silva, A. A., Pires, M. L. N., Poyares, D., Tufik, S., & Calil, H. M. (2003). Further validation of actigraphy for sleep studies. *Sleep, 26(1)*, 81–85.

Destexhe, A., Hughes, S. W., Rudolph, M., & Crunelli, V. (2007). Are corticothalamic "up" states fragments of wakefulness? *Trends in Neurosciences*, *30*(7), 334–342.

Diekelmann, S., Wilhelm, I., & Born, J. (2009). The whats and whens of sleep-dependent memory consolidation. *Sleep Medicine Reviews*, *13*(5), 309–321.

Doyon, J., Bellec, P., Amsel, R., Penhune, V., Monchi, O., Carrier, J., Benali, H. (2009). Contributions of the basal ganglia and functionally related brain structures to motor learning. *Behavioural Brain Research*, *199*(1), 61–75.

Doyon, J., Song, A. W., Karni, A., Lalonde, F., Adams, M. M., Ungerleider, L. G. (2002). Experience-dependent changes in cerebellar contributions to motor sequence learning. *Proc. Natl. Acad. Sci., 99*(2), 1017–1022.

Ehlers, C. L., Kupfer, D. J. (1989). Effects of age on delta and REM sleep parameters. *Electroencephalogr Clin Neurophysio, 72*, 118-25.

Ehlers, C., & Kupfer, D. (1997). Slow-wave sleep: do young adult men and women age differently? *Journal of Sleep Research*, *6*(3), 211–215.

Feinberg, I. (1974). Changes in sleep cycle patterns with age. *J Psychiatr Res, 10*, 283-306.

Feinberg, I., & Campbell, I. G. (2003). Kinetics of non-rapid eye movement delta production across sleep and waking in young and elderly normal subjects: theoretical implications. *Sleep*, *26*(2), 192–200.

Fischer, S., Hallschmid, M., Elsner, A. L., & Born, J. (2002). Sleep forms memory for finger skills. *Proceedings of the National Academy of Sciences*, *99*(18), 11987–11991.

Fishbein, W. (1971). Disruptive effects of rapid eye movement sleep deprivation on long-term memory. *Physiol. Behav., 6*, 279–282.

Floyer-Lea, A., Matthews, P. M., 2005. Distinguishable brain activation networks for short- and long-term motor skill learning. *J. Neurophysiol., 94*(1), 512–518.

Floyer-Lea, A., Wylezinska, M., Kincses, T., & Matthews, P. M. (2006). Rapid Modulation of GABA Concentration in Human Sensorimotor Cortex During Motor Learning. *Journal of Neurophysiology*, *95*(3), 1639–1644.

Fogel, S. M., & Smith, C. T. (2006). Learning-dependent changes in sleep spindles and Stage 2 sleep. *Journal of Sleep Research*, *15*(3), 250–255.

Fogel, S. M., Smith, C. T., & Cote, K. A. (2007). Dissociable learning-dependent changes in REM and non-REM sleep in declarative and procedural memory systems. *Behavioural Brain Research*, *180*(1), 48–61.

Fogel, S., Martin, N., Lafortune, M., Barakat, M., Debas, K., Laventure, S., Carrier, J. (2012). NREM sleep oscillations and brain plasticity in aging. *Frontiers in Sleep and Chronobiology*, *3*, 176.

Fraser, S. A., Li, K. Z. H., & Penhune, V. B. (2009). A comparison of motor skill learning and retention in younger and older adults. *Exp Brain Res, 195*, 419-427.

Gais, S., Plihal, W., Wagner, U., & Born, J. (2000). Early sleep triggers memory for early visual discrimination skills. *Nature Neuroscience*, *3*(12), 1335–1339.

Gallicchio, L., & Kalesan, B. (2009). Sleep duration and mortality: a systematic review and meta-analysis. *Journal of Sleep Research*, *18*(2), 148–158.

Genzel, L., Kroes, M. C. W., Dresler, M., & Battaglia, F. P. (2013). Light sleep versus slow wave sleep in memory consolidation: a question of global versus local processes? *Trends in Neurosciences*, 1-10.

Girardeau, G., Benchenane, K., Wiener, S. I., Buzsáki, G., & Zugaro, M. B. (2009). Selective suppression of hippocampal ripples impairs spatial memory. *Nature Neuroscience*, *12*(10), 1222–1223.

Good, C. D., Johnsrude, I. S., Ashburner, J., Henson, R. N. A., Friston, K. J., & Frackowiak, R. S. J. (2001). A Voxel-Based Morphometric Study of Ageing in 465 Normal Adult Human Brains. *NeuroImage*, *14*(1), 21–36.

Graf, P., & Schacter, D. L. (1985). Implicit and explicit memory for new associations in normal and amnesic subjects. Journal of Experimental Psychology: *Learning, Memory and Cognition, 13*, 45–53.

Grafton, S. T., Hazeltine, E., & Ivry, R. B. (1998). Abstract and Effector-Specific Representations of Motor Sequences Identified with PET. *The Journal of Neuroscience*, *18*(22), 9420–9428.

Hamming, W. (1989). *Digital filters*, 3rd edn. Englewood Cliffs: Prentice Hall.

Hanlon, E. C., Faraguna, U., Vyazovskiy, V. V., Tononi, G., & Cirelli, C. (2009). Effects of Skilled Training on Sleep Slow Wave Activity and Cortical Gene Expression in the Rat. *Sleep*, *32*(6), 719–729.

Hebb, D. O. 1942. The effects of early and late brain injury upon test scores, and the nature of normal adult intelligence. *Proc. Am. Philos. Soc.*, 85, 275–292.

Helmholtz, H. L. F. (1853). Ueber einige Gesetze der Vertheilung elektrischer Strome in korperlichen Leitern mit Anwendung aud die thierisch-elektrischen Versuche. *Ann Physik und Chemie*, *9*, 211–33.

Hikosaka, O., Nakahara, H., Rand, M. K., Sakai, K., Lu, X., Nakamura, K., Doya, K. (1999). Parallel neural networks for learning sequential procedures. *Trends in Neurosciences*, *22*(10), 464–471.

Hikosaka, O., Nakamura, K., Sakai, K., & Nakahara, H. (2002). Central mechanisms of motor skill learning. *Current Opinion in Neurobiology*, *12*(2), 217–222.

Hishikawa, Y., & Shimizu, T. (1995). Physiology of REM sleep, cataplexy, and sleep paralysis. *Advances in Neurology*, *67*, 245–271.

Hoddes, E., Zarcone, V., Smythe, H., Phillips, R., & Dement, W. C. (1973). Quantification of Sleepiness: A New Approach. *Psychophysiology*, *10*(4), 431–436.

Hoffman, K. L., Battaglia, F. P., Harris, K., MacLean, J. N., Marshall, L., & Mehta, M. R. (2007). The Upshot of Up States in the Neocortex: From Slow Oscillations to Memory Formation. *The Journal of Neuroscience*, *27*(44), 11838–11841.

Huang, Y.-L., Liu, R.-Y., Wang, Q.-S., Van Someren, E. J. W., Xu, H., & Zhou, J.-N. (2002). Age-associated difference in circadian sleep–wake and rest–activity rhythms. *Physiology & Behavior*, *76*(4–5), 597–603.

Huber, R., Ghilardi, M. F., Massimini, M., Ferrarelli, F., Riedner, B. A., Peterson, M. J., & Tononi, G. (2006). Arm immobilization causes cortical plastic changes and locally decreases sleep slow wave activity. *Nature Neuroscience*, *9*(9), 1169–1176.

Huber, R., Ghilardi, M. F., Massimini, M., & Tononi, G. (2004). Local sleep and learning. *Nature*, *430*(6995), 78–81.

James, W. (1890). *Principles of psychology*. New York: Holt.

Jasper, H. H. (1999). The ten twenty electrode system of the international federation. *Electroencephalography and Clinical Neurophysiology*, *52*, 371-375.

Ji, D. and Wilson, M. A. (2007). Coordinated memory replay in the visual cortex and hippocampus during sleep. *Nat. Neurosci., 10*, 100–107

Kahana, M. J., Sekuler, R., Caplan., J. B., Kirschen, M., Madsen, J. R. (1999). Human theta oscillations exhibit dependence during virtual maze navigation. *Nature*, *399*(24), 781-784.

Karni, A., Meyer, G., Jezzard, P., Adams, M. M., Turner, R., & Ungerleider, L. G. (1995). Functional MRI evidence for adult motor cortex plasticity during motor skill learning. *Nature*, *377*(6545), 155–158.

Karni, A., Tanne, D., Rubenstein, B.S., Askenasy, J.J., and Sagi, D. 1994. Dependence on REM sleep of overnight improvement of a perceptual skill. *Science, 265*, 679–682.

Keisler, A., & Willingham, D. T. (2007). Non-Declarative Sequence Learning does not Show Savings in Relearning. *Human Movement Science*, *26*(2), 247–256.

Kemp, N., & Bashir, Z. I. (1997). A role for adenosine in the regulation of long-term depression in the adult rat hippocampus in vitro. *Neuroscience Letters*, *225*(3), 189–192.

Kim, A., Latchoumane, C., Lee, S., Kim, G. B., Cheong, E., Augustine, G. J., & Shin, H.-S. (2012). Optogenetically induced sleep spindle rhythms alter sleep architectures in mice. *Proceedings of the National Academy of Sciences*, *109*(50), 20673–20678.

King, B. R., Fogel, S. M., Albouy, G., & Doyon, J. (2013). Neural correlates of the age-related changes in motor sequence learning and motor adaptation in older adults. *Frontiers in Human Neuroscience*, *7*, 1-13.

Klass, M., Baudry, S., & Duchateau, J. (2007). Voluntary activation during maximal contraction with advancing age: a brief review. *European Journal of Applied Physiology*, *100*(5), 543–551.

Knott, G. W., Quairiaux, C., Genoud, C., & Welker, E. (2002). Formation of dendritic spines with GABAergic synapses induced by whisker stimulation in adult mice. *Neuron*, *34*(2), 265–273.

Korman, M., Doyon, J., Doljansky, J., Carrier, J., Dagan, Y., & Karni, A. (2007). Daytime sleep condenses the time course of motor memory consolidation. *Nature Neuroscience*, *10*(9), 1206–1213.

Krueger, J. M., & Obál, F. (1993). A neuronal group theory of sleep function. *Journal of Sleep Research*, *2*(2), 63–69.

Kuriyama, K., Stickgold, R., & Walker, M. P. (2004). Sleep-dependent learning and motor-skill complexity. *Learning & Memory*, *11*(6), 705–713.

Lancel, M. (1999). Role of GABAA receptors in the regulation of sleep: initial sleep responses to peripherally administered modulators and agonists. *Sleep*, *22*(1), 33–42.

Landsness, E. C., Crupi, D., Hulse, B. K., Peterson, M. J., Huber, R., Ansari, H., Tononi, G. (2009). Sleep-dependent improvement in visuomotor learning: a causal role for slow waves. *Sleep*, *32*(10), 1273–1284.

Lee, A. K. and Wilson, M. A. (2002). Memory of sequential experience in the hippocampus during slow wave sleep. *Neuron, 36*, 1183–1194.

Lehéricy, S., Benali, H., Van deMoortele, P.-F., Pélégrini-Issac,M., Waechter, T., Ugurbil, K., Doyon, J. (2005). Distinct basal ganglia territories are engaged in early and advanced motor sequence learning. *Proc. Natl. Acad. Sci., 102*(35), 12566–12571.

Lemaître, H., Crivello, F., Grassiot, B., Alpérovitch, A., Tzourio, C., & Mazoyer, B. (2005). Age- and sex-related effects on the neuroanatomy of healthy elderly. *NeuroImage*, *26*(3), 900–911.

Lemaitre, H., Goldman, A. L., Sambataro, F., Verchinski, B. A., Meyer-Lindenberg, A., Weinberger, D. R., & Mattay, V. S. (2012). Normal age-related brain

morphometric changes: nonuniformity across cortical thickness, surface area and gray matter volume? *Neurobiology of Aging*, *33*(3), 617.e1–617.e9.

Linden, E. R., Bern, D., and Fishbein, W. (1975). Retrograde amnesia: Prolonging the fixation phase of memory consolidation by paradoxical sleep deprivation. *Physiol*. *Behav*., *14*, 409–412.

Loomis, A. L, Harvey, E. N., Hobart, G. A. (1937). Cerebral states during sleep, as studied by human brain potentials. *J Exp Psychol, 21*, 127–44.

Maine de Biran, F. P. G. (1929). *The influence of habit on the faculty of thinking*. Baltimore: Williams & Wilkins.

Mander, B. A., Rao, V., Lu, B., Saletin, J. M., Lindquist, J. R., Ancoli-Israel, S., Walker, M. P. (2013). Prefrontal atrophy, disrupted NREM slow waves and impaired hippocampal-dependent memory in aging. *Nature Neuroscience,* 1-10.

Marchand, W. R., Lee, J. N., Suchy, Y., Garn, C., Johnson, S., Wood, N., & Chelune, G. (2011). Age-related changes of the functional architecture of the cortico-basal ganglia circuitry during motor task execution. *NeuroImage*, *55*(1), 194–203.

Marr, D. (1971). Simple memory: a theory for archicortex. *Philos Trans R Soc Lond B Biol Sci, 262*, 23– 81.

Marmon, A. R., Pascoe, M. A., Schwartz, R. S., & Enoka, R. M. (2011). Associations among strength, steadiness, and hand function across the adult life span. *Medicine & Science in Sports & Exercise*, *43*(4), 560–567.

Marneweck, M., Loftus, A., & Hammond, G. (2011). Short-interval intracortical inhibition and manual dexterity in healthy aging. *Neuroscience Research*, *70*(4), 408–414.

Marshall, L., Helgadóttir, H., Mölle, M., & Born, J. (2006). Boosting slow oscillations during sleep potentiates memory. *Nature*, *444*(7119), 610–613.

Massimini, M., Ferrarelli, F., Esser, S. K., Riedner, B. A., Huber, R., Murphy, M., Peterson, M. J., Tononi, G. (2007). Triggering sleep slow waves by transcranial magnetic stimulation. *Proc Natl Acad Sci USA, 104*, 8496–8501.

Massimini, M., Huber, R., Ferrarelli, F., Hill, S., & Tononi, G. (2004). The Sleep Slow Oscillation as a Traveling Wave. *The Journal of Neuroscience*, *24*(31), 6862–6870.

Mattay, V. S., Fera, F., Tessitore, A., Hariri, A. R., Das, S., Callicott, J. H., & Weinberger, D. R. (2002). Neurophysiological correlates of age-related changes in human motor function. *Neurology*, *58*(4), 630–635.

McClelland, J. L., McNaughton, B. L., O'Reilly, R. C. (1995). Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. *Psychol Rev, 102*, 419–457.

McCormick, L., Nielsen, T., Nicolas, A., Ptito, M., Montplaisir, J. (1997). Topographical distribution of spindles and K-complexes in normal subjects. *Sleep*, *20*, 939-41.

Mednick, S., Nakayama, K., & Stickgold, R. (2003). Sleep-dependent learning: a nap is as good as a night. *Nature Neuroscience*, *6*(7), 697–698.

Meerlo, P., de Bruin, E. A., Strijkstra, A. M., & Daan, S. (2001). A social conflict increases EEG slow-wave activity during subsequent sleep. *Physiology & Behavior*, *73*(3), 331–335.

Mescher, M., Tannus, A., Johnson, M. O., Garwood, M. (1996). Solvent suppression using selective echo dephasing. *J. Magn. Reson. Ser., 123*(2), 226–229.

Michel, C. M., Murray, M. M., Lantz, G., Gonzalez, S., Spinelli, L., & Grave de Peralta, R. (2004). EEG source imaging. *Clinical Neurophysiology*, *115*(10), 2195–2222.

Milner, B. (1962). *Les troubles de la memoire accompagnant des lesions hippocampiques bilaterales*. In Physiologie de hippocampe (pp. 257–272). Paris: Centre National de la Recherche Scientifique. English translation: B. Milner & S. Glickman (Eds.). Princeton: Van Nostrand, 1965 (pp. 97–111).

Mullins, P. G., McGonigle, D. J., O'Gorman, R. L., Puts, N. A. J., Vidyasagar, R., Evans, C. J., Edden. (2012). Current practice in the use of MEGA-PRESS spectroscopy for the detection of GABA. *NeuroImage*, *86*, 43-52.

Murphy, M., Riedner, B. A., Huber, R., Massimini, M., Ferrarelli, F., & Tononi, G. (2009). Source modeling sleep slow waves. *Proceedings of the National Academy of Sciences*, *106*(5), 1608–1613.

Naccarato, M., Calautti, C., Jones, P. S., Day, D. J., Carpenter, T. A., & Baron, J.-C. (2006). Does healthy aging affect the hemispheric activation balance during paced index-to-thumb opposition task? An fMRI study. *NeuroImage*, *32*(3), 1250–1256.

Nicolas, A., Petit, D., Rompré, S., & Montplaisir, J. (2001). Sleep spindle characteristics in healthy subjects of different age groups. *Clinical Neurophysiology*, *112*(3), 521–527.

Nishida, M., & Walker, M. P. (2007). Daytime Naps, Motor Memory Consolidation and Regionally Specific Sleep Spindles. *PLoS One*, *2*(4), e341.

Nitsche, M. A., Jakoubkova, M., Thirugnanasambandam, N., Schmalfuss, L., Hullemann, S., Sonka, K., Happe, S. (2010). Contribution of the Premotor Cortex to Consolidation of Motor Sequence Learning in Humans During Sleep. *Journal of Neurophysiology*, *104*(5), 2603–2614.

Ohayon, M. M., Carskadon, M. A., Guilleminault, C., & Vitiello, M. V. (2004). Meta-analysis of quantitative sleep parameters from childhood to old age in healthy

individuals: developing normative sleep values across the human lifespan. *Sleep*, *27*(7), 1255–1273.

O'Neill, J. et al. (2008) Reactivation of experience-dependent cell assembly patterns in the hippocampus. *Nat. Neurosci., 11*, 209–215

Orban, P., Peigneux, P., Lungu, O., Debas, K., Barakat, M., Bellec, P., Doyon, J. (2011). Functional neuroanatomy associated with the expression of distinct movement kinematics in motor sequence learning. *Neuroscience*, *179*, 94–103.

Pace-Schott,. E. F. (2009). *Sleep architecture*. In The Neuroscience of Sleep (pp 1-337), Eds Stickgold, R. and Walker, M. 2009. San Diego: Academic Press.

Packard, M. G., & McGaugh, J. L. (1996). Inactivation of Hippocampus or Caudate Nucleus with Lidocaine Differentially Affects Expression of Place and Response Learning. *Neurobiology of Learning and Memory*, *65*(1), 65–72.

Paquet, J., Kawinska, A., & Carrier, J. (2007). Wake Detection Capacity of Actigraphy During Sleep. *Sleep, 30(10)*, 1362–1369.

Pascual-Marqui, R. D. (1999). Review of methods for solving the EEG inverse problem. *International Journal of Bioelectromagnetism, 1*, 75–86.

Peigneux, P., Orban, P., Balteau, E., Degueldre, C., Luxen, A., et al. (2006). Offline persistence of memory-related cerebral activity during active wakefulness. *PLoS Biol, 4*(4): e100.

Peyrache, A., Khamassi, M., Benchenane, K., Wiener, S. I., & Battaglia, F. P. (2009). Replay of rule-learning related neural patterns in the prefrontal cortex during sleep. *Nature Neuroscience*, *12*(7), 919–926.

Phillips, B., & Ancoli-Israel, S. (2001). Sleep disorders in the elderly. *Sleep Medicine*, *2*(2), 99–114.

Plagenhoef, S., Evans, F. G., & Abdelnour, T. (1983). Anatomical Data for Analyzing Human Motion. *Research Quarterly for Exercise and Sport*, *54*(2), 169–178.

Ranganathan, V. K., Siemionow, V., Sahgal, V., & Yue, G. H. (2001). Effects of Aging on Hand Function. *Journal of the American Geriatrics Society*, *49*(11), 1478–1484.

Rasch, B., & Born, J. (2013). About sleep's role in memory. *Physiological Reviews*, *93*(2), 681–766.

Rasch, B., Buchel, C., Gais, S., & Born, J. (2007). Odor cues during slow-wave sleep prompt declarative memory consolidation. *Science*, *315*(5817), 1426–1429.

Raz, N., Gunning, F. M., Head, D., Dupuis, J. H., McQuain, J., Briggs, S. D., … Acker, J. D. (1997). Selective aging of the human cerebral cortex observed in Vivo: Differential vulnerability of the prefrontal gray matter. *Cerebral Cortex*, *7*(3), 268–282.

Raz, N., Lindenberger, U., Rodrigue, K. M., Kennedy, K. M., Head, D., Williamson, A., … Acker, J. D. (2005). Regional brain changes in aging healthy adults: general trends, individual differences and modifiers. *Cerebral Cortex*, *15*(11), 1676–1689.

Ribeiro, S., Goyal, V., Mello, C. V., & Pavlides, C. (1999). Brain gene expression during rem sleep depends on prior waking experience. *Learning & Memory*, *6*(5), 500–508.

Ribeiro, S., & Nicolelis, M. A. L. (2004). Reverberation, storage, and postsynaptic propagation of memories during sleep. *Learning & Memory*, *11*(6), 686–696.

Riecker, A., Gröschel, K., Ackermann, H., Steinbrink, C., Witte, O., & Kastrup, A. (2006). Functional significance of age-related differences in motor activation patterns. *NeuroImage*, *32*(3), 1345–1354.

Robertson, E. (2009). From creation to consolidation: a novel framework for memory processing. PLoS Biology, 7(1), 0011-0019.

Robertson, E. M., & Cohen, D. A. (2006). Understanding Consolidation through the Architecture of Memories. *The Neuroscientist*, *12*(3), 261–271.

Robertson, E. M., Pascual-Leone, A., & Press, D. Z. (2004). Awareness modifies the skill-learning benefits of sleep. *Current Biology: CB*, *14*(3), 208–212.

Rogers, A. E., Caruso, C. C., & Aldrich, M. S. (1993). Reliability of sleep diaries for assessment of sleep/wake patterns. *Nursing Research*, *42*(6), 368–372.

Sadeh, A., & Acebo, C. (2002). The role of actigraphy in sleep medicine. *Sleep Medicine Reviews*, *6*(2), 113–124.

Salat, D. H., Buckner, R. L., Snyder, A. Z., Greve, D. N., Desikan, R. S. R., Busa, E., Fischl, B. (2004). Thinning of the Cerebral Cortex in Aging. *Cerebral Cortex*, *14*(7), 721–730.

Sandyk, R. & Gillman, M. A. (1985). Baclofen-induced memory impairment. *Clin Neuropharmacol*, *8*, 194–195.

Sanei, S., & Chambers., J. A. (2007). *EEG signal processing*. West Sussex: John Wiley & Sons, Ltd.

Sarajishvili, P. M., Geladze, T. S., Bibileishvili, S. I., Shubladze, G. N., Squire, L. R. (1992). Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psychol Rev*, *99*, 195–231.

Sederberg, P. B., Kahana, M. J., Howard, M. W., Donner, E. J., & Madsen, J. R. (2003). Theta and gamma oscillations during encoding predict subsequent recall. *J. Neurosci*., *23*, 10809–10814.

Seidler, R. D., Bernard, J. A., Burutolu, T. B., Fling, B. W., Gordon, M. T., Gwin, J. T., Lipps, D. B. (2010). Motor control and aging: Links to age-related brain

structural, functional, and biochemical effects. *Neuroscience & Biobehavioral Reviews*, *34*(5), 721–733.

Silber, M. H., Ancoli-Israel, S., Bonnet, M. H., Chokroverty, S., Grigg-Damberger, M. M., Hirshkowitz, M., Iber, C. (2007). The visual scoring of sleep in adults. *Journal of Clinical Sleep Medicine: JCSM: Official Publication of the American Academy of Sleep Medicine*, *3*(2), 121–131.

Silva, A. J. (2003). Molecular and cellular cognitive studies of the role of synaptic plasticity in memory. *Journal of Neurobiology*, *54*(1), 224–237.

Smith, C. D., Chebrolu, H., Wekstein, D. R., Schmitt, F. A., & Markesbery, W. R. (2007). Age and gender effects on human brain anatomy: A voxel-based morphometric study in healthy elderly. *Neurobiology of Aging*, *28*(7), 1075–1087.

Smith, J. R., Karacan, I., & Yang, M. (1977). Ontogeny of delta activity during human sleep. *Electroencephalography and Clinical Neurophysiology*, *43*(2), 229–237.

Smith, C., & Kelly, G. (1988). Paradoxical sleep deprivation applied 2 days after end of training retards learning. *Physiol. Behav.*, *43*, 213–216.

Soer, R., Brouwer, S., Geertzen, J. H., van der Schans, C. P., Groothoff, J. W., & Reneman, M. F. (2012). Decline of Functional Capacity in Healthy Aging Workers. *Archives of Physical Medicine and Rehabilitation*, *93*(12), 2326–2332.

Sohn, Y. H., & Hallett, M. (2004). Surround inhibition in human motor system. *Experimental Brain Research*, *158*(4), 397–404.

Spencer, R. M. C., Gouw, A. M., & Ivry, R. B. (2007). Age-related decline of sleep-dependent consolidation. *Learning & Memory*, *14*(7), 480–484.

Spoormaker, V. I., Schröter, M. S., Gleiser, P. M., Andrade, K. C., Dresler, M., Wehrle, R., Czisch, M. (2010). Development of a Large-Scale Functional Brain Network

during Human Non-Rapid Eye Movement Sleep. *The Journal of Neuroscience*, *30*(34), 11379–11387.

Stagg, C. J., Bachtiar, V., & Johansen-Berg, H. (2011). What are we measuring with GABA magnetic resonance spectroscopy? *Communicative & Integrative Biology*, *4*(5), 573–575.

Steele, C. J., Scholz, J., Douaud, G., Johansen-Berg, H., & Penhune, V. B. (2012). Structural correlates of skilled performance on a motor sequence task. *Frontiers in Human Neuroscience*, *6*, 289.

Steele, C. J., & Penhune, V. B. (2010). Specific increases within global decreases: a functional magnetic resonance imaging investigation of five days of motor sequence learning. *J. Neurosci., 30*, 8332–8341.

Stefan, D., Di Cesare, F., Andrasescu, A., Popa, E., Lazariev, A., Vescovo, E., Strbak, O., Williams, S., Starcuk, Z., Cabanas, M., van Ormondt, D., Graveron-Demilly. D. (2009). Quantitation of magnetic resonance spectroscopy signals: the jMRUI software package. *Measurement Science and Technology, 20*, 104035-104043.

Steriade, M., Nunez, A., & Amzica, F. (1993). A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. *Journal of Neuroscience*, *13*(8), 3252–3265.

Stickgold, R. (2009). How do I remember? Let me count the ways. *Sleep Medicine Reviews, 13*, 305-308.

Stickgold, R., James, L., & Hobson, A. J. (2000). Visual discrimination learning requires sleep after training. *Nature Neuroscience*, *3*(12), 1237–1238.

Stickgold, R., & Walker, M. P. (2009). *The neuroscience of sleep*. San Diego: Academic Press.

Sumner, P., Edden, R. A. E., Bompas, A., Evans, C. J., & Singh, K. D. (2010). More GABA, less distraction: a neurochemical predictor of motor decision speed. *Nature Neuroscience*, *13*(7), 825–827.

Swartzwelder, H. S., Tilson, H. A., McLamb, R. L., & Wilson, W. A. (1987). Baclofen disrupts passive avoidance retention in rats. *Psychopharmacology*, *9*, 398–401.

Tononi, G., & Cirelli, C. (2003). Sleep and synaptic homeostasis: a hypothesis. *Brain Research Bulletin*, *62*(2), 143–150.

Tononi, G., & Cirelli, C. (2006). Sleep function and synaptic homeostasis. *Sleep Medicine Reviews*, *10*(1), 49–62.

Trepel, C., & Racine, R. J. (2000). GABAergic modulation of neocortical long-term potentiation in the freely moving rat. *Synapse*, *35*(2), 120–128.

Tucker, M., McKinley, S., & Stickgold, R. (2011). Sleep optimizes motor skill in older adults. *Journal of the American Geriatrics Society*, *59*(4), 603–609.

Van Der Werf, Y. D., Altena, E., Vis, J. C., Koene, T., & Van Someren, E. J. W. (2011). Reduction of nocturnal slow-wave activity affects daytime vigilance lapses and memory encoding but not reaction time or implicit learning. *Progress in Brain Research*, *193*, 245–255.

Ventouras, E. M., Ktonas, P. Y., Tsekou, H., Paparrigopoulos, T., Kalatzis, I., & Soldatos, C. R. (2010). Independent Component Analysis for Source Localization of EEG Sleep Spindle Components. *Computational Intelligence and Neuroscience*, *2010*.

Verwey, W. B., & Wright, D. L. (2004). Effector-independent and effector-dependent learning in the discrete sequence production task. *Psychological Research*, *68*(1), 64–70.

Voineskos, A. N., Rajji, T. K., Lobaugh, N. J., Miranda, D., Shenton, M. E., Kennedy, J. L., Mulsant, B. H. (2012). Age-related decline in white matter tract integrity and cognitive performance: A DTI tractography and structural equation modeling study. *Neurobiology of Aging*, *33*(1), 21–34.

Wagner, J. J., & Alger, B. E. (1995). GABAergic and developmental influences on homosynaptic LTD and depotentiation in rat hippocampus. *The Journal of Neuroscience*, *15*(2), 1577–1586.

Walker, M. P., Brakefield, T., Hobson, A. J., & Stickgold, R. (2003). Dissociable stages of human memory consolidation and reconsolidation. *Nature*, *425*(6958), 616–620.

Walker, M. P., Brakefield, T., Morgan, A., Hobson, A. J., & Stickgold, R. (2002). Practice with Sleep Makes Perfect. *Neuron*, *35*(1), 205–211.

Walker, M. P., Stickgold, R., Alsop, D., Gaab, N., & Schlaug, G. (2005). Sleep-dependent motor memory plasticity in the human brain. *Neuroscience*, *133*(4), 911–917.

Whitney, C. W., Gottlieb, D. J., Redline, S., et al. (1998). Reliability of scoring respiratory disturbance indices and sleep staging. *Sleep, 21*, 749-57.

Wilson, J. K., Baran, B., Pace-Schott, E. F., Ivry, R. B., & Spencer, R. (2012). Sleep modulates word-pair learning but not motor sequence learning in healthy older adults. *Neurobiology of Aging, 33*, 991-1000.

Yousry, T. A., Schmid, U. D., Alkadhi, H., Peraud, A., Buettner, A., & Winkler, P. (1997). Localization of the motor hand area to a knob on the precentral gyrus: a new landmark. *Brain, 120*, 141-157.

Appendix

Pittsburgh Sleep Quality Index

Subject code:_____ STUDY: _____

Date: _____

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 the questions.

1) During the past month, when have you usually gone to bed at night?

Usual bedtime:

2) During the past month, how long (in minutes) has it usually take you to fall asleep each night?

Number of minutes:

3) During the past month, when have you usually got up in the morning?

Usual getting up time:

4) During the past month, how many hours of actual sleep did you get at night? (This may be different from the number of hours spent in bed.)

Hours of sleep per night:

For each of the remaining questions, tick the ONE best response. Please answer all questions.

5) During the past month, how often have you had trouble sleeping because you…

a) Cannot get to sleep within 30 minutes

Not during the past month____	Less than once a week____	Once or twice a week____	Three or more times a week____

b) Wake up in the middle of the night or early morning

Not during the past month____	Less than once a week____	Once or twice a week____	Three or more times a week____

c) Have to get up to use the bathroom

Not during the past month____	Less than once a week____	Once or twice a week____	Three or more times a week____

d) Cannot breathe comfortably

Not during the past month____	Less than once a week____	Once or twice a week____	Three or more times a week____

e) Cough or snore loudly

Not during the past month____	Less than once a week____	Once or twice a week____	Three or more times a week____

f) Feel too cold

Not during the past month____	Less than once a week____	Once or twice a week____	Three or more times a week____

g) Feel too hot

Not during the past month____	Less than once a week____	Once or twice a week____	Three or more times a week____

h) Had bad dreams

Not during the past month____	Less than once a week____	Once or twice a week____	Three or more times a week____

i) Have pain

Not during the past month____	Less than once a week____	Once or twice a week____	Three or more times a week____

j) Other reason(s), please describe

_____

_____

How often during the past month have you had trouble sleeping because of this?

Not during the past month____	Less than once a week____	Once or twice a week____	Three or more times a week____

6) During the past month, how would you rate your sleep quality overall?

Very good_____

Fairly good_____

Fairly bad_____

Very bad_____

7) During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?

Not during the past month____	Less than once a week____	Once or twice a week____	Three or more times a week____

8) During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?

Not during the past month____	Less than once a week____	Once or twice a week____	Three or more times a week____

9) During the past month, how much of a problem has it been for you to show enthusiasm to get things done?

No problem at all_____

Only a very slight problem_____

Somewhat of a problem_____

A very big problem_____

10) Do you have a bed partner or roommate?

No bed partner or roommate?_____

Partner/roommate in other room_____

Partner in same room, but not same bed_____

Partner in same bed_____

If you have a roommate or bed partner, ask him/her how often in the past month you have had…

a) Loud snoring

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

b) Long pauses between breaths while asleep

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

c) Legs twitching or jerking while you sleep

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

d) Episodes of disorientation or confusion during sleep?

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

e) Other restlessness while you sleep; please describe

_____

_____

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

Important details for the use of the ACTIWATCH

- Wear the ACTIWATCH on your non-dominant wrist:
- Right handers wear it on their left wrist
- Left handers wear it on their right wrist.

- Wear the ACTIWATCH continuously day and night.
- Take the ACTIWATCH off when having a bath or a shower.
- Don't forget to put it on again.

- Indoors: Don't cover the ACTIWATCH with your sleeve.
- Outdoors: Don't cover the ACTIWATCH with your sleeves, except when it will get very wet.

- Be careful and protect it from any damage.

Completion of the diary

IMPORTANT TO NOTE:

- Indicate time of removal of the ACTIWATCH and reason (e.g. shower). Keep removal time to a minimum (no longer than 20 min).

- Indicate time when the ACTIWATCH was put on again.

- Indicate Bedtime / Lights off
- Indicate Get-up time / Lights on
- Indicate when Awake in bed
- Indicate Naps

- Indicate calm activities such as reading, computer games, watching TV

- Indicate Parties and Sports e.g. jogging, rowing

- Indicate when unwell or sick or stressed

- Indicate time of medication intake

- Indicate time of meals

ACTIVITY MONITORING - SLEEP/WAKE PROTOCOL

Code: _____ Date: _____ Page: _____

Please remark: Get-up time, Bedtime, Actiwatch off/on, Have a shower, Lights off/on, Awake in bed, Medication, Go out (to see friends, take a walk), Reading, Watching TV, Meals.

TIME		REMARKS	TIME		REMARKS	TIME		REMARKS
12 AM (00)	**00**	**Midnight**	**08** AM	**00**		**4** PM (16)	**00**	
	10			10			10	
	20			20			20	
	30			30			30	
	40			40			40	
	50			50			50	
01 AM	**00**		**09** AM	**00**		**5** PM (17)	**00**	
	10			10			10	
	20			20			20	
	30			30			30	
	40			40			40	
	50			50			50	
02 AM	**00**		**10** AM	**00**		**6** PM (18)	**00**	
	10			10			10	
	20			20			20	
	30			30			30	
	40			40			40	
	50			50			50	
03 AM	**00**		**11** AM	**00**		**7** PM (19)	**00**	
	10			10			10	
	20			20			20	
	30			30			30	
	40			40			40	
	50			50			50	
04 AM	**00**		**12** PM	**00**	**Noon**	**8** PM (20)	**00**	
	10			10			10	
	20			20			20	
	30			30			30	
	40			40			40	
	50			50			50	
05 AM	**00**		**1** PM (13)	**00**		**9** PM (21)	**00**	
	10			10			10	
	20			20			20	
	30			30			30	
	40			40			40	
	50			50			50	
06 AM	**00**		**2** PM (14)	**00**		**10** PM (22)	**00**	
	10			10			10	
	20			20			20	
	30			30			30	
	40			40			40	
	50			50			50	
07 AM	**00**		**3** PM (15)	**00**		**11** PM (23)	**00**	
	10			10			10	
	20			20			20	
	30			30			30	
	40			40			40	
	50			50			50	

Stanford Sleepiness Scale

An introspective measure of sleepiness

This is a quick way to assess how alert you are feeling right now.

This simple seven –point Likert-type scale has descriptors ranging from "feeling active, vital alert, or wide awake" (score = 1) to "no longer fighting sleep, sleep onset soon and having dream-like thoughts" (score = 7). Choose the set of descriptors that best describes your feeling of sleepiness at the time this scale is administered (at the time of the sleep assessment/right now). If you go below a three (3-7) when you should be feeling alert, this is an indication that you have a serious sleep debt and you need more sleep.

Circle the number that best describes how you are feeling right now > your sleepiness rating

Degree of Sleepiness	Scale Rating
Feeling active, vital, alert, or wide awake	1
Functioning at high levels, but not at peak; able to concentrate	2
Awake, but relaxed; responsive but not fully alert	3
Somewhat foggy, let down	4
Foggy; losing interest in remaining awake; slowed down	5
Sleepy, woozy, fighting sleep; prefer to lie down	6
No longer fighting sleep, sleep onset soon; having dream-like thoughts	7
Asleep	X

2009 May ML van der Horst www.rgpc.ca
References: http://www.stanford.edu/~dement/sss.html
go to **www.rgpc.ca** for complete Reference List and Resources