

Neurodegeneration and brain cancer: A longitudinal field study of rest-activity and sleep



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(Main Thesis)

'The progress should go on.
Anything I can do, let me do it now, as I shall not pass this way again'
Ella Wiltshire, Alzheimer's disease patient brain donor
www.mindovermatterproject.co.uk

'The advantage of a bad memory is that one enjoys several times
the same good things for the first time.'
Friedrich Nietzsche

'That some good can be derived from every event is a better proposition than
that everything happens for the best, which it assuredly does not.'
James K. Feibleman

Abstract

This thesis investigates rest-activity and sleep profiles in neurodegeneration and brain cancer. Study 1 comprised longitudinal field assessments of rest-activity, sleep and memory in controls and memory-impairment individuals with: subjective memory complaint (SMC), amnesic mild cognitive impairment (aMCI), mild and moderate Alzheimer's disease (AD). Four questions were addressed: (1) is SMC a prodromal stage of AD? (2) do characteristics of SMC predict future decline? (3) does cholinergic medication (ChEI) impact rest-activity and sleep of moderate AD patients? and (4) are there factors predicting response to ChEI? Study 2 assessed rest-activity and melatonin rhythms in a brain cancer patient (JJB), and post-mortem analysis of brain tissue assessed infiltration of cancer cells on the circadian clock (SCN). Both studies used questionnaires, cognitive tests, electroencephalography and actigraphy simultaneously at patients' homes. In Study 1, the SMC group showed a reduced activity amplitude to be correlated with increasing memory impairment severity, lower sleep quality and efficiency. Increased sleep fragmentation was observed in all memory-impaired groups, although not correlated to impairment severity. Increased fragmentation of rest-activity rhythm correlated with increasing memory impairment severity in all groups except SMC. Following ChEI medication with donepezil, moderate AD patients showed increased sleep fragmentation, probably due to potentiation of available acetylcholine known to maintain arousal. Higher daytime-activity and lower activity in the rest-phase, when drug-naïve, predicted improved cognition following ChEIs. In Study 2, cancer cell infiltration of the patient's SCN was confirmed. However, a robust circadian rest-activity period with a misaligned melatonin phase, was recorded, indicating that the effects of partial SCN lesions in humans are complex and this result was possibly in part due to the masking effect of social behaviour.

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Abbreviations

Abbreviation	Descriptions
Classification	
SMC	Subjective Memory Complaint
aMCI	Amnesic Mild Cognitive Impairment
mild AD	Mild Alzheimer's Disease
mAD	Moderate Alzheimer's Disease (all methodologies)
qmAD	Moderate Alzheimer's Disease (questionnaires only)
JJB	Case study patient initials
Questionnaires	
MMSE	The Mini-Mental State Examination
HQ	The Health Questionnaire
HAD	The Hospital Anxiety and Depression (Scale)
PSQI	The Pittsburgh Sleep Quality Index
JSQ	The Jupiter Sleep Questionnaire
MEQ	The Morningness-Eveningness Questionnaire
ADCS-ADL	The Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory
SFS	The Social Functioning Scale
CANTABeclipse tests	
BLC	Big/Little Circle
CRT	Choice Reaction Time
PAL	Paired-Associates Learning
SSP	Spatial Span length
SST	Stop-Signal Task
GNT	Graded Naming Test
Other methods	
EEG	Electroencephalography
EOG	Electrooculography
EMG	Electromyography

Abbreviation	Descriptions
Analyses	
NPCRA	Non-parametric circadian rhythm analysis
IV	Intra-daily Variability
IS	Inter-daily Stability
M10	Most active ten hours
L5	Least active five hours
RA	Relative amplitude between M10 and L5
Dunn-Šidák correction	Dunn-Šidák correction for multiple comparisons
Collaborating bodies	
NHS	National Health Service (UK)
OPTIMA	The Oxford Project To Investigate Memory and Ageing
DeNDRoN	The Dementias & Neurodegenerative Diseases Research Network
NICE	National Institute for health and Clinical Excellence
Diagnostic criteria	
DSM	Diagnostic Statistical Manual
NINCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association
Other abbreviations	
ChEI	Cholinesterase Inhibitors
SCN	Suprachiasmatic nucleus
ADLs	Activities of Daily Living
aMT6s	6-sulphatoxymelatonin (melatonin sulphate)
REM	Rapid-Eye Movement sleep
SWS	Slow Wave Sleep
OD	Once daily (used in medication dose regimes)

1. Introduction

Circadian rhythms, for example rest-activity patterns, are known to interact with sleep regulation and structure. Circadian rhythms and sleep in turn influence cognition, in particular memory. Longitudinal research assessing the interaction between rest-activity, sleep and memory is limited and rarely takes place outside of laboratory settings.

This thesis will seek to further elucidate the relationship between sleep, circadian rhythms and memory by assessing individuals longitudinally at home in Study 1. Alzheimer's disease (AD), a neurodegenerative disease, is a good example of a multiple stages of severity condition, with severity of progressing overtime. Alzheimer's disease has been previously associated with deficits in rest-activity, sleep and memory. Rest-activity, sleep and memory was assessed by actigraphy (watch-like device that measures rest-activity rhythms), questionnaires, computerised memory and attention tests, and electroencephalography (EEG, the gold-standard method for studying sleep macro-architecture) in Study 1. This thesis is the first to report the longitudinal, concomitant use of these methods in the field to assess the progression of memory impairment from a healthy, older population with healthy memory to moderate severity AD.

The second study of the thesis, a case study of an individual diagnosed with terminal brain cancer is also presented. This case provided a further opportunity to assess rest-activity and sleep longitudinally in the field. Dramatic changes in brain anatomy caused by the cancer and resection surgery limited the assessment of sleep to subjective questionnaires.

The two studies of this thesis will be presented as two examples of how rest-activity and sleep can be researched longitudinally in the field (i.e. in the community). The background research and development of research questions are described in each data chapter. In order to

place these studies in context with the research areas of circadian rhythms, sleep and their relation to memory, an overview is provided here.

1.1. Circadian rhythms

Biological rhythms vary in duration (period). Mammals have rhythms which can last seconds, minutes, days or months. Biological rhythms that have a period of less than 24 hours, for example a heartbeat, are named ultradian rhythms [1]. Rhythms with a period of approximately 24 hours, for example the rhythm of rest-activity, are categorised as circadian rhythms (*'circa'* meaning 'about', *'diem'* meaning 'a day'). Human circadian rhythms have periods between 24.2 and 25.1 hours when the self-sustained rhythm of the individual is not entrained to environmental cues (called free-running) [2]. Rhythms that have a period longer than 24 hours are called infradian rhythms [1]. An example of infradian rhythms is the annual rhythm of animal migration [2].

The phase of the circadian rhythm of rest-activity (timing state of the oscillation) enables rest to occur in night-time hours and activity to occur in daylight hours (and the reverse for nocturnal animals). To be able to anticipate dusk and dawn, by the regulation of hormonal and molecular cycles, circadian rhythms are understood to be key to a species' survival [3]. Circadian rhythms affect temperature regulation [4], metabolism [5] and the sleep/wake timings [6]. Disruptions of circadian rhythms in humans have been linked to many conditions including cardiovascular disease, cancer, psychiatric disorders and neurodegenerative diseases [7].

1.1.1. Circadian rhythm generation

Circadian rhythms are generated by the suprachiasmatic nuclei (SCN), a group of approximately 20,000 neurons located in the anterior hypothalamus, found to generate circadian rhythms even when isolated [8]. This group of neurons are known to be the circadian pacemaker (named the circadian clock) due to studies finding ablation of the SCN results in the loss of

circadian rhythmicity. Implantation of a functionally intact rhythmic SCN into an arrhythmic animal has been found to restore rhythmicity maintaining the rhythm of the donor, demonstrating the SCN itself generated the rhythm [9].

Since initial lesion studies determined that circadian rhythms were generated by the SCN, the effects of disrupting SCN function were studied. Many studies have replicated the first findings of circadian rhythmicity being abolished when the SCN is ablated in a variety of species [10-13]. Rhythms of feeding, drinking, rest-activity and entrainment to auditory time-of-day cues were some of the factors investigated in those studies. The circadian marker, melatonin, a hormone secreted by the pineal gland in-phase and with the same period as the SCN, was also investigated [11]. In this study, when the SCN was ablated, melatonin rhythmicity was lost, reinforcing the overriding view that melatonin is an easily measured marker of the phase and period of the SCN. More detail of SCN lesion studies and the human cases of SCN lesions and their resultant effects will be provided in Chapter 6 (p.224).

The essence of molecular circadian clock function is a transcriptional-translational negative feedback loop, involving several central clock genes and many other secondary genes [3]. There are five key proteins involved in this cycle: Period (PER) 1, 2, 3 and Cryptochromes (CRY) 1 and 2. The products of these proteins oscillate over a period of about 24 hours involving a key negative loop by inhibiting their own promoters. A heterodimer of CLOCK and BMAL1 is permanently attached to these promoters of PER and CRY proteins [14]. *Bmal1* mRNA is expressed in the middle of the subjective night and is delayed in expression due to *Rev-erba*, a gene controlled by the BMAL1/CLOCK complex. This occurs by the REV-ERB α protein influencing the *Bmal1* promoter and therefore delaying its expression [15]. When the activation of the *Per* and *Cry* promoters is undisturbed there is an accumulation of *Per* and *Cry* mRNA late in the subjective day or in the early night. This mRNA is transported to the cytoplasm where

these mRNA transcripts are translated into PER 1, 2, 3 and CRY 1 and 2 proteins. This translation leads to peak protein concentrations in the middle of the subjective night. These PER and CRY proteins form a trimeric complex of PER, CRY and CKI (casein kinase is a protein kinase that functions as a regulator of signal transduction pathways in cells). CK I has two variants: delta (δ); and epsilon (ϵ) which are both interchangeably involved in this PER/CRY/CKI δ/ϵ complex. The trimeric complex enters the suprachiasmatic nucleus and binds to the CLOCK/BMAL1 complex which prevents the transcription of *Per* and *Cry* mRNA. CASEIN KINASE (CK I) phosphorylates the clock gene proteins leading to their transport into the nucleus and eventually their degradation. This degradation prevents PER and CRY acting on their own promoters, leading to the undisturbed activation of the CLOCK/BMAL1 heterodimer of *Per* and *Cry* promoters. This activation by the heterodimer on these promoters commences the cycle again [14]. This cycle is demonstrated in Figure 1 (p.14).

This oscillation is observed in the majority of mammalian cells and are synchronised by output from the SCN. This is why the SCN is referred to as the 'conductor of the orchestra' or the 'master clock'. *In vitro* research has found that the neurons of the SCN have the same circadian period but different phases, therefore, needing some signal to synchronise. It has been suggested that the synchronising signal is related to the expression of gamma-aminobutyric acid (GABA) as most, if not all, SCN cells express GABA [16].

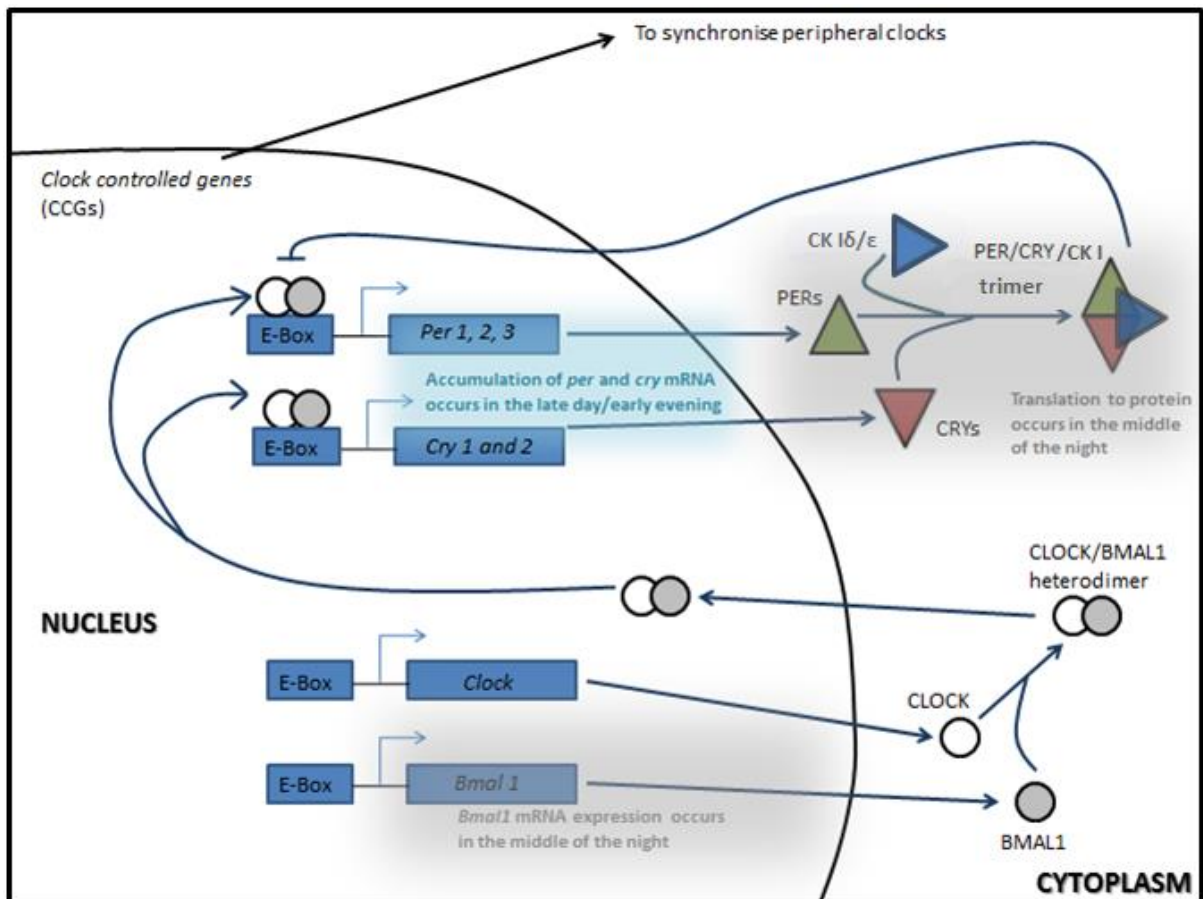


Figure 1. Schematic shows the key negative feedback loop of the SCN molecular clock. Timings of *Bmal1*, *Per* and *Cry* mRNA accumulation and protein translation are displayed in blue and grey. Clock controlled genes (CCGs) then influence cascade pathways to disseminate the circadian rhythm of the SCN to other clock cells in the periphery to synchronise them [14].

1.1.2. Entrainment of the SCN

In order to set itself to the environmental light-dark cycle, the inner core of the SCN receives projections from the retino-hypothalamic tract, whilst the neurons in the outer shell remain intrinsically rhythmic without light pulses [17]. The entrainment property of light on circadian rhythms is known to be very marginally due to the rods or cones found in the mammalian eye [18]. This was discovered due to rodless/coneless mice showing only little or no attenuation of their circadian rhythm to a light stimulus [18]. Non-rod and non-cone photoreceptors have been found which contain a retinal pigment called melanopsin (Opn4) [19]. Melanopsin has been observed to cause the retinal ganglion cells, in which it is found, to be photoreceptive

confirmed by knock-out of *Opn4* leaving these cells insensitive to light [20]. These retinal ganglion cells are known to be linked with the SCN via the retino-hypothalamic tract through the optic chiasm, providing a pathway of entrainment [9]. Bright light pulses have been found to reset the circadian clock by delaying or advancing the circadian phase depending at which phase the light is administered [8]. Circadian rhythms, for example rest-activity, can also be synchronised to other environmental cues like social pressure, though these cues do not directly affect the SCN [21].

1.1.3. Diurnal preference and chronotype

Research of sleep behaviour has revealed that there are preferences of the timing of sleep/wake [22]. The time of day when an individual prefers to sleep or be awake is called diurnal preference [23], which can be classified from extremely morning types ('larks') to extremely evening types ('owls'). This personal sleep-wake preference remains regardless of social pressures, for example to get up early for work [24]. In order to determine actual sleep timing, work and free-day activity where the individual can choose when they wake, can be considered to determine chronotype (actual sleep timing behaviour) [25].

There are different ways to calculate preference for sleep timing. One method is the Morningness-Eveningness Questionnaire (MEQ) developed by Horne and Östberg (1976) [26] with categorises individuals into types of diurnal preference. Another method is the Munich Chronotype Questionnaire (MCTQ) [25] which determines chronotype by comparing the mid-sleep time on free and work days.

The difference of mid-sleep times on work and free days has been named 'social jetlag' [21]. Late types who have to get up early for work following a short sleep period due to a preference for a late bedtime have a large amount of social jetlag. Social jetlag is, therefore, a

calculation of how much of a sleep deficit an individual has due to conflict between their chronotype and social pressure [27]. Notably, chronotype and diurnal preference vary over an individual's lifetime. Adolescents and young adults are more owl-like than young children and adults [27]. In terms of their progression over time, young children have earlier chronotypes which progressively become later (delay) until about 20 years old and then become gradually earlier (advance) with increasing age [23].

1.2. Sleep

In order to discuss sleep thoroughly, firstly sleep architecture should be described. There is a well-known pattern of sleep stages observed in humans, however, there are of course variations in percentages and distinct alterations when observing diseased or mentally ill individuals. Interestingly, certain disorders or illnesses exhibit characteristic sleep patterns. Normal sleep consists of two main states: rapid eye movement sleep (REM) usually involving motor atonia (muscle paralysis) and non-rapid eye movement sleep (NREM). Initially the individual will move through the first four stages of NREM sleep and then move back through them in reverse leading to a period of REM sleep, ending the cycle. Most adults go through this cycle of sleep stages four to five times every night, with each cycle lasting approximately 80-110 minutes [28].

When an individual is awake or asleep the pattern of their electrical brain activity can be observed using electroencephalography (EEG). When awake, the electroencephalogram shows a mixture of different wave frequencies but is mainly dominated by fast frequency waves (more than 15-20 Hz) with low amplitude, called beta-activity. When the individual relaxes and closes their eyes, the wave rhythm changes to a recurring pattern with a frequency of about 9 to 12Hz, known as the alpha rhythm. As the individual gets closer to sleep the percentage of time spent in alpha activity is reduced and smaller amplitude waves with irregular frequency appear, as well as sharp waves called vertex spikes; this is defined as stage one sleep. After a few minutes this leads to stage two sleep, which is characterised by theta waves of 4-8Hz and waves of 12-14Hz called sleep spindles which occur periodically in bursts. Also, during stage two, a waveform called a 'K-complex' appears which is a negative sharp wave followed immediately by a slower positive wave [29]. Stage 2 sleep leads to stage three sleep, which is the beginning of slow wave sleep (SWS) within these four non-REM (NREM) sleep stages [30].

Stage three is defined by the appearance of slow, large amplitude waves called delta waves (about 1Hz). After stage three stage four commences and is characterised by an increase in the percentage of time spent in delta waves. After progression of the cycle described above, rapid eye movement sleep (REM) begins with small amplitude, high frequency waves, very similar to wakefulness [30]. REM sleep was first properly defined in 1953 by the observation of cycles of eye movement and body inaction during sleep [31]. REM sleep is known to be an active process, regulated by a loop between the caudal midbrain and the Pons [29].

1.2.1. Sleep generation

Sleep is known to be regulated by two regulatory systems: sleep timing (driven by the circadian drive); and wake and sleep promotion. Sleep timing will be discussed in section 1.3 (p.20).

Wake-promoting areas of the brain promote arousal and, therefore, wakefulness when activated. In these wake-promoting areas, projections from the pontine and midbrain wake-promoting cells dorsally activate the thalamo-cortical system whilst ventrally activating the hypothalamo-cortical and basalo-cortical systems [29]. There are several neurotransmitter pathways and hormones involved in wake-promotion. These include: acetylcholine; GABA (γ -aminobutyric acid); norepinephrine; serotonin; glutamate; dopamine; histamine; and hypocretin/orexin. The acetylcholine neurotransmitter pathway of the cholinergic system, and therefore cholinergic neurons, are involved in wake-promotion. The PPT (pedunculo-pontine tegmentum) is a key area of the brain involved in arousal, containing a large proportion of cholinergic neurons. Cholinergic cells also produce NO (nitric oxide), a gaseous neuromodulator [32]. Nitric oxide does not itself affect wakefulness, although it acts as a signal to regulate activity of the PPT cholinergic neurons affecting wakefulness. These cholinergic neurons project to the suprachiasmatic nucleus (SCN), the basalo-thalamo, thalamo-cortical, hypothalamo-

cortical and amygdaloid wake-promoting structures [29]. The PPT appears to be involved in REM sleep and wakefulness [33]. In fact 60% of the cells within the cholinergic cell compartment of the PPT are related to wakefulness and REM sleep [34]. These cells have been found to increase neuronal activity before wakefulness and remain active until 5-8 seconds before sleep commences [34]. Cholinergic neurons of the basal forebrain are implicated in behaviours that require wakefulness, for example attention, learning and memory [35]. Other areas known to have cholinergic neurons involved in wakefulness are the neocortex and the hippocampus [36].

Sleep promotion functions of cholinergic neurons are well researched, especially in terms of REM sleep initiation and maintenance. As an individual progresses through the sleep stages their cholinergic tone increases in the signal generators of REM sleep [29]. Anatomical studies show projections from the PPT resulting in REM sleep signal generating nuclei [37]. Other studies have shown the activation of the PPT cholinergic cells results in an increase in the amount of REM sleep, whereas their inhibition causes a decrease [38]. Two types of cholinergic neurons in the brainstem, REM-on type and Wake-REM-on type neurons were found to have different firing rates. The REM-on type firing correlate to the initiation of REM sleep and the Wake-REM-on neuronal firing correlate to REM maintenance [29].

1.3. The interaction between sleep and circadian drives

Melatonin synthesis, as mentioned above, has a circadian rhythm. This hormone is involved in thermoregulation and is produced by the pineal gland which appears to have GABAergic neuronal connections to the SCN [39]. Melatonin is a good indicator of an individual's endogenous circadian rhythm (a circadian phase marker) as it cycles in a sinusoidal manner across the circadian period [40]. Melatonin levels have also been shown to be different between long and short sleepers, suggesting a neurobiological interaction between sleep and circadian processes [41].

Sleep is regulated through processes of sleep timing (circadian driven) and neurobiological wake and sleep promotion [42]. Sleep promotion is understood to be influenced by the duration of previous sleep and wake episodes, determining the 'sleep pressure' of an individual (homeostatically driven). Therefore, sleep can be described as being regulated by two main mechanisms: the circadian pacemaker (C process); and homeostatic drive (S process). Their interaction has been hypothesised in the two-process model [6, 42]. The circadian pacemaker, the SCN, gates the timing of sleep onset and offset so that sleep is ecologically appropriate [43]. The homeostatic drive causes a build in sleep pressure throughout the day leading to sleepiness and the need to sleep [44]. The individual will fall asleep due to the opening of a circadian window for sleep and the homeostatic sleep pressure both aligning correctly, and will wake when the circadian drive for wakefulness increases and homeostatic sleep pressure decreases [42]. During sleep there is also the ultradian stage changes moving between REM and NREM sleep [43]. Slow wave sleep is a type of NREM sleep which has been strongly linked to the homeostatic control of sleep, with the amount of SWS relating to sleep pressure [43]. While the homeostatic sleep pressure increases through wakefulness, the circadian pacemaker counteracts this need for sleep by delaying the sleep

episode until appropriate (habitual sleep time/night in humans) [40]. Adenosine, a neurotransmitter produced by the basal forebrain, is understood to be involved in the homeostatic control of sleep, with levels in the basal forebrain increasing after prolonged wakefulness suggesting this is the mechanism for sleep pressure [40]. Also when adenosine is added to this brain region, sleep is induced [40].

Circadian and sleep systems are neuronally linked with cholinergic and serotonergic neurons linking key sleep areas with the SCN [45]. To exemplify this, cholinergic neurons afferently link the nucleus basalis and pedunculopontine tegmental nucleus and the SCN, showing a clear connection between the homeostatic drive and the circadian pacemaker [45]. The SCN activity has been shown to vary with the stages of sleep, by observing rat EEG readings of the sleep stages and by measuring the electrical activity of the SCN simultaneously. When the SCN electrical activity levels were heightened, the rats were in REM sleep and conversely when the electrical activity of the SCN was lower, the rats were confirmed to be in NREM sleep, according to EEG readings. EEG readings also showed increased SCN neuronal activity when the rats were awake [45]. This shows that sleep states feedback to regulate SCN activity, and is reviewed well by Colwell and Michel (2003), however, this interaction still needs to be investigated more fully [46].

1.4. Sleep-related memory

As cholinergic systems in the brain are known to be involved in sleep as well as memory, the link between these two processes is particularly worthy of discussion. A possible mechanism of this interaction has been described in the 'synaptic homeostasis hypothesis' [44].

The synaptic homeostasis hypothesis suggests that synaptic strength (potentiation) increases throughout the day, reaching its maximum just before sleep and then decreasing (synaptic downscaling) throughout sleep[44]. The synaptic homeostasis hypothesis appears to complement the homeostatic process (Process S) of sleep regulation, described more thoroughly in Section 1.3 [42] (p.20). The synaptic homeostasis hypothesis suggests that learning, or information storage, through the process of LTP (long term potentiation: two neurons are simultaneously activated, which augments the transmission of future signals between them) occurs during wakefulness [44]. As the amount of slow wave activity (SWA, the power density of low frequency delta activity during sleep measured by electroencephalography) in sleep is related to the homeostatic process of sleep pressure, the hypothesis states that slow wave activity is also related to the amount of synaptic potentiation that occurred during the previous period of wakefulness. The amount of SWA has been determined to be related not only to duration of wakefulness but also the quality of wakefulness (amount of stimulation). In order for synaptic downscaling to occur during sleep so that potentiation can reoccur in the next period of wakefulness, long term depression (LTD: reduction in the efficacy of a synapse, either due to a reduction in receptors or neurotransmitter availability, caused by either constant weak or strong stimulation, depending on the brain area involved) is thought to occur. Reduced cholinergic transmission, as well as other neurotransmitters, during NREM sleep is thought to enable LTD, as high levels of acetylcholine and other neuromodulatory agents are known to prevent LTD. The synaptic

homeostasis hypothesis, therefore, suggests a biological link between memory (learning) and sleep based on actions carried out during wakefulness [44].

Sleep has been thought to play a substantial part in memory consolidation, by protecting memories from interference and promoting storage [47]. This is a theory from Jenkins and Dallenbach (1924) [48] who found that people can remember nonsense syllables better after sleep and assumed this was due to reduction in interference experienced during sleep. Sleep does appear to be more than just a passive state in its interaction with memory [47]. One study found that sleep improved declarative memory recall regardless of the amount of time spent awake, therefore, suggesting that sleep does not just passively protect memory consolidation [49].

The neurobiological nature of sleep could lead directly to the consolidation of memories. Early observation found increased slow wave sleep (SWS) to be correlated with more consolidated memories, in comparison to wakefulness and reduced SWS conditions [50]. Later studies determined that levels of hippocampal activity were correlated with spatial navigation learning improvement, thus higher activity resulting in more consolidated memories [51]. The hippocampus is also thought to have an alternating relationship with the neocortex governed by the cycling between NREM and REM stages of sleep. This alternating relationship is understood to strengthen synaptic connections (as discussed in the synaptic hypothesis) in storing memories. Animal studies have led to the hypothesis that sleep spindles (as described in Section 1.4, p.22) may represent the mechanism of communication between the hippocampus and the neocortex. This is discussed being primarily due to the increase in hippocampal activity observed when sleep spindles are present [47, 52].

1.5. Methods for assessing rest-activity and sleep

1.5.1. Actigraphy

The most reliable method currently available to assess a person's rest-activity profile is actigraphy, also called actimetry. This measure has been used for over 20 years and can be used 24 hours a day, for days to months. Actigraphy data can be analysed for circadian rhythm parameters such as period, amplitude and acrophase [53]. There are two main ways to use actigraphy: wrist and waist, of which wrist actigraphy is most commonly used currently, mainly due to convenience. Wrist actigraphy involves the individual wearing an accelerometer on their wrist, usually on their non-dominant wrist. There are two commonly used varieties, although many companies offer variations on the same theme. The older version is a wrist accelerometer which detects both wrist movements and light intensity. This is the most commonly used method, however, it does have the problem of not being resistant to water and needs to be removed, with reliance on the wearer to remember to replace it back on their wrist after washing. Some newer versions of the accelerometer are waterproof, which is preferable especially when studying memory impaired individuals who would have difficulty remembering to remove or replace the watch. However, this watch does require a separate light sensor but this can be attached easily to the outside of clothing.

When the data from the accelerometer are downloaded the resulting actogram shows each day's activity and light levels, indicating sleep onset and duration. The researcher indicates sleep and wake timings based on observation of light levels, activity intensity, and the individual's sleep diary. These data can be used to calculate differences between groups. Practice parameters have been developed for the use of actigraphy in the clinical assessment of sleep disorders. Since these parameters were developed in 1995, evidence has accumulated about the effectiveness of actigraphy. It has been found to be reliable and also a valid method

of evaluating an individual's sleep [53]. More specifically, it is very reliable in healthy populations but less reliable in individuals who have disturbed sleep as the accuracy of actigraphy has been found to diminish with reduced sleep efficiency [53]. The main use of actigraphy has been the assessment of daytime somnolence, sleep-wake patterns and longitudinal observation of rest-activity patterns. Parameters about sleep quality derived from actigraph recordings have been used as outcome measures in the study of children and demented individuals [54] and well as many other areas of research. Actigraphy has since been used to evaluate many populations; one example is the use of wrist actigraphy to evaluate the sleep of healthy elderly women. One study found that their sleep efficiency and sleep duration was significantly reduced in comparison to younger women [55]. Actigraphy has also been found to be reliable in monitoring treatment success and can distinguish between placebo and medicated groups, which is a measure required by many studies [56].

1.5.2. Electroencephalography (EEG)

When considering an individual's sleep macro and micro structure, electroencephalography (EEG) is commonly used and is highly reliable when the electrodes are fitted correctly to the scalp. Attenuation of the neuronal electrical signal by the skull is high. EEG was first developed by a German psychiatrist called Hans Berger who published his findings in 1929 [29]. He realised that the electrical wave frequency of the brain altered throughout sleep and he developed a device to record these alterations [29].

Nowadays, EEG involves placing electrodes on various parts of the individuals' scalp using a conductive gel. The places where the electrodes are attached are specific to certain areas of the brain. Despite low spatial resolution of where the signal is generated from, the time resolution of EEG is high. Further electrodes measure the electrical activity of the muscles

near the eye, assisting in the detection of rapid-eye movement (REM) sleep and also under the chin to measure muscle tone, known to decrease in REM sleep. These, as well as the cortical electrodes, help to indicate when each stage of sleep is occurring and specific characteristics related to these stages, such as K-complexes and sleep spindles. This use of cortical (EEG) and eye (EOG) and muscle (EMG) electrodes as well as respiratory measures is called polysomnography. This is very useful, as many disorders or diseases show distinct sleep architecture or characteristic changes.

Despite the usefulness of both techniques, EEG and actigraphy have been found to be even more valuable in combination. The main limitation of comparing these two techniques is the varying size of data sampling epoch; actigraphy being one minute and EEG being thirty seconds. However, actigraphy has been found to agree with EEG 91% of the time, suggesting that they are quite comparable in respect to measures they both evaluate. Evidence suggests that these measures should be used in combination in order to get a broader understanding of an individual's sleep, in terms of their overall sleep patterns over a longer duration and more specific characteristics in a few nights testing [57].

1.6. Study overviews and research questions

Circadian rhythms have an impact on many aspects of life from core body temperature to rest-activity rhythms. The circadian drive is known to regulate sleep timing and structure. Sleep is thought to feedback on the suprachiasmatic nucleus (SCN) to regulate circadian rhythms, however, the mechanism of this feedback is still under investigation. The performance of memory consolidation and recall are known to alter throughout the day [58], based on a circadian rhythm of attention and cognitive functioning. In addition, sleep duration and structure is known to impact the storage of memories. Although circadian rhythms, sleep and memory have been investigated previously in laboratories, longitudinal simultaneous assessment of these processes in the field has not been conducted, in particular not in the context of a progressive disease, such as Alzheimer's disease, where disorientation and agitation increases in unfamiliar environments. Therefore, home studies are a good way to study these patients.

Alzheimer's disease (AD) is understood to progress from a subjective memory complaint, a mild amnesic cognitive impairment, mild AD, moderate AD through to severe AD. Further information about AD, its characteristics, sleep and circadian impairments and its stages can be found in [Chapter 4](#) (p.106). The progression of AD and how this progression relates to rest-activity and sleep are the subject of Study 1 of this thesis.

The first chapter of this study, [Chapter 3](#) (p.68) in this thesis, addresses the research question:

- ***Are the cognitive abilities, rest-activity and sleep patterns different between older people who have or do not have a subjective memory complaint?***

How each further stage of AD-related progression compares to non-memory impaired controls of a similar age is described in [Chapter 4](#) (p.106), and concentrates on the research questions:

- ***With each prodromal and progressive stage of Alzheimer's disease, to what extent do rest-activity and sleep profiles change in patients opposed to non-memory impaired controls?***

and

- ***How do rest-activity and sleep progress across the spectrum of increasing severity of Alzheimer's disease and prodromal stages?***

The most severe stage included in this thesis were the moderate stage of AD who, in order to maintain cognitive function, were administered cholinergic medication. Details of previous research of this medication and its relation to memory and sleep are given in [Chapter 5](#) (p.168).

This chapter addresses the research questions:

- ***Does cholinergic medication alter objective measures of rest-activity or sleep pattern in moderate Alzheimer's disease patients when observed in the field?***
- ***Are there predictors of future cognitive benefits of taking cholinergic medication? And are there any factors that predict who will tolerate higher doses of donepezil?***
- ***Are there any predictive factors of future sleep problems associated with taking cholinergic medication?***

- ***Finally, are there factors of sleep or rest-activity rhythms at baseline that predict who can tolerate donepezil and who will need to change to a different type of cholinesterase inhibitor?***

The final part of this thesis, Study 2 (p.224), details the long-term field observation of circadian behaviour and its relation to the brain anatomy of a patient with brain cancer, named JJB. The observations of JJB for approximately five months until the end of his terminal illness, with the brain tissue of his suprachiasmatic nucleus analysed post-mortem, are described in Chapter 6 (p.224). Therefore this chapter focuses on the research questions:

- ***Do rest-activity patterns in a terminal brain cancer patient relate to underlying circadian disruption and if so, how were the suprachiasmatic nuclei affected by cancer cell infiltration?***

The final chapter of this thesis, Chapter 7 (p.260), details future directions of the research described herein and assesses the validity of using certain methodologies in these patient groups. The methods considered are: actigraphy, electroencephalography, questionnaires and computerised memory and attention tests.

2. Methods

This thesis consists of two studies sharing similar methodologies. The design of each study will be discussed in turn. Following these study descriptions, details of each methodology will be given and of the statistical analyses performed. For reference throughout the thesis, specific methods sections will be provided with page numbers to ease the understanding of the methods in all data chapters.

2.1. Study Overviews and Design

The first study (Study 1) addresses sleep and rest-activity across the spectrum of Alzheimer's disease (AD) and its related memory impairment. The data for this study are presented in Chapters 3 to 5. Older adults were divided into five diagnostic groups based on the nature and severity of their memory impairment. Those without memory impairment were labelled 'controls', those with subjective memory complaints that could not be verified objectively on testing were named 'subjective memory complaint (SMC) individuals', those with an amnesic mild cognitive impairment were labelled aMCI (as an acronym), individuals diagnosed with AD by National Health Service (NHS) memory clinics were subdivided into mild and moderate severity, determined by memory impairment severity, daily functioning and brain atrophy. All categorisation of individuals was conducted by trained Old Age Psychiatrists or Gerontologists (and in collaboration with the research nurses of OPTIMA (see details below) for all groups except the AD patients) based on their own assessments, interviews and interpretation of brain images (CT or MRI). At the time of the study only moderate AD patients were eligible for treatment with cholinesterase inhibitors (for more information, see Chapter 5, (p.168). The

effects of cholinesterase inhibitor medication on the sleep of moderate AD patients are investigated and presented in Chapter 5 (p.168).

All participants followed the same time-course of assessment shown in Figure 2 (p.32), except for a subgroup of moderate Alzheimer's disease (qmAD) patients who only completed questionnaires, their timeline is also shown in Figure 2. All methodologies used in this study are described in further detail throughout this chapter. As shown in Figure 2, participants were assessed initially with three weeks of actigraphy to assess rest-activity rhythm at baseline. At the end of this three week period, two nights of electroencephalography (EEG) were performed and all questionnaires and computerised testing were carried out on these nights. The moderate AD (mAD) patients participating in all methodologies commenced their cholinesterase inhibitors (ChEIs) at the end of this three week baseline assessment period. All participants had a rest period of six weeks to allow for ChEI stabilisation. Details of dosage increases and how long stabilisation took are provided in Chapter 5 (p.168). After this rest period, a further three weeks of actigraphy were conducted with two further nights of EEG at the end of the three week period, again with questionnaires and computerised memory tests performed on these nights. This second assessment enabled the observation of ChEI effects on sleep and circadian rhythmicity, and to determine what non-therapeutic changes could be observed in the other groups over the same time period.

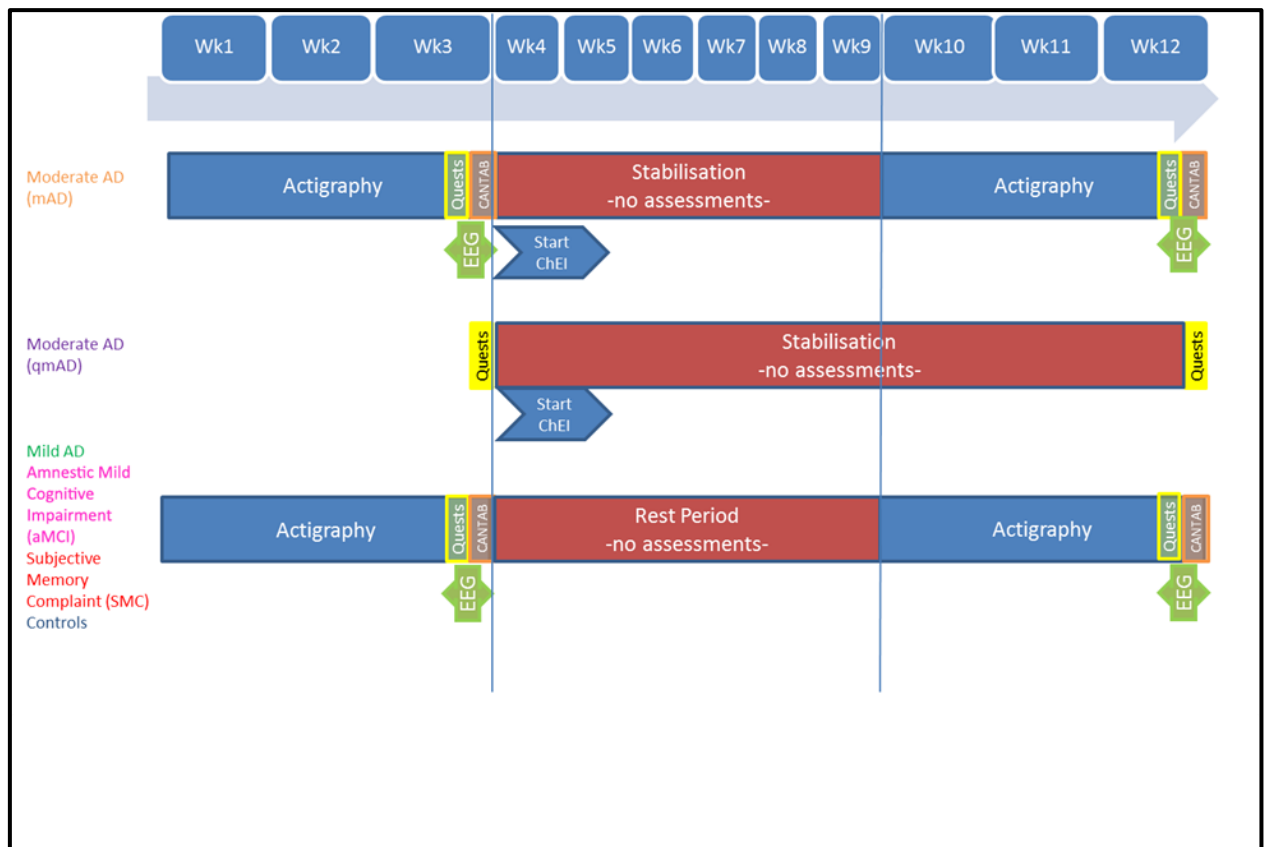


Figure 2. Timeline of Study 1 groups. The timelines indicate three different assessment schedules. The majority of groups (Control to Mild AD) were not taking medication and therefore had a rest period of no assessments whilst the moderate AD groups (top two timelines) stabilised on cholinesterase inhibitor (ChEI) medication. The moderate AD groups were divided into those completing all assessments and those only completing questionnaires. The timelines show that despite only completing questionnaires, assessments were scheduled in order to compare the same time difference between questionnaires in all groups.

The second study (Study 2) presented in this thesis is the case study of suprachiasmatic nuclei (see Introduction, Chapter 1 (p.9) for details) cancer cell infiltration due to brain cancer, described in Chapter 6 (p.224). The assessment of patient JJB in this case study shares common methodology with the memory impairment study described above. This is the first time to my knowledge that a cancer patient has been assessed using actigraphy for six months (including one month without assessment) up until the moment of death. As patient JJB presented with temporal misperception, initially memory and cognition tests were performed to determine whether memory impairment was present. In addition to actigraphy, a SCN phase marker, a

urinary melatonin metabolite was analysed to assess the phase of patient JJB's circadian rhythm as produced by the SCN (for more details on SCN circadian rhythm production see Introduction Chapter, 1 (p.9).

Details of common methodologies and study-specific methodologies are presented in this chapter.

2.2. Overview of Methodologies

		Study 1						Study 2
		Group Type						JJB
Assessment Type		Controls	SMC	aMCI	Mild AD	Moderate AD Group A	Moderate AD Group B	
Participant Demographics, Chronotype and Screening	Questions							
	General Health Questionnaire (GHQ)	✓	✓	✓	✓	✓	✓	
	Hospital Anxiety and Depression Scale (HAD)	✓	✓	✓	✓	✓	✓	
	Morningness-Eveningness Questionnaire (MEQ)	✓	✓	✓	✓	✓	✓	✓
	Medical records check	✓	✓	✓	✓	✓	✓	✓
	MRI + CT scan review							✓
Memory, Attention and Cognitive Profile	CANTAB							
	Mini-Mental State Examination (MMSE)	✓	✓	✓	✓	✓	✓	✓
	Big/Little Circle test (BLC)	✓	✓	✓	✓	✓	✓	✓
	Choice Reaction test (CRT)	✓	✓	✓	✓	✓	✓	✓
	Paired Associates Learning test (PAL)	✓	✓	✓	✓	✓	✓	✓
	Spatial Span Length test (SSP)	✓	✓	✓	✓	✓	✓	✓
	Stop Signal Task (SST)	✓	✓	✓	✓	✓	✓	✓
	Graded Naming test (GNT)	✓	✓	✓	✓	✓	✓	✓
Activities of Daily Living and Social Functioning	Questions							
	Social Functioning Scale (SFS)	✓	✓	✓	✓	✓	✓	
	Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory (ADCS-ADL)	✓	✓	✓	✓	✓	✓	
	Familial report							✓
Outcome measure Circadian profile	Rest-Activity Actigraphy							
	Averaged activity across 24h	✓	✓	✓	✓		✓	✓
	Activity periodogram analysis	✓	✓	✓	✓		✓	✓
	Non-parametric circadian rhythm analysis (NPCRA)	✓	✓	✓	✓		✓	
Outcome measure Sleep Profile and Characteristics	SCN phase marker Melatonin							
	Rhythm analysis							✓
	Questions							
	Pittsburgh Sleep Quality Index (PSQI)	✓	✓	✓	✓	✓	✓	✓
Outcome measure Sleep Profile and Characteristics	Actigraphy							
	Jupiter Sleep (JSQ)	✓	✓	✓	✓	✓	✓	✓
	Sleep analysis	✓	✓	✓	✓		✓	
	EEG Scoring	✓	✓	✓	✓		✓	
Outcome measure SCN Localisation	EEG spectral frequency analysis	✓	✓	✓	✓		✓	
	Brain collection							✓
	Tissue storage							✓
	SCN localisation							✓
Outcome measure Cancer Cell localisation	Immuno							
	Vasopressin							✓
Outcome measure Cancer Cell localisation	Immuno							
	Luxol-fast-blue-cresyl-violet							✓
	Haematoxylin + eosin							✓

Figure 3. Schematic of assessments conducted for both studies separated into assessment types and groups. Blue assessments indicate demographic screening with memory and functioning profiling. Outcome measures for both studies are shown in the red boxes.

2.3. Participant Demographics and Screening

2.3.1. Participant Recruitment

Study 1

There were five different diagnostic groups observed in Study 1: the controls, subjective memory complaint (SMC) individuals, amnesic mild cognitive impairment (aMCI) individuals, mild Alzheimer's disease (mild AD) and moderate Alzheimer's disease. The moderate Alzheimer's disease group was divided into two groups based on inclusion criteria, those meeting age, clinical diagnosis and medication criteria were classified as mAD and were given the opportunity to complete all methodologies. The inclusion criteria were as follows: cholinesterase inhibitor-naïve, retired with no regular volunteering, living in the community, aged between 65-85, no heart arrhythmia or severe asthma (or significant respiratory problems), had either mild binocular cataracts or monocular cataract (includes those who have had cataract surgery on one eye) [those with moderate to severe binocular cataracts, retinal detachment, macular degeneration or had had recent cataract surgery were excluded], no sleep disorder diagnoses or sleep medication use, no head injury or a diagnosed stroke, psychiatric illness or epilepsy present in the past 3 years. Those who did not meet these criteria but were diagnosed with moderate AD, living in the community, beginning a cholinesterase inhibitor (ChEI) and had a study partner, completed the questionnaires only (qmAD). Only qmAD participants were allowed a concomitant diagnosis of vascular dementia.

There were two modes of recruitment into the study: through NHS memory clinics of Oxford Health and Oxford Radcliffe Hospitals' NHS Trusts (approximately 13 clinics); or through a longitudinal cohort called the Oxford Project to Investigate Memory and Aging (OPTIMA). The groups recruited through the clinics were the aMCI, Mild AD and both mAD and qmAD groups. Controls and SMC groups were recruited solely through OPTIMA, with aMCI and mild AD

recruited through OPTIMA where possible. Clinical recruitment involved doctor referral with a signature from the patient to authorise contact details to be provided to me. Following referral, participant information sheets were provided with an indication if medication was to be withheld until after baseline assessment, in accordance with ethical approval. Participants recruited through OPTIMA were pre-screened by OPTIMA nurses and, if eligible, invited to hear more about this study, and information sheets were provided. Diagnosis was either determined by referring clinicians from Oxfordshire and Buckinghamshire memory clinics or through the team of research nurses and research clinicians in OPTIMA. OPTIMA diagnoses are routinely checked with new MRI scans and participant report. Alzheimer's disease patients were diagnosed according to the NINCDS-ADRDA criteria [59] and aMCI patients were diagnosed by Petersen criteria [60].

Study 2

Patient JJB was referred to our department by his wife due to activity that was not time-of-day appropriate. Upon discussion, a study plan was created, the oncologist in charge of JJB's case agreed to the testing and consent forms for access to medical notes, and agreements to collect and keep data provided were signed by JJB and his wife.

2.3.2. Questionnaire: Data Collection

Questionnaires were conducted at the participant's home with both the patient and carer present. The Mini-Mental State Examination (MMSE) [61], Section 2.4.1. (p.40), was carried out with the participant in interview. The carer was asked to remain quiet and not indicate answers to the participant. The Health Questionnaire (HQ) [62], Section 2.3.2.1. (p.37), the Hospital Anxiety and Depression (HAD) Scale [63], Section 2.3.2.2. (p.38), the Pittsburgh Sleep Quality

Index (PSQI) [64], Section 2.7.2. (p.51), and the Jupiter Sleep Questionnaire (JSQ) [65], Section 2.7.1. (p.51), were conducted with the participant, but also taking into account carer/study partner comments and factual corrections. The Morningness-Eveningness Questionnaire (MEQ) [26], Section 2.3.2.3. (p.38), was completed solely by the participant with questions and possible answers read aloud and responses recorded. The Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory (ADCS-ADL) [66], Section 2.5.1. (p.41), and the Social Functioning Scale (SFS) [67], Section 2.5.2. (p.41), were completed in interview with the carer/study partner wherever possible. The MMSE, PSQI, JSQ, ADCS-ADL and SFS were completed at both assessments whereas the HQ, HAD scale and MEQ were only completed at baseline assessment.

Each questionnaire is described below in terms of its composition, outcome measures and scoring if appropriate. All questionnaires apart from the ADCS-ADL inventory were adapted to have a large font size to improve legibility. Questionnaires presented in the appendices of this thesis are the adapted versions used in the study (although with font size decreased to conserve space).

2.3.2.1. Questionnaire: Health Questionnaire (HQ)

The Health Questionnaire (HQ) used in this thesis was adapted from the Primary Care Evaluation of Mental Disorders (PRIME-MD) Patient Health Questionnaire (PHQ) developed by Spitzer *et al.* [62]. The adapted version used in this thesis consists of thirty-six questions about demographic information (for example age, gender and level of education); past and present medical conditions, allergies and hospital appointments; current medication; alcohol and caffeine intake; smoking frequency; and visual conditions. A separate sheet was used to

determine whether the participant had risk factors for restless legs syndrome. For the questionnaire in full and the restless legs syndrome sheet, see Appendix 4.7. (p.84).

2.3.2.2. Questionnaire: Hospital Anxiety and Depression (HAD) Scale

The Hospital Anxiety and Depression (HAD) scale was designed by Zigmond and Snaith [63]. The HAD Scale consists of fourteen questions separated into seven questions assessing anxiety symptoms and seven questions assessing depressive symptoms. The HAD scale provides an indication of anxiety or depressive tendencies. All questions were multiple-choice with answers assigned scores between 0-4. Total subscale scores for depression and anxiety were found in the original validation tests to account for how certain a diagnosis was [63]. Scores of 7 or less indicated no diagnosis, scores of 8-10 accounted for those with doubtful cases and scores of 11 or more accounted for those with a certain diagnosis [63]. Total scores for anxiety and depression tendency were calculated and used in Study 1 of this thesis to determine if differences between groups were present and, if so, to act as a covariate for further analyses where possible. For the questionnaire in full see Appendix 4.6 (p.83).

2.3.2.3. Questionnaire: Morningness-Eveningness Questionnaire (MEQ)

Diurnal preference (sleep time preference) was assessed by Morningness-Eveningness 'Lark-Owl' Questionnaire (MEQ) [23]. The MEQ includes nineteen questions designed to assess the timing preference of the individual's actions. Questions were scored as to standard criteria and then summated to form a total test score, and then the individual was characterised as: definitely a morning type, rather more a morning than an evening type, rather more an evening than a morning type, or definitely an evening type. For the questionnaire in full see Appendix 4.3 (p.73).

2.3.3. Medical notes check

In Study 1, due to the short time frame between diagnosis and commencing the study, and the busy lifestyle of healthier individuals, medical record checks occurred during the assessment period. Medical records were checked for previous illnesses, current medication and notes from the clinic where they were diagnosed. Medical records could not be read until informed consent was given, records were then obtained from the relevant clinic. All controls and participants recruited from the OPTIMA cohort had their OPTIMA study records checked before commencing the study. All other conditions and medications were noted for comparison between groups.

For Study 2, medical records were obtained from the diagnostic clinic and the oncology department. Details of treatments, biopsy and scan results and surgery dictations were recorded.

2.3.4. MRI and CT scan review

For Study 2, after reading medical record notes, MRI and CT reports were checked for radiographer findings. MRI and CT scans were studied with the help of Dr. Olaf Ansorge, a neuropathologist in the University of Oxford who directs the Thomas Willis Brain Bank, who captured the image in Figure 35 in Chapter 6 (p.252).

2.4. Subject Variable: Memory, Attention and Cognitive Profile

2.4.1. Questionnaire: Mini-Mental State Examination (MMSE)

The mini-mental state examination (MMSE) is a 30 point scale with orientation, recall, praxis, language, attention, repetition, registration and comprehension questions [61]. The MMSE was administered to assess memory impairment extent. For questionnaire in full see Appendix 4.2 (p.71).

2.4.2. Cambridge Cognition's Cambridge Neuropsychological Test Automated Battery (CANTAB)

Computerized tests developed by Cambridge Cognition were administered (descriptions are provided in Appendix 4.11. (p.96). The CANTAB tests were as follows: Big/Little circle (BLC) familiarizing participants to the equipment, choice reaction time (CRT) testing attention and paired-associates learning (PAL) assessing visual memory. Additionally, there were: the spatial span (SSP) test assessing executive function; the stop signal test (SST) assessing decision making and ability to inhibit responses; and the graded-naming test (GNT) assessing verbal memory. These tests were always performed in the same order, where possible parallel forms of the test were used in order to have different sequences at each assessment to avoid a learning effect. The PAL test was the only one available in clinical mode. These tests were chosen as they have been previously validated to differentiate between controls and Alzheimer's disease patients [68-70]. Furthermore the tests have been previously used to investigate drug effects [71, 72].

2.5. Subject Variable: Activities of Daily Living and Social Functioning

2.5.1. Questionnaire: Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory (ADCS-ADL)

The Alzheimer's Disease Cooperative Study of Activity of Daily Living inventory ADCS-ADL consists of twenty-three questions with multiple-choice answers which relate to the individual's functioning in activities of daily living [66]. The first six questions summate to form the basic activities of daily living (basic ADLs) component score, and the last seventeen questions summate to form the instrumental activities of daily living (instrumental ADLs) component score. Basic ADLs include the ability of the individual to eat, walk, use the toilet, wash and dress oneself. Instrumental ADLs range from ability to maintain a conversation to selecting television programmes to watch. This questionnaire is validated for use in Alzheimer's disease patients [66]. Permission was provided by the NIA Alzheimer's Disease Cooperative Study (NIA Grant AG10483) to administer the questionnaire. For the questionnaire in full, see Appendix 4.9 (p.88).

2.5.2. Questionnaire: Social Functioning Scale (SFS)

The Social Functioning Scale (SFS) was used to assess the social functioning and independence of participants. The SFS was initially designed for use in schizophrenia, a group for which it is validated [67]. Section 2.5.2.1 (p.42) describes the validation of this questionnaire for the moderate Alzheimer's disease participants of this thesis. The SFS contains twelve questions with multiple options to indicate level of independence and sociability. The questions are then divided into six components; the seventh component of occupational ability was removed due to irrelevancy. The six components are: Withdrawal; Interpersonal Communication;

Independence Performance; Independence Competence, Recreation and Prosocial. Questions regarding ability to find a job were omitted. Permission was given by Prof. Max Birchwood, University of Birmingham, to administer the questionnaire. For questionnaire in full see Appendix 4.8 (p.85).

2.5.2.1. Validation of Social Functioning Scale for Alzheimer's disease patients

In order to validate the Social Functioning Scale (SFS) for use in an older population, internal consistency of the scale was measured with a Chronbach alpha test and by determining how well the components correlated with each other with a Pearson correlation. Then the SFS was compared, component by component, to the scores from the ADCS-ADL inventory using a Pearson correlation. These analyses were conducted initially with the baseline data of the control group and then with the baseline (cholinesterase inhibitor-naïve) data of the moderate Alzheimer's disease group. Results from these tests are presented in Appendix 3.1., (p.65) which show the validity of the SFS for use in both of these groups.

2.6. Outcome Measure: Circadian Profile

2.6.1. Actigraphy: Data Collection

Rest-activity rhythms were measured with an AW7 wrist accelerometer, and environmental light intensity was measured with a mini-L Actilight™ from CamNTEch Ltd. The AW7 watches were chosen as they are waterproof, so participants could wear the watches continuously without having to remember to replace the watch after bathing. For Study 1 the AW7 was worn in accordance with the timeline in Figure 2 (p.32), three weeks at baseline and after a six week rest period again for three weeks. Participants in the mAD group were given the option of only two weeks of actigraphy in each assessment period, the second assessment was then timed to control for the same number of weeks of ChEI stabilisation as the other mAD participants. For Study 2 the AW7 was worn initially for approximately one month, then there was a break of approximately one month after which the Actiwatch™ was worn continuously until death. All AW7 watches were worn on the non-dominant wrist. Watches were set up, downloaded and analysed using the Actiwatch Activity & Sleep Analysis 7™, version 7.45 from CamNTEch Ltd., except for the periodogram analysis.

Activity levels were sampled in one minute epochs. Due to the long battery life (approx.. 2000 days) the AW7 batteries were not changed during the research. Watches were calibrated by CamNTEch Ltd. before first use. Any faulty watches were returned to CamNTEch Ltd. to be fixed. Watch usage was monitored so that each participant had the same watch for both assessments and that each watch was worn by a least one control, aMCI individual and one of the other groups to control for watch variations. Watches were cleaned thoroughly with antiseptic between uses.

For Study 1 missing data was replaced by daily average activity level if the missing data period lasted for less than three hours. Days where data was missing for more than three hours

were rejected from analysis. For Study 2, as no diary was kept, data were only marked as missing when informed of the removal of the watch. As there was caregiver assistance towards the end of JJB's life, the times and dates were noted and analysis re-performed after the data from these periods was removed and marked as missing, to ensure only JJB's inherent activity remained. For Periodogram analysis, missing data were converted to a value of minus one for the Lomb-Scargle analysis [73] instead of daily average activity, as the test does not require data extrapolation (see Section 2.6.1.4. (p.46) for more details). The Sokolove-Bushell method [74] of periodogram analysis was conducted after missing data were replaced with the day average activity level. All data were edited for British Summer Time clock changes manually. A comparison of the two methods for the Study 2 data is provided in Appendix 2.1. (p.61).

2.6.1.1. Actigraphy: Validation for Mild Cognitive Impairment Individuals

As the use of actigraphy to measure sleep characteristics has not been validated previously in amnesic mild cognitive impairment individuals (aMCI). Actigraphic recordings were compared in this thesis to electroencephalography (EEG) recordings (for more detail on how the EEG recordings were collected see Section 2.7.4 (p.52). Activity data derived from actigraphy was isolated for the period of EEG recording for the same night. As no treatment or intervention was given to the aMCI group the night of sleep used in this comparison was chosen from either assessment to increase the number of comparisons possible. One comparison per participant (if the recordings were available) was conducted in order not to bias results. In order to determine which activity counts indicated a sleep epoch and which indicated a wake epoch, an algorithm was applied to the data. Two types of algorithms for determining sleep/wake epochs in actigraph data were implemented and compared [75, 76]. To prepare the EEG recordings for comparison, first the 30 second epochs were converted into 1 minute epochs. This was

conducted according to the procedure of Cole and colleagues [76]. Epochs of the EEG recording were then marked as sleep or wake. Sleep encompassed all epochs scored as Stage 1, 2, 3 or 4 sleep, as well as REM sleep using the scoring criteria of Rechtschaffen and Kales [77]. Wake encompassed epochs marked as wake or movement according to the same scoring criteria. After epochs for both actigraphy and EEG recordings were scored as sleep or wake, the two methods were compared epoch by epoch for agreement rates. Agreement was also divided into the agreement of the detection of sleep and wake epochs separately. The results for the validation of actigraphy in the amnesic mild cognitive impairment group are presented in Appendix 3.2. (p.66).

2.6.1.2. Actigraphy: Non-Parametric Circadian Rhythm Analysis (NPCRA)

Non-parametric circadian rhythm analysis was conducted to determine the circadian nature of the rest-activity rhythm derived from actigraphy data [78]. The NPCRA was conducted by converting 1 minute epochs into hourly data before calculating the NPCRA parameters, implemented in MS Excel (courtesy of Prof. van Someren). The NPCRA variables are: Inter-daily Stability (IS), the lack of variability between days indicating the strength of link between external environmental cues, and the rhythm calculated using the Sokolove and Bushell chi-square periodogram [74]; Intra-daily Variability (IV), the fragmentation of the activity rhythm, how many transitions between rest and activity occur and their extent calculated from the mean squares of the difference between all consecutive days and the overall variance; the least five hours of activity average activity count (L5); the most active ten hours of activity average activity count (M10); and their respective onset times; amplitude of activity; and relative amplitude (RA, the difference between L5 and M10 hours). For details of how these parameters were calculated and where they arose from, see van Someren *et al.*, 1999 [78]. Missing data

was skipped when data were missing for longer than 3 hours. Missing values were provided on the hour with the following 24h removed from analysis as recommended by the program.

2.6.1.3. Actigraphy: Average data calculated for 24 h profile of activity

Within the Actiwatch Activity & Sleep Analysis 7™, version 7.45 from CamNTEch Ltd. software the actigraphy files were cut to end and begin at midnight and any missing data were replaced with the day average activity. An average graph was created and raw data exported which provided an average activity count for each minute of a 24h day averaged from all days included. These values were then averaged per hour and plots averaged across groups per assessment. Results were graphed and are presented in the relevant data chapters.

2.6.1.4. Actigraphy: Periodogram Analysis

Periodogram analysis of rest-activity data was conducted for both studies. For Study 1, each actigraphic recording was analysed in Chronoshop version 1.01 (courtesy of Dr. Spoelstra) with missing data treated appropriately.

Study 2 involved dividing the data into bins determined by steroid (dexamethasone) dosage, due to known dosage-dependent sleep-altering effect of the drug [16]; bins are marked in Figure 30a in Chapter 6 (p.239).

Periodogram analyses to assess periodicity in the activity data for both studies were conducted using both Lomb-Scargle's analysis and Chi-square periodogram analysis developed by Sokolove and Bushell [18]. Both types of periodogram analysis were compared, as each has its own merit. The Chi-square periodogram analysis is applicable to activity data which does not contain missing data and, since most rest-activity data are evenly spaced, this is the method most used for periodogram analysis in circadian studies. The Lomb-Scargle method was also

performed as it is sensitive to rhythms in the presence of missing data without the need for data interpolation, and additionally is sensitive in the presence of noise and low amplitude data [19], as in this case. All periodogram analyses were performed in Chronoshop version 1.01 at a significance level of $p=0.001$, and one minute bin size to match the one minute sampling epoch of the actigraphy. The $p=0.001$ significance level was chosen here as a higher level than usually recommended ($p=0.01$) hourly data-sets, because a more conservative significance level was thought to act as a post-hoc threshold for multiple comparisons.

Results from both types of periodogram analyses are presented for Study 1 in the data chapters. For Study 2, to ease understanding, only the Lomb-Scargle periodogram results are presented in Chapter 6 (p.224). The Chi-square periodogram results are presented in Appendix 2.1. (p.61).

2.6.2. Melatonin Rhythm: Data Collection

In Study 2, two sets of urine samples were collected however, the first set was rejected due to urinary incontinence and the inability to collect all urine over the 53h study duration. The second time all urine excreted over a 50h period was collected, with times noted and aliquots from each sample were taken for analysis. For each sample melatonin sulphate (6-sulphatoxylmelatonin or aMT6s) concentration was determined by radioimmunoassay by Stock Grant Ltd., and excretion rates (ng/h) were then calculated [17].

2.6.2.1. Melatonin Rhythm Analysis

In order to determine whether JJB had an endogenous circadian rhythm, a circadian clock marker, melatonin was assessed. The first sample taken was rejected and one sample was removed from analysis due to a fault in determining aMT6s concentration, out of a total of 14

samples analysed. In order to determine the acrophase and amplitude of melatonin peak a non-linear regression model (as defined in [18]) was fitted to the data implemented with Solver in MS Excel, altering the constant, acrophase and amplitude to provide the smallest p-value (courtesy of Roelof Hut). Then, linear cosinor analysis was conducted to determine the correct tau value, using CircWave version 1.4 to generate the fitted curve, with curve parameters generated using CircWave Batch version 50 (both courtesy of Roelof Hut). The fitted curve was calculated by fixing tau at 0.1 iterations between eighteen and twenty-six hours, until the best fit was determined. As both methods have been reported in circadian studies previously, they are compared in this chapter. There are several parameters required to assess the rhythmicity of a melatonin profile, these are shown in Figure 4.

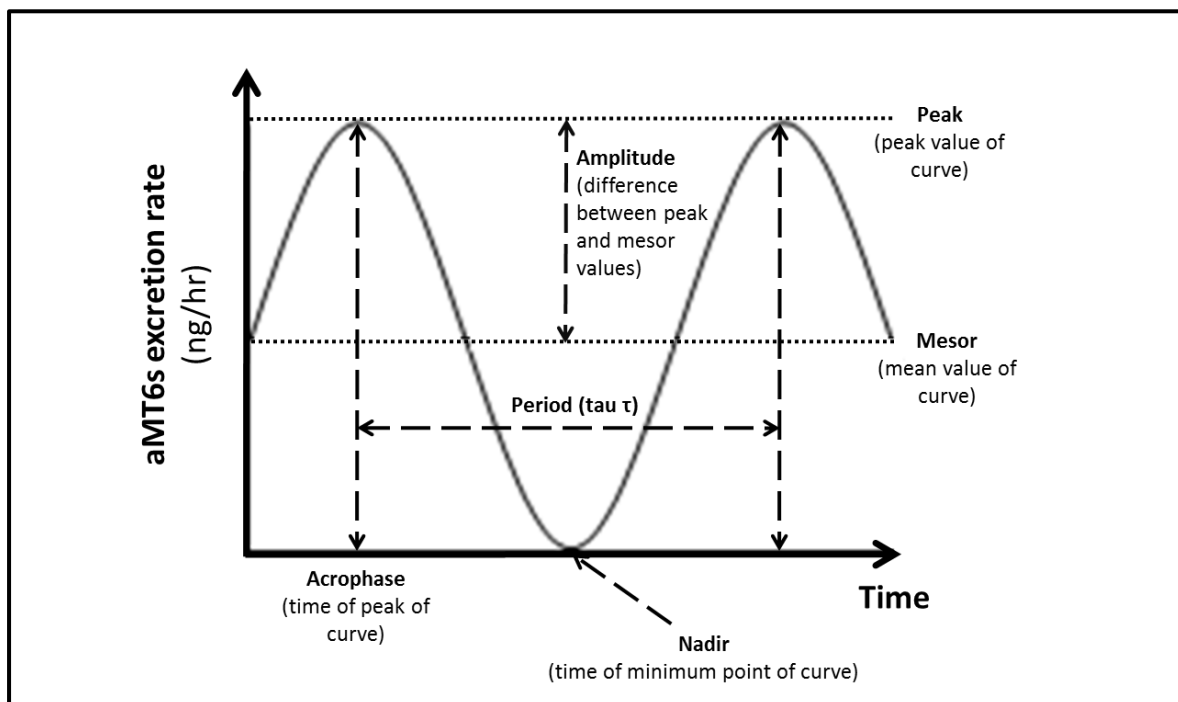


Figure 4. Schematic depicts a circadian rhythm of excretion rate against time. Acrophase is the time that the curve reaches its Peak Value. The Nadir is the minimum value the curve reaches and the time when this occurs. The time between each acrophase is the Period or Tau (τ). The Mesor is the mean excretion rate value between the peak value and the nadir of the curve. The Amplitude of the fitted curve is the difference between the peak and mesor values.

Non-linear regression model

This method uses a non-linear regression to assess data that is unequally spaced, like aMT6s samples [79]. The equation for this method is:

$$MT = c + (Amp \times \cos(2\pi \times ((t - tc)/T)))$$

Where MT is the excretion rate (ng/h), t is time, c is the mesor (rhythm adjusted mean), tc is the phase angle, Amp is the amplitude of the rhythm and T is the period.

Linear cosinor analysis

Cosinor analysis assesses the fit of the excretion rates to a circadian rhythm by determining whether the data are best described by a straight line or a cosine wave [80]. Urinary samples of aMT6s are routinely analysed with cosinor analysis which is especially recommended when data points are not equidistant [81-85]. The cosinor analysis in this case uses a linear regression fit, as used in CircWave software (courtesy of Roelof Hut). The software has been previously used to process excretion rates and genetic circadian rhythm profiles [86-92].

Cosinor analysis involves fitting a sine wave to the data points to determine amplitude and period length of the melatonin metabolite (aMT6s) rhythm. A cosine wave is additionally fitted in order to determine the phase of the sine wave. Therefore, cosinor analysis assumes that the data points are sinusoidal and fits the cosinor curve by calculating the sum of squares. The linear cosinor analysis equation used in this analysis is as follows:

$$F(t) = c + a.\sin \left[2\pi t/24 \right] + b.\cos \left[2\pi t/24 \right]$$

Using this equation the following parameters are calculated: the f-statistic, sum of squares and the f-statistics significance value (p-value), the peak value, trough value (nadir), amplitude of the cosinor wave and the equation constants (a, b, and c). The definitions of these values can be best comprehended by the figure of a labelled circadian rhythm (Figure 4, p.48). The values of the “a” and “b” constants describe how the curve fits the data points, with a larger constant “a” leading to a fitted curve more similar to a sine wave, and a larger constant “b” leading to the wave being more like a cosine wave.

2.7. Outcome Measure: Sleep Profile

2.7.1. Questionnaire: Jupiter Sleep Questionnaire (JSQ)

The Jupiter Sleep Questionnaire (JSQ) is a questionnaire designed by the Jupiter Medical Center sleep clinic in Jupiter, Florida, USA [65]. Permission was given by the clinic for the use of this questionnaire. The questionnaire was adapted for use in this study and now consists of twenty-four questions detailing when the participant went to bed, sleep latency, reasons for difficulty initiating or maintaining sleep, when they woke up and their napping behaviour. The questionnaire also has questions to determine if the participant has risk factors for sleep apnea. For questionnaire in full and the adapted version see Appendix 4.1 (p.68).

2.7.2. Questionnaire: Pittsburgh Sleep Quality Index (PSQI)

Subjective sleep quality was assessed by the Pittsburgh Sleep Quality Index (PSQI) which consists of 10 questions and generates 6 component scores and one summated 'global' score [64]. Each component has a 4-point scale from 0 to 3. A global PSQI score >5 indicates disturbed sleep. Components include: subjective sleep quality; sleep latency; sleep duration; habitual sleep efficiency; sleep disturbances; use of sleeping medication; and daytime dysfunction. As the same questions were in both the JSQ and the PSQI, questions were only asked once and then scoring of the PSQI carried out by using both questionnaires to answer the original PSQI questions. For the questionnaire used in this study and the original see Appendix 4.4. (p.77) and 1.4.5. (p.80).

2.7.3. Actigraphy: Sleep Analysis

Analysis to determine the characteristics of the sleep period from rest-activity data was carried out in the Actiwatch Activity and Sleep Analysis software™ version 7.45 from CamNTEch Ltd.

Every whole sleep period that was recorded was included in the analysis. Sleep onset and offset were decided by comparing the sleep diaries to reduction of activity times near to the reported bedtime in the diary. Lights off information from the sleep diaries was preferred but both lights off and bedtimes were not always reported accurately. Unfortunately, due to non-compliance light data from the Actilights™ was rejected due to its low reliability. From the manually set sleep start and sleep end times, the software then calculates the sleep latency by accounting for the amount of activity after the manual marker was set. Due to light information being unavailable, sleep latencies were not highly reliable. Whenever lights off times were provided they were used instead of bedtime to make sleep latencies more accurate, however, this information was not routinely given. Parameters of bedtime, getup time, sleep start and end times, sleep latency, sleep efficiency $[(\text{amount of sleep/hours in bed}) \times 100]$, number of sleep and wake bouts, number of immobile minutes, mean length of immobility, one minute immobility (number of times only immobile for one minute, with surrounding epochs being moving minutes), and fragmentation index $[(\text{one minute immobility/time asleep}) \times 100]$ were then calculated and averaged over the assessment period. Sleep analysis parameters were then averaged for each individual per assessment for further analyses. The validity of actigraphy to measure sleep in aMCI individuals was tested, results are shown in Appendix 3.2. (p.66)

2.7.4. Electroencephalography (EEG): Data Collection

A light-weight, small Actiwave™ system (from CamNTEch Ltd.) was used in this study to minimise discomfort and to enable the devices to be worn at home by memory impaired

individuals. This lightweight device was a prototype of a large channel version of an imbedded animal EEG system. The Actiwave™ system consisted of three boxes, a two-channel electrooculogram (EOG) with a reference channel, a two channel electromyogram (EMG) with a reference channel and a four channel EEG with a reference channel. Gold plated 0.5mm electrodes were used, the placement of these electrodes and the physical set up of the boxes can be seen in Figure 5 (p.55). The placement of the EEG electrodes were according to a standard 10-20 system set up using only positions Fz (frontal lobe), Cz (central sulcus), Pz (parietal lobe) and Oz (occipital lobe). The head was measured from Nasion (centre of eyebrows) to Inion (inward dent under occipital lobe) and electrodes placed at 20% intervals along this line. The centre of the head was found by locating the midpoint between the ears. The EEG electrodes were referenced to the right mastoid, the EOG electrodes to the left mastoid and the EMG electrodes to the central chin. The skin was prepared with abrasive gel and the electrodes held in place by the electrode paste with micropore over the top.

Due to the size of the electrodes, although different systems and adapters were trialled, I was unable to get impedance readings. I did set up one person with an alternative system which confirmed that my preparation of the head and electrode placement was correct. The system was required to be set up in advance of recording and due to this being a lengthy process it had to be conducted before travelling to participants' homes. Therefore, calibration of the device with scheduled eye blinks, eyes closed etc. was not able to be carried out. As two identical systems were used, each system was kept separate with boxes not interchanged between systems. Furthermore, it was ensured that participants wore the same system on both nights of assessment in each period.

A fault with the hardware was discovered on processing the EEG data after the data collection period had ended. This fault involved all channels appearing to have recorded data at

very low amplitudes. During the study this was put down to individual differences but on discussion with an EEG specialist the fault was discovered. The fault was determined to be a hardware or download issue as all channels were affected; therefore, all boxes would have had to become faulty at the same time. Other alternatives were ruled out as the same system attached to the participant on two consecutive nights could work correctly one night and not the next. This ruled out that one system was faulty or that the fault was due to participant differences such as interference from electrical household objects. For details of the number of recordings rejected due the fault and the number of recordings, therefore, available for analysis see the methods sections of Chapters 3-5.

The systems were set up with EEG channels sampling at 128Hz at 8 bits, EOG at 256Hz and 8 bits and EMG at 256Hz and 8 bits, according to standard set up procedure with an epoch length of 30 seconds. No EEG filters could be set up at this stage, all filtering was carried out after download of data, however, the left EMG was given a low pass filter and the right EMG was given a high pass filter for comparison. After use each system required immediate download as battery life was only 13 hours. Once downloaded and backed up, the recording had to be deleted from the system to allow for future recordings. Each box was downloaded separately and then the three edf+ files were joined using an edf+ joining utility from CamNTEch Ltd. to create a single file containing the EEG, EOG and EMG channels.

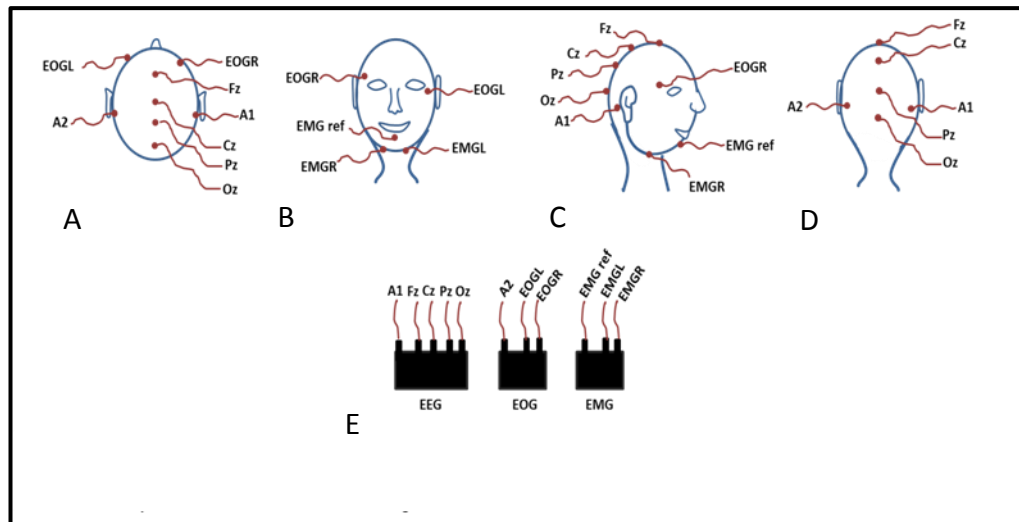


Figure 5. Schematic of electroencephalography (EEG), electrooculography (EOG) and electromyography (EMG) set up. Images A to D show positioning of electrodes. Electrode positions are as follows: Fz: Frontal lobe midline; Cz: Central sulcus midline; Pz: Parietal lobe midline; Oz: Occipital lobe midline; EOGL: left EOG; EOGR: right EOG; EMGL: left EMG; EMGR: right EMG; EMG ref: EMG reference; A1: EEG reference on right mastoid; and A2: EOG reference on left mastoid. The set-up of the Actiwave™ boxes is shown in Image E.

2.7.5. Electroencephalography: Sleep Macro-architecture (Stage Scoring)

After a single edf+ file for each recording night was created the files were imported to SomnoScreen DOMINO version 2.5.0. (SomnoMedics Ltd. , Randersacker, Germany) for analysis. At each assessment there were two nights of EEG recordings, due to such a lightweight, small system both nights were considered valid recording nights. In order to choose which of the recordings to analyse certain criteria were considered: whether it was a faulty recording (this accounted for 17 recordings out of a possible first assessment total of 60 recordings, and 12 recordings out of a possible second assessment total of 51 recordings); and if any electrodes fell off during the night (4 at first assessment and 2 at second assessment). All faulty recordings were rejected. If both recordings of the assessment had electrodes that fell off, the one with the least artefact due to this was analysed. If both recordings did not have these problems, the second night was analysed.

Chosen recordings were then filtered with the following filters: EOG had a high pass filter of 0.3Hz and a low pass filter of 10Hz, EEG had a high pass filter of 0.3Hz and a low pass filter of 30Hz and EMG had additional filters of a high pass filter of 10Hz and a low pass filter of 256Hz. Each recording was then scored manually according to scoring criteria of Rechtschaffen and Kales [77] on the central channel (Cz). Lights on was determined by the beginning of the final consistent period of wake at the end of the recording. Lights off was determined by finding the last period of relaxed wakefulness before the first sleep stage was scored, that persisted as sleep changing sleep stage again before a wake bout. Relaxed wakefulness was generally taken from after the last large movement before sleep onset. However, where difficult to determine, the sleep diary of actigraphy was consulted for guidance. At no time was actigraphy data compared to EEG data to determine staging or lights off/on and sleep onset times. Sleep scoring parameters such as wake after sleep onset and percentages of stages in the night were calculated. Sleep latency was determined by the time between lights off and sleep onset. In order to assess when rapid eye movement (REM) sleep periods occurred in the night, and to account for different sleep durations, the recording was divided into thirds and stage percentages were calculated for each section of the recording. Due to the program used for sleep scoring, files did not have artefact removed before sleep scoring parameters were calculated.

2.7.5.1. Scoring validation

In order to assess my scoring accuracy a subset of files, all of the MCI valid recordings, were rescored blind by an independent expert, Dr. Alpar Lazar, University of Cambridge. Following rescored, the hypnograms from my original scoring and Dr. Lazar's scoring were compared epoch by epoch. This was done by first classifying all epochs of stages 3 and 4 sleep as 3, as

both can be categorised as Slow Wave Sleep (SWS). Then each epoch was given a score of 1 if the epoch was scored the same by both of us or 0 if the epochs were scored differently. Then a frequency test was conducted and expressed in percentage terms. Concordance between scoring was at a minimum 69.7% and at a maximum 91.4% with an average agreement rate of 80.1%. Results are presented in Appendix 3.3. (p.66).

2.8. Outcome Measure: Suprachiasmatic Nucleus Localisation

2.8.1. Brain Collection

The brain donation was carried out as per the procedures of the Thomas Willis Brain Bank, Neuropathology Department, University of Oxford, UK. Signed permission was collected from JJB before death and after from the family to confirm continuation of desire to donate the brain. The body was stored at a funeral director's property under usual conditions for approximately 60 hours before post-mortem removal of the brain was carried out at the John Radcliffe Hospital, Oxford, UK under the supervision of Dr. Ansorge, collaborating Neuropathologist from the above department. Tissue was stored in formalin at room temperature until dissection.

2.8.2. Suprachiasmatic Nucleus Localisation

The suprachiasmatic nuclei (SCN) were located through 5mm coronal sections using a human brain anatomy atlas [33]. A 2cm² section was taken around the SCN including the optic chiasm and paraffin embedded for immunohistochemistry to be conducted.

2.8.3. Immunohistochemistry: Confirmation of SCN Location and Cancer Cell Observation

Sections were stained with haematoxylin and eosin (H&E). Immunohistochemical analyses were performed on 6µm sections from the FFPE brain sections. After de-waxing, endogenous peroxidase activity was blocked (10% H₂O₂ in PBS, pH 7.3, 30 minutes). Antigen retrieval was achieved by autoclaving the sections in sodium citrate, (10mM pH 6.0, 10 minutes), 2x5min microwaving, or incubation with protease (Menarini, 10 minutes). Sections were incubated with the following primary antibodies: GFAP - Dako polyclonal rabbit (1/4000 dilution); MIB-1 - Dako

monoclonal mouse (1/100 dilution). Luxol-fast blue and cresyl violet stains were conducted to observe neuronal structures (myelin). This was conducted according to the same procedure set out in Bäumer *et al.* [93].

To further define SCN areas we used monoclonal clone PS41 against Vasopressin neurophysin in a dilution of 1:25 (gift from Prof John Morris, Anatomy, Oxford [94]). Sections were incubated at 25°C for 40 minutes with primary antibody and at 25°C for 35 minutes with secondary antibody (Dako REAL EnVision/HRP, Rabbit/Mouse (ENV) kit). Dako REAL DAB+ chromagen was applied for 10 minutes and sections were counterstained with Harris's haematoxylin.

These methodology details were provided by Dr. Ansorge, Neuropathology department, University of Oxford who conducted this work.

2.9. Ethical approval

Study 1

Following a lengthy process of gaining research passports for those involved from the three initial NHS trusts (John Radcliffe Hospital, Oxford; Research Institute for Care of the Elderly, Bath; and Kingshill Research Centre, Swindon), financial and university accountability was sought and insurance and indemnity secured for the study from Clinical Trials Research and Governance, University of Oxford. All forms were completed for NHS national ethics approval and SSI forms submitted for each NHS Trust. The application procedure included scientific review of the study by peers, study design and methodology, as well as information and consent form creation. I then submitted all of these documents to the NHS ethics committee and was assigned a meeting in London (South East NHS Ethics committee) which specialised in research conducted with vulnerable people with issue of mental capacity. I had addressed the issue of mental capacity and informed consent by having study partners co-sign consent forms and by creating a form that would register any consultation conducted with the study partner to determine the willingness of the participant to continue the study if mental capacity was in doubt after beginning the study. All forms were submitted in December 2008 after beginning my DPhil research in October 2008. I was invited with Dr. Wulff to attend the meeting of the South East NHS Ethics Committee which took place on 14th January 2009. Unfortunately the application was rejected due to a misunderstanding about how the EEG equipment was to be attached.

After this unfavourable opinion I went about addressing the issues of the committee, recreating all of the forms and resubmitting to a different committee at the end of January 2009. It was decided not to appeal the previous committee decision, due to another meeting with a different committee being available quickly. The meeting for this application with

Berkshire NHS Ethics Committee occurred on 10th March 2009. I attended the meeting with both Dr. Wulff and Professor Wilcock. This time our application was received favourably, so with a few slight changes to information sheets (mainly to remove details required by South East Ethics Committee) the study was approved in May 2009. Following approval from the National Ethics Committee, each NHS Trust individually dealt with the respective R&D application. This application involved approval for approaching patients and approval for use of NHS facilities and staff. A substantial amendment to two questionnaires was submitted on 5th May 2009 and a further amendment to add the ADCS-ADL inventory was submitted on 16th June 2009. After these amendments were then approved by the relevant R&D committees recruitment into the study began, with the first person commencing the study at the end of July 2009. This was at the same time as recruitment began through OPTIMA, which was ethically approved through the same process as the NHS National Ethics.

In August 2009 a substantial amendment was made to add another subset of clinics of the then Oxford and Buckinghamshire Mental Health NHS Trust (OBMH) (now Oxford Health). Following this approval and that of the respective R&D committees, as well as a new honorary contract to conduct research within their NHS Trust, recruitment began through the Dementia Research Network (DeNDRoN) Thames Office in order to recruit from all of the 13 mental health clinics and advertise the study widely.

In August 2010 the Bath clinic was removed from the study as no participants had been recruited from this location. In this amendment a further two participant groups of MCI and Mild AD were added to improve the number of participants in the study. This amendment received approval from the relevant R&D committees at the end of November 2010. Recruitment began with the first mild AD participant beginning in January 2011 and the first MCI participant beginning in February 2011.

The final amendment to the study occurred in March 2011 to enable Mild AD patients, who had just begun to be allowed to be prescribed ChEIs, due to changes to NICE guidelines, to have the ChEI effect on their rest-activity and sleep monitored. This approval was granted and, although no mild AD participants recruited were beginning ChEI treatment, this enabled future researchers working on this study to recruit from this group.

Study 2

Approval from leading oncologist consultant, Dr. Blesing, Churchill Hospital, Oxford and a signed informed-consent form from JJB and next of kin were provided. All Brain donation forms were provided by Thomas Willis Brain Bank and were re-signed after death by next of kin.

2.10. Statistical Analysis

As power calculations determined sample sizes required for valid statistical analyses to be larger than any found in Study 1, the cut off of a sample size of at least four individuals per group was used. Comparison to groups of lower than four individuals are described in the text of results sections. This arbitrary cut off was used as required sample sizes from power calculations were higher than achieved for most methodologies and groups (apart from the qmAD group). Power calculations are as follows (calculated using G*Power version 3.1.5. developed by Faul, University of Kiel, Germany): For questionnaires, the PSQI was used as an example from the study Wulff *et al.* (2012 [79]) with a power of 0.8 and an effect size of 1.1, a sample size of 11 per group was calculated from Global PSQI scores of those with and without schizophrenia. For actigraphy, van Someren *et al.* (1997 [95]) was used with a power of 0.8 and an effect size of 2.4, a sample size of 4 per group was required for statistically significant results, from the variable of Intra-daily Variability between two repeated measures assessments after bright light therapy. For CANTAB, Blackwell *et al.* (2004 [68]) with a power of 0.8 and an effect size of 1.7 showed a sample size of 6 per group was required from the GNT total score between converters and non-converters to AD. The sample size for EEG comparisons was obtained by analysis of the means of data from Bliwise *et al.* (1989 [96]) for Stages 3 and 4 sleep combined (%) at baseline between AD patients and controls with a power of 0.8 and an effect size of 0.9 indicating a required sample size of 16.

2.10.1. Parametric Tests

All nominal data was tested for parametric assumptions of normal distribution and homogeneity of variance. All testing to determine whether data was parametric was conducted in SPSS version 20. A Kolmogorov-Smirnov test was used to determine whether data were

normally distributed. If nominal data were found to be normally distributed, the Levene's test for homogeneity of variance was carried out within the parametric testing of the data.

Due to the low sample size of the mild AD group in Study 1, no statistical tests were conducted but instead means and standard deviations compared. In line with this, Study 2 did not involve statistical tests between patient JJB and the control group.

All statistical analyses had a 95% confidence level with results being classified as significant if the p-value was below 0.05. A multiple comparisons correction was applied, details of which are below and exact calculations for each methodology are presented in Appendix p.15, 25, 29, 36 & 60).

2.10.2. Determination of Covariates

For Study 1, in order to statistically compare groups, covariates needed to be taken into consideration where possible. To be able to do this the first parameters assessed were those in the participant demographics, chronotype and screening assessment category in Figure 3 (p.34). All medical conditions, medications, age, gender, anxiety and depression tendencies and diurnal preference were compared between groups. These assessments were based on the type of data, either nominal (parametric or non-parametric), binomial or categorical. Those who were significantly different between the groups being compared were used as covariates in further statistical analyses where possible. Unfortunately, non-parametric nominal and categorical data could not be analysed with covariates taken into account.

2.10.3. Parametric Data

When comparing baseline data, a multivariate general linear model (normal distribution), taking into account covariates where necessary, was conducted. Where covariates did not need to be taken into account a one-way ANOVA was conducted.

When assessing the change over time or change due to ChEI administration, a repeated measures general linear model (normal distribution) was conducted, taking into account covariates where necessary.

In the progression of memory impairments chapter (Chapter 4, p.106), to determine whether there was an effect of memory impairment severity on circadian and sleep parameters a Pearson bivariate correlation was conducted using the MMSE total score as a measure of memory impairment severity (found to decrease progressively with increases in memory impairment severity, see Chapter 4, Table 15 p.125).

All statistical testing was conducted in IBM SPSS Statistics version 20, except for binomial data which were analysed in 'R' (copyright of Kurt Hornik).

2.10.4. Non-Parametric Data

Cross-sectional baseline statistical analysis of non-parametric data was conducted with a Mann-Whitney U-test corrected for ties in the data. To assess change over time or medication effects, individual groups were analysed with a Wilcoxon test. In order to compare repeated measures between groups, where possible (component scores etc.) the first assessment was subtracted from the second assessment and the change over time was then compared between groups using a Mann-Whitney U-test. Only two groups at a time could be analysed when dealing with non-parametric data.

2.10.5. Binomial Data

Binomial data (yes or no answers) were analysed in “R” (copyright of Kurt Hornik) firstly compared between groups cross-sectionally at baseline, using the code found in Appendix 3.4. (p.67) to conduct a binomial regression between groups. Change over time or medication effects were then compared binomially in “R” using a repeated measure binomial analysis using lmer, for the code see Appendix 3.4. (p.67). Covariates were added to each model as required. Only two groups could be assessed with the binomial analysis in one test.

2.10.6. Multiple Categorical Data

Categorical data with more than two categories (two categories were analysed binomially) were analysed with a Pearson’s Chi-Square test. If the Pearson Chi-Square test was significant, the Pearson r correlation associated with the categorical data analysis was observed to determine a true significant difference between groups. Categorical data was only analysed cross-sectionally for baseline assessment, although data from multiple groups could be compared simultaneously.

2.10.7. Multiple Comparisons Correction

For each statistical test conducted for each type of assessment, the number of variables were noted and a Dunn-Sidak (D-S) correction of the p-value was manually calculated. The corrected significance levels are provided in the Appendix.

All values provided in results tables are according to the sample sizes stated. Repeated measures statistical analysis results are based on only those participants that had data for both timepoints.

2.10.8. Funding

Funding was provided for the studies of this thesis by the NIHR Oxford Biomedical Research Centre (under Chronobiology and sleep within the Brain Theme) and the Nuffield Laboratory of Ophthalmology, University of Oxford.

3. Study 1: Subjective memory complaint

3.1. Introduction

3.1.1. Ageing

Many aspects of life are affected by ageing processes as, for example, older people are more likely to develop cancer due to a lifetime of cell division leading to an increased risk of malignant mutations [97]. In addition, brain atrophy leads to reduced cognitive function and the body reaps the rewards of a lifetime's movement with bone and joint problems and reduced muscle mass [98, 99]. Getting older can also lead to social isolation with retirement from work and friends moving or dying, leading to a more compact social environment [100]. Finally, seemingly small issues like increased nocturnal urination (nocturia) can strongly impact sleep, leading to sleep deprivation, which, with increased daytime napping, can exacerbate sleep problems [101].

3.1.2. Sleep disruption in Ageing

Sleep patterns and sleep architecture change over the lifespan. Of special interest in this thesis are the sleep changes of older people in comparison to younger populations. Such studies may enable a fuller understanding of sleep changes related to aging and in turn could enable a better understanding of how sleep of Alzheimer's disease patients differs from healthy elderly (addressed in the following three chapters).

Older people (over the age of 65 years) often complain about difficulty initiating or maintaining their sleep with 20-60% reporting insufficient sleep [102]. Reports of insufficient sleep are also thought to be related to an increased risk of falls, difficulties with concentration and memory, and overall decreased quality of life [103]. Insufficient sleep has been considered

by some [103] not to be an inevitable part of aging but the result of the multitude of medications many older people generally take. In a study of melatonin 5mg OD administration [104], researchers found that older people had more nocturnal arousals, increased daytime napping with earlier bedtime and wake up times and more time spent in bed. The same study found that whilst total sleep time remained unchanged, this increase in duration in bed led to lower sleep efficiency scores. Furthermore, phase shifts induced by 5mg melatonin administration were found to be less tolerated by older people than younger populations [104].

When considering sleep and especially sleep architecture changes, Ohayon and colleagues [105] found from a study on adults (ranging from 15 years old to over 75 years old) reported that total sleep time, sleep efficiency, percentages of REM sleep slow wave sleep and REM sleep latency were found to decrease with age. The percentage of stage 1 and stage 2 sleep, and the amount of wake after sleep onset, were found to significantly increase with age.

3.1.3. Circadian disruption in Aging

Circadian period has been found to not change across the lifespan with both younger men (average age 23.7 years) and older men and women (average age 67.4 years) having comparable period lengths of $24.18 \text{ hours} \pm 0.04$ [2]. Despite this consistency in circadian period, changes in circadian rhythms for older people compared to younger populations have been found [106]. This study by Walters *et al.*, researching endothelial function in premenopausal women compared to those who had already passed through menopause, found that the circadian rhythm of endothelium-dependent flow mediated dilation was flattened in post-menopausal women. This suggests that the natural processes of ageing, such as menopause, can have an effect on circadian rhythms. Furthermore a gradual decrease in

activity amplitude has been observed as ageing progresses [107-109], another alteration of the circadian rhythm as individuals get older.

3.1.4. Memory problems related to Ageing

Memory is known to alter over the 24h day and also alter throughout an individual's lifetime [110, 111]. In a sleep deprivation protocol, healthy older people (57-74 years old) were found to have slower reaction times in comparison to healthy younger people (20-31 years old) during their biological day but not during their biological night [112]. Although age-related changes in memory occur, some people experience memory impairments associated to aging that are more advanced than normal age-related memory changes. One such group of people are those with subjective memory complaints (SMC). SMC sufferers experience memory problems, however, they are not normally detectable on testing [113]. Research into this phenomenon is increasing, with some studies suggesting that this could be used as an indicator of developing an amnesic mild cognitive impairment (aMCI) and progressing to an AD diagnosis [114, 115]. There is still much controversy as to whether these individuals do have testable memory problems, however, delayed free recall is impaired in SMC versus healthy elderly individuals [114]. Therefore, testing and determining the clinical significance of subjective memory complaints remains an important focus of current research. This chapter will seek to investigate the differences, if any, between healthy elderly and SMC individuals. Differences in their sleep and rest-activity pattern will be assessed, as sleep spectral frequency changes have already been noted in this group [115]. This chapter will therefore seek to answer the research question:

- ***Are the cognitive abilities, rest-activity and sleep patterns different between older people who have or do not have a subjective memory complaint?***

3.2. Methods

3.2.1. Participant recruitment

Participants were recruited through the Oxford Project To Investigate Memory and Aging (OPTIMA) cohort by research nurses; more details are provided in Chapter 2, Section 2.3.1. (p.35). Controls (n=9, 4 females) mean age: 73.2 ± 4.9 years, Subjective memory complaint (SMC, n=5, 2 females) mean age: 73.2 ± 5.4 years. All participants were routinely checked for memory and medical conditions with MRI/CT scans taken regularly. Individuals were determined to be either controls (those with no subjective or objective memory complaint) or subjective memory complaint (SMC) individuals by a team of research nurses and doctors within OPTIMA. All participants were required to have a study partner, however, they did not necessarily have to live with the participant; for other study criteria see Chapter 2, Section 2.3.1. (p.35). An initial sample of 13 controls were recruited. Two were excluded because of abnormal breathing (one with chronic asthma and the other with diagnosed sleep apnea) a further two were excluded due to full-time work. Therefore, data are presented in this chapter which were collected from nine controls, of which one did not complete the second assessment. An initial sample of six SMC individuals were recruited. One was excluded from data collection and analysis due to use of diazepam as an hypnotic. Therefore, data collected from five SMC individuals were analysed and are presented in this chapter, of which one completed only baseline assessment (due to cataract surgery within two weeks of second assessment) resulting in four participants being followed over time.

3.2.2. Determination of demographics

Participants underwent baseline assessment of medical condition screening, functioning, memory, sleep and rest-activity; for details of the study timeline see Chapter 2, Figure 2 (p.32). Details from medical notes of medications and conditions, as well as the Health Questionnaire (HQ) adapted from the Patient Health Questionnaire [62], the Hospital Anxiety and Depression (HAD) Scale [63] (to determine anxiety or depression tendencies), the Morningness-Eveningness Questionnaire (MEQ) [26] (to determine diurnal preference), were compared between the control and SMC groups to determine if any differences were present. Details of the questionnaires can be found in Chapter 2 (p.36). BMI was calculated using height and weight details collected in the HQ with an online BMI calculator [116]. To ensure the visual system was capable of receiving entrainment information, details of participant's vision, especially presence of cataracts or having had eye surgery, were collected. Participants with early signs of cataract and those who had recovered from cataract removal from one eye were permitted into the study. All participants were free from visual impairments such as blindness, macular degeneration and retinal detachment. No participant was currently being treated for a psychological illness such as anxiety or depression.

3.2.3. Assessment of memory

Memory was assessed by the Mini-Mental State Examination (MMSE) [61], a questionnaire used clinically to measure memory impairment, and some other aspects of cognition, and also by computerised memory and attention tests from Cambridge Cognition Ltd. called the CANTABclipse™ battery. For details of the tests and the questionnaire see Chapter 2 (p.40). Memory was assessed at baseline and nine weeks later to determine any longitudinal changes that were different between the two groups. Longitudinal changes were thought to be

important to measure as, if SMC is a stage in the progression from healthy to Alzheimer's disease, progressive change may be possible to observe [114].

3.2.4. Assessment of functioning

In order to assess functioning, both social functioning and activities of daily living were assessed using questionnaires: Social Functioning Scale (SFS) [67] and the ADCS-Activities of Daily Living Scale (ADCS-ADL) [66]. For details of these questionnaires see Chapter 2 (p.41). Functioning was assessed at baseline and nine weeks later to assess potential progressive change.

3.2.5. Circadian measurements

Rest-activity patterns were assessed using actigraphy, for three weeks at baseline and six weeks later. Non-parametric circadian rhythm analysis (NPCRA) [78] and average daily activity was evaluated for each assessment period, as well as periodogram analysis was conducted to determine activity period length for each assessment. All details of the measurement using actigraphy and the analyses carried out of the resultant data are found in Chapter 2 (p.43).

3.2.6. Sleep measurements

Sleep was measured by three techniques: questionnaires, actigraphy and electroencephalography (EEG), for details of each see Chapter 2 (p.51 &52). All three techniques were utilised at both assessments, with sleep analysis being conducted on the rest-activity derived from the actigraphy of both assessment periods. The sleep questionnaires were the Pittsburgh Sleep Quality Index (PSQI) [64] and selected questions from the Jupiter Sleep Questionnaire (JSQ) [65], details of both are given in Chapter 2 (p.51). EEG data were scored according to the scoring criteria of Rechtschaffen and Kales [77]. Due to a technical fault

(detailed in methods section x) 7 out of 9 controls had recordings analysed at baseline and 5 out of 8 at second assessment, and 4 out of 5 SMC individuals had recordings analysed at baseline, and 3 out of 4 at second assessment. Following scoring, the sleep period was divided into three sections ('thirds') to compare the sleep stage durations and percentages and how they change across the sleep period.

3.2.7. Statistical analysis

All data were divided into parametric or non-parametric scale data (by means of a Kolmogorov-Smirnov method to test for normal distribution, and by Levene's method to test for homogeneity of variance), binominal data or categorical data. Each type of data was statistically analysed as detailed in Chapter 2 (p.63). All data were compared between groups at baseline assessment and for change over time. Correlation of memory scores with sleep and circadian parameters are presented in Chapter 4 (p.106) in the context of all tested possible prodromal stages of Alzheimer's disease, as well as mild and moderate severity Alzheimer's disease.

Multiple comparisons correction was conducted using the Dunn-Šidák correction. Exact resultant significance levels are provided in the Appendix section for each test. Throughout the chapters, if a result is stated as 'still significant after Dunn-Šidák correction' this means the result met multiple comparisons correction for significance. If this is not stated, the result did not remain significant after the correction. All test statistics are provided in the relevant Appendix section.

3.2.8. Ethical approval

Ethical approval for this project was given by the NHS Berkshire Ethics Committee, for further details see Chapter 2 (p.60). Funding was provided by the NIHR Brain Theme.

3.3. Results

3.3.1. Demographics

Time between assessments was analysed and both groups did not differ significantly ($p>0.05$), control mean: 64.6 ± 6.4 days, SMC mean: 69.8 ± 7.8 days. Participant characteristics did not differ between the control and SMC groups in terms of age, sex, or anxiety and depression tendencies (Table 1). As no variables from the screening questionnaires (Health Questionnaire (HQ), Hospital Anxiety and Depression (HAD) scale and review of medical and OPTIMA records) differed significantly between the control and SMC groups after Dunn-Šidák correction for multiple comparisons, no variables were used as covariates for subsequent analyses.

Table 1. Demographic and screening parameters for control and subjective memory complaint (SMC) groups.

Parameter		Control (n=9)	SMC (n=5)
Age (yrs.)		73.2 ± 4.9	73.2 ± 5.4
Sex	Number of females (%)	4 (44.4)	2 (40.0)
	Number of males (%)	5 (55.6)	3 (60.0)
Body Mass Index (BMI)		25.0 ± 4.0	25.1 ± 2.1
Number of individuals with collar size in normal range (%)		7 (77.7)	4 (80)
Average units of alcohol consumed per week		11.6 ± 11.2	13.3 ± 9.6
Years of education		14.7 ± 4.9	15.3 ± 4.7
Anxiety tendency score (HAD)		2.8 ± 2.5	1.6 ± 1.5
Depression tendency score (HAD)		1.7 ± 1.2	1.4 ± 1.1

No significant differences.

Data are mean $\pm$ SD unless otherwise stated.

Full data, test statistics and Dunn-Šidák calculations are presented in Appendix 1.3. (p.7).

For both groups body mass index (BMI) was calculated for each individual, groups had comparable means with the majority of participants on the border between healthy weight and being overweight (BMI ≥ 25.0) (Table 1). In order to determine tendency for sleep apnea, collar

size was collected in the HQ and compared to breathing information collected in the Jupiter Sleep Questionnaire (Table 1, p.75).

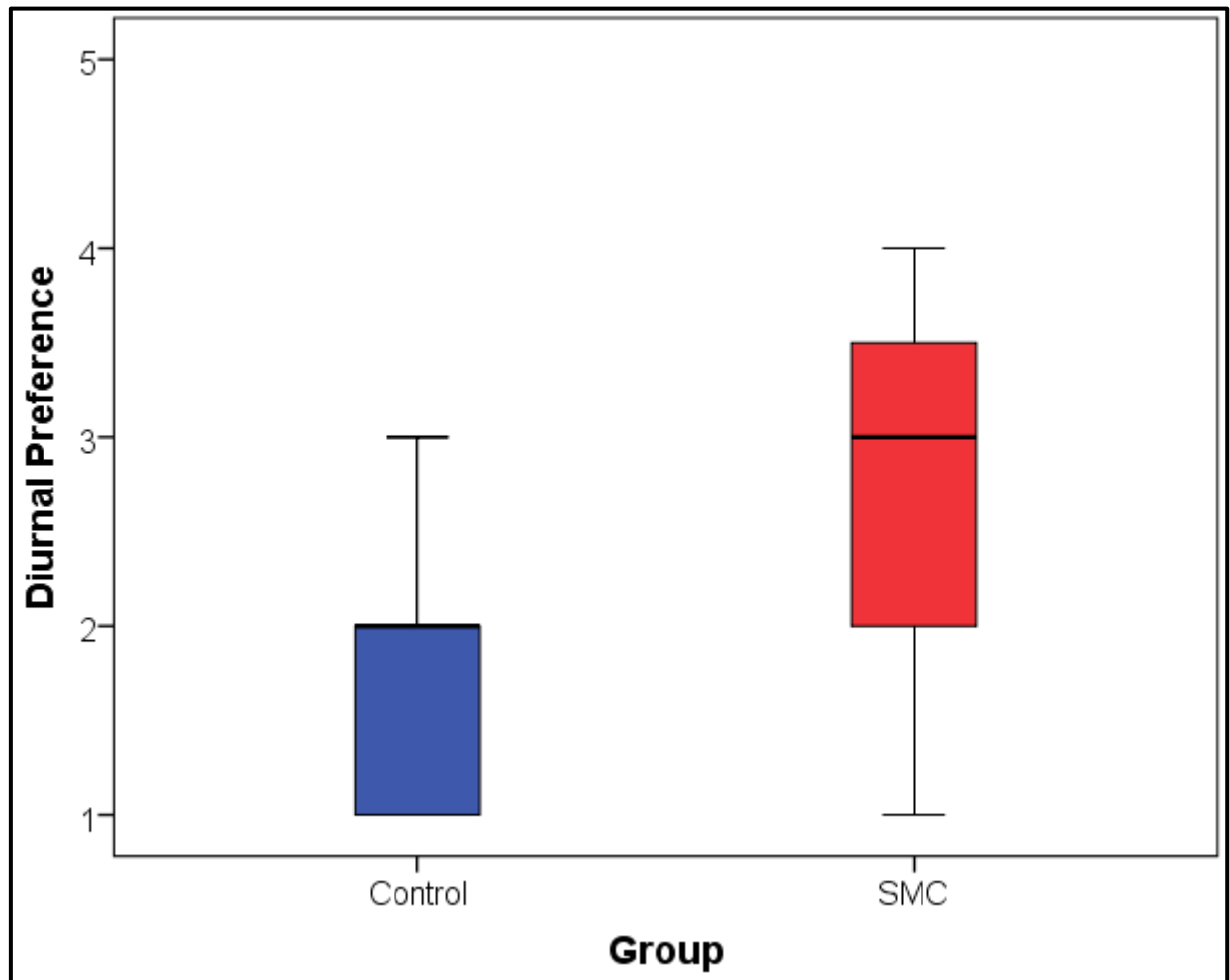


Figure 6. Diurnal preference distribution in control and SMC groups as measured by the Morningness-Eveningness Questionnaire (MEQ). Each gradation of the y-axis shows diurnal preference types, ascending numerical values denote increasing eveningness of sleep timing preference, 1: Definitely morning type, 2: Moderately morning type, 3: Neither type, 4: Moderately evening type and 5: Definitely evening type. Boxed area indicates 50 percentile of type distribution, horizontal line shows median for group and error bars indicate 95% to 5% range of types. Diurnal preference does not significantly differ between groups (one-way ANOVA, $p>0.05$). However controls tend towards morning types with SMC individuals displaying a larger range across types. No individuals had a definitely evening type. All data and Dunn-Šidák correction are presented in Appendix 1.3 (p.14).

The majority (93%) of participants showed no symptoms of sleep apnea and none analysed were officially diagnosed with sleep apnea according to medical records. No statistical difference in symptoms were found between groups ($p>0.05$).

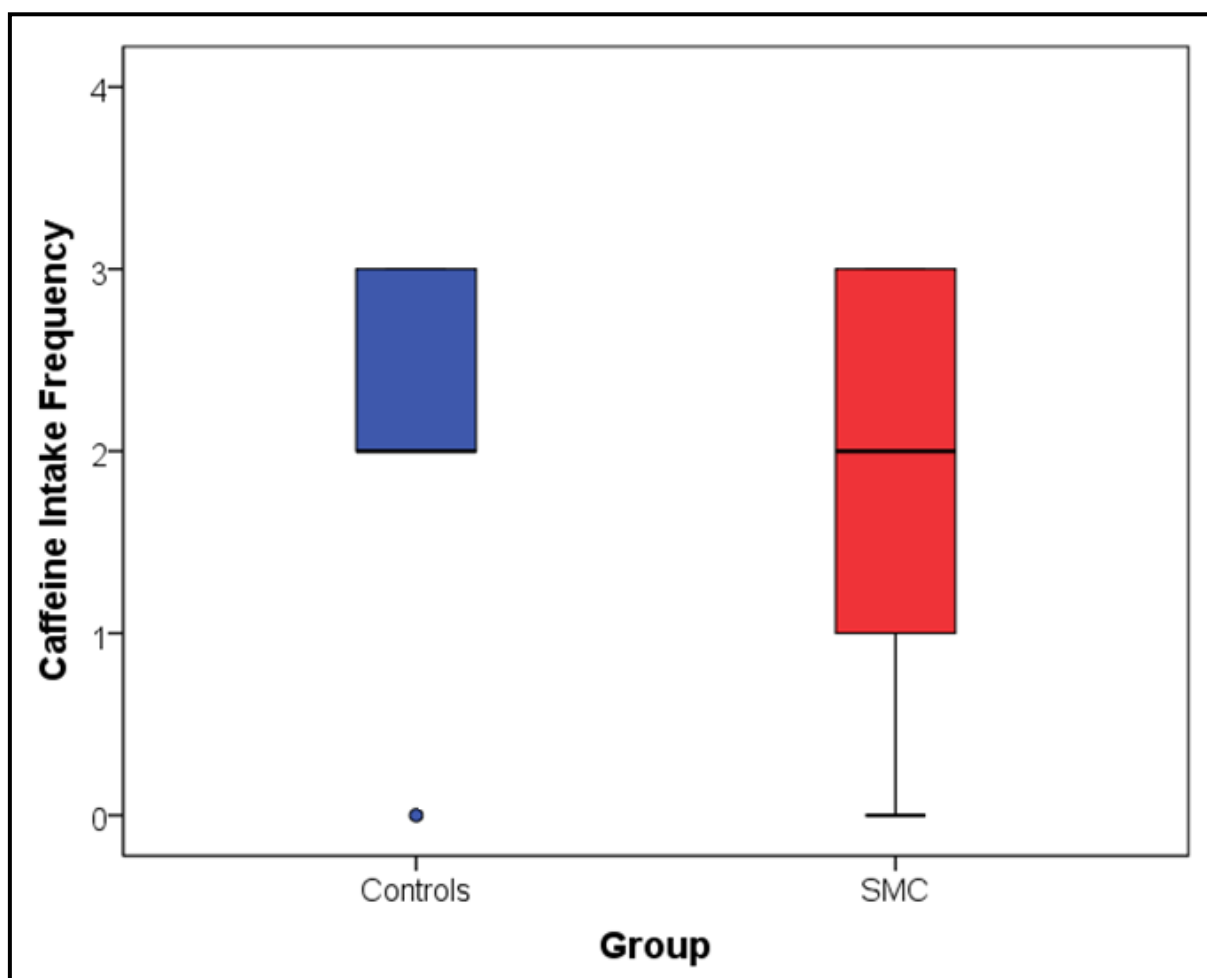


Figure 7. Caffeine intake frequency in control and SMC groups. Each gradation of the y-axis shows the amount of usual caffeine intake per day (p/d), ascending numerical values denote increasing frequency of caffeine intake (taken from the HQ), 0: Never (0 p/d), 1: Occasionally (<1 p/d), 2: Sometimes (1-3 p/d), 3: Frequently (3-7 p/d) and 5: Often (>7 p/d). Boxed area indicates 50 percentile of type distribution, horizontal line shows median for group and error bars indicate 95% to 5% range of types, smaller circle denotes an outlier within the colour-corresponded group. Caffeine intake does not significantly differ between groups (Pearson chi-square, $p>0.05$). Both groups ranged over frequencies of caffeine consumption however the majority of controls consumed caffeine sometimes or frequently. No individuals consumed caffeine often. All data and Dunn-Šidák correction are presented in Appendix 1.3 (p.8).

Symptoms of restless legs syndrome (RLS) were evaluated with information provided from questions within the HQ and the Pittsburgh Sleep Quality Index (PSQI). No participants included for analysis had a diagnosed RLS or sleep disorder and no statistical difference in possible symptoms (such as leg twitching when initiating sleep) was found between the groups ($p>0.05$).

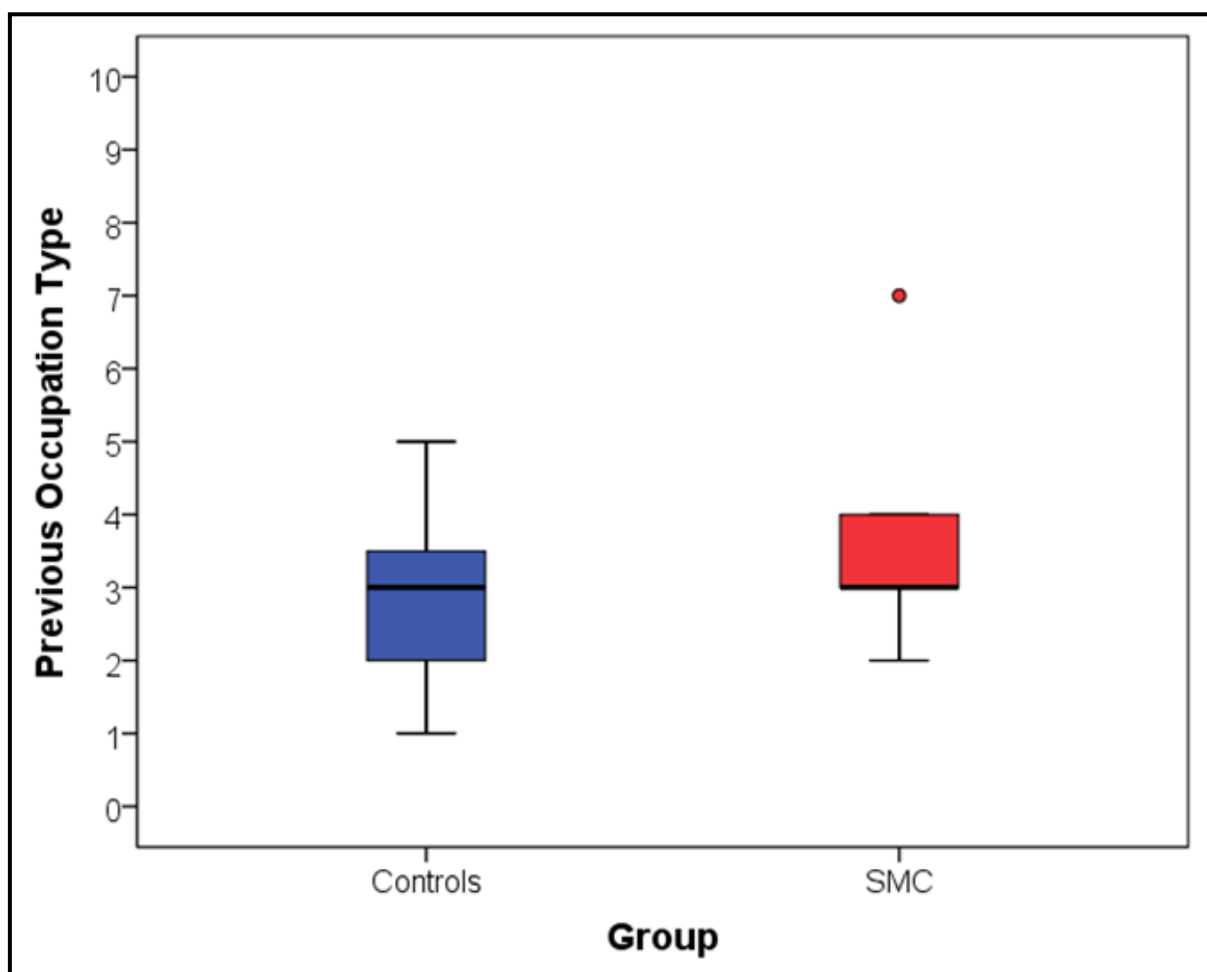


Figure 8. Previous occupation type of control and SMC groups taken from OPTIMA study notes. Each gradation of the y-axis is an arbitrary number representing a category of employment, 1: Care-related, 2: Educational, 3: Medical, 4: Vocational, 5: Administration, 6: Sales, 7: Homemaker, 8: Research, 9: Law enforcement, 10: Manual labour. Boxed area indicates 50 percentile of type distribution, horizontal line shows median for group and error bars indicate 95% to 5% range of types, smaller circle denotes an outlier within the colour-corresponded group. Previous occupations of both groups are centred around care, educational, medical, vocational and administrative previous occupations with one SMC individual having been a homemaker. Previous occupation prior to retirement was not significantly different between groups (Pearson's chi-squared, $p>0.05$). All data and Dunn-Šidák correction are presented in Appendix 1.3 (p.8).

In terms of lifestyle habits, none of the participants currently smoked and average alcohol intake was of moderate levels and was not significantly different between groups ($p>0.05$). No participant had undergone cataract surgery within the previous three months before the study and no significant difference in corrected vision was found between groups ($p>0.05$).

To further define the groups' diurnal preference (Figure 6, p.76), caffeine intake (Figure

7, p.77) and previous occupation before retirement type (Figure 8, p.78) were compared. No significant differences were found, with a wide range of highest level of education and previous occupation types observed ($p>0.05$) (Figures 8&9, p.78-79).

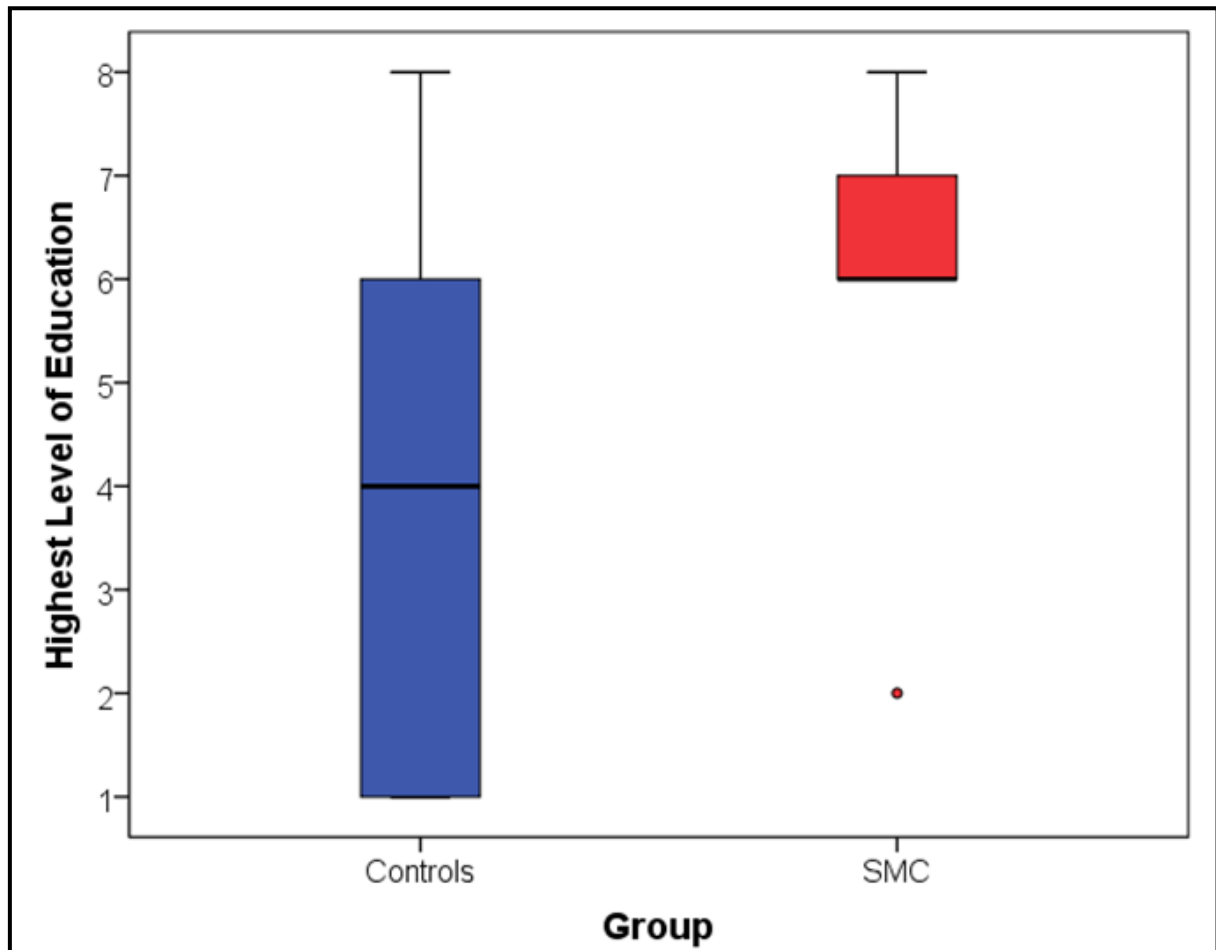


Figure 9. Highest level of education of control and SMC groups (from the HQ). Each gradation of the y-axis is an arbitrary number representing a level of education, 1: Secondary school, 2: Grammar school, 3: Apprenticeship, 4: Diploma, 5: Polytechnic degree, 6: Bachelor degree, 7: Masters degree, 8: Doctoral degree. Boxed area indicates 50 percentile of type distribution, horizontal line shows median for group and error bars indicate 95% to 5% range of types, smaller circle denotes an outlier within the colour-corresponded group. Controls have all levels of highest education type whereas the SMC group centres around higher education degrees with one individual with secondary school as their highest level of education. There was no significant difference between the groups (Pearson's chi-squared, $p>0.05$). All data and Dunn-Šidák correction are presented in Appendix 1.3 (p.8).

3.3.2. Memory

The control and the subjective memory complaint (SMC) groups were compared in terms of objectively testable cognitive abilities. Global memory, as assessed by the MMSE, was comparable between groups at all assessments ($p>0.05$) (Table 2). This similarity extended throughout MMSE components measuring short term memory (Recall), ability to attend and manipulate information (Attention and Praxis) and orientation to time and space (Orientation) ($p>0.05$) (Table 2).

Table 2. Mini-Mental State Examination (MMSE) components for control and SMC groups for each session of testing.

MMSE component	Baseline Assessment		Second Assessment	
	Control (n=9)	SMC (n=5)	Control (n=8)	SMC (n=4)
Orientation	9.9 $\pm$ 0.3	9.8 $\pm$ 0.4	10.0 $\pm$ 0.0	9.8 $\pm$ 0.5
Language	2.9 $\pm$ 0.3	2.8 $\pm$ 0.4	2.9 $\pm$ 0.4	3.0 $\pm$ 0.0
Repetition	1.0 $\pm$ 0.0	1.0 $\pm$ 0.0	1.0 $\pm$ 0.0	1.0 $\pm$ 0.0
Registration	3.0 $\pm$ 0.0	3.0 $\pm$ 0.0	3.0 $\pm$ 0.0	3.0 $\pm$ 0.0
Attention	5.0 $\pm$ 0.0	5.0 $\pm$ 0.0	4.9 $\pm$ 0.4	4.8 $\pm$ 0.5
Recall	2.6 $\pm$ 0.5	2.8 $\pm$ 0.4	2.9 $\pm$ 0.4	3.0 $\pm$ 0.0
Praxis	4.9 $\pm$ 0.3	5.0 $\pm$ 0.0	4.9 $\pm$ 0.4	4.8 $\pm$ 0.5
Test total	29.2 $\pm$ 1.0	29.4 $\pm$ 0.5	29.5 $\pm$ 0.8	29.3 $\pm$ 1.0

No significant differences.

Data are presented as mean $\pm$ SD.

Dunn-Šidák calculations are presented in Appendix 1.5 (p.16).

In order to test participants memory and attention in more detail computerised memory and attention tests were conducted (Table 2 and Appendix 1.5 p.17). Further to the results of the MMSE, no significant differences were found between control and SMC groups in terms of baseline memory ability ($p>0.05$). The number of errors made on the Spatial Span (SSP) test

(assessing executive function) was found to be significantly different between groups for change over time ($p < 0.005$, Table 3). The control group made more errors over time whilst the SMC group improved over time and made fewer errors. No differences in reaction times were noted across tests due to large variation between individuals, as shown by the standard deviations in Table 3. Ability to name objects were comparable between groups and stable over time.

Table 3. Selected CANTABclipse battery of memory and attention tests for control and SMC groups at each testing session.

CANTABclipse variable	Baseline Assessment		Second Assessment		Statistical difference P
	Control (n=9)	SMC (n=5)	Control (n=8)	SMC (n=4)	
BLC correct (%)	99.7 $\pm$ 0.9	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	
BLC mean correct latency (ms)	771.6 $\pm$ 75.0	768.8 $\pm$ 102.4	751.6 $\pm$ 89.1	819.4 $\pm$ 176.6	
CRT mean correct latency (ms)	390.4 $\pm$ 60.7	364.5 $\pm$ 35.2	380.5 $\pm$ 48.1	356.5 $\pm$ 29.5	
CRT correct trials (%)	99.5 $\pm$ 1.1	99.5 $\pm$ 1.0	98.8 $\pm$ 1.9	99.8 $\pm$ 0.5	
PAL stages completed	4.5 $\pm$ 0.5	4.8 $\pm$ 0.5	4.8 $\pm$ 0.7	5.0 $\pm$ 0.0	
SSP span length	5.4 $\pm$ 0.7	5.8 $\pm$ 1.5	5.5 $\pm$ 0.8	5.5 $\pm$ 0.6	
SSP total errors	11.5 $\pm$ 2.5	15.5 $\pm$ 4.04	11.9 $\pm$ 4.6	14.8 $\pm$ 7.9	Δ^{***}
SST median correct reaction time on 'go' trials (ms)	643.6 $\pm$ 111.5	591.5 $\pm$ 80.1	637.6 $\pm$ 260.5	476.3 $\pm$ 87.3	
GNT correct (%)	89.6 $\pm$ 9.2	91.7 $\pm$ 6.4	92.5 $\pm$ 6.1	93.3 $\pm$ 3.9	

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, Δ indicates change over time between groups.

Data are presented as mean $\pm$ SD.

Full dataset, test statistics and Dunn-Šidák calculations are presented in Appendix 1.5. (p.17).

3.4.1. Functioning

Functioning in terms of performance on activities of daily living (Table 4) and social functioning (Table 5, p.83) were measured for both groups at baseline and for changes over time. No significant differences in performance on activities of daily living (ADL) were observed with scores on basic ADLs such as bathing and eating independently, and instrumental ADLs such as selecting television channels and keeping up with current news ($p>0.05$, Table 4). Furthermore, scores for both groups remained stable over the 9 week period between assessments.

Table 4. Component and total scores of the ADCS-ADL functioning scale for control and SMC groups at each testing session.

ADCS-ADL component	Baseline Assessment		Second Assessment	
	Control (n=9)	SMC (n=5)	Control (n=8)	SMC (n=4)
Basic ADLs	22.0 $\pm$ 0.0	22.0 $\pm$ 0.0	22.0 $\pm$ 0.0	22.0 $\pm$ 0.0
Instrumental ADLs	54.3 $\pm$ 2.8 (7)	55.0 $\pm$ 1.0 (3)	54.8 $\pm$ 1.0 (4)	55.5 $\pm$ 0.7 (2)
Total score	76.3 $\pm$ 2.8 (7)	77.0 $\pm$ 1.0 (3)	76.8 $\pm$ 1.0 (4)	77.5 $\pm$ 0.7 (2)

No significant differences.

Data presented as mean $\pm$ SD. Sample sizes varying from those given, are provided in parentheses. Dunn-Šidák calculations are presented in Appendix 1.7 (p.26).

Social functioning was also found to be comparable between groups at both assessments and over time ($p>0.05$, Table 5, p.83). Large variation in score in both groups limited the ability to find statistical differences between groups, however, there does appear to be a trend of the total score decreasing more for the SMC group than the control group over time (non-significant, $p>0.05$). Overall functioning appears to be the same at baseline between groups and to remain stable over time.

Table 5. Social Functioning Scale components for control and SMC groups for both testing sessions.

SFS component score	Baseline Assessment		Second Assessment	
	Control (n=9)	SMC (n=5)	Control (n=8)	SMC (n=4)
Withdrawal	12.2 ± 2.5	13.4 ± 1.1	12.5 ± 2.4	13.5 ± 1.3
Interpersonal communication	8.7 ± 0.7	9.0 ± 0.0	8.9 ± 0.4	9.0 ± 0.0
Independence Performance	31.7 ± 4.3 (7)	32.0 ± 4.9 (4)	31.5 ± 2.7	29.0 ± 5.0
Recreation	30.1 ± 5.8 (8)	28.6 ± 8.1	27.4 ± 6.9 (7)	28.3 ± 5.9
Prosocial	29.5 ± 13.8 (8)	33.4 ± 5.9	27.9 ± 13.3 (7)	29.3 ± 6.2
Independence competence	35.7 ± 1.0	34.6 ± 1.5	36.0 ± 0.0	35.3 ± 1.2(3)
Total	145.7 ± 23.0 (6)	150.5 ± 20.5 (4)	141.5 ± 16.8 (6)	139.3 ± 13.6 (3)

No significant differences.

Data presented as mean ± SD. Sample sizes varying from those given, are provided in parentheses. Dunn-Šidák calculations are presented in Appendix 1.7. (p.26).

3.4.2. Circadian assessment

Non-parametric circadian rhythm analyses (NPCRA) were performed to determine the circadian characteristics of the rest-activity data (Table 6). In addition, two different types of periodogram analysis have been performed to determine the period of the rest-activity data and to compare between methodologies (Table 7, p.87). By averaging the rest-activity data for each assessment over 24h, I determined whether activity was at the same circadian phase when comparing the groups (Figure 11, p.89). Example actograms of rest-activity profiles for one control and one SC individual at baseline are presented in Figure 10 (p.85).

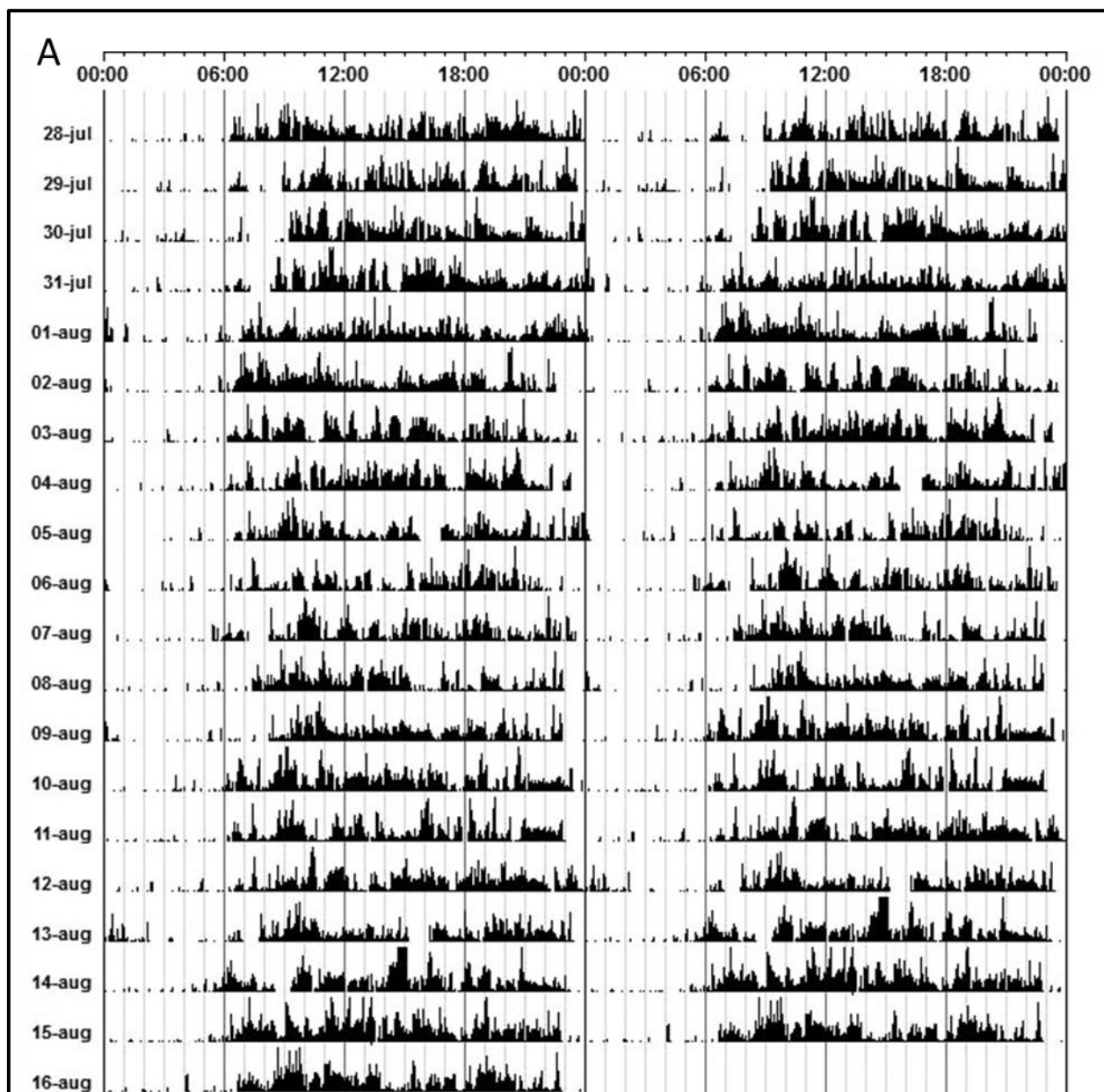
Table 6. Parameters from a non-parametric circadian rhythm analysis (NPCRA) of rest-activity data derived from actigraphy.

NPCRA parameter	Baseline Assessment		Second Assessment		Statistical difference P
	Control (n=9)	SMC (n=5)	Control (n=7)	SMC (n=4)	
Average activity count	195.9 ± 92.6	207.6 ± 48.3	186.7 ± 72.8	203.4 ± 26.4	
Inter-daily stability (IS)	0.6 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	
Intra-daily variability (IV)	0.8 ± 0.3	0.7 ± 0.0	0.8 ± 0.2	0.8 ± 0.1	
Least active five hours (L5)	11.5 ± 4.4	15.9 ± 5.5	13.1 ± 7.3	17.8 ± 3.5	
L5-onset phase (h)	00:42 ± 00:58	00:46 ± 00:36	00:26 ± 00:58	00:58 ± 00:35	
Most active ten hours (M10)	338.9 ± 161.1	356.0 ± 70.8	314.3 ± 112.6	336.8 ± 52.8	Δ*
M10-onset phase (h)	08:16 ± 00:52	08:29 ± 00:21	08:13 ± 00:52	08:29 ± 00:19	
Amplitude of activity	327.3 ± 158.1	340.1 ± 66.8	301.2 ± 107.1	319.0 ± 56.5	Δ*
Relative Amplitude (RA)	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	

* $p < 0.05$, Δ indicates change over time between groups.

Data are presented as mean ± SD. h represents hh:mm time format.

Full data, test statistics and Dunn-Šidák calculations are presented in Appendix 1.9. (p.30).



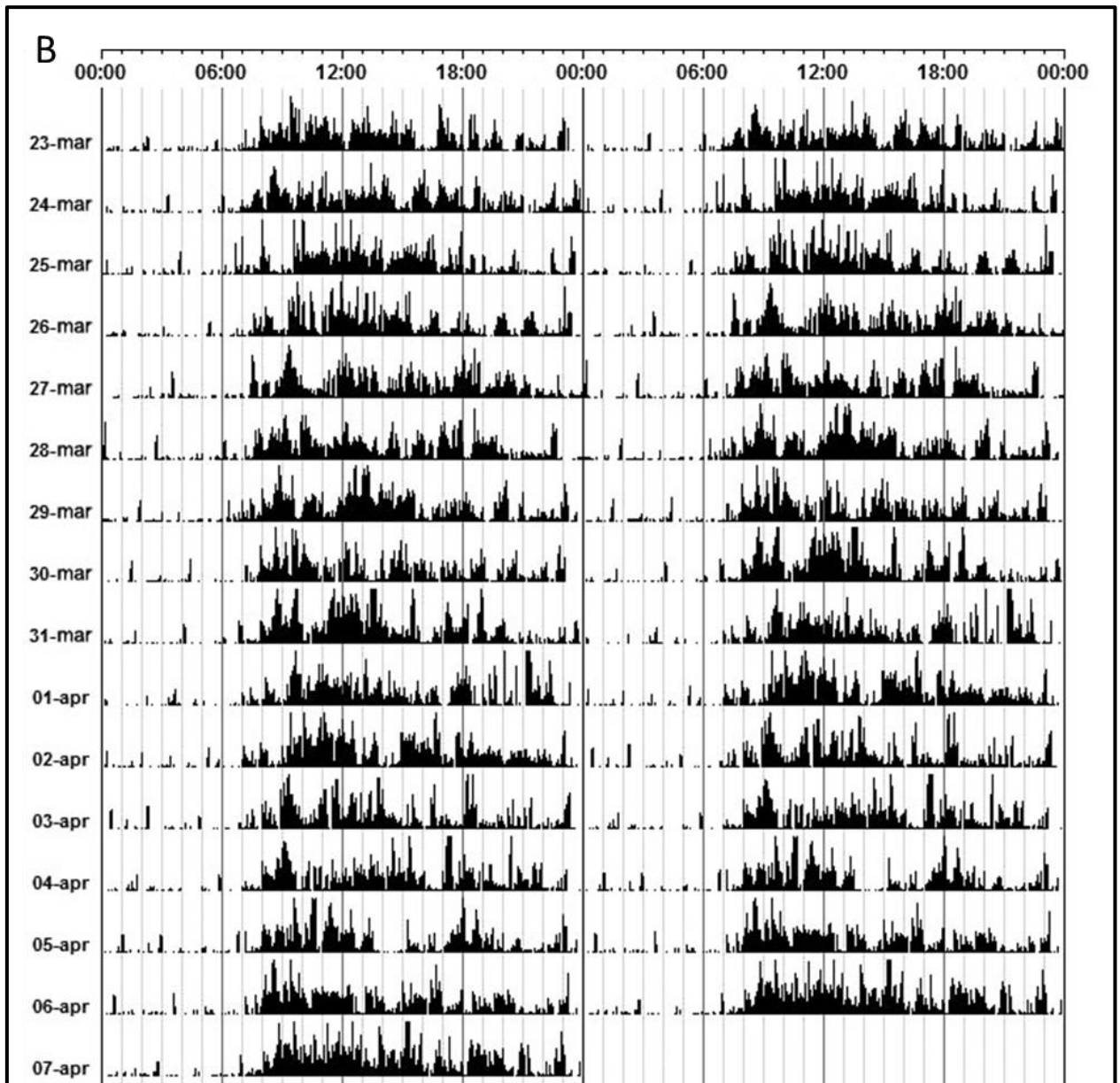


Figure 10. Exemplar baseline assessment actograms for the control and SMC groups.
Actogram A: Control and Actogram B: SMC individuals. Black lines indicate activity and rest-activity is double plotted to view sleep periods. Activity is scaled to 1550 activity counts. Actograms show comparable rest-activity however more activity bouts are present in the sleep period of the SMC actogram (Actogram B).

At baseline both groups had comparable circadian characteristics to their rest-activity profile, with comparable amplitude of activity and relative amplitude between least active five hours (L5) and most active ten hours (M10) (Table 6, p.84). The SMC group did have higher levels of L5 activity at both assessments in comparison to controls ($p>0.05$). When analysing change over

time between groups more differences became apparent. The amplitude of the activity profile was found to change significantly over time in both groups ($p < 0.05$) with the amplitude decreasing over time in both groups, which was more pronounced in the control group (Table 6, p.84). Another statistically significant difference between groups for change over time was the M10 variable ($p < 0.05$) again with both groups having lower activity counts in their M10 but with more pronounced decrease in the control group data (Table 6, p.84). Further to these observations, trends were found in the average 24h activity count ($p = 0.067$) and inter-daily stability (IS) ($p = 0.093$) over time with controls reducing in activity and having a less stable inter-daily pattern (Table 6, p.84). A trend in Inter-daily variability (IV) ($p = 0.088$) was also observed with controls maintaining the same level of variability over time, with the SMC group increasing in variability over time. This indicates that their rest-activity rhythm became more fragmented (Table 6, p.84). This increase in fragmentation was minor reaching the same level as controls.

No differences were found in the period length of the rest-activity rhythm between groups at baseline or for change over time ($p > 0.05$, Table 7, p.87). Furthermore no difference was found between periodogram analysis types ($p > 0.05$).

Table 7. Period length of activity rhythm calculated by two types of periodogram analyses from actigraphy data.

	Baseline Assessment		Second Assessment	
	Control (n=9)	SMC (n=5)	Control (n=7)	SMC (n=4)
Average period length				
Lomb-Scargle method	23.98 ± 0.04	24.01 ± 0.05	24.02 ± 0.03	24.01 ± 0.02
Sokolove-Bushell method	23.96 ± 0.07	23.99 ± 0.05	24.02 ± 0.05	23.93 ± 0.11

No significant differences.

Data presented as mean ± SD in hh:mm time format.

Dunn-Šidák calculations are presented in Appendix 1.9. (p.31).

To determine the circadian phase of the rest-activity pattern for each group at each assessment, data averaged across each assessment was plotted on a 24h basis (Figure 11, p.89). Both groups had comparable phases of rest-activity profiles. The difference over time for each group individually as well as between groups over time were only marginal (Figure 11, p.89). The differences in activity counts in the afternoon hours were reduced in significance due to large variability between individuals resulting in large standard deviations. Analysis of these averaged activity profile found that there was no difference in phase of activity between control and SMC groups, and that the phase of activity did not alter over the nine weeks gap between assessment periods (Figure 11 p.89). An afternoon dip in activity was observed between 13:30 and 14:30 for both groups at both assessments.

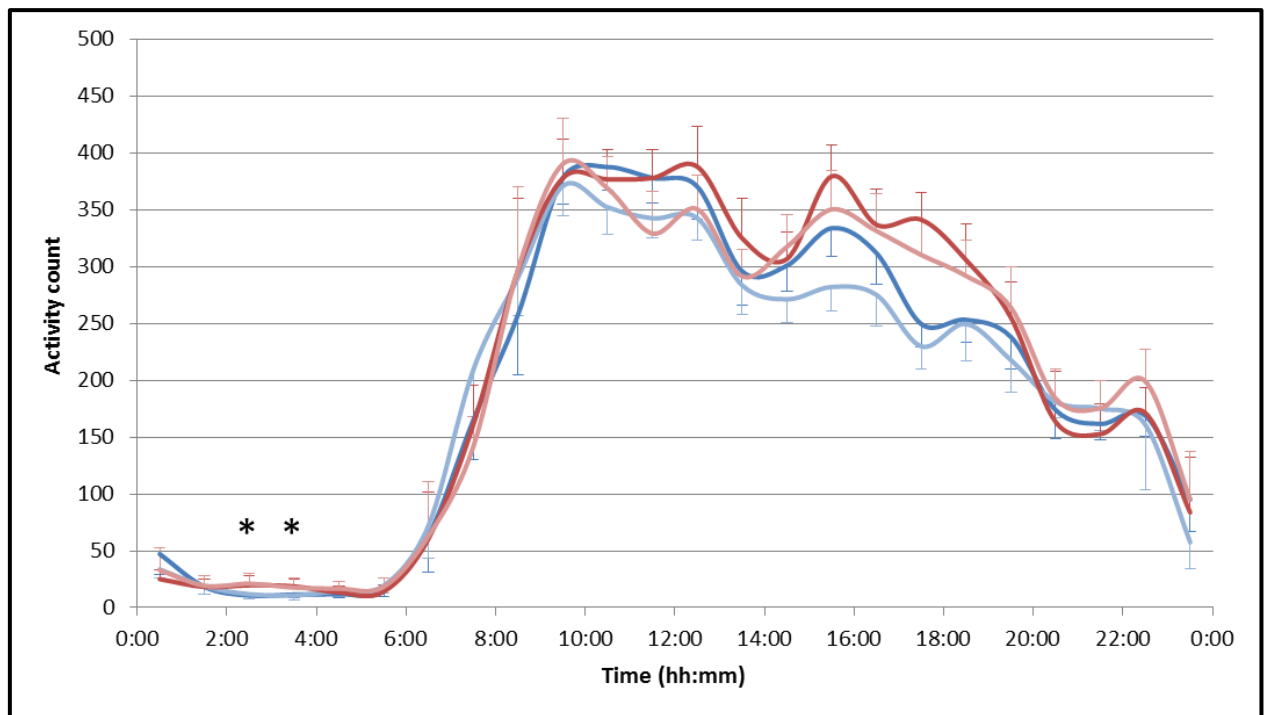


Figure 11. Averaged activity count per hour for control and SMC group at both assessments. Graph shows the averaged activity data on a 24h scale, blue lines indicate control data and red lines indicate SMC data, lighter shades indicate second assessment data with standard deviation error bars shown. The average activity count per hour is displayed on the half hour in the graph. Activity appears time of day appropriate for all groups at both assessments with SMC data appearing of higher amplitude though with comparable time-of-day activity. The second assessment of control data appears lower in amplitude than at baseline. Baseline data was compared between groups with a one-way ANOVA, a trend of $p=0.064$ was found for the third hour (not significant, $p>0.05$). Over time data were compared with a repeated measures ANOVA general linear model which found significant difference between groups over time at hours three ($p=0.011$, displayed at 02:30 with *) and four ($p=0.037$, not significant after D-S correction, displayed at 03:30 with *) and trends found at hours eighteen ($p>0.05$, displayed at 17:30) and twenty-four ($p>0.05$, displayed at 23:30). All data, test statistics and Dunn-Šidák correction are presented in Appendix 1.9. (p.30).

3.4.3. Sleep assessment

Sleep characteristics were assessed subjectively with the Pittsburgh Sleep Quality Index (PSQI) (Table 8) and the Jupiter Medical Center Sleep Questionnaire (JSQ) (Table 9, p.92) and objectively by analysis of the sleep period of actigraphy data (Table 10, p.93), and stage scoring of electroencephalography (EEG) data (Table 11, p.96).

No statistically significant differences between groups in PSQI component scores at baseline or over time were found ($p>0.05$, Table 8).

Table 8. Pittsburgh Sleep Quality Index (PSQI) component scores for control and SMC groups.

PSQI component score	Baseline Assessment		Second Assessment	
	Control (n=9)	SMC (n=5)	Control (n=8)	SMC (n=4)
1: Subjective sleep quality	0.3 ± 0.5	1.0 ± 1.2	0.5 ± 0.5	0.8 ± 1.0
2: Sleep latency	0.2 ± 0.7	0.4 ± 0.5	0.5 ± 1.1	0.3 ± 0.5
3: Sleep duration	0.4 ± 1.0	0.6 ± 1.3	0.1 ± 0.4	1.0 ± 0.8
4: Habitual sleep efficiency	0.6 ± 1.0	1.2 ± 0.8	0.1 ± 0.4	1.0 ± 0.0 (3)
5: Sleep disturbances	1.2 ± 0.4	1.2 ± 0.4	1.0 ± 0.5	1.0 ± 0.0
6: Use of sleep medication	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
7: Daytime dysfunction	0.2 ± 0.4	0.2 ± 0.4	0.5 ± 0.5	0.0 ± 0.0
Global PSQI score	3.0 ± 2.4	4.6 ± 3.0	2.8 ± 2.3	4.7 ± 2.1 (3)

No significant differences.

Data presented as mean ± SD. Sample sizes varying from those given, are provided in parentheses. Dunn-Šidák calculations are presented in Appendix 1.11. (p.37).

Subjective quality of sleep was consistently reported being worse by the SMC group in comparison to controls at both assessments. The SMC group reported shorter sleep (component 3) with reduced sleep efficiency in comparison to controls (component 4). The degree of sleep disturbance was comparable in both groups (component 5). In terms of global

sleep, the SMC group reported higher levels of disturbance across components observed in the global PSQI score. The SMC group were close to the cut off of poor sleep (>5) with their global PSQI scores approaching a score of 5 (Table 8. p.90). When observing the raw data, at baseline two controls achieved global scores of over 5, one achieving a score of 6 and the other a score of 8. One SMC individual achieved a score 10. At second assessment, two controls scored 6 and one SMC scored 7, again with all other controls not reaching scores above 3. Neither group appeared to change in the reporting of their sleep over the two assessment periods.

Further subjective reporting of sleep characteristics, such as sleep timings and napping behaviour, were measured with the JSQ (Table 9, p.92). Sleep onset times for week and weekend nights were comparable between both groups, remaining stable over time as were awakening times (Table 9, p.92). Problems that bothered the participants when trying to sleep were assessed binomially between groups. How bothered individuals were by light entering the bedroom showed a trend at baseline ($p=0.075$) with controls less bothered by light than the SMC group.

Table 9. Jupiter sleep questionnaire (JSQ) sleep characteristics of both groups for both assessments.

JSQ parameter	Baseline Assessment		Second Assessment	
	Control (n=9)	SMC (n=5)	Control (n=8)	SMC (n=4)
Week sleep-onset	22:53 ± 00:37	22:49 ± 00:13	22:58 ± 00:44	22:52 ± 00:29
Weekend sleep-onset	22:53 ± 00:37	22:49 ± 00:13	23:05 ± 00:34	22:52 ± 00:29
Usual wake time	06:51 ± 00:44	06:50 ± 00:32	06:58 ± 00:48	06:33 ± 00:08
Sleep per night (h)	07:09 ± 01:45	07:01 ± 01:15	07:36 ± 00:29	06:46 ± 00:51 (3)
No. of awakenings	0.9 ± 0.4	1.9 ± 0.9	1.1 ± 0.6	2.3 ± 1.0
Wake per night (h)	00:38 ± 01:17	00:44 ± 00:47	00:13 ± 00:08	00:40 ± 00:54
No. of naps per week	2.7 ± 3.0	2.5 ± 2.5 (3)	4.0 ± 4.4 (7)	1.3 ± 2.3 (3)

No significant differences.

Data presented as mean ± SD. h indicates hh:mm time format.

Sample sizes varying from those given, are provided in parentheses.

Full dataset and Dunn-Šidák calculations are presented in Appendix 1.11. (p.38).

Objective assessment of sleep characteristics of the sleep period of rest-activity data was conducted. As shown through the subjective sleep questionnaires, bed time, get up time and sleep durations were very comparable between groups both at baseline and in change over time (Table 10, p.93). Higher activity during the night was observed in the SMC group at both assessments, with lower sleep efficiency observed in the SMC group than the controls ($p>0.05$). The SMC group also had less immobile minutes during sleep than controls and increased moving time in both assessments. At baseline moving time percentage showed a trend of being statistically higher ($p=0.093$) and number of immobile minutes being lower ($p=0.069$) in the SMC group than controls. One minute immobility was found to have a trend ($p=0.070$) between groups at baseline, with the SMC group having more fragmented sleep with more of these one minute immobility epochs than controls. The SMC group also had more of these epochs at second assessment than controls, although this variable was relatively stable in both groups

over time (Table 10). Fragmentation index, a calculation of the number of one minute immobility epochs over sleep period duration multiplied by one hundred, also appeared different between groups. At baseline a significantly higher fragmentation index was observed for the SMC group in comparison to controls ($p=0.043$). This difference is also apparent at second assessment, however, groups remained stable over time (Table 10).

Table 10. Selected sleep analysis parameters derived from actigraphy for control and SMC groups.

Parameter	Baseline Assessment		Stat. diff. P	Second Assessment	
	Control (n=9)	SMC (n=5)		Control (n=7)	SMC (n=4)
Bed time (h)	23:35 ± 00:50	23:26 ± 00:20		23:11 ± 00:56	23:29 ± 00:15
Get up time (h)	07:14 ± 00:38	06:48 ± 00:10		07:14 ± 00:47	06:55 ± 00:23
Time in bed (h)	07:39 ± 00:37	07:21 ± 00:15		07:51 ± 00:28	07:26 ± 00:31
Sleep start (h)	23:40 ± 00:49	23:33 ± 00:22		23:25 ± 00:46	23:34 ± 00:16
Sleep end (h)	07:14 ± 00:38	06:47 ± 00:09		07:13 ± 00:47	06:55 ± 00:23
Mean activity score	10.5 ± 4.6	14.7 ± 4.9		12.8 ± 6.4	17.4 ± 4.2
Av. wake movement	279.2 ± 134.6	285.7 ± 63.9		268.9 ± 106.6	280.2 ± 42.0
Sleep efficiency (%)	90.0 ± 4.3	86.7 ± 3.6		88.8 ± 5.4	85.4 ± 4.3
Sleep latency (h)	00:05 ± 00:04	00:06 ± 00:03		00:02 ± 00:01	00:05 ± 00:04
Wake bouts	20.7 ± 9.4	24.0 ± 4.1		23.1 ± 10.5	25.0 ± 7.6
Immobile minutes	398.7 ± 35.6	361.5 ± 28.5		404.8 ± 26.4	359.4 ± 34.0
Moving time (%)	11.9 ± 5.0	17.0 ± 4.9		13.3 ± 3.4	18.6 ± 7.2
One Min. immobility	5.6 ± 3.9	10.4 ± 5.1		6.7 ± 3.4	11.2 ± 6.7
One Min. immobility (%)	13.6 ± 4.8	21.6 ± 6.3	*	15.0 ± 4.3	22.1 ± 7.4
Fragmentation index	25.8 ± 9.6	38.6 ± 10.9	*	28.6 ± 7.6	40.6 ± 14.6

Stat. diff. indicates statistical difference at baseline between groups, * $p<0.05$.

Data presented as mean ± SD. h indicates hh:mm time format.

Full dataset, test statistics and Dunn-Šidák calculations are presented in Appendix 1.11. (p.47).

Due to the low sample size of the SMC group only descriptive results are shown from EEG derived sleep data. Sleep and wake durations in the periods between lights off and lights on were comparable between groups at baseline and in change over time (Table 11, p.96). Rapid-eye movement (REM) sleep duration and percentage were comparable between groups, although REM latency is shorter for the SMC group at both assessments. In addition, the latency for deep sleep was shorter for the SMC group at both assessments in comparison to controls. When considering these latencies, the cycle of stages in the SMC group were altered from that of controls (this can be further elucidated by comparing thirds of the sleep period dividing time in bed into three thirds of equal duration for observation of the beginning, middle and end of the sleep period (Figure 12, p. 97). When analysing deeper stages of sleep, the SMC group had a higher percentage of stage 3 and stage 4 sleep at baseline (Table 11, p.96). Whilst controls remained relatively stable in the percentages of stage 3 and 4 sleep, the SMC group over time had a large reduction in stage 3 percentage, dropping an average of 8.2% compared to a reduction of 1.5% in control's sleep.

In order to analyse progression of stage durations during the sleep period each EEG recording was divided into thirds as described above. The results are displayed in the graphs in Figure 12 (p.97). Durations of sleep can be observed in Graphs A and B, Figure 12 (p.97), showing varying patterns between groups and at each assessment. Controls had relatively stable amounts of wakefulness throughout the sleep period which remained comparable over time (Graphs A and B, Figure 12, p.97). The SMC group had more wakefulness towards the end of the night at the second assessment. As Graph I (Figure 12, p.101) illustrates this is an extended period of wakefulness as less stage changes are observed in the final third of the second assessment in the SMC group. Stage 1 sleep duration was stable across the night from both groups at both assessments (Graph C, Figure 12, p.98). Stage 2 sleep duration had a

different pattern between groups, with the control group having increasingly more stage 2 sleep through the night, diminishing slightly in the final third of the night to enable more REM sleep at this time (Graphs D and G, Figure 12, p.98&100). In contrast the SMC group had increasing stage 2 sleep throughout the night with no reduction in the final third of the night (Graphs D and G, Figure 12, p.98&100). Rapid-eye movement sleep was comparable to control sleep at first assessment, although less REM sleep was found for the SMC group at second assessment.

Table 11. Selected electroencephalography (EEG) derived sleep staging parameters for control and SMC groups for both assessments.

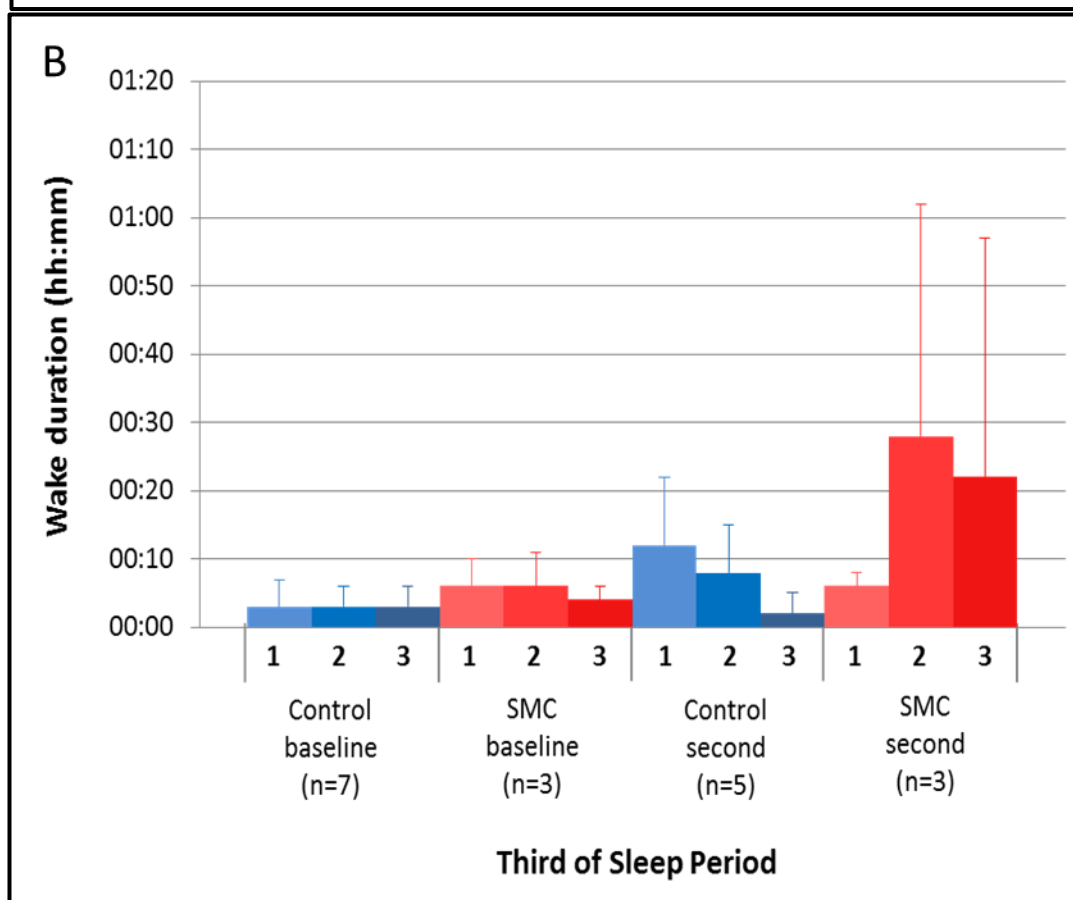
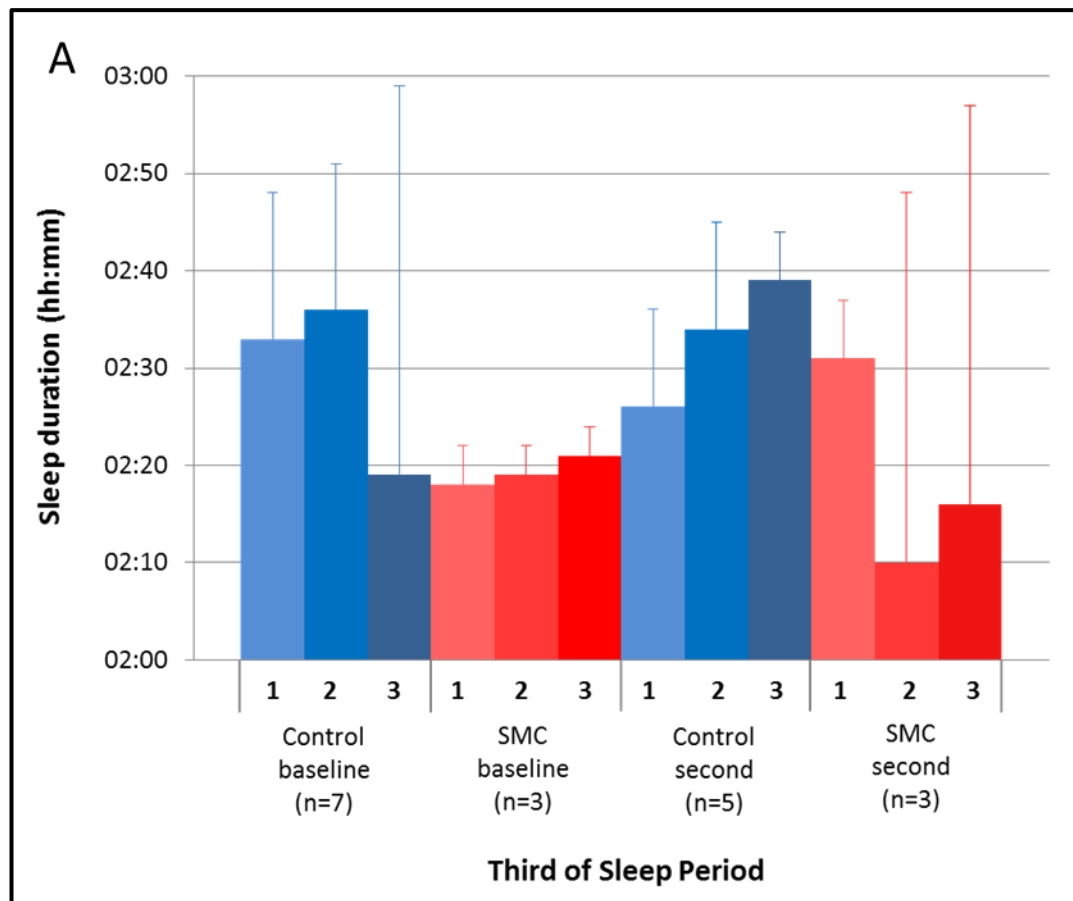
EEG staging parameter	Baseline Assessment		Second Assessment	
	Control (n=7)	SMC (n=3)	Control (n=5)	SMC (n=3)
Time in bed	08:04 ± 00:37	07:18 ± 00:13	08:08 ± 00:23	07:57 ± 00:14
Sleep duration	07:44 ± 00:43	06:57 ± 00:07	07:39 ± 00:23	06:56 ± 01:25
Wake duration	00:12 ± 00:07	00:15 ± 00:07	00:23 ± 00:09	00:56 ± 01:09
REM sleep duration	01:45 ± 00:21	01:38 ± 00:12	01:36 ± 00:32	01:24 ± 00:48
REM sleep (%)	21.7 ± 4.3	22.5 ± 3.0	19.5 ± 6.1	17.3 ± 15.1
NREM sleep duration	05:59 ± 0:45	05:19 ± 00:18	06:03 ± 00:25	05:33 ± 00:41
NREM sleep (%)	74.1 ± 5.3	72.9 ± 3.5	74.5 ± 5.3	69.7 ± 6.8
REM latency	01:10 ± 01:01	00:59 ± 00:04	01:28 ± 01:06	00:52 ± 00:28
NREM latency	00:03 ± 00:02	00:05 ± 00:03	00:08 ± 00:11	00:04 ± 00:00
Deep sleep latency (stages 3 and 4)	00:18 ± 00:10	00:11 ± 00:06	00:24 ± 00:13	00:16 ± 00:15
Sleep efficiency (%)	95.9 ± 1.8	95.3 ± 2.1	94.0 ± 2.0	87.1 ± 15.4
Stage 1 sleep duration	01:12 ± 00:23	00:41 ± 00:41	01:20 ± 00:33	00:49 ± 00:33
Stage 1 sleep (%)	14.8 ± 4.2	9.2 ± 9.1	16.5 ± 7.2	10.3 ± 6.8
Stage2 sleep duration	03:13 ± 00:50	02:41 ± 00:31	03:12 ± 1:16	03:17 ± 00:52
Stage 2 sleep (%)	39.8 ± 9.0	36.8 ± 6.6	38.9 ± 13.8	41.2 ± 10.0
Stage 3 sleep duration	01:26 ± 00:46	01:42 ± 00:40	01:19 ± 00:32	01:12 ± 00:41
Stage 3 sleep (%)	17.8 ± 9.2	23.4 ± 9.8	16.3 ± 7.0	15.2 ± 8.5
Stage 4 sleep duration	00:08 ± 00:11	00:15 ± 00:08	00:13 ± 00:18	00:14 ± 00:18
Stage 4 sleep (%)	1.7 ± 2.4	3.5 ± 1.8	2.8 ± 4.0	3.1 ± 3.8
Movement duration	00:08 ± 00:03	00:06 ± 00:04	00:07 ± 00:03	00:04 ± 00:02
Movement (%)	1.7 ± 0.9	1.2 ± 0.8	1.4 ± 0.6	0.8 ± 0.3
Wake after sleep onset	00:08 ± 00:05	00:10 ± 00:04	00:15 ± 00:12	00:52 ± 01:09

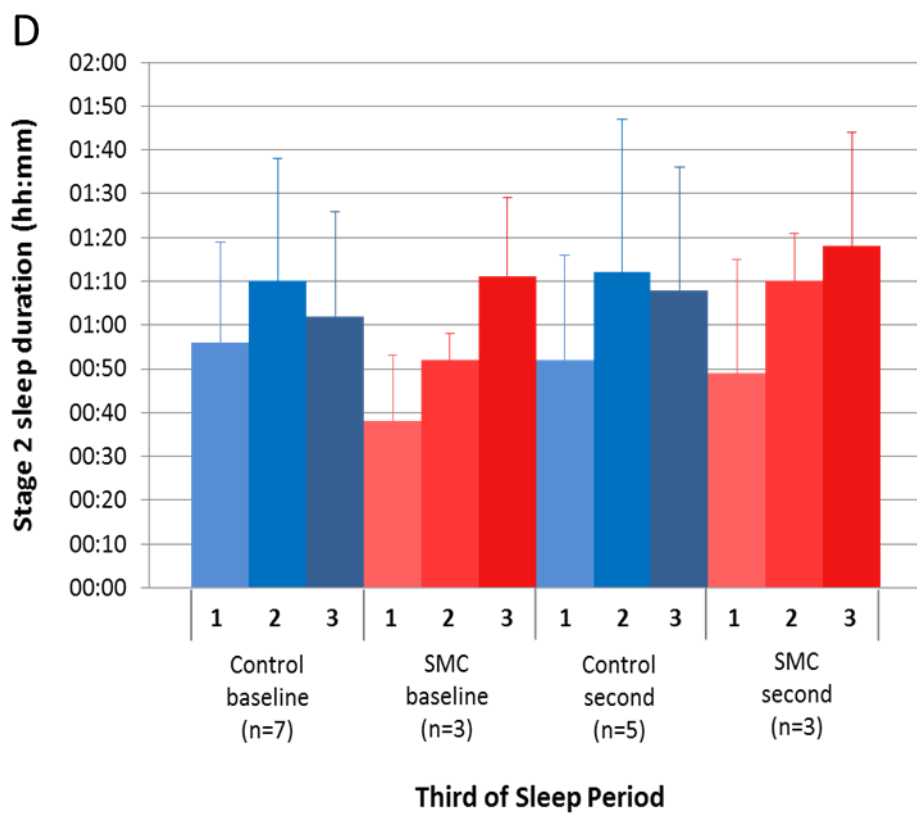
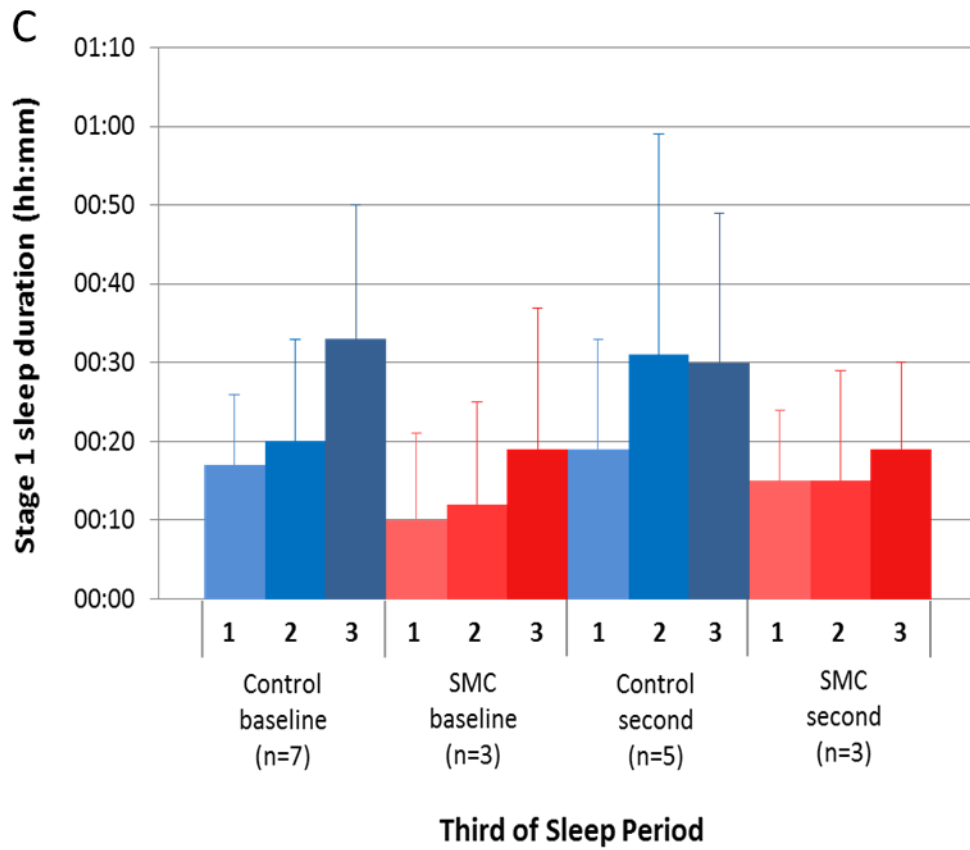
No statistical analyses conducted.

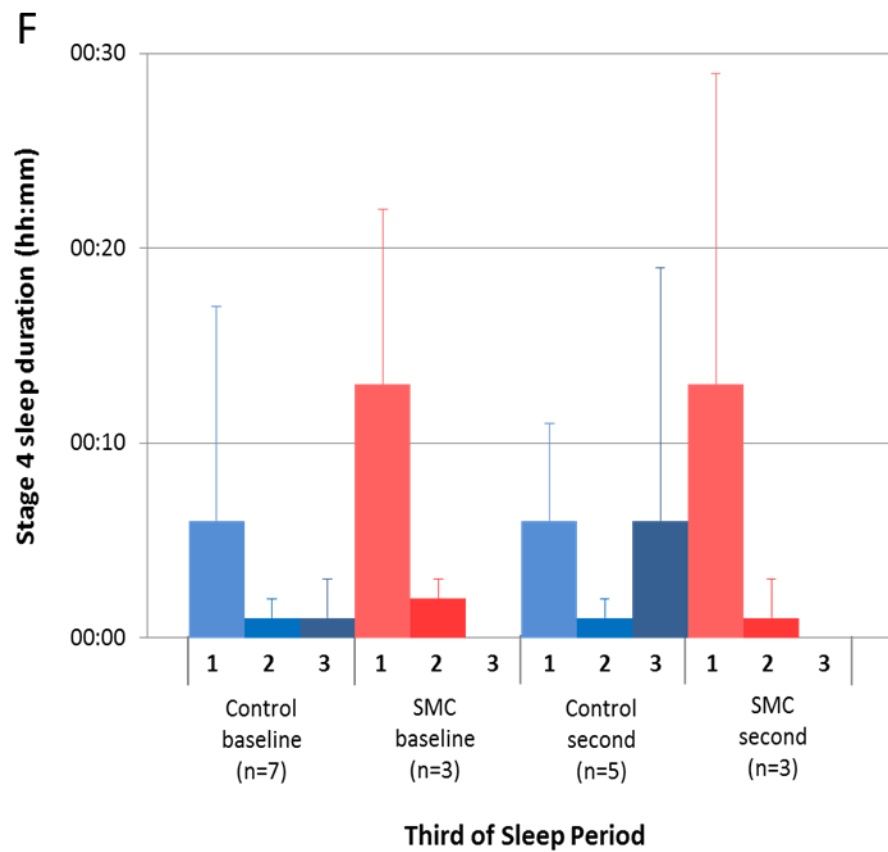
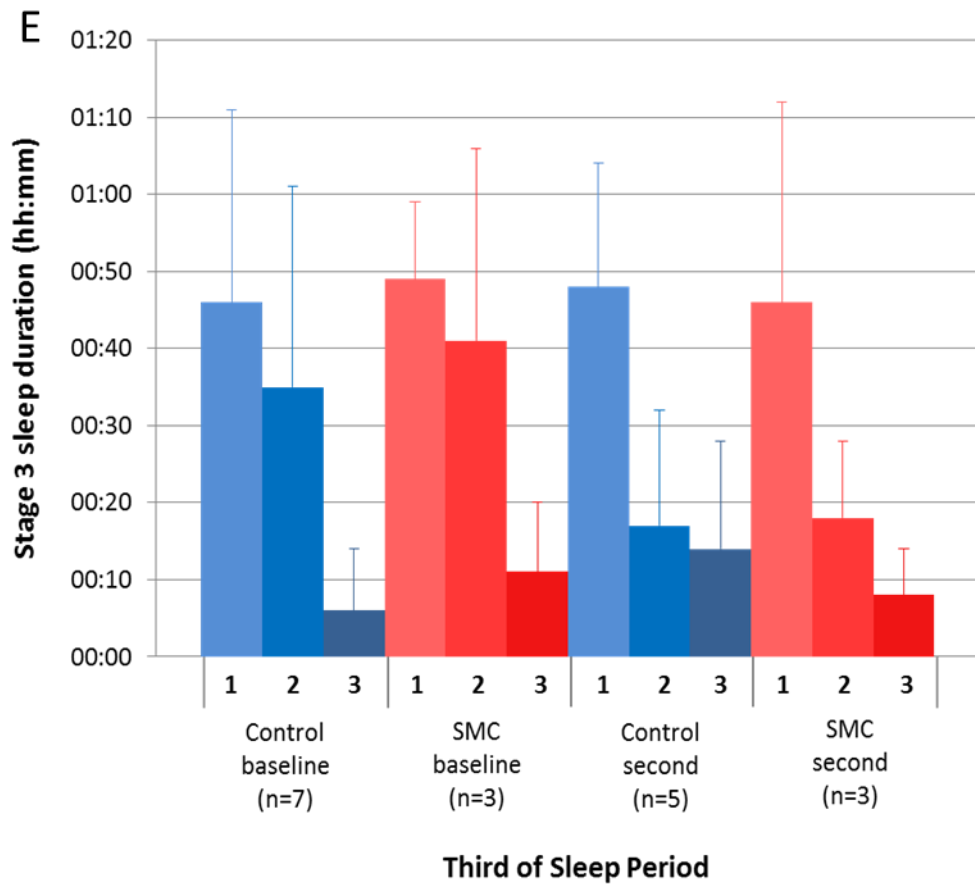
Data presented as mean ± SD. Unless stated variable is in hh:mm time format.

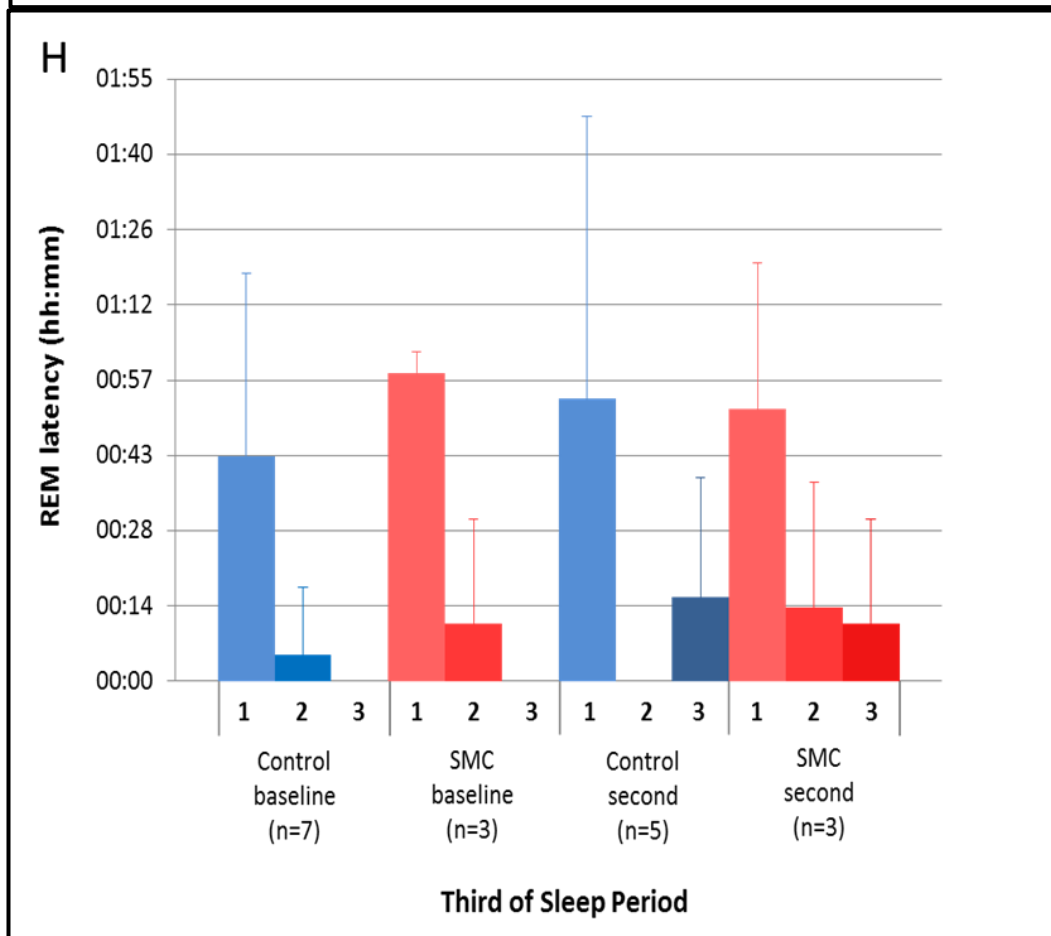
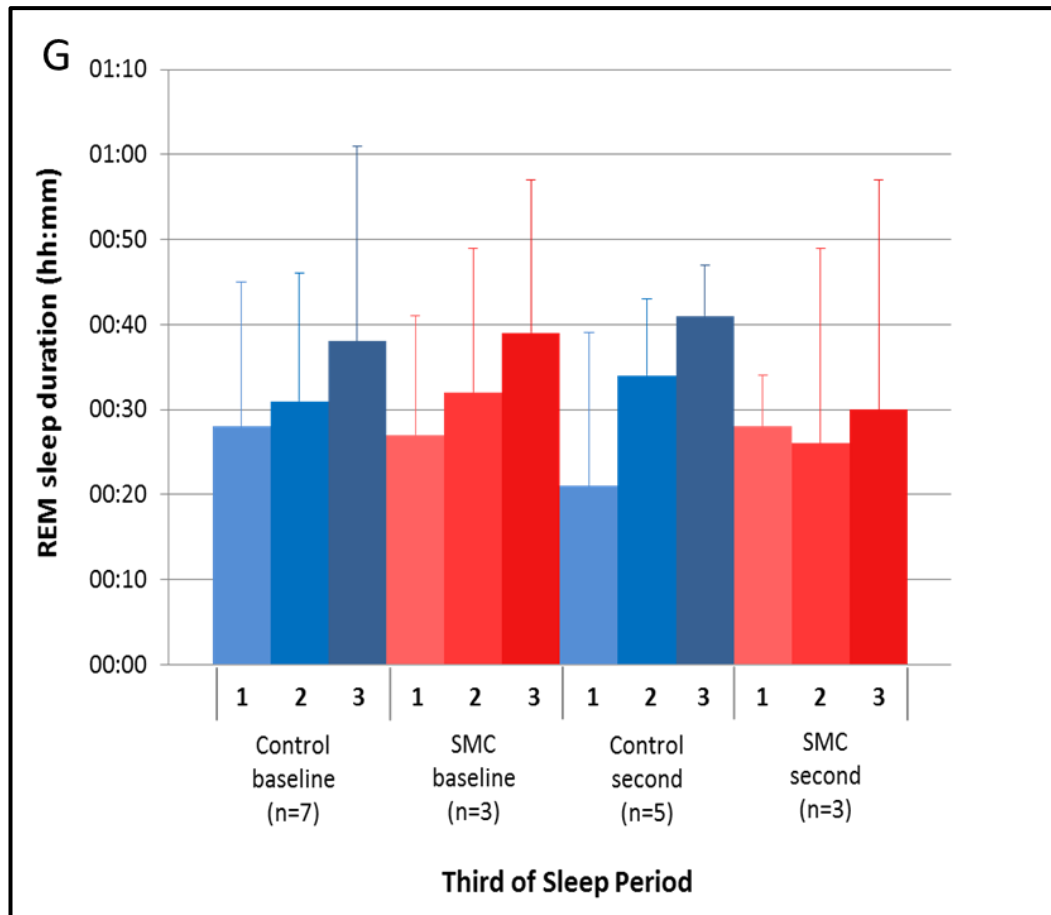
% indicates % of Time in bed.

Full dataset is presented in Appendix 1.11. (p.52).









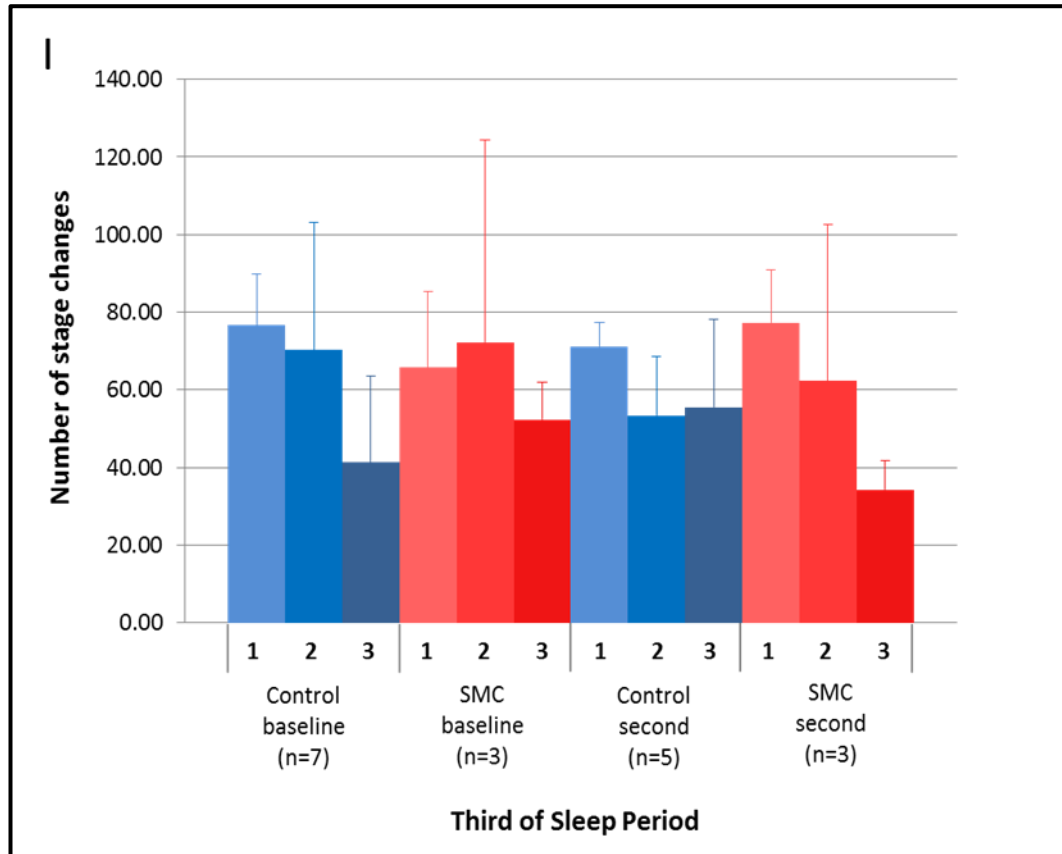


Figure 12. EEG scoring for each third of the sleep period. Graphs are displayed as means for each group at both assessments with the three thirds of each night clustered per group. Blue indicates the control group with red indicating the SMC group. Colours darken through the sleep period. Error bars indicate standard deviation of the mean. Graphs are as follows: A: REM/NREM Sleep duration, B: Wake duration, C: Stage 1 sleep duration, D: Stage 2 sleep duration, E: Stage 3 sleep duration, F: Stage 4 sleep duration, G: Rapid eye movement (REM) sleep duration, H: REM latency and I: Number of stages changes. Sample sizes are provided on the x-axis. All data are presented in Appendix 1.11. (p.54).

Rapid-eye movement sleep duration decreased in at end of the night over time for the SMC group with a longer REM sleep latency (Graph H, Figure 12, p.100). The REM latency of controls, for the final third of the night at second assessment was longer than at first assessment (Graph H, Figure 12, p.100). Deeper stages of sleep (stages 3 and 4 sleep) diminished across the night for all groups at both assessments. The exception to this was observed in the second assessment of controls who had comparable levels of stage 4 sleep duration both at the beginning and end of the night (Graphs E and F, Figure 12, p.99).

3.5. Discussion

3.5.1. Demographics and Screening

The individuals from the control group and subjective memory complaint (SMC) group did not differ in sex, age, education history, caffeine intake and diurnal preference. There were no differences in levels of anxiety and depression tendencies between the groups. This is particularly important, as individuals complaining about memory problems could be thought to have increased anxiety about their memory or underlying depressive symptoms [117]. The majority of research comparing SMC individuals to healthy controls report depressive symptoms as being correlated to subjective memory complaints [117-119]. In the current thesis, no differences were found between groups in terms of levels of anxiety or depression tendencies, and no participants were formally diagnosed with a psychological/psychiatric condition according to medical notes. Therefore, any differences between controls and the SMC group cannot be attributed to altered affect.

3.5.2. Memory and Functioning

Subjective memory complaints are commonly found in the older population, with some studies quoting figures of up to 50% complaining of memory impairment [120, 121]. As SMC has been reported to be a prodromal stage of developing Alzheimer's disease (AD), research has focused on detecting indicators of this progression to aid the development of new treatments [122]. For this reason SMC individuals recruited into this study were first assessed in terms of memory, as well as daily living and social functioning to confirm their memory category. Subjective memory complaint individuals, by definition, complain of a memory impairment, however, functioning and objective memory testing do not enable a diagnosis of mild cognitive impairment [123]. In the present study no memory or attention differences were found between controls and SMC

individuals. Furthermore, no differences in daily living or social functioning were found between groups. Inability to conduct activities of daily living has been thought to be a first sign of memory decline affecting daily life [121]. However, such signs are not noticeable in this sample. These data, therefore, suggests that, other than in the presence of subjective complaints of memory problems, SMC individuals did not differ from controls demographically, cognitively or functionally.

3.5.3. Rest-activity and Sleep

Differences were found in circadian and sleep parameters between the SMC group and controls. SMC individuals were found to have a larger amplitude of activity, however, they also exhibited more fragmentation in their circadian rhythm of rest-activity (seen in IV and IS differences) in comparison to controls. Sleep differences were more pronounced, with a trend of higher global sleep disturbance scores and lower habitual sleep efficiency on PSQI. Objective measures of activity during the sleep period, confirmed the lower sleep efficiency of the SMC group and found more sleep disturbance. The SMC group were observed to have more fragmentation of rest during the sleep period, with higher fragmentation index scores as well as a higher percentage of unbroken durations of immobility (one minute immobility percentage). Sleep EEG analyses revealed that the SMC group had a shorter REM sleep latency and a reduction of REM sleep over time. The SMC group also had a large reduction in the amount of Stage 3 and 4 sleep over time. When the sleep period was divided into thirds, it was observed that at the second assessment, more wakefulness and stage 2 sleep were found towards the end of the night when an increase of rapid-eye movement (REM) sleep would usually be expected [124]. This could be an indication of future changes in sleep architecture. These results will be compared in the following chapter with the later prodromal stage of AD, mild

cognitive impairment, as well as mild and moderate AD, to determine if these observations are indicative of sleep alterations found in these stages of AD-related progression.

3.5.4. Strengths and Limitations

Overall, the only observed differences between older adults, with and without subjective memory complaints, were in rest-activity pattern fragmentation, sleep efficiency, architecture and consolidation. These data indicate that such changes might be early signs of a prodromal phase of AD, however, further progression of these differences would need to be observed in later prodromal phases. There are limitations to this study, in particular the low sample size observed. Therefore, in order to determine the true validity of these data presented in this chapter a larger cohort of at least 16 people per group (according to power calculations provided in the methods chapter, Chapter 2, p.30) would need to be recruited. Despite this limitation, diagnostic categorisation of SMC individuals can be relied upon, as the longitudinal cohort researchers were highly experienced in differentiating between SMC and mild cognitive impairments (MCI) individuals. Furthermore, this study found no difference in memory between the SMC and control groups, indicating that the misdiagnosis of SMC in the place of MCI is less likely. A key difficulty with this study was the debate in the field as to whether SMC is truly a prodromal phase of AD. Some studies have determined that there appears to be no association between having SMC and future cognitive decline [120, 125], however, others found that SMC was another stage in the progression to AD [119, 121, 122]. These differences in findings probably indicate that like MCI, only a proportion of SMC individuals will go on to progress through MCI to AD. Some will probably remain with a non-objectively testable memory complaint that does not progress, and others will develop a mild cognitive impairment that is not AD-related. Although the research of prodromal stages for possible AD biomarkers is

important, it should also be remembered that not all individuals classified in these prodromal categories will develop Alzheimer's disease.

3.5.5. Conclusions

Although the validation of SMC being a prodromal phase cannot be determined in the remit of this study and no differences in objective memory and demographics and functioning were found. Differences were observed in sleep and rest-activity profiles when comparing the SMC group to non-memory complaint controls. This study would suggest that there are subtle differences between those reporting memory complaints and those who do not. Whether these differences are also observed at later phases of the progression to moderate Alzheimer's disease will be addressed in the following chapter. However, even if no progression can be observed, the current study has found evidence of sleep and rest-activity differences associated with a subjective memory complaint. This in itself is an interesting and important finding and warrants future investigations, as this result was not related to increased anxiety or depressive tendencies in this sample.

4. Study 1: Memory impairment severity

4.1. Introduction

4.1.1. Alzheimer's disease

Sleep, circadian rhythms and memory are interlinked and change over the lifespan. A disease that is distinctly coupled to ageing and has disruptions in all of these three processes is Alzheimer's disease (AD). This disease is named after Alois Alzheimer who, in 1907, first characterised Alzheimer's disease by describing the protein tangles and plaques in the post-mortem brains of patients with memory problems [126]. Later research determined these tangles to be hyperphosphorylated tau protein, neurofibrillary tangles (NFTs) and the plaques to be amyloid- β peptide plaques. Amyloid- β in particular has been the focus of recent research, as it has been considered to be neurotoxic, and a possible mechanism of the cell degeneration observed in AD [127, 128]. However, this theory is still debated, with some research finding only a weak correlation between amyloid- β plaques and memory impairment in transgenic mice [129].

Currently, AD is the most frequent reason for irreversible dementia in the older population [126]. However, it can also affect much younger individuals. A key feature of AD is the gradual and progressive decline in cognitive functioning and memory, due to the gradual degeneration of neurons (brain atrophy). This neuronal loss begins in the hippocampus, entorhinal cortex and associated areas of the neocortex, leading to degeneration in the temporal, parietal lobes and parts of the frontal and cingulate cortices. As neuronal degeneration progresses from the entorhinal cortex to the neocortex, the severity of AD increases; each stage is defined by Braak stages [130]. As Braak stages are not dealt with within this thesis, see Braak and Braak, 1995 for more details [130].

Approximately 80% of AD patients show neuropsychological impairment [131], which is considered to be partly due to the dysfunction of the cholinergic system. Additional symptoms and characteristics have been associated with a cholinergic dysfunction (as well as other neurotransmitters) in AD including: psychosis; agitation; depression; anxiety and personality alterations [132]. Agitation, aggression and psychosis are more frequent in the later stages of the disease, however, symptoms of depressed mood, low self-esteem, anxiety and hopelessness seem more associated with earlier stages [133]. More specifically, depressive symptoms have been found to be related to monoamine changes, which is currently being researched as a potential therapeutic target [131]. Some patients with AD also experience a phenomenon called sundowning, the exacerbation of behavioural symptoms (such as, agitation and aggression) in the afternoon and evening [134]. These patients were found to have less activity in daylight hours with more nocturnal activity, though this rhythm of activity was unstable.

In the clinic diagnosis of Alzheimer's disease is complex. There are six categories of criteria (A-F) required by the Diagnostic statistical manual (DSM-IV-TR[135]), Table 12 (p.108). For example, when criteria are met but the patient does not have advanced memory problems and minimal brain atrophy, the patient will be diagnosed with mild AD. Mild AD is a different diagnosis to early onset AD, as the 'early' refers to the person's age of onset rather than the severity of the disease.

Table 12. Diagnostic criteria for Alzheimer's disease from DSM-IV-TR.

Category	Main criterion	Further details
A	Memory impairment (impaired ability to learn new or recall previously learnt information) AND one or more cognitive disturbances	Cognitive disturbances: -Aphasia (language disturbance) -Apraxia (impaired ability to do motor tasks although with an intact motor system) -Agnosia (inability to recognise or identify objects despite intact visual system) -Disturbance of executive function (inability to organise, sequence, plan or think abstractly)
B	Cognitive disturbances must cause significant social or occupational impairment in functioning	Must be significantly worse than the individuals' previous functioning
C	Gradual onset with a continuing cognitive decline	
D	The impairments must not be due to other reasons	They cannot be due to: -Other central nervous system conditions (causing progressive deficits in cognition and memory, for example, Huntington's disease and Parkinson's disease) -Systemic conditions known to cause dementia (for example, HIV infection and folic acid deficiency) -Conditions induced by substance abuse
E	Deficits must not occur solely when the individual is delirious	
F	Impairments observed must not be better accounted for by an Axis 1 disorder (such as Major Depressive disorder)	

Another set of criteria used to diagnose probable Alzheimer's disease is the NINCDS-ADRDA, modified by Dubois *et al.* [136]. The NINCDS-ADRDA criteria centre on the need for the individual to have early and significant episodic memory impairment with gradual and

progressive change over more than 6 months. In addition to this there must be at least one or more supportive features, and these are shown in Table 13 as well as exclusion criteria.

Table 13. Supportive criteria for the diagnosis of probable Alzheimer's disease from the NINCDS-ADRDA guidelines.

Supportive features	Further details
B. Medial temporal lobe atrophy observed on MRI scan	Volume loss of hippocampi, amygdala and entorhinal cortex using visual scoring or quantification of volume loss
C. Abnormal cerebrospinal fluid Biomarker	Low amyloid- β concentrations, increased tau concentrations or increased phospho-tau concentrations or a combination of the above
D. A specific pattern observed through PET functional neuroimaging	Reduction in glucose metabolism in bilateral temporal parietal regions or other validated ligands
E. AD gene mutation	Autosomal dominant mutation within the patient's immediate family
Exclusion criteria	Further details
- Patient history	Sudden onset of symptoms and early occurrence of gait disturbances, seizures or behavioural changes
- Clinical features	Neurological features of hemiparesis, sensory loss and visual field deficits or early signs of movement disorder
- Memory and related symptoms accounted for by other medical disorders	These disorders include: Non-AD dementia; major depression; cerebrovascular disease; toxic and metabolic abnormalities; and MRI FLAIR or T2 signal abnormalities in the medial temporal lobe consistent with infectious or vascular insults

Whilst diagnosing probable Alzheimer's disease of various severities is highly accurate, using these criteria, diagnosis cannot be certain unless either: (a) post-mortem study of the individual's brain has been conducted to determine histological evidence of AD or (b) genetic evidence of an AD mutation on chromosomes 1, 14 or 21 has been found as well as the above clinical evidence of AD.

4.1.2. Memory problems associated with Alzheimer's disease

The main feature of the cognitive impairment found in Alzheimer's disease (AD) patients is the gradual decline of declarative memory (the conscious memory of facts and everyday experiences). AD patients also specifically have a lower ability than age-matched controls to carry out recall tasks, as the ability to bind the features of an object appears to be diminished in AD [137]. This is probably due to the degeneration of brain regions associated with holding integrated features together so the object can be recalled (verbal short term memory).

Precise levels of acetylcholine are needed for memory and learning [138]. Not only are levels of acetylcholine reduced but the cholinergic areas of the brain are degenerated early in the progression of AD. Acetylcholine appears to have a dual role in memory, as slow wave sleep (SWS), characterised by low acetylcholine levels, has been found to be required for the consolidation of declarative memories [138]. These memories are dependent on the hippocampus and neocortex and in these areas high levels of acetylcholine assist with memory encoding during wakefulness and low levels assist with consolidation of these memories during sleep [138]. Using muscarinic and nicotinic antagonists, it was found that acetylcholine seems to act as a switch between encoding and consolidation of memories [138]. There are further disturbances to the sleep of AD patients which will be discussed in more detail later (Section 4.1.5. p.112).

4.1.3. The spectrum of Alzheimer's disease memory impairments

Alzheimer's disease is progressive and is known to take many years to develop. Therefore, a large proportion of current research seeks to determine who will go on to develop AD in order to provide interventions earlier. One categorisation of memory problem, called amnesic mild cognitive impairment (aMCI), is known to progress into AD in over 15% of sufferers per year

[60, 139]. By using a word repetition paradigm, researchers appear more capable of predicting which aMCI patients will develop AD, with the 'congruous word effect' accounting for almost all the non-converters from MCI to AD [140]. However, this is not a definitive tool, and other approaches are available. Research into degeneration of synapses using post-mortem tissue has shown that MCI can be classified in some people as a transition stage from healthy ageing to AD [139]. In fact, across the range of cognitive impairment, from no cognitive impairment to AD, the number of synapses per section significantly decreases in post-mortem tissue. The number of synapses per section correlate well with scores of cognitive impairment from the mini-mental state examination (MMSE), which involves delayed recall [139]. As explained previously, subjective memory complaint (SMC) could be a precursor to MCI showing a spectrum of AD-related disorders. However, more research needs to be conducted into elderly people with SMC.

4.1.4. Circadian disturbances in Alzheimer's disease

Patients with AD have disrupted circadian rhythms as shown by the dysregulation of melatonin, a circadian phase marker [141]. In AD patients, blood plasma melatonin levels are often considerably reduced from older controls (controls mean amplitude: 9.26 ± 1.28 pg/ml, AD patients mean amplitude: 7.78 ± 1.43 pg/ml) [142]. There is also an increase in melatonin 1a receptors (MT_1), as shown by increased intensity in immunohistochemistry staining, in AD cases compared to age-matched healthy controls. This up-regulation presumably is a compensatory response to the reduction in levels of melatonin, considered to be necessary to augment melatonin's neuroprotective properties [141].

Frequently, increased nocturnal activity and significant phase delay in terms of core body temperature and rest-activity rhythms are also found in AD patients [143]. This delay has

been quantified by actigraphy (rest-activity monitoring) finding an average delay of 4.5 hours to the activity acrophase [144]. These observations appear to be quite consistent with the amplitude of the rest-activity rhythms being reduced [145]. Interestingly, the degree of circadian rhythm disturbance does not appear to correlate with disease severity.

Researchers have investigated rest-activity rhythms before and after increased environmental illumination, and found that increased illumination results in more stable rest-activity rhythms in AD patients [95].

4.1.5. Sleep disruption in Alzheimer's disease

Sleep disturbances are a common complaint for Alzheimer's disease (AD) patients and their carers. Caregivers often experience exhaustion as a consequence of a patient's sleep disturbance and rest-activity rhythm disturbances. This is deemed to be the primary causal factor for institutionalisation of the AD patients [143]. In particular, aggressiveness as a global rating on the BEHAVE-AD, a questionnaire about behavioural symptoms, has been found to be a significant predictor of sleep disturbances in AD [146].

When comparing the sleep architecture of AD patients to age-matched healthy controls, both groups show significant reduction of sleep spindles in comparison to younger participants [147]. The mean intensity of the fast spindles was positively correlated with the immediate recall performance in AD patients. The percentage of REM sleep from time in bed was also found to be decreased in AD patients, with a decrease in the mean duration of REM episodes [147]. Latency to the first REM sleep episode could also be a marker for AD [96]. A longer latency has been observed to relate to overall sleep disturbances and the selective deterioration of the REM cholinergic system in these patients [96]. Since the cholinergic system is essential for REM sleep generation, this system is worth investigating in the context of the

progression of AD and memory. This is even more important when considering that the cholinergic system is the only treatment target available to AD patients at the moderate stage of the disease (the first medicated stage). This will be discussed further in Chapter 5 (p.168).

Sleep disturbances have also been found to occur earlier in the prodromal stage of AD, in patients categorised as having a mild cognitive impairment (MCI) [60]. Yu *et al.*, [148] found that sleep efficiency was lower in MCI individuals than healthy controls. Despite no statistically significant differences in sleep architecture, they did observe a trend towards less REM sleep being present during the sleep of MCI individuals than controls [148]. This same study also found the same trend for the mild AD individuals, however, as this study was not conducted in the United Kingdom these patients were already medicated with cholinesterase inhibitors (the effects of this medication on sleep will be addressed in Chapter 5 (p.168). A review of the literature found that sleep disturbance was one of the four most prevalent neuropsychiatric symptoms of MCI with up to 59% of MCI individuals reporting them [149].

The current study seeks to elucidate further the characteristics of sleep and rest-activity pattern in MCI, mild AD and moderate AD before initiation of cholinergic medication, both in comparison to controls and to determine whether any progressive changes in rest-activity or sleep can be attributed to increasing memory impairment severity. The research questions addressed by this chapter are, therefore, the following:

- ***With each prodromal and progressive stage of Alzheimer's disease, to what extent do rest-activity and sleep profiles change in patients opposed to non-memory impaired controls?***

and

- *How do rest-activity and sleep progress across the spectrum of increasing severity of Alzheimer's disease and prodromal stages?*

4.2. Methods

4.2.1. Participant recruitment

Participants were recruited either through the OPTIMA cohort or through memory clinics located in Oxfordshire and Buckinghamshire; for details see Chapter 2 (p.35). Controls (n=9, 4 females) mean age: 73.2 ± 4.9 years, Subjective Memory Complaint (SMC, n=5, 2 females) mean age: 73.2 ± 5.4 years, Amnesic Mild Cognitive Impairment (aMCI, n=9, 4 females) mean age: 76.8 ± 3.9 years, Mild Alzheimer's disease (Mild AD, n=3, 1 female) mean age: 77.0 ± 8.5 years, and Moderate AD that completed questionnaires (qmAD, n=31, 14 females) (subset did all methodologies (mAD), n=5) qmAD mean age: 78.5 ± 6.2 years. All participants were required to be community dwelling, have a study partner and be acetylcholinesterase inhibitor naïve. Except for the qmAD group (questionnaires only moderate Alzheimer's disease) participants were excluded if they were currently diagnosed with a sleep condition, or psychiatric illness, or were taking hypnotic medication. One aMCI individual was excluded due to full-time work and one only completed first assessment due to a hip operation. One Mild AD individual did not complete the second assessment due to illness. Of the 31 qmAD patients who began the study, two did not have baseline assessments due to starting the medication earlier than planned.

4.2.2. Determination of demographics

To determine if any difference in demographics, diurnal preference and anxiety or depression tendencies was present between groups medical and OPTIMA study records were viewed and a variety of self-rated scale were administered including the Health Questionnaire (HQ) derived from the PRIME-MD questionnaire developed by Spitzer *et al.*, [62], medical and OPTIMA study records, the Morningness-Eveningness Questionnaire (MEQ) [26] and the Hospital Anxiety and Depression (HAD) Scale [63] were conducted. For more information about each of these

questionnaires, see Methods Chapter 2 (p.36). Grouping of medical conditions and medications into categories can be found in Appendix 1.1 (p.3). BMI was calculated using height and weight details collected in the HQ with an online BMI calculator [116]. Participants of the mAD group are included in qmAD sample at all times, as mAD are a subset of the qmAD participants. No comments are made in the chapter comparing control to subjective memory complaint (SMC) groups, as these data are presented in Chapter 3 (p.68).

4.2.3. Assessment of memory

Memory was assessed by the Mini-Mental State Examination (MMSE) [61], a questionnaire used clinically to measure memory and some other aspects of cognitive impairment, and with six computerised memory and attention tests from Cambridge Cognition called the CANTABeclipse battery [150]. For details of the tests and the questionnaire see Chapter 2 (p.40). Memory was assessed at baseline and nine weeks later to determine any longitudinal changes that were different between the two groups. Longitudinal changes were measured in order to observe, if possible, whether SMC forms a stage in the progression from healthy older people to Alzheimer's disease.

4.2.4. Assessment of functioning

In order to assess functioning, both social functioning and activities of daily living were assessed using questionnaires: Social Functioning Scale (SFS) [67] and the ADCS-Activities of Daily Living Scale (ADCS-ADL) [66]. For details of these questionnaires see Chapter 2 (p.41). Functioning was assessed at baseline and nine weeks later for the same reasons as above.

4.2.5. Circadian measurements

Rest-activity was assessed using actigraphy, for three weeks at baseline and six weeks later. For non-parametric circadian rhythm analysis, average daily activity was evaluated for each assessment period as well as periodogram analysis conducted to determine activity tau for each assessment. All details of the measurement using actigraphy and the analyses performed on the resultant data are found in Chapter 2 (p.43).

4.2.6. Sleep measurements

Sleep was measured by three techniques: questionnaires, actigraphy and electroencephalography (EEG); for details of each see Chapter 2 (p.52). All were measured at both assessments, with sleep analysis being conducted on the rest-activity derived from the actigraphy of both assessment periods. The sleep questionnaires were the Pittsburgh Sleep Quality Index (PSQI) [64] and selected questions from the Jupiter Sleep Questionnaire (JSQ) [65]; details of both are given in Chapter 2 (p.51). EEG data were scored according to the scoring criteria of Rechtschaffen and Kales [77]. Following scoring the sleep period was divided into three sections (or 'thirds') to compare the sleep stage durations and percentages and how they change across the sleep period. These thirds were calculated per recording to take into account the individual's sleep duration. Due to a technical fault some EEG recordings were unable to be scored, for more details see Chapter 2 (p.53). Due to the technical fault 7 out of 9 controls had recordings analysed at baseline and 5 out of 8 at second assessment, and 4 out of 5 SMC individuals had recordings analysed at baseline, and 3 out of 4 at second assessment. For the aMCI group, 6 out of 9 recordings were analysed at baseline and 5 out of 8 recordings were analysed at second assessment. For the mild AD group all 3 recordings were analysed at baseline and 2 recordings were analysed at second assessment. For the mAD group, 1 out of 3

recordings were analysed at baseline and 2 out of 4 recordings were analysed at second assessment (results shown in Chapter 5, p. 168).

4.2.7. Statistical analysis

All data were divided into parametric or non-parametric scale data, binominal data or categorical data. Each type of data was analysed statistically as detailed in Chapter 2 (p.63). All data were compared between groups, with differences between time-points compared between groups also. Statistical comparisons of change over time between groups were restricted to controls, SMC and aMCI. Comparisons of change over time for controls and change following cholinergic medication for qmAD and mAD groups are described in Chapter 5 (p.168). Only baseline comparison is shown in this chapter, as qmAD and mAD were acetylcholinesterase inhibitor (ChEI) naïve at baseline, but participants began ChEI intake after this assessment. Details of statistical tests and the corrected significance level, through the Dunn-Šidák method of correcting for multiple comparisons, are provided in the Appendix section for the relevant data chapter. In the text of this chapter if a result was significant after multiple comparisons correction 'significant after Dunn-Šidák correction' will be stated.

In order to determine progression of circadian or sleep characteristics across increasing memory impairment severity Pearson correlations were performed.

4.2.8. Ethical approval

Ethical approval for this study was given by the NHS Berkshire Ethics Committee, for further details see Chapter 2 (p.60). Funding was provided by the NIHR Brain Theme.

4.3. Results

4.3.1. Demographics

Time between assessment did not differ between groups significantly ($p>0.05$), control mean: 64.6 ± 6.4 days, SMC mean: 69.8 ± 7.8 days, aMCI mean: 65.1 ± 6.5 days, Mild AD mean: 64.5 ± 2.1 days, Moderate AD mean: 68.5 ± 9.5 days. There was no significant difference in age or sex found between the controls and all groups ($p>0.05$), except that the moderate Alzheimer's disease group participating in questionnaires only (qmAD) had a significantly higher average age than controls ($p<0.05$), Table 14 (p.121). No statistics were conducted with the Mild AD group as the sample size was too small, however, average age and sex proportions did not appear different from other groups, Table 14 (p.121).

When determining differences using the Health Questionnaire (HQ), the only significant difference was in the number of visual conditions, with controls and the qmAD group having more visual conditions than the amnesic mild cognitive impairment (aMCI) group ($p<0.05$). Body mass indices (BMI) were comparable with all groups having an average BMI bordering between healthy and overweight ranges (≥ 25).

In terms of medication, controls were found to take significantly less analgesics ($p<0.05$) than the qmAD group ($p<0.05$) as well as less diabetic medication ($p<0.05$) and less diuretic medication ($p<0.05$). No difference were found in the use of anti-hypertensives; beta-blockers or other types of anti-hypertensives, as well as psychiatric medication (for example, selective serotonin re-uptake inhibitors), $p>0.05$. The SMC group had taken significantly more corticosteroids than the aMCI group ($p<0.05$) and significantly more eye medications ($p<0.05$) and allergy medications ($p<0.05$) than the qmAD group. The aMCI group took significantly more hormone replacement medication ($p<0.01$) and eye medication ($p<0.01$) than the qmAD. The mild AD group appeared consistent in medication use in comparison to other groups.

The qmAD group had higher depression tendency scores (from the HAD scale) than the control group ($p < 0.05$). The SMC group had a significantly lower anxiety tendency total than the aMCI group ($p < 0.05$) and the aMCI had a significantly higher anxiety score than the mAD group ($p < 0.01$, significant after Dunn-Šidák correction). Due to this finding, when comparing the SMC and aMCI groups, and the aMCI and mAD groups, where possible, anxiety score was used as a covariate (Table 14, p121).

Tendency for sleep apnea, as determined through collar size and caffeine intake frequency ($p > 0.05$) (Figure 14, p.123), were not significantly different between the groups. However, the SMC group normally consumed more units of alcohol per week ($p < 0.05$) in comparison to the qmAD group.

Diurnal preference was found not to be significantly different between any of the groups ($p > 0.05$), Figure 13 (p.122). No participants had definitely evening types, Figure 13 (p.122).

Highest level of education was not significantly different between groups and controls ($p > 0.05$) with mild AD groups appearing in the same range as other groups (Figure 15, p.124). However, the SMC group had a significantly different higher level of education than the qmAD group ($p < 0.05$). Most groups have a large range of highest level of education however the mAD group were restricted to school and apprenticeships as their highest level ($p > 0.05$), Figure 15, (p.124). Years of education are provided in Table 14 (p.121), with no significant difference found between groups, except for the SMC group having had more years of education ($p < 0.05$) in comparison to the qmAD group.

Table 14. Demographic and screening parameters for all groups.

Parameter	Control (n=9)	SMC (n=5)	aMCI (n=9)	Mild AD (n=3)	qmAD (n=31)
Age (yrs.)	73.2 ± 4.9	73.2 ± 5.4	76.8 ± 3.9	77.0 ± 8.5	78.5 ± 6.2 ¹
Sex	Female (%)	4 (44.4)	2 (40.0)	4 (44.4)	1 (33.3)
	Male (%)	5 (55.6)	3 (60.0)	5 (55.6)	2 (66.6)
Body Mass Index (BMI)	25.0 ± 4.0	25.1 ± 2.1	25.6 ± 4.4	22.6 ± 2.7	25.2 ± 4.1 (n=21)
No. of individuals with collar size in normal range (%)	7 (77.7)	4 (80.0)	8 (88.8)	3 (100.0)	19 (82.6)
Av. units of alcohol consumed per week	11.6 ± 11.2	13.3 ± 9.6 ³	6.0 ± 3.9	7.0 ± 1.0	5.3 ± 7.6
Years of education	14.7 ± 4.9	15.3 ± 4.7 ³	13.8 ± 3.3	14.0 (n=1)	11.3 ± 2.0
Anxiety tendency score (HAD)	2.8 ± 2.5	1.6 ± 1.5	4.4 ± 2.1 ²	1.3 ± 1.5	3.7 ± 3.2
Depression tendency score (HAD)	1.7 ± 1.2	1.4 ± 1.1	2.6 ± 1.7	2.0 ± 2.6	3.7 ± 2.6 ¹

¹ p<0.05 group and controls.

² p<0.05 group and SMC.

³ p<0.05 group and qmAD.

Data are mean ± SD unless otherwise stated.

Full dataset, test statistics and Dunn-Šidák calculations are presented in Appendix 1.3. (p.7).

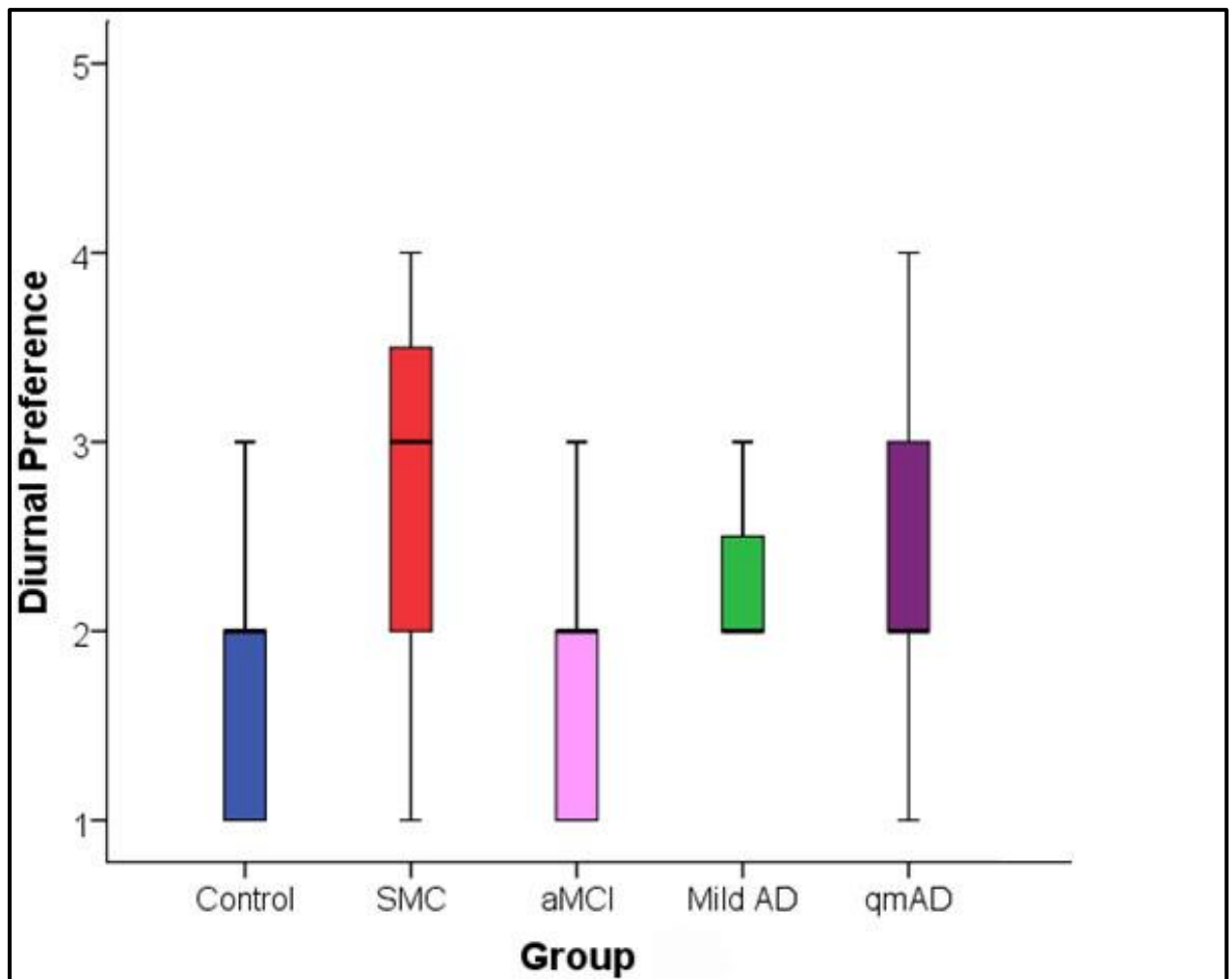


Figure 13. Diurnal preference distribution for all groups at baseline as measured by the Morningness-Eveningness Questionnaire (MEQ).

Each gradation of the y-axis shows diurnal preference types, ascending numerical values denote increasing eveningness of sleep timing preference, 1: Definitely morning type, 2: Moderately morning type, 3: Neither type, 4: Moderately evening type and 5: Definitely evening type. Boxed area indicates 50 percentile of type distribution, horizontal line shows median for group and error bars indicate 95% to 5% range of types. Groups are as follows: controls (n=9); subjective memory complaint (SMC, n=4); amnesic mild cognitive impairment (aMCI, n=9); mild Alzheimer's disease (AD, n=3); and moderate AD questionnaires only (qmAD, n=29). Memory impairment severity increases from left to right (qmAD and mAD are equal severity). No significant differences were found between any of the groups ($p>0.05$). All data and Dunn-Šidák correction calculation is presented in Appendix 1.3. (p.14).

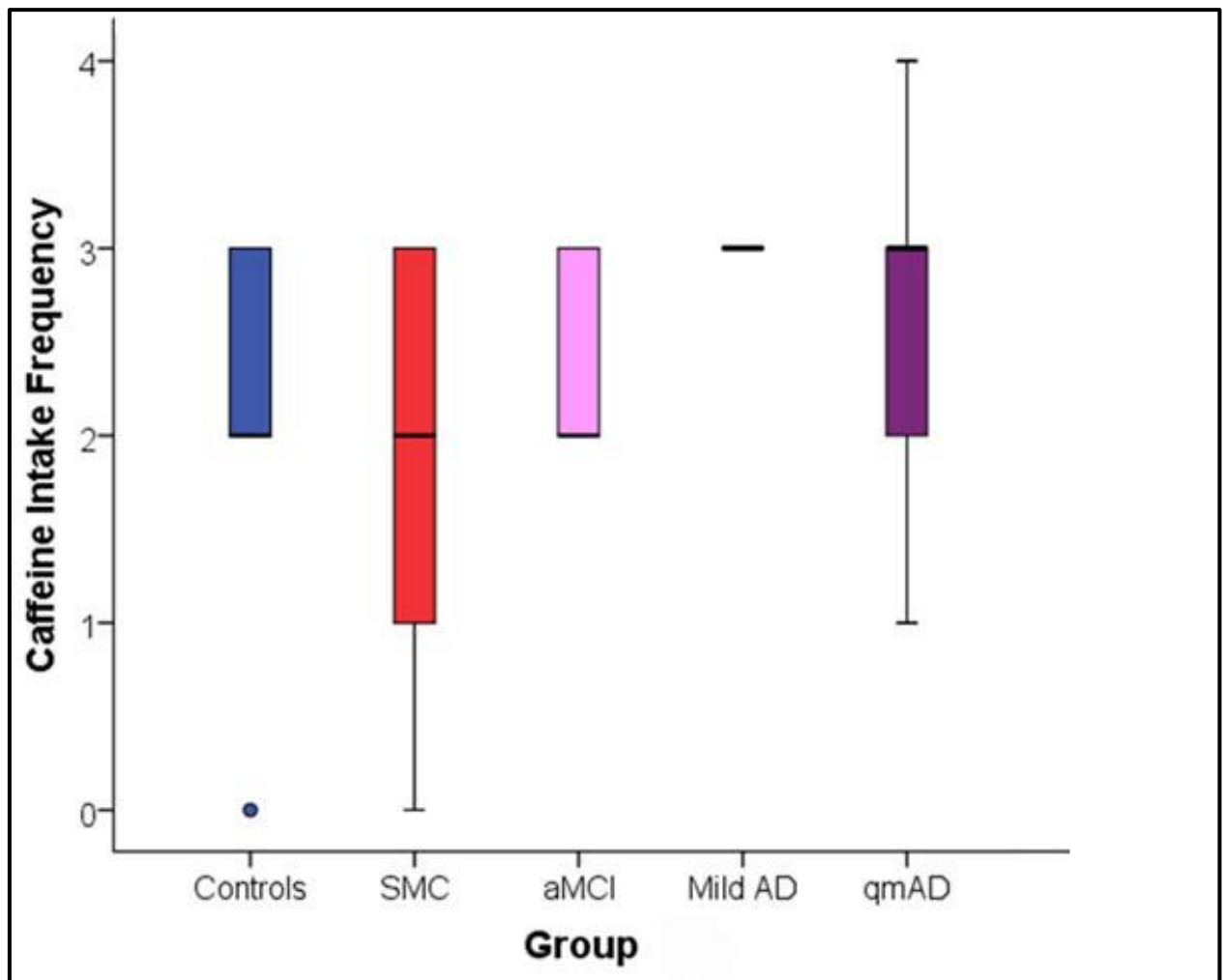


Figure 14. Caffeine intake frequency for all groups at baseline as determined through completion of the HQ.

Each gradation of the y-axis shows the amount of usual caffeine intake per day (p/d), ascending numerical values denote increasing frequency of caffeine intake (taken from the HQ), 0: Never (0 p/d), 1: Occasionally (<1 p/d), 2: Sometimes (1-3 p/d), 3: Frequently (3-7 p/d) and 5: Often (>7 p/d). Boxed area indicates 50 percentile of type distribution, horizontal line shows median for group and error bars indicate 95% to 5% range of types, smaller circle denotes an outlier within the colour-corresponded group. Groups are as follows: controls (n=9); subjective memory complaint (SMC, n=5); amnesic mild cognitive impairment (aMCI, n=9); mild Alzheimer's disease (AD, n=3) and moderate AD questionnaires only (qmAD, n=29). Memory impairment severity increases from left to right (qmAD and mAD are equal severity). Caffeine intake frequency was calculated with a Pearson chi-square. There were no significant differences between any groups ($p>0.05$). All data, test statistics and Dunn-Šidák correction calculation are presented in Appendix 1.3. (p.8).

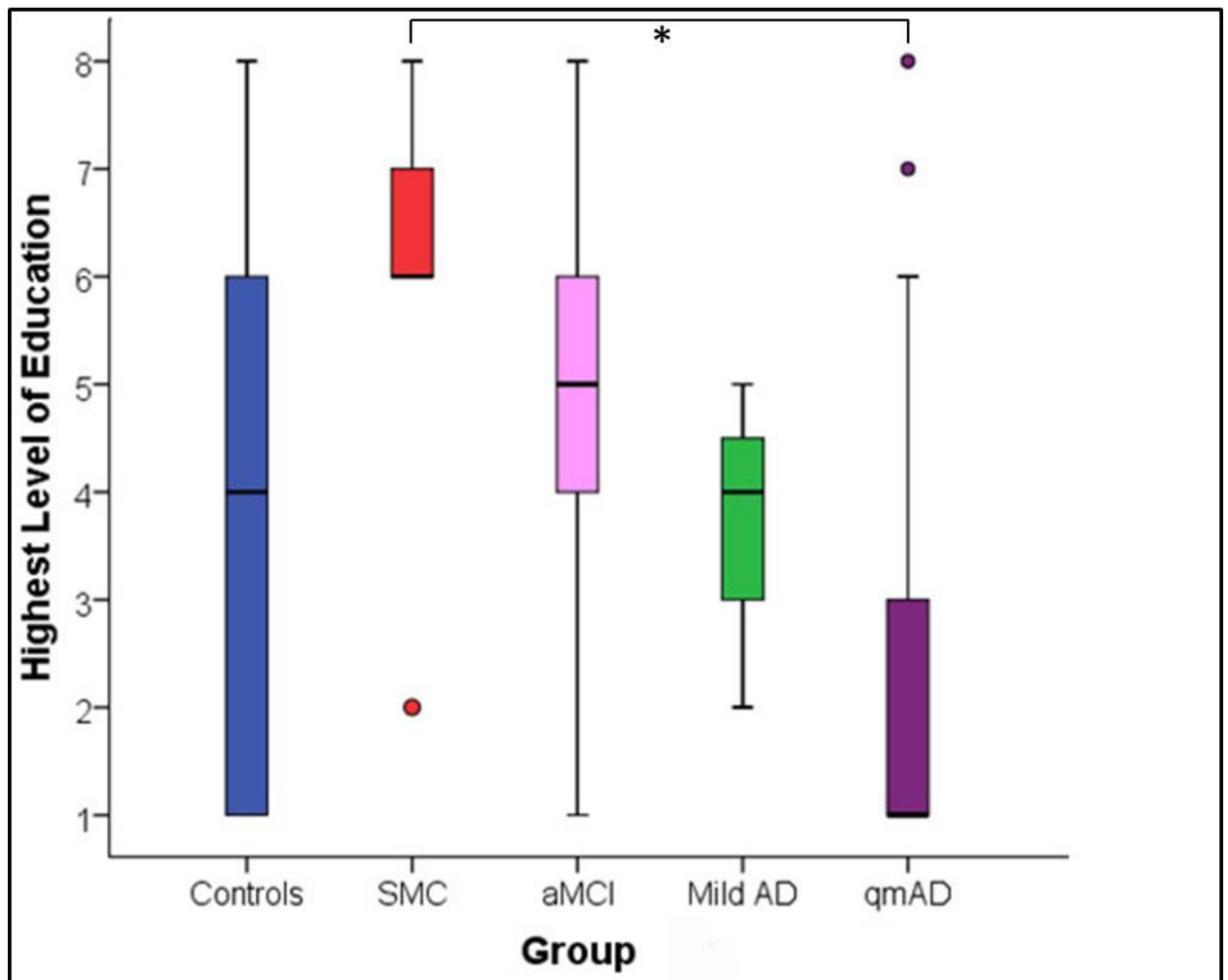


Figure 15. Highest level of education of all groups at baseline as assessed by the HQ. Each gradation of the y-axis is an arbitrary number representing a level of education, 1: Secondary school, 2: Grammar school, 3: Apprenticeship, 4: Diploma, 5: Polytechnic degree, 6: Bachelor degree, 7: Masters degree, 8: Doctoral degree. Boxed area indicates 50 percentile of type distribution, horizontal line shows median for group and error bars indicate 95% to 5% range of types, smaller circle denotes an outlier within the colour-corresponded group. Groups are as follows: controls (n=9); subjective memory complaint (SMC, n=5); amnesic mild cognitive impairment (aMCI, n=9); mild Alzheimer's disease (AD, n=3); and moderate AD questionnaires only (qmAD, n=28). Memory impairment severity increases from left to right (qmAD and mAD are equal severity). Highest level of education was compared by Pearson chi-square. * $p < 0.05$, significant. All data, test statistics and Dunn-Šidák correction are presented in Appendix 1.3. (p.8).

4.3.2. Memory

4.3.2.1. Comparison of groups

The aMCI group had significantly lower recall ($p<0.01$) and total scores ($p<0.001$) than controls (significant after Dunn-Šidák correction) on the Mini-Mental State Examination (MMSE), Table 15. The mild AD group had lower orientation, repetition, recall and total scores when compared to controls, Table 15. The qmAD group had significantly lower orientation ($p<0.001$), language ($p<0.001$), recall ($p<0.001$) and total scores ($p<0.001$, all significant after Dunn-Šidák correction) as well as lower praxis ($p<0.05$) and attention ($p<0.05$) scores than controls, Table 15. No significant differences were found between groups for change of score overtime ($p>0.05$, Appendix 1.5. p.16).

Table 15. Mini-Mental State Examination (MMSE) component scores for all groups in order of severity at Baseline Assessment.

Component score	Control (n=9)	SMC (n=5)	aMCI (n=9)	Mild AD (n=3)	qmAD (n=29)
Orientation	9.9 ± 0.3^{4d}	9.8 ± 0.4^{4d}	9.7 ± 0.5^{4d}	8.0 ± 2.0	$6.8 \pm 2.3^{1d, 2d, 3d}$
Language	2.9 ± 0.3^{4d}	2.8 ± 0.4^{4a}	2.6 ± 0.5	2.3 ± 0.6	$2.3 \pm 0.5^{1d, 2a}$
Repetition	1.0 ± 0.0	1.0 ± 0.0	0.6 ± 0.5	0.3 ± 0.6	0.3 ± 0.5
Registration	3.0 ± 0.0	3.0 ± 0.0	2.6 ± 0.9	3.0 ± 0.0	2.9 ± 0.4 (27)
Attention	5.0 ± 0.0^{4a}	5.0 ± 0.0	4.4 ± 0.5	5.0 ± 0.0	4.0 ± 1.4^{1a}
Recall	$2.6 \pm 0.5^{3b, 4d}$	$2.8 \pm 0.4^{3a, 4d}$	1.4 ± 1.0^{1b}	0.3 ± 0.6	$0.5 \pm 0.8^{1d, 2d}$
Reading	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	0.9 ± 0.4
Praxis	4.9 ± 0.3^{4a}	5.0 ± 0.0^{4a}	4.8 ± 0.0	5.0 ± 0.0	$4.3 \pm 0.9^{1a, 2a}$
Test total	$29.2 \pm 1.0^{3d, 4d}$	$29.4 \pm 0.5^{3a, 4d}$	$26.4 \pm 1.8^{1d, 4d}$	24.7 ± 3.1	$22.4 \pm 2.5^{1d, 2d, 3d}$
Memory impairment severity increases					→

^a $p<0.05$, ^b $p<0.01$, ^c $p<0.005$, ^d $p<0.001$. ¹ group and controls, ² group and SMC, ³ group and aMCI, ⁴ group and qmAD. No statistics were computed with the Mild AD group.

Sample sizes provided in parentheses if different from header values. Data are presented as mean $\pm$ SD. Full dataset, test statistics and Dunn-Šidák calculations are presented in Appendix 1.5. (p.16).

Table 16. Selected CANTABclipse test parameters for all groups at Baseline Assessment.

Parameter	Control (n=8)	SMC (n=4)	aMCI (n=9)	Mild AD (n=3)	mAD (n=4)
<i>Big/Little Circle (BLC) Test</i>					
Total correct (%)	99.7 ± 0.9	100.0 ± 0.0	99.7 ± 0.8	100.0 ± 0.0	98.1 ± 2.4
Correct latency	771.6 ± 75.0 ^{4d}	768.8 ± 102.4 ^{4c}	966.1 ± 323.6	851.2 ± 282.6	1110.4 ± 103.1 ^{1d, 2c}
<i>Choice Reaction Time (CRT) Test</i>					
Correct latency	390.4 ± 60.7 ^{4a}	364.5 ± 35.2	432.4 ± 81.7	391.7 ± 29.3	512.0 ± 118.9 ^{1a}
Correct trials (%)	99.5 ± 1.1	99.5 ± 1.0	99.2 ± 1.2	99.0 ± 1.7	98.5 ± 1.9
Incorrect trials (%)	0.4 ± 0.7	0.5 ± 1.0	0.6 ± 0.9	0.7 ± 1.2	1.5 ± 1.9
<i>Paired Associates Learning (PAL) Test</i>					
Stages completed	4.5 ± 0.5 ^{4a}	4.8 ± 0.5	4.0 ± 0.9	3.3 ± 0.6	3.0 ± 1.4 ^{1a}
Trails to success	3.6 ± 1.6 ^{4c, Δ3a}	3.1 ± 1.4 ^{4b}	3.9 ± 1.2 ^{Δ1a, 4a}	4.2 ± 1.1	7.9 ± 2.1 ^{1c, 2b, 3a}
Trials completed on first trial	2.6 ± 0.5	2.8 ± 0.5 ^{4a}	2.1 ± 0.9 ^{4a}	2.0 ± 0.0	1.0 ± 0.8 ^{2a, 3a}
First trial memory score	11.9 ± 2.2 ^{4c}	10.3 ± 1.9	10.1 ± 3.3 ^{4c}	5.7 ± 2.1	6.0 ± 2.6 ^{1c, 3c}
Total errors	35.9 ± 21.9 ^{4c}	34.3 ± 18.5 ^{4c}	34.4 ± 14.7	54.3 ± 43.2	65.5 ± 35.6 ^{1c, 2c}
<i>Spatial Span (SSP) Test</i>					
Span length	5.4 ± 0.7 ^{3b, 4c}	5.8 ± 1.5 ^{4a}	4.3 ± 0.7 ^{1b}	5.0 ± 1.0	3.5 ± 1.0 ^{1c, 2a}
Total errors	11.5 ± 2.5	15.5 ± 4.04 ^{3a}	11.5 ± 5.5 ^{2a}	15.7 ± 2.9	12.3 ± 5.1
Time to last response (span 2)	3278.5 ± 291.9 ^{4a}	3190.8 ± 447.2	4941.7 ± 3118.3	4020.0 ± 919.2	4425.3 ± 1451.0 ^{1a}
<i>Stop Signal Task (SST)</i>					
	n=6	n=2	n=5	n=2	n=3
Median correct RT on 'go' trials	643.6 ± 111.5	591.5 ± 80.1	796.5 ± 209.1	859.0 ± 124.5	800.3 ± 211.8
Direction errors	1.9 ± 2.2	0.8 ± 1.5	5.0 ± 4.2	1.0 ± 1.4	5.0 ± 5.9
<i>Graded Naming Test (GNT)</i>					
Total correct	26.9 ± 2.7 ^{3a, 4c}	27.5 ± 1.9 ^{4c}	22.3 ± 3.7 ^{1a}	13.3 ± 13.6	21.3 ± 2.1 ^{1c, 2c}
Total correct (%)	89.6 ± 9.2 ^{4c}	91.7 ± 6.4 ^{4c}	74.4 ± 12.2	44.4 ± 45.3	70.8 ± 6.9 ^{1c, 2c}
Total errors	3.1 ± 2.7 ^{3a}	2.5 ± 1.9 ^{4a}	7.7 ± 3.7 ^{1a}	16.7 ± 13.6	8.8 ± 2.1 ^{2a}
<i>Memory impairment severity increases</i> →					

^a p<0.05, ^b p<0.01, ^c p<0.005, ^d p<0.001. ¹ group and controls, ² group and SMC, ³ group and aMCI, ⁴ group and mAD. No statistics were computed with the Mild AD group.

Data are mean ± SD. RT is reaction time. Latencies and RTs are in ms.

Sample sizes for SST test are reduced due to a technical fault.

Full dataset, test statistics and Dunn-Šidák calculations are presented in Appendix 1.5. (p.17).

A further assessment of memory, through the use of computerised memory and attention tests of the CANTABeclipse battery was conducted. As this was not a questionnaire, only the mAD subgroup, not the whole qmAD participated. The mAD group had significantly longer mean correct latency for the BLC test ($p < 0.001$, significant after Dunn-Šidák correction) than controls. Furthermore, the mAD group had a significantly longer choice reaction time (CRT) mean correct latency ($p < 0.05$), lower first trial memory score for the PAL test ($p < 0.005$), more errors on the PAL test ($p < 0.005$), took more trials to succeed on the PAL test ($p < 0.005$), completed less PAL stages ($p < 0.05$), shorter span length on the SSP test ($p < 0.005$), longer mean time to last response on the SSP test ($p < 0.05$), lower ability to name objects on the GNT test for total correct ($p < 0.005$) and percentage correct ($p < 0.005$) with more errors on the GNT test ($p < 0.005$) in comparison to the control group.

The aMCI group had a significantly shorter span length ($p < 0.01$) with less ability to name objects (Graded naming test (GNT) total correct lower and GNT total errors higher, $p < 0.05$) than controls.

In comparison to controls, the mild AD group had longer reaction times on the Big/Little Circle (BLC) test, the Spatial span (SSP) test and Stop signal test (SST), Table 16 (p.126). The mild AD group completed fewer stages, had a lower first trial memory score and took more trials to be successful on the paired associates learning (PAL) test than the controls, Table 16 (p.126). The mild AD group also named fewer objects in the graded naming test (GNT) than controls.

Change over time was also calculated for each of the CANTABeclipse variables. For the PAL test mean number of trials to success, the control group improved over time (needing fewer trials), whereas the aMCI group performed less well at second assessment ($p < 0.05$). The total trials for the whole of the PAL test again improved over time for controls and the aMCI group performance worsened over time, taking more trials to complete the test ($p < 0.05$). For

the SSP test, mean time to first response worsened for both the controls and the aMCI group, however, the aMCI change over time was larger, becoming slower than controls ($p < 0.05$).

In contrast to the mild AD group, controls improved in the number of stages completed on first trial on the PAL test, with both groups having a large reduction in number of errors over time. The mild AD group had the same span length (SSP test) stability over time as seen in the controls, although their mean time to first response shortened over time, whereas controls lengthened slightly. Controls were stable in their number of errors and usage errors over time whereas the mild AD group made fewer errors on the SSP test but more usage errors over time. Due to an on-going technical fault at the time of some assessments with the SST test, none of the mild AD group were administered this test at second assessment. In terms of naming objects, on the GNT test, controls performed better over time, whereas the mild AD group named fewer objects correctly over time.

4.3.2.2. Progression of memory characteristics as impairment severity increases

Total score on the MMSE shows a clear progression in average score with a decrease in score associated with an increase in the memory impairment severity, Table 15 (p.125). The SMC total score was significantly higher than the aMCI total score ($p < 0.05$). The aMCI and SMC groups' total score were significantly higher than the qmAD group (both $p < 0.001$, both significant after Dunn-Šidák correction), Table 15 (p.125). These significant results confirm the overall trend observed in the raw total scores decreasing as memory impairment severity increases.

Orientation score also showed a trend to decrease as memory impairment severity increased, Table 15.(p.125) Orientation score was found to be significantly reduced in qmAD in

comparison to the SMC group ($p < 0.001$) and the aMCI group ($p < 0.001$, both significant after Dunn-Šidák correction).

In terms of the Mild AD group, scores were in-line with the above trends and were comparable to control scores (where no trend was present), except for recall score which was lower than the average score for the qmAD group, Table 15 (p.125).

When observing the progression of memory deficits in CANTABeclipse variables as memory impairment severity increased, the number of choice reaction time (CRT) test incorrect trials percentage also increased, Table 16 (p.126).

In addition, the paired-associates learning (PAL) mean number of trials to success was significantly higher for the mAD group than the SMC group ($p < 0.01$) and significantly higher for the mAD group than the aMCI group ($p < 0.05$). The mean number of PAL errors was significantly higher from the mAD group than the SMC group ($p < 0.005$). The number of PAL stages completed on first trial decreased as memory impairment severity increased, apart from the SMC and control groups which were comparable, Table 16 (p.126). The mAD group was found to complete significantly fewer stages on first trial than the SMC group ($p < 0.05$) and the aMCI group ($p < 0.05$).

Trends in the word naming ability (graded naming test [GNT] total correct and percentage correct) of groups were found with ability decreasing as memory impairment severity increased, except for the mild AD group which performed worse than the mAD group, Table 16. (p.126) The total correct and percentage correct were significantly higher in the SMC group than the mAD group ($p < 0.005$ for both), and the total errors on the GNT test were significantly higher in the mAD group than the SMC group ($p < 0.05$).

4.3.3. Functioning

4.3.3.1. Comparison of groups

No significant differences in ADCS-ADL component scores were found between the control group and the aMCI group, Table 17 (p.131). The qmAD group was observed to have lower functioning on Instrumental activities of daily living (ADLs) and total functioning than controls (both $p < 0.001$, both significant after Dunn-Šidák correction). For basic ADLs, the mild AD group had the same average score as the control and SMC groups, higher than that of the qmAD group, Table 17 (p.131) No component scores were observed to have significant changes over time for any group in comparison to the control group ($p > 0.05$) (Appendix 1.7.,p.26) Scores for the mild AD group for basic ADLs remained stable over time with no difference from the control group, Appendix 1.7. (p.26).

When assessing social functioning using the social functioning scale (SFS), the aMCI group had no significant differences in comparison to controls, Table 18 (p.131). The qmAD group was observed to have significantly lower Independence Performance ($p < 0.001$), Recreation ($p < 0.001$), Total ($p < 0.001$, all significant after Dunn-Šidák correction) and Prosocial component scores ($p < 0.01$) than controls. Interpersonal Communication score ($p < 0.05$) and Independence Competence score ($p < 0.001$, significant after Dunn-Šidák correction) were significantly lower in the qmAD group than controls. For social functioning, no SFS variables were found to change significantly over time for any groups in comparison to controls, Appendix 1.7. (p.26). The mild AD group appeared to remain stable in their average score over time, Appendix 1.7. (p.26).

Table 17. Component and total scores of the ADCS-ADL functioning scale for all groups at Baseline Assessment.

Component	Control (n=9)	SMC (n=5)	aMCI (n=9)	Mild AD (n=3)	qmAD (n=27)
Basic ADLs	22.0 ± 0.0	22.0 ± 0.0	21.8 ± 0.7	22.0 ± 0.0	18.7 ± 3.5
Instrumental ADLs	54.3 ± 2.8 ^{4d} (7)	55.0 ± 1.0 (3)	55.0 ± 1.2 ^{4d} (4)	Incomplete	35.0 ± 9.7 ^{1d, 3d} (15)
Total score	76.3 ± 2.8 ^{4d} (7)	77.0 ± 1.0 (3)	77.0 ± 1.2 ^{4c} (4)	Incomplete	55.1 ± 12.0 ^{1d, 3d} (15)
<i>Memory impairment severity increases</i>					➔

^a p<0.05, ^b p<0.01, ^c p<0.005, ^d p<0.001.

¹ group and controls, ² group and SMC, ³ group and aMCI, ⁴ group and qmAD.

No statistics were computed with the Mild AD group.

Data are mean ± SD. Sample sizes are as given unless in parentheses.

Full dataset, test statistics and Dunn-Šidák calculations are presented in Appendix 1.8. (p.26).

Table 18. Social Functioning Scale components for all groups at Baseline Assessment.

Component score	Control (n=9)	SMC (n=5)	aMCI (n=9)	Mild AD (n=3)	qmAD (n=27)
Withdrawal	12.2 ± 2.5	13.4 ± 1.1	13.3 ± 1.1 ^{4a}	12.0 ± 2.6	11.4 ± 2.3 ^{3a}
Interpersonal communication	8.7 ± 0.7 ^{4a}	9.0 ± 0.0	8.7 ± 0.5 ^{4a}	9.0 ± 0.0 (2)	7.4 ± 1.7 ^{1a, 3a}
Independence Performance	31.7 ± 4.3 ^{4d} (7)	32.0 ± 4.9 ^{4c} (4)	33.4 ± 2.8 ^{4d}	21.0 ± 12.1	18.0 ± 8.7 ^{1d, 2c, 3d} (26)
Recreation	30.1 ± 5.8 ^{4d} (8)	28.6 ± 8.1 ^{4d}	29.9 ± 5.2 ^{4d} (7)	22.0 ± 2.6	14.0 ± 6.2 ^{1d, 2d, 3d} (26)
Prosocial	29.5 ± 13.8 ^{4b} (8)	33.4 ± 5.9 ^{4d}	32.0 ± 15.3 ^{4b} (6)	19.5 ± 9.2 (2)	17.6 ± 8.8 ^{1b, 2d, 3b} (22)
Independence competence	35.7 ± 1.0 ^{4d}	34.6 ± 1.5	35.3 ± 1.4 ^{4d} (8)	26.3 ± 7.5	25.4 ± 6.3 ^{1d, 3d} (22)
Total	145.7 ± 23.0 ^{4d} (6)	150.5 ± 20.5 ^{4d} (4)	151.2 ± 20.9 ^{4d} (6)	94.0 (1)	93.8 ± 24.7 ^{1d, 2d, 3d} (14)
<i>Memory impairment severity increases</i>					➔

^a p<0.05, ^b p<0.01, ^c p<0.005, ^d p<0.001.

¹ group and controls, ² group and SMC, ³ group and aMCI, ⁴ group and qmAD.

No statistics were computed with the Mild AD group.

Data are mean ± SD. Sample sizes are as given unless in parentheses.

Full dataset, test statistics and Dunn-Šidák calculations are presented in Appendix 1.8. (p.26).

4.3.3.2. Progression of functioning characteristics as impairment severity increases

For all components of the ADCS-ADL, all groups appear comparable except the qmAD group which had the lowest component scores on all components, Table 17 (p.131). Furthermore, the aMCI group was found statistically to have higher scores in the instrumental ($p<0.001$) and total scores than the qmAD group ($p<0.005$, both significant after Dunn-Šidák correction). The Instrumental and Total scores for the mild AD group were not available due to missing values so it cannot be determined whether the reduction in scores observed in the qmAD group were also present in the mild AD group.

In terms of the social functioning scale, a decrease in withdrawal score was observed as memory impairment severity increased, except that the control group showed a mean score equivalent to mild AD, Table 18 (p.131). The qmAD group was found to have a significantly lower withdrawal score ($p<0.05$) than the aMCI group.

Recreation score showed a trend of decreasing score with increasing memory impairment severity, except for the aMCI group which scored marginally higher than the SMC group, Table 18 (p.131). The qmAD group had a significantly lower score than the SMC group ($p<0.001$) and the aMCI group ($p<0.001$, both significant after Dunn-Šidák correction). The Prosocial component score decreased as memory impairment severity increased, apart from the control group who scored less than the aMCI group, Table 18 (p.131). A significantly lower score for the qmAD group was observed in comparison to the SMC group ($p<0.001$) and the aMCI group ($p<0.01$, both significant after Dunn-Šidák correction). Although a clear trend of progressive decline in the total score values cannot be observed, there were two levels of total functioning: the first with comparable scores between the control, SMC and aMCI groups; and the second with the mild AD and qmAD groups being comparable, Table 18 (p.131). The qmAD

group were determined to have a significantly lower total score than the SMC group ($p < 0.001$) and the aMCI group ($p < 0.001$, both significant after Dunn-Šidák correction).

4.3.4. Circadian assessment

4.3.4.1. Comparison between groups

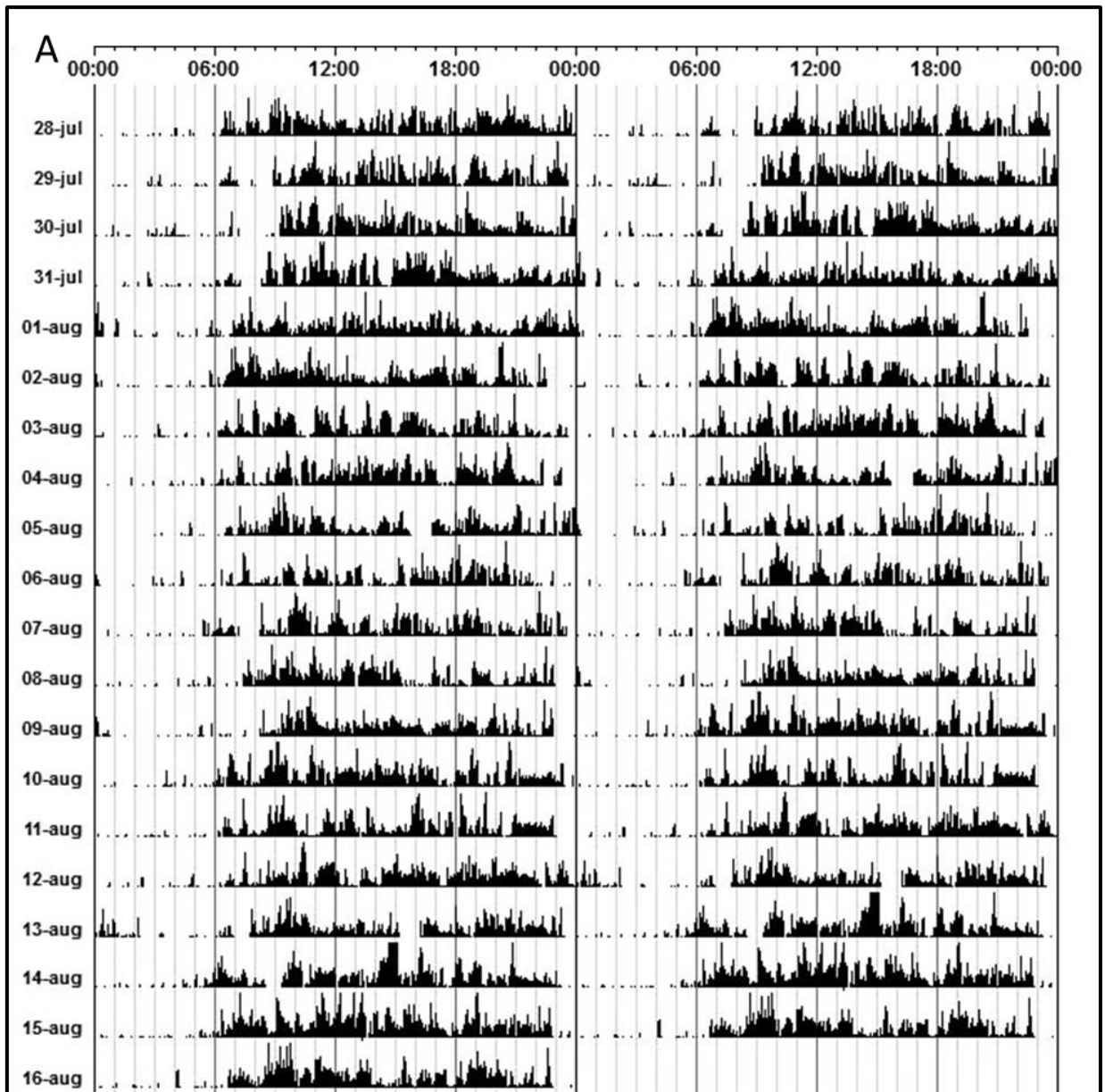
Representative rest-activity profiles (actograms) of five individuals, one from each diagnostic group are provided in Figure 16 (p.135). Rest-activity data were analysed with non-parametric circadian rhythm analysis (NPCRA), two methods of periodogram analyses, and by calculating differences between groups for phase of activity. The NPCRA found no significant difference between the aMCI group and the control group at baseline ($p > 0.05$). Controls were found to have a larger average activity count ($p < 0.05$), larger activity counts in the most active ten hours (M10, $p < 0.05$), larger amplitude of activity ($p < 0.05$) and a higher relative amplitude between M10 and least active 5 hours (L5, $p < 0.01$) than the mAD group. The mAD group was determined to have significantly lower activity amplitude and M10 counts than the SMC group ($p < 0.005$ and $p < 0.005$ the latter was significant after Dunn-Šidák correction) and lower activity amplitude than the aMCI group ($p < 0.005$, both significant after Dunn-Šidák correction). The mAD group also had a significantly higher IV score than the SMC group ($p < 0.05$) and the aMCI group ($p < 0.05$). The aMCI group was determined to have a significantly higher IV score than the SMC group ($p < 0.05$). The mild AD group had higher average activity, L5 and M10 activity counts, and amplitude of activity than controls, Table 19 (p.140).

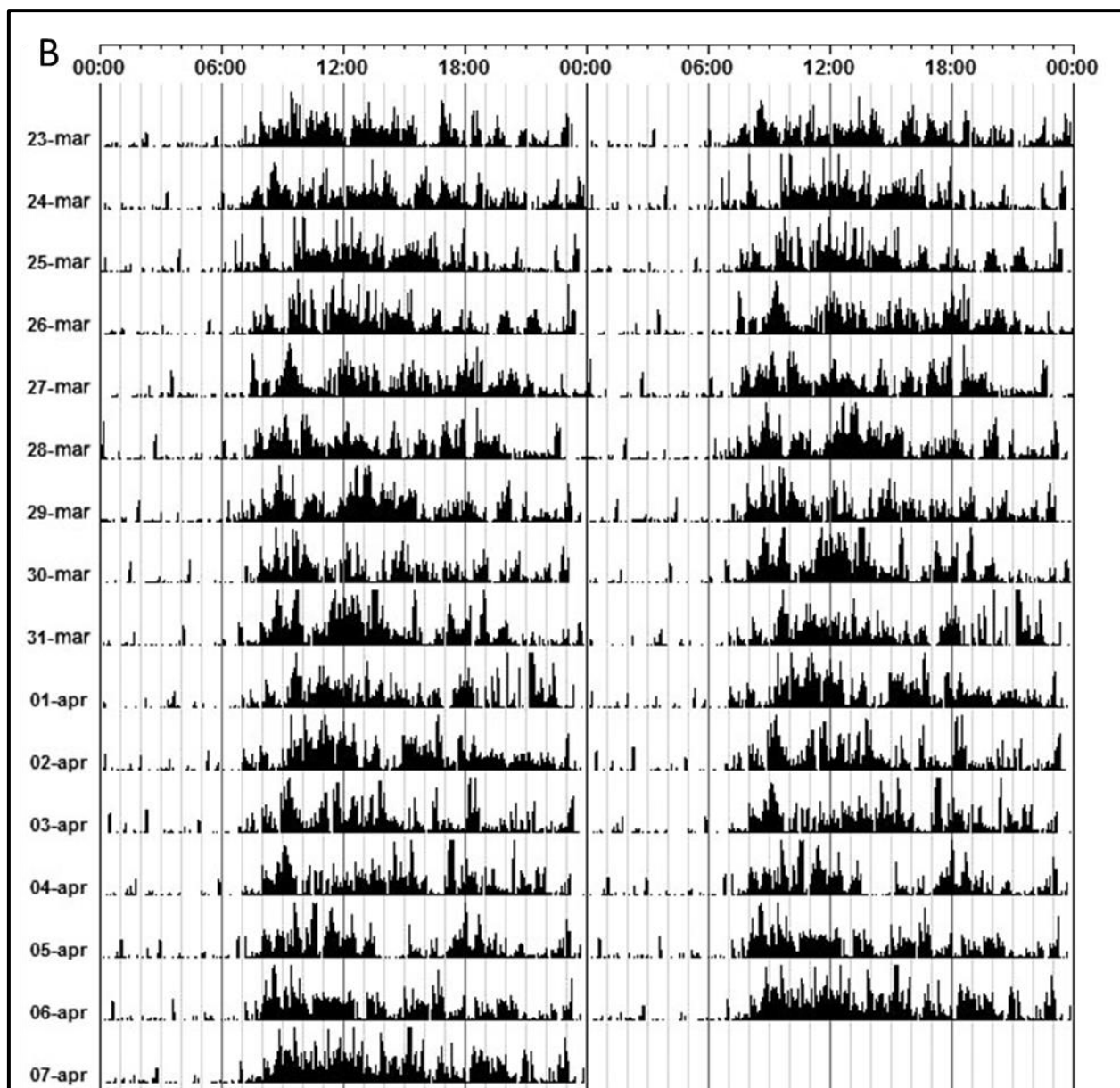
No significant differences were found for the period length of activity between all groups for either of the periodogram analyses ($p > 0.05$), Table 20 (p.141). The mild AD group had period lengths comparable to the other groups, Table 20 (p.141).

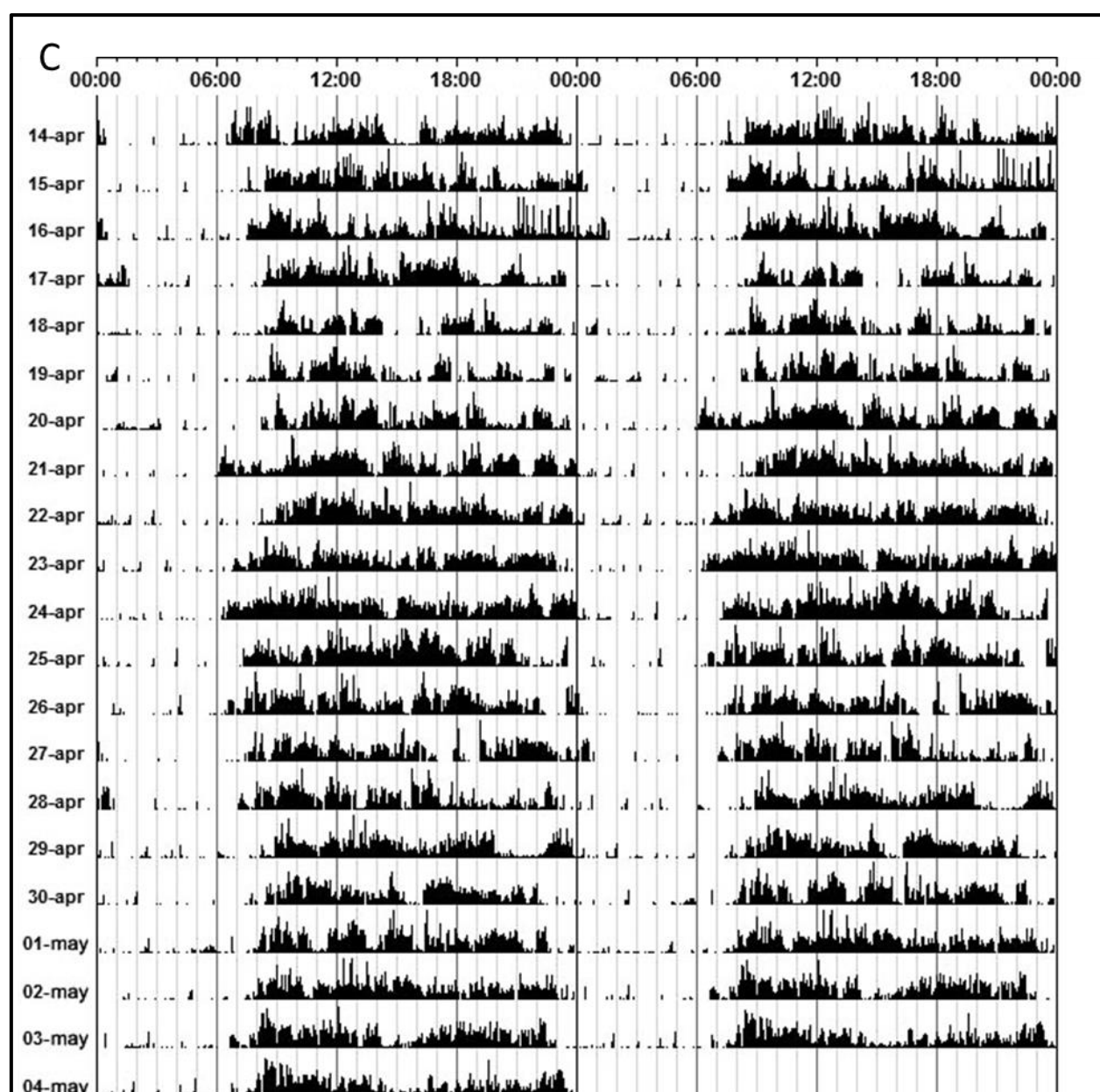
Activity counts averaged for each actigraph recording per hour over a 24h timescale were compared between controls and the other groups at baseline, shown in Figure 17 (p.142).

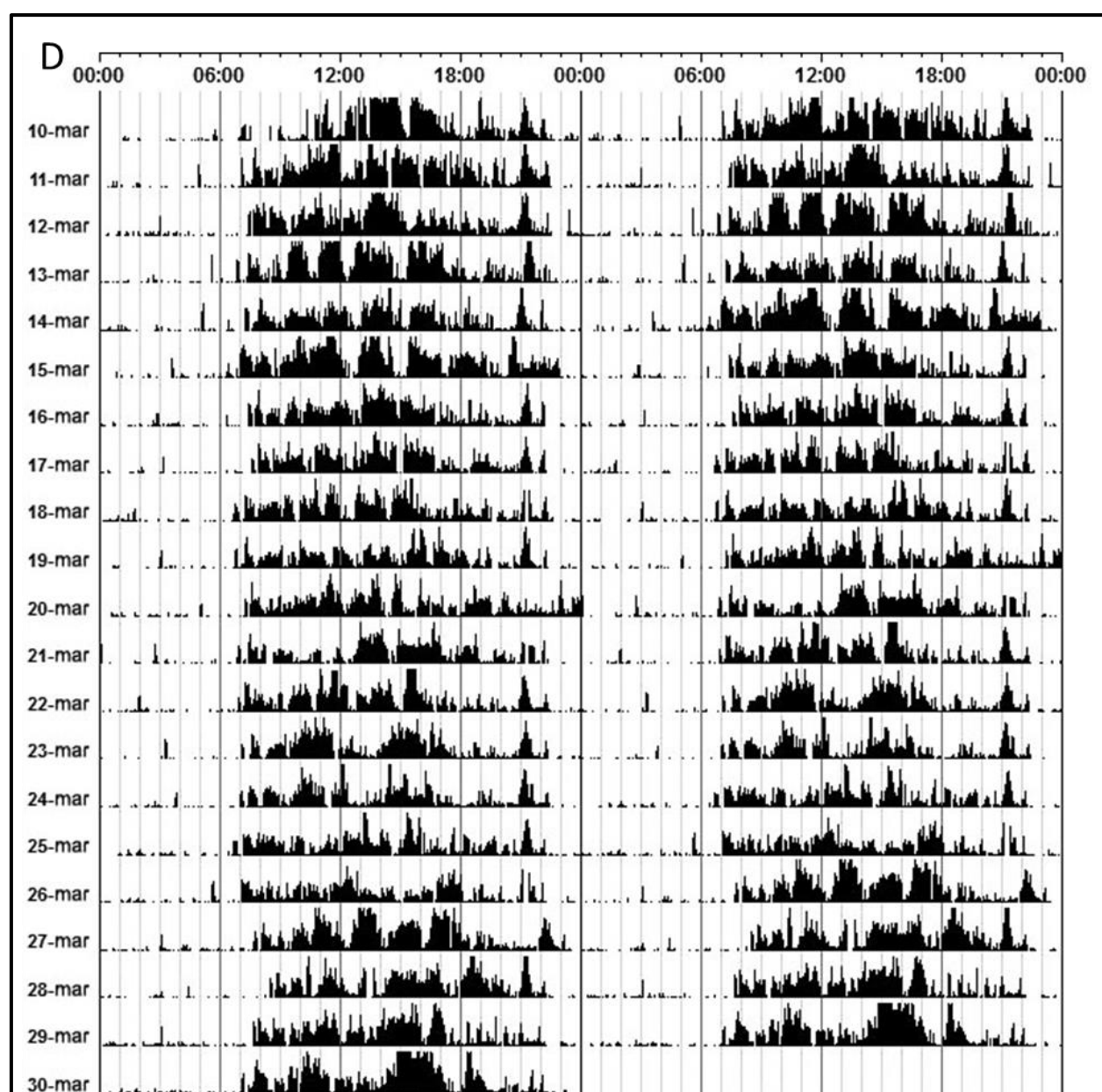
There were no significant differences between the changes over time for the NPCRA analysis for the aMCI, mAD and control groups. Despite mild AD group activity counts remaining higher than controls throughout both assessments, controls increased in average activity counts, M10 activity counts and activity amplitude whereas the mild AD group's activity in these variables diminished over time, Appendix 1.9.(p.30). Intra-daily variability (IV) was observed to decrease in controls over time in contrast to the large increase in the mild AD group over time, Appendix 1.9. (p.30).

Period length remained stable for all groups over time (Appendix 1.9., p.31). Change over time of averaged activity data are provided in Appendix 1.9. (p.31).









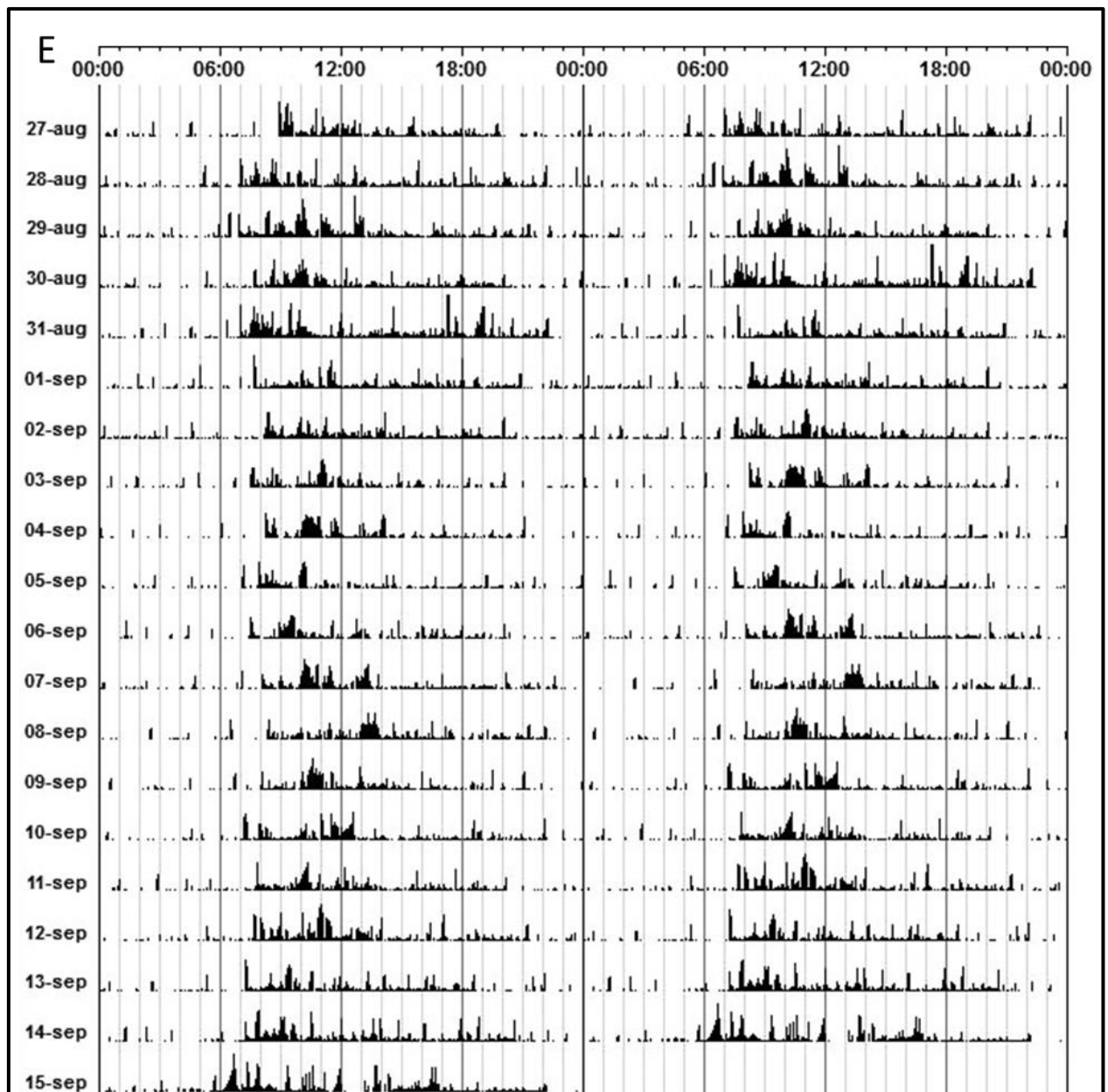


Figure 16. Exemplar baseline assessment actograms for all of the diagnostic groups. Actogram A: Control, Actogram B: SMC, Actogram C: aMCI, Actogram D: Mild AD and Actogram E: Moderate AD (cholinergic medication-naïve) individuals. Black lines indicate the level of activity. Rest-activity is double plotted to view sleep periods. Activity is scaled to 1550 activity counts.

Table 19. Parameters from a non-parametric circadian rhythm analysis (NPCRA) of rest-activity data derived from actigraphy for all groups at Baseline Assessment.

NPCRA parameter	Control (n=9)	SMC (n=5)	aMCI (n=9)	Mild AD (n=3)	mAD (n=5)
Average activity count	195.9 ± 92.6 ^{4a}	207.6 ± 48.3 ^{4b}	170.8 ± 37.7 ^{4b}	232.7 ± 98.1	94.9 ± 53.5 ^{1a, 2a, 3a}
Inter-daily stability (IS)	0.55 ± 0.15	0.62 ± 0.15	0.60 ± 0.55	0.54 ± 0.20	0.53 ± 0.18
Intra-daily variability (IV)	0.80 ± 0.27	0.65 ± 0.04 ^{3a, 4a}	0.86 ± 0.14 ^{2a, 4a}	0.88 ± 0.24	1.19 ± 0.39
Least active five hours (L5)	11.5 ± 4.4	15.9 ± 5.5	13.0 ± 6.8	16.9 ± 4.7	14.2 ± 2.9
L5-onset phase (h)	00:42 ± 00:58	00:46 ± 00:36	00:32 ± 01:05	00:14 ± 00:56	00:11 ± 01:18
Most active ten hours (M10)	338.9 ± 161.1 ^{4a}	356.0 ± 70.8 ^{4c}	302.7 ± 65.2	409.7 ± 167.1	159.4 ± 91.8 ^{1a, 2c}
M10-onset phase (h)	08:16 ± 00:52	08:29 ± 00:21	07:52 ± 00:40	09:01 ± 02:43	07:52 ± 00:54
Amplitude of activity	327.3 ± 158.1 ^{4a}	340.1 ± 66.8 ^{4c}	289.6 ± 64.9 ^{4c}	392.8 ± 162.3	145.3 ± 89.3 ^{1a, 2c}
Relative amplitude (RA)	0.93 ± 0.04 ^{4b}	0.92 ± 0.03	0.91 ± 0.04 ^{4a}	0.92 ± 0.02	0.79 ± 0.13 ^{1b}
<i>Memory impairment severity increases</i>					→

^a p<0.05, ^b p<0.01, ^c p<0.005, ^d p<0.001.

¹ group and controls, ² group and SMC, ³ group and aMCI, ⁴ group and mAD.

No statistics were computed with the Mild AD group. h represents hh:mm time format.

Data are mean ± SD. Sample sizes are as given unless in parentheses.

Full dataset, test statistics and Dunn-Šidák calculations are presented in Appendix 1.9. (p.30).

Table 20. Period length of activity rhythm calculated by two types of periodogram analyses from actigraphy data for all groups at Baseline Assessment.

Average period length	Control (n=9)	SMC (n=5)	aMCI (n=9)	Mild AD (n=3)	mAD (n=5)
Lomb-Scargle method	23.98 ± 0.04	24.01 ± 0.05	24.00 ± 0.02	24.02 ± 0.04	24.00 ± 0.02
Sokolove-Bushell method	23.96 ± 0.07	23.99 ± 0.05	23.98 ± 0.05	24.04 ± 0.03	23.93 ± 0.08
<i>Memory impairment severity increases</i>					→

No significant differences.

No statistics were computed with the Mild AD group.

Data are mean ± SD in hh:mm time format.

Full dataset and Dunn-Šidák calculations are presented in Appendix 1.9. (p.31).

4.3.4.2. Progression of circadian characteristics as memory impairment severity increases

At baseline certain trends across the progression of memory impairment severity can be observed. In terms of the NPCRA variables, the average activity count decreased as impairment severity increased, with the exceptions of the SMC and Mild AD groups (Pearson correlation, $p>0.05$). Inter-daily stability was observed to decrease (reduced stability) as memory impairment severity increased with the exception of the control group which was more stable than the Mild AD group and the mAD group, Table 19 (p.140). With the exception of the low SMC score the intra-daily variability (IV) increased as memory impairment increased (significant Pearson correlation, $p<0.05$), Table 19 (p.140). A trend in L5 onset phase was observed with the onset time becoming progressively earlier as memory impairment increased, however the control and SMC group times are comparable, Table 19 (p.140). With the exception of the mild AD group there was a general trend in M10 activity counts reducing as memory impairment severity increased (Pearson correlation, $p>0.05$), Table 19 (p.140). Relative amplitude (RA) decreased as memory impairment severity increased (Pearson correlation, $p>0.05$) with the mAD group having a lower RA than the SMC ($p>0.05$) as well as the aMCI group ($p<0.05$).

As stated previously all period lengths for both periodogram analyses for all groups were comparable and no significant correlation was found between period length and MMSE total score (Pearson correlation, $p > 0.05$).

The trends observed in the NPCRA variables of amplitude of activity and average activity counts were also present with the averaged activity over 24h profile data. As the memory impairment severity increased the amplitude decreased except for the Mild AD group which has a much higher amplitude than all other groups, Figure 17. This trend was only observed between 08:30 and 22:30 with night-time hours being comparable in activity counts between groups, Figure 17.

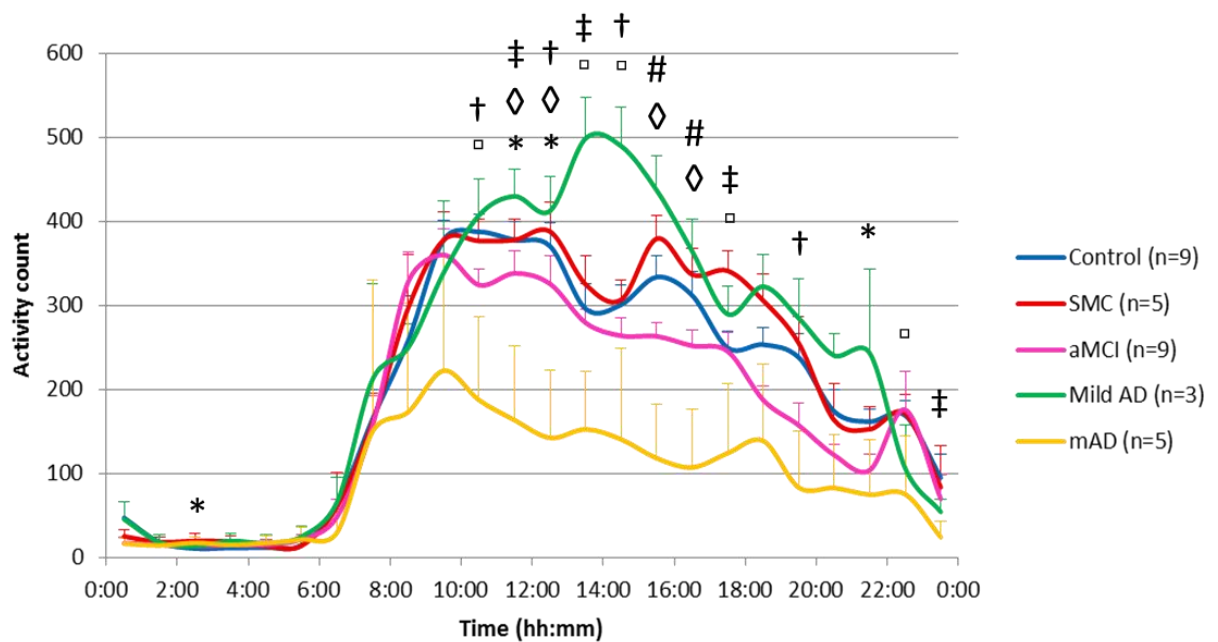


Figure 17. Averaged actigraphy data over 24h timescale for all groups at baseline assessment. Groups are as follows: controls; subjective memory complaint (SMC); amnesic mild cognitive impairment (aMCI); mild Alzheimer's disease (AD); moderate AD (mAD). Significant differences are as follows: * $p < 0.05$ between mAD and controls; □ $p < 0.05$ between mAD and aMCI groups; ◇ $p < 0.005$ between mAD and aMCI group; † $p < 0.05$ between mAD and SMC groups; ‡ $p < 0.01$ between mAD and SMC groups and # $p < 0.001$ between mAD and SMC groups. Memory impairment severity increases from top to bottom of colour key with sample sizes provided. Data is presented as mean and standard deviation error bars. Full dataset, test statistics and Dunn-Šidák correction calculation are presented in Appendix 1.9. (p.32).

4.3.5. Sleep assessment

4.3.5.1. Comparison between groups

In comparison to controls, only subjective sleep quality from the PSQI was found to be significantly different for the aMCI group with a higher score (more disturbance) than controls ($p < 0.05$), Table 21. At baseline no other groups or components were significantly different from that of the control group. For all of the components, except for daytime dysfunction, the mild AD group scored either less or the same value as the control group, Table 21. Change in PSQI component 3 (sleep duration) score was significantly different over time between the aMCI and control group, with a decrease in score for the control group and an increase in score for the aMCI group ($p < 0.05$). The mild AD group also showed an increase in component 5 (sleep disturbances) score in contrast to the slight decrease observed in controls, Appendix (p.37).

Table 21. Pittsburgh Sleep Quality Index (PSQI) component scores for all groups at Baseline Assessment.

Component score	Control (n=9)	SMC (n=5)	aMCI (n=9)	Mild AD (n=3)	qmAD (n=29)
1: Subjective sleep quality	0.3 ± 0.5^{3a}	1.0 ± 1.2	$1.0 \pm 0.5^{1a, 4a}$	0.3 ± 0.6	0.5 ± 0.7^{3a}
2: Sleep latency	0.2 ± 0.7	0.4 ± 0.5	1.2 ± 1.3	0.0 ± 0.0	0.8 ± 1.1
3: Sleep duration	$0.4 \pm 1.0^{\Delta 3a, \Delta 4a}$	0.6 ± 1.3	$0.8 \pm 1.1^{\Delta 1a, 4a}$	0.0 ± 0.0	$0.2 \pm 0.6^{3a, \Delta 1a}$
4: Habitual sleep efficiency	$0.6 \pm 1.0^{\Delta 4a}$	1.2 ± 0.8^{4a}	1.2 ± 1.3	0.3 ± 0.6	$0.5 \pm 0.9^{2a, \Delta 1a}$
5: Sleep disturbances	1.2 ± 0.4	1.2 ± 0.4	1.2 ± 0.4	0.0 ± 0.0	1.1 ± 0.4
6: Use of sleep medication	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.8
7: Daytime dysfunction	0.2 ± 0.4	0.2 ± 0.4	0.6 ± 0.5	0.3 ± 0.6	0.7 ± 0.7
Global PSQI score	3.0 ± 2.4^{3a}	4.6 ± 3.0	6.0 ± 3.8^{1a}	2.0 ± 1.0	4.0 ± 3.2
Memory impairment severity increases					➔

^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.005$, ^d $p < 0.001$. ¹ group and controls, ² group and SMC, ³ group and aMCI, ⁴ group and qmAD. No statistics were computed with the Mild AD group. Data are mean $\pm$ SD. Full dataset, test statistics and Dunn-Šidák calculations are presented in Appendix 1.7 (p.).

When comparing the Jupiter sleep questionnaire (JSQ) variables to controls at baseline, the aMCI group woke up significantly more times ($p < 0.05$), Table 22 (p.145). The qmAD group had significantly larger minimum amount of hours sleep ($p < 0.005$), more hours of sleep per night ($p < 0.05$), and earlier bedtimes on weekdays and at the weekend ($p < 0.05$ for both). The mild AD group had earlier bedtimes, more hours of sleep, woke more during the night, less duration of wakefulness in the night and napped more during the day than controls, Table 22 (p.145).

Over time a trend was observed in JSQ variables for the aMCI group in comparison to controls, with the aMCI group having an decrease in the number of hours of sleep per night in comparison to an increase for controls ($p > 0.05$, Appendix 1.11. (p.38). The same trend was observed with the usual awakening time becoming earlier in the aMCI group and becoming later in the control group ($p > 0.05$, Appendix, p.38). The mild AD group decreased in the number of times woken in the night, whereas controls remained stable, Appendix (p.38). The mild AD group increased in the number of hours they remained in bed after waking, with the control group decreasing, Appendix (p.38).

Table 22. Jupiter sleep questionnaire (JSQ) sleep characteristics of all groups for Baseline Assessment.

Parameter	Control (n=9)	SMC (n=5)	aMCI (n=9)	Mild AD (n=3)	qmAD (n=29)
Bedtime (weekdays)	22:53 ± 00:37 ^{4a}	22:51 ± 00:13	22:31 ± 00:29	22:00 ± 00:30	22:09 ± 00:56 ^{1a}
Bedtime (weekend)	22:53 ± 00:37 ^{4a}	22:51 ± 00:13	22:31 ± 00:29	22:00 ± 00:30	22:18 ± 00:55 ^{1a}
Earliest bedtime	22:06 ± 01:01	22:15 ± 00:42	21:23 ± 00:47	21:40 ± 00:46	21:29 ± 01:23
Latest bedtime	00:16 ± 01:43	23:54 ± 00:23 ^{4a}	23:41 ± 00:44	23:30 ± 00:30	23:16 ± 01:15 ^{2a}
Usual wake up time	06:51 ± 00:44 _{Δ3a}	06:52 ± 00:33	06:38 ± 01:02 _{Δ1a, 4a}	07:05 ± 00:53	07:20 ± 00:52 ^{3a}
Earliest wake up time	06:05 ± 00:50	06:19 ± 00:34	05:51 ± 01:29 ^{4b}	06:13 ± 00:42	07:01 ± 00:52 ^{3b}
Latest wake up time	08:05 ± 01:19	07:32 ± 00:53	07:43 ± 01:06	07:35 ± 01:23	08:24 ± 01:27
Hrs. of sleep per night	07:09 ± 01:45 ^{4a, Δ3a, Δ4a}	07:01 ± 01:15	07:10 ± 01:28 ^{Δ1a, 4a}	08:47 ± 00:32	08:36 ± 01:42 ^{1a, 3a, Δ1a}
Min. amount of sleep	04:59 ± 02:35 ^{4c, Δ4a}	05:25 ± 00:54	05:14 ± 01:58 ^{4a}	06:25 ± 00:43	07:09 ± 01:52 ^{1c, 3a, Δ1a}
Max. amount of sleep	09:09 ± 02:06	08:17 ± 01:49	09:24 ± 02:00	09:37 ± 00:48	10:19 ± 02:16
No. of times woke in night	0.9 ± 0.4 ^{2a, 3a}	1.9 ± 0.9 ^{1a}	1.9 ± 0.9 ^{1a}	1.8 ± 0.8	1.6 ± 1.1
Longest period of wakefulness	00:37 ± 01:17	00:40 ± 00:50 ^{4a}	00:20 ± 00:38	00:05 ± 00:00	00:07 ± 00:16 ^{2a}
Total wake time per night	00:38 ± 01:17	00:44 ± 00:47 ^{4a}	00:25 ± 00:36	00:09 ± 00:03	00:14 ± 00:40 ^{2a}
Hrs. stay in bed after waking	00:34 ± 00:34	00:45 ± 00:49	01:04 ± 00:39	00:36 ± 00:47	00:43 ± 00:39
No. of naps per week	2.7 ± 3.0	2.5 ± 2.5	4.1 ± 3.4	3.5 ± 5.0	9.4 ± 8.2 (27)
<i>Memory impairment severity increases</i>					→

^a p<0.05, ^b p<0.01, ^c p<0.005, ^d p<0.001.

¹ group and controls, ² group and SMC, ³ group and aMCI, ⁴ group and qmAD.

No statistics were computed with the Mild AD group. Data are presented as hh:mm time format.

Data are mean ± SD. Sample sizes are as given unless in parentheses.

Full dataset, test statistics and Dunn-Šidák calculations are presented in Appendix 1.11. (p.38).

From the sleep analysis of the actigraphy data, no significant differences were found between the control group and the aMCI group for any component ($p>0.05$), Table 23 (p.147). The mAD group in contrast had a significantly longer time in bed ($p<0.05$), more moving minutes ($p<0.05$), larger number of one minute immobilities ($p<0.05$), a higher percentage of one minute immobilities ($p<0.05$) and a higher fragmentation index ($p<0.05$) in comparison to controls. The mild AD group had a higher mean activity score in the sleep episode, reduced sleep efficiency, more moving minutes, more one minute immobilities and higher fragmentation index than the control group, Table 23 (p.147).

The number of immobile minutes increased over time for the mild AD group in comparison to the decrease observed in the control group ($p>0.05$), Appendix 1.11. (p.47). Fragmentation index decreased over time for the mild AD group, in comparison to the minimal increase in the control group ($p>0.05$), Appendix (p.47).

Table 23. Selected sleep analysis parameters derived from actigraphy for all groups at Baseline Assessment.

Parameter	Control (n=9)	SMC (n=5)	aMCI (n=9)	Mild AD (n=3)	mAD (n=5)
Bed time	23:35 ± 00:50 ^{4a}	23:26 ± 00:20 ^{4a}	23:12 ± 00:37 ^{4a}	23:10 ± 00:51	22:14 ± 00:52 ^{1a,} 2a, 3a
Get up time	07:14 ± 00:38	06:48 ± 00:10	07:03 ± 00:36	07:13 ± 01:17	07:48 ± 01:51
Time in bed	07:39 ± 00:37 ^{4a}	07:21 ± 00:15	07:50 ± 00:40 ^{4a}	08:03 ± 00:50	09:34 ± 02:12 ^{1a,} 3a
Actual wake time	00:40 ± 00:19	00:51 00:13	00:43 ± 00:18	01:01 ± 00:20	00:59 ± 00:09
Mean activity score	10.5 ± 4.6	14.7 ± 4.9	12.1 ± 5.3	15.3 ± 4.7	14.1 ± 3.4
Average wake movement	279.2 ± 134.6	285.7 ± 63.9 ^{4b,} Δ4a	241.8 ± 51.8 ^{4b}	328.3 ± 129.9	136.8 ± 72.7 ^{2b,} 3b, Δ2a
Total activity score	4904.1 ± 2218.9 ^{4a}	6427.9 ± 1996.8	5818.2 ± 2904.4	7252.9 ± 1776.9	7749.5 ± 1232.6 ^{1a}
Sleep efficiency (%)	90.0 ± 4.3	86.7 ± 3.6	90.0 ± 3.4	86.0 ± 4.4	87.8 ± 1.4
Sleep latency	00:05 ± 00:04	00:06 ± 00:03	00:03 ± 00:01	00:06 ± 00:03	00:09 ± 00:08
Mean no. of sleep bouts	21.0 ± 9.4	24.4 ± 4.1	19.2 ± 5.9	27.6 ± 7.4	24.4 ± 5.0
Wake bouts	20.7 ± 9.4	24.0 ± 4.1	18.8 ± 5.9	27.5 ± 7.7	24.2 ± 5.1
Immobile minutes	398.7 ± 35.6	361.5 ± 28.5 ^{4a}	399.6 ± 26.0 ^{4a}	379.0 ± 31.4	469.2 ± 89.9 ^{2a,} 3a
No. of moving minutes	55.4 ± 23.6 ^{4a}	73.6 ± 20.2	67.1 ± 26.3	97.7 ± 24.9	95.0 ± 41.5 ^{1a}
Moving time (%)	11.9 ± 5.0	17.0 ± 4.9	14.0 ± 4.6	20.4 ± 3.5	16.4 ± 3.9
One Minute immobility	5.6 ± 3.9 ^{4a}	10.4 ± 5.1	7.4 ± 3.7	14.1 ± 4.7	11.8 ± 6.8 ^{1a}
One Min immobility (%)	13.6 ± 4.8 ^{4a}	21.6 ± 6.3	17.9 ± 5.5	26.0 ± 7.2	21.1 ± 4.9 ^{1a}
Fragmentation index	25.8 ± 9.6 ^{4a}	38.6 ± 10.9	31.9 ± 9.8	46.3 ± 10.7	37.5 ± 8.7 ^{1a}
<i>Memory impairment severity increases</i>					➔

^a p<0.05, ^b p<0.01, ^c p<0.005, ^d p<0.001.

¹ group and controls, ² group and SMC, ³ group and aMCI, ⁴ group and mAD.

No statistics were computed with the Mild AD group. Time data are presented as hh:mm time format.

Data are mean ± SD. Sample sizes are as given unless in parentheses.

Full dataset, test statistics and Dunn-Šidák calculations are presented in Appendix 1.11. (p.47).

Statistical analysis was only conducted on the EEG data from the control and aMCI groups due to group sample sizes. The aMCI group at baseline had significantly shorted sleep duration ($p<0.05$), reduced REM sleep duration ($p<0.005$), reduced REM sleep percentage ($p<0.05$), shortened stage 1 sleep duration ($p<0.05$) and percentage ($p<0.05$) than controls. In addition the aMCI group had significantly reduced sleep efficiency ($p<0.001$), movement duration ($p<0.001$) as well as a longer duration of wake after sleep onset ($p<0.001$, all significant after Dunn-Šidák correction). In comparison to controls, the mild AD group had less REM sleep, lower sleep efficiency, less stage 3 sleep, less movement duration and more wake after sleep onset, Table 24 (p.150). The mAD individual spent longer time in bed, had longer sleep duration, less REM sleep duration and percentage, reduced sleep efficiency, less stage 2 sleep and more wake after sleep onset than controls, Table 24 (p.150).

In the first third of the night the aMCI group had significantly lower sleep duration ($p<0.05$), higher wake duration ($p<0.005$), decreased sleep efficiency ($p<0.005$) and lower movement duration ($p<0.005$) than controls, Figure 18 (p.152). The mild AD group had shorter sleep duration, longer wake duration, less movement duration, less REM sleep and lower sleep efficiency than the control group, Figure 18 (p.152). The mAD individual had longer sleep duration, longer wake duration, less movement duration, longer stage 3 and 4 sleep durations, less REM sleep, longer REM sleep latency, reduced sleep efficiency and increased number of sleep stage changes in comparison to controls, Figure 18 (p.152).

In the second third of the night the aMCI group had significantly lower sleep duration ($p<0.05$), lower wake duration ($p<0.01$), reduced sleep efficiency ($p<0.01$) and shorter movement duration ($p<0.001$, significant after Dunn-Šidák correction) in comparison to controls. The mild AD group had shorter sleep duration, longer wake duration, less movement duration, reduced stage 3 sleep, a longer REM latency, reduced sleep efficiency and less sleep

stage changes in comparison to controls, Figure 18 (p.152). The mAD individual had longer sleep duration, less movement duration, more stage 3 and 4 sleep, more REM sleep and more sleep stage changes than the control groups, Figure 18 (p.152).

No statistically significant differences were found between the changes in EEG scoring over time of the aMCI and control groups, Appendix 1.11. (p.52). The mild AD group had an increase over time of time in bed in contrast to the minimal increase of the control group, Appendix (p.37). Stage 3 sleep duration was observed to increase in the sleep of the mild AD group over time, but remained stable in the control group, Appendix (p.52).

Table 24. EEG staging parameters for all groups at baseline assessment.

EEG scoring parameter	Control group (n=7)	SMC group (n=3)	aMCI group (n=6)	Mild AD group (n=3)	mAD group (n=1)
Time in bed	08:04 ± 00:37	07:18 ± 00:13	07:58 ± 01:17	07:49 ± 00:24	09:24
Sleep duration	07:44 ± 00:43 ^{3a}	06:57 ± 00:07	06:34 ± 00:57 ^{1a}	06:43 ± 00:24	08:38
Wake duration	00:12 ± 00:07 ^{3d}	00:15 ± 00:07	01:24 ± 00:36 ^{1d}	01:05 ± 00:44	00:45
Wake duration (%)	2.4 ± 1.4 ^{3d}	3.4 ± 1.6	17.2 ± 6.2 ^{1d}	13.6 ± 8.4	8.0
REM duration	01:45 ± 00:21 ^{3c}	01:38 ± 00:12	01:05 ± 00:16 ^{1c}	01:07 ± 00:49	01:24
REM sleep (%)	21.7 ± 4.3 ^{3a}	22.5 ± 3.0	14.0 ± 4.9 ^{1a}	14.2 ± 10.5	14.9
REM latency	01:10 ± 01:01	00:59 ± 00:04	01:08 ± 00:42	01:49 ± 01:02	01:23
NREM latency	00:03 ± 00:02	00:05 ± 00:03	00:06 ± 00:04	00:04 ± 00:05	00:08
Deep sleep latency (NREM stages 3 and 4)	00:18 ± 00:10	00:11 ± 00:06	00:40 ± 00:35	00:24 ± 00:11	00:20
Sleep efficiency (%)	95.9 ± 1.8 ^{3d}	95.3 ± 2.1	82.6 ± 6.0 ^{1d}	86.1 ± 8.3	91.8
Stage 1 duration	01:12 ± 00:23 ^{3a}	00:41 ± 00:41	00:44 ± 00:17 ^{1a}	01:14 ± 00:53	01:42
Stage 1 sleep (%)	14.8 ± 4.2 ^{3a}	9.2 ± 9.1	9.2 ± 3.9 ^{1a}	16.0 ± 12.0	18.1
Stage 2 duration	03:13 ± 00:50	02:41 ± 00:31	03:14 ± 00:30	03:25 ± 00:29	02:46
Stage 2 sleep (%)	39.8 ± 9.0	36.8 ± 6.6	41.6 ± 9.5	43.8 ± 7.2	29.4
Stage 3 duration	01:26 ± 00:46	01:42 ± 00:40	00:58 ± 00:29	00:53 ± 00:12	02:27
Stage 3 sleep (%)	17.8 ± 9.2	23.4 ± 9.8	11.8 ± 5.0	11.3 ± 2.4	26.1
Stage 4 duration	00:08 ± 00:11	00:15 ± 00:08	00:34 ± 00:48	00:04 ± 00:04	00:19
Stage 4 sleep (%)	1.7 ± 2.4	3.5 ± 1.8	6.0 ± 8.4	0.9 ± 0.8	3.4
Movement duration	00:08 ± 00:03 ^{3d}	00:06 ± 00:04	00:01 ± 00:02 ^{1d}	00:01 ± 00:02	00:01
Movement (%)	1.7 ± 0.9 ^{3c}	1.2 ± 0.8	0.2 ± 0.3 ^{1c}	0.3 ± 0.5	0.2
Wake after sleep onset	00:08 ± 00:05 ^{3d}	00:10 ± 00:04	01:15 ± 00:38 ^{1d}	00:58 ± 00:33	00:36
<i>Memory impairment severity increases</i>					→

^a p<0.05, ^b p<0.01, ^c p<0.005, ^d p<0.001.

¹ group and controls, ² group and SMC, ³ group and aMCI.

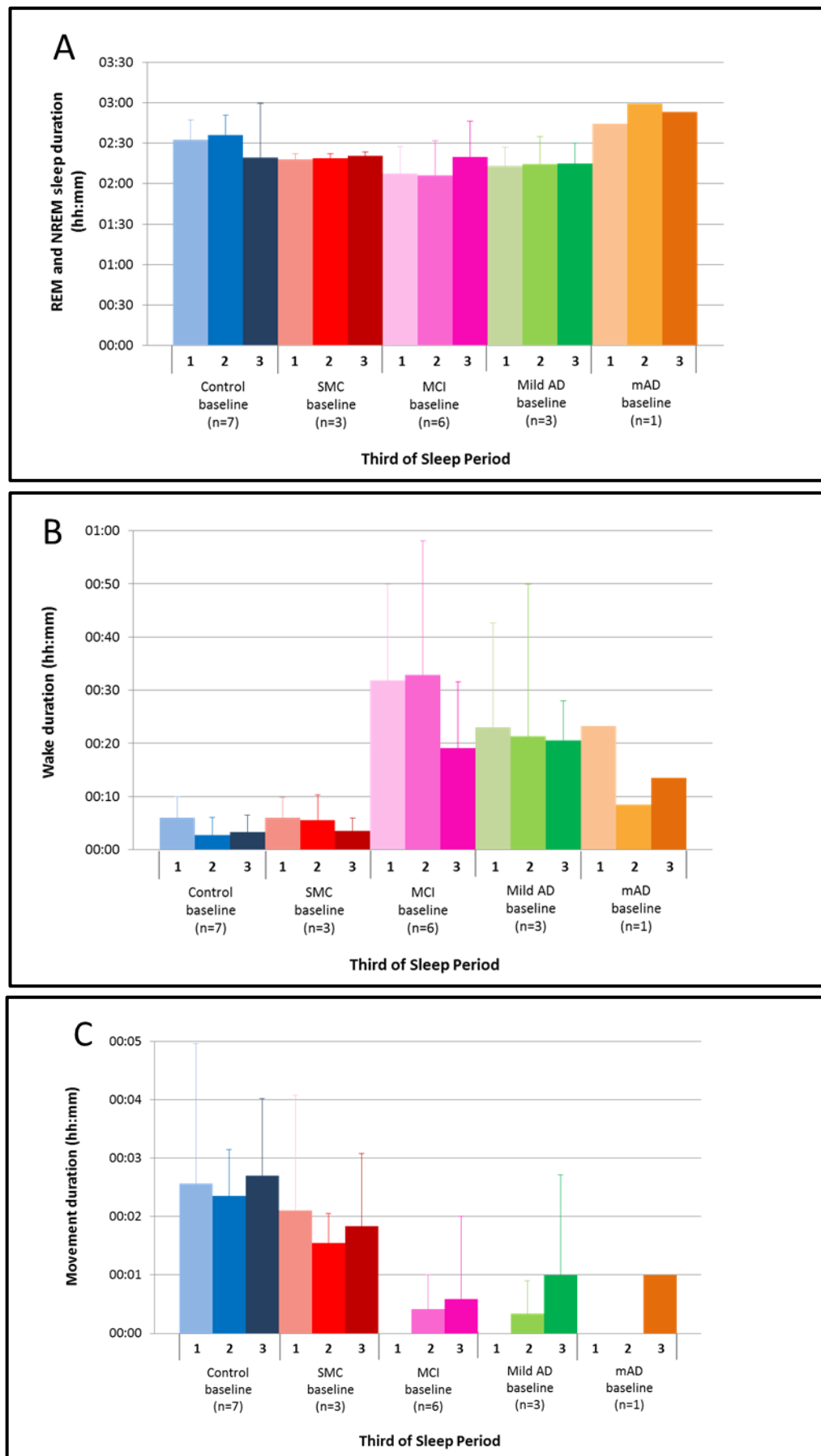
No statistics were computed with the Mild AD or mAD groups. % indicates % of Time in bed.

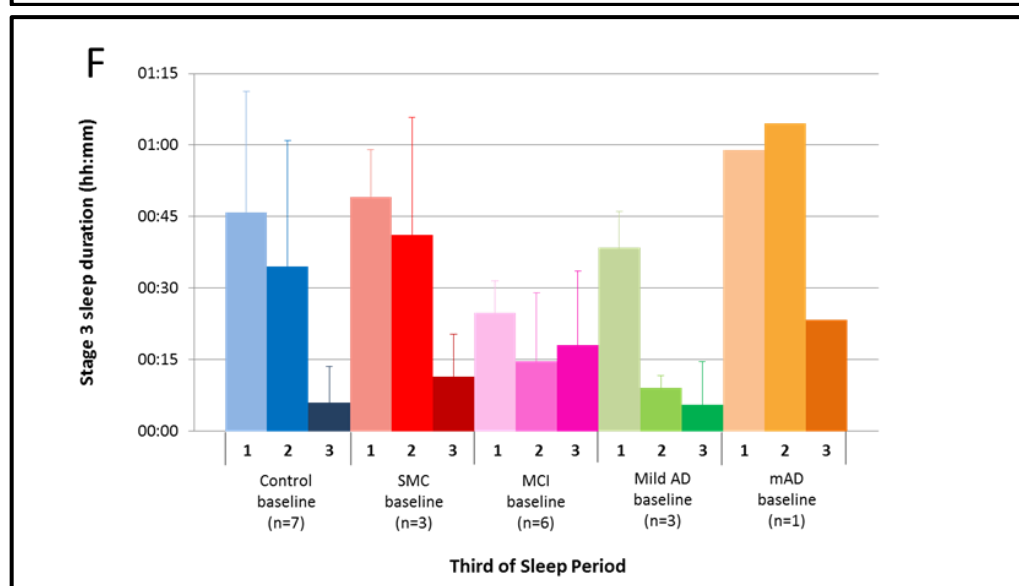
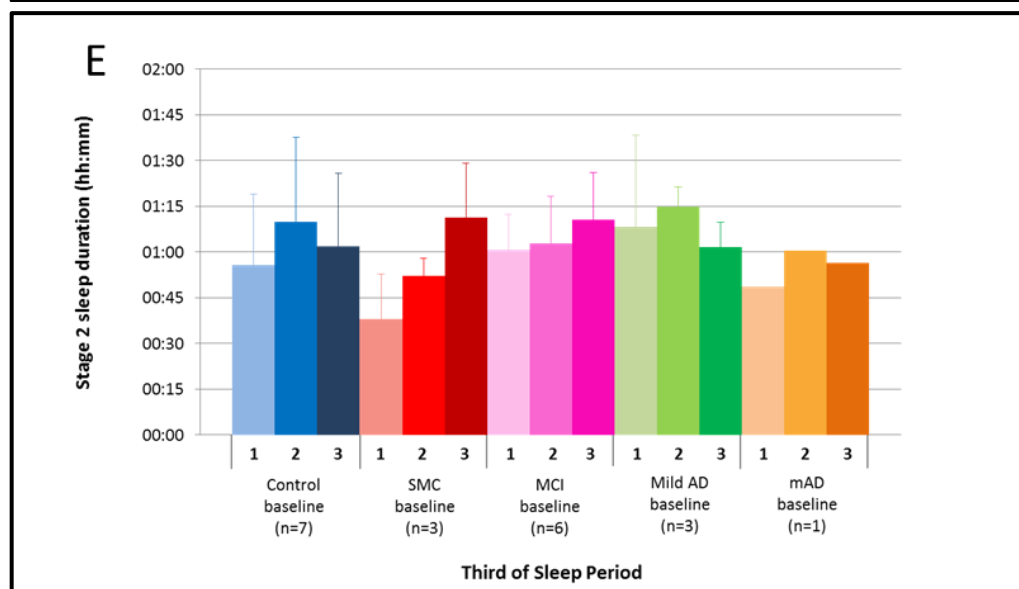
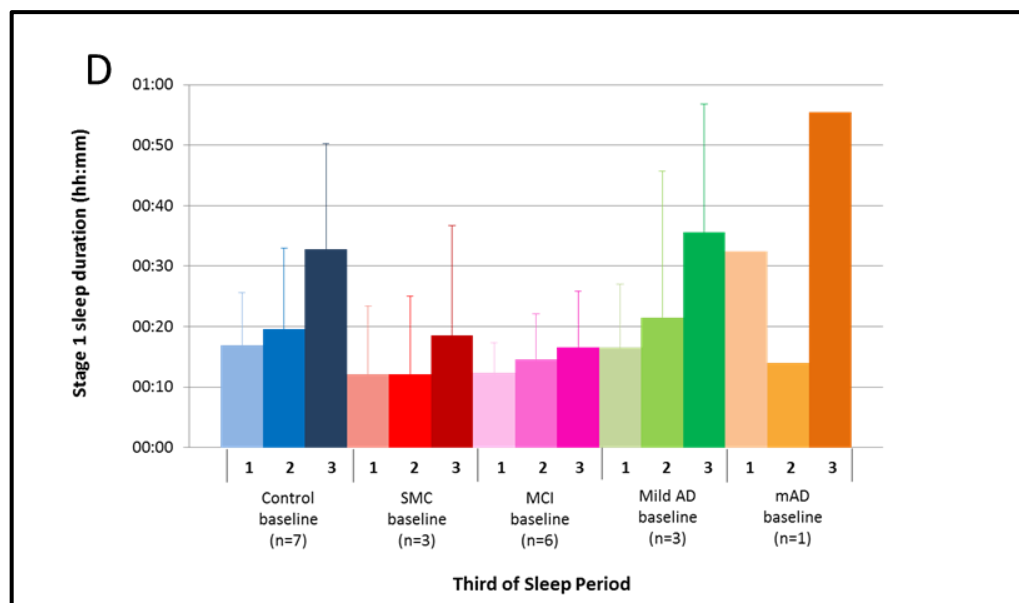
Data are mean ± SD. Time data are presented as hh:mm time format.

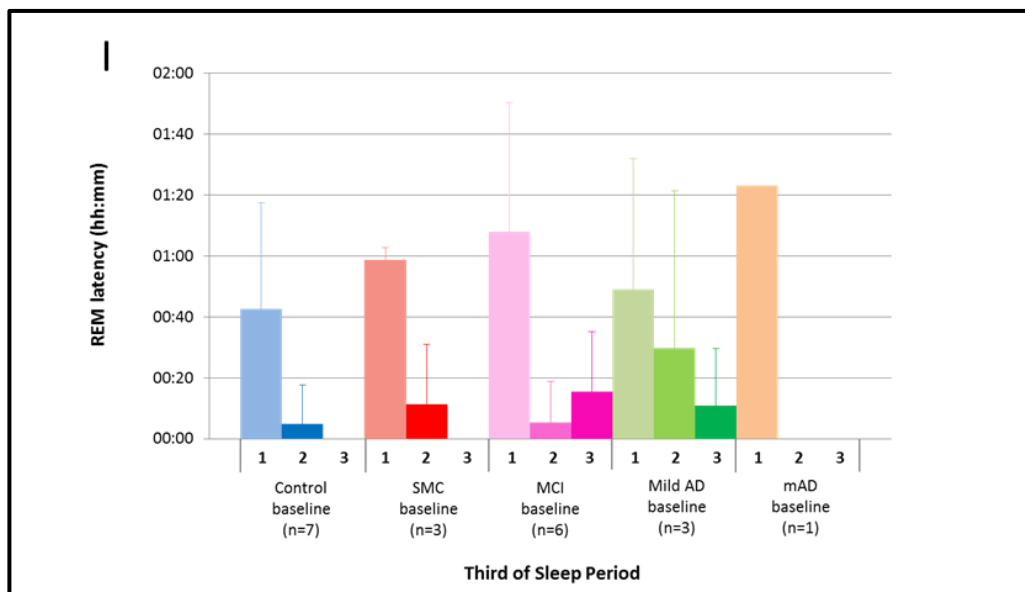
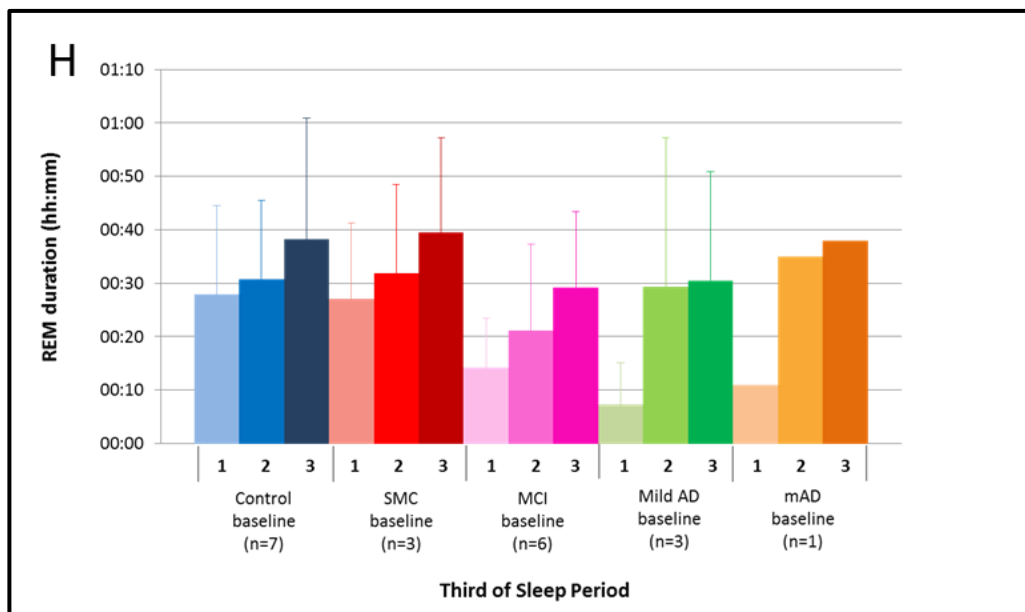
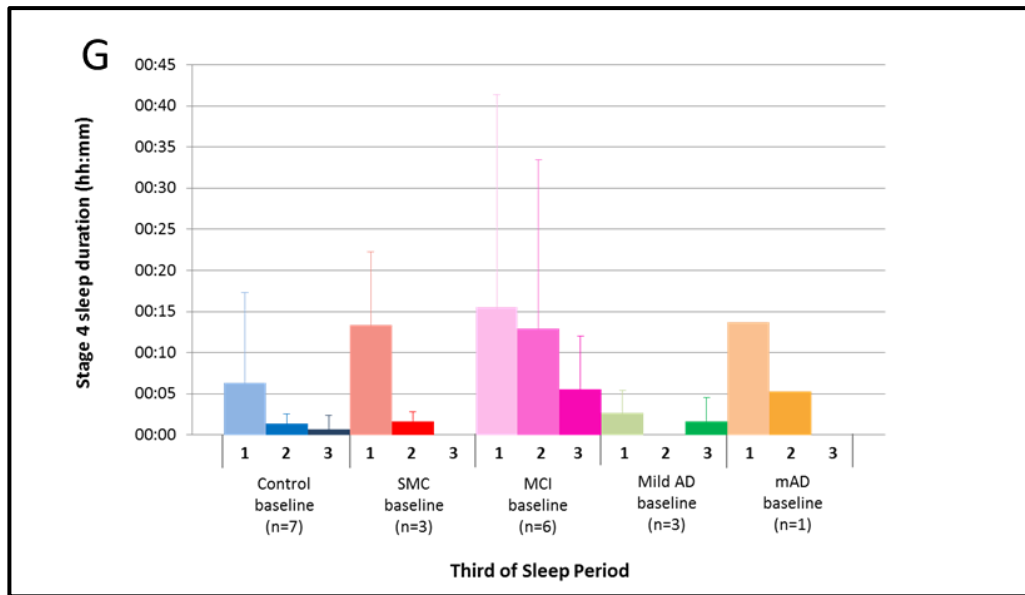
Full dataset, test statistics and Dunn-Šidák calculations are presented in Appendix 1.11. (p.52).

When the EEG recordings were divided into thirds, in the final third of the night, the aMCI group had a longer wake duration ($p < 0.01$), reduced movement duration ($p < 0.05$), reduced sleep efficiency ($p < 0.05$), a higher number of sleep stage changes ($p < 0.01$) and a longer REM sleep latency ($p < 0.05$) in comparison to controls. The mild AD group had a longer wake duration, reduced movement duration, reduced REM sleep duration, a longer REM sleep latency and reduced sleep efficiency in comparison to controls, Figure 18 (p.152). The mAD individual had longer sleep and wake duration, reduced movement duration, increased stage 1 and 3 sleep and increased number of sleep stage changes in comparison to controls, Figure 18 (p.152).

Few differences were evident in terms of sleep scoring across the three thirds of the night when assessed for change over time ($p > 0.05$). In the final third of the night, stage 3 duration over time significantly decreased in the aMCI group in comparison to the increase observed in the control group, Appendix 1.11.(p.55). In the second third of the night, the number of sleep stage changes increased over time for the mild AD group, in comparison to the decrease observed over time in the control group, Appendix (p.55). In the final third of the night, the mild AD group had a decrease in REM sleep duration compared to the increase in that of the control group over time, Appendix (p.55). The stage 3 sleep duration decreased by half in the mild AD group over time, but the control group increased over time, Appendix (p.37). The sleep efficiency of the mild AD group reduced over time whereas that of the control group increased over time, Appendix (p.55). The number of the sleep stage changes decreased over time for the mild AD group, however, the control group increased in the number of sleep stage changes over time, Appendix (p.55).







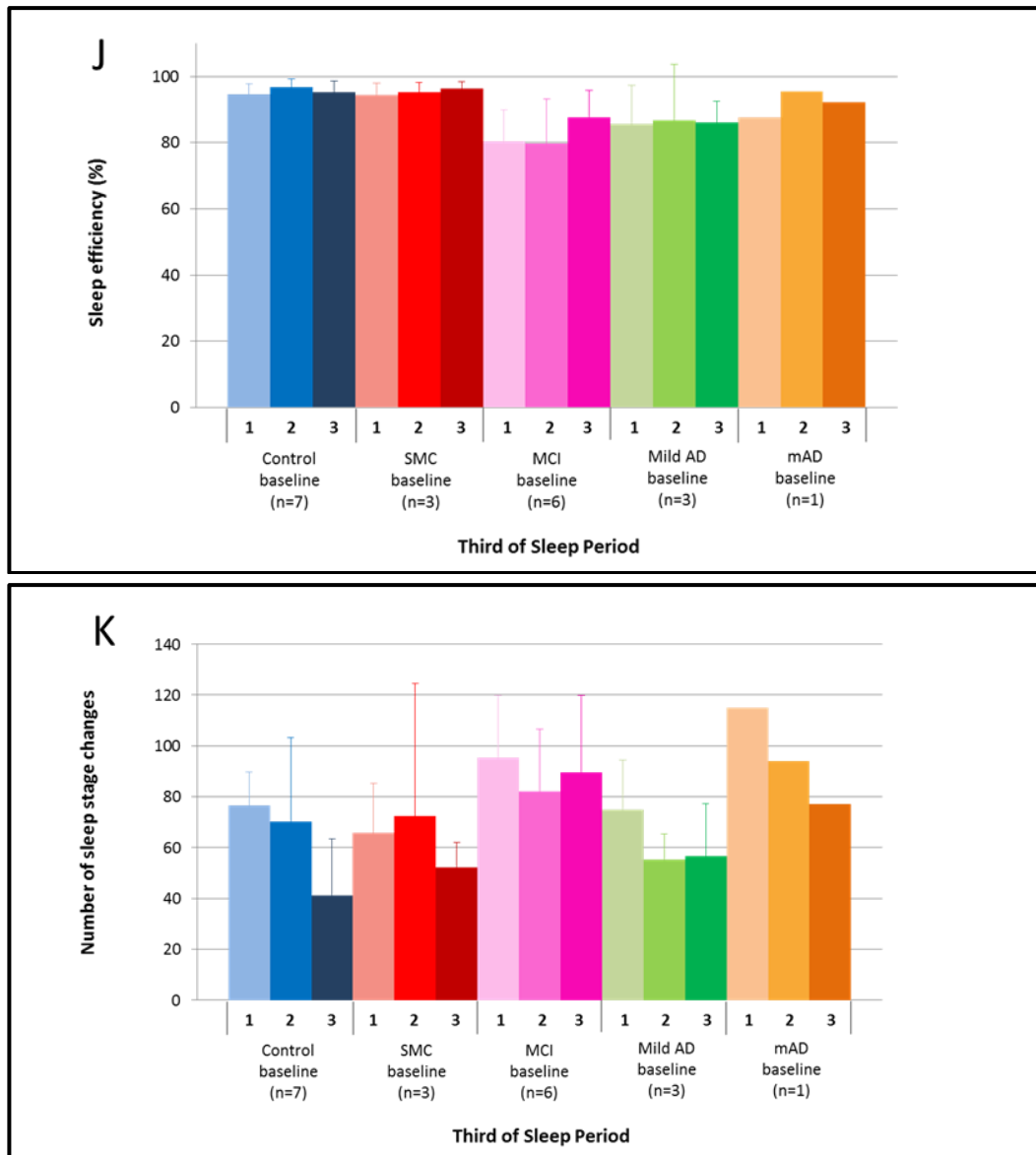


Figure 18. Sleep staging parameters for each third of the night for each group at baseline assessment. All parameters for both assessments can be found in Appendix 1.11.(p.55). Groups are as follows: controls; subjective memory complaint (SMC); amnesic mild cognitive impairment (MCI); mild Alzheimer's disease (AD); moderate AD (mAD). Memory impairment severity increases from left to right. For all statistical analyses Dunn-Šidák (D-S) correction was calculated to determine significance level (39 variables) $p=0.001314$. All data are presented as mean with standard deviation error bars. Sample sizes are provided in brackets on the x-axis of each graph. Graphs are as follows: A=REM and Non-REM sleep duration; B=Wake duration; C=Movement duration; D=Stage 1 sleep duration; E=Stage 2 sleep duration; F=Stage 3 sleep duration; G=Stage 4 sleep duration; H=REM sleep duration; I=REM latency; J=Sleep efficiency percentage; and K=Number of sleep stage changes.

4.3.5.2. Progression of sleep characteristics as memory impairment severity increases

When considering the PSQI variables, the only significant correlation between memory impairment severity (MMSE total score) and a component score was that of component 6 (sleep medication use) which was found only in the qmAD group (Pearson correlation, $p < 0.01$).

The JSQ indicated more trends in sleep characteristics that were progressively altered as memory impairment severity increased. The number of hours of sleep per night significantly correlated with MMSE total score, with increasing duration observed as memory deficits increased (Pearson correlation, $p < 0.05$). The same direction of trend was found in the minimum amount of sleep ($p < 0.001$, significant after Dunn-Šidák correction) and maximum amount of sleep ($p < 0.005$) increasing with increasing memory impairment severity. The number of naps significantly increased as memory impairment severity increased ($p < 0.05$). The latest bedtime became earlier as memory deficit increased ($p < 0.05$) and usual wake up time became later as memory deficit increased ($p < 0.005$). Earliest ($p < 0.001$) and latest ($p < 0.005$, both significant after Dunn-Šidák correction) awakening times also became significantly later as memory deficit increased.

From the sleep analysis conducted on the actigraphy data, bedtime was found to become significantly earlier as memory deficit increased (Pearson correlation, $p < 0.05$) as did sleep start time ($p < 0.05$). Time in bed significantly increased in duration as memory impairment severity increased ($p < 0.005$) as did assumed sleep time ($p < 0.005$) and actual sleep time ($p < 0.01$). The number of immobile minutes increased as memory deficit increased ($p < 0.05$). Although a clear progressive trend in one minute immobility percentage and fragmentation index was not present, all groups had higher scores than the control group, Table x (p.).

Increasing memory impairment severity was found to be correlated with EEG scoring parameters in terms of increasing sleep duration ($p < 0.05$) and reduced movement duration

($p < 0.05$) (with all groups combined and MMSE total scores compared to EEG scoring parameters). Furthermore, increasing memory deficits correlated with increasing wake duration ($p < 0.001$) and percentage ($p < 0.001$), decreasing REM sleep duration ($p < 0.001$) and percentage ($p < 0.001$), decreasing sleep efficiency ($p < 0.001$) and increasing wake after sleep onset ($p < 0.001$, all significant after Dunn-Šidák correction).

Few variables of timing of sleep stages in the night (through analysis of each third of the sleep episode) were found to change according to memory impairment severity. REM sleep duration was found to increase progressively through the night except for the mild AD and mAD groups which appeared to have almost equal REM sleep durations for both the second and final thirds of the night. This finding, along with an earlier REM latency observed in the mAD group for the second third of the night, indicate earlier REM sleep for the mAD group.

4.4. Discussion

4.4.1. Alzheimer's disease and mild cognitive impairment in comparison to controls

Sleep and rest-activity differences were observed between the prodromal stages and two severities of Alzheimer's disease. Progressive changes in sleep and rest-activity characteristics were observed with increasing memory impairment severity. These findings indicate that certain sleep or rest-activity characteristics could be predictive of future cognitive decline.

In terms of demographics and clinical history, there were no differences in age or sex for the amnesic mild cognitive impairment (aMCI), mild Alzheimer's disease (AD) or the moderate AD group (qmAD) in comparison to controls. Diurnal preference was found not to be different from that of the controls with all types represented, except for extremely evening types. This finding is in-line with current knowledge of diurnal preference becoming earlier as ageing progresses [23]. Caffeine intake frequency was found not to differ between groups indicating that any sleep differences between groups were probably not due to differing caffeine intake. Furthermore, as groups were not different in the use of hypnotics (apart from the qmAD individual), these are unlikely to be the cause of the reported sleep disturbances.

4.4.1.1. Assessment of memory

Memory assessment confirmed that the aMCI, mild AD and moderate AD groups had significantly more memory deficits than controls. In particular orientation, recall and total scores on the Mini-mental state examination (MMSE) were determined to be lower than controls for all of the above groups. Spatial span length was a good CANTAB battery indicator of memory deficit in aMCI in comparison to controls, with reaction times on most tests, reduced ability on the paired-associates learning (PAL) test, a visual short term memory test, and

reduced ability to name objects (GNT test) differentiating between AD patients of both levels of severity and the aMCI and control groups. These results are in-keeping with working memory deficits previously observed in aMCI individuals [151] and visual memory [70] and word naming deficits [152] found in Alzheimer's disease.

4.4.1.2. Assessment of functioning

No differences were found between the aMCI group and the controls in terms of functioning for either the ADCS-ADL or the SFS. This confirms diagnosis of these individuals as having a memory impairment with no functioning deficit [153]. Basic and instrumental activities of daily living (ADLs) were observed to be reduced for the moderate AD patients, as were independence-performance, recreation, prosocial, interpersonal communication and independence-competence scores on the SFS in comparison to controls. This reduction in functioning ability has been noted previously [154]. However, the specific changes in social functioning ability have not previously been focused upon in a greater range of social interactions than simply how well patients can communicate [154]. For details of the validation of the SFS in the qmAD group see Appendix 3.1. (p.65) and the validation of methodologies chapter, Chapter 7 (p.260).

4.4.1.3. Assessment of the circadian rhythm of rest-activity

No differences were found at baseline between the aMCI group and controls in their rest-activity profiles. The mild AD group were observed to have higher average activity, least five hours (L5) of activity and most active ten hours (M10) activity counts. In contrast the mAD group had significantly lower activity counts for the M10 and average activity counts with lower activity amplitude. The mAD also had a lower relative amplitude between the L5 and M10

activity counts, indicating less difference between daytime and night-time activity levels in comparison to controls. Reduced amplitude and activity levels have been previously noted in moderate AD patients [155] but the high levels of activity observed in the present mild AD group have not been reported previously. It appears unlikely that increased activity is in some way, compensating for memory loss, but this behaviour could be related to the high level of functioning of the particular individuals who participated in this study. In terms of the circadian period of activity profile, all groups were comparable. The phase of the activity profile confirmed the above alterations in amplitude of activity, and showed that the activity profiles of all groups were in phase with each other. This is important, as it has been noted previously that moderate Alzheimer's disease patients can display phase-delay of their core body temperature (an endogenous circadian marker) and disruption of phase of activity [145].

4.4.1.4. Assessment of Sleep

Subjective sleep quality was reported as being poorer in the aMCI group in comparison to controls. No other PSQI variables were found to differ from the control data for any group. Little systematic research has been conducted on the sleep of aMCI individuals, and to my knowledge poorer subjective sleep quality has not been reported previously. This could be an early sign of future sleep disturbance associated with cognitive decline. Due to the sleep disturbance observed from sleep analysis of actigraphy data and EEG, it could be that before memory impairment reduces awareness of sleep disturbance, aMCI individuals are able to report accurately their lower sleep quality. The JSQ observed that aMCI individuals woke more times during the sleep period than controls and, although it was not statistically significant, fragmentation of sleep (fragmentation index and one minute immobility percentage), derived from the sleep analysis of activity data, was more fragmented in aMCI individuals than the

control group. Observation of sleep architecture by EEG measurement showed that aMCI individuals slept less, had less REM sleep percentage and more wake after sleep onset than controls. These findings replicate previous observations of aMCI sleep [156], although a reduction in slow wave sleep was not observed in my study. What until now has been unknown, is how these alterations in sleep architecture alter throughout the sleep period for aMCI individuals. My study found that wake duration was higher throughout the whole night in comparison to controls, but did reduce almost by half in the final third of the night. Furthermore, REM sleep duration increased progressively through the night, as expected, in-line with the pattern observed in the controls, but just to a lower extent.

The mild AD group had comparable scores on all PSQI components to controls, but the JSQ revealed that they reported sleeping for longer, going to bed earlier and napped more during the day than controls. The increased napping behaviour can be an indicator of poor sleep [157] but it is still questionable whether it can also be a causal factor in worsening sleep quality [158, 159]. From rest-activity data derived sleep analysis, the mild AD group were observed to have reduced sleep efficiency and higher fragmentation of the sleep period (one minute immobility and fragmentation index) than the control group. In addition, the mild AD group had reduced sleep duration, REM sleep duration and less stage 3 sleep from EEG measures, with these data also confirming reduced sleep efficiency in comparison to controls. Less than half the REM sleep observed in the control group was observed in the first third of sleep period in the mild AD group, with REM sleep having longer latencies in the final two thirds of the night than for any other group. This alteration in REM sleep could be due to the AD-related degeneration of cholinergic systems known to be involved in the generation and maintenance of REM sleep [29].

The moderate AD group reported no differences in sleep characteristics measured by the PSQI in comparison to controls. However, they reported a longer sleep duration and earlier bedtimes than controls in the JSQ. These differences were confirmed objectively by sleep analysis of activity data. The mAD group also had significantly more fragmented sleep (one minute immobility percentage and fragmentation index) than controls. Fragmentation of sleep in moderate AD patients has been reported previously [160], although a significantly higher fragmentation index has not. Although only one individual moderate AD patient was observed by EEG, longer sleep duration was again confirmed as well as reduced sleep efficiency, less stage 2 and REM sleep, and more wake after sleep onset. Surprisingly, the mAD individual was observed to have more stage 3 and 4 sleep, unusually for AD patients, whereas a large decrease would often be observed [161]. Movements were only present in the final third of the night, as well as a large increase in stage 1 sleep. Stage 3 and 4 sleep were present in all thirds of the sleep period, but reduced by more than half in the final third of the night. REM sleep duration increased during the night, although little difference was observed between the second and final thirds of the night. REM sleep duration decrease in comparison to the sleep of controls, has been noted on numerous occasions for moderate AD patients [161-163]. These findings are of particular interest as disrupted sleep of AD patients has been observed to have such a large impact on the quality of life of caregivers that it is one of the primary reasons for institutionalisation [164].

4.4.2. Progression of characteristics with increasing memory impairment severity

Although aMCI, mild and moderate Alzheimer's disease (AD) groups have been compared previously to healthy controls, in many studies analysis of the progression of rest-activity or

sleep characteristics through the prodromal stage of AD, aMCI, and then progressing through the severity of AD have not been reported previously. The data from this study showed a progressive decrease in MMSE total score as memory impairment severity increased, except for the subjective memory complaint (SMC) group, which was found to have comparable memory scores to the controls in the previous chapter. MMSE total score was, therefore, used as a marker of memory deficit severity and correlated against rest-activity and sleep parameters, to determine any parameters that altered as memory impairment severity increased.

Amplitude of activity, and most active ten hours activity count, were both found to decrease as memory impairment severity increased. Although decreased amplitude has been observed before in aMCI, mild and moderate AD individuals in comparison to controls [155, 165] this is the first study to show a progressive decline correlated to memory impairment severity. This is an important finding, as a study by Tranah *et al.* [165], found that low amplitude of activity in aMCI individuals predicted future progression to an Alzheimer's disease diagnosis. The relative amplitude (RA) between most active 10 hours and least active five hours was also found to decrease as memory deficit severity increased. This indicates a reduction in the distinction between sleep and wake periods. Although this reduction has been previously noted when comparing AD patients to controls [166], the progression of RA decrease with increasing memory deficit severity has not been found previously. Another activity-derived circadian variable of Intra-daily variability (IV) was found to have a significant correlation with MMSE total score, with IV increasing as memory deficit severity increased. This means that as memory impairment severity increased, so did the fragmentation of the circadian rhythm with increasing transitions from restfulness to activity. A previous study by Hatfield and colleagues found that when comparing controls, mild AD and moderate AD patients IV was not found to correlate significantly with MMSE total score [166]. Perhaps due to the inclusion of more stages

of impairment severity, the progression of IV and RA were able to be observed in my study when not found by Hatfield and colleagues.

When correlating MMSE total score and JSQ variables, it was observed that sleep duration increased as memory impairment severity increased. As this is reported subjective sleep duration, it only includes the duration of the sleep period without accounting for any wakefulness occurring within this period. The sleep actigraphic variable of bedtime was also found to correlate with MMSE total score, with bedtimes becoming earlier as memory impairment severity increased. Several sleep EEG variables were found to correlate with memory deficit severity, with sleep duration increasing and movement duration decreasing as memory deficit severity increased. Again, although AD patients have been reported to have longer sleep durations than controls [167], the progression of the characteristic through prodromal stages of the disease, as well as in relation to AD severity has not been reported previously. Electroencephalography variables highly correlated to increasing memory deficit severity were: increasing wake duration after sleep onset (WASO); decreasing REM sleep duration; percentage of time in bed; and decreasing sleep efficiency. The decrease in REM sleep has been found previously when comparing AD patients to controls [162], but this is the first study to directly correlate this variable with increasing memory impairment severity. On the other hand, this study's finding of increasing WASO with increasing memory deficit severity confirms the previous finding of Yesavage and colleagues [168].

Although the issue of sleep and circadian rhythms in relation to memory impairment (especially in Alzheimer's disease) has been likened to the problem of the 'chicken and the egg', this debate is gaining more evidence [169, 170]. Research supports the assertion that although sleep and rest-activity dysfunction are related to AD they could also be involved in promoting or exacerbating the development of AD [170]. This idea is supported by the findings of

Moghekar et al., who observed increased amyloid- β in the cerebro-spinal fluid (CSF) after acute sleep suppression and also found a circadian rhythm of CSF levels. These studies, therefore support the idea that dysfunction of rest-activity and sleep in AD patients may be causative and not just correlational. The other side of the debate hypothesises is that circadian and sleep dysfunction are aggravated by the large amount of medications that older people take routinely [171]. A recent study of a mouse AD model found that when amyloid beta plaques had built up, sleep disturbances began, yet nearly complete elimination of the plaques resulted in the sleep-wake cycle returning to normal [172]. This finding illuminates the debate concerning a direct link between amyloid beta plaque build-up and sleep disturbance. This finding would suggest that sleep disturbance could get progressively worse with increase plaque build-up. Roh *et al.*'s mouse model findings would suggest that sleep disturbance could be a reliable measure of amyloid build-up and, therefore, Alzheimer's disease progression [172].

Therefore sleep markers linked to progressive memory impairment of increased sleep duration, decreased REM sleep duration and percentage, increased wake after sleep onset and decreased sleep efficiency are important. As well as the rest-activity markers of decreasing amplitude of activity, increased Intra-daily variability and decreased relative amplitude which could hold the key to predicting progression to Alzheimer's disease. However, it was not in the scope of this thesis to firstly determine whether these changes are predictive and/or causal, and secondly, to follow-up individuals later to assess who progressed to AD.

4.4.3. Limitations and strengths of study

Although little research has been conducted into progression of rest-activity and sleep in relation to increasing memory impairment severity, the findings of this study did corroborate previous results and additionally found correlations with both relative amplitude and intra-daily

variability of rest-activity data which was not found in previous research [166]. These findings may be due to the inclusion of more stages of memory impairment severity in this current study.

A major limitation of this study was the low sample size of the mild and moderate Alzheimer's disease patient groups. This was due to recruitment difficulties in finding cholinesterase inhibitor naïve patients. Furthermore, a technical fault with the EEG equipment led to a reduced number of usable files (details in methods, chapter 2, p.52). The low sample size did not enable statistical analysis of the mild AD group for all methodologies, and the SMC and mAD groups for EEG-derived variables.

A further limitation was that, despite using a less conservative multiple comparisons correction than the Bonferroni method, the Dunn-Šidák method still decreased the significance level of many variables considerably. This was due in part to the large number of variables assessed when dealing with questionnaires without component scores (such as the health questionnaire and Jupiter sleep medicine questionnaire). However, despite this limitation some variables remained statistically significant and some variables which were found to be significant prior to Dunn-Šidák correction were still reported, to enable future directions of research to be undertaken.

4.4.4. Future directions of research

As with all research, future replication of the findings of this study are required before conclusions can be properly validated. In particular, the results of this study warrant investigation with a larger sample of participants in every group. When repeating measurement of the rest-activity and sleep parameters, found to correlate with progression of memory impairment severity, individuals need to be observed over longer periods of time, as only small

differences over time were found in the three month assessment period of this study. Focus could then be given to individuals who do progress from healthy memory through the stages of subjective memory complaint, amnesic mild cognitive impairment, mild Alzheimer's disease and moderate Alzheimer's disease to determine whether the correlated progression of sleep and activity characteristics are detectable and reproducible in real-time. Although it was not possible to include them in this study, there are severe AD patients who are community-dwelling who could be assessed to determine whether the progressive markers found in this study are evident at the most severe stage of AD (although co-operation with testing and measurements would be more difficult in this group). Finally, it would also be profitable to analyse the EEG data in terms of spectral frequency differences between groups, to determine any possible spectral markers for progressive memory impairment severity. Unfortunately, time did not allow for analysis of the data in this way, but future spectral analysis of the data will be conducted.

In conclusion, this study found rest-activity and sleep differences between amnesic mild cognitive impairment, mild and moderate Alzheimer's disease patients in comparison to controls, in a community dwelling, retired population. Markers of amplitude of activity, intra-daily variability of rest-activity and relative amplitude between rest and activity, were found to correlate with increasing memory impairment severity. Further markers of total sleep duration, REM sleep duration and percentage of time in bed, wake after sleep onset and sleep efficiency, were also found to correlate with increasing memory impairment severity. These markers may predict future progression to Alzheimer's disease before definitive symptoms become apparent, however, future research to confirm and further elucidate these findings is required.

5. Study 1: Cholinesterase inhibitors

5.1. Introduction

5.1.1. Alzheimer's disease medications

Alzheimer's patients are given different medications to assist with their different presentations of the disease. One type of commonly prescribed medication is cholinesterase inhibitors, which are thought to improve cholinergic transmission and, therefore, decelerate cognitive decline [173]. The cholinergic neural transmission system is also known to be involved in REM sleep and wakefulness, as well as cognition, through basal forebrain projections mediating arousal [29]. This thesis focuses on one type of cholinesterase inhibitor, donepezil, the most prescribed by clinicians treating AD-related cognitive problems in the UK.

Donepezil is a cholinesterase inhibitor promoting the neurotransmission of acetylcholine (ACh) by inhibiting the post-synaptic enzymes that hydrolyse it in the synaptic cleft therefore increasing availability of acetylcholine for neuronal transmission [174]. This interaction is demonstrated in Figure 19 (p.169) (adapted from Siegler and Reidenberg, 2003) [174].

Other benefits of cholinesterase inhibitors are their psychotropic effects. They play an important role in controlling neuropsychiatric symptoms of AD, in particular behavioural disturbances [175]. These benefits may occur due to improvements in cholinergic limbic system functioning, known to deteriorate in AD. Unfortunately, the effects of donepezil do not appear to be beneficial for more severe cases of AD, with results only slightly favouring donepezil over placebo [176, 177].

Other cholinesterase inhibitors are also prescribed to AD patients, such as galantamine and rivastigmine. Both have been observed to improve AD symptoms, although little has been

researched in terms of their effects on sleep. Galantamine has been observed to have stabilising effects on cognition by binding to cholinergic receptors, with cognitive ability staying close to baseline for 12 months [178]. In terms of its effects on sleep, galantamine is known to be neither detrimental nor beneficial, maintaining sleep quality [179]. Rivastigmine reduces the agitation and excessive motor activity associated with AD, as shown by actigraphy, however, sleep has not been fully researched for this medication [180].

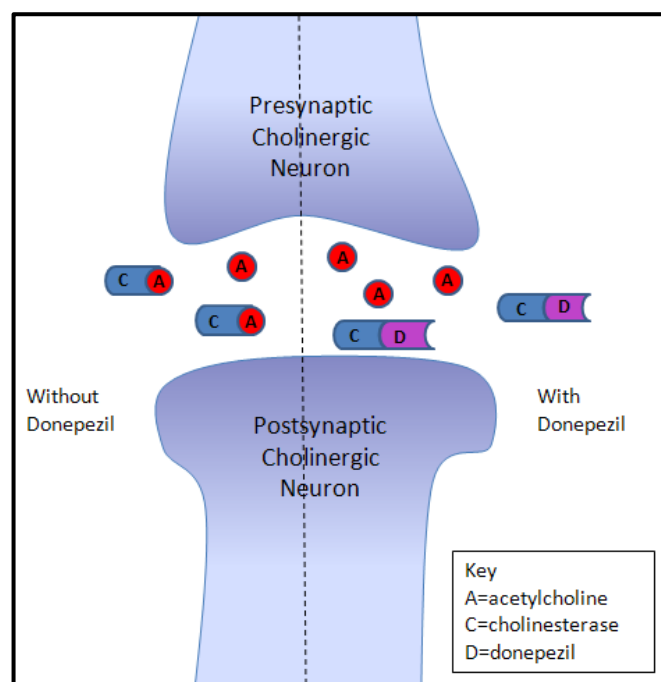


Figure 19. Schematic depicts the action of donepezil, a cholinesterase inhibitor on the cholinesterase enzyme at the synaptic cleft of two cholinergic neurons. In Alzheimer’s disease acetylcholine (Ach) neurotransmission is reduced, thought to be due to a reduction in the number of cholinergic neurons and as well the increased activity of cholinesterase enzymes that hydrolyse Ach. Donepezil addresses this by inhibiting the cholinesterase enzymes allowing increased concentrations of Ach to be available for neurotransmission [174].

In the UK, National Institute for Health and Clinical Excellence (NICE) guidelines only allow Alzheimer’s patients to be prescribed donepezil once they achieve a score as low as 20 on the MMSE test (Mini Mental State Examination range 0-30, with 30 indicating high ability) [181]. Changes to these guidelines have occurred during the course of this study, details are

provided in the discussion section (p. 219). Healthy controls usually score between 30 and 25 on the 30 point test that considers orientation, language, naming and repeating, registration, attention, concentration and calculation, recall, reading comprehension and praxis. Donepezil has been considered to be effective and safe with no clinically significant drug-drug interactions, whilst significantly increasing the global functioning and maintaining cognitive ability of the individual [182]. Cognitive improvements are modest and temporary, as this medication cannot cure the underlying neurodegeneration that continues to progress. This medication only benefits approximately 50% of patients, and finding out who will benefit from the medication is the other focus of this chapter.

5.1.2. Interaction between Sleep and Donepezil

According to American Hospital Formulary Service (AHFS) guidelines for donepezil, its side effects strongly affect sleep as this drug has been shown to lead to drowsiness, excessive tiredness, abnormal dreams and problems falling asleep or staying asleep [183].

Due to the high frequency of sleep disturbances observed in Alzheimer's disease (AD) patients independent of medication, and the likely effects on sleep of donepezil, this interaction is subject to current investigation. One recent study comparing donepezil and placebo in mild/moderate AD patients found increased REM sleep duration and increased sleep apnea index score from baseline after 3 months of donepezil administration [184]. Improvement in cognitive scores did not appear to be correlated with sleep apnea score or with REM sleep duration [184]. There is, however, the hypothesis that the medication is working on the pontine cholinergic neurons, that affect REM sleep and through projections to the thalamus, affecting respiratory drive [184]. Currently this hypothesis has not been either supported or disproved.

Further studies into the effect of donepezil on REM sleep observed there was an increase in duration of REM sleep and an increase in the number of REM sleep episodes after treatment [185, 186]. Additional studies have found increased sleep efficiency and a shortening of sleep latency with polysomnographic measurements after treatment with 5mg OD donepezil at breakfast [186]. This shows clear improvements in sleep structure and sleep quality for AD patients when the medication is taken at the beginning of the day. As cholinergic medication has been found to alter sleep, this study seeks to add further understanding and detail to this relationship. Elucidating this relationship requires home-based study of the drug-associated changes rather than laboratory based study. This chapter seeks to answer the following research questions:

- ***Does cholinergic medication alter objective measures of rest-activity or sleep pattern in moderate Alzheimer's disease patients when observed in the field?***
- ***Are there predictors of future cognitive benefits of taking cholinergic medication? And are there any factors that predict who will tolerate higher doses of donepezil?***
- ***Are there any predictive factors of future sleep problems associated with taking cholinergic medication?***
- ***Finally, are there factors of sleep or rest-activity rhythms at baseline that predict who can tolerate donepezil and who will need to change to a different type of cholinesterase inhibitor?***

5.2. Methods

5.2.1. Participant recruitment

The control group (healthy older people with no subjective or objective memory deficits) were recruited as described in previous chapters and the methods chapter, Chapter 2 (p.35).

Moderate Alzheimer's disease (AD) patients were recruited through memory clinics located in Oxfordshire and Buckinghamshire, for details see Chapter 2 (p.35). Controls (n=9, 4 females) mean age: 73.2 ± 4.9 years and Moderate AD that completed questionnaires (qmAD, n=31, 14 females) (subset did all methodologies (mAD), n=5) qmAD mean age: 78.5 ± 6.2 years.

All participants were required to be community dwelling, have a study partner and be acetylcholinesterase inhibitor naïve. Except for the qmAD group (questionnaires only moderate Alzheimer's disease), participants were not included if diagnosed with a sleep condition or psychiatric illness or being on hypnotic medication. Diagnosis was determined by memory clinic staff, neuropsychological testing and observation of CT or MRI scans of brain atrophy.

Moderate AD patients were divided into two groups based on inclusion criteria, details of which are provided in the methods chapter, Chapter 2 (p.35). The first group included all participants eligible for the study, named qmAD. These participants completed only the questionnaires; the study design is provided in Chapter 2 (p.32). A subset of the qmAD participants were measured with objective measures of memory testing, circadian rest-activity rhythm assessment and sleep analysis, this group was named mAD.

All patients were first prescribed 5mg donepezil, and after four weeks of once-daily oral administration of 5mg donepezil, a telephone check-up was conducted by a memory clinic nurse. At this check-up any adverse reactions to the medication were discussed with the patient's carer (usually a spouse) and a decision was made to either increase dosage to 10mg once daily, remain on 5mg once daily or change to a different type of cholinesterase inhibitor

(ChEI). Timing of ChEI tablet intake was determined by advice from the memory clinic prescribing doctor or nurse. Standard medication guidance provided with the medication recommended taking the tablet with the largest meal of the day or in the evening. All, but two participants (n=31) in the qmAD group of this study either increased to 10mg once daily or changed to a different type of ChEI. Of the two drop-outs, one patient received a diagnosis of Parkinson's disease and medication was altered, the other one refused to take the medication as she believed it was disturbing her sleep to a large extent. Of the 31 patients who began the study, two did not have baseline assessments due to starting the medication earlier than planned, and 22 patients completed second assessment. Therefore only 20 participants having both assessments were included in analyses of change following medication. Participants left the study due to either ending medication use or health problems. Medical records of which participants remained on donepezil or did not, were collected despite whether assessments for both sessions were conducted. In assessing baseline differences between these, those who remained on donepezil and those who changed ChEI, 25 individuals were included (4 changed from donepezil to another ChEI) who had baseline assessments and information on medication changes.

Participants who remained on donepezil 10mg once daily (n=17) after initial dosage increase were compared to those who reverted back to 5mg once daily (n=4) at baseline assessment.

From the 18 participants with MMSE test total scores for both assessments, six reported sleep problems associated with taking the ChEIs. Those who did and those who did not complain of sleep problems associated with ChEI administration, were compared at baseline assessment.

All comparisons of baseline assessment data were conducted to determine if any characteristics predicted future sleep problems, or a requirement to change medication, or lack of tolerance to higher dosage of donepezil. The most important comparison was that of those who were classified as benefitting cognitively from the ChEI medication and those who did not. Classification of whether individuals benefitted from ChEI administration cognitively was determined by observing whether participants stabilised or increased in MMSE total scores between assessments, indicating a cognitive benefit, or decreased in MMSE total scores between assessments indicating no cognitive benefit from ChEI initiation. Participants in the qmAD group were, therefore, divided into those who benefitted (n=11) and those who did not (n=7) and were compared at baseline to determine any predictors of future benefit of ChEI medication.

5.2.2. Determination of demographics

To determine if any differences in demographics, diurnal preference and anxiety or depression tendencies were present between groups the Health Questionnaire (HQ) derived from the PRIME-D questionnaire developed by Spitzer *et al.*, [62], medical and OPTIMA study records, the Morningness-Eveningness Questionnaire (MEQ) [26] and the Hospital Anxiety and Depression (HAD) Scale [63] were conducted. For more information about each of these measures see methods Chapter 2 (p.36). Grouping of medical conditions and medications into categories can be found in Appendix 1.1.(p.3-5). BMI was calculated using height and weight details collected in the HQ with an online BMI calculator [116]. Participants in the mAD group are included in qmAD sample at all times as mAD are a subset of the qmAD participants.

5.2.3. Assessment of memory

Memory was assessed by the Mini-Mental State Examination (MMSE) [61], a questionnaire used clinically to measure memory and some other aspects of cognitive impairment and by computerised memory and attention tests from Cambridge Cognition called the CANTABeclipse battery [150]. For details of the tests and the questionnaire see Chapter 2 (p.40). Memory was assessed at baseline and nine weeks later, to determine any longitudinal changes that occurred between the control group over time and the effect of ChEI initiation in the mAD and qmAD groups.

5.2.4. Assessment of functioning

In order to assess functioning, both social functioning and activities of daily living two questionnaires were used: Social Functioning Scale (SFS) [67] and the ADCS-Activities of Daily Living Scale (ADCS-ADL) [66]. For details of these questionnaires see Chapter 2 (p.41).

Functioning was assessed at baseline and nine weeks later to determine any effects of medication administration.

5.2.5. Circadian measurements

Rest-activity behaviour was assessed for three weeks first at baseline and again six weeks later using actigraphy. For non-parametric circadian rhythm analysis, average daily activity was evaluated for each assessment period, as well as periodogram analysis conducted to determine activity period length for each assessment. All details of the measurement using actigraphy and the resultant data analyses are found in Chapter 2 (p.43).

5.2.6. Sleep measurements

Sleep was measured by three techniques: questionnaires, actigraphy and electroencephalography (EEG); for details of each method see Chapter 2 (p.52) All techniques were applied at both assessments. The sleep questionnaires were the Pittsburgh Sleep Quality Index (PSQI) [64] and selected questions from the Jupiter Sleep Questionnaire (JSQ) [65]; details of both are given in Chapter 2 (p.51) EEG data were scored according to the scoring criteria of Rechtschaffen and Kales [77]. Following scoring each individual's sleep period was divided into three sections to compare durations and percentages of each sleep stage between groups, and how they changed across the sleep period. These 'thirds' were calculated per recording as a proportion of the individual's time in bed. Due to a technical fault some EEG recordings were unable to be scored, for more details see Chapter 2 (p.53). Due to the technical fault 7 out of 9 controls had recordings analysed at baseline and 5 out of 8 at second assessment, and for the mAD group, 1 out of 3 recordings were analysed at baseline and 2 out of 4 recordings were analysed at second assessment

5.2.7. Statistical analysis

All data were divided into parametric or non-parametric scale data, binominal data or categorical data. Each type of data was statistically analysed as detailed in Chapter 2 (p.63). All data were compared between groups, with differences between time-points compared between groups also. Details of which statistical test and the Dunn-Šidák (D-S) multiple comparisons correction calculations, are provided in the Appendix section for the relevant data. In the text of this chapter if a result was significant after multiple comparisons correction 'significant after Dunn-Šidák correction' will be stated.

5.2.8. Ethical approval

Ethical approval for this project was given by the NHS Berkshire Ethics Committee, for further details see Chapter 2 (p.60). Funding was provided by the NIHR Brain Theme.

5.3. Results

5.3.1. Demographics

Time between assessment did not differ between groups significantly ($p>0.05$), control mean: 64.6 ± 6.4 days, qmAD mean: 68.5 ± 9.5 days. The effect of cholinesterase inhibitor (ChEI) medication was determined by the difference between baseline drug-naïve assessment and after ChEI initiation assessment. The time between control assessments was the same as the moderate AD individuals (control mean: 64.6 ± 6.4 days, qmAD mean: 68.5 ± 9.5 days). Details of the demographic differences between the qmAD and the control group are provided in Chapter 4 (p.115). To summarise, the qmAD group had a higher mean age (control mean age: 73.2 ± 4.9 years, qmAD mean age: 78.5 ± 6.2 years) ($p<0.05$) than the control group. No significant differences in sex, diurnal preference, caffeine intake frequency, previous occupation, highest level of education and years of education were found between the control group and either of the moderate AD groups ($p>0.05$). There were some differences in types of medications and medical conditions between the controls and the moderate AD groups however, none were significant after Dunn-Šidák multiple comparisons correction (for a more detailed description see Chapter 4 (p.115)). From the Hospital anxiety and depression scale, total depression tendency score was found to be significantly higher for the qmAD in comparison to the control group, however, it was not significant after D-S correction ($p<0.05$).

5.3.2. Memory

5.3.2.1. Effect of medication

In comparison to change over time in controls, the moderate AD group was found to have a no significant change in MMSE scores due to ChEI medication initiation ($p>0.05$), Table 25.

Table 25. Mini-Mental State Examination (MMSE) components for control and qmAD groups for each session of testing.

Component score	Baseline Assessment (drug-naïve)		Second Assessment (after ChEI initiation for qmAD)	
	Control (n=9)	qmAD (n=29)	Control (n=8)	qmAD (n=20)
Orientation	9.9 ± 0.3	6.8 ± 2.3	10.0 ± 0.0	6.8 ± 1.5
Language	2.9 ± 0.3	2.3 ± 0.5	2.9 ± 0.4	2.6 ± 0.5
Repetition	1.0 ± 0.0	0.3 ± 0.5	1.0 ± 0.0	1.1 ± 0.4
Registration	3.0 ± 0.0	2.9 ± 0.4 (27)	3.0 ± 0.0	2.7 ± 0.8
Attention	5.0 ± 0.0	4.0 ± 1.4	4.9 ± 0.4	4.5 ± 0.9
Recall	2.6 ± 0.5	0.5 ± 0.8	2.9 ± 0.4	0.5 ± 0.8
Praxis	4.9 ± 0.3	4.3 ± 0.9	4.9 ± 0.4	4.6 ± 0.6
Test total	29.2 ± 1.0	22.4 ± 2.5	29.5 ± 0.8	22.4 ± 2.6

No significant differences between assessments.

Data are presented as mean ± SD.

Any alteration of sample sizes are shown in parentheses.

Dunn-Šidák calculations are presented in Appendix 1.5.(p.16).

Due to the small sample size of the mAD group at the second session, no statistical analyses of CANTABeclipse parameters were conducted when assessing change due to ChEI medication initiation. The mAD group showed fewer stages completed on the PAL test (visual memory), using short term memory, therefore, worsening after ChEI medication initiation in comparison to the control group who improved over time, Table 26 (p.180). The mAD group improved their reaction times including the BLC test mean correct latency, the CRT test (assessing attention) mean correct latency and the SST test (assessing decision making) median correction reaction

time on 'go' trials after ChEI medication initiation, with controls remaining stable over time,

Table 26.

Table 26. Selected CANTABeclipse battery of memory and attention tests for control and mAD groups at each testing session.

CANTABeclipse variable	Baseline Assessment (drug-naïve)		Second Assessment (after ChEI initiation for qmAD)	
	Control (n=8)	mAD (n=4)	Control (n=8)	mAD (n=3)
BLC correct (%)	99.7 ± 0.9	98.1 ± 2.4	100.0 ± 0.0	100.0 ± 0.0
BLC mean correct latency (ms)	771.6 ± 75.0	1110.4 ± 103.1	751.6 ± 89.1	1037.5 ± 188.4
CRT mean correct latency (ms)	390.4 ± 60.7	512.0 ± 118.9	380.5 ± 48.1	444.6 ± 95.8
CRT correct trials (%)	99.5 ± 1.1	98.5 ± 1.9	98.8 ± 1.9	98.0 ± 2.0
PAL stages completed	4.5 ± 0.5	3.0 ± 1.4	4.8 ± 0.7	2.3 ± 1.2
SSP span length	5.4 ± 0.7	3.5 ± 1.0	5.5 ± 0.8	3.7 ± 0.6
SSP total errors	11.5 ± 2.5	12.3 ± 5.1	11.88 ± 4.6	13.3 ± 3.5
SST median correct reaction time on 'go' trials (ms)	643.6 ± 111.5	800.3 ± 211.8	637.6 ± 260.5	767.0 ± 256.9
GNT correct (%)	89.6 ± 9.2	70.8 ± 6.9	92.5 ± 6.1	68.9 ± 8.4

No statistical analyses conducted between assessments.

Data are presented as mean ± SD.

Full dataset and Dunn-Šidák calculations are presented in Appendix 1.5. (p.17).

5.3.2.2. Cognitive benefit of ChEI administration

In order to determine which patients benefitted from taking the ChEI medication, change in total MMSE score was analysed. If patients remained the same or increased in MMSE total score they were classified as having benefitted from ChEI medication; if their score decreased after initiating ChEI medication they were classified as not benefitting from the medication.

Mean MMSE total score for each group are shown in Table 27. Those who were classified as not benefitting from ChEI initiation had higher baseline MMSE scores, Table 27.

Table 27. Mini-Mental State Examination (MMSE) total scores for those who benefitted from ChEI medication and those who did not.

MMSE Test Total Score	Benefit group (n=11)	No benefit group (n=7)
ChEI-naïve baseline session	21.0 ± 2.6	24.0 ± 1.3
Test total score after ChEI medication initiation	23.8 ± 2.0	21.4 ± 1.7

No significant differences between assessments.

Data are presented as mean ± SD.

Full dataset and Dunn-Šidák calculations are presented in Appendix 1.5. (p.16).

5.3.2.3. Maintaining at 10mg OD donepezil or reverting back to 5mg OD

There were no significant differences in baseline MMSE scores between those who maintained a once-daily dosage of 10mg donepezil (n=17) and those who increased their dosage to 10mg but then reverted back to 5mg (n=4) due to adverse reactions (p>0.05). Again, no participants of the mAD group reverted back to 5mg donepezil in the course of the study (all data in Appendix (p.16).

5.3.2.4. Changing to other types of cholinesterase inhibitors

Those patients who maintained on donepezil throughout the study had a lower MMSE attention score (mean (n=21) 3.67 ± 1.43) than those who changed medication (mean (n=4) 5.00 ± 0.00, p<0.05). In addition patients remaining on donepezil had lower baseline MMSE total score (mean 22.11 ± 2.68) than those who changed medication (mean 24.00 ± 1.63, p>0.05). None of the participants of the mAD group changed from donepezil to a different cholinesterase inhibitor during the course of the study (all data in Appendix, p.16).

5.3.2.5. Sleep problems related to taking the ChEI medication

The patients who reported sleep problems due to the medication did not score differently on any MMSE components at baseline compared with those patients who did not report sleep problems following medication. None of the individuals that reported sleep problems were in the subset of moderate AD patients that completed the CANTAB memory and attention test battery (the mAD group). Further details of data can be found in the Appendix (p.16).

5.3.3. Functioning

5.3.3.1. Effect of medication

In terms of general functioning as measured by the ADCS-ADL and social functioning as measured by the Social Functioning Scale (SFS), no differences were found for any components due to medication initiation in the qmAD group in comparison to change over time in controls ($p>0.05$). For component scores, see Appendix 1.7.(p.26).

5.3.3.2. Cognitive benefit of ChEI administration

There were no significant differences for the component scores on the ADCS-ADL for general functioning or the SFS for social functioning between those classified as benefitting from taking the ChEI medication and those who did not benefit ($p>0.05$, all data in Appendix, p.26). In terms of change in ADCS-ADL variables between assessments, no difference was found for the basic ADLs component between those who benefitted and those who did not ($p>0.05$, Appendix, p.26). For SFS variables the independence-performance component was found to be significantly increased in those who did not benefit cognitively from ChEI medication, whereas, a decrease in score was observed for those who did benefit cognitively ($p<0.05$). Other components are presented in Appendix 1.7. (p.26).

5.3.3.3. Maintaining at 10mg OD donepezil or reverting back to 5mg OD

For the ADCS-ADL measurement of general functioning, those who stayed on 10mg donepezil had lower instrumental ADLs and test total scores at baseline (mean 32.2 ± 9.6 and mean 51.3 ± 12.7 respectively) than those who reverted back to 5mg due to not tolerating the 10mg dosage regime (mean 47.5 ± 0.7 and mean 69.5 ± 0.7 respectively, $p>0.05$). No differences were

found at baseline between the groups in terms of social functioning as measured by the SFS ($p>0.05$, Appendix, p.26).

5.3.3.4. Changing to other types of cholinesterase inhibitors

No baseline functioning component scores were different between individuals maintained on donepezil and those who changed to other types of ChEIs ($p>0.05$, all data in Appendix, p.26).

5.3.3.5. Sleep problems related to taking the ChEI medication

None of the baseline functioning component scores were different between individuals that reported sleep problems related to taking ChEI medication and those who did not ($p>0.05$, all data in Appendix, p.26).

5.3.4. Circadian assessment

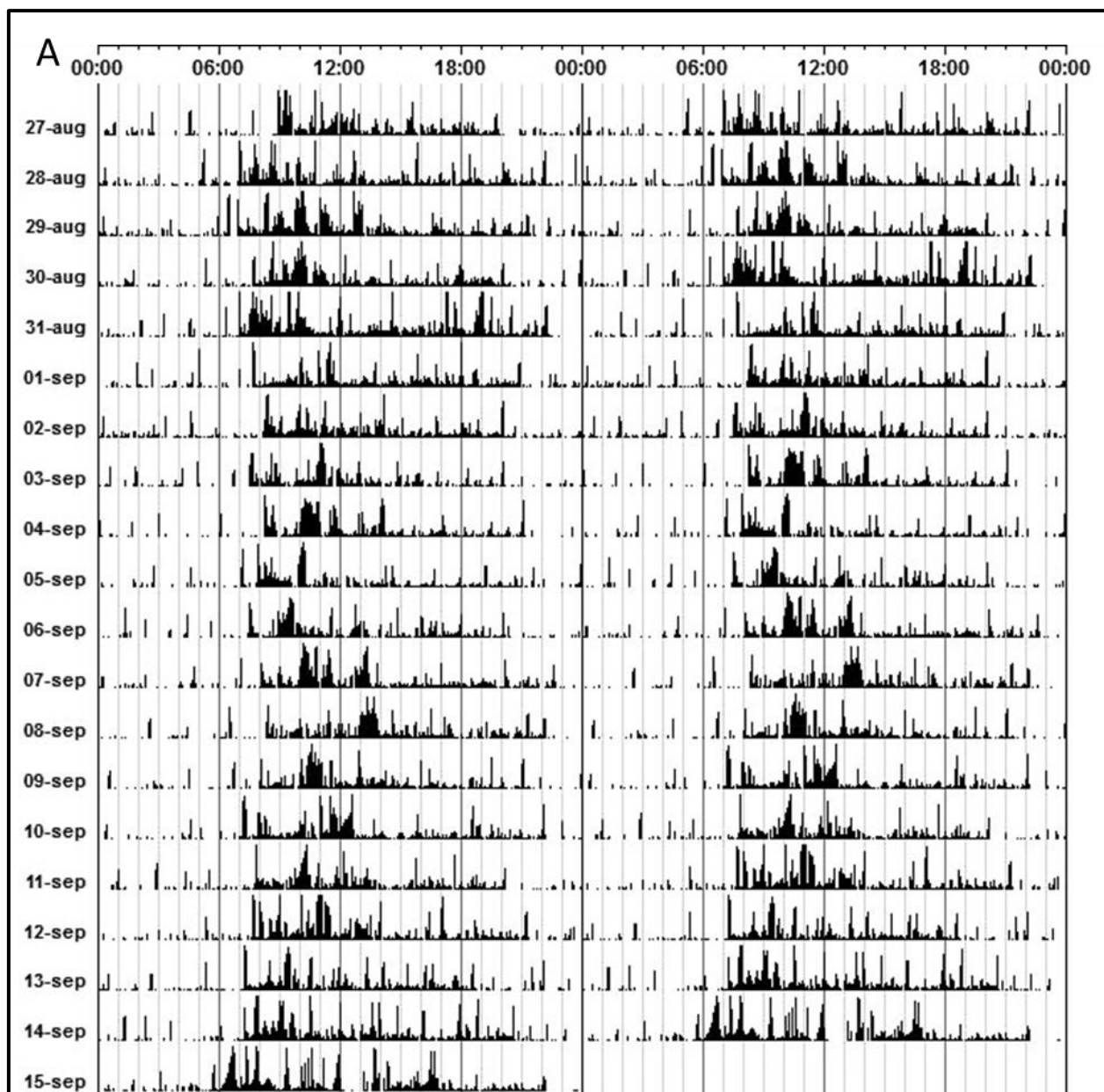
5.3.4.1. Effect of medication

Although no significant change due to ChEI initiation could be determined when analysing the NPCRA components in comparison to change over time in controls, two trends were observed ($p>0.05$). The onset of the phase of the least active five hours (L5-onset) was observed to become earlier in both groups at the second assessment, with the mAD group advancing in phase more than controls' change over time, Table 28 (p.188). In addition, the relative amplitude in activity between L5 and most active 10 hours (M10) remained stable over time in the control group but increased due to ChEI medication initiation in the mAD group ($p>0.05$, Table 28, p.188).

No change due to ChEI initiation in the mAD group in comparison to change over time in controls in period length (calculated from actigraph data) was found in either the Lomb-Scargle or the Sokolove-Bushell Chi-square methods of conducting a periodogram analysis ($p>0.05$, Appendix 1.9., p.31).

Rest-activity data were averaged over the whole assessment to determine a 24-hour profile of activity for each assessment, for the controls showing change over time and for the mAD group showing before and after ChEI medication initiation, Figure 21 (p.189). There were no differences due to ChEI initiation with no altering of the phase of activity. Despite an apparent increase in activity levels on average in the mAD group, large variance led to non-significant results (Figure 21, p.189). For the majority of the 24 hour profile the mAD group had lower activity counts for both sessions than the control group.

To show change following ChEI initiation the actograms of before and after ChEI initiation of a mAD patient are provided in Figure 20 (p.186) as an example.



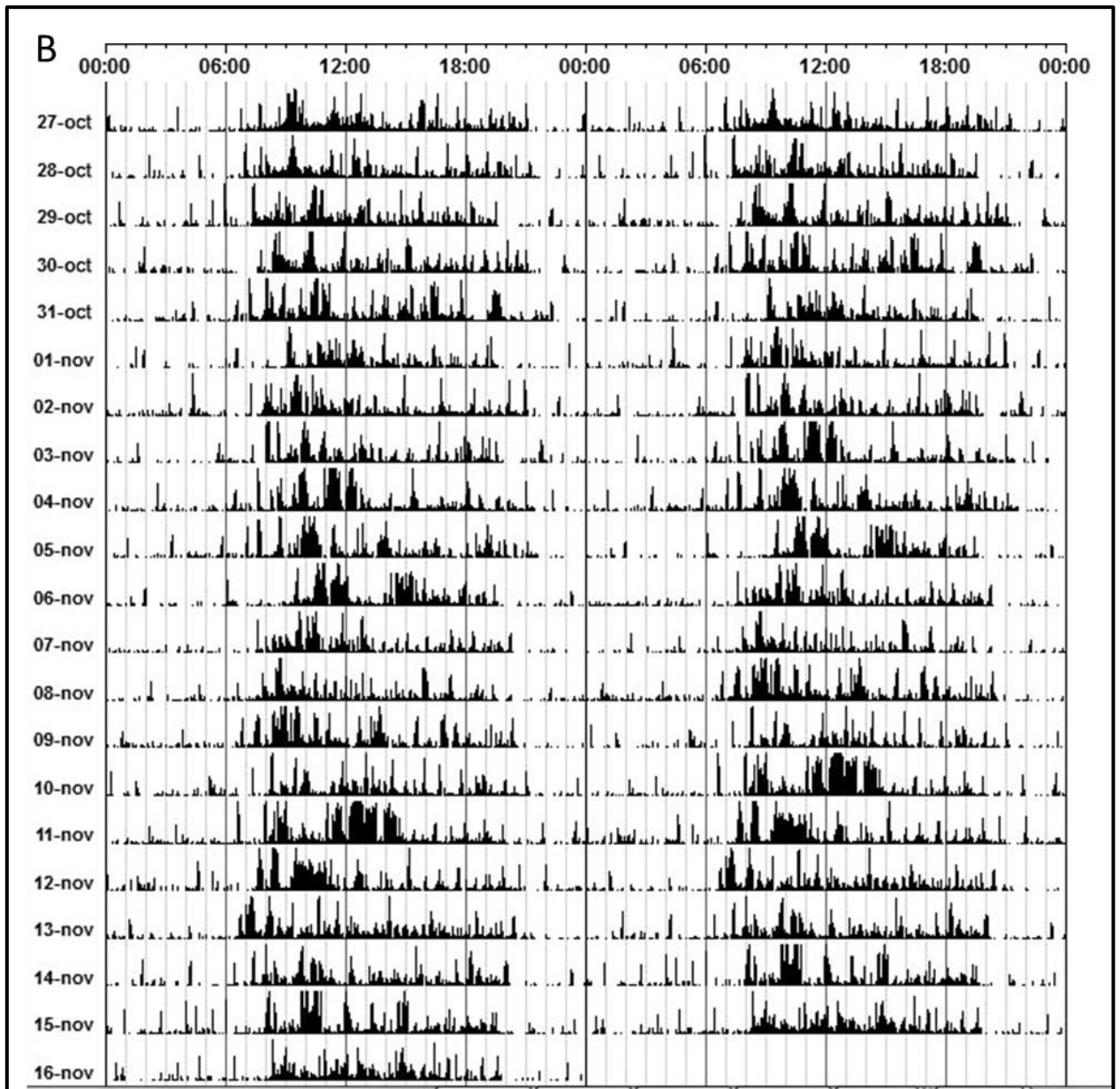


Figure 20. Exemplar actograms for change in rest-activity following ChEI initiation.
Actogram A: mAD individual before ChEI initiation and Actogram B: same mAD individual following ChEI initiation (6 weeks after initiation of 5mg and 2 weeks after dose was raised to 10mg OD). Black lines indicate activity and rest-activity is double plotted to view sleep periods. Activity is scaled to 950 activity counts.

Table 28. Parameters from a non-parametric circadian rhythm analysis (NPCRA) of rest-activity data derived from actigraphy for controls and mAD groups over time.

NPCRA parameter	Baseline Assessment (drug-naïve)		Second Assessment (after ChEI initiation for qmAD)	
	Control (n=9)	mAD (n=5)	Control (n=7)	mAD (n=6)
Average activity count	195.9 ± 92.6	94.9 ± 53.5	186.7 ± 72.8	113.3 ± 76.6
Inter-daily stability (IS)	0.55 ± 0.15	0.53 ± 0.18	0.54 ± 0.14	0.54 ± 0.19
Intra-daily variability (IV)	0.80 ± 0.27	1.19 ± 0.39	0.83 ± 0.17	1.05 ± 0.24
Least active five hours (L5)	11.5 ± 4.4	14.2 ± 2.9	13.1 ± 7.3	11.7 ± 5.0
L5-onset phase (h)	00:42 ± 00:58	00:11 ± 01:18	00:26 ± 00:58	22:49 ± 00:55
Most active ten hours (M10)	338.9 ± 161.1	159.4 ± 91.8	314.3 ± 112.6	199.0 ± 138.6
M10-onset phase (h)	08:16 ± 00:52	07:52 ± 00:54	08:13 ± 00:52	07:22 ± 00:31
Amplitude of activity	327.3 ± 158.1	145.3 ± 89.3	301.2 ± 107.1	187.2 ± 134.8
Relative amplitude	0.93 ± 0.04	0.79 ± 0.13	0.92 ± 0.04	0.86 ± 0.06

No significant differences between assessments.

Data are presented as mean ± SD. h represents hh:mm time format.

Full dataset, test statistics and Dunn-Šidák calculations are presented in Appendix 1.9. (p.30).

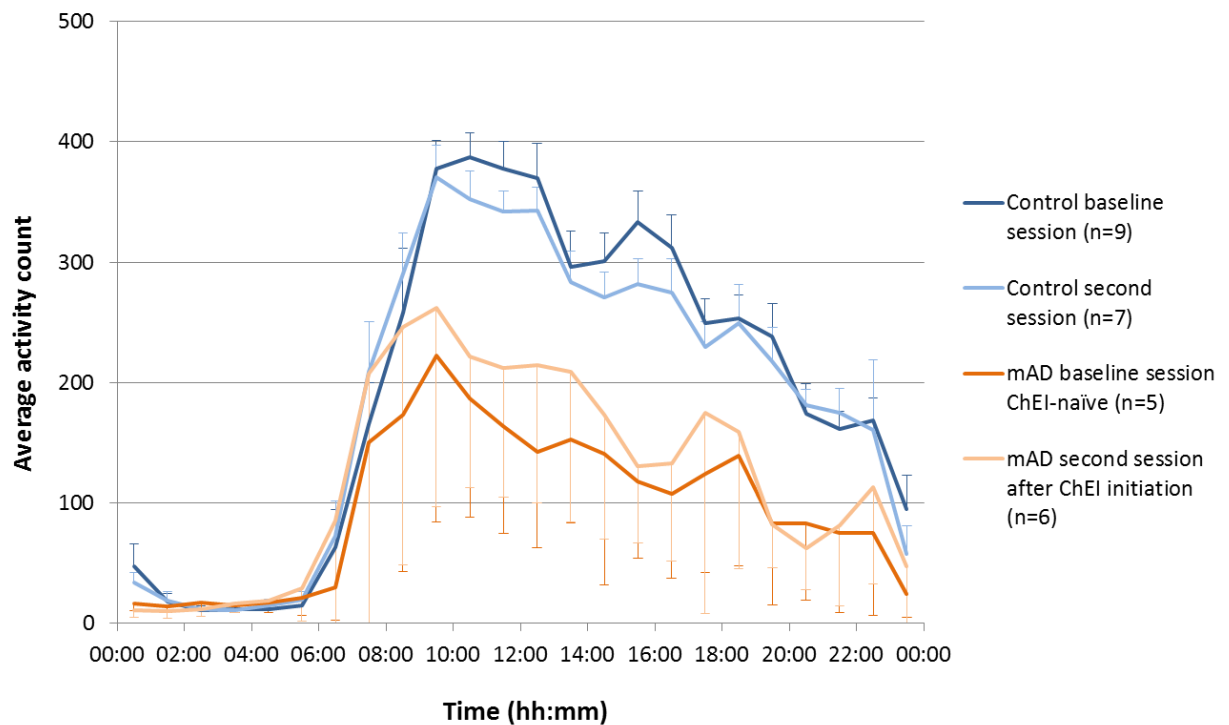


Figure 21. Averaged activity counts over 24h timescale for controls over time and the mAD group at baseline when ChEI –naïve and at second assessment after ChEI initiation. The control group are presented in blue and the mAD group in orange with lighter colours indicating second assessment for that group. Data are shown as mean with standard deviation error bars. Full dataset, test statistics and Dunn-Šidák calculations are presented in Appendix 1.9. (p.31).

One individual from the mAD group was assessed weekly with actigraphy commencing from when he began medication and for eight weeks afterwards. The progression of each of the NPCRA analyses is displayed in Figure 23 (p.191)., and increase of once-daily dosage from 5mg to 10mg donepezil occurred in week 5. Inter-daily stability (IS) and Intra-daily variability (IV) did not show a pattern of progression across the weeks of ChEI administration. Average amplitude of activity, average activity count (Figure 23, Graph C, p.192), and relative amplitude (Figure 23, Graph D, p.192) had stable scores across the weeks with a reduction in activity count occurring in the week when dosage increased. Relative amplitude also remained stable across the weeks of donepezil administration with a decrease in week 5. The average M10 activity counts increased gradually over the weeks with a decrease at week 5 before again increasing again (Figure 23., Graph A, p.191). The average L5 activity counts showed an increasing trend up to

week 5 with activity counts in the rest-phase then decreasing in the following weeks (Figure 23., Graph B, p.191). The phase of onset of L5 and M10 showed no trend over the eight weeks of ChEI administration.

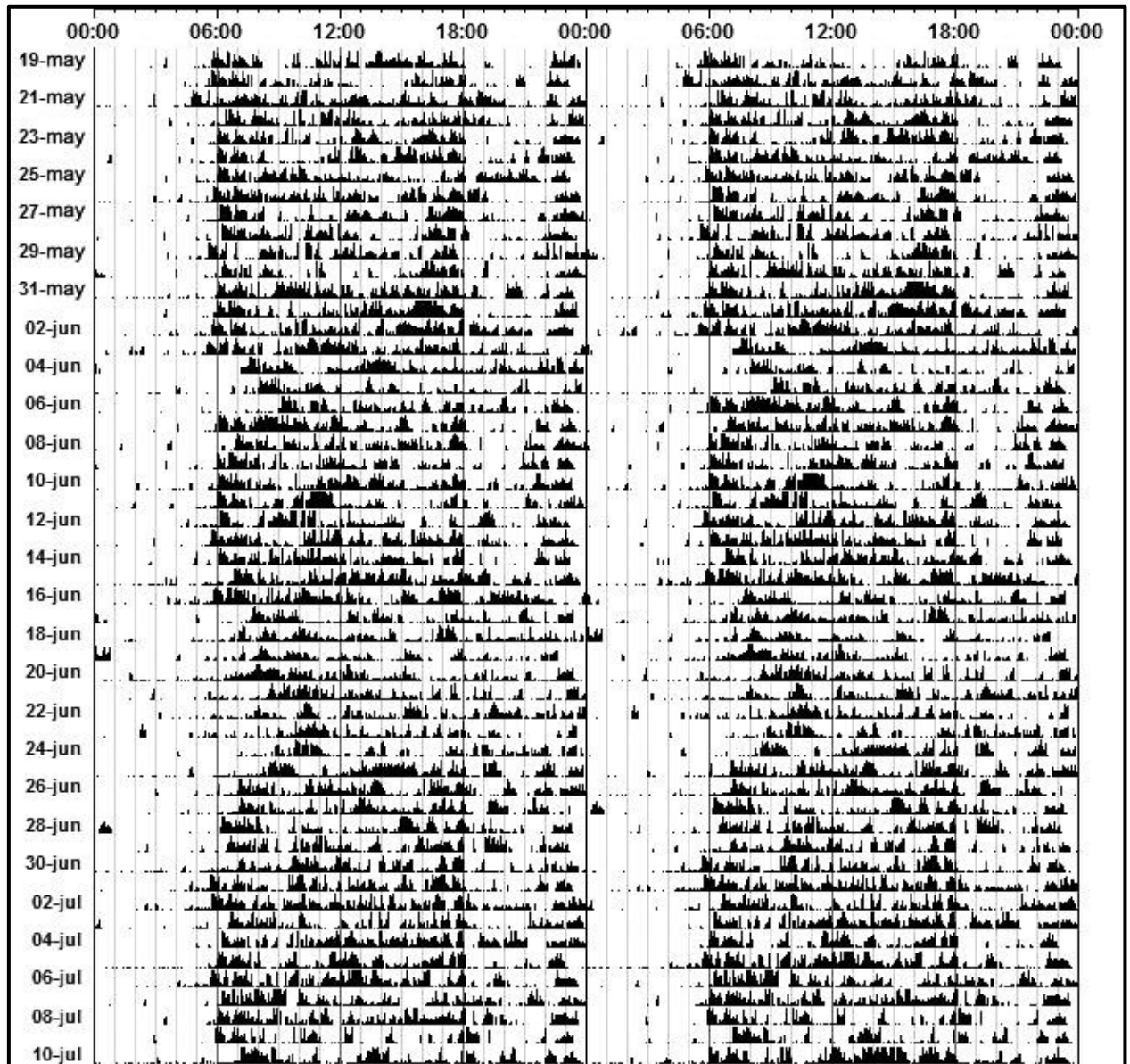
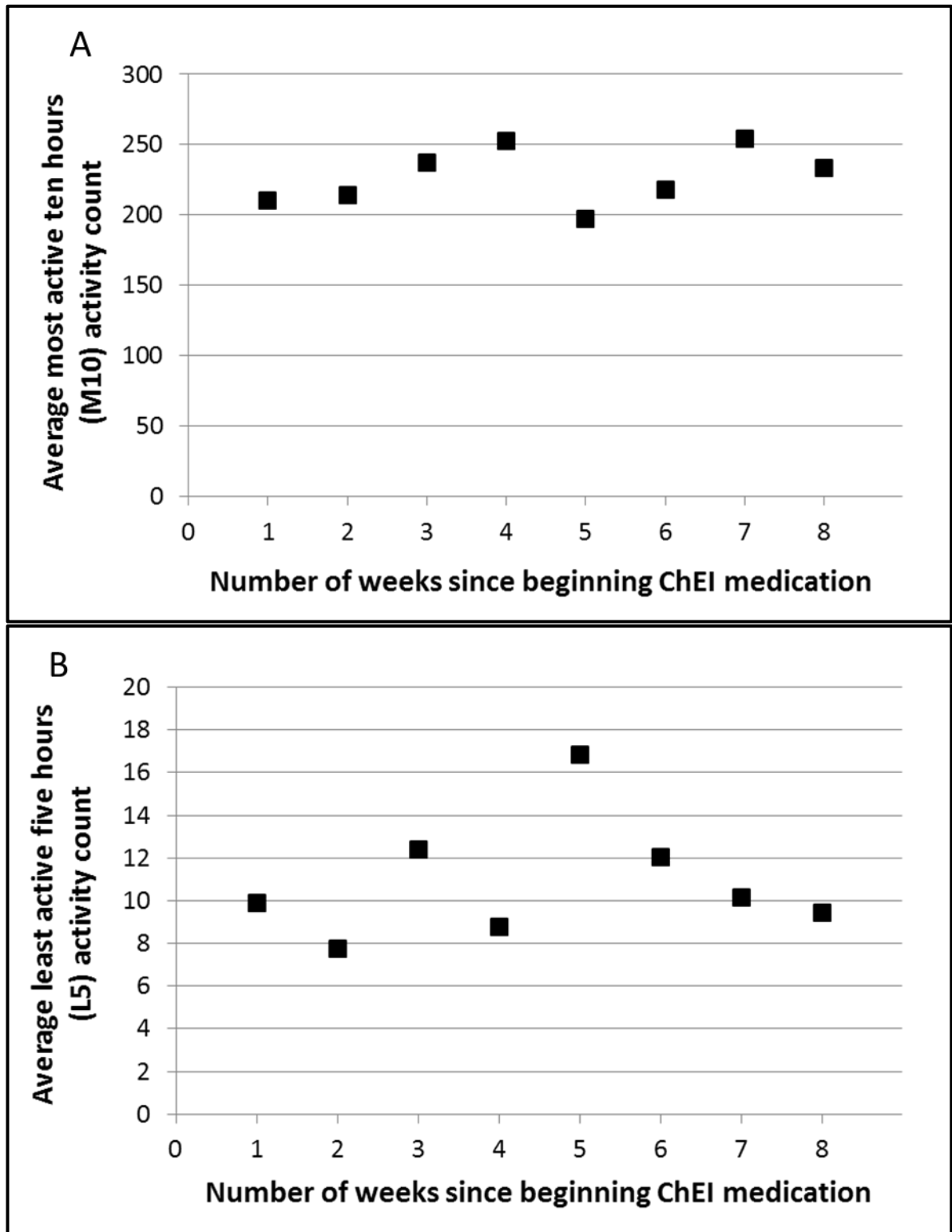


Figure 22. Actogram of rest-activity profile of the individual followed for eight weeks after ChEI initiation. Black lines indicate activity and rest-activity is double plotted to view sleep periods. Activity is scaled to 1550 activity counts. The first alteration in sleep timing occurred at 4th June (no explanation found in sleep log). The second alteration in sleep timing occurred at the beginning of the fifth week of assessment, the week that donepezil dosage was increased from 5mg to 10mg OD (week beginning 16th June).

An actogram showing the individual's rest-activity profile for the whole assessment period is shown in Figure 22 (p.190). The Actogram indicates that there were two alterations in sleep timing. The first could not be explained by the sleep log but the second alteration occurred in the fifth week of assessment, when donepezil dosage was increased from 5mg to 10mg OD.



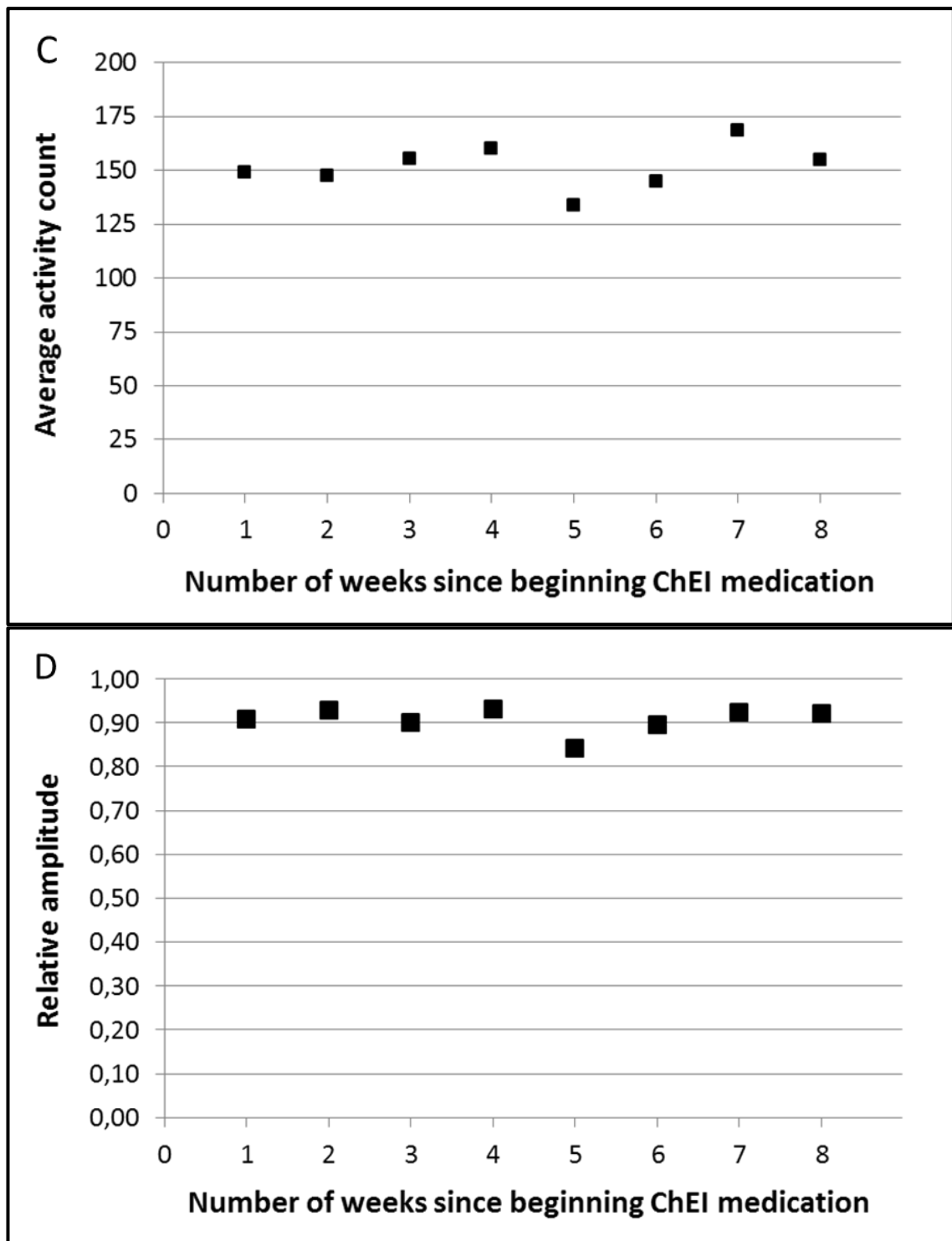


Figure 23. Graphs displaying selected NPCRA parameters derived from actigraphy data for a mAD individual with weekly analysis produced to track progression for each week on ChEI medication from being drug-naïve. At week 5 dosage was changed from 5mg donepezil to 10mg donepezil. Graphs are as follows: A: Average most active ten hours (M10) activity count; B: Average least active five hours (L5) activity count; C: Average activity count; and D: Relative amplitude. Data points represent the mean score for each week.

Alteration of rest-activity due to dosage increase for the individual observed for eight weeks from initiating donepezil was observed. When activity was averaged for every week (Figure 24) it could be observed that activity began later for the two weeks following dosage increase before returning to the same phase of activity onset as before dosage increase.

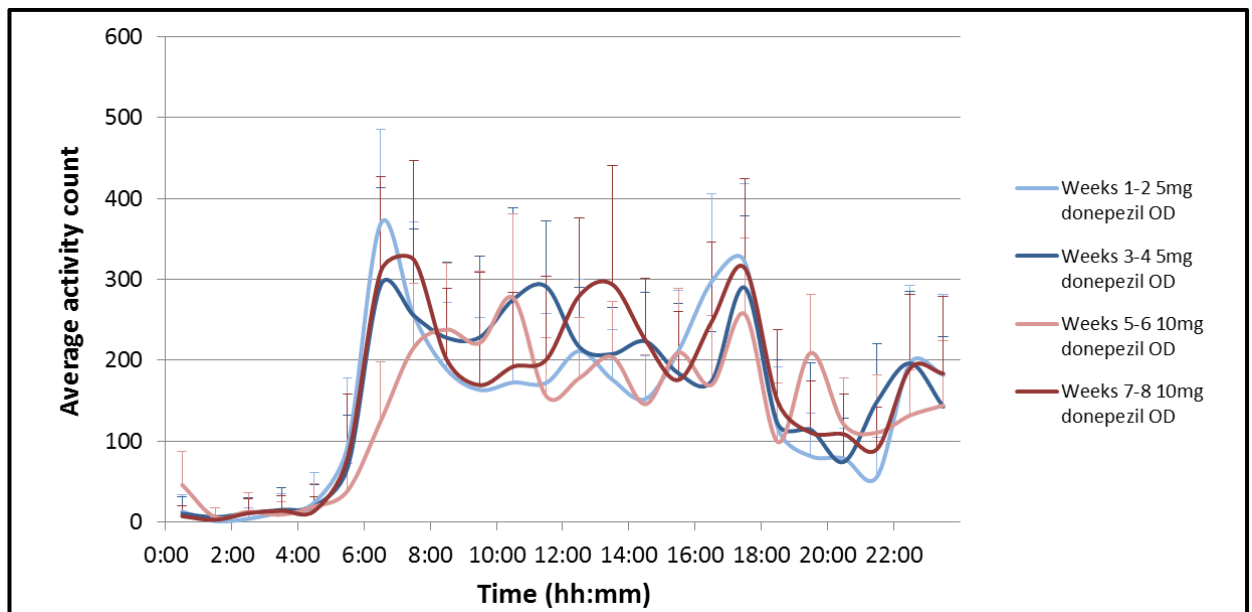


Figure 24. Bi-weekly averaged activity for the individual AD patient that was monitored for eight weeks after donepezil initiation.
Data are averaged per week over the 24h day. Every two weeks were then averaged so that four periods remained; two periods of 5mg donepezil OD (blue lines) and two periods of 10mg donepezil OD (red lines). Data are presented as mean activity count with standard deviation error bars.

5.3.4.2. Cognitive benefit of ChEI administration

As only one individual from the mAD group was classified as having not benefitted from ChEI initiation, no statistical analyses of comparisons were possible between those who benefitted (n=3) and the one who did not benefit from ChEI medication, for the NPCRA analyses. Data at baseline assessment are presented in Table 29 (p.193). Those participants who went on to benefit from the ChEI medication at baseline assessment had higher average activity counts

and activity amplitude, lower intra-daily variability, earlier L5 and M10 onset phases than the individual who did not benefit from the ChEI medication cognitively, Table 29.

Table 29. Parameters from a non-parametric circadian rhythm analysis (NPCRA) of rest-activity data derived from actigraphy for the benefit and no benefit groups at baseline assessment.

NPCRA parameter	Benefit group (n=3)	No benefit group (n=1)
Average activity count	117.97 ± 49.32	96.20
Inter-daily stability (IS)	0.61 ± 0.09	0.60
Intra-daily variability (IV)	0.92 ± 0.07	1.44
Least active five hours (L5)	15.33 ± 2.70	14.80
L5-onset phase (h)	00:15 ± 01:31	01:08
Most active ten hours (M10)	198.33 ± 84.09	165.00
M10-onset phase (h)	07:13 ± 00:03	08:50
Amplitude of activity	183.10 ± 82.27	150.30
Relative amplitude	0.84 ± 0.05	0.84

No statistical analysis was conducted.

Data are presented as mean ± SD. h represents hh:mm time format.

Full dataset is presented in Appendix 1.9. (p.30).

In terms of period length of rest-activity rhythm there were no evident differences with either methodology at baseline assessment between those who did and did not benefit cognitively from taking donepezil, Table 30 (p.195).

Although sample sizes were small, the mAD group that benefitted cognitively from donepezil administration, showed a larger difference between average activity counts (averaged over 24h) following medication initiation than was observed for the benefit group, Figure 25 (p.195). The benefit group was observed to increase activity counts after ChEI initiation between 08:00 and 14:00, whereas, little difference was observed between assessments for the individual who did not benefit cognitively from donepezil administration, Figure 25.

Table 30. Period length of activity calculated by two types of periodogram analyses from actigraphy data for benefit and no benefit of ChEI groups at baseline.

Period length	Benefit group (n=3)	No benefit group (n=1)
Lomb-Scargle method	23.98 ± 0.04	23.82
Sokolove-Bushell method	24.00 ± 0.02	24.02

No statistical analysis was conducted.

Data are presented as mean ± SD in hh:mm time format.

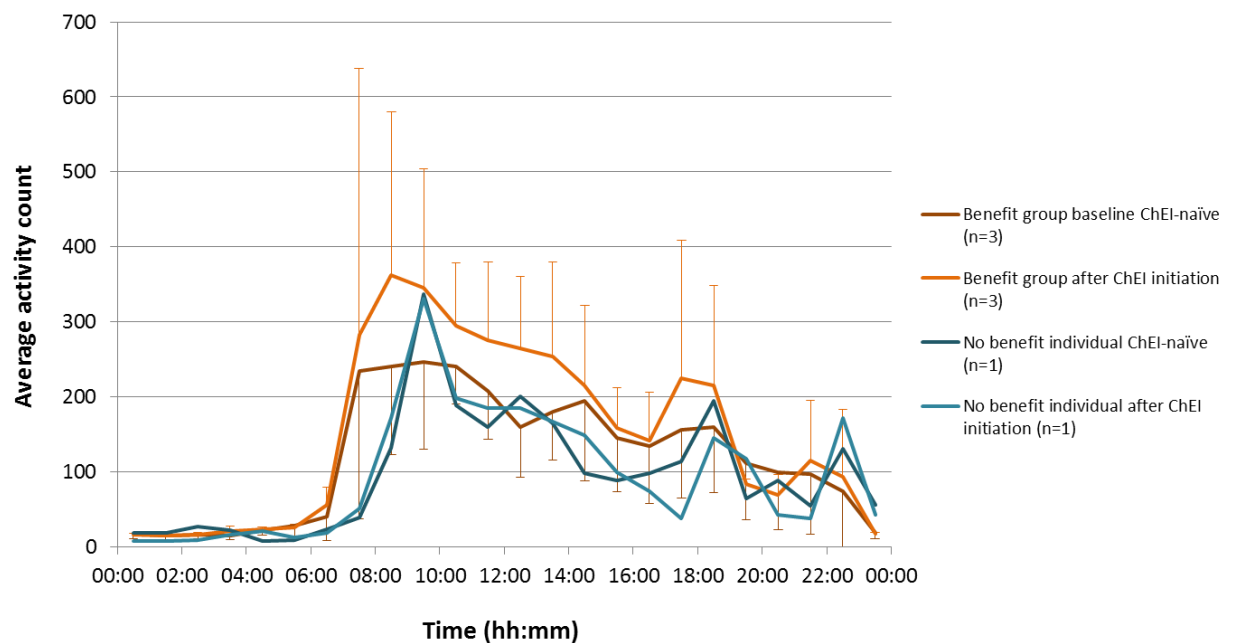


Figure 25. Averaged activity counts over 24h timescale for those who cognitively benefitted from ChEI medication and the individual that did not from the mAD group.

The benefit group are presented in orange and the no benefit individual in turquoise with lighter colours indicating second assessment for that group. No statistical analysis was conducted due to low sample sizes. Data are shown as mean with standard deviation error bars.

None of the individuals in the mAD group changed from donepezil to another cholinesterase inhibitor, none of the reverted back to a 5mg after initial dosage increase to 10mg, and they did not report sleep problems associated with taking the medication. Therefore, no rest-activity data comparisons of cholinesterase inhibitor type, presence of reported sleep problems or with dosage change of donepezil, could be carried out.

5.3.5. Sleep assessment

5.3.5.1. Effect of medication

Data comparing control change over time to change due to ChEI initiation in the qmAD group are displayed in Table 31. A significant difference in change between assessments was found in scores for PSQI component 3 (sleep duration) and component 4 (habitual sleep efficiency, $p < 0.05$).

Table 31. Pittsburgh Sleep Quality Index (PSQI) component scores for controls over time and the qmAD group when ChEI-naïve and after ChEI initiation.

PSQI component score	Baseline Assessment (drug-naïve)		Second Assessment (After ChEI initiation for qmAD group)		Statistical difference p
	Control (n=9)	qmAD (n=29)	Control (n=8)	qmAD (n=22)	
1: Subjective sleep quality	0.3 ± 0.5	0.5 ± 0.7	0.5 ± 0.5	0.6 ± 0.6	
2: Sleep latency	0.2 ± 0.7	0.8 ± 1.1	0.5 ± 1.1	0.8 ± 1.2	
3: Sleep duration	0.4 ± 1.0	0.2 ± 0.6	0.1 ± 0.4	0.5 ± 1.0	Δ*
4: Habitual sleep efficiency	0.6 ± 1.0	0.5 ± 0.9	0.1 ± 0.4	0.7 ± 1.1	Δ*
5: Sleep disturbances	1.2 ± 0.4	1.1 ± 0.4	1.0 ± 0.5	1.2 ± 0.5	
6: Use of sleep medication	0.0 ± 0.0	0.2 ± 0.8	0.0 ± 0.0	0.0 ± 0.0	
7: Daytime dysfunction	0.2 ± 0.4	0.7 ± 0.7	0.5 ± 0.5	0.5 ± 0.6	
Global PSQI score	3.0 ± 2.4	4.0 ± 3.2	2.8 ± 2.3	4.3 ± 3.1	

* $p < 0.05$, Δ indicates change over time between groups.

Data are presented as mean ± SD.

Full data, test statistics and Dunn-Šidák calculations are presented in Appendix 1.11. (p.37).

To investigate in more detail, the Jupiter sleep medical questionnaire (JSQ) was conducted; data are presented in Table 32 (p.197) comparing control change over time and qmAD change due to ChEI initiation. A significant difference was found in change of number of

hours of sleep per night and the minimum amount of sleep per night between groups between assessments ($p < 0.05$, binomial data are shown in Appendix, p.38), Table 32.

Table 32. Jupiter sleep questionnaire (JSQ) sleep characteristics of both groups for both assessments.

JSQ parameter	Baseline Assessment (drug-naïve)		Second Assessment (after ChEI initiation for qmAD group)		Stat. Diff. P
	Control (n=9)	qmAD (n=29)	Control (n=8)	qmAD (n=22)	
Sleep-onset time (week)	22:53 ± 00:37	22:09 ± 00:56	22:58 ± 00:44	22:10 ± 01:19	
Sleep-onset time (weekend)	22:53 ± 00:37	22:18 ± 00:55	23:05 ± 00:34	23:20 ± 05:20	
Usual awoken time	06:51 ± 00:44	07:20 ± 00:52	06:58 ± 00:48	07:12 ± 01:04	
Hrs. of sleep per night	07:09 ± 01:45	08:36 ± 01:42	07:36 ± 00:29	08:13 ± 02:02	Δ*
Min. amount of sleep per night	04:59 ± 02:35	07:09 ± 01:52	06:14 ± 01:19	06:51 ± 01:52	Δ*
Max. amount of sleep per night	09:09 ± 02:06	10:19 ± 02:16	09:14 ± 01:06	09:38 ± 01:56	
No. of times woke in night	2.4 ± 4.7	1.6 ± 1.1	4.5 ± 6.5	1.4 ± 1.3	
Total wake time per night	00:38 ± 01:17	00:14 ± 00:40	00:13 ± 00:08	00:08 ± 00:09	
No. of naps per week	2.7 ± 3.0	9.4 ± 8.2 (27)	4.0 ± 4.4 (7)	9.0 ± 8.7	

* $p < 0.05$, Δ indicates change over time between groups.

Data are presented as mean ± SD. Sample sizes if different from state are shown in parentheses. Full data, test statistics and Dunn-Šidák calculations are presented in Appendix 1.11. (p.38).

Sleep analysis of the rest period for actigraphy derived rest-activity data was conducted, and the results are displayed in Table 33 (p.198). No significant differences were found between assessments before and after ChEI initiation, in comparison to change over the same timeframe

in controls ($p>0.05$). However, a trend in mean activity score was observed with controls increasing in mean activity and the mAD group decreasing in mean activity in the rest period ($p>0.05$, Table 33).

Table 33. Selected sleep analysis parameters derived from actigraphy for control and mAD groups.

Parameter	Baseline Assessment (drug-naïve)		Second Assessment (After ChEI initiation for mAD group)	
	Control (n=9)	mAD (n=5)	Control (n=7)	mAD (n=6)
Bed time (h)	23:35 ± 00:50	22:14 ± 00:52	23:11 ± 00:56	21:44 ± 01:23
Get up time (h)	07:14 ± 00:38	07:48 ± 01:51	07:14 ± 00:47	07:09 ± 01:17
Time in bed (h)	07:39 ± 00:37	09:34 ± 02:12	07:51 ± 00:28	08:44 ± 02:02
Sleep start (h)	23:40 ± 00:49	22:24 ± 00:49	23:25 ± 00:46	21:31 ± 02:04
Sleep end (h)	07:14 ± 00:38	07:48 ± 01:52	07:13 ± 00:47	07:09 ± 01:17
Mean activity score	10.5 ± 4.6	14.1 ± 3.4	12.8 ± 6.4	12.8 ± 4.8
Average wake movement	279.2 ± 134.6	136.8 ± 72.7	268.9 ± 106.6	172.0 ± 91.7
Sleep efficiency (%)	90.0 ± 4.3	87.8 ± 1.4	88.8 ± 5.4	88.6 ± 3.9
Sleep latency (h)	00:05 ± 00:04	00:09 ± 00:08	00:02 ± 00:01	00:06 ± 00:02
Wake bouts	20.7 ± 9.4	24.2 ± 5.1	23.1 ± 10.5	21.7 ± 12.9
Immobile minutes	398.7 ± 35.6	469.2 ± 89.9	404.8 ± 26.4	427.0 ± 91.5
Moving time (%)	11.9 ± 5.0	16.4 ± 3.9	13.3 ± 3.4	17.0 ± 5.7
One Minute immobility	5.6 ± 3.9	11.8 ± 6.8	6.7 ± 3.4	12.2 ± 9.1
One Min immobility (%)	13.6 ± 4.8	21.1 ± 4.9	15.0 ± 4.3	23.1 ± 5.6
Fragmentation index	25.8 ± 9.6	37.5 ± 8.7	28.6 ± 7.6	40.1 ± 10.8

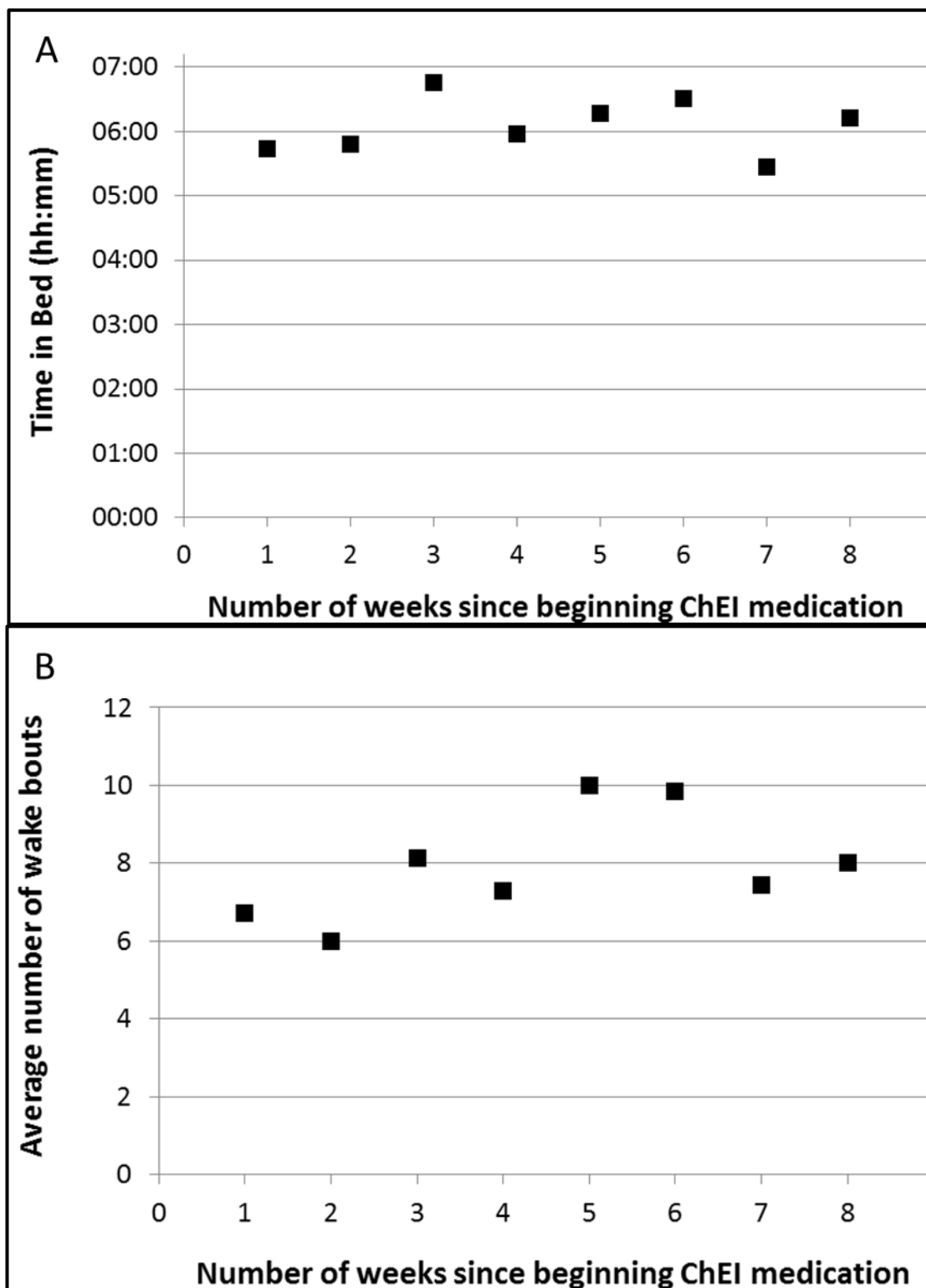
No significant differences between assessments.

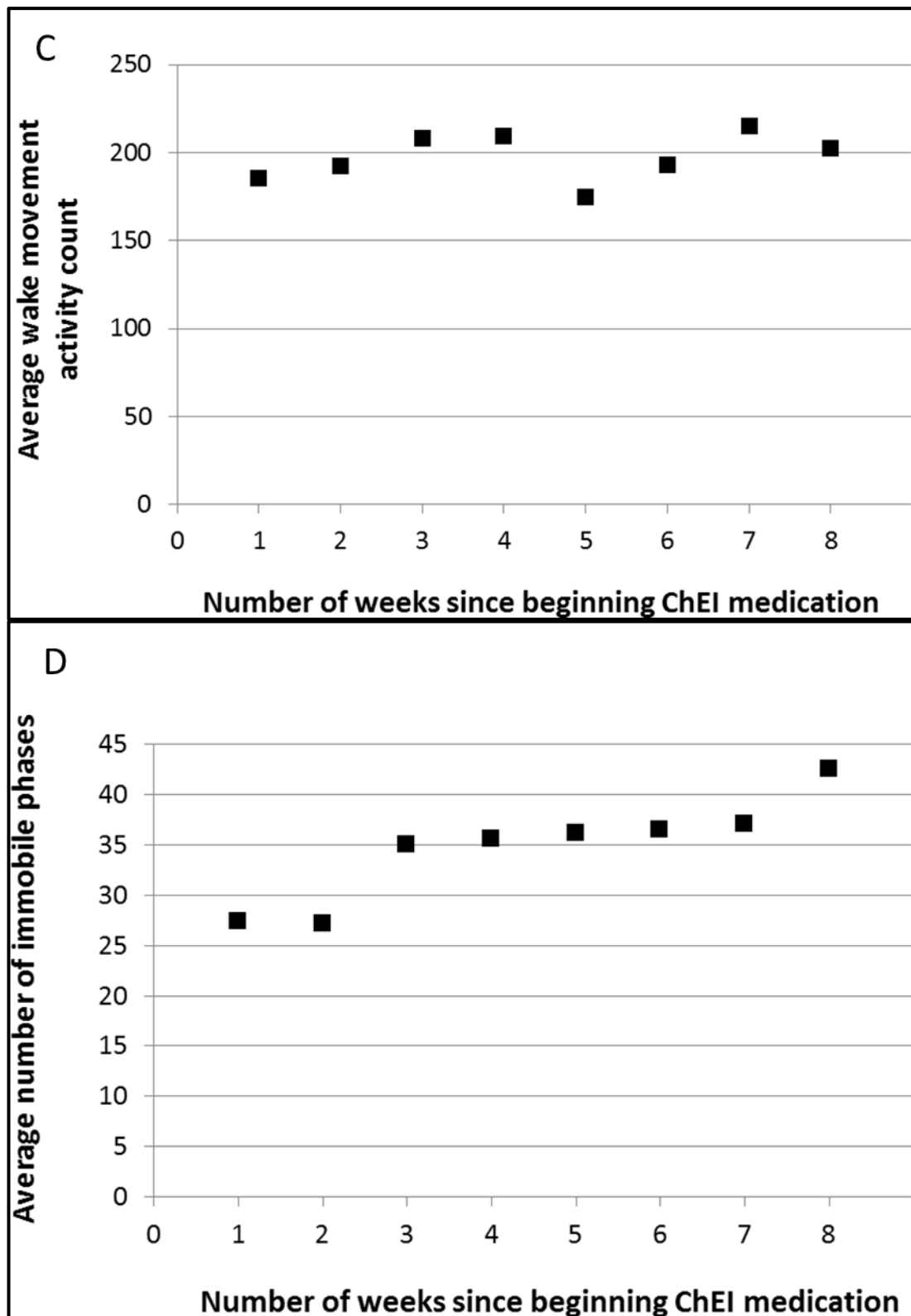
Data are presented as mean ± SD. h represents hh:mm time format.

Full dataset and Dunn-Šidák calculations are presented in Appendix 1.11. (p.47).

One individual from the mAD group was assessed with actigraphy after initiating donepezil and for the following eight weeks. Sleep analysis of each week's data are presented in Figure 33.

Time in bed (Figure 26, Graph A, p.200) remained stable across all weeks of observation and the number of wake bouts (Figure 26, Graph B, p.200) increased with increasing duration of donepezil administration. Average wake movement activity (Figure 26, Graph C, p.201) was stable with increasing duration of donepezil administration, however, a reduction in activity was observed at week 5 before increasing again. The average number of immobile phases (Figure 26, Graph D, p.201) were observed to increase gradually with duration of donepezil administration. The average number of one minute immobilities were observed to increase until the dosage was increased to 10mg, which resulted in the stabilisation of the number of one minute immobilities (Figure 26, Graph E, p.202). The same pattern was observed in the individual's fragmentation index with increasing duration of donepezil administration. There was a gradual initial increase of fragmentation, stabilising from week five after dosage increase (Figure 26, Graph F, p.202).





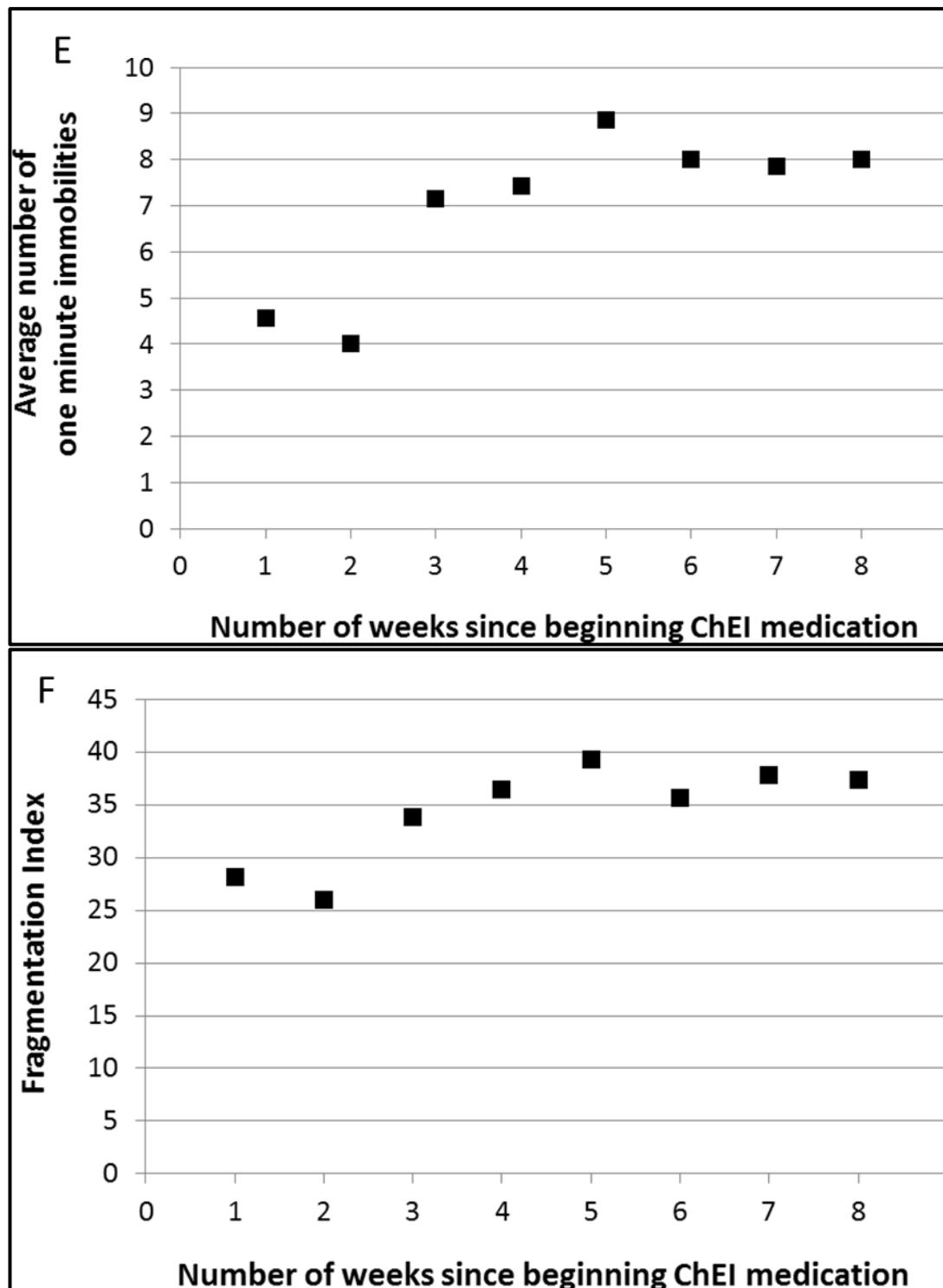


Figure 26. Weekly selected sleep analysis parameters derived from rest-activity data for a mAD individual.

Weekly analyses were conducted to track progression of each parameter for each week on donepezil from being drug-naïve. At week 5 dosage was changed from 5mg donepezil to 10mg donepezil. Graphs are as follows: A: Time in Bed; B: Average number of wake bouts; C: Average wake movement activity counts; D: Average number of immobile phases; E: Average number of one minute immobilities and F: Fragmentation index. Data points represent the mean score for each week.

Due to the small sample size, parameters of sleep macro-architecture could not be compared statistically between the controls over time and the change after ChEI initiation in the mAD group, Table 34 (p.204). Wake duration and wake after sleep onset (WASO) increased after ChEI initiation in the mAD group, with the controls also increasing over time but to a lesser extent, Table 34 (p.204). Rapid eye movement (REM) sleep duration and percentage of time in bed (TIB) decreased in both groups' sleep over time for controls and following ChEI initiation in the mAD group, Table 24 (p.204). The latency of REM sleep remained relatively stable for the control group over time, but was delayed by approximately one hour after ChEI initiation for the mAD group, although variance was high, Table 34 (p.204). Non-REM sleep duration and as a percentage of TIB remained stable in controls' sleep but decreased for the mAD after ChEI initiation, Table 34 (p.204). Sleep efficiency remained stable in the control group over time but decreased by 10% in the mAD group after ChEI initiation, although again variance was high, Table 34 (p.204). Stage 3 sleep duration and percentage decreased after ChEI initiation for the mAD group but remained stable in the controls' sleep, Table 34 (p.204).

When observing the duration of REM sleep in each third of the sleep period durations were consistent across the sleep period for both assessments in the control group's sleep, Table 35 (p.205). The mAD group showed consistent REM sleep durations in the first two thirds of the sleep period for both before and after ChEI initiation, Table 35 (p.205). However, the REM sleep duration of the final third of the sleep period after ChEI initiation was decreased from that observed at ChEI-naïve baseline assessment, Table 35 (p.205).

The individual mAD patient who was observed with EEG at both assessments showed an advance of REM sleep across the night after ChEI initiation, although no differences in REM sleep duration were seen in the hypnograms displayed in Figure 27 (p.205).

Table 34. EEG macro-architecture parameters for control and mAD groups for both assessments.

EEG parameter	Baseline Assessment (drug-naïve)		Second Assessment (After ChEI initiation for mAD group)	
	Control (n=7)	mAD (n=1)	Control (n=5)	mAD (n=2)
Time in bed (TIB)	08:04 ± 00:37	09:24	08:08 ± 00:23	08:59 ± 00:05
Sleep duration	07:44 ± 00:43	08:38	07:39 ± 00:23	07:20 ± 01:16
Wake duration	00:12 ± 00:07	00:45	00:23 ± 00:09	01:37 ± 01:08
REM duration	01:45 ± 00:21	01:24	01:36 ± 00:32	01:05 ± 00:19
REM sleep (% of TIB)	21.7 ± 4.3	14.9	19.5 ± 6.1	12.0 ± 3.5
NREM duration	05:59 ± 0:45	07:14	06:03 ± 00:25	06:15 ± 00:56
NREM sleep (% of TIB)	74.1 ± 5.3	76.9	74.5 ± 5.3	69.5 ± 9.8
REM latency	01:10 ± 01:01	01:23	01:28 ± 01:06	02:13 ± 01:21
NREM latency	00:03 ± 00:02	00:08	00:08 ± 00:11	00:03 ± 00:03
Deep sleep latency (NREM stages 3 and 4)	00:18 ± 00:10	00:20	00:24 ± 00:13	00:27 ± 00:19
Sleep efficiency (%)	95.9 ± 1.8	91.8	94.0 ± 2.0	81.5 ± 13.3
Stage 1 duration	01:12 ± 00:23	01:42	01:20 ± 00:33	01:55 ± 00:03
Stage 1 sleep (% of TIB)	14.8 ± 4.2	18.1	16.5 ± 7.2	21.3 ± 0.4
Stage2 duration	03:13 ± 00:50	02:46	03:12 ± 1:16	02:41 ± 00:24
Stage 2 sleep (% of TIB)	39.8 ± 9.0	29.4	38.9 ± 13.8	29.8 ± 4.2
Stage 3 duration	01:26 ± 00:46	02:27	01:19 ± 00:32	01:26 ± 00:28
Stage 3 sleep (% of TIB)	17.8 ± 9.2	26.1	16.3 ± 7.0	15.9 ± 5.0
Stage 4 duration	00:08 ± 00:11	00:19	00:13 ± 00:18	00:13 ± 00:01
Stage 4 sleep (% of TIB)	1.7 ± 2.4	3.4	2.8 ± 4.0	2.5 ± 0.2
Movement duration	00:08 ± 00:03	00:01	00:07 ± 00:03	00:03 ± 00:03
Movement duration (% of TIB)	1.7 ± 0.9	0.2	1.4 ± 0.6	0.6 ± 0.5
Wake after sleep onset	00:08 ± 00:05	00:36	00:15 ± 00:12	01:30 ± 01:08

**No statistical analyses conducted. Full dataset is presented in Appendix 1.11. (p.52).
Data presented as mean ± SD. Unless stated otherwise, variable is in hh:mm time format.**

Table 35. Rapid-eye movement (REM) sleep duration across the sleep period (divided into thirds) for both groups at both assessments.

Third of EEG sleep period	Baseline Assessment (drug-naïve)		Second Assessment (After ChEI initiation for mAD group)	
	Control (n=7)	mAD (n=1)	Control (n=5)	mAD (n=2)
First third REM sleep duration	00:28 ± 00:17	00:11	00:21 ± 00:18	00:15 ± 00:21
Second third REM sleep duration	00:31 ± 00:15	00:35	00:34 ± 00:09	00:32 ± 00:15
Final third REM sleep duration	00:38 ± 00:23	00:38	00:41 ± 00:06	00:18 ± 00:13

No statistical analyses conducted.

Full dataset is presented in Appendix 1.11. (p.55).

Data presented as mean ± SD in hh:mm time format.

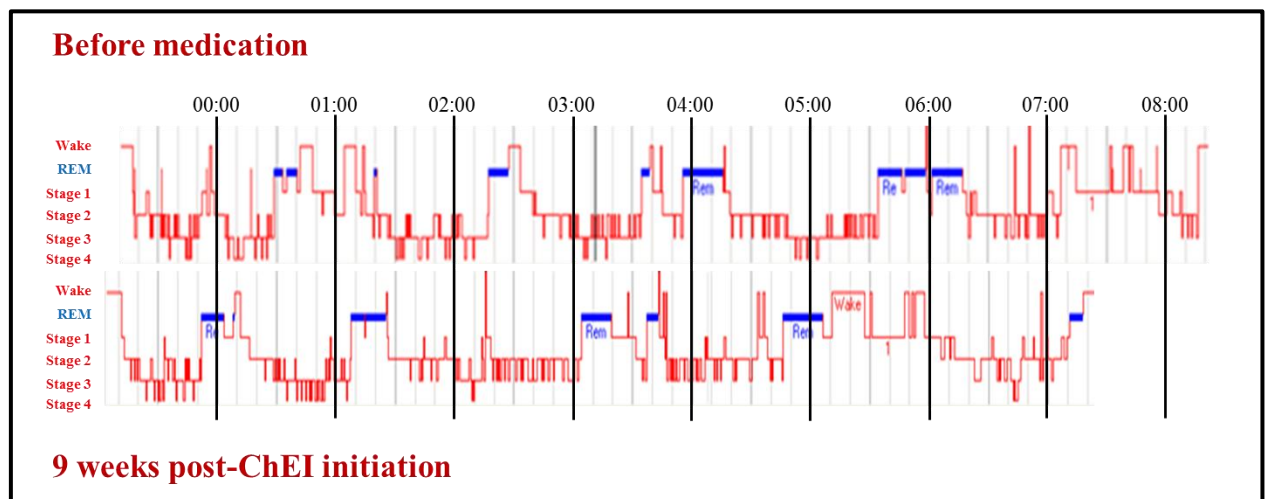


Figure 27. Hypnograms for the mAD individual assessed at baseline when ChEI-naïve and after ChEI initiation. Hypnograms depict sleep stages throughout the sleep period with stages 1-4 illustrating increasing deep sleep and with REM sleep indicated in blue. Times for both are provided above the hypnograms. The post-ChEI hypnogram shows a reduction in sleep duration, an advancement of REM sleep in the sleep period from baseline assessment (upper trace) to post-ChEI initiation assessment (lower trace) but also the presence of REM sleep at the end of the sleep period which was not present before donepezil medication was initiated. The patient maintained on donepezil throughout the course of the study, increasing dosage from 5mg to 10mg during week 5 of assessment. The patient was classified as not benefitting cognitively from the ChEI medication according to change in MMSE total score. Sleep macro-architecture is altered after ChEI medication initiation.

5.3.5.2. Cognitive benefit of ChEI administration

Those who showed benefit cognitively were found not to have any differences in sleep measured by the PSQI at baseline compared with those who did not benefit cognitively from ChEI administration, Appendix (p.37). At baseline, those who benefitted cognitively from ChEI initiation were observed to have a larger maximum amount of sleep (from the JSQ) at baseline than those who did not ($p<0.01$), Table 36. In comparison to those who did not benefit cognitively, those who did benefit had less reduction in the number of hours of sleep per night due to ChEI initiation ($p>0.05$, Table 36). The total amount of wake time per night decreased following ChEI administration in those who benefitted cognitively, whereas, those who did not benefit had increased wake duration per night after ChEI initiation ($p>0.05$, Table 36).

Table 36. Jupiter sleep questionnaire (JSQ) sleep characteristics of those who benefitted cognitively and those who did not benefit from ChEI medication.

JSQ Parameter	Baseline Assessment (drug-naïve)		Statistical difference P	Second Assessment (After ChEI Initiation)	
	Benefit group (n=11)	No benefit group (n=7)		Benefit group (n=11)	No benefit group (n=7)
Hrs. of sleep per night	09:20 ± 01:45	08:11 ± 00:49		09:01 ± 01:56	06:35 ± 01:34
Total wake time per night	00:08 ± 00:06	00:06 ± 00:02		00:06 ± 00:06	00:13 ± 00:13
Max. amount of sleep	11:53 ± 01:25	09:09 ± 02:27	*	10:25 ± 01:50	08:07 ± 01:27

* $p<0.05$ at baseline assessment.

Data presented as mean ± SD in hh:mm time format. Full dataset, test statistics and Dunn-Šidák correction calculation are presented in Appendix1.11. (p.38).

Due to the low sample sizes, no statistical comparison could be conducted of those who benefitted cognitively from ChEI administration and those who did not. However, those who benefitted went to bed and got up approximately one hour earlier, had more wake during the

sleep period, had lower activity levels, more wake bouts, more moving minutes, higher one minute immobility percentage and a higher fragmentation index at baseline (ChEI-naïve) than those who did not benefit cognitively from ChEI administration, Table 37 (p.208). In terms of change due to ChEI initiation, wake percentage increased, activity increased, mean number of wake bouts increased, number of moving minutes increased, one minute immobility percentage increased as did fragmentation index in those who benefitted cognitively, Table 37 (p.208). In comparison, those who did not benefit from ChEI initiation did not show any change from baseline levels in most sleep parameters after ChEI administration, apart from decreased activity levels, Table 37 (p.208). As only one mAD patient was assessed with EEG for sleep macro-architecture information from both assessments could not be compared between groups.

Table 37. Selected sleep analysis parameters derived from actigraphy for benefit and no benefit from ChEI medication groups at baseline.

Sleep summary parameter	Baseline Assessment (drug-naïve)		Second Assessment (After ChEI Initiation)	
	Benefit group (n=3)	Benefit group (n=3)	No benefit group (n=1)	No benefit group (n=1)
Bed time	22:01 ± 01:06	21:49 ± 01:14	22:56	23:07
Get up time	06:47 ± 01:01	06:55 ± 00:58	07:54	07:52
Time in bed	08:46 ± 02:02	09:06 ± 02:05	08:58	08:45
Sleep start	22:09 ± 01:02	21:56 ± 01:13	23:00	23:11
Sleep end	06:47 ± 01:01	06:55 ± 00:57	07:54	07:52
Assumed sleep	08:38 ± 01:58	08:58 ± 02:03	08:54	08:40
Actual sleep (%)	88.3 ± 1.1	86.9 ± 2.8	90.4	90.3
Actual wake (%)	11.7 ± 1.1	13.1 ± 2.8	9.6	9.7
Av. activity score	15.0 ± 2.1	16.2 ± 2.9	16.7	13.3
Av. wake movement	171.3 ± 63.2	217.0 ± 86.5	130.7	138.0
Total activity score	7714.8 ± 1239.5	9053.2 ± 3188.3	9025.4	6938.1
Sleep efficiency (%)	87.0 ± 1.0	85.6 ± 2.9	89.8	89.5
Sleep latency	00:07 ± 00:03	00:07 ± 00:03	00:03	00:04
Av. number of sleep bouts	24.9 ± 6.1	28.1 ± 15.6	20.2	21.5
Av. number of wake bouts	24.6 ± 6.4	28.2 ± 15.6	20.2	21.4
Immobile minutes	435.1 ± 96.2	430.5 ± 64.5	470.2	457.7
Number of moving minutes	83.4 ± 25.3	108.1 ± 66.2	64.6	62.8
Moving time (%)	15.9 ± 1.9	18.9 ± 7.9	12.0	12.1
One Minute immobility	10.0 ± 3.9	15.1 ± 13.1	6.6	7.0
One Min immobility (%)	19.6 ± 1.5	24.9 ± 6.7	17.2	17.3
Fragmentation index	35.5 ± 3.4	43.7 ± 14.6	29.2	29.4

No statistical analyses were conducted.

Data presented as mean ± SD.

Full dataset is presented in Appendix 1.11. (p.47).

5.3.5.3. Maintaining at 10mg OD donepezil or reverting back to 5mg OD

At baseline assessment, those who maintained a once-daily intake of 10mg donepezil for the duration of the study had a higher PSQI score for component five (more sleep disturbances) than those who reverted back to 5mg donepezil ($p>0.05$, Appendix 1.11., p. 37). No differences were found in sleep parameters recorded by the JSQ ($p>0.05$, Appendix, p.38) and no comparison could be made for other sleep measures as none of the mAD group reverted back to 5mg donepezil during the course of the study.

5.3.5.4. Changing to other types of cholinesterase inhibitors

Subjective sleep differences recorded with the PSQI indicated that those who remained on donepezil for the whole of the experimental period had a higher habitual sleep efficiency (lower score on PSQI component score, $p>0.05$, Appendix, p.37) at baseline than those who changed to other cholinesterase inhibitors. Analysis of JSQ parameters found at baseline a significantly larger minimum duration of sleep ($p<0.05$) and greater number of weekly naps ($p<0.05$) for those who remained on donepezil, in comparison to those who changed cholinesterase inhibitors, Appendix 1.11. (p.38). As none of the individuals who changed to other cholinesterase inhibitors were in the mAD group, no comparison could be made with the objective measures of sleep.

5.3.5.5. Sleep problems related to taking the ChEI medication

Subjective reports of sleep problems as being related to ChEI administration according to the AD patients were not detected by the subjective sleep questionnaires of the PSQI and the JSQ in change due to ChEI administration ($p>0.05$, Appendix 1.11., p.37) with no differences found between those who reported sleep problems and those who did not. None of the individuals

who reported sleep problems were in the mAD group, so no comparison could be made with the objective measures of sleep.

5.4. Discussion

5.4.1. Effect of medication

Changes in memory characteristics due to cholinesterase inhibitor (ChEI) initiation were not observed when compared to change over the same amount of time in controls. Although not significant, the mAD group did improve their reaction times for the BLC, CRT, and SST tests of the CANTABclipse test battery after initiation of ChEI treatment. The improvement of cognitive ability due to ChEI treatment has been documented previously [182] but the absence of such a result in this study may be due to the lack of a placebo-controlled group. The results presented in this chapter also show that no apparent change due to ChEI treatment was observed for either global functioning (measured by the ADCS-ADL) or social functioning (as measured by the SFS). Little change, or a slight decline in functioning has been reported previously [173] in a study which compared patients treated with donepezil *versus* a placebo group for over 24 weeks. This study showed that, for patients on donepezil functioning declined to a lesser extent than for those on placebo. Therefore, the results presented in this chapter are aligned with previous findings, but a longer assessment period may have been required to detect significant differences.

In terms of rest-activity, the relative amplitude of the least active five and most active ten hours increased after ChEI initiation, leading to an improved distinction between rest and activity phases (relative amplitude). This suggests an improvement in time-appropriate activity levels, with individuals being active during the daytime and reducing their movement during the sleep period. The individual patient who was followed every week after beginning ChEI medication did not show this change in relative amplitude, however, when the donepezil dosage was increased from 5 to 10mg this appeared to disrupt the relative amplitude with scores decreasing. This finding, therefore, could indicate that patients should only be treated

with ChEIs if the balance between cognitive benefit and rhythm disruption is favourable to the patient. To my knowledge, no previous research has studied the circadian effects of taking ChEI medication in moderate AD through actigraph measurement of rest-activity, so this is a novel finding which warrants further investigation.

Sleep duration increased and habitual sleep efficiency improved due to ChEI administration, as measured by the PSQI. Ancoli-Israel and colleagues [187] found decreases in PSQI score due to donepezil administration, a result that could not be replicated in the current study. This may be due to a lower sample size of patients not including those whose sleep improved due to the medication. However, subjectively reported sleep duration, using the JSQ, was found to decrease due to ChEI medication. This leaves a rather confused picture and adds evidence to support the need for objective measures of sleep in such studies, as discussed by Most and colleagues [188]. Sleep analysis of actigraph-derived rest-activity data did not, however, reveal any changes due to ChEI administration for the moderate AD patients. Minimal alterations in sleep percentage, derived from actigraphy in AD patients treated with donepezil, have been reported previously [187]. Possibly due to the small sample size, it was not possible to replicate these results. The individual AD patient who was followed weekly for eight weeks with actigraphy after beginning donepezil treatment, showed a gradual increase in the number of immobile phases, indicating an increasingly more fragmented sleep period. This was confirmed by gradual increases of one minute immobilities and fragmentation index score with increasing duration of donepezil administration. These findings would suggest a negative impact of donepezil on the consolidation of sleep. This negative impact on sleep consolidation might be due to the prolonged increase in circulating acetylcholine brought about by donepezil administration, which would lead to an increase in acetylcholine which was not physiologically beneficial for the regulation of sleep [189]. This is not physiologically beneficial as an increase in

acetylcholine in the evening, the time recommended by most clinicians in Oxfordshire and Buckinghamshire to take this ChEI medication, is in opposition to the normal reduction in cholinergic activity at night (except for periods of Rapid-eye movement (REM) sleep) [189]. Results from the analysis of macro-architecture derived from EEG recordings in my study, confirm the alteration of sleep due to donepezil administration. Wake duration increased due to donepezil treatment, which compliments actigraph findings of more fragmented sleep. As a neurotransmitter involved in wakefulness, increases in acetylcholine at night can be understood to increase night-time activity and wakefulness [29]. Since acetylcholine increases are known to lead to REM sleep enhancement in controls [190, 191] and in AD patients [192], it is surprising that my study found decreases in REM sleep duration and an increase in REM latency, which is the opposite of published findings. When observing the one individual patient who completed EEG measurements at both assessments, no REM sleep duration changes could be observed and there was a slight shortening of REM sleep latency in-line with previous findings [192]. This suggests that the above results showing a decrease in REM sleep duration and increase in REM sleep latency show the high variability associated with this medication acting on sleep.

Measurements of the effects of cholinergic medication on rest-activity and sleep need to be developed further. Rest-activity patterns improved somewhat, showing an increase in activity during the day and a reduction at night. However, changes in sleep showed a decline in sleep quality with increased fragmentation of the sleep period observed. Increased acetylcholine availability appeared to lead to reduced REM latency, although latencies varied largely between the two individuals observed. This study indicates that sleep alterations due to cholinergic medication could be observed in the field. However, recruitment of such patients was extremely difficult, and in order to increase sample size, more centres are needed.

5.4.1.1. Cognitive benefit of ChEI administration

A stable score, or an increase in MMSE score, was classified as cognitively benefitting and a decrease in score was classified as not benefitting. Baseline data were then analysed to determine if any parameters could predict who would benefit cognitively from the medication.

At baseline those who benefitted from the ChEI medication had higher MMSE total scores with no difference in global or social functioning observed. Those who benefitted at baseline also had higher activity counts and amplitude, and lower intra-daily variability, than those who did not benefit cognitively. This indicates that being more active generally and having a less fragmented rest-activity rhythm may predict future cognitive benefit from ChEI medication. Subjective measures of sleep, except for longer duration of maximum amount of sleep, did not indicate any factors predictive of future cognitive benefit of ChEI medication. Objective measures of sleep were analysed at baseline, with lower activity levels in the sleep period and more fragmentation over the entire sleep period, predicting future cognitive benefit of ChEI administration. Although less activity during the sleep period would suggest that 'better sleep' could predict future ChEI benefit, the overall fragmentation across the sleep period paradoxically seemed to be a more predictive factor. However, small sample size in the objective measures of sleep means interpretation of these results needs to be made with caution.

5.4.1.2. Maintaining at 10mg OD donepezil or reverting back to 5mg OD

As it is standard clinical procedure to increase the dosage of donepezil after 4 weeks stabilisation on 5mg once daily (OD) to 10mg OD, predicting in advance who will tolerate and benefit from this higher dose could be beneficial for clinicians prescribing and the wellbeing of the patients. This is of particular interest as there is currently little evidence for a proportional

increase of benefit from the dosage regimen being doubled, and increased adverse reactions are often observed with the higher dose [182]. There were no differences in terms of memory scores at baseline assessment between those who tolerated and those who did not tolerate an increase of dosage regimen to 10mg OD. Those who tolerated donepezil 10mg OD had lower instrumental and ADL total scores on the ADCS-ADL at baseline, indicating they had a greater impairment of functioning when drug-naïve than those who did not tolerate the higher dose of donepezil. Unfortunately, due to low sample sizes, objective measures of rest-activity and sleep could not be compared with confidence between those who tolerated and those who did not tolerate the higher dose of donepezil. In terms of subjective sleep measures, those who tolerated a higher dose of donepezil had more sleep disturbances, measured by the PSQI, at baseline. Therefore, it suggests that lower functioning when drug-naïve may predict future tolerance of 10mg OD donepezil. The finding of more sleep disturbances at baseline being associated with future tolerance suggests that the cholinergic system reacts better to higher doses of donepezil, possibly due to an already impaired cholinergic system leading to sleep problems [189].

5.4.1.3. Changing to other types of cholinesterase inhibitors

As time is precious to patients with neurodegenerative diseases, it is important not to delay beneficial treatment any longer than necessary. Therefore, having an adverse reaction to donepezil before being administered an alternative cholinesterase inhibitor (that may be better tolerated by the individual) can delay effective treatment and cause unnecessary discomfort to the patient. Analysis of baseline data was, therefore, conducted in this study to determine what predisposing factors could predict lack of tolerance to donepezil.

At baseline those who did not tolerate donepezil, and therefore changed to a different

type of cholinesterase inhibitor, had higher MMSE test total scores. No difference in drug-naïve functioning scores could be observed. In terms of subjective sleep reports, those who did not tolerate donepezil had lower habitual sleep efficiency (PSQI), shorter minimum duration of sleep (JSQ) and less naps per week (JSQ) at drug-naïve assessment than those who tolerated donepezil. There appears, therefore, to be little evidence of predictive factors in the data collected from this study to indicate tolerance of donepezil. This is, however, an area of research that warrants future investigation to improve quality of life and minimise adverse symptoms for Alzheimer's disease patients.

5.4.1.4. Sleep problems related to taking the ChEI medication

One particular adverse effect, sleep disturbance, due to taking cholinesterase inhibitors has been reported anecdotally. In this study, those who subjectively reported sleep problems related to beginning ChEI medication were compared at baseline to those who did not, to determine if any predictive factors could be observed.

At baseline, no differences in terms of memory ability or functioning were found between those who reported sleep disturbances related to taking ChEIs and those who did not. Unfortunately, none of the individuals whose sleep was assessed objectively reported sleep problems related to taking ChEIs. Subjective measures of sleep change due to medication, however, did not highlight any differences between those reporting sleep problems and those who did not. This finding confirms that subjective measures of sleep should be combined with objective measures, as has been previously recommended [188]. Furthermore, it appears that subjective reports of sleep disturbances at memory clinic consultations do not necessarily indicate that a severe problem is present, otherwise it would be expected that such sleep problems would be reported in the following research assessment. There is also the possibility

that, due to the subjective report of sleep disturbance to the clinical team, action was taken to reduce the problem before the research second assessment was conducted. A common response of memory clinics to reported sleep problems is to either recommend taking the medication earlier in the day (to avoid peak drug levels affecting sleep) or changing the cholinesterase inhibitor administered.

5.4.2. Strengths and limitations

The simultaneous assessment of rest-activity and sleep quality of medicated moderate Alzheimer's disease (AD) patients in the field has not been reported previously, with the combination of subjective and objective measures present in the current study. Data collection in the homes of patients is an advantage in terms of real-world conditions and new findings, but also leads to methodological limitations.

The main limitation of this study is the low sample size of moderate AD patients especially of those participating in objective measures of rest-activity and sleep. This low recruitment rate was due to the minimal period between diagnosis and prescription to approach patients and the reluctance of next of kin to delaying medication initiation. A further, more specific limitation is that the moderate AD group were observed to be older and have higher depression tendency scores than the control group, although not significant after multiple comparisons correction. As age is known to be a predictor of sleep disturbance [102, 103] this is an important consideration. Despite these considerations, alteration of sleep over a short timeframe of a couple of months and changing at a different rate due to age has not been reported previously. Depression is also understood to alter cognitive ability and impact the quality of sleep [193]. Furthermore, although depression is a common complaint associated with Alzheimer's disease, the above finding was only a tendency of showing depressive

symptoms score and no significant difference was found in the number of individuals clinically diagnosed with depression between the AD patient and control groups.

A further limitation of the study is that AD patients were classified as either benefitting or not benefitting cognitively from cholinesterase inhibitor medication, based on MMSE scores rather than on clinical assessment. This was due to large variance in the detail of medical notes and that the majority of patients deemed to not benefit remained on cholinesterase inhibitors anyway, as in the UK ChEIs are the only approved medication aimed at maintaining cognition for moderate Alzheimer's disease patients.

Another limitation of this research was that the project was constrained to comparing the effect of ChEI over time against the same period of time in a non-memory impaired healthy elderly group. A better design would have been to compare against a control group of AD patients over the same timeframe given a placebo. This, however, raises ethical issues of not providing medication to individuals with a progressive disease.

Finally, the control group recruited were not pre-analysed to refine the group to those without sleep disturbances and, in order to have a fair representation of healthy older individuals this did lead to large variance in the control group. With a larger sample size this would have been a strength of the study, as any significant effects of the medication would need to be well outside the normal variance of older people. However, with a small sample size this was a limitation to statistical analysis for the current project.

Despite the limited number of participants, this study assessing the effects of cholinesterase inhibitors on rest-activity and sleep provides future directions of potentially fruitful research.

5.4.3. Future research directions

This study indicates that a project with a larger recruitment of moderate Alzheimer's disease (AD) patients is feasible, and could result in elucidating more accurately the cholinergic effects of cholinesterase inhibitors on sleep in participants' familiar home surroundings. Furthermore, it appears that with further research employing a larger sample size, predictive factors determining who may benefit cognitively from cholinesterase inhibitors (ChEIs), and who may tolerate donepezil, could be determined. This would aid clinicians in reducing adverse reactions to medications for those who do not tolerate donepezil, as well as balancing the cognitive benefit *versus* the possible sleep disturbance on patients' quality of life. To this end, it would be advantageous to follow AD patients for a longer period of time than the 9 weeks in this study, to assess longer term effects of the medication.

During the course of the study (March 2011) the National Institute for Clinical Excellence (NICE), who dictate to clinicians which medications can be prescribed by the NHS at each stage of a disease, altered their guidelines to enable mild AD patients to be prescribed cholinesterase inhibitors [194]. This was a reversion back to their original recommendation (in operation until 2005) which will now enable more people access to the medication. None of the participants in the mild AD group of this study were prescribed a ChEI, but the ability in the future to recruit more participants with mild to moderate AD prescribed ChEIs will assist in the elucidation of the issues raised in this thesis.

Although it was not in the scope of the current project to do so, an investigation of how the timing of ingesting the ChEI tablet should be researched. It has already been discussed that due to the long half-life values of ChEIs (70h for donepezil), taking the medication just before bed is the 'perfect storm' to induce sleep disturbances as the medication increases cholinergic neurotransmission at the wrong stage of sleep [189]. Therefore, a thorough investigation is

required of how the time of day of administration of the ChEIs impacts resultant sleep disturbances.

A further research refinement (with consideration of the ethical issues) would be to include a placebo group of AD patients to compare with the ChEI medicated AD patients, rather than comparing with a non-memory impaired control group. This would, therefore, control for underlying brain atrophy changes, which alone could lead progressively to worsened sleep or rest-activity rhythm fragmentation.

Finally, the observation of the individual patient who was followed every week immediately from beginning donepezil, showed how progression of characteristics over time and due to dosage regimen changes could be studied. This method of study, though time consuming, could lead to clearer findings than from assessments performed over a month apart, especially if acute changes after starting donepezil require observation.

5.4.4. Chapter conclusions

This study has indicated the necessity of further research into determining changes in sleep and rest-activity patterns in Alzheimer's disease patients treated with cholinesterase inhibitors. Predictive factors of future tolerance of this type of medication could lead to a more streamlined prescription protocol in memory clinics and result in reduced adverse reactions and improved quality of life of Alzheimer's disease patients.

5.5. Study1 Conclusions

This project sought to further investigate the rest-activity and sleep patterns of individuals diagnosed with Alzheimer's disease of various severities and its probable prodromal stages. Rest-activity patterns and sleep have been investigated previously in Alzheimer's disease [155], although to my knowledge never concurrently in the field. Despite biomarkers of genetic predisposition [195], MRI atrophy patterns [196] and proportions of amyloid- β and phosphorylation tau in cerebrospinal fluid [197] being researched the predictive power of rest-activity characteristics or sleep abnormalities in prodromal stages have not been investigated previously.

In this project five groups of retired individuals, living in the community had their memory, daily general and social functioning, rest-activity rhythm and sleep profile assessed. The groups were as follows: control group (no subjective or objective memory problems); subjective memory impairment (SMC) group (no objective memory problem); amnesic mild cognitive impairment (aMCI) group (categorised according to Petersen criteria [60]); mild Alzheimer's disease (AD) group (diagnosed by clinical teams at memory clinics); and moderate AD group (also diagnosed by clinical teams).

Firstly, each group was compared to the control group to categorise each group's rest-activity and sleep profiles (Chapters 3 and 4, p.68 & 106). As all groups were assessed at two time-points, changes over time were calculated and all groups were again compared to the control group (Chapters 3 and 4). Baseline assessment data were then compared between all groups to determine if any parameters gradually changed as memory severity increased (Chapter 4). These parameters could then be suggested for future research as possible biomarkers for development of Alzheimer's disease. In particular, as it is still under debate whether SMC individuals are at a prodromal stage of AD, or even any different from controls

[122, 123, 198], rest-activity and sleep assessment of these two groups were compared (Chapter 3). A further facet to the project was the investigation of the effects of cholinergic medication on sleep and rest-activity in moderate Alzheimer's disease patients (Chapter 5). Data were analysed for rest-activity or sleep characteristics that, when the patient was drug-naïve could predict future tolerance of donepezil (a commonly prescribed cholinesterase inhibitor to stabilise cognition for AD patients [199]) (Chapter 5).

The data presented in this thesis leads to the following conclusions. There are differences between retired individuals who do report (SMC) and do not report (controls) memory problems despite no objective finding of a memory impairment. The assessment of SMC individuals in comparison to controls showed that the SMC group had a reduction in activity amplitude over time, subjective reports of lower sleep quality and efficiency, and higher fragmentation of sleep (actigraph-derived) in terms of more one minute immobilities and a higher fragmentation index.

Furthermore, and of special interest, is the finding that amplitude of activity was observed to decrease with increasing memory impairment, suggesting that if this is a predictor of future cognitive decline, it appears to be already present when individuals just subjectively acknowledge a memory impairment. Increasing fragmentation of rest-activity rhythm was observed as memory impairment severity increased, however, any difference between controls and SMC individuals was not observed. This indicates that this predictor may only appear at a later prodromal stage of Alzheimer's disease (AD). Although fragmentation of sleep was not observed to increase progressively with increasing memory impairment severity, all groups had higher fragmentation indexes than the control group, including the SMC group. Despite not being necessarily a progressive factor, it is still possible that this sleep disturbance, is an early predictor already present at the early prodromal stage of subjective memory complaint.

Finally, this thesis has shown that, following the administration of cholinesterase inhibitors, improvements to rest and activity definition and sleep efficiency were observed. An individual patient had his rest-activity rhythm recorded from the onset of the medication and for the following eight weeks. From his data a gradual increase in the fragmentation of sleep and an effect of dose increase, which lowered the rest-activity amplitude were detected. Overall, those patients who went on to tolerate the medication well and benefit cognitively from its administration, had previously shown higher levels of activity during the day-time and lower levels of activity during the sleep period when drug-naïve.

There were two major limitations to this projects' data collection. Firstly, recruitment was very difficult with a low sample size obtained in all groups despite over ten recruitment centres across Oxfordshire and Buckinghamshire being involved in the project. This all impacted the second limitation of this project which was the malfunction of the electroencephalography hardware. This led to a reduced dataset, resulting from the limitations to the objective measurement of sleep in this project.

Despite these limitations, this study has shown interesting results which warrant further investigation. The study results contribute to the understanding of not only sleep and rest-activity in these patient groups, but also of how the progressive increase of memory impairment severity is associated with rest-activity and sleep.

6. Study 2: Terminal Brain Cancer

6.1. Introduction

The region of the circadian pacemaker was first described in rats by Richter in 1965 when ablating areas of the body to determine what lesion resulted in the loss of circadian rhythmicity [200]. Since this seminal work the suprachiasmatic nucleus (SCN) has been ablated in many studies to assess resultant behavioural changes [10, 201, 202]. The first experiments were conducted in rodents [201]. Ablation of the bilateral SCN resulted in the loss of the circadian rhythms of rest-activity, feeding and drinking behaviour [10]. Following these experiments non-human primates were investigated [11]. This study showed that Rhesus monkeys with complete SCN ablation lost their melatonin rhythmicity. However, the same study also showed that monkeys with partially ablated SCN exhibited melatonin rhythmicity but the usual effect of the light-dark cycle in entrainment and melatonin suppression was absent. This could have been a result due to the finding that when only the posterior SCN of squirrel monkeys are ablated the saliency of external stimuli to act as a zeitgeber is altered [12]. A further study investigating partial SCN lesions assessed the effect of auditory time-of-day cues on squirrel monkeys with partial or complete lesions of the SCN [13]. Monkeys with full lesions could not be entrained to these auditory cues of time-of-day, however, those with partial SCN lesions could [13]. Another study in the effects of hamster unilateral SCN lesions and the extent of partial lesion affecting SCN rhythmicity indicated that full ablation of the SCN is required to alter rhythmicity, whilst partial lesions seemed to have no or only minor effects [203].

It is unknown whether the same conclusions can be drawn about human SCN ablation. The majority of studies in humans have been historical reports of the consequences of natural

or surgical lesions of the SCN [204]. These lesions were linked to symptoms of drowsiness, hypersomnia, insomnia and restlessness. It could therefore be inferred that these lesions were affecting the SCN – directly or indirectly. One particular study found disruption to circadian rhythmicity to be linked to a discrete lesion of the SCN [205] . Most other studies, however, preceded the precise localization of the SCN in man. Therefore, it is unknown whether humans' circadian rhythmicity could be affected by only partial ablation/lesion of the SCN.

There are many other factors that affect the rest-activity rhythm in humans, for example ill health, medications and social obligations [21]. Choice in bedtime or get-up-time can be influenced by personal preference (chronotype), obligations and possibly time perception [21]. An initial study of temporal perception determined that time processing was independent of light intensity and core body temperature but correlated with duration of preceding wakefulness [206]. There appear to be two different neural mechanisms one which processes activities over millisecond intervals and another that processes longer durations of time [207]. Although difficult to investigate, MRI data suggest that time processing for durations longer than seconds is cognitively controlled by areas in the thalamo-cortical-striatal circuit [208, 209]. Although it is difficult to assess subjective time perception, objective measures such as personal and familial reports, as well as objective measures of cognitive ability and rest-activity rhythmicity, can be utilised.

In this chapter the case of JJB is presented, a 62 year old man, who presented with sleep-wake rhythm abnormalities after right temporal lobe resection due to a temporal lobe glioblastoma grade IV. Glioblastoma (GBM) is the most aggressive and common type of brain tumour [210]. The tumour consists of malignant glial cells with areas of necrosis and microvascular proliferation. Diffuse infiltration of grey and white matter remote from the main tumour bulk is characteristic of this neoplasm [211]. Very low survival is associated with GBMs

with the majority of patients not surviving after 12 months, despite resection surgery [212]. Patients with GBM, as well as other types of cancer, frequently report sleep disturbance and fatigue which is thought to reduce quality of life [213]. Furthermore, steroidal use, often part of treatment post-surgery, is known to alter sleep patterns, therefore, influencing rest-activity pattern [214]. Family reports of JJB documented temporal misperception of up to five months before resection surgery, continuing for the remainder of his life. In order to assess the impact of JJB's temporal misperception, an understanding of glioma location and the state of JJB's cognition needed to be determined. It is unclear whether his abnormal sleep-wake rhythm was due to a distorted signal of his biological clock, therefore, this was investigated. This chapter describes the assessment of JJB's rest-activity profile, melatonin rhythm as a circadian rhythmicity marker, cognition and post-mortem brain tissue. This chapter seeks to address the research question:

- ***Do rest-activity patterns in a terminal brain cancer patient relate to underlying circadian disruption and if so, how were the suprachiasmatic nuclei affected by cancer cell infiltration?***

6.2. Methods

We had approval from all clinicians involved in the care of JJB and his family to conduct our investigation into his circadian profile and cognition. The brain donation was carried out as per the procedures of the Thomas Willis Brain Bank, Oxford, UK.

6.2.1. Patient Characteristics and Presentation

Patient JJB was a 62 year old, male academic. Prior to diagnosis with glioblastoma grade IV, JJB had no on-going conditions or medications. A timeline of presentation, diagnosis and observations are displayed in Table 38 (Date=dd/mm/yyyy).

Table 38. Assessment and presentation timeline of this study.

Date	Aspect of presentation
04/07/2009	Short term memory problems first noticed
17/09/2009	Temporal misperception first noticed (waking for breakfast after an afternoon nap)
27/10/2009	Initial medical presentation after fall
27/10/2009	First CT scan indicating tumour and midline shift
02/11/2009	Resection surgery
10/11/2009	Post-op MRI scan
18/11/2009	Eye exam finds nothing, short term memory problems noted
08/12/2009	Concomitant Temozolomide (TMZ) (160mg o.d.) and Radiotherapy 2Gy per session (weekdays)
22/12/2009	Eye exam finds some change, diplopia when tired
21/01/2010	First session of Temozolomide ends, Dexamethasone (1.5mg o.d.)
24/02/2010	Second session of Temozolomide (daily 320mg)
25/02/2010	<i>Our investigation of the case begins measuring rest-activity rhythm</i>
28/02/2010	Second session of Temozolomide ends
05/03/2010	<i>Cognitive testing conducted</i>
15/03/2010	Dexamethasone (8.0mg o.d.) due to significant deterioration
22/03/2010	MRI scan shows progressive disease (possibly pseudo-progression due to TMZ)

Date	Aspect of presentation
24/03/2010	Third session of Temozolomide (daily 320mg)
28/03/2010	Third session of Temozolomide ends
21/04/2010	Dexamethasone reduced (now 6.0mg o.d.)
28/04/2010	Decision to stop Temozolomide made due to allergic reaction (rash, treated with topical steroid)
<i>07/05/2010</i>	<i>Urine collection to assess melatonin rhythm begun</i>
<i>10/05/2010</i>	<i>Urine collection ends</i>
20/05/2010	MRI scan shows stable tumour mass, reduced swelling and midline shift since 22/3/2010
02/07/2010	Time of death 16:45pm
05/07/2010	Post-mortem brain, spinal cord and pineal gland removed

Times and details of medication, chemotherapy and radiotherapy sessions, scans and observations. Observations carried out are indicated in bold italics.

Patient JJB initially presented due to fall and inability to regain standing position. Diagnosis of brain tumour was made following a CT scan showing a heterogeneous lesion in the right cerebral hemisphere involving most of the temporal and frontal lobes, basal ganglia and thalamus crossing the anterior midline, with a significant midline shift. Furthermore, the effects of a head injury the previous week were also apparent as the brain was swollen with areas of bleeding. Following diagnosis resection surgery was conducted to remove the right temporal lobe. In surgery the brain was discovered swollen, with the tumour consisting of areas of necrosis and xanthochomil fluid. Tissue samples concluded that the tumour was in fact a high-grade glioma containing gemistocytes and pleomorphic astrocytes, many of which had mitoses. The glioma was found to infiltrate the right posterior temporal area, right insula and right frontal lobe as well as deeper brain structures. Resection was conducted from the anterior of a large temporal draining posterior vein towards the transverse sinus. Resection was limited due to the extensive nature of the tumour infiltration. Post-op JJB recovered well with no evidence of hematoma or neurological

deficits. Details and timing of post-operative medication, chemotherapy and radiotherapy as well as observations are displayed in Table 38 (p.227). Also indicated in Table 38 (p.227), JJB experienced temporal misperception months before glioma diagnosis. These temporal misperceptions are detailed and put into the context of JJB's diagnosis and treatment in Figure 38 (p.227). Patient JJB presented to the authors by family referral due to his misperception of time leading to behaviour that did not appear to be entrained to a light-dark cycle, details of recruitment are provided in Chapter 2 (p.36)

Dexamethasone was administered as the adjuvant steroid doses altered throughout study, as noted in Figure 30 (p.239). Chemotherapy and radiotherapy were given concomitantly after surgery with 3 cycles of chemotherapy alone (temozolomide), for details see Table 1. A topical steroid was administered to treat the allergic reaction to chemotherapy and omeprazole was administered orally for nausea. No anti-convulsants were administered throughout.

All assessments of JJB carried out in this study are shown in Figure 3 in Chapter 2 (p.34).

6.2.2. Cognition and temporal perception

Cognitive assessment of JJB was compared to the healthy control group of Study 1 to determine the extent of his memory impairment (mean age: 73.2 yrs $\pm$ 4.9yrs, 9 individuals: 4 females), more information and further assessment of these controls can be found in Chapter 3 (p.68) with recruitment details provided in Chapter 2 (p.36). The Mini-Mental State Examination (MMSE) was administered to assess memory impairment extent, details in Chapter 2 (p.40) [17]. Furthermore the CANTAB battery of cognitive and attention tests was administered as described in Chapter 2 (p.40). In order to assess JJB's temporal misperception familial reports were collected, these are shown in Figure 28 (p.234).

6.2.3. Circadian assessment

6.2.3.1. Diurnal preference

Diurnal preference (sleep timing preference) was assessed by Morningness-Eveningness Questionnaire (MEQ) [23], details can be found in Chapter 2 (p.38).

6.2.3.2. Rest-activity rhythms

Rest-activity rhythms were measured with an AW7 Actiwatch from CamnTech Ltd, for details see Chapter 2 (p.43). Data were collected over two periods, shown in Figure 30 (p.239), in one minute epochs. Data were divided into bins determined by steroid (dexamethasone) dosage due to known dosage-dependent sleep-altering effect of the drug [16]; bins are marked in Figure 30 (p.239) indicating each bin length. Also, in order to determine whether a stable rhythm was found throughout the observation, the activity was divided further into periods of 6 days each; these periods can be observed in figure 30 (p.239). Periodogram analysis to assess periodicity in the activity data was conducted using both Lomb-Scargle's analysis, and Chi-square periodogram analysis developed by Sokolove and Bushell [73, 74]. Both types of periodogram analysis were compared, details can be found in Chapter 2 (p.46). In order to determine the phase of activity onset for each period, minute-by-minute data over 24 h was calculated and graphed; methods can be found in Chapter 2 (p.46).

6.2.3.3. Circadian Profile using melatonin data

In order to determine whether or not JJB had an endogenous circadian rhythm, a circadian clock marker, melatonin was assessed. Details of the urine collection procedure are provided in Chapter 2 (p.47). In order to determine the acrophase and amplitude of the melatonin peak, a non-linear regression model (as defined in [79]) was fitted to the data in Microsoft

Excel 2010. Then linear cosinor analysis was conducted for the correct period length using CircWave version 1.4 to generate the fitted curve with curve parameters generated using CircWave Batch version 50 (both courtesy of Roelof Hut). Both of these methods are fully described in Chapter 2 (p.47).

6.2.3.4. Sleep quality and characteristics

Subjective sleep quality was assessed by the Pittsburgh Sleep Quality Index (PSQI) [64] and further sleep information was collected using the Jupiter Sleep Questionnaire (JSQ) [65]; details can be found in Chapter 2 (p.51).

6.2.4. Assessment of Suprachiasmatic nucleus infiltration

6.2.4.1. Tissue collection and storage

After cold storage the brain and spinal cord were removed 63 hours post-mortem under the supervision of Dr. Olaf Ansorge, Neuropathology Group, Nuffield Department of Clinical Neurosciences, University of Oxford. They were then stored in formalin and kept in a cool storage room until dissection.

6.2.4.2. Immunocytochemistry

The SCN was located through 5mm coronal sections using a human brain anatomy atlas [215] carried out by Dr. Ansorge with location advice from me. Sections were taken from paraffin embedded blocks and processed for haematoxylin and eosin and Luxol-fast-blue-cresyl-violet staining. Immunohistochemistry for vasopressin was used to further define the SCN in serial sections, further details of all immunohistochemistry can be found in Chapter 2 (p.58). [216, 217]. All sections, embedding and immunohistochemistry was carried out by

the Neuropathology group, Nuffield Department of Clinical Neurosciences, University of Oxford. Post-mortem findings were correlated with magnetic resonance images of the brain four months prior to death (22/03/2010).

6.3. Results

6.3.1. Cognition and Temporal Perception

In order to assess the temporal misperception that JJB experienced we first noted familial subjective reports of his behaviour. These reports are summarised in the timeline shown in Figure 28 (p.234) in the context of his diagnosis, surgery and our assessment of his cognition. Reports suggest his inability to have perceived the difference between night and day leading to unusual time-of-day behaviour. On assessment JJB was found to have mild memory impairment restricted primarily to reaction time, orientation to time and space and short-term memory impairments (Figure 29, p.235). There appeared to be no difference in age between JJB and the control group, enabling comparisons to be made. Global memory function measured by the MMSE was found to be impaired in comparison to control. However JJB appears not to have largely impaired orientation to time and space in comparison to controls.

Reaction times were compared by the computerised cognition battery (CANTAB) and for tests BLC, CRT and SSP (see methods for details) JJB had longer latencies (BLC: 1301.2ms, CRT: 673.4ms and SSP: 3253.0ms) in comparison to controls (BLC mean: 776.2ms $\pm$ 71.6 and CRT mean: 395.2ms $\pm$ 58.6) as shown in Figure 29 (p.235). In addition JJB was impaired in visual memory and new learning as shown by the inability to complete the first stage of the PAL test on first attempt, as seen in Figure 29 (p.235).

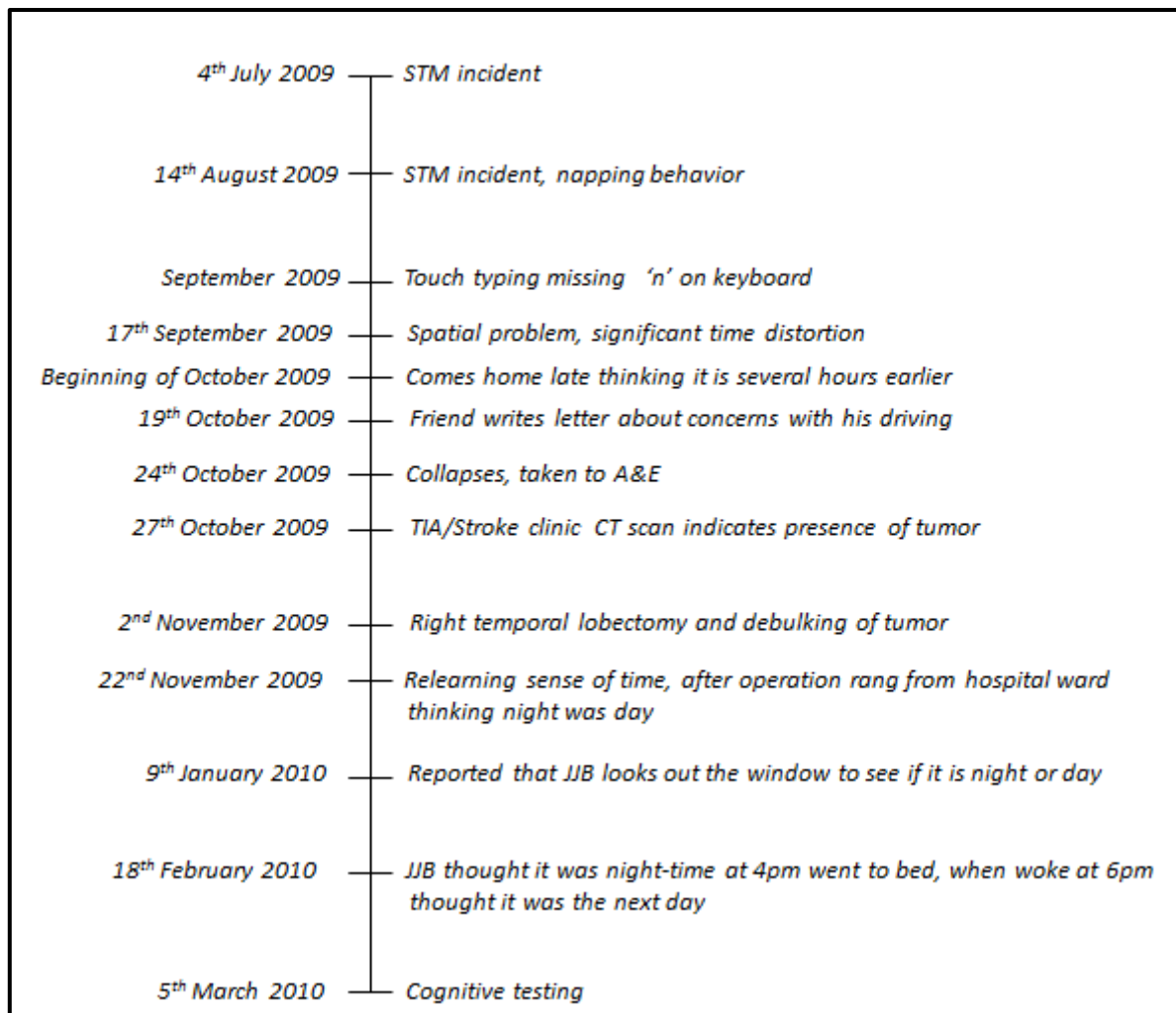
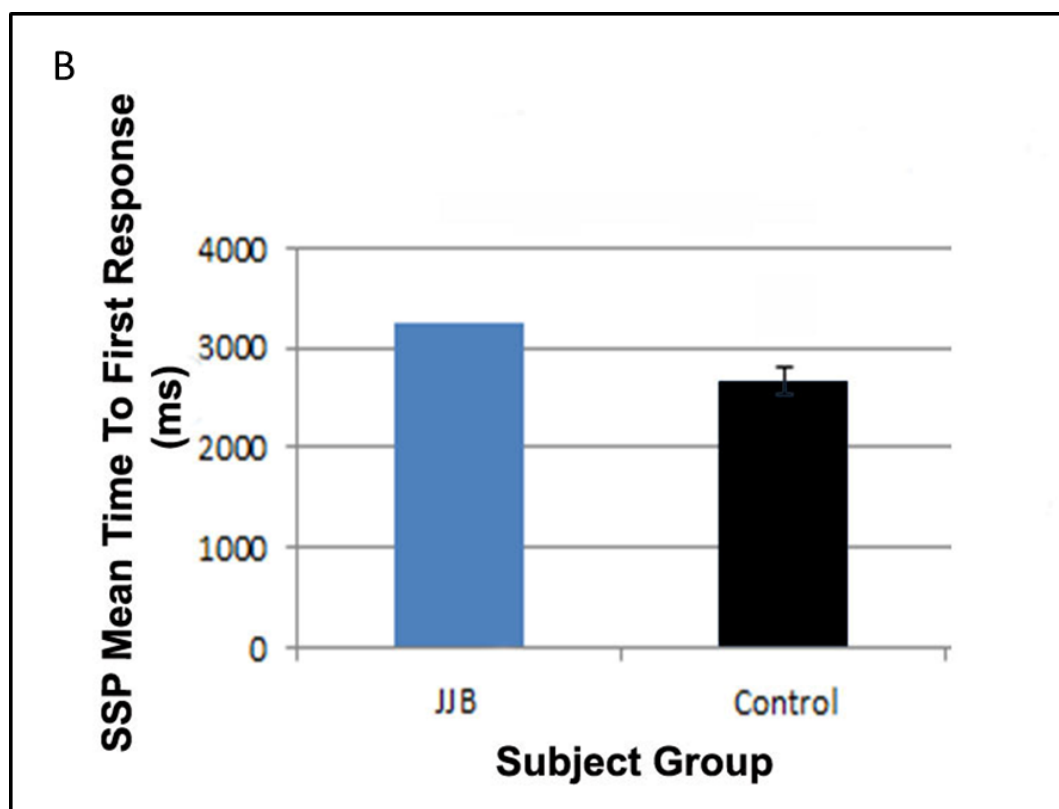
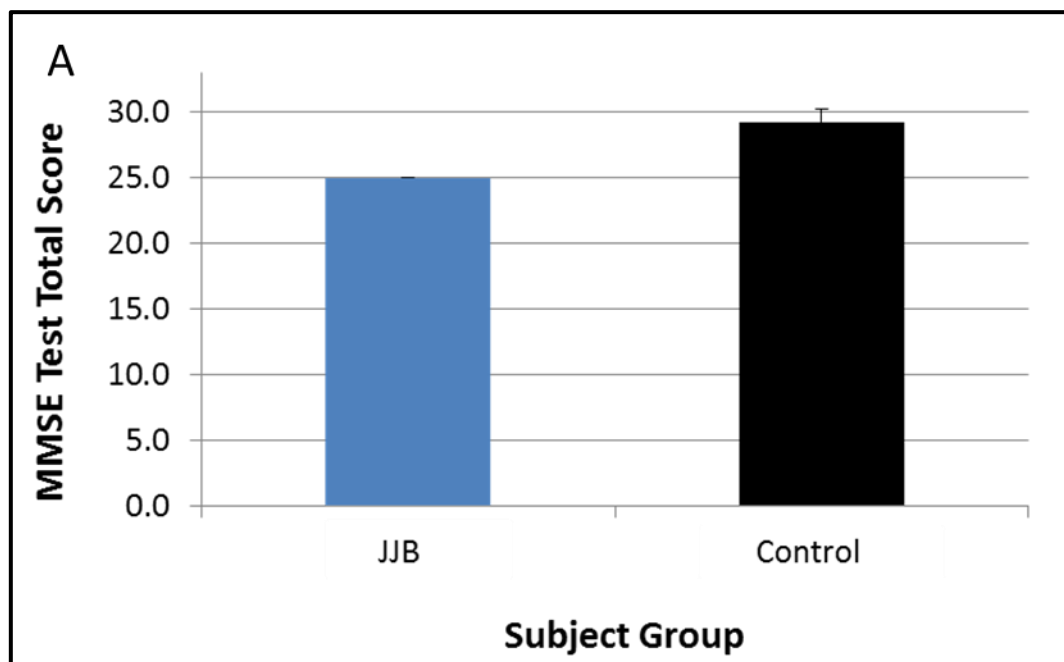


Figure 28. Timeline of temporal misperceptions in the context of JJB's diagnosis and treatment. All temporal misperceptions are that of familial reports as JJB denied any misperception. STM stands for short term memory



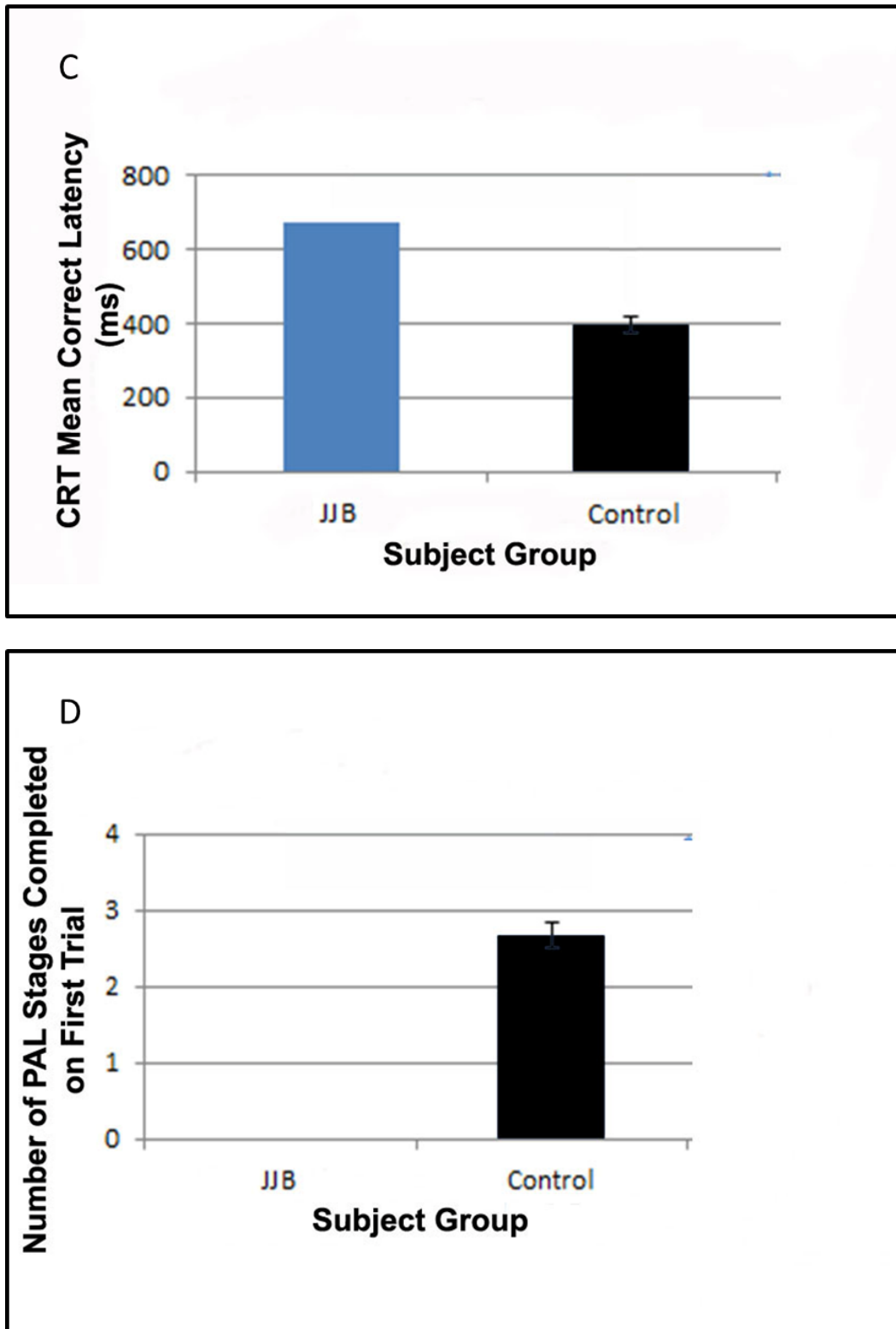


Figure 29. Cognitive parameters that were altered from healthy controls. The Mini Mental State Examination (MMSE) test total is shown in graph A. Graphs B-D show a selection of parameters from the CANTAB computerised cognition battery. SSP is the spatial span test assessing executive function; CRT is the choice reaction test assessing attention and the PAL is the paired associates learning test assessing visual memory. Patient JJB is found to have a longer reaction time (latency) on a variety of tests (B and C) and is unable to match two pairs of diagrams on first attempt indicating impairment in visual memory and new learning (D). All graphs are mean $\pm$ SD for the control group.

6.3.2. Circadian Functioning

At the outset of the study JJB's diurnal preference (preference for late or early waking and going to bed) was assessed. It was found that JJB had a moderately morning type (MEQ score: 64) which when compared to the control group was not different (control mean MEQ score: 66.7 ± 6.8 [moderately morning]).

Rest-activity was plotted in actograms as shown in Figure 30 (p.239), marking missing data, chemotherapy sessions, medication doses and observations (MRI scans, cognitive testing and urine collection for melatonin rhythm analysis). All activity was divided into bins based upon steroidal dosage (bins in purple and doses in blue, Figure 30, p.239). Averaged whole bin activity levels are of low amplitude throughout assessment starting at 2231 activity counts in bin 1 and reaching 919 activity counts in bin 3 before raising slightly in bin 4 to 1225 activity counts, even when caregiver-induced activity counts (marked in Figure 30, p.239) are removed. Activity appears consolidated to daylight hours, however, differences between activity levels in daylight and night-time become harder to distinguish. Each bin was assessed with periodogram analysis to determine whether a significant circadian rhythm could be found in the data. The periodogram analyses are graphed in Figure 31 (p.242). Lomb-Scargle analysis found a significant circadian period for all bins, $p < 0.001$. Bin 4 was re-analysed after caregiver induced activity was removed, remaining activity was still determined to be rhythmic, however, the period length changed from 23.9h to 24.03h.

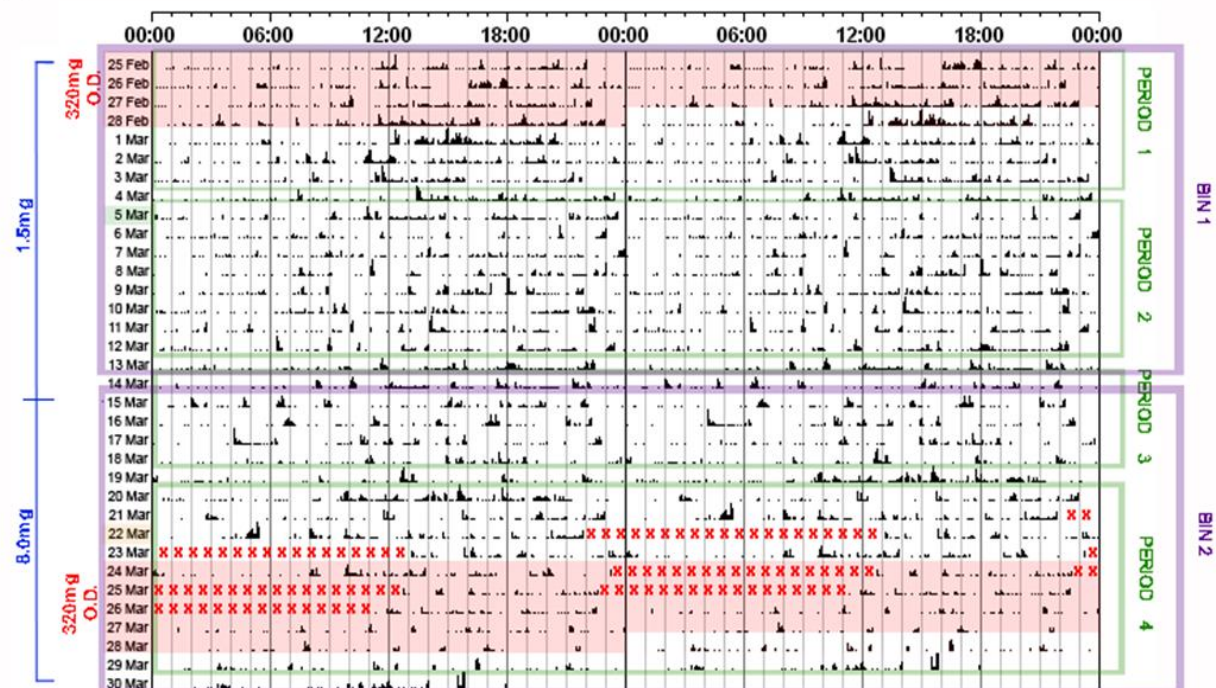
In order to assess the periodicity of the rest-activity at a higher magnification smaller periods of data were created containing a minimum of six days (Figures 32, p.247). As with the bins of data, amplitude of activity was observed to decrease throughout the course of assessment (Figures 32, p.247) with the highest activity count of 2231 occurring in the second period decreasing to 548 activity counts in the last period of JJB's life (Figure 32,

p.247). All but one of the periods were significantly rhythmic as assessed by the Lomb-Scargle method, with a period of approximately 24 hours (ranging from 23.7h to 24.85h). There was one exception, and that was the last period of JJB's life when the period was found to be 22.55h when caregiver-induced activity was left in the data set and 22.05h without caregiver-induced activity.

In order to assess the ability of the SCN to generate 24h rhythmicity, melatonin excretion rate was used as a phase marker and fitted to a non-linear regression model, plotted in Figure 34 (p.249). The regression model found a significant rhythm in melatonin excretion rates with a period of 24.0h (Table 39, p.249). Once period was determined, data were analysed with linear cosinor analysis which confirmed the rhythm characteristics (Table 40, p.249). From this analysis the curve was fitted to the data as seen in Figure 34 (p.249), showing an advanced acrophase of aMT6s excretion. A comparison of parameters can be found in Table 41 (p.249) showing very little difference between the results of both methods.

The averaged 24h activity profiles are shown in Figure 33 (p.248) to indicate that activity was time-of-day appropriate. As can be seen in Figure 33 (p.248), amplitude begins relatively high at the beginning of assessment and gradually decreases as the disease progresses. Activity in period 1 appears consolidated to daylight hours however even by period 2 this distinction is less noticeable. Despite the larger spread of activity throughout the 24h day, activity does remain higher during daylight hours than nighttime hours throughout and no shift (advance or delay) can be observed. Therefore despite activity decreasing in amplitude and spreading more throughout the 24h day, activity does not appear to shift in phase.

A



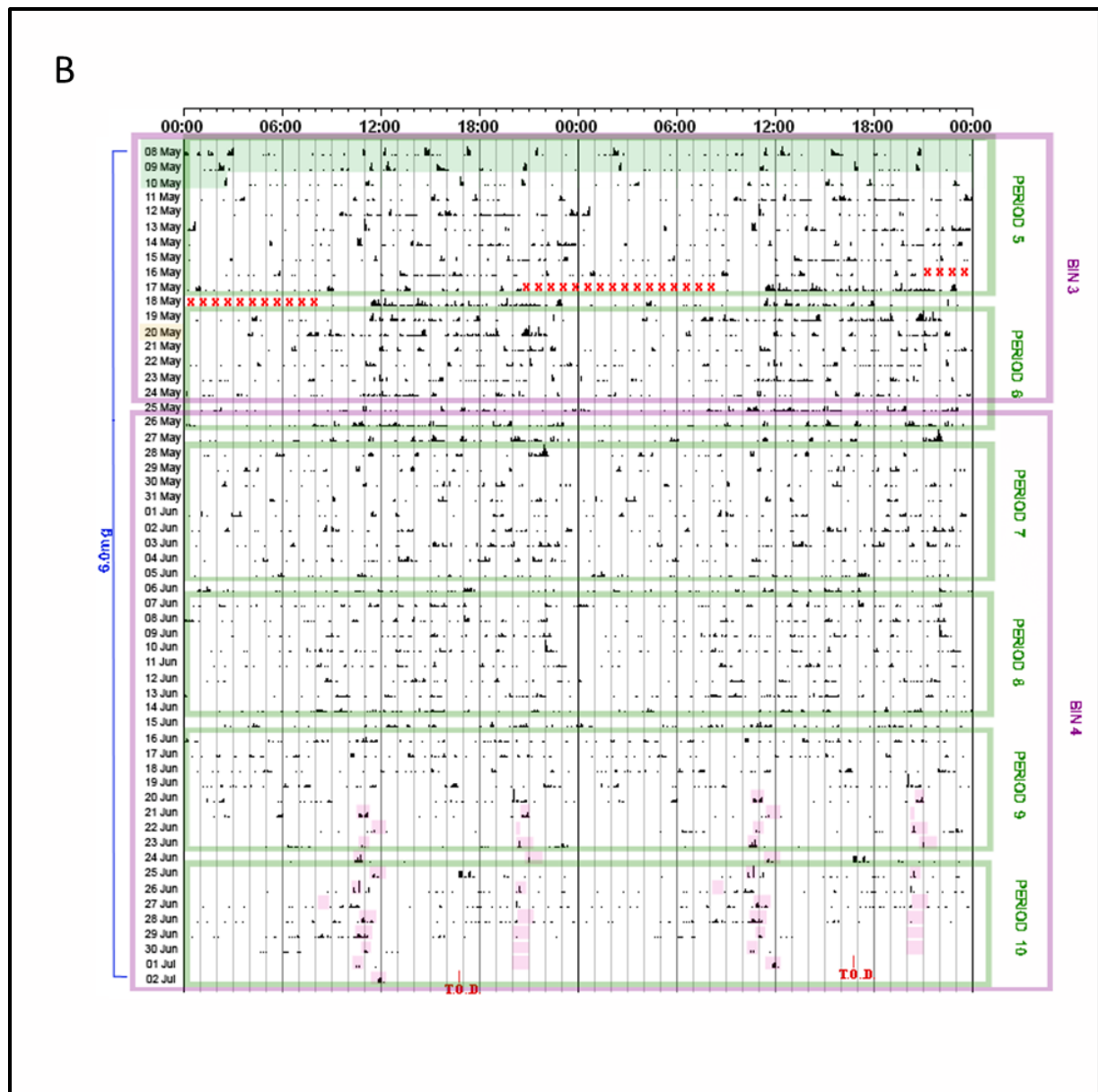
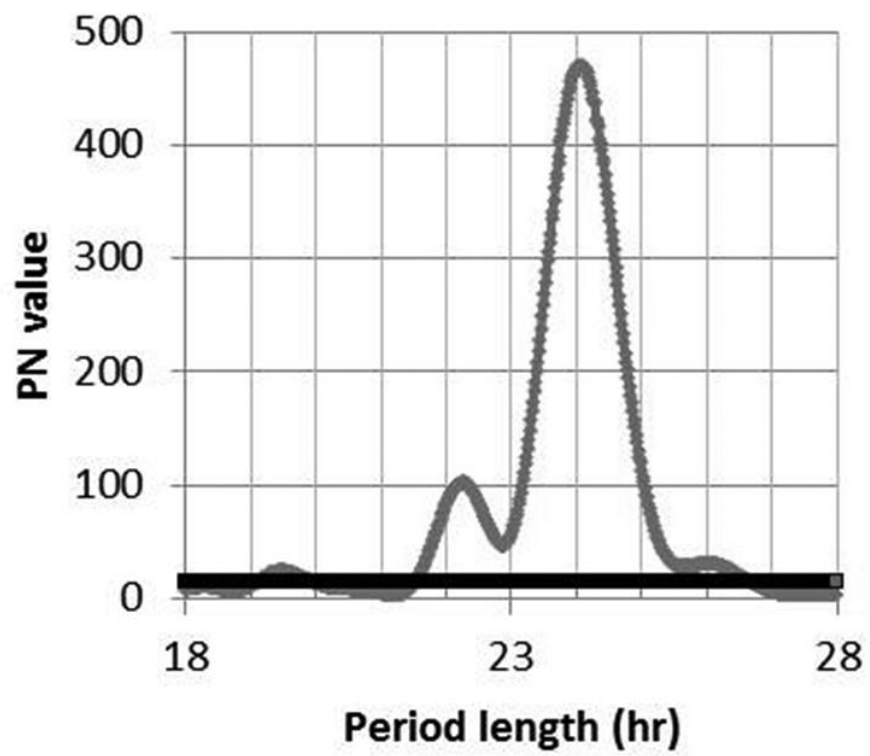
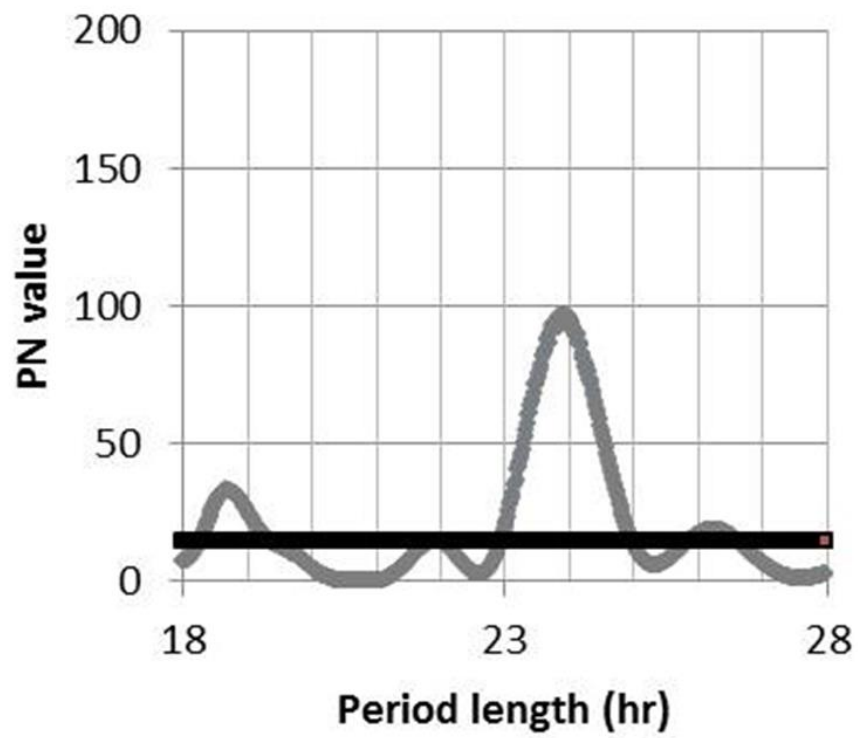


Figure 30. Two assessment periods of rest-activity monitoring. The actograms are double plotted with black lines indicating activity count for each minute. The actograms are scaled to 1200 activity counts. Red highlights indicate periods of chemotherapy (temozolomide) with the dosage also in red. The division of the rest-activity into bins is indicated by the purple boxes based upon dosage of steroid (dexamethasone) indicated in blue to the left of the actograms. Activity data are further divided into periods of 6-9 days shown in the green boxes in order to assess progressive alterations over time. The day of cognitive testing is highlighted in green and the date of MRI scans are highlighted in orange. Missing data are indicated by red crosses. The urine collection period is highlighted in green and the date of MRI scan is highlighted in orange. The acrophase of melatonin is indicated by orange stars during the urine collection period. Caregiver assistance possibly influencing JJB's activity levels are marked by pink highlights. Time of death is indicated by the red line and 'T.O.D.' Actogram A indicates rhythmicity in the rest-activity of JJB at the beginning of our observations with little alteration due to chemotherapy. Activity remains clustered to daylight hours throughout however amplitude is reduced from that of healthy controls and decreases throughout our observations. Actogram B shows continued rhythmicity of the rest-activity pattern. Amplitude continues to reduce over time and more activity is observed during night-time hours.

A: BIN 1



B: BIN 2



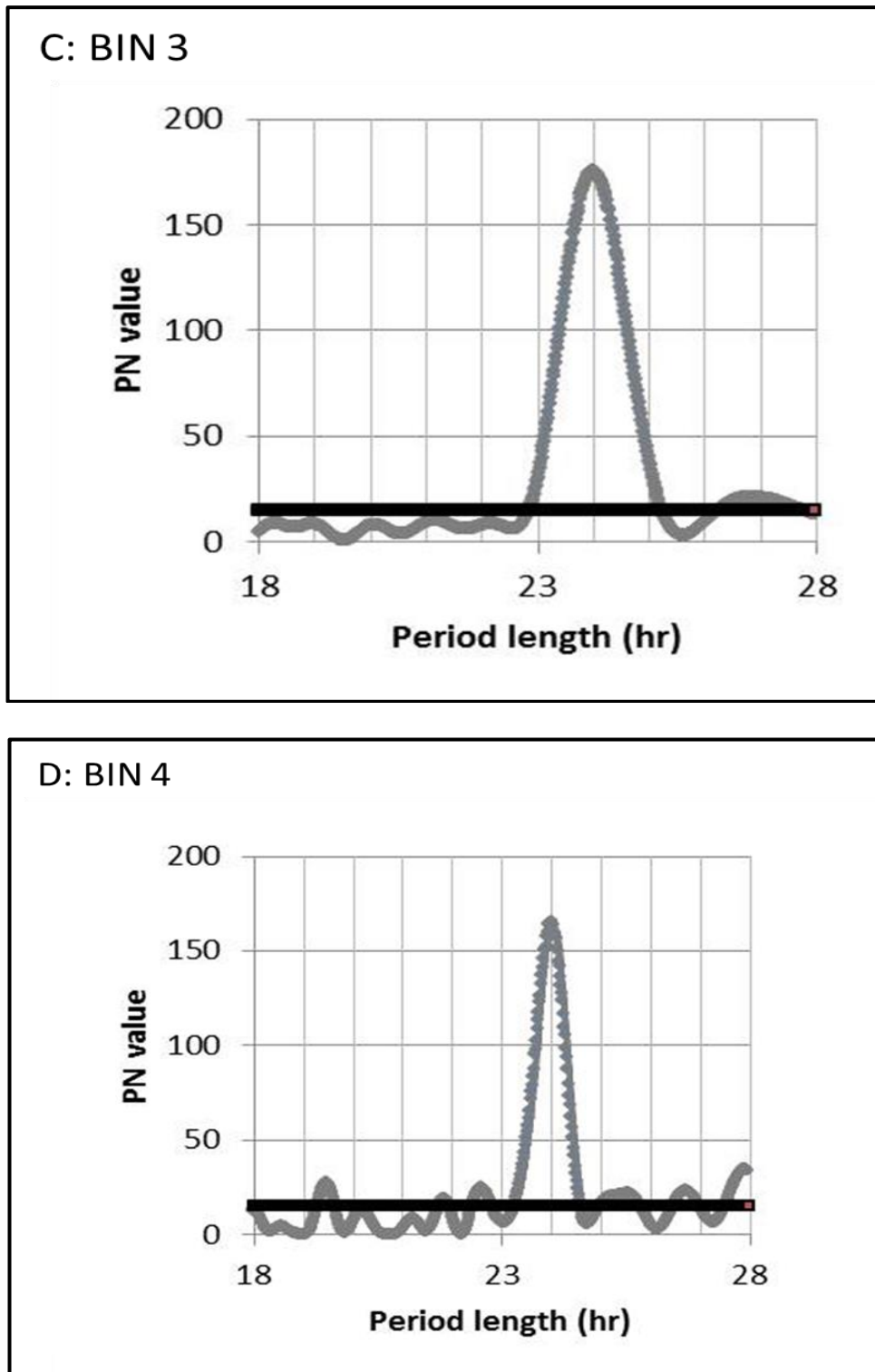
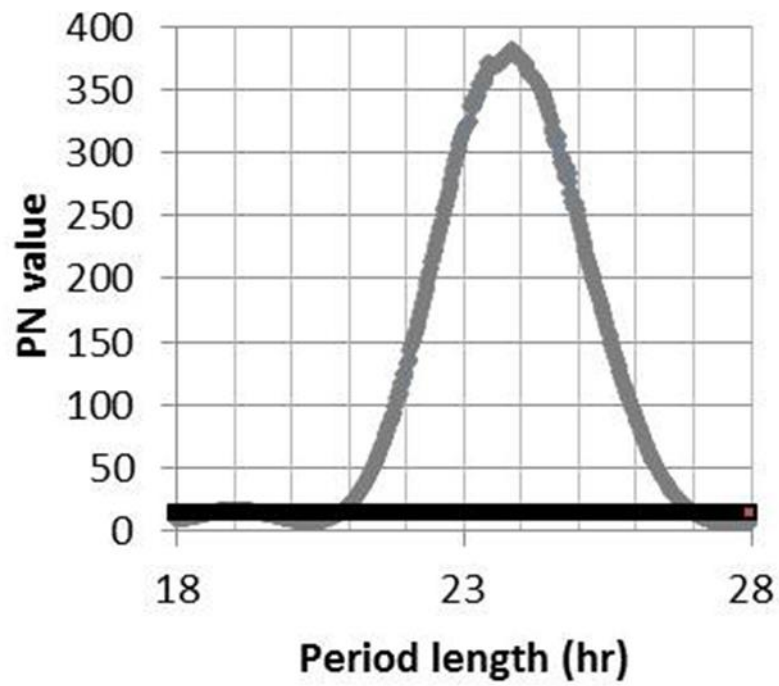
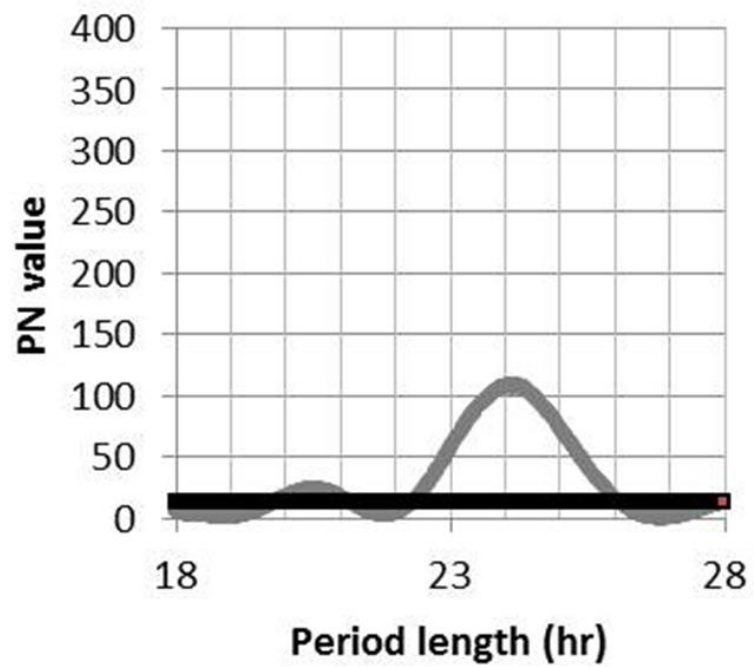


Figure 31. Periodogram analysis conducted on each bin of actigraphy data. The graphs (A-E) show Lomb & Scargle periodogram analyses. Black data points indicate PN (normalised power) values with grey lines showing significance level. All periodogram analyses were conducted at $p=0.001$. Graphs are as follows: A: Bin 1, B: Bin 2, C: Bin 3 and D: Bin 4 with caregiver-induced activity removed. Lomb-Scargle periodogram analysis found a significant rhythm in every bin of the data. All data were found to be rhythmic to a period of approximately 24h (range of 23.88h-24.05h).

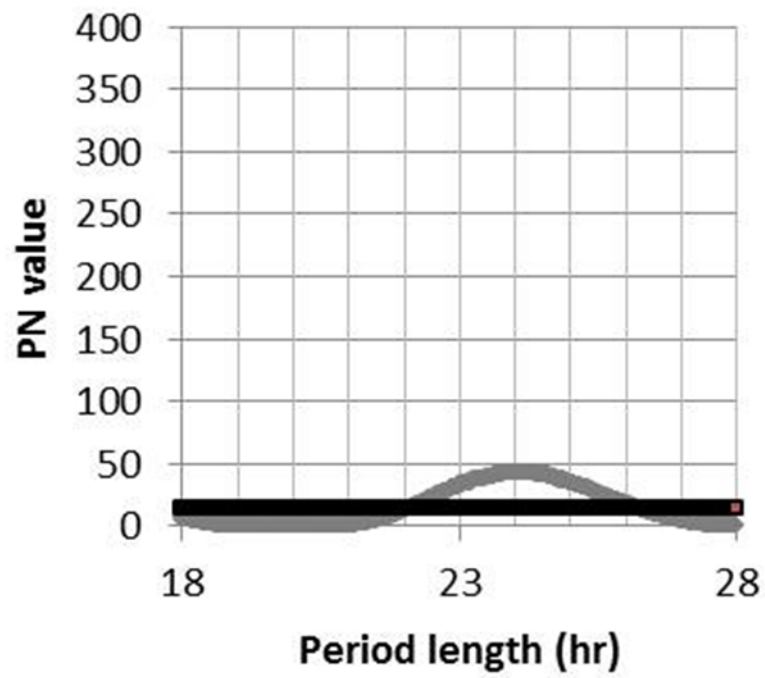
A: Period 1



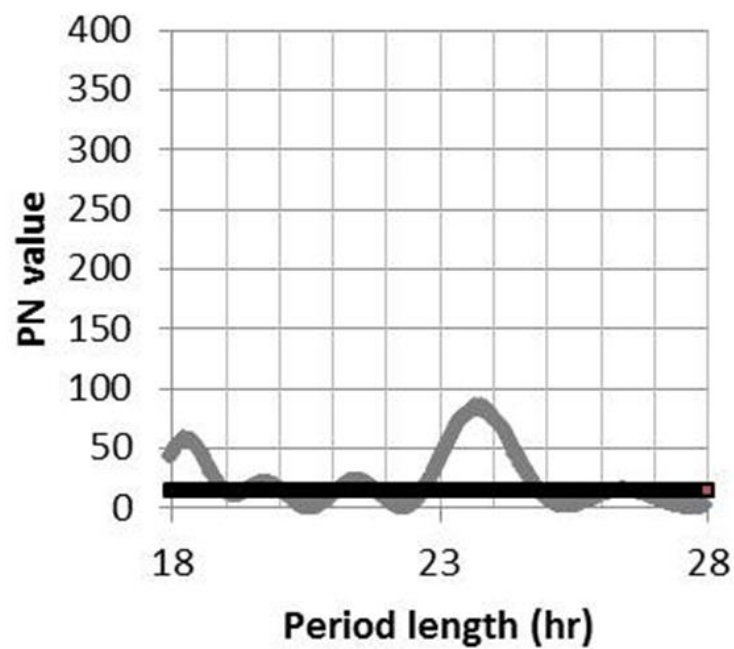
B: Period 2



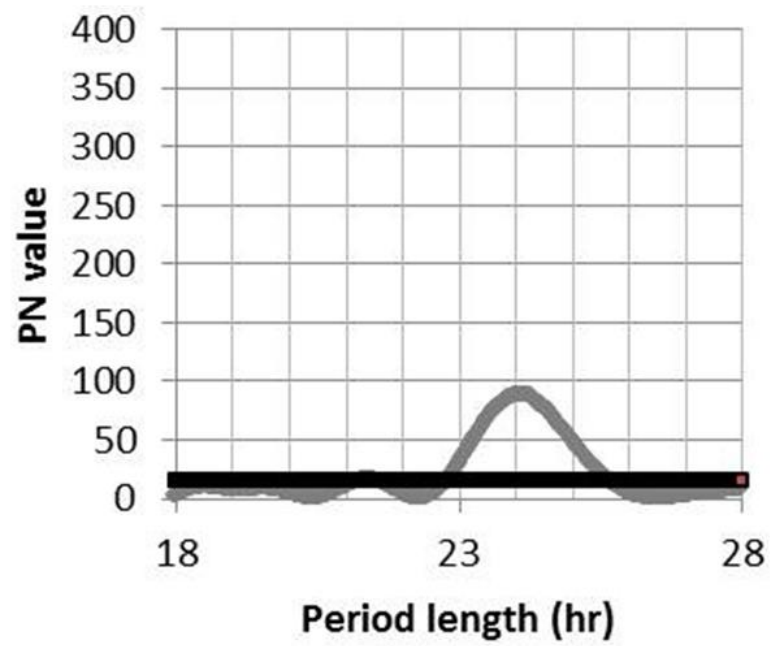
C: Period 3



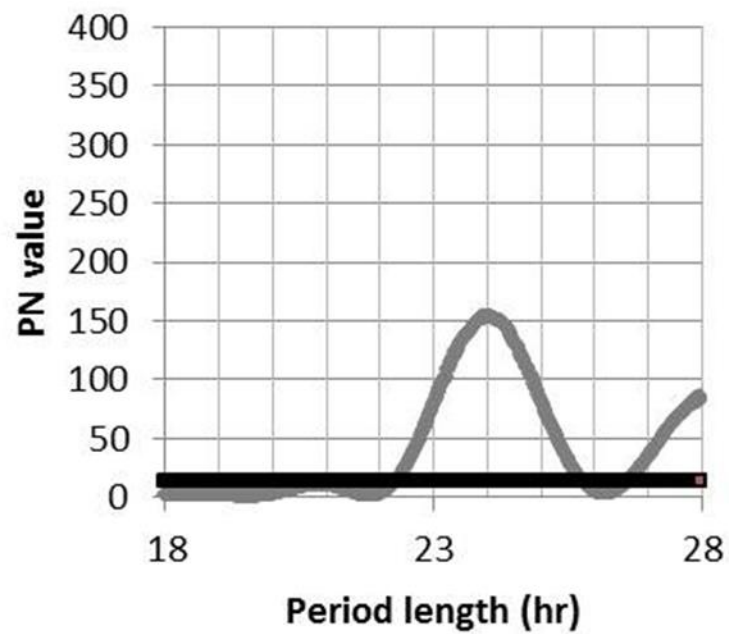
D: Period 4



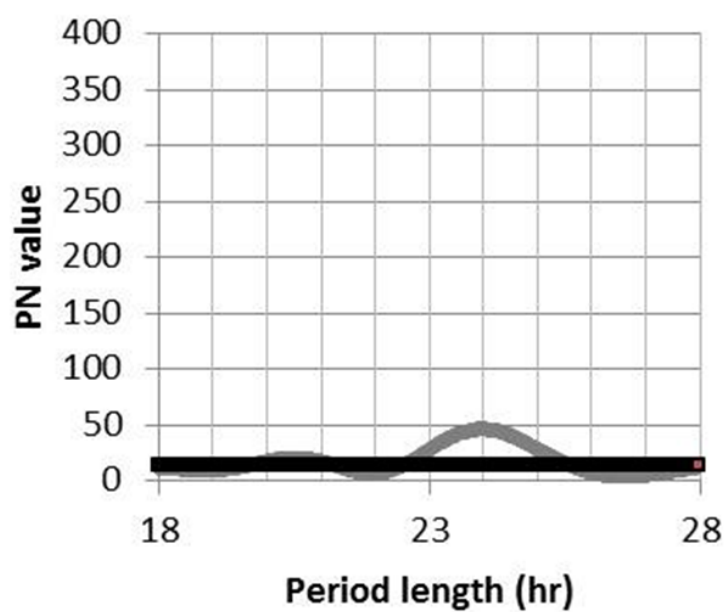
E: Period 5



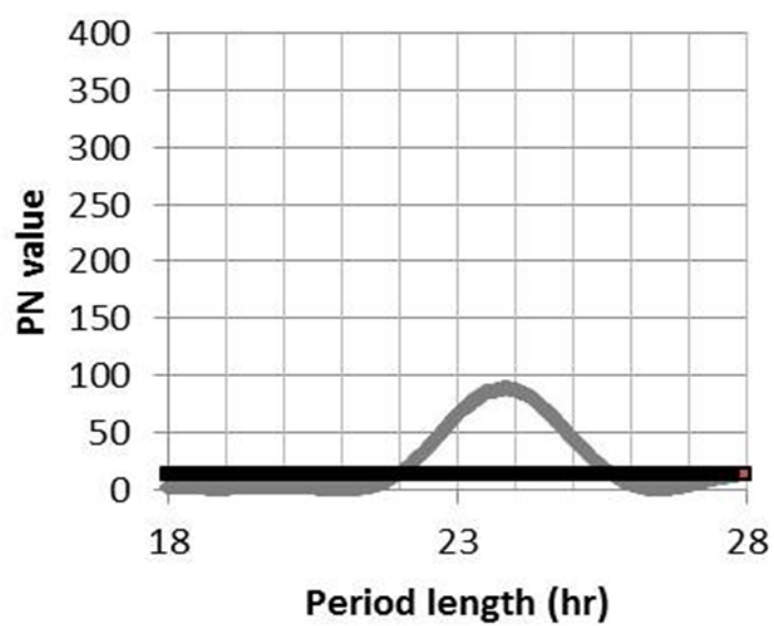
F: Period 6



G: Period 7



H: Period 8



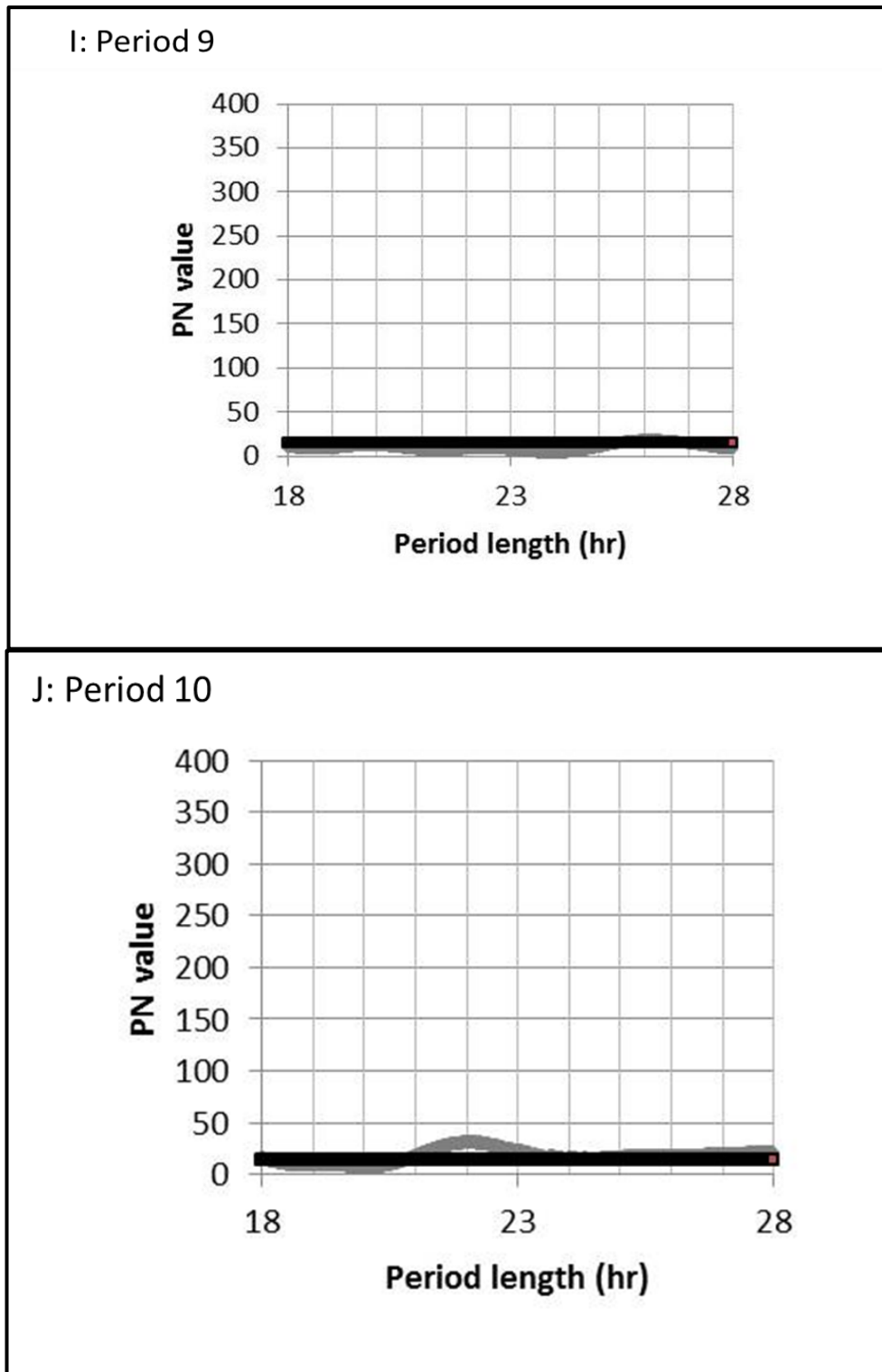


Figure 32. Periodogram analysis conducted on each period of actigraphy data. The graphs (A-J) show Lomb & Scargle periodogram analyses. Black data points indicate PN (normalised power) values with grey lines showing significance level. All periodogram analyses were conducted at $p=0.001$. Graphs are as follows: A: Period 1 (period=23.85h), B: Period 2 (period=24.07h), C: Period 3 (period=24.10h), D: Period 4 (period=23.70h), E: Period 5 (period=24.17h), F: Period 6 (period=24.07h), G: Period 7 (period=23.90h), H: Period 8 (period=23.87h), I: Period 9 with caregiver-induced activity removed (period=26.12h) and J: Period 10 with caregiver-induced activity removed (period=22.05h).

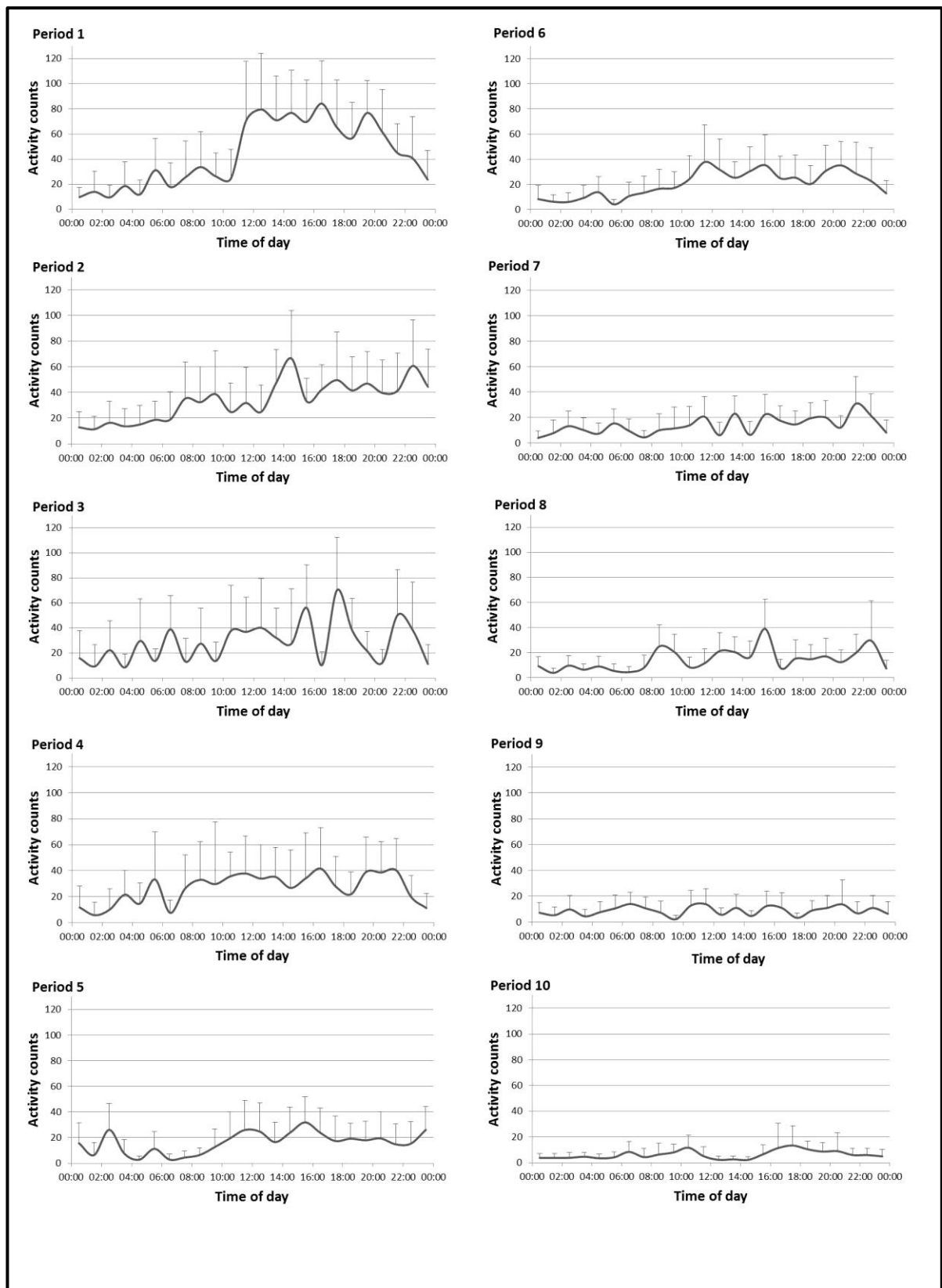


Figure 33. Averaged 24h activity profile for each period of actigraphy. Each period is marked in Figure 30 (p.239) for reference with numbering indicating the progression of time of the observation period. Periods 9 and 10 are shown with caregiver activity removed. All graphs do not include the days of missing data. As can be seen, amplitude begins relatively high at the beginning of assessment and gradually decreases as the disease progresses. Error bars are standard deviation.

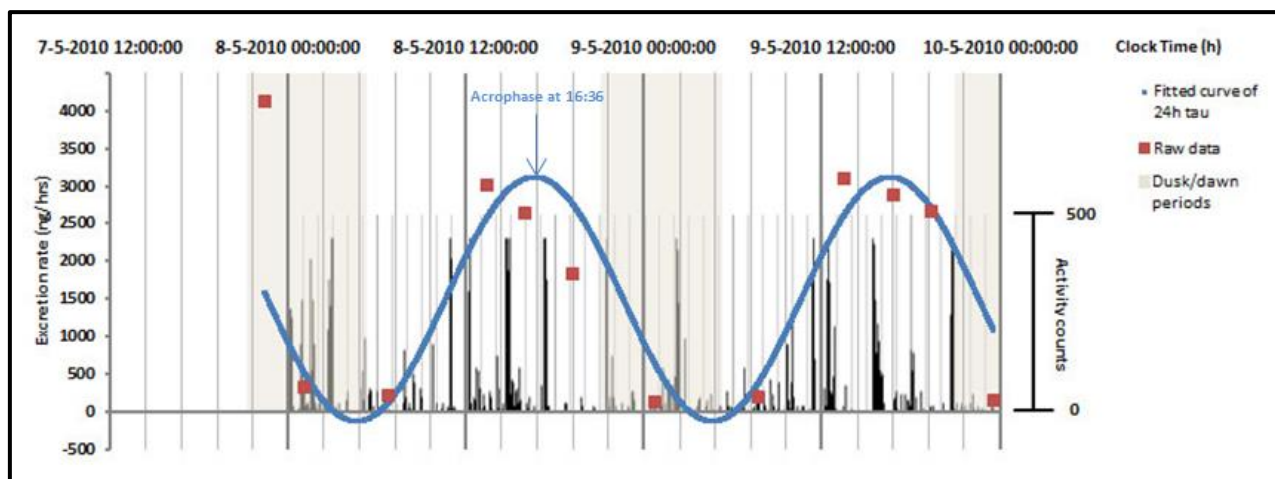


Figure 34. Melatonin excretion rate over time (red squares) sampled at every urination over 48hours. The blue line shows the curve fitted to the raw data by cosinor analysis for a tau of 24hours. The acrophase of the melatonin rhythm is indicated showing a peak at 16:36. The rest-activity data (black vertical lines) for the same days are superimposed with the height of line indicating the amount of activity for each 1 minute epoch. Actual dawn and dusk times are indicated in grey.

Table 39. Non-linear regression model F-statistic table.

Source	df	Sum of squares	Mean Square	F value	approx P>F
Model	2	14774592.8	7387296.417	7.245784066	0.013334823
Error	9	9175772.7	1019530.302		
Corrected total	11	23950365.55			

Table 40. Linear cosinor analysis model F-statistic table.

Source	df	Sum of squares	Mean Square	F value	approx P>F	r ²
Model (wave fit)	2	14774595.39	7387297.7	7.25	0.013335	0.6169
Error (residual)	9	9175781.91	1019531.32			
Corrected total	11	23950377.30				

Table 41. Comparison of circadian parameter output of both models.

Type of melatonin rhythm analysis	Period (h)	Amplitude of melatonin rhythm	Constant (c)	Other constants		Acrophase
Non-linear regression model	24.0	1623.07	1496.63	none		16:36
Linear cosinor analysis	24.0	1623.07	1496.63	(a) -1515.83	(b) -580.18	16:36

6.3.3. Sleep Quality and Characteristics

Characteristics of sleep were compared against the control group. Both the PSQI and the Jupiter sleep questionnaires concurred that JJB spent longer in bed, having earlier bedtime and later get up times (Tables 42 and 43, p.250-251). Furthermore JJB spent more hours asleep, though due to more nocturnal awakenings of longer duration and increased sensitivity to light and thirst his sleep efficiency was lower than the control group. JJB was also more disorientated and had more problems staying awake during daytime. These problems led to increased daytime dysfunction with an overall global PSQI score of 6. A score of 6 indicates disturbed sleep, as the cut-off for normal sleep is a score of 5 or below.

Table 42. Sleep characteristics from Pittsburgh Sleep Quality Index (PSQI) questionnaire.

PSQI variable	JJB	Controls (mean $\pm$ SD)
Usual Bed Time (hh:mm)	20:30	22:53 $\pm$ 00:36
Usual Get Up Time (hh:mm)	09:00	07:25 $\pm$ 00:36
Number of hours in bed (hh:mm)	12:30	08:32 $\pm$ 00:33
Number of hours of sleep (hh:mm)	12:02	07:09 $\pm$ 01:45
Minutes To Fall Asleep (hh:mm)	00:03	00:10 $\pm$ 00:13
Disorientation or Confusion	3.00	00.11 $\pm$ 00.33
Level of problem to show enthusiasm	2.00	0.33 $\pm$ 0.71
Ability to stay awake and to show enthusiasm	5.00	0.33 $\pm$ 0.71
Daytime dysfunction	3.00	0.22 $\pm$ 0.44
Habitual Sleep Efficiency (%)	75.2	83.82 $\pm$ 20.09
PSQI Global Score	6.00	3.00 $\pm$ 2.40

Data are presented as mean $\pm$ SD for the control group.

JJB experienced waking earlier than planned more than the control group (in Table 43 (p.251). Despite no age difference between the control group and JJB, one factor that should be highlighted is that until diagnosis JJB was not retired whereas the control group consisted of

only retired individuals. This is worth highlighting that controls reported no difference between sleep on weekday nights or weekend nights. In contrast JJB reports a difference of two hours between weekday and weekend bedtimes. This indicates that JJB was still socially influenced by familial work obligations and schedules, which was not present in the control group.

Table 43. Sleep characteristics from Jupiter Sleep Questionnaire (JSQ).

Jupiter variable	JJB	Control (mean $\pm$ SD)
Usual time go to bed on weeknights (hh:mm)	20:30	22:53 $\pm$ 00:37
Usual time go to bed on weekend nights (hh:mm)	22:30	22:53 $\pm$ 00:37
Earliest bedtime (hh:mm)	19:00	22:06 $\pm$ 01:01
Latest bedtime (hh:mm)	23:30	00:16 $\pm$ 01:42
Usual awakening time (hh:mm)	09:00	06:51 $\pm$ 00:44
Earliest awakening time (hh:mm)	07:30	06:05 $\pm$ 00:50
Latest awakening time (hh:mm)	13:00	08:05 $\pm$ 01:19
Hours in bed once woken (hh:mm)	03:30	00:34 $\pm$ 00:34
Hours of sleep per night (hh:mm)	12:02	07:09 $\pm$ 01:45
Maximum amount of usual sleep (hh:mm)	17:32	09:09 $\pm$ 02:06
Minimum amount of usual sleep (hh:mm)	07:32	04:59 $\pm$ 02:35
Number of times wake up during night	5.00	0.88 $\pm$ 0.35
Problem of waking earlier than wanted	1.00	0.11 $\pm$ 0.33
Frequently bothered by light in the bedroom	1.00	0.11 $\pm$ 0.33
Frequently bothered by hunger or thirst during night	1.00	0.11 $\pm$ 0.33

Data for the control group are presented as mean $\pm$ SD.

6.3.4. Cancer Cell Localisation

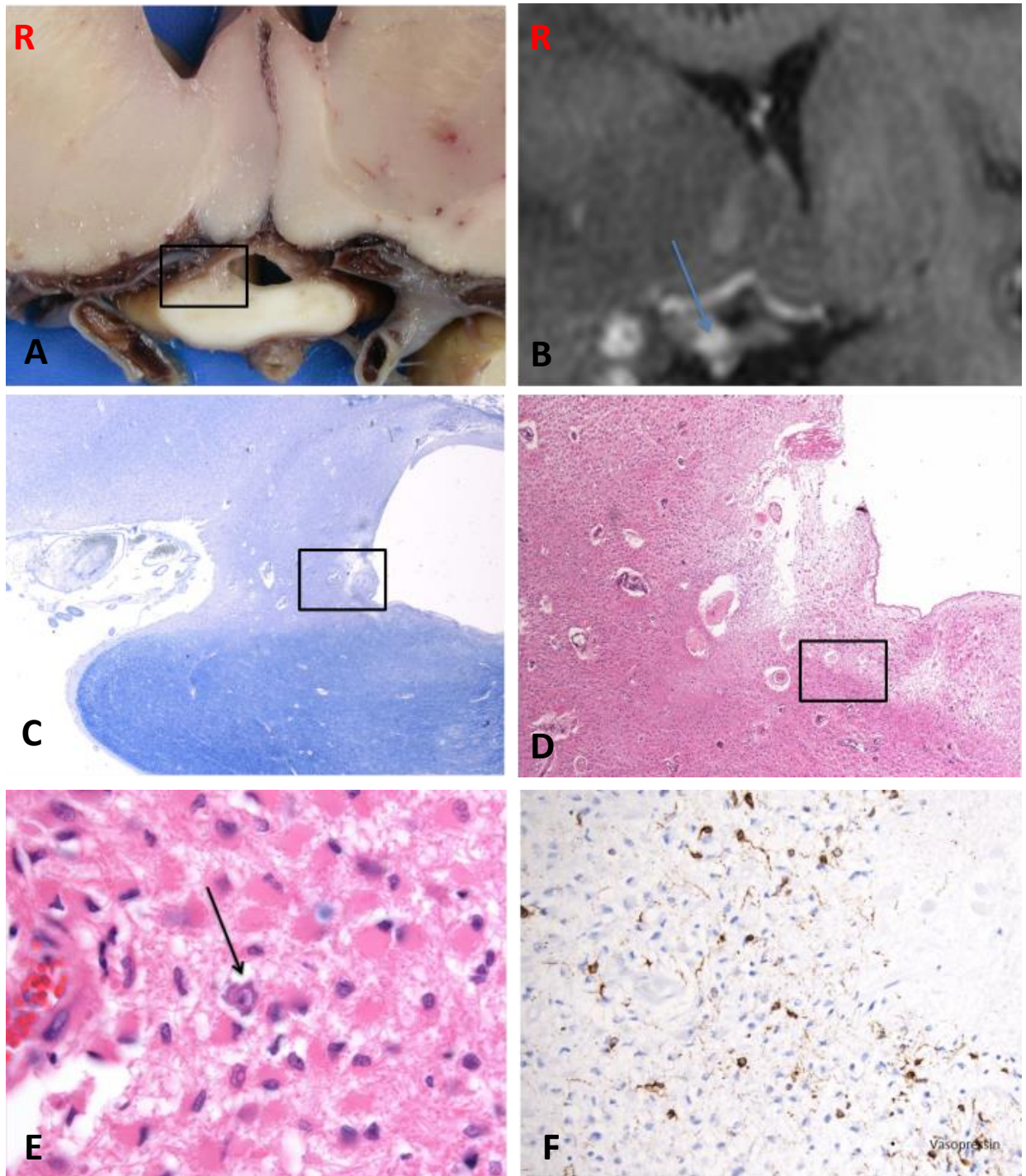


Figure 35. Invasion of the SCN by glioma cells (GBM). (A) Coronal brain section at the level of the SCN (box) shows SCN appearing distended with brown discolouration. The same level is shown in a gadolinium-enhanced MRI 4 months earlier (B), SCN shown by arrow appears enhanced. 'R' indicates right hemisphere. (C) Luxol-fast-blue-cresyl-violet histological section corresponds to macroscopic view. The SCN is boxed in (D) confirming tumour invasion at higher magnification (haematoxylin and eosin). The boxed section of (D) is further magnified as seen in (E) where neurons of the SCN (arrow) are surrounded by glioma cells. (F) demonstrates that these neurons express Vasopressin, (brown reactive product). Topography, morphology and immunoprofile of these neurons are consistent with the SCN.

On initial CT scan (27/10/2009) a lesion was discovered in the right hemisphere involving the majority of the temporal and frontal lobes, thalamus and basal ganglia and was seen to cross the anterior midline. With contrast rim enhancing lesions were observed to be most prominent in the temporal lobe. There was no evidence of hydrocephalus and basal cisternal spaces were preserved. Significant midline shift was observed and surgery booked instantly. Pre-op MRI (02/11/2009) confirmed a mass centred on the right temporal lobe and right basal ganglia. Large infiltration of non-enhancing tumour was noted in the right inferior frontal lobe. Only a relatively small amount of vasogenic oedema was observed surrounding the tumour. Midline shift was calculated at 1.1cm shift with uncal herniation. In surgery (02/11/2009) the tumour infiltration was confirmed as above with the addition of right insula infiltration. The right temporal lobe was resected en bloc, however debulking was limited due to the extent of the tumour infiltration. Biopsy confirmed diagnosis.

In order to assess the success of chemotherapy cycles a further MRI scan was obtained (22/03/2010), shown in Figure 35B (p.252). As seen in the MRI scan the area of the SCN is enhanced and clear midline shift can be observed. It was concluded that this scan confirmed progression of the disease though it was noted this could represent pseudo-progression associated with temozolomide use. A final MRI scan (20/05/2010) was taken approximately 1.5 months before death. The tumour progression appeared to have stopped and appearances remained consistent with the previous MRI scan. The effect of the tumour shifting surrounding brain areas, in particular the tumour's effect of reducing the right lateral ventricle causing distortion were reduced. Furthermore the midline shift was reduced and the corpus callosum was less swollen. Enhancement was localised to the surgical bed, right hippocampus and right orbitofrontal lobe.

JJB agreed to brain donation and the post-mortem was carried out 3 days after death. The brain was sliced and the SCN localised macroscopically as shown in Figure 35A (p.252). Luxol-fast-blue-cresyl-violet staining was administered to localise the SCN microscopically, Figure 35C (p.252). At a further magnification the SCN was stained with haematoxylin and eosin and confirmed cancer infiltration in the area of the SCN, Figure 35D (p.252). This stain was magnified to observe glioma infiltration more clearly, Figure 35E (p.252). Finally the subsequent section was stained to show vasopressin localisation, Figure 35F (p.252). Vasopressin was found to be present in the cells, confirming that the cells infiltrated by tumour were indeed SCN cells.

6.4. Discussion

Initially patient JJB presented with unusual time-of-day behaviour thought to be associated with his lack of temporal perception. On investigation it was determined that JJB had a dissociation to time both cognitively, displaying impairment in orientation to time and space in comparison to controls, and behaviourally when considering familial reports. When considering pre-mortem MRI scan reports it became clear that many areas of his brain were infiltrated by the tumour and as cognition generally relies on a network of brain areas it is unsurprising that deficits were noticed. Furthermore the final MRI scan showed definite infiltration of the hippocampus, a brain area known to be involved in memory consolidation [27]. These global brain changes might therefore explain JJB's memory impairment and worsened cognition. In terms of temporal misperception, the thalamo-cortical-striatal circuit has been implicated, which were all affected brain areas in this case [10, 11]. Furthermore, right hemispheric lesions have been previously linked to time misperception [218]. Another explanation could be that generalised encephalopathy has been shown to alter time perception and due to the nature of JJB's diagnosis, surgery and treatment this is an important factor [28]. It seems that the temporal misperception observed relates to the cortical infiltration of cancer cells, and not due solely to SCN lesion, as the cancer was too-wide spread to conclude this.

Measurement of JJB's rest-activity pattern showed low amplitude activity, decreasing in amplitude with time, but remaining rhythmic to an approximately circadian period. Endogenous melatonin rhythm, an SCN rhythmicity marker, confirmed a period of 24h that further was misaligned in comparison to healthy individuals [219]. The peak of melatonin excretion was found to be at 16:36 whereas the usual acrophase in healthy individuals is between 22:00 and 04:00 [219]. This misalignment was not evident in the activity data suggesting internal desynchrony between these two SCN driven outputs. This led me to

consider the involvement of the SCN in JJB's behavioural changes. On close inspection of CT and MRI scans the right temporal and frontal lobes were found infiltrated by the tumour, with infiltration extending to the basal ganglia and thalamus. These scans confirmed our suspicions of global brain tumour infiltration including lesioning of the SCN area. When investigated, brain sections of the SCN area validated this conclusion, finding glioma between SCN cells.

6.4.1. Interpretations of results

It appears there could be several interpretations of JJB's behaviour. Firstly, that there was a visual defect leading to difficulty of entraining to the light/dark cycle. Projections from the eye to the SCN could have been disrupted leading to an inability for the SCN to entrain to the light-dark cycle. In terms of eye problems, the only visual problem reported in eye exams was mild diplopia when drowsy suggesting an intact eye. However, this still leaves open the possibility that the non-visual pathway from the eye to the SCN, which entrains the SCN to the light-dark cycle, was disrupted and that rest-activity only remained entrained due to social familial cues.

A second hypothesis is that the SCN lesion was too severe to generate sufficient rhythmic output to stimulate the pineal gland to excrete melatonin. Since his rest-activity rhythm was rhythmic with a period of about 24h we can conclude that the SCN maintained its rhythmicity. However, extra-SCN projections could have been lesioned by the tumour leading to a difference of phase between the SCN and pineal gland. When considering animal SCN-lesion studies, behaviour remains rhythmic unless the SCN is completely ablated [2]. This finding has been shown in non-human primate studies showing that melatonin rhythmicity in rhesus monkeys only becomes arrhythmic when the SCN is completely ablated [3].

A further hypothesis can be that JJB had a free-running circadian rhythm, which due to sampling melatonin excretion rates only once, appeared to be phase misaligned.

As the rest-activity of JJB was found to be rhythmic and, at the time of sampling, his melatonin rhythm had the same period but was phase-misaligned, a further hypothesis can be made. The difference in phase of the rest-activity pattern (averaged activity over 24h) and melatonin excretion could be due to the SCN itself being only partially lesioned and not fully ablated. Some reports of the behavioural changes associated with partial lesions do exist [13]. For example, Fuller et al. investigated the effect of auditory time-of-day cues from non-lesioned entrained squirrel monkeys with partial or complete lesions of the SCN [5]. Monkeys with full lesions could not be entrained to these auditory cues of time-of-day, however, those with partial SCN lesions could [5]. This could indicate that, although partial lesioning can disrupt circadian rhythmicity, the remaining SCN can be entrained if the appropriate stimulus is provided. Another study of squirrel monkeys found that the animal with <50% SCN lesion kept a significant free-running rhythm of activity in stark contrast to the arrhythmicity of fully lesioned animals [220]. From these studies we can conclude that in order to maintain rhythmicity only a small portion of the SCN needs to remain. This could explain how JJB maintained a significant circadian rhythm of activity despite partial SCN damage.

The misalignment between the SCN according to the measured melatonin rhythm and the rest-activity rhythm could be two-fold. The partial SCN lesion could have shortened the period of the SCN and, due to there being only one melatonin excretion rate measurement, the altered phase detected could be showing a 'snap-shot' of this change in period. This shortening of the SCN period resulting from a lesion to either side of the SCN has been found previously in a study on unilateral lesions of the SCN in the hamster [203]. The rest-activity of JJB may then have been masked by social cues of family activities and the work schedule of his spouse to maintain an entrained rhythm, albeit with a low amplitude.

Although this is the first reported case of temporal misperception and circadian abnormalities to involve behavioural and post-mortem tissue analysis, related cases have been reported [205]. One such case was that of a 55-year old woman with a discrete metastasis in the ventral hypothalamus and optic chiasm [205, 221]. She had temperature rhythm disruption, though unfortunately there were no objective measures of sleep-wake rhythmicity. Post-mortem tissue indicated cancer infiltration of the ventral hypothalamus, infundibulum, the optic chiasm and tracts. The SCN region was determined to have a reduction in neurons. However, no cancer cell infiltration was reported. The case of a 34-year old woman that had a tumour resected resulting in SCN lesioning is also worth noting [221]. She was reported to have no sleep problems beforehand but following surgery considerable disruption of the circadian rhythm was reported. She had a very disturbed sleep-wake rhythm with daytime hypersomnolence and frequent awakenings at night. Despite polysomnography to observe her sleep, no objective measures of circadian rhythm were conducted, although nurse sleep diaries and oral temperature rhythm were recorded. There was no post-mortem assessment of the tissue to assess the true extent of SCN damage. Therefore, no conclusions can be made about the lack of sleep-wake rhythmicity resulting from SCN lesion and/or loss of circadian drive. Despite these case studies indicating cancer infiltration of the SCN, our work is the first case study to report temporal misperception and SCN rhythmicity despite the large infiltration of the SCN by glioma. These findings appear to show that, as with non-human primates, humans are capable of maintaining rhythmicity although possibly with a shortened period with a partial SCN lesion.

6.5. Study 2 Conclusions

This study observed patient JJB, who presented with a temporal lobe glioma and an unusual time perception resulting in an abnormal rest-activity rhythm subjectively. This study sought to elucidate whether an underlying circadian abnormality was the cause of these behaviours. As only the right side SCN was investigated with immunohistochemistry it is unknown what proportion of the SCN was affected by cancer cell infiltration. However, this study found distortion of both SCN and cancer cell infiltration of the right side. Along with the assessment of rest-activity profile as well as investigation of melatonin rhythm, a misalignment of the two rhythms was found suggesting some disruption of the circadian system. However, rest-activity retained a circadian rhythm. It can therefore be concluded that a shortening in period of the SCN could have occurred due to the partial nature of the lesion. Although this period shortening was not evident in the rest-activity rhythm this may have been due to social cues. Therefore, this study shows that when partial lesioning of the SCN occurs circadian rhythmicity is affected. However, rest-activity behaviour alterations could be masked by the social environment of the individual. Despite the global nature of brain abnormalities due to infiltration of the glioma, these data shed new light on the reaction of human behaviour to SCN lesioning.

7. Discussion

7.1. Overview of thesis

This thesis consists of two studies both aimed at longitudinally assessing rest-activity and sleep concomitantly in the field. The first studies concentrated on evaluating the rest-activity and sleep in Alzheimer's disease and its prodromal stages in comparison with non-memory impaired controls. All participants lived in the community and were measured at home with both subjective and objective measures of sleep. The second study sought to investigate rest-activity rhythms in more detail over a longer period of time, in the terminal illness of brain cancer patient JJB. In particular, the recording of the rest-activity of JJB, as well as the post-mortem brain tissue analysis, enabled the elucidation of the effects of the infiltration of cancer on his circadian clock (the suprachiasmatic nucleus [SCN]). Specific conclusions from each study were noted at the end of Chapter 5 (p.221) for the Alzheimer's disease related study (Study 1) and at the end of Chapter 6 (p.259) for the brain cancer study (Study 2). A critical consideration of the usefulness of the methodological approaches applied in these studies is provided in this chapter, as well as the validation of certain methods never used previously in these patient groups.

7.2. Assessment of methodologies

7.2.1. Questionnaires

7.2.1.1. General practicalities of administering questionnaires

Implementing questionnaires in the home has its benefits and its weaknesses compared with bringing patients into the laboratory or posting questionnaires to them. The individual being questioned in their home leads to more comfortable and more familiar surroundings for the participant. This is exceptionally important in Alzheimer's disease patients as unfamiliar surroundings are known to disturb patients [222].

Despite these benefits, practicalities of home measurements can raise certain problems. A key problem in Study 1 was that the majority of participants wished their study partner to stay with them during testing. Steps were needed to be taken to reduce distraction of the participant by the study partner. Most study partners complied with remaining quiet and not answering or prompting the participant for answers. Issues arose when the participant and the study partner disagreed on answers, however, solutions were usually found quickly with a consensus of the correct answer decided on between the participant and study partner. Particular difficulty arose with administering memory questions to the patient/participant. Cognitive testing of the patient at the memory clinic is handled in a way that the caregiver is kept busy providing a recent history of the patient's behaviour to the clinician. In this study it was the first time that the caregiver witnessed first-hand the impairment of the participant's memory. This was distressing for some caregivers and details were provided for carer support, if requested. For Study 2, JJB was tested alone to avoid distractions and his answers to questions about usual bed and get up times were checked with the family later.

All questionnaires were administered by myself, as participants and study partners alike found the questionnaires difficult to read despite the increased printed font size. I believe this

difficulty was due to the volume of questions asked, which, could have been overcome if more days of assessment could have been arranged.

7.2.1.2. The Health Questionnaire (HQ)

The Health Questionnaire (HQ) was adapted from the Primary Care Evaluation of Mental Disorders (PRIME-MD) Patient Health Questionnaire (PHQ) developed by Spitzer and colleagues [62]. In the original publication, information from the PHQ was compared with information from mental health clinicians, measures of functioning, reports of health care usage and treatment choices [62]. After comparison in this way with 3000 individuals, the PHQ was found to have an accuracy of 85% for diagnoses. The adaptation of this validated questionnaire enabled a good overview of participants' health to be recorded to check eligibility criteria and compare between groups. A benefit of using the HQ was that a standard set of conditions could be compared between groups and often filled the gaps in medical notes.

7.2.1.3. The Hospital Anxiety and Depression (HAD) Scale

The Hospital Anxiety and Depression (HAD) Scale was developed by Zigmond and Snaith [63]. The scale was validated in groups of 100 individuals and had internal consistency of up to 0.76 using Spearman correlations when the weakest item for both, the Anxiety and Depression scales, were removed [63]. Both scales correlated significantly with interview ratings of anxiety and depression symptoms in the validation study [63]. This scale has been used in a variety of other studies [223-225] and is a well-validated method of determining anxiety or depression. In the current study, scores were used only to compare between groups, in order for any differences to be controlled for in future statistical analyses.

7.2.1.4. The Morningness-Eveningness Questionnaire (MEQ)

The Morningness-Eveningness Questionnaire (MEQ) was designed to evaluate the diurnal preference of individuals [26]. It was first validated against the circadian rhythm of oral temperature in individuals, with 'earlier types' having earlier peaks in oral temperature rhythm [26]. The published validation of the questionnaire was carried out with a group of 150 individuals, however, the maximum age was only 32 years [26]. This thesis involved participants who were much older than this population, however, other studies have used the MEQ successfully in older populations [226-229]. For both studies, participants found this the most difficult questionnaire to answer, due to the 'preference' aspect of the questions. Participants from all groups found it difficult to separate preference from usual habits. This does limit somewhat the results from this questionnaire, as participants, especially those with memory problems, answered questions based on the previous few days' activities. 'What if' type questions were especially difficult, as all participants were retired from work and had no fixed daily schedule and, for example, moderate Alzheimer's disease patients could not comprehend having to decide when to sleep in order to do a night shift.

An alternative questionnaire, called the Munich Chronotype Questionnaire (MCTQ) [23] seemed at first to be more suitable. However, it does not assess diurnal preference but calculates mid-sleep point based on daily sleep times [23]. The MCTQ has been validated against the MEQ [25] but not against any physiological measures. In addition, the MCTQ extracts mid-sleep from actual sleep times, which were collected with questionnaires and actigraphy for this thesis. Therefore the use of the MCTQ in this thesis would have resulted in a repetition of questions. Furthermore, although mid-sleep may correlate with chronotype and can be compared between groups, there is no categorical system of defining someone as a 'morning type' or 'evening type' in relation to the internal time and time of day. The main

reason for using the MEQ in these studies was that currently no data has been published correlating MCTQ scores with objective circadian measures [23] unlike the MEQ [26].

7.2.1.5. Assessment of risk factors for restless legs syndrome (RLS) and sleep apnea

The screening tools for restless legs syndrome (RLS) [230] and sleep apnea [231] were both questionnaires designed by sleep clinics for the determination of risk factors, evaluating which patients would require further examination. Both of these screening tools were used with the other questionnaires. For the restless legs questionnaire, if a participant did not meet the first two criteria, then the rest of the questionnaire was not asked. Although no diagnosis can be made from the information collected, these screening tools did enable the tendency towards these disorders to be determined and to rule out these being the primary cause of participants' sleep disturbances (apart from the qmAD group).

7.2.1.6. The Mini-Mental State Examination (MMSE)

As the main cognitive scale used in UK memory clinics, the Mini-Mental State Examination (MMSE) [61] was conducted with all participants. Although the MMSE is unable to assess all aspects of memory, a wide range of cognitive abilities are measured. The MMSE has been found to have good internal consistency and has been validated [232] against Diagnostic Statistical Manual criteria (version 3R) and NINCDS-ADRDA criteria [59] for the diagnosis of Alzheimer's disease. The scores of the MMSE needed to be controlled for by age, education and cultural background [232]. In this thesis it was important, therefore, to determine if any age, education or cultural differences were present between groups before comparing MMSE scores. Where differences were found they acted as covariates in further statistical analyses.

One practical problem of administering the MMSE in both studies was the recent use of the MMSE by patients at their last clinical visit. Therefore, the three words in the recall section were changed from 'Apple, table and penny' to 'Tree, bag and spoon' to minimise learning. This was equally important for the control group, with individuals, who had never been to a memory clinic, as they were participants of the OPTIMA project, a longitudinal monitoring of ageing and memory with regular assessments, including the MMSE.

Although the MMSE does not enable the diagnosis of a dementia on its own, it does provide validated scores of cognition in multiple components and was the appropriate cognitive questionnaire. Finally, and most importantly, due to the MMSE's wide use [158, 176, 233, 234], comparability to other studies was a large benefit of using this method of memory assessment.

7.2.1.7. The Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory (ADCS-ADL)

This inventory was designed in 1997 [66] and has since been used in many other studies [235-237] to determine the effect of illness and medication on functioning in terms of Activities of Daily Living (ADLs). The ADCS-ADL appears to have become the 'gold'-standard of measuring functioning of people diagnosed with Alzheimer's disease, using a single questionnaire. The ADCS-ADL was validated for data collected in interview with carers of Alzheimer's disease patients [66]. Due to the nature of this research being conducted in people's homes in the evenings it was not always possible to separate the participant and the study partner. For some cases there was only one room where testing could be conducted and for other cases the patient could not be left alone. This resulted in the study partner completing the inventory which did lead in some cases to missing data. The necessity of the study partner completing the inventory in other participant groups was due to the discomfort for some study partners in

answering the questions in front of the participant. Wherever possible the inventory was conducted in interview, however, instances existed in all groups where this was not possible. Although the resulting amount of missing data was reduced by checking the inventory, human error and the number of questions led to some being missed. As component scores and the total score were the summation of scores from many questions, component and total scores were marked as missing when just one answer was not provided correctly.

As this inventory is usually conducted in a facility rather than in people's homes, two researchers are normally present and facilities enable the separation of the participant and the study partner. In future research, this questionnaire should be completed with the presence of two researchers whether the research is carried out in a facility or at a participant's home. This would also reduce the time taken for these assessments to be conducted, improving the experience for all involved. Failing this, the ADCS-ADL should be validated for use by the study partner directly so that data collection could be consistent for all participants.

7.2.1.8. The Social Functioning Scale (SFS)

Even though the ADCS-ADL is a comprehensive inventory of functioning, the social impact of a worsening memory was also an important aspect which required measurement. To do this effectively the Social Functioning Scale (SFS) was administered. This questionnaire was designed to assess social functioning in terms of withdrawal from social situations, recreational hobbies as well as self-motivation and interaction with others in patients with schizophrenia [67]. The questionnaire was validated in a sample of 334 patients diagnosed with schizophrenia and 100 healthy controls [67]. Issues of missing data due to human error and the volume of questions were the same as described above for the ADCS-ADL.

As the SFS was not designed for, and to my knowledge has not been previously used in Alzheimer's disease (AD) patients, I sought to validate the questionnaire in comparison to the ADCS-ADL, the most used AD inventory for measuring functioning, in Study 1's baseline assessment (Cholinesterase inhibitor naïve) of the moderate AD group (all details and results in Appendix, p.65). Internal consistency scores (Appendix, p.65) were comparable with the original validation of the scale both for patients and controls [67]. Correlations within the scale between components were also consistent with the original validation in patients diagnosed with schizophrenia [67] (Appendix 3.1., p.65). To compare between the SFS and ADCS-ADL for the moderate AD group data Pearson correlations were conducted between components. This was done with the exception of the Withdrawal and Interpersonal Communication components of the SFS, as these type of questions were not present in the ADCS-ADL and were the main reason for the addition of another questionnaire. When comparing the SFS components to the two components and total test score of the ADCS-ADL, all included components correlated to at least the ADCS-ADL total score (correlation coefficients of 0.5 to 0.9, further details provided in Appendix, p.65). Additionally, only the Prosocial component did not correlate to ADCS-ADL total score, but still had a high correlation with the ADCS-ADL instrumental ADLs component score (all details in Appendix, p.65). These results indicate that the two questionnaires were highly correlated. Although a larger sample would be needed to confirm these findings, the results indicate that the SFS is a valid and reliable method for assessing social functioning in moderate Alzheimer's disease patients. Furthermore, the much younger age of the original control group used for validating the SFS in people diagnosed with schizophrenia [67], required validation in an older population anyway. Internal consistency, correlation between components and comparison to ADCS-ADL component scores, all indicated that the SFS is also valid for the use in an older, non-memory impaired, population (Appendix 3.1., p.65).

7.2.1.9. The Pittsburgh Sleep Quality Index (PSQI)

The Pittsburgh Sleep Quality Index (PSQI) was first developed by Buysse and colleagues to assess the prevalence of sleep complaints in controls, patients with sleep disorders and psychiatric patients (major depressive disorder) [64]. Since its conception, the PSQI has become a standard in the subjective self-rated assessment of sleep for a diverse range of research [223, 238, 239]. It was used here to enable comparability between studies. It was simple to administer, with the benefit of 7 component scores and an overall score as outcome measures. The PSQI was, therefore, a good measure of subjective sleep complaints, however, it is not a replacement for objective measures of sleep through actigraphy or polysomnography, as the PSQI has been found to correlate poorly with these objective measures [240]. Therefore, the PSQI is a valid measure to distinguish between self-rated poor or good sleep [240].

7.2.1.10. The Jupiter Sleep Questionnaire (JSQ)

The Jupiter Sleep Questionnaire (JSQ, so named in this thesis) was developed by the Jupiter Medical Center in Florida, USA to assess the sleep of patients on initial consultation [65]. As this questionnaire was designed for clinical use and not research purposes, it is not validated and does not have a scoring system. The JSQ enabled the collection of information not possible with the PSQI, providing more detail about reasons for having problems initiating and maintaining sleep. Napping behaviour was also only assessed with the use of the JSQ. These details provided more detail of the reasons for poor sleep found by the PSQI. Despite providing more information about participants' subjective sleep than the PSQI alone, the lack of a scoring system and components was a major flaw of using this questionnaire. Therefore the JSQ variables were used descriptively except for the scale questions included in the questionnaire.

7.2.2. CANTABeclipse battery

The CANTABeclipse battery of tests of memory and attention used in this thesis was compiled based on guidance from the manufacturer and observation of previously used tests in Alzheimer's disease [68-70]. A test was selected from each of the five cognitive modalities (visuo-spatial memory, executive function, attention, verbal memory and response control) to provide a range of cognitive measures and a warm-up test (BLC) to test reaction times and familiarise the participant to the set-up. Outcome measures were much more rigorous and standardised for cognitive assessment compared to that of the MMSE. In terms of the practicalities of conducting the tests, the test-script was followed for all participants and, despite initial worries, all participants found the touchscreen computer easy to use. The only flaw of the equipment was the poorly designed tripod used to rest the touchscreen against. However this was overcome by careful set-up of the equipment.

7.2.3. Actigraphy

The waterproof AW7™ actiwatches were a great design for these studies. Due to the participants' memory problems, it was a very good design feature that the watches were waterproof and had not to be removed during the monitoring period. One control and one aMCI participant did remove the watch for bathing, but noted the times clearly on the sleep diary so the gap in data could be filled with day average data. Initially, the study design involved use of the mini-L™ light sensors to detect light levels that the participants were exposed to. This was organised due to previous research indicating that lower light exposure led to disrupted circadian rhythms of rest-activity in institutionalised Alzheimer's disease patients [95]. Unfortunately, the practicalities of measuring this in the field led to discontinuation of assessment with the mini-L actilights™. The reason was that the light sensors needed to be on

the outside layer of clothing all the time, and the participants, patients and controls alike, forgot to move the sensor outward, so it was often left on previously worn clothing. Changing to the wrist, the mini-L Actilight™ was often covered by a sleeve. Therefore, the data recorded were very unreliable as to whether light exposure was due to actually being exposed to the dark or light at eye level or to the watch being covered up. It appears, therefore, that in order to test light exposure, it may be necessary for a light sensor to be in a clearer view, for example as a finger ring.

7.2.3.1. Actigraph-derived sleep analysis (validation with EEG)

Rest-activity rhythms have not been widely investigated in amnesic mild cognitive impairment individuals (only 3 studies, see [165, 241, 242] and sleep analysis derived from actigraphy has been assessed in only one study previously [242]. Here, I have validated the use of actigraphy for the sleep assessment of mild cognitive impairment individuals, Appendix (p.66). Sleep staging of electroencephalography (EEG) data was confirmed by Dr. Lazar, details provided in Chapter 2 (p.52). The EEG data were condensed into one minute epochs to compare against actigraph epochs (see methods Chapter 2, p.53). Actigraphic data were then treated with two algorithms separately: (1) to determine sleep and wake epochs, and (2) to compare epoch by epoch to the EEG-derived data.

By using the Sadeh *et al.* [75] method of calculating sleep information from actigraphy on the aMCI group's actigraphy data, agreement rates varied between 69.7 and 83.6% and between 65.9 and 88.3% for the Cole *et al.* method [76] (see Appendix 3.2., p.66 for details). The results of the validation of actigraphy for the assessment of sleep conducted in this thesis provide evidence for the valid use of actigraphy to measure sleep in aMCI individuals. Analysis indicated that using the Sadeh *et al.* [75] method actigraphy underestimated sleep in

comparison to EEG data, whereas, the Cole et al.[76] method overestimated sleep (mean minutes of sleep: EEG 402.3 ± 63.2 ; Sadeh et al. method 380.8 ± 45.5 and Cole et al. method 505.8 ± 51.4). A larger sample will be needed to confirm these findings, as currently indirect sleep outcome measures (e.g. sleep percentage and sleep efficiency) do not correlate well between actigraphic and EEG data. This is understood to be due to the low sample size and low variance in the data between individuals. In terms of accuracy of algorithms, although the Cole *et al.* method had higher agreement in sleep measurement than the Sadeh *et al.* method, this was due to its overestimation of sleep. This is evident by the complete lack of correlation in the detection of wake epochs, Appendix (p.66).

7.2.3.2. Actigraph-derived circadian analysis

The analysis of actigraph-derived rest-activity rhythms was conducted using a non-parametric circadian rhythm analysis [78] implemented in MS Excel, which enabled high throughput of the data. The outcome measures have been published in many studies in Alzheimer's disease patients, those with delirium and those diagnosed with schizophrenia [78, 79, 155, 166, 243-245] enabled easy comparisons to be made to previous Alzheimer's disease research.

The determination of period length of rest-activity rhythm, especially important in Study 2, was carried out by running a periodogram analysis on the activity data. Two methods were used and compared (see Appendix 2.1., p.61), the Sokolove-Bushell chi-square periodogram analysis [74] and the Lomb-Scargle periodogram analysis [73]. However, for Study 2 due to the nature of such low amplitude data, and with some missing data, the Lomb-Scargle method was much more suited [73] and was sensitive enough to find a circadian rhythm of the activity that the Sokolove-Bushell missed. This is thought to be due to the necessity for data extrapolation with the chi-square method, whereas, the Lomb-Scargle does not require such extrapolation

[73]. It appears from the Study 2 data presented in Appendix (p.61), that for low amplitude data with missing segments the Lomb-Scargle should be the method of choice of analysis for determining period length. In order to determine whether the Lomb-Scargle method had any differences with the Sokolove-Bushell method with normal amplitude data, comparison of periodogram analyses of the Study 1 data were conducted. For these comparisons, no differences were found between the two methods, therefore, as commonly used in circadian rhythm studies, for comparability the Sokolove-Bushell should be used, except for instances of very low amplitude data as found in Study 2.

7.2.4. Electroencephalography (EEG)

Electroencephalography (EEG) was used to measure the architecture of sleep objectively and to determine any medication related effects on sleep architecture in Study 1. This was very important as the medication was known to alter cholinergic functioning in the brain [192] and cholinergic functioning is especially involved in rapid-eye movement sleep and wake promotion [29]. Therefore, the architecture of sleep needed to be assessed before and after medication initiation. Using EEG came with some practical considerations: firstly whether patients, especially moderate Alzheimer's disease patients, would tolerate the equipment; and secondly whether a system for easy field assessment could be used. A simple four channel EEG system was decided upon from Actiwave™ (CamnTech Ltd.). This small, lightweight system was a prototype adaptation of implantable EEG systems previously used in animal work. This system overcame the issues of user tolerance and field use, due to its lightweight nature.

To adapt the recorders for our purpose, I designed and developed a holder for the individual boxes. The manufacturing company took up my prototype and built copies for their customers. This holder could be attached to the back of the upper garment worn in bed by the

participant. With some participants this was not desirable, as they were then required to wear a top in bed which they would not usually do. This led in a small number of cases to participants being too hot, leading to sweating. Secondly, no available impedance monitor fitted the cable size of the electrodes. Adapters were offered but none of the impedance monitors produced reliable readings. As impedance could not be measured externally, and was not an internal feature of the Actiwave™ system, special attention was paid to the procedure of attaching the electrodes to the head. This was tested with another system (where an impedance monitor worked) to ensure the attachment was carried out correctly and did not result in impedance problems. Finally, due to a technical fault of still unknown origin, a large number of recordings (1 at each assessment) had to be rejected (percentage of assessments unable to be included due to technical fault per group: control: 29%, SMC: 33%, aMCI: 21%, mild AD: 0% and moderate AD: 57%). The recordings still showed wave formations, however, they were very low amplitude, with low amplitude of the EOG and EMG channels also. None of the waves could have reached a high enough amplitude for the scoring of Stage 3 sleep. Steps were taken to ensure the same boxes measured all recordings from each participant, however, sometimes 1 out of the 4 recordings had a fault. As all channels from one system were observed to be faulty, an issue of downloading the data is probably the cause as boxes were not combined until this stage. As to whether the fault was related to impedance, it is not possible to say, however, the system was fitted the same every night and the same participant often had good and faulty recordings.

Overall, participants tolerated the system well with only one control feeling claustrophobic but still going on to complete the second assessment. This system has proven to work in home sleep studies of Alzheimer's disease patients, due to its miniature and lightweight nature.

Finally, the analysis of EEG data must be commented on. The scoring criteria of Rechtschaffen and Kales [77] was utilised due to the separation of levels of deep sleep stages (Stages 3 and 4). The new AASM criteria combines these two stages [246]. It was thought that due to aging alterations in deep sleep [103] it would be important to differentiate between stage 3 and 4 sleep.

Future investigation of sleep EEG in Alzheimer's disease and its prodromal stages should be conducted in the field, to gain a better understanding of the natural sleep of Alzheimer's disease patients in their familiar surroundings.

7.2.5. Melatonin rhythm analysis

For Study 2, in order to analyse the rhythm of the endogeneous circadian clock, a clock rhythm phase marker, melatonin, was analysed. The sulphate metabolite of melatonin was assayed from sequential urine samples over a 3 day period and concentrations per hour were determined (for details see Chapter 2, p. 47). Two analyses of the data were then conducted: one using a non-linear regression model to best-fit the data [79]; and the other using a linear cosinor analysis (unpublished, Roelof Hut). Despite different methods of analysing the data, both methods yielded identical results (Chapter 6, p.249). From the data it can be concluded that both analyses are valid for the rhythm analysis of melatonin as a circadian marker.

7.2.6. Brain donation

Due to help of the Neuropathology Department staff, University of Oxford, and the family of JJB, the process of brain donation was conducted easily. Post-mortem tissue sections were collected with guidance from a brain atlas to determine the location of the relatively newly

located suprachiasmatic nuclei in the anterior hypothalamus [215] and no problems were experienced in this process. Further anatomical and imaging analysis was conducted by Dr. Olaf Ansorge from the Neuropathology department.

7.2.7. Statistical analysis

Statistical analyses of the data were limited due to missing data and small sample sizes per group for some, but not all comparisons. As explained in the methods chapter (Chapter 2, p.63), power calculations were conducted for each of the included methods. For the majority of groups and methods the sample sizes for good statistical power were not reached. However, an arbitrary cut-off of a minimum of four participants per group being compared was decided upon which allowed preliminary statistical analyses.

Calculations of change overtime or medication effects were further limited by missing data and due to the time elapsed from starting medication, this did not allow for repetition of the data collection.

Finally, those results which met a $p < 0.05$ significance level were usually found to not be significant after correction for multiple comparisons using the Dunn-Šidák test. This correction was used instead of the more conservative Bonferroni correction, due to the large number of variables collected from the questionnaire data. However, when analysing questionnaires, especially the JSQ which had no components or scoring system, the Dunn-Šidák was still very conservative, as even large differences between groups were deemed not to meet the corrected significance level due to the large number of variables.

7.3. Final conclusions

In Study 1, this thesis highlights the need for further research of the rest-activity and sleep of Alzheimer's disease and its prodromal stages in order to predict future progression of the disease. From the data presented it can be concluded that there is evidence that having a subjective memory complaint has an impact on the rest-activity and sleep of individuals. Assessment of the prodromal stages of Alzheimer's disease with regards to sleep and rest-activity patterns provides evidence that a subjective memory complaint may be an early prodromal stage of Alzheimer's disease. There is also evidence of a progression of rest-activity and sleep characteristics becoming altered in parallel with increasing memory impairment severity, which requires future investigation. Since cholinesterase inhibitors are the only medication prescribed for cognition maintenance in Alzheimer's disease in the UK, and have been shown to impact sleep, medication effects were investigated in more detail. Changes in sleep were noted with the medication when compared to treatment-naïve baseline assessment, and certain rest-activity and sleep parameters were found to predict tolerance and benefit from the medication. All of these findings of Study 1 warrant further research with a larger sample size and the addition of weekly assessments from the onset of medication.

Study 2 was the first of its kind to longitudinally measure the rest-activity pattern of a terminally ill cancer patient until death. Due to the nature of the brain cancer, the infiltration of the brain with cancer cells was diffuse. A mismatch between melatonin peak (a circadian rhythm phase marker) and rest-activity rhythms was found, which was probably related to cancer cell infiltration into the suprachiasmatic nucleus (SCN). Although cases such as this are unique the findings add to current knowledge of the human SCN.

Many lessons have been learnt from the limitations, strengths and practicalities of using each method in each of the groups observed in this thesis. Results here show that longitudinal

field studies of concomitant objective rest-activity, sleep and subjective measurements can be conducted in neurodegeneration and brain cancer. Although considered preliminary results, this thesis forms the basis for future investigation into sleep/circadian phenotypes in ageing populations, with and without Alzheimer's disease.

8. References

1. Ali, M.A., Boujard, T. and Gerkema, M., *Terminology in biological rhythms*, in *Rhythms in Fish*, M.A. Ali, Editor. 1992, Plenum Press: New York. p. 7-10.
2. Czeisler, C.A., et al., *Stability, precision, and near-24-hour period of the human circadian pacemaker*. Science, 1999. 284(5423): p. 2177-81.
3. Langmesser, S. and U. Albrecht, *Life time-circadian clocks, mitochondria and metabolism*. Chronobiol Int, 2006. 23(1-2): p. 151-7.
4. Romeijn, N., et al., *Sleep, vigilance, and thermosensitivity*. Pflugers Arch, 2012. 463(1): p. 169-76.
5. Albrecht, U., *Timing to perfection: the biology of central and peripheral circadian clocks*. Neuron, 2012. 74(2): p. 246-60.
6. Daan, S., D.G. Beersma, and A.A. Borbely, *Timing of human sleep: recovery process gated by a circadian pacemaker*. Am J Physiol, 1984. 246(2 Pt 2): p. R161-83.
7. Klerman, E.B., *Clinical aspects of human circadian rhythms*. J Biol Rhythms, 2005. 20(4): p. 375-86.
8. Hastings, M., *The brain, circadian rhythms, and clock genes*. BMJ, 1998. 317(7174): p. 1704-7.
9. Moore, R.Y., *Circadian rhythms: basic neurobiology and clinical applications*. Annu Rev Med, 1997. 48: p. 253-66.
10. Stephan, F.K. and I. Zucker, *Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions*. Proc Natl Acad Sci U S A, 1972. 69(6): p. 1583-6.
11. Reppert, S.M., et al., *Effects of damage to the suprachiasmatic area of the anterior hypothalamus on the daily melatonin and cortisol rhythms in the rhesus monkey*. J Neurosci, 1981. 1(12): p. 1414-25.
12. Lydic, R. and M.C. Moore-Ede, *Three dimensional structure of the suprachiasmatic nuclei in the diurnal squirrel monkey (Saimiri sciureus)*. Neurosci Lett, 1980. 17(3): p. 295-9.
13. Fuller, C.A., et al., *Auditory entrainment of primate drinking rhythms following partial suprachiasmatic nuclei lesions*. Physiol Behav, 1983. 31(4): p. 573-6.
14. von Schantz, M., *Clocks, genes and sleep*. Journal of the Royal Society of Medicine, 2003. 96: p. 486-9.
15. Hastings, M.H., A.B. Reddy, and E.S. Maywood, *A clockwork web: circadian timing in brain and periphery, in health and disease*. Nat Rev Neurosci, 2003. 4(8): p. 649-61.
16. Aton, S.J. and E.D. Herzog, *Come together, right...now: synchronization of rhythms in a mammalian circadian clock*. Neuron, 2005. 48(4): p. 531-4.
17. Antle, M.C. and R. Silver, *Orchestrating time: arrangements of the brain circadian clock*. Trends Neurosci, 2005. 28(3): p. 145-51.
18. Freedman, M.S., et al., *Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors*. Science, 1999. 284(5413): p. 502-4.
19. Foster, R.G., *Neurobiology: bright blue times*. Nature, 2005. 433(7027): p. 698-9.
20. Melyan, Z., et al., *Addition of human melanopsin renders mammalian cells photoresponsive*. Nature, 2005. 433(7027): p. 741-5.

21. Wittmann, M., et al., *Social jetlag: misalignment of biological and social time*. Chronobiol Int, 2006. 23(1-2): p. 497-509.
22. Roenneberg, T., A. Wirz-Justice, and M. Mewes, *Life between clocks: daily temporal patterns of human chronotypes*. J Biol Rhythms, 2003. 18(1): p. 80-90.
23. Roenneberg, T., et al., *Epidemiology of the human circadian clock*. Sleep Med Rev, 2007. 11(6): p. 429-38.
24. Schmidt, C., et al., *A time to think: circadian rhythms in human cognition*. Cogn Neuropsychol, 2007. 24(7): p. 755-89.
25. Zavada, A., et al., *Comparison of the Munich Chronotype Questionnaire with the Horne-Ostberg's Morningness-Eveningness Score*. Chronobiol Int, 2005. 22(2): p. 267-78.
26. Horne, J.A. and O. Ostberg, *A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms*. Int J Chronobiol, 1976. 4(2): p. 97-110.
27. Phillips, M.L., *Circadian rhythms: Of owls, larks and alarm clocks*. Nature, 2009. 458(7235): p. 142-4.
28. Wulff, K., et al., *Sleep and circadian rhythm disturbances: multiple genes and multiple phenotypes*. Curr Opin Genet Dev, 2009.
29. Datta, S. and R.R. Maclean, *Neurobiological mechanisms for the regulation of mammalian sleep-wake behavior: reinterpretation of historical evidence and inclusion of contemporary cellular and molecular evidence*. Neurosci Biobehav Rev, 2007. 31(5): p. 775-824.
30. Rosenzweig, *Biological Psychology*. 2005.
31. Aserinsky, E. and N. Kleitman, *Regularly occurring periods of eye motility, and concomitant phenomena, during sleep*. Science, 1953. 118(3062): p. 273-4.
32. Datta, S., E.H. Patterson, and D.F. Siwek, *Endogenous and exogenous nitric oxide in the pedunculopontine tegmentum induces sleep*. Synapse, 1997. 27(1): p. 69-78.
33. Datta, S. and J.A. Hobson, *Suppression of ponto-geniculo-occipital waves by neurotoxic lesions of pontine caudo-lateral peribrachial cells*. Neuroscience, 1995. 67(3): p. 703-12.
34. Datta, S. and D.F. Siwek, *Single cell activity patterns of pedunculopontine tegmentum neurons across the sleep-wake cycle in the freely moving rats*. J Neurosci Res, 2002. 70(4): p. 611-21.
35. Sarter, M. and J.P. Bruno, *Cognitive functions of cortical acetylcholine: toward a unifying hypothesis*. Brain Res Brain Res Rev, 1997. 23(1-2): p. 28-46.
36. Giovannini, M.G., et al., *Effect of subchronic treatment with metrifonate and tacrine on brain cholinergic function in aged F344 rats*. Eur J Pharmacol, 1998. 354(1): p. 17-24.
37. Woolf, N.J., J.B. Harrison, and J.S. Buchwald, *Cholinergic neurons of the feline pontomesencephalon. II. Ascending anatomical projections*. Brain Res, 1990. 520(1-2): p. 55-72.
38. Ulfloor, J., et al., *Spontaneous REM sleep is modulated by the activation of the pedunculopontine tegmental GABAB receptors in the freely moving rat*. J Neurophysiol, 2004. 91(4): p. 1822-31.
39. Kalsbeek, A., S. Perreau-Lenz, and R.M. Buijs, *A network of (autonomic) clock outputs*. Chronobiol Int, 2006. 23(3): p. 521-35.

40. Dijk, D.J. and S.W. Lockley, *Integration of human sleep-wake regulation and circadian rhythmicity*. J Appl Physiol, 2002. 92(2): p. 852-62.
41. Aeschbach, D., et al., *A longer biological night in long sleepers than in short sleepers*. J Clin Endocrinol Metab, 2003. 88(1): p. 26-30.
42. Borbely, A.A., *A two process model of sleep regulation*. Hum Neurobiol, 1982. 1(3): p. 195-204.
43. Borbely, A.A. and P. Achermann, *Sleep homeostasis and models of sleep regulation*. J Biol Rhythms, 1999. 14(6): p. 557-68.
44. Tononi, G. and C. Cirelli, *Sleep function and synaptic homeostasis*. Sleep Med Rev, 2006. 10(1): p. 49-62.
45. Deboer, T., et al., *Sleep states alter activity of suprachiasmatic nucleus neurons*. Nat Neurosci, 2003. 6(10): p. 1086-90.
46. Colwell, C.S. and S. Michel, *Sleep and circadian rhythms: do sleep centers talk back to the clock?* Nat Neurosci, 2003. 6(10): p. 1005-6.
47. Ellenbogen, J.M., J.D. Payne, and R. Stickgold, *The role of sleep in declarative memory consolidation: passive, permissive, active or none?* Curr Opin Neurobiol, 2006. 16(6): p. 716-22.
48. Jenkins, J.G.a.D., K.M., *Obliviscence during sleep and waking*. Am J Psychol, 1924. 35: p. 605-612.
49. Gais, S., B. Lucas, and J. Born, *Sleep after learning aids memory recall*. Learn Mem, 2006. 13(3): p. 259-62.
50. Barrett, T.R. and B.R. Ekstrand, *Effect of sleep on memory. 3. Controlling for time-of-day effects*. J Exp Psychol, 1972. 96(2): p. 321-7.
51. Peigneux, P., et al., *Are spatial memories strengthened in the human hippocampus during slow wave sleep?* Neuron, 2004. 44(3): p. 535-45.
52. Steriade, M., *Corticothalamic resonance, states of vigilance and mentation*. Neuroscience, 2000. 101(2): p. 243-76.
53. Ancoli-Israel, S., et al., *The role of actigraphy in the study of sleep and circadian rhythms*. Sleep, 2003. 26(3): p. 342-92.
54. Littner, M., et al., *Practice parameters for the role of actigraphy in the study of sleep and circadian rhythms: an update for 2002*. Sleep, 2003. 26(3): p. 337-41.
55. Mehra, R., et al., *Interpreting wrist actigraphic indices of sleep in epidemiologic studies of the elderly: the Study of Osteoporotic Fractures*. Sleep, 2008. 31(11): p. 1569-76.
56. Mahlberg, R. and S. Walther, *Actigraphy in agitated patients with dementia : Monitoring treatment outcomes*. Z Gerontol Geriatr, 2007. 40(3): p. 178-184.
57. de Souza, L., et al., *Further validation of actigraphy for sleep studies*. Sleep, 2003. 26(1): p. 81-5.
58. Blatter, K. and C. Cajochen, *Circadian rhythms in cognitive performance: methodological constraints, protocols, theoretical underpinnings*. Physiol Behav, 2007. 90(2-3): p. 196-208.
59. McKhann, G., et al., *Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease*. Neurology, 1984. 34(7): p. 939-44.
60. Petersen, R.C., et al., *Mild cognitive impairment: clinical characterization and outcome*. Arch Neurol, 1999. 56(3): p. 303-8.

61. Folstein, M.F., S.E. Folstein, and P.R. McHugh, *"Mini-mental state". A practical method for grading the cognitive state of patients for the clinician.* J Psychiatr Res, 1975. 12(3): p. 189-98.
62. Spitzer, R.L., K. Kroenke, and J.B. Williams, *Validation and utility of a self-report version of PRIME-MD: the PHQ primary care study. Primary Care Evaluation of Mental Disorders. Patient Health Questionnaire.* JAMA, 1999. 282(18): p. 1737-44.
63. Zigmond, A.S. and R.P. Snaith, *The hospital anxiety and depression scale.* Acta Psychiatr Scand, 1983. 67(6): p. 361-70.
64. Buysse, D.J., et al., *The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research.* Psychiatry Res, 1989. 28(2): p. 193-213.
65. Jupiter Medical Centre, Available from www.jupitermed.com/workfiles/.../sleep%20questionnaire.pdf. 2009.
66. Galasko, D., et al., *An inventory to assess activities of daily living for clinical trials in Alzheimer's disease. The Alzheimer's Disease Cooperative Study.* Alzheimer Dis Assoc Disord, 1997. 11 Suppl 2: p. S33-9.
67. Birchwood, M., et al., *The Social Functioning Scale. The development and validation of a new scale of social adjustment for use in family intervention programmes with schizophrenic patients.* Br J Psychiatry, 1990. 157: p. 853-9.
68. Blackwell, A.D., et al., *Detecting dementia: novel neuropsychological markers of preclinical Alzheimer's disease.* Dement Geriatr Cogn Disord, 2004. 17(1-2): p. 42-8.
69. De Jager, C., et al., *Predicting cognitive decline in healthy older adults.* Am J Geriatr Psychiatry, 2005. 13(8): p. 735-40.
70. Egerhazi, A., et al., *Automated Neuropsychological Test Battery (CANTAB) in mild cognitive impairment and in Alzheimer's disease.* Prog Neuropsychopharmacol Biol Psychiatry, 2007. 31(3): p. 746-51.
71. Sahakian, B.J. and J.T. Coull, *Tetrahydroaminoacridine (THA) in Alzheimer's disease: an assessment of attentional and mnemonic function using CANTAB.* Acta Neurol Scand Suppl, 1993. 149: p. 29-35.
72. Riekkinen, M., M.P. Laakso, and P. Jakala, *Clonidine impairs sustained attention and memory in Alzheimer's disease.* Neuroscience, 1999. 92(3): p. 975-82.
73. Ruf, T., *The Lomb-Scargle periodogram in biological rhythm research: Analysis of incomplete and unequally spaced time-series.* Biological Rhythm Research, 1999. 30(2): p. 178-201.
74. Sokolove, P.G. and W.N. Bushell, *The chi square periodogram: its utility for analysis of circadian rhythms.* J Theor Biol, 1978. 72(1): p. 131-60.
75. Sadeh, A., K.M. Sharkey, and M.A. Carskadon, *Activity-based sleep-wake identification: an empirical test of methodological issues.* Sleep, 1994. 17(3): p. 201-7.
76. Cole, R.J., et al., *Automatic sleep/wake identification from wrist activity.* Sleep, 1992. 15(5): p. 461-9.
77. Rechtschaffen, A., *A manual of standardized terminology, techniques and scoring system of sleep stages in human subjects.* 1968, Los Angeles: Brain Information Service/Brain Research Institute, University of California.
78. Van Someren, E.J., et al., *Bright light therapy: improved sensitivity to its effects on rest-activity rhythms in Alzheimer patients by application of nonparametric methods.* Chronobiol Int, 1999. 16(4): p. 505-18.
79. Wulff, K., et al., *Sleep and circadian rhythm disruption in schizophrenia.* Br J Psychiatry, 2012. 200(4): p. 308-16.

80. Redfern, P.H., J.M. Waterhouse, and D.S. Minors, *Circadian rhythms: principles and measurement*. Pharmacol Ther, 1991. 49(3): p. 311-27.
81. Minors, D.S. and J.M. Waterhouse, *Mathematical and statistical analysis of circadian rhythms*. Psychoneuroendocrinology, 1988. 13(6): p. 443-64.
82. Lockley, S.W., et al., *Relationship between napping and melatonin in the blind*. J Biol Rhythms, 1997. 12(1): p. 16-25.
83. Howatson, G., et al., *Effect of tart cherry juice (Prunus cerasus) on melatonin levels and enhanced sleep quality*. Eur J Nutr.
84. Bartsch, C., et al., *Melatonin and 6-sulfatoxymelatonin circadian rhythms in serum and urine of primary prostate cancer patients: evidence for reduced pineal activity and relevance of urinary determinations*. Clin Chim Acta, 1992. 209(3): p. 153-67.
85. Pina, G., et al., *Long-term alteration of daily melatonin, 6-sulfatoxymelatonin, cortisol, and temperature profiles in burn patients: a preliminary report*. Chronobiol Int, 2010. 27(2): p. 378-92.
86. Keller, M., et al., *A circadian clock in macrophages controls inflammatory immune responses*. Proc Natl Acad Sci U S A, 2009. 106(50): p. 21407-12.
87. Gilbert, M.R., et al., *Nocturnin expression is induced by fasting in the white adipose tissue of restricted fed mice*. PLoS One, 2011. 6(2): p. e17051.
88. Sporl, F., et al., *A circadian clock in HaCaT keratinocytes*. J Invest Dermatol, 2011. 131(2): p. 338-48.
89. Oster, H., et al., *Transcriptional profiling in the adrenal gland reveals circadian regulation of hormone biosynthesis genes and nucleosome assembly genes*. J Biol Rhythms, 2006. 21(5): p. 350-61.
90. Kiessling, S., G. Eichele, and H. Oster, *Adrenal glucocorticoids have a key role in circadian resynchronization in a mouse model of jet lag*. J Clin Invest, 2010. 120(7): p. 2600-9.
91. Jasper, I., et al., *Circadian rhythm in handwriting*. J Sleep Res, 2009. 18(2): p. 264-71.
92. Sellix, M.T., T. Yoshikawa, and M. Menaker, *A circadian egg timer gates ovulation*. Curr Biol, 2010. 20(6): p. R266-7.
93. Baumer, D., et al., *Juvenile ALS with basophilic inclusions is a FUS proteinopathy with FUS mutations*. Neurology, 2010. 75(7): p. 611-8.
94. Ben-Barak, Y., et al., *Neurophysin in the hypothalamo-neurohypophysial system. I. Production and characterization of monoclonal antibodies*. J Neurosci, 1985. 5(1): p. 81-97.
95. Van Someren, E.J., et al., *Indirect bright light improves circadian rest-activity rhythm disturbances in demented patients*. Biol Psychiatry, 1997. 41(9): p. 955-63.
96. Bliwise, D.L., et al., *REM latency in Alzheimer's disease*. Biol Psychiatry, 1989. 25(3): p. 320-8.
97. Benz, C.C. and C. Yau, *Ageing, oxidative stress and cancer: paradigms in parallax*. Nat Rev Cancer, 2008. 8(11): p. 875-9.
98. Rossi, P., et al., *Human skeletal muscle aging and the oxidative system: cellular events*. Curr Aging Sci, 2008. 1(3): p. 182-91.
99. Brodtmann, A., et al., *Regional fMRI brain activation does correlate with global brain volume*. Brain Res, 2009. 1259: p. 17-25.
100. Mandal, B. and B. Roe, *Job loss, retirement and the mental health of older Americans*. J Ment Health Policy Econ, 2008. 11(4): p. 167-76.

101. Bliwise, D.L., et al., *Nocturia and disturbed sleep in the elderly*. Sleep Med, 2009. 10(5): p. 540-8.
102. Ancoli-Israel, S. and L. Ayalon, *Diagnosis and treatment of sleep disorders in older adults*. Am J Geriatr Psychiatry, 2006. 14(2): p. 95-103.
103. Ancoli-Israel, S., L. Ayalon, and C. Salzman, *Sleep in the elderly: normal variations and common sleep disorders*. Harv Rev Psychiatry, 2008. 16(5): p. 279-86.
104. Baskett, J.J., et al., *Does melatonin improve sleep in older people? A randomised crossover trial*. Age Ageing, 2003. 32(2): p. 164-70.
105. Ohayon, M.M., *Sleep and the elderly*. J Psychosom Res, 2004. 56(5): p. 463-4.
106. Walters, J.F., et al., *Circadian variation in endothelial function is attenuated in postmenopausal women*. Maturitas, 2006. 54(3): p. 294-303.
107. Jean-Louis, G., et al., *Circadian sleep, illumination, and activity patterns in women: influences of aging and time reference*. Physiol Behav, 2000. 68(3): p. 347-52.
108. Maggi, S., et al., *Sleep complaints in community-dwelling older persons: prevalence, associated factors, and reported causes*. J Am Geriatr Soc, 1998. 46(2): p. 161-8.
109. Vitiello, M.V., et al., *Circadian temperature rhythms in young adult and aged men*. Neurobiol Aging, 1986. 7(2): p. 97-100.
110. Bjerner, B., A. Holm, and A. Swensson, *Diurnal variation in mental performance; a study of three-shift workers*. Br J Ind Med, 1955. 12(2): p. 103-10.
111. Wilkinson, R.T. and S. Allison, *Age and simple reaction time: decade differences for 5,325 subjects*. J Gerontol, 1989. 44(2): p. P29-35.
112. Blatter, K., et al., *Gender and age differences in psychomotor vigilance performance under differential sleep pressure conditions*. Behav Brain Res, 2006. 168(2): p. 312-7.
113. Jorm, A.F., et al., *Do cognitive complaints either predict future cognitive decline or reflect past cognitive decline? A longitudinal study of an elderly community sample*. Psychol Med, 1997. 27(1): p. 91-8.
114. Mattos, P., et al., *Memory complaints and test performance in healthy elderly persons*. Arq Neuropsiquiatr, 2003. 61(4): p. 920-4.
115. Alexander, D.M., et al., *EEG markers for cognitive decline in elderly subjects with subjective memory complaints*. J Integr Neurosci, 2006. 5(1): p. 49-74.
116. BMI index calculator, Available from:
http://www.weightlossresources.co.uk/body_weight/healthy_weight/bmi_calculator.htm.
117. Jorm, A.F., et al., *Complaints of cognitive decline in the elderly: a comparison of reports by subjects and informants in a community survey*. Psychol Med, 1994. 24(2): p. 365-74.
118. Mol, M., et al., *The effect of perceived forgetfulness on quality of life in older adults; a qualitative review*. Int J Geriatr Psychiatry, 2007. 22(5): p. 393-400.
119. Jorm, A.F., et al., *Memory complaints as a precursor of memory impairment in older people: a longitudinal analysis over 7-8 years*. Psychol Med, 2001. 31(3): p. 441-9.
120. Blazer, D.G., et al., *Memory complaint as a predictor of cognitive decline: a comparison of African American and White elders*. J Aging Health, 1997. 9(2): p. 171-84.
121. Peres, K., et al., *Gender differences in the prodromal signs of dementia: memory complaint and IADL-restriction. a prospective population-based cohort*. J Alzheimers Dis, 2011. 27(1): p. 39-47.

122. Glodzik-Sobanska, L., et al., *Subjective memory complaints: presence, severity and future outcome in normal older subjects*. Dement Geriatr Cogn Disord, 2007. 24(3): p. 177-84.
123. Lenihan, M.E., S.Z. Klekociuk, and M.J. Summers, *Absence of a relationship between subjective memory complaint and objective memory impairment in mild cognitive impairment (MCI): is it time to abandon subjective memory complaint as an MCI diagnostic criterion?* Int Psychogeriatr, 2012. 24(9): p. 1505-14.
124. Klemm, W.R., *Why does rem sleep occur? A wake-up hypothesis*. Front Syst Neurosci, 2011. 5: p. 73.
125. Flicker, C., S.H. Ferris, and B. Reisberg, *A longitudinal study of cognitive function in elderly persons with subjective memory complaints*. J Am Geriatr Soc, 1993. 41(10): p. 1029-32.
126. Martin, J.B., *Molecular basis of the neurodegenerative disorders*. N Engl J Med, 1999. 340(25): p. 1970-80.
127. Yan, S.D., et al., *An intracellular protein that binds amyloid-beta peptide and mediates neurotoxicity in Alzheimer's disease*. Nature, 1997. 389(6652): p. 689-95.
128. Yan, S.D., et al., *RAGE and amyloid-beta peptide neurotoxicity in Alzheimer's disease*. Nature, 1996. 382(6593): p. 685-91.
129. Cleary, J.P., et al., *Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function*. Nat Neurosci, 2005. 8(1): p. 79-84.
130. Braak, H. and E. Braak, *Staging of Alzheimer's disease-related neurofibrillary changes*. Neurobiol Aging, 1995. 16(3): p. 271-8; discussion 278-84.
131. Meeks, T.W., S.A. Ropacki, and D.V. Jeste, *The neurobiology of neuropsychiatric syndromes in dementia*. Curr Opin Psychiatry, 2006. 19(6): p. 581-6.
132. Cummings, J.L. and D. Kaufer, *Neuropsychiatric aspects of Alzheimer's disease: the cholinergic hypothesis revisited*. Neurology, 1996. 47(4): p. 876-83.
133. Lopez, O.L., et al., *Psychiatric symptoms vary with the severity of dementia in probable Alzheimer's disease*. J Neuropsychiatry Clin Neurosci, 2003. 15(3): p. 346-53.
134. Volicer, L., et al., *Sundowning and circadian rhythms in Alzheimer's disease*. Am J Psychiatry, 2001. 158(5): p. 704-11.
135. American Psychiatric Association, *Diagnostic Statistical Manual Version IV-T-R*. 1994.
136. Dubois, B., et al., *Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria*. Lancet Neurol, 2007. 6(8): p. 734-46.
137. Parra, M.A., et al., *Short-term memory binding deficits in Alzheimer's disease*. Brain, 2009. 132(Pt 4): p. 1057-66.
138. Rasch, B.H., J. Born, and S. Gais, *Combined blockade of cholinergic receptors shifts the brain from stimulus encoding to memory consolidation*. J Cogn Neurosci, 2006. 18(5): p. 793-802.
139. Scheff, S.W., et al., *Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment*. Neurobiol Aging, 2006. 27(10): p. 1372-84.
140. Olichney, J.M., et al., *Abnormal verbal event related potentials in mild cognitive impairment and incipient Alzheimer's disease*. J Neurol Neurosurg Psychiatry, 2002. 73(4): p. 377-84.
141. Savaskan, E., et al., *Increased melatonin 1a-receptor immunoreactivity in the hippocampus of Alzheimer's disease patients*. J Pineal Res, 2002. 32(1): p. 59-62.
142. Ferrari, E., et al., *Pineal and pituitary-adrenocortical function in physiological aging and in senile dementia*. Exp Gerontol, 2000. 35(9-10): p. 1239-50.

143. Harper, D.G., et al., *Differential circadian rhythm disturbances in men with Alzheimer disease and frontotemporal degeneration*. Arch Gen Psychiatry, 2001. 58(4): p. 353-60.
144. Satlin, A., et al., *Circadian locomotor activity and core-body temperature rhythms in Alzheimer's disease*. Neurobiol Aging, 1995. 16(5): p. 765-71.
145. Harper, D.G., et al., *Disturbance of endogenous circadian rhythm in aging and Alzheimer disease*. Am J Geriatr Psychiatry, 2005. 13(5): p. 359-68.
146. Moran, M., et al., *Sleep disturbance in mild to moderate Alzheimer's disease*. Sleep Med, 2005. 6(4): p. 347-52.
147. Rauchs, G., et al., *Is there a link between sleep changes and memory in Alzheimer's disease?* Neuroreport, 2008. 19(11): p. 1159-62.
148. Yu, J.M., et al., *Low sleep efficiency in patients with cognitive impairment*. Acta Neurol Taiwan, 2009. 18(2): p. 91-7.
149. Beaulieu-Bonneau, S. and C. Hudon, *Sleep disturbances in older adults with mild cognitive impairment*. Int Psychogeriatr, 2009. 21(4): p. 654-66.
150. Ltd., C.C., *CANTABclipse*, 2006: Cambridge.
151. Bombin, I., et al., *Functional impairment as a defining feature of: amnesic MCI cognitive, emotional, and demographic correlates*. Int Psychogeriatr, 2012. 24(9): p. 1494-504.
152. Swainson, R., et al., *Early detection and differential diagnosis of Alzheimer's disease and depression with neuropsychological tasks*. Dement Geriatr Cogn Disord, 2001. 12(4): p. 265-80.
153. Petersen, R.C., et al., *Apolipoprotein E status as a predictor of the development of Alzheimer's disease in memory-impaired individuals*. JAMA, 1995. 273(16): p. 1274-8.
154. Maki, Y., et al., *Anosognosia: Patients' Distress and Self-awareness of Deficits in Alzheimer's Disease*. Am J Alzheimers Dis Other Dement, 2012. 27(5): p. 339-45.
155. Pollak, C.P. and P.E. Stokes, *Circadian rest-activity rhythms in demented and nondemented older community residents and their caregivers*. J Am Geriatr Soc, 1997. 45(4): p. 446-52.
156. Westerberg, C.E., et al., *Concurrent impairments in sleep and memory in amnesic mild cognitive impairment*. J Int Neuropsychol Soc, 2012. 18(3): p. 490-500.
157. Bliwise, D.L., *Sleep in normal aging and dementia*. Sleep, 1993. 16(1): p. 40-81.
158. Bonanni, E., et al., *Daytime sleepiness in mild and moderate Alzheimer's disease and its relationship with cognitive impairment*. J Sleep Res, 2005. 14(3): p. 311-7.
159. Campbell, S.S., et al., *Effects of a month-long napping regimen in older individuals*. J Am Geriatr Soc, 2011. 59(2): p. 224-32.
160. Song, Y., et al., *Sleep in older adults with Alzheimer's disease*. J Neurosci Nurs, 2010. 42(4): p. 190-8; quiz 199-200.
161. Prinz, P.N., et al., *Sleep, EEG and mental function changes in senile dementia of the Alzheimer's type*. Neurobiol Aging, 1982. 3(4): p. 361-70.
162. Montplaisir, J., et al., *Sleep disturbances and eeg slowing in alzheimer's disease*. Sleep Res Online, 1998. 1(4): p. 147-51.
163. Montplaisir, J., et al., *Sleep in Alzheimer's disease: further considerations on the role of brainstem and forebrain cholinergic populations in sleep-wake mechanisms*. Sleep, 1995. 18(3): p. 145-8.
164. Pollak, C.P. and D. Perlick, *Sleep problems and institutionalization of the elderly*. J Geriatr Psychiatry Neurol, 1991. 4(4): p. 204-10.

165. Tranah, G.J., et al., *Circadian activity rhythms and risk of incident dementia and mild cognitive impairment in older women*. Ann Neurol, 2011. 70(5): p. 722-32.
166. Hatfield, C.F., et al., *Disrupted daily activity/rest cycles in relation to daily cortisol rhythms of home-dwelling patients with early Alzheimer's dementia*. Brain, 2004. 127(Pt 5): p. 1061-74.
167. Tractenberg, R.E., C.M. Singer, and J.A. Kaye, *Characterizing sleep problems in persons with Alzheimer's disease and normal elderly*. J Sleep Res, 2006. 15(1): p. 97-103.
168. Yesavage, J.A., et al., *Sleep/wake disruption in Alzheimer's disease: APOE status and longitudinal course*. J Geriatr Psychiatry Neurol, 2004. 17(1): p. 20-4.
169. Moghekar, A. and R.J. O'Brien, *Con: Alzheimer's disease and circadian dysfunction: chicken or egg?* Alzheimers Res Ther, 2012. 4(4): p. 26.
170. Bedrosian, T.A. and R.J. Nelson, *Pro: Alzheimer's disease and circadian dysfunction: chicken or egg?* Alzheimers Res Ther, 2012. 4(4): p. 25.
171. Deschenes, C.L. and S.M. McCurry, *Current treatments for sleep disturbances in individuals with dementia*. Curr Psychiatry Rep, 2009. 11(1): p. 20-6.
172. Roh, J.H., et al., *Disruption of the Sleep-Wake Cycle and Diurnal Fluctuation of beta-Amyloid in Mice with Alzheimer's Disease Pathology*. Sci Transl Med, 2012. 4(150): p. 150ra122.
173. Feldman, H., et al., *Efficacy of donepezil on maintenance of activities of daily living in patients with moderate to severe Alzheimer's disease and the effect on caregiver burden*. J Am Geriatr Soc, 2003. 51(6): p. 737-44.
174. Siegler, E.L. and M. Reidenberg, *Treatment of urinary incontinence with anticholinergics in patients taking cholinesterase inhibitors for dementia*. Clin Pharmacol Ther, 2004. 75(5): p. 484-8.
175. Cummings, J.L., *Cholinesterase inhibitors: A new class of psychotropic compounds*. Am J Psychiatry, 2000. 157(1): p. 4-15.
176. Black, S.E., et al., *Donepezil preserves cognition and global function in patients with severe Alzheimer disease*. Neurology, 2007. 69(5): p. 459-69.
177. Howard, R.J., et al., *Donepezil for the treatment of agitation in Alzheimer's disease*. N Engl J Med, 2007. 357(14): p. 1382-92.
178. Lyketsos, C.G., et al., *Long-term outcomes of galantamine treatment in patients with Alzheimer disease*. Am J Geriatr Psychiatry, 2004. 12(5): p. 473-82.
179. Markowitz, J.S., et al., *Sleep-related outcomes in persons with mild to moderate Alzheimer disease in a placebo-controlled trial of galantamine*. Sleep, 2003. 26(5): p. 602-6.
180. Mahlberg, R., et al., *Effects of rivastigmine on actigraphically monitored motor activity in severe agitation related to Alzheimer's disease: a placebo-controlled pilot study*. Arch Gerontol Geriatr, 2007. 45(1): p. 19-26.
181. National Institute for Health and Clinical Excellence, *Dementia: Supporting people with dementia and their carers in health and social care*, in NICE clinical guideline 42, 2006.
182. Burns, A., et al., *The effects of donepezil in Alzheimer's disease - results from a multinational trial*. Dement Geriatr Cogn Disord, 1999. 10(3): p. 237-44.
183. American Society of Health-System Pharmacists, *AHFS Consumer Medication Information: Donepezil*. Available from: <http://www.nlm.nih.gov/medlineplus/druginfo/meds/a697032.html>.

184. Moraes, W., et al., *Donepezil improves obstructive sleep apnea in Alzheimer disease: a double-blind, placebo-controlled study*. Chest, 2008. 133(3): p. 677-83.
185. Nissen, C., et al., *Differential effects of the muscarinic M1 receptor agonist RS-86 and the acetylcholine-esterase inhibitor donepezil on REM sleep regulation in healthy volunteers*. Neuropsychopharmacology, 2006. 31(6): p. 1294-300.
186. Mizuno, S., et al., *Effects of donepezil on Alzheimer's disease: the relationship between cognitive function and rapid eye movement sleep*. Psychiatry Clin Neurosci, 2004. 58(6): p. 660-5.
187. Ancoli-Israel, S., et al., *Effects of galantamine versus donepezil on sleep in patients with mild to moderate Alzheimer disease and their caregivers: a double-blind, head-to-head, randomized pilot study*. Alzheimer Dis Assoc Disord, 2005. 19(4): p. 240-5.
188. Most, E.I., et al., *Discrepancy between subjective and objective sleep disturbances in early- and moderate-stage Alzheimer disease*. Am J Geriatr Psychiatry, 2012. 20(6): p. 460-7.
189. Davis, B. and K. Sadik, *Circadian cholinergic rhythms: implications for cholinesterase inhibitor therapy*. Dement Geriatr Cogn Disord, 2006. 21(2): p. 120-9.
190. Schredl, M., et al., *The effect of donepezil on sleep in elderly, healthy persons: a double-blind placebo-controlled study*. Pharmacopsychiatry, 2006. 39(6): p. 205-8.
191. Kanbayashi, T., et al., *Effects of donepezil (Aricept) on the rapid eye movement sleep of normal subjects*. Psychiatry Clin Neurosci, 2002. 56(3): p. 307-8.
192. Moraes Wdos, S., et al., *The effect of donepezil on sleep and REM sleep EEG in patients with Alzheimer disease: a double-blind placebo-controlled study*. Sleep, 2006. 29(2): p. 199-205.
193. Sutter, C., et al., *Sleep Quality and Cognitive Function in Healthy Old Age: The Moderating Role of Subclinical Depression*. Neuropsychology, 2012.
194. National Institute for Health and Clinical Excellence, *TA217 Donepezil, galantamine, rivastigmine and memantine for the treatment of Alzheimer's disease*, 2011: publications.nice.org.uk/donepezil-galantamine-rivastigmine-and-memantine-for-the-treatment-of-alzheimers-disease-ta217.
195. Diedrich, J., et al., *Identifying and mapping changes in gene expression involved in the neuropathology of scrapie and Alzheimer's disease*. Curr Top Microbiol Immunol, 1991. 172: p. 259-74.
196. Tondelli, M., et al., *Structural MRI changes detectable up to ten years before clinical Alzheimer's disease*. Neurobiol Aging, 2012. 33(4): p. 825 e25-36.
197. Blennow, K., H. Zetterberg, and A.M. Fagan, *Fluid biomarkers in Alzheimer disease*. Cold Spring Harb Perspect Med, 2012. 2(9).
198. Lautenschlager, N.T., et al., *Subjective memory complaints with and without objective memory impairment: relationship with risk factors for dementia*. Am J Geriatr Psychiatry, 2005. 13(8): p. 731-4.
199. Holmes, C., et al., *The efficacy of donepezil in the treatment of neuropsychiatric symptoms in Alzheimer disease*. Neurology, 2004. 63(2): p. 214-9.
200. Richter, C., *Sleep and activity: their relation to the 24 hour clock.*, in *Sleep and Altered States of Consciousness*, S.S. Kety, Evarts, E.V. & Williams, H.L., Editor. 1967, Williams & Williams Company: Baltimore. p. 9-29.
201. Rusak, B. and I. Zucker, *Neural regulation of circadian rhythms*. Physiol Rev, 1979. 59(3): p. 449-526.

202. Stephan, F.K., *Circadian rhythm dissociation induced by periodic feeding in rats with suprachiasmatic lesions*. Behav Brain Res, 1983. 7(1): p. 81-98.
203. Davis, F.C. and R.A. Gorski, *Unilateral Lesions of the Hamster Suprachiasmatic Nuclei - Evidence for Redundant Control of Circadian-Rhythms*. Journal of Comparative Physiology, 1984. 154(2): p. 221-232.
204. Fulton, J.a.B., P, *Tumors in the region of the third ventricle: their diagnosis and relation to pathological sleep*. J Nerv Ment Dis, 1929. 69: p. 145-164.
205. Schwartz, W.J., N.A. Busis, and E.T. Hedley-Whyte, *A discrete lesion of ventral hypothalamus and optic chiasm that disturbed the daily temperature rhythm*. J Neurol, 1986. 233(1): p. 1-4.
206. Aschoff, J. and S. Daan, *Human time perception in temporal isolation: effects of illumination intensity*. Chronobiol Int, 1997. 14(6): p. 585-96.
207. Aschoff, J., *Human perception of short and long time intervals: its correlation with body temperature and the duration of wake time*. J Biol Rhythms, 1998. 13(5): p. 437-42.
208. Lewis, P.A. and R.C. Miall, *Distinct systems for automatic and cognitively controlled time measurement: evidence from neuroimaging*. Curr Opin Neurobiol, 2003. 13(2): p. 250-5.
209. Buhusi, C.V. and W.H. Meck, *What makes us tick? Functional and neural mechanisms of interval timing*. Nat Rev Neurosci, 2005. 6(10): p. 755-65.
210. Dresemann, G., *Temozolomide in malignant glioma*. Onco Targets Ther. 3: p. 139-46.
211. Holland, E.C., *Glioblastoma multiforme: the terminator*. Proc Natl Acad Sci U S A, 2000. 97(12): p. 6242-4.
212. Lassman A.B., H.E.C., *Glioblastoma mutlifforme-Past, present, and future*. Business Briefing US Neurology Review, 2005: p. 1-6.
213. Morrow, G.R., et al., *Fatigue associated with cancer and its treatment*. Support Care Cancer, 2002. 10(5): p. 389-98.
214. Hinds, P.S., et al., *Dexamethasone alters sleep and fatigue in pediatric patients with acute lymphoblastic leukemia*. Cancer, 2007. 110(10): p. 2321-30.
215. Mai JK, A.J., Paxinos G, *Atlas of the Human Brain Book and CD-ROM*. 1998: Academic Press.
216. Stopa, E.G., et al., *Human brain contains vasopressin and vasoactive intestinal polypeptide neuronal subpopulations in the suprachiasmatic region*. Brain Res, 1984. 297(1): p. 159-63.
217. Swaab, D.F., M.A. Hofman, and M.B. Honnebier, *Development of vasopressin neurons in the human suprachiasmatic nucleus in relation to birth*. Brain Res Dev Brain Res, 1990. 52(1-2): p. 289-93.
218. Harrington, D.L., K.Y. Haaland, and R.T. Knight, *Cortical networks underlying mechanisms of time perception*. J Neurosci, 1998. 18(3): p. 1085-95.
219. Arendt, J., *Melatonin and the pineal gland: influence on mammalian seasonal and circadian physiology*. Rev Reprod, 1998. 3(1): p. 13-22.
220. Albers, H.E., et al., *Role of the suprachiasmatic nuclei in the circadian timing system of the squirrel monkey. I. The generation of rhythmicity*. Brain Res, 1984. 300(2): p. 275-84.
221. Cohen, R.A. and H.E. Albers, *Disruption of human circadian and cognitive regulation following a discrete hypothalamic lesion: a case study*. Neurology, 1991. 41(5): p. 726-9.

222. *Round-the-clock resources for Dementia*. 2012; Available from: http://www.psychiatry24x7.com/bgdisplay.jhtml?itemname=dementia_symptoms.
223. Calandre, E.P., et al., *Trazodone plus pregabalin combination in the treatment of fibromyalgia: a two-phase, 24-week, open-label uncontrolled study*. BMC Musculoskelet Disord, 2011. 12: p. 95.
224. Carroll, B.T., et al., *Screening for depression and anxiety in cancer patients using the Hospital Anxiety and Depression Scale*. Gen Hosp Psychiatry, 1993. 15(2): p. 69-74.
225. Lisspers, J., A. Nygren, and E. Soderman, *Hospital Anxiety and Depression Scale (HAD): some psychometric data for a Swedish sample*. Acta Psychiatr Scand, 1997. 96(4): p. 281-6.
226. Meliska, C.J., et al., *Relationship of morningness-eveningness questionnaire score to melatonin and sleep timing, body mass index and atypical depressive symptoms in peri- and post-menopausal women*. Psychiatry Res, 2011. 188(1): p. 88-95.
227. Hofstra, W.A., et al., *Chronotypes and subjective sleep parameters in epilepsy patients: a large questionnaire study*. Chronobiol Int, 2010. 27(6): p. 1271-86.
228. Kim, S.J., et al., *Age as a moderator of the association between depressive symptoms and morningness-eveningness*. J Psychosom Res, 2010. 68(2): p. 159-64.
229. Taillard, J., et al., *Validation of Horne and Ostberg morningness-eveningness questionnaire in a middle-aged population of French workers*. J Biol Rhythms, 2004. 19(1): p. 76-86.
230. Athens Center for Sleep Disorders, T., USA. *Screening Questionnaire: Restless Legs Syndrome*. [cited 2012].
231. White, D. *Screening for Sleep Apnea*. 1998.
232. Tombaugh, T.N. and N.J. McIntyre, *The mini-mental state examination: a comprehensive review*. J Am Geriatr Soc, 1992. 40(9): p. 922-35.
233. Doody, R.S., et al., *Effect of donepezil on cognition, activities of daily living, behaviour, and global function in patients with mild-to-moderate Alzheimer's disease: a randomised, double-blind, placebo-controlled study*. Lancet, 2008. 372(9634): p. 207-15.
234. Rogers, S.L., et al., *A 24-week, double-blind, placebo-controlled trial of donepezil in patients with Alzheimer's disease*. Donepezil Study Group. Neurology, 1998. 50(1): p. 136-45.
235. Lewis, J.E., et al., *The Effect of an Aloe Polymannose Multinutrient Complex on Cognitive and Immune Functioning in Alzheimer's Disease*. J Alzheimers Dis, 2012.
236. Gordon, M.L., et al., *An Open-Label Exploratory Study with Memantine: Correlation between Proton Magnetic Resonance Spectroscopy and Cognition in Patients with Mild to Moderate Alzheimer's Disease*. Dement Geriatr Cogn Dis Extra, 2012. 2(1): p. 312-20.
237. Farlow, M.R., et al., *The effect of vascular risk factors on the efficacy of rivastigmine patch and capsule treatment in Alzheimer's disease*. Dement Geriatr Cogn Dis Extra, 2011. 1(1): p. 150-62.
238. Carpenter, J.S. and M.A. Andrykowski, *Psychometric evaluation of the Pittsburgh Sleep Quality Index*. J Psychosom Res, 1998. 45(1 Spec No): p. 5-13.
239. Picarsic, J.L., et al., *Self-reported napping and duration and quality of sleep in the lifestyle interventions and independence for elders pilot study*. J Am Geriatr Soc, 2008. 56(9): p. 1674-80.

240. Buysse, D.J., et al., *Relationships between the Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS), and clinical/polysomnographic measures in a community sample.* J Clin Sleep Med, 2008. 4(6): p. 563-71.
241. Kuhlmei, A., et al., *Actigraphic daytime activity is reduced in patients with cognitive impairment and apathy.* Eur Psychiatry, 2011.
242. Naismith, S.L., et al., *Sleep well, think well: sleep-wake disturbance in mild cognitive impairment.* J Geriatr Psychiatry Neurol, 2010. 23(2): p. 123-30.
243. Tortorese, D.J., et al., *Experimental jetlag disrupts circadian clock genes but improves performance in racehorses after light-dependent rapid resetting of neuroendocrine systems and the rest-activity cycle.* J Neuroendocrinol, 2011. 23(12): p. 1263-72.
244. van Uiter, M., et al., *Rest-activity patterns in patients with delirium.* Rejuvenation Res, 2011. 14(5): p. 483-90.
245. Bromundt, V., et al., *Sleep-wake cycles and cognitive functioning in schizophrenia.* Br J Psychiatry, 2011. 198(4): p. 269-76.
246. Iber, C., Ancoli-Israel, S., Chesson, A.L. Jr. and Quan, S.F., *The AASM manual for the scoring of sleep and associated events: rules, terminology and technical specifications.* 2007, Westchester, IL: American Academy of Sleep Medicine.

Neurodegeneration and brain cancer: A longitudinal field study of rest-activity and sleep



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Appendix

1. Study 1

1.1. List of Medication Categories

Calcium Channel Blockers

Lercanidipine
Adalat
Amlodipine Maleate
Felodipine
Diltiazem Hydrochloride

Beta Blockers

Levobunolol Hydrochloride
Atenolol
Bisoprolol

Corticosteroids

Pregnisolone
Fluticasone Propionate
Budesonide
Becloethasone

Hormone Reduction Medication

Goserelin
Tamoxifen

Hormone Replacement Therapy

Premarin
Ovestin

Vitamins

Hydroxocobalamin
Glucosamine
Alendronic Acid
Ginseng
Ginkgo Biloba
Vitamin C
Zinc
Folic Acid
Vitamin B
Adcal D3
Cu10
Red Vine Leaf Extract
Fish Oil

Proton Pump Inhibitors

Omeprazole
Lansoprazole

Statins

Atorvastatin
Simvastatin

Non-Steroidal Anti-Inflammatory Drugs

Naproxen
Dicloflex
Ibuprofen

Anti-Coagulants

Warfarin

Cholesterol Lowering Drugs

Ezetimibe

ACE Inhibitors

Lisinopril
Ramipril
Enalapril Maleate

Analgesics

Paracetamol
Tramadol
Parecoxib
Cocodomol
Aspirin

Thyroxine

Synthetic Thyroxine

Anti-Glaucoma Medications

Latanoprost
Dorzololmide Hydrochloride

Selective Serotonin Re-Uptake Inhibitors

Citalopram
Seroxat
Paroxetine

Anti-Diabetics

Glibenclamide
Gliclazide
Pioglitazone
Metformin

Anti-Cholinergics

Ipratropium Bromide
Oxybutynin Hydrochloride

Other Hypertensives

Losartan
Valsartan
Candesartan

Bisphosphates

Alendronic Acid

Beta-2 Adrenergic Receptor Agonists

Salmeterol
Atenolol
Timolol
Salbutamol

Alpha Blockers

Doxazosin
Tabphyn

Hypnotics

Zopiclone

Anti-Muscarinics

Tolterodine

Eye Lubricants or Medications

Viscotears
Retinax
Hypromellose

Laxatives

Laxido
Regulan
Lactulose

Anti-Platelet Medications

Clopidogrel

Allergy Medications

Prevalin
Fexofenadine

Diuretics

Bendroflumethiazide
Furosemide
Amiloride

Antacids

Gaviscon

Anti-Angina Medications

Glyceryl Trinitrate

1.2. List of Clinical Condition Categories

Weight Conditions

Overweight
Obesity
Appetite issues

Neurological Conditions

Delirium after operation
Carpel Tunnel
Synaesthesia
Tinnitus
Bell's palsy
Raymon-Céstan syndrome
Aphonia
Confusion

Past Head injury/Loss of Consciousness/Falls

Respiration Issues

Problems on exercise
Dispnea

Hypertension

Past Cancer

Breast cancer
Breast diverticulitis

Urinary Conditions

Current Cancer

Prostate cancer

Allergies

Arthritis/Bone Conditions

Perthe's disease
Cervical spondylosis
Bone fracture
Laminectomy

Viral Diseases

Shingles

Past Epilepsy

Pain Conditions

Diabetes

Alzheimer's disease

Vascular Dementia

Blood Conditions

Gout
Past malaria
Anaemia

Reproductive Conditions

Removal of reproductive organs

Thyroid Conditions

Gastro-Intestinal Conditions

Gastric ulcer
HP gastritis
Gall-bladder problems
Hernia

Kidney Conditions

Chronic kidney disease

Bacterial Diseases

Autoimmune Conditions

Psoriasis

Skin Conditions

Eczema

High Cholesterol

Hypercholesterolemia

Sleep Conditions

Nightmares

Past Stroke

Past transient ischaemic attack

Heart Conditions

Ischaemic heart disease

Angina

Triple heart bypass

Myocardial infarction

1.3. Demographics and Screening Data

Parameter	Age (yrs.)	No. of females (%)	No. of males (%)	Body Mass Index (BMI)	Collar size in normal range (%)	Weight (kg)	Height (cm)	Av. units of alcohol per week	Years of education
Control (n=9)	73.2 ± 4.9	4 (44.4)	5 (55.6)	25.0 ± 4.0	7 (77.7)	70.8 ± 14.0	168.0 ± 10.8	11.6 ± 11.2	14.7 ± 4.9
SMC (n=5)	73.2 ± 5.4	2 (40.0)	3 (60.0)	25.1 ± 2.1	4 (80.0)	75.2 ± 9.4	171.8 ± 5.5	13.3 ± 9.6	15.3 ± 4.7
aMCI (n=9)	76.8 ± 3.9	4 (44.4)	5 (55.6)	25.6 ± 4.4	8 (88.8)	75.7 ± 18.9	171.2 ± 10.2	6.0 ± 3.9	13.8 ± 3.3
Mild AD (n=3)	77.0 ± 8.5	1 (33.3)	2 (66.6)	22.6 ± 2.7	3 (100.0)	68.8 ± 11.1	174.3 ± 4.0	7.0 ± 1.0	14.0 (1)
qmAD (n=31)	78.5 ± 6.2	14 (45.2)	17 (54.8)	25.2 ± 4.1 (21)	19 (82.6)	69.4 ± 12.9	167.3 ± 10.0	5.3 ± 7.6	11.3 ± 2.0
Control vs. qmAD test statistic (p-value)	F=5.3 (0.027)								
SMC vs. qmAD test statistic (p-value)	F=4.4 (0.045) F=5.5 (0.037)								

Parameter	Marital Status Married (%)	Living Situation Live with partner (%)	No. of people that have children (%)	Accommodati on type House (%)	Travel across time zones last 3 months (%)	Ever >14 units of alcohol per week (%)	Smoking frequency none (%)	Caffeine intake frequency Frequently (%)
Control (n=9)	8 (88.9)	9 (100.0)	9 (100.0)	8 (88.9)	0 (0.0)	3 (33.3)	9 (100.0)	4 (44.4)
SMC (n=5)	4 (80.0)	4 (80.0)	5 (100.0)	5 (100.0)	1 (20.0)	1 (20.0)	5 (100.0)	2 (40.0)
aMCI (n=9)	7 (77.8)	7 (77.8)	8 (88.9)	7 (77.8)	1 (12.5)	2 (22.2)	9 (100.0)	4 (44.4)

Parameter	Marital Status Married (%)	Living Situation Live with partner (%)	No. of people that have children (%)	Accommodati on type House (%)	Travel across time zones last 3 months (%)	Ever >14 units of alcohol per week (%)	Smoking frequency none (%)	Caffeine intake frequency Frequently (%)
Mild AD (n=3)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	0 (0.0)	1 (33.3)	3 (100.0)	3 (100.0)
qmAD (n=31)	25 (80.6)	24 (77.4)	29 (96.7)	29 (93.5)	0 (0.0)	6 (21.4)	26 (89.7)	18 (62.1)

Parameter	Highest level of education Secondary school (%)	Occupation Type Care sector (%)	Shift work in lifetime (%)	Handedness Right (%)	Past mental health diagnosis Yes (%)	Heart medication Yes (%)	Chest pain or blood pressure medication Yes (%)	Asthma Medication Yes (%)
Control (n=9)	4 (44.4)	1 (12.5)	1 (11.1)	6 (85.7)	1 (11.1)	1 (12.5)	2 (25.0)	0 (0.0)
SMC (n=5)	0 (0.0)	0 (0.0)	0 (0.0)	3 (100.0)	2 (40.0)	1 (25.0)	2 (40.0)	0 (0.0)
aMCI (n=9)	1 (11.1)	0 (0.0)	1 (11.1)	2 (50.0)	1 (11.1)	3 (37.5)	7 (77.8)	1 (16.7)
Mild AD (n=3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	1 (33.3)	0 (0.0)	1 (33.3)	1 (33.3)
qmAD (n=31)	18 (64.3)	1 (7.7)	2 (6.9)	8 (100.0)	6 (22.2)	5 (20.0)	9 (37.5)	3 (12.5)

Chi-square=16.1
square=16.1
(0.024)
test statistic (p-value)
Pearson's R=-
0.5 (0.002)

Parameter		Had					Diagnosed with high blood pressure Yes (%)	Heart problems Yes (%)	Family history of heart disease/stroke Yes (%)	History or family history of epilepsy Yes (%)	Diagnosis of asthma Yes (%)
		Have a disability Yes (%)	complications from previous trials or treatments Yes (%)	Allergies Yes (%)							
Control	(n=9)	3 (33.3)	1 (11.1)	1 (11.1)			3 (33.3)	2 (22.2)	5 (55.6)	0 (0.0)	1 (11.1)
SMC	(n=5)	1 (20.0)	0 (0.0)	1 (20.0)			2 (40.0)	0 (0.0)	4 (80.0)	1 (20.0)	0 (0.0)
aMCI	(n=9)	1 (11.1)	1 (11.1)	2 (22.2)			7 (77.8)	2 (22.2)	3 (33.3)	1 (11.1)	2 (22.2)
Mild AD	(n=3)	0 (0.0)	1 (33.3)	0 (0.0)			1 (33.3)	0 (0.0)	1 (33.3)	0 (0.0)	1 (33.3)
qmAD	(n=31)	8 (28.6)	2 (6.9)	2 (7.1)			15 (53.6)	5 (17.9)	8 (29.6)	3 (11.1)	2 (7.1)

Parameter		Wheezy chest Yes (%)	Pains in heart or chest Yes (%)	Feel faint or dizzy spells Yes (%)	Bone or joint problems Yes (%)	Hospital in last 2 years Yes (%)	Operation in last 6 months Yes (%)	Checked for glaucoma Yes (%)	Diagnosis of glaucoma Yes (%)
Control	(n=9)	0 (0.0)	0 (0.0)	0 (0.0)	3 (33.3)	2 (22.2)	1 (11.1)	4 (44.4)	0 (0.0)
SMC	(n=5)	0 (0.0)	1 (20.0)	0 (0.0)	1 (20.0)	1 (20.0)	1 (20.0)	5 (100.0)	0 (0.0)
aMCI	(n=9)	1 (12.5)	2 (22.2)	3 (33.3)	3 (37.5)	1 (11.1)	0 (0.0)	6 (66.7)	0 (0.0)
Mild AD	(n=3)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)
qmAD	(n=31)	3 (11.1)	4 (14.3)	12 (42.9)	9 (32.1)	13 (46.4)	2 (6.9)	20 (50.0)	3 (12.5)

Parameter	Presence of visual problems Yes (%)	Corrected vision Yes (%)	Calcium channel blockers	Beta blockers	Corticosteroids	Hormone reduction medication	Hormone Replacement therapy	Vitamins
Control (n=9)	8 (88.9)	8 (100.0)	1 (11.1)	1 (11.1)	0 (0.0)	1 (11.1)	0 (0.0)	3 (33.3)
SMC (n=5)	5 (100.0)	4 (100.0)	0 (0.0)	0 (0.0)	2 (40.0)	0 (0.0)	0 (0.0)	1 (20.0)
aMCI (n=9)	4 (44.4)	4 (100.0)	3 (33.3)	1 (11.1)	0 (0.0)	0 (0.0)	2 (22.2)	3 (33.3)
Mild AD (n=3)	2 (66.6)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
qmAD (n=31)	22 (78.6)	16 (94.1)	6 (20.0)	4 (13.3)	3 (10.0)	1 (3.3)	0 (0.0)	11 (36.7)
Control vs. SMC test statistic (p-value)	U=13.5 (0.049)							
Control vs. aMCI test statistic (p-value)	Z=-2.0 (0.045)							
SMC vs. aMCI test statistic (p-value)	U=13.5 (0.049)							
aMCI vs. qmAD test statistic (p-value)	U=105.0 (0.009)							
Parameter	Proton pump inhibitors	Statins	Non-steroidal anti-inflammatory drugs	Anti-coagulants	Cholesterol lowering drugs	ACE inhibitors	Analgesics	Thyroxine
Control (n=9)	0 (0.0)	1 (11.1)	1 (11.1)	1 (11.1)	0 (0.0)	1 (11.1)	1 (11.1)	0 (0.0)
SMC (n=5)	1 (20.0)	2 (40.0)	1 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (20.0)	0 (0.0)
aMCI (n=9)	3 (33.3)	2 (22.2)	1 (11.1)	1 (11.1)	2 (22.2)	3 (33.3)	3 (33.3)	0 (0.0)
Mild AD (n=3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	1 (33.3)	1 (33.3)
qmAD (n=31)	5 (16.7)	13 (43.3)	1 (3.3)	1 (3.3)	0 (0.0)	8 (26.7)	16 (53.3)	2 (6.7)

Parameter	Proton pump inhibitors	Statins	Non-steroidal anti-inflammatory drugs	Anti-coagulants	Cholesterol lowering drugs	ACE inhibitors	Analgesics	Thyroxine
Control vs. qmAD test statistic (p-value)							U=77.5 (0.027)	
aMCI vs. qmAD test statistic (p-value)					U=105.0 (0.009)			

Parameter	Anti-glaucoma medications	Selective serotonin re-uptake inhibitors	Anti-diabetics	Anti-cholinergics	Other hypertensives	Bisphosphates	Beta-2 adrenergic receptor agonists	Alpha blockers
Control (n=9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)
SMC (n=5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (40.0)	0 (0.0)	0 (0.0)	0 (0.0)
aMCI (n=9)	0 (0.0)	1 (11.1)	1 (11.1)	0 (0.0)	3 (33.3)	0 (0.0)	0 (0.0)	1 (11.1)
Mild AD (n=3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	1 (33.3)	0 (0.0)
qmAD (n=31)	2 (6.7)	6 (20.0)	5 (16.7)	2 (6.7)	7 (23.3)	1 (3.3)	3 (10.0)	0 (0.0)

Parameter	Hypnotics	Anti-muscarinics	Eye lubricants or medications	Laxatives	Anti-platelet medications	Allergy medications	Diuretics	Antacids
Control (n=9)	0 (0.0)	0 (0.0)	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (11.1)
SMC (n=5)	0 (0.0)	1 (20.0)	1 (20.0)	1 (20.0)	0 (0.0)	1 (20.0)	0 (0.0)	0 (0.0)
aMCI (n=9)	0 (0.0)	0 (0.0)	2 (22.2)	2 (22.2)	0 (0.0)	1 (11.1)	1 (11.1)	1 (11.1)
Mild AD (n=3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Parameter	Hypnotics	Anti-muscarinics	Eye lubricants or medications	Laxatives	Anti-platelet medications	Allergy medications	Diuretics	Antacids
qmAD (n=31)	1 (3.3)	1 (3.3)	0 (0.0)	1 (3.3)	1 (3.3)	0 (0.0)	8 (26.7)	1 (3.3)
SMC vs. qmAD test statistic (p-value)			U=60.0 (0.014)			U=60.0 (0.014)		
aMCI vs. qmAD test statistic (p-value)			U=105.0 (0.009)					

Parameter	Anti-angina medications	Weight conditions	Neurological conditions	Past Head injury/LOC/Falls	Respiration Issues	Hypertension	Past cancer	Current cancer
Control (n=9)	0 (0.0)	1 (11.1)	2 (22.2)	2 (22.2)	2 (22.2)	4 (44.4)	2 (22.2)	0 (0.0)
SMC (n=5)	0 (0.0)	0 (0.0)	2 (40.0)	0 (0.0)	0 (0.0)	1 (20.0)	1 (20.0)	0 (0.0)
aMCI (n=9)	0 (0.0)	0 (0.0)	2 (22.2)	1 (11.1)	2 (22.2)	3 (33.3)	1 (11.1)	0 (0.0)
Mild AD (n=3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	1 (33.3)	1 (33.3)	0 (0.0)
qmAD (n=31)	1 (3.3)	2 (6.7)	8 (26.7)	5 (16.7)	3 (10.0)	10 (33.3)	2 (6.7)	2 (6.7)

Parameter	Allergies	Arthritis/Bone conditions	Viral diseases	Past epilepsy	Pain conditions	Diabetes	Alzheimer's disease	Vascular dementia
Control (n=9)	2 (22.2)	5 (55.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
SMC (n=5)	0 (0.0)	2 (40.0)	1 (20.0)	1 (20.0)	2 (40.0)	0 (0.0)	0 (0.0)	0 (0.0)
aMCI (n=9)	1 (11.1)	5 (55.6)	0 (0.0)	0 (0.0)	2 (22.2)	1 (11.1)	0 (0.0)	0 (0.0)
Mild AD (n=3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)
qmAD (n=31)	2 (6.7)	11 (36.7)	0 (0.0)	1 (3.3)	5 (16.7)	4 (13.3)	31 (100.0)	7 (23.3)

Parameter	Allergies	Arthritis/Bone conditions	Viral diseases	Past epilepsy	Pain conditions	Diabetes	Alzheimer's disease	Vascular dementia
Control vs. SMC test statistic (p-value)					U=13.5 (0.048)			
Control vs. qmAD test statistic (p-value)							U=0.0 (0.000)	
SMC vs. qmAD test statistic (p-value)			U=60.0 (0.014)				U=0.0 (0.000)	
aMCI vs. qmAD test statistic (p-value)							U=0.0 (0.000)	

Parameter	Blood conditions	Reproductive conditions	Thyroid conditions	Gastro-intestinal conditions	Kidney conditions	Heart conditions	Autoimmune conditions	Skin conditions
Control (n=9)	1 (11.1)	1 (11.1)	0 (0.0)	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)	2 (22.2)
SMC (n=5)	1 (20.0)	1 (20.0)	0 (0.0)	2 (40.0)	1 (20.0)	1 (20.0)	0 (0.0)	0 (0.0)
aMCI (n=9)	1 (11.1)	3 (33.3)	0 (0.0)	1 (11.1)	0 (0.0)	1 (11.1)	0 (0.0)	2 (22.2)
Mild AD (n=3)	0 (0.0)	2 (66.6)	1 (33.3)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)
qmAD (n=31)	3 (10.0)	6 (20.0)	3 (10.0)	10 (33.3)	2 (6.7)	5 (16.7)	3 (10.0)	2 (6.7)

Parameter	High cholesterol	Past stroke	Urinary conditions	Bacterial diseases	RLS growing pains or creeping feelings in legs in the past (%)	RLS Uncomfortable or creeping feelings in legs Never (%)
Control (n=9)	0 (0.0)	0 (0.0)	0 (0.0)	3 (33.3)	3 (33.3)	7 (77.8)
SMC (n=5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (20.0)	0 (0.0)	4 (80.0)

Parameter	High cholesterol	Past stroke	Urinary conditions	Bacterial diseases	RLS growing pains Only in the past (%)	RLS Uncomfortable or creeping feelings in legs Never (%)
aMCI (n=9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (33.3)	9 (100.0)
Mild AD (n=3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (100.0)
qmAD (n=31)	4 (13.3)	2 (6.7)	2 (6.7)	1 (3.3)	6 (22.2)	26 (96.3)
Control vs. qmAD test statistic (p-value)				U=94.5 (0.010)		
SMC vs. qmAD test statistic (p-value)					Chi-square=12.1 (0.002) Pearson's R=-0.5 (0.009)	

Parameter	Anxiety score (HAD)	Depression score (HAD)	MEQ Total Score (diurnal preference)
Control (n=9)	2.8 ± 2.5	1.7 ± 1.2	66.7 ± 6.8
SMC (n=5)	1.6 ± 1.5	1.4 ± 1.1	59.8 ± 8.7 (4)
aMCI (n=9)	4.4 ± 2.1	2.6 ± 1.7	67.3 ± 6.3
Mild AD (n=3)	1.3 ± 1.5	2.0 ± 2.6	64.3 ± 3.1
qmAD (n=31)	3.7 ± 3.2	3.7 ± 2.6	64.7 ± 7.1 (29)
Control vs. qmAD test statistic (p-value)		F=5.0 (0.031)	
SMC vs. aMCI test statistic (p-value)	F=6.9 (0.022)		

All data are presented as mean $\pm$ SD unless otherwise stated. Only significant comparisons are shown, results are test statistic (p-value). If a result was significant after Dunn-Šidák correction for multiple comparisons (see calculations below) the result is marked with *.

1.4. Demographics Dunn-Šidák correction for multiple comparisons calculations

Heath Questionnaire variables, 45 variables, corrected significance level of 0.001.

Medication information, 35 variables, corrected significance level of 0.001.

Medical conditions information, 29 variables, corrected significance level of 0.002.

HAD Scale anxiety and depression scores, 2 variables, corrected significance level of 0.025.

MEQ diurnal preference, 2 variables, corrected significance level of 0.025.

1.5. Memory Assessment Data

Parameter	MMSE Orientatio n score	MMSE Language score	MMSE Repetitio n score	MSE Registratio n score	MMSE Attention score	MMSE Recall score	MMSE Praxis score	MMSE Test Total score
Control first session	(n=9) 9.9 ± 0.3	2.9 ± 0.3	1.0 ± 0.0	3.0 ± 0.0	5.0 ± 0.0	2.6 ± 0.5	4.9 ± 0.3	29.2 ± 1.0
Control second session	(n=8) 10.0 ± 0.0	2.9 ± 0.4	1.0 ± 0.0	3.0 ± 0.0	4.9 ± 0.4	2.9 ± 0.4	4.9 ± 0.4	29.5 ± 0.8
SMC first session	(n=5) 9.8 ± 0.4	2.8 ± 0.4	1.0 ± 0.0	3.0 ± 0.0	5.0 ± 0.0	2.8 ± 0.4	5.0 ± 0.0	29.4 ± 0.5
SMC second session	(n=4) 9.8 ± 0.5	3.0 ± 0.0	1.0 ± 0.0	3.0 ± 0.0	4.8 ± 0.5	3.0 ± 0.0	4.8 ± 0.5	29.3 ± 1.0
aMCI first session	(n=9) 9.7 ± 0.5	2.6 ± 0.5	0.6 ± 0.5	2.6 ± 0.9	4.4 ± 0.5	1.4 ± 1.0	4.8 ± 0.0	26.4 ± 1.8
aMCI second session	(n=8) 9.4 ± 0.7	2.8 ± 0.5	1.0 ± 0.0 (7)	3.0 ± 0.0	4.8 ± 0.7	1.9 ± 1.1	4.5 ± 0.8	27.3 ± 1.8
Mild AD first session	(n=3) 8.0 ± 2.0	2.3 ± 0.6	0.3 ± 0.6	3.0 ± 0.0	5.0 ± 0.0	0.3 ± 0.6	5.0 ± 0.0	24.7 ± 3.1
Mild AD second session	(n=2) 8.5 ± 0.7	2.5 ± 0.7	0.5 ± 0.7	3.0 ± 0.0	5.0 ± 0.0	0.5 ± 0.7	5.0 ± 0.0	25.0 ± 2.8
qAD ChEI-naïve	(n=29) 6.8 ± 2.3	2.3 ± 0.5	0.3 ± 0.5	2.9 ± 0.4 (27)	4.0 ± 1.4	0.5 ± 0.8	4.3 ± 0.9	22.4 ± 2.5
qAD 9wks post ChEI initiation	(n=20) 6.8 ± 1.5	2.6 ± 0.5	1.1 ± 0.4	2.7 ± 0.8	4.5 ± 0.9	0.5 ± 0.8	4.6 ± 0.6	22.4 ± 2.6
qAD benefit group	(n=11) 6.4 ± 2.4	2.5 ± 0.5	0.5 ± 0.5	2.8 ± 0.6	3.5 ± 1.5	0.3 ± 0.6	4.0 ± 1.1	21.0 ± 2.6
qAD no benefit group	(n=7) 8.0 ± 1.0	2.0 ± 0.0	0.0 ± 0.0	3.0 ± 0.0	4.7 ± 0.8	0.4 ± 0.5	4.9 ± 0.4	24.0 ± 1.3
qAD sleep problems	(n=6) 7.5 ± 1.0	2.3 ± 0.5	1.2 ± 0.4 (5)	2.7 ± 0.8	4.5 ± 0.8	0.7 ± 0.8	4.2 ± 1.2	23.0 ± 1.1
qAD no sleep problems	(n=12) 6.8 ± 1.0	2.3 ± 0.5	1.0 ± 0.0 (11)	3.0 ± 0.0	3.8 ± 1.5	0.2 ± 0.4	4.4 ± 0.9	21.8 ± 3.0
qAD maintained on donepezil	(n=21) 6.6 ± 2.6	2.3 ± 0.5	1.3 ± 0.7 (19)	2.8 ± 0.5 (19)	3.7 ± 1.4	0.5 ± 0.9	4.3 ± 0.8	22.1 ± 2.7 (18)

Parameter		MMSE Orientatio n score	MMSE Language score	MMSE Repetitio n score	MSE Registratio n score	MMSE Attention score	MMSE Recall score	MMSE Praxis score	MMSE Test Total score
qmAD changed to other ChEI	(n=4)	7.8 ± 1.3	2.0 ± 0.0	1.0 ± 0.0 (3)	3.0 ± 0.0	5.0 ± 0.0	0.3 ± 0.5	4.8 ± 0.5	24.0 ± 1.6
mAD maintained 10mg OD donepezil	(n=17)	6.2 ± 2.7	2.4 ± 0.5	0.4 ± 0.5	2.9 ± 0.3 (15)	3.6 ± 1.5	0.5 ± 0.9	4.2 ± 0.9	21.8 ± 2.9 (14)
mAD reverted back to 5mg OD donepezil	(n=4)	8.5 ± 1.0	2.3 ± 0.5	0.3 ± 0.5	2.5 ± 1.0	3.8 ± 1.0	0.8 ± 1.0	4.5 ± 0.6	23.3 ± 1.5
Control vs. aMCI test statistic (p-value)						U=18.0 (0.011)	F=8.5 (0.01)		F=16.4 (0.001)*
Control vs. qmAD test statistic (p-value)		U=3.5 (0.000)*	U=50.5 (0.001)*			U=72.0 (0.017)	U=10.5 (0.000)*	U=75.5 (0.032)	F=65.4 (0.000)*
SMC vs. aMCI test statistic (p-value)							F=5.3 (0.048)		F=5.5 (0.043)
SMC vs. qmAD test statistic (p-value)		U=3.5 (0.001)*	U=34.5 (0.026)				U=4.0 (0.000)*	U=35.0 (0.04)	F=39.5 (0.000)*
aMCI vs. qmAD test statistic (p-value)		U=10.5 (0.000)*					U=60.5 (0.008)		F=20.6 (0.000)*

Parameter		BLC Correct (%)	BLC correct latency (ms)	BLC Total attempts	BLC Total correct	BLC Total errors	CRT max. correct latency (ms)	CRT mean correct latency (ms)	CRT median correct latency (ms)
Control first session	(n=8)	99.7 ± 0.9	771.6 ± 75.0	40.0 ± 0.0	39.9 ± 0.4	0.1 ± 0.4	826.4 ± 303.4	390.4 ± 60.7	377.1 ± 58.8
Control second session	(n=8)	100.0 ± 0.0	751.6 ± 89.1	40.0 ± 0.0	40.0 ± 0.0	0.0 ± 0.0	681.0 ± 159.9	390.5 ± 48.1	371.6 ± 49.3
SMC first session	(n=4)	100.0 ± 0.0	768.8 ± 102.4	40.0 ± 0.0	40.0 ± 0.0	0.0 ± 0.0	877.5 ± 301.9	364.5 ± 35.2	351.0 ± 31.5

Parameter	BLC Correct (%)	BLC correct latency (ms)	BLC Total attempts	BLC Total correct	BLC Total errors	CRT max. correct latency (ms)	CRT mean correct latency (ms)	CRT median correct latency (ms)
SMC second session	(n=4)	100.0 ± 0.0	40.0 ± 0.0	40.0 ± 0.0	0.0 ± 0.0	713.5 ± 179.8	356.5 ± 29.5	342.3 ± 36.4
aMCI first session	(n=9)	99.7 ± 0.8	40.0 ± 0.0	39.9 ± 0.3	0.1 ± 0.3	1010.9 ± 275.9	432.4 ± 81.7	413.1 ± 77.0
aMCI second session	(n=7)	100.0 ± 0.0	40.0 ± 0.0	40.0 ± 0.0	0.0 ± 0.0	1286.3 ± 595.0	435.5 ± 87.3	402.1 ± 67.9
Mild AD first session	(n=3)	100.0 ± 0.0	40.0 ± 0.0	40.0 ± 0.0	0.0 ± 0.0	979.3 ± 368.6	391.7 ± 29.3	374.8 ± 28.3
Mild AD second session	(n=2)	100.0 ± 0.0	40.0 ± 0.0	40.0 ± 0.0	0.0 ± 0.0	961.5 ± 460.3	393.1 ± 19.4	365.8 ± 26.5
mAD ChEI-naïve	(n=4)	98.1 ± 2.4	40.0 ± 0.0	39.3 ± 1.0	0.8 ± 1.0	1353.0 ± 598.5	512.0 ± 118.9	477.6 ± 107.0
mAD 9wks post ChEI initiation	(n=4)	100.0 ± 0.0	40.0 ± 0.0	40.0 ± 0.0	0.0 ± 0.0	1138.0 ± 344.3	448.8 ± 78.7	430.9 ± 71.1
mAD benefit group	(n=2)	98.8 ± 1.8	40.0 ± 0.0	39.5 ± 0.7	0.5 ± 0.7	1522.5 ± 811.1	597.4 ± 112.0	557.3 ± 92.3
mAD no benefit group	(n=1)	100.0	40.0	40.0	0.0	1572.0	408.5	383.0
Control vs. mAD test statistic (p-value)		F=42.9 (0.000)*					F=5.8 (0.037)	
SMC vs. mAD test statistic (p-value)		F=22.1 (0.003)						
Overtime SMC vs. aMCI test statistic (p-value)		F=29.0 (0.033)						
Overtime aMCI vs. mAD test statistic (p-value)							F=16.3 (0.027)	

Parameter		CRT min. correct latency (ms)	CRT correct trials (%)	CRT Incorrect trials (%)	CRT SD correct latency (ms)	CRT total correct trials	PAL first trial memory score	PAL mean errors to success	PAL mean trials to success
Control first session	(n=8)	254.4 ± 37.4	99.5 ± 1.1	0.4 ± 0.7	87.1 ± 30.0	99.5 ± 1.1	11.9 ± 2.2	8.5 ± 5.8	3.6 ± 1.6
Control second session	(n=8)	249.0 ± 35.3	98.8 ± 1.9	1.1 ± 1.9	77.0 ± 20.5	98.8 ± 1.9	13.4 ± 3.3	5.0 ± 3.4	2.8 ± 1.2
SMC first session	(n=4)	243.5 ± 19.5	99.5 ± 1.0	0.5 ± 1.0	86.5 ± 21.9	99.5 ± 1.0	10.3 ± 1.9	7.6 ± 4.9	3.1 ± 1.4
SMC second session	(n=4)	220.0 ± 22.6	99.8 ± 0.5	0.3 ± 0.5	84.6 ± 19.5	99.8 ± 0.5	14.0 ± 2.3	3.0 ± 1.3	2.1 ± 0.4
aMCI first session	(n=9)	252.7 ± 41.8	99.2 ± 1.2	0.6 ± 0.9	112.3 ± 36.6	99.2 ± 1.2	10.1 ± 3.3	9.3 ± 4.4	3.9 ± 1.2
aMCI second session	(n=7)	264.4 ± 46.2	99.0 ± 1.4	0.7 ± 0.8	153.5 ± 109.3	99.0 ± 1.4	8.9 ± 3.3	10.1 ± 4.3	4.0 ± 0.9
Mild AD first session	(n=3)	281.0 ± 25.5	99.0 ± 1.7	0.7 ± 1.2	91.4 ± 29.3	99.0 ± 1.7	5.7 ± 2.1	15.3 ± 10.2	4.2 ± 1.1
Mild AD second session	(n=2)	271.0 ± 15.6	99.5 ± 0.7	0.5 ± 0.7	105.6 ± 36.0	99.5 ± 0.7	6.0 ± 5.7	15.7 ± 1.4	4.8 ± 0.7
mAD ChEI-naïve	(n=4)	264.0 ± 39.4	98.5 ± 1.9	1.5 ± 1.9	153.0 ± 56.3	98.5 ± 1.9	6.0 ± 2.6	21.0 ± 2.7	7.9 ± 2.1
mAD 9wks post ChEI initiation	(n=4)	262.8 ± 41.3	98.5 ± 1.9	1.0 ± 1.2	121.7 ± 40.3	98.5 ± 1.9	3.5 ± 2.9	18.0 ± 4.5	7.9 ± 3.6
mAD benefit group	(n=2)	278.5 ± 53.0	98.0 ± 2.8	2.0 ± 2.8	179.1 ± 73.4	98.0 ± 2.8	5.0 ± 2.8	18.8 ± 1.2	8.7 ± 3.3
mAD no benefit group	(n=1)	227.0	98.0	2.0	153.3	98.0	5.0	22.0	7.0
Control vs. mAD test statistic (p-value)					F=7.3 (0.022)		F=16.8 (0.002)	F=16.0 (0.003)	F=16.3 (0.002)
SMC vs. mAD test statistic (p-value)							F=7.0 (0.038)	F=22.6 (0.003)	F=14.4 (0.009)

Parameter	CRT min. correct latency (ms)	CRT correct trials (%)	CRT Incorrect trials (%)	CRT SD correct latency (ms)	CRT total correct trials	PAL first trial memory score	PAL mean errors to success	PAL mean trials to success
aMCI vs. mAD test statistic (p-value)						F=28.8 (0.003)		F=8.0 (0.036)
Overtime control vs. aMCI test statistic (p-value)								F=10.7 (0.014)
Overtime control vs. mAD ChEI-naïve to 9wks post initiation of ChEI test statistic (p-value)						F=7.6 (0.025)		
Overtime SMC vs. aMCI test statistic (p-value)							F=39.8 (0.024)	
Overtime SMC vs. mAD test statistic (p-value)						F=7.8 (0.049)		
Overtime aMCI vs. mAD test statistic (p-value)			F=73.7 (0.003)					

Parameter	PAL stages completed	PAL stages completed on first trial	PAL total errors	PAL total errors on 1 shape	PAL total trials	PAL total trials on 1 shape	SSP span length	SSP mean time to first response (span length 2)
Control first session (n=8)	4.5 ± 0.5	2.6 ± 0.5	35.9 ± 21.9	0.0 ± 0.0	15.0 ± 0.5	0.0 ± 0.0	5.4 ± 0.7	2534.4 ± 177.1
Control second session (n=8)	4.8 ± 0.7	2.9 ± 0.8	21.8 ± 11.1	0.0 ± 0.0	12.5 ± 3.3	0.0 ± 0.0	5.5 ± 0.8	2851.6 ± 1025.4
SMC first session (n=4)	4.8 ± 0.5	2.8 ± 0.5	34.3 ± 18.5	0.0 ± 0.0	14.3 ± 4.8	0.0 ± 0.0	5.8 ± 1.5	2427.8 ± 165.6
SMC second session (n=4)	5.0 ± 0.0	2.8 ± 0.5	14.8 ± 6.4	0.0 ± 0.0	10.5 ± 1.9	0.0 ± 0.0	5.5 ± 0.6	8103.5 ± 10829.7
aMCI first session (n=9)	4.0 ± 0.9	2.1 ± 0.9	34.4 ± 14.7	0.0 ± 0.0	15.1 ± 4.3	0.0 ± 0.0	4.3 ± 0.7 (8)	2524.0 ± 298.1 (8)

Parameter	PAL stages completed	PAL stages completed on first trial	PAL total errors	PAL total errors on 1 shape	PAL total trials	PAL total trials on 1 shape	SSP span length	SSP mean time to first response (span length 2)	Parameter
aMCI second session	(n=8)	4.1 ± 0.9	1.6 ± 0.8	39.3 ± 12.4	0.0 ± 0.0	16.1 ± 1.8	0.0 ± 0.0	4.1 ± 1.1	3191.6 ± 1208.2
Mild AD first session	(n=3)	3.3 ± 0.6	2.0 ± 0.0	54.3 ± 43.2	0.0 ± 0.0	14.3 ± 5.5	0.0 ± 0.0	5.0 ± 1.0	3358.7 ± 927.0
Mild AD second session	(n=2)	3.0 ± 0.0	1.5 ± 2.1	47.0 ± 4.2	0.0 ± 0.0	14.5 ± 2.1	0.0 ± 0.0	5.5 ± 0.7	3066.5 ± 159.1
mAD ChEI-naïve	(n=4)	3.0 ± 1.4	1.0 ± 0.8	65.5 ± 35.6	0.0 ± 0.0	21.8 ± 8.5	0.0 ± 0.0	3.5 ± 1.0	3560.1 ± 1429.4
mAD 9wks post ChEI initiation	(n=4)	2.5 ± 1.0	0.8 ± 1.0	42.0 ± 13.4	0.0 ± 0.0	17.3 ± 4.3	0.0 ± 0.0	3.8 ± 0.5	3869.8 ± 1482.6
mAD benefit group	(n=2)	2.0 ± 1.4	1.5 ± 0.7	38.5 ± 29.0	0.0 ± 0.0	15.0 ± 5.7	0.0 ± 0.0	3.0 ± .4	4141.3 ± 2094.8
mAD no benefit group	(n=1)	4.0	0.0	88.0	0.0	28.0	0.0	4.0	2537.0
Control vs. aMCI test statistic (p-value)							F=9.6 (0.008)		
Control vs. mAD test statistic (p-value)	F=7.5 (0.021)	F=18.2 (0.002)					F=13.6 (0.004)		
SMC vs. mAD test statistic (p-value)		F=13.4 (0.011)					F=6.2 (0.047)		
aMCI vs. mAD test statistic (p-value)	F=8.7 (0.032)								
Overtime control vs. aMCI test statistic (p-value)								F=6.8 (0.035)	
Overtime control vs. mAD ChEI-naïve to 9wks post initiation of ChEI test statistic (p-value)	F=7.7 (0.024)								

Parameter	PAL stages completed	PAL stages completed on first trial	PAL total errors	PAL total errors on 1 shape	PAL total trials	PAL total trials on 1 shape	SSP span length	SSP mean time to first response (span length 2)	Parameter
Overtime SMC vs. aMCI test statistic (p-value)		F=22.1 (0.042)							
Overtime aMCI vs. mAD test statistic (p-value)								F=17.0 (0.026)	
Parameter	PAL stages completed	SSP mean time to last response (span length 2)	SSP no. of attempts (span length 2)	SSP total errors	SSP total usage errors	SSP direction errors on stop and go trials	SSP proportion of successful stops (last half)	SSP median correct RT on go trials	SSP SSD (last half)
Control first session	(n=8)	3278.4 ± 291.9	1.0 ± 0.0	11.5 ± 2.5	2.9 ± 1.8	1.9 ± 2.2	0.5 ± 0.2	642.6 ± 111.5	441.7 ± 91.4
Control second session	(n=8)	3544.9 ± 1120.6	1.0 ± 0.0	11.8 ± 4.6	2.4 ± 1.1	4.0 ± 6.7 (6)	0.6 ± 0.1 (6)	637.6 ± 260.5 (6)	468.8 ± 252.7 (6)
SMC first session	(n=4)	3190.8 ± 447.2	1.0 ± 0.0	15.5 ± 4.0	3.0 ± 0.8	0.8 ± 1.5	0.5 ± 0.1	591.5 ± 80.1	439.2 ± 69.4
SMC second session	(n=4)	9913.1 ± 13080.1	1.3 ± 0.5	14.8 ± 7.9	3.5 ± 2.1	2.5 ± 2.1 (2)	0.5 ± 0.0 (2)	476.3 ± 87.3 (2)	303.5 ± 92.8 (2)
aMCI first session	(n=8)	4941.7 ± 3118.3 (8)	1.3 ± 0.5 (8)	11.5 ± 5.5 (8)	3.4 ± 2.3 (8)	5.0 ± 4.2 (5)	0.6 ± 0.1 (5)	796.5 ± 209.1 (5)	567.1 ± 125.3 (5)
aMCI second session	(n=8)	5161.2 ± 3246.1	1.3 ± 0.8	11.9 ± 4.8	3.9 ± 2.0	7.0 ± 4.0 (3)	0.6 ± 0.1 (3)	843.8 ± 252.0 (3)	575.9 ± 104.1 (3)
Mild AD first session	(n=3)	4020.0 ± 919.2	1.0 ± 0.0	15.7 ± 2.9	1.7 ± 0.6	1.0 ± 1.4 (2)	0.7 ± 0.1 (2)	859.0 ± 124.5 (2)	638.7 ± 65.1 (2)
Mild AD second session	(n=2)	3744.5 ± 29.0	1.0 ± 0.0	14.0 ± 2.8	3.0 ± 1.4				

Parameter	SSP mean time to last response (span length 2)	SSP no. of attempts (span length 2)	SSP total errors	SSP total usage errors	SSP direction errors on stop and go trials	SSP proportion of successful stops (last half)	SSP median correct RT on go trials	SSP SSD (last half)
mAD ChEI-naïve (n=4)	4425.3 ± 1451.0	1.3 ± 0.5	12.3 ± 5.1	3.8 ± 3.0	5.0 ± 5.9	0.7 ± 0.1	800.3 ± 211.8	525.7 ± 159.9
mAD 9wks post ChEI initiation (n=4)	5061.6 ± 1913.4	1.3 ± 0.5	12.5 ± 3.3	3.5 ± 1.7	3.5 ± 4.7	0.7 ± 0.2	859.6 ± 279.8	514.6 ± 146.5
mAD benefit group (n=2)	4975.0 ± 1955.9	1.5 ± 0.7	13.0 ± 8.5	5.5 ± 3.5	3.0 ± 4.2	0.7 ± 0.1	893.3 ± 199.1	609.7 ± 142.1
mAD no benefit group (n=1)	3075.0	1.0	10.0	1.0	13.0	0.6	533.5	322.7
Control vs. mAD test statistic (p-value)	F=5.1 (0.048)							
SMC vs. aMCI test statistic (p-value)	F=9.5 (0.021)							
Overtime control vs. SMC test statistic (p-value)	F=19.2 (0.005)							
Overtime SMC vs. mAD test statistic (p-value)	F=9.0 (0.040)							
Parameter	GNT correct (%)		GNT total attempts		GNT total correct		GNT total errors	
Control first session (n=8)	89.6 ± 9.2		30.0 ± 0.0		26.9 ± 2.7		3.1 ± 2.7	
Control second session (n=8)	89.6 ± 9.2		30.0 ± 0.0		26.9 ± 2.7		3.1 ± 2.7	
SMC first session (n=4)	91.7 ± 6.4		30.0 ± 0.0		27.5 ± 1.9		2.5 ± 1.9	
SMC second session (n=4)	93.3 ± 3.9		30.0 ± 0.0		28.0 ± 1.2		2.0 ± 1.2	
aMCI first session (n=9)	74.4 ± 12.2		30.0 ± 0.0		22.3 ± 3.7		7.7 ± 3.7	

aMCI second session	(n=8)	77.6 ± 14.0	30.0 ± 0.0	22.3 ± 4.2	6.7 ± 4.2
Mild AD first session	(n=3)	44.4 ± 45.3	30.0 ± 0.0	13.3 ± 13.6	16.7 ± 13.6
Mild AD second session	(n=2)	56.7 ± 47.1	30.0 ± 0.0	17.0 ± 14.1	13.0 ± 14.1
mAD ChEI-naïve	(n=4)	70.8 ± 6.9	30.0 ± 0.0	21.3 ± 2.1	8.8 ± 2.1
mAD 9wks post ChEI initiation	(n=4)	70.8 ± 7.9	30.0 ± 0.0	21.3 ± 2.4	8.8 ± 2.4
mAD benefit group	(n=2)	71.7 ± 7.1	30.0 ± 0.0	21.5 ± 2.1	8.5 ± 2.1
mAD no benefit group	(n=1)	76.7	30.0	23.0	7.0
Control vs. aMCI test statistic (p-value)		F=8.1 (0.012)		F=8.1 (0.012)	F=8.1 (0.012)
Control vs. mAD test statistic (p-value)		F=12.9 (0.005)		F=12.9 (0.005)	F=12.9 (0.005)
SMC vs. mAD test statistic (p-value)		F=19.7 (0.004)		F=19.7 (0.004)	F=19.7 (0.004)
Overtime SMC vs. aMCI test statistic (p-value)				F=26.6 (0.036)	F=26.6 (0.036)

All data are presented as mean $\pm$ SD unless otherwise stated. Only significant comparisons are shown, results are test statistic (p-value). If a result was significant after Dunn-Šidák correction for multiple comparisons (see calculations below) the result is marked with *.

8.1.1. Memory Dunn-Šidák correction for multiple comparisons calculations

MMSE variables, 8 variables, corrected significance level of 0.006.

CANTABeclipse variables, 43 variables, corrected significance level of 0.001.

1.2. Functioning Assessment Data

.1.2.1.

Parameter		ADCS-ADL Basic ADLs Component score	ADCS-ADL Instrumental ADLs Component score	ADCS-ADL Total score	SFS Withdrawal Component score	SFS Interpersonal Communication component score	SFS Independence Performance component score
Control first session	(n=9)	22.0 ± 0.0	54.3 ± 2.8 (7)	76.3 ± 2.8 (7)	12.2 ± 2.5	8.7 ± 0.7	31.7 ± 4.3 (7)
Control second session	(n=8)	22.0 ± 0.0	54.8 ± 1.0 (4)	76.8 ± 1.0 (4)	12.5 ± 2.4	8.9 ± 0.4	31.5 ± 2.7
SMC first session	(n=5)	22.0 ± 0.0	55.0 ± 1.0 (3)	77.0 ± 1.0 (3)	13.4 ± 1.1	9.0 ± 0.0	32.0 ± 4.9 (4)
SMC second session	(n=4)	22.0 ± 0.0	55.5 ± 0.7 (2)	77.5 ± 0.7 (2)	13.5 ± 1.3	9.0 ± 0.0	29.0 ± 5.0
aMCI first session	(n=9)	21.8 ± 0.7	55.0 ± 1.2 (4)	77.0 ± 1.2 (4)	13.3 ± 1.1	8.7 ± 0.5	33.4 ± 2.8
aMCI second session	(n=8)	22.0 ± 0.0	55.2 ± 1.1 (5)	77.2 ± 1.1 (5)	12.8 ± 0.9	8.9 ± 0.4	33.4 ± 2.1 (7)
Mild AD first session	(n=3)	22.0 ± 0.0			12.0 ± 2.6	9.0 ± 0.0 (2)	21.0 ± 12.1
Mild AD second session	(n=2)	22.0 ± 0.0	55.0 ± 0.0 (1)	77.0 ± 0.0 (1)	13.0 ± 1.4	9.0 ± 0.0	35.0 (1)
qmAD ChEI-naïve	(n=27)	18.7 ± 3.5	35.0 ± 9.7 (15)	55.1 ± 12.0 (15)	11.4 ± 2.3	7.4 ± 1.7	18.0 ± 8.7 (26)
qmAD 9wks post ChEI initiation	(n=23)	18.1 ± 4.8 (22)	38.2 ± 7.1 (13)	57.8 ± 10.6 (12)	11.4 ± 2.0	8.0 ± 1.1	17.8 ± 9.5 (18)
qmAD benefit group	(n=11)	17.7 ± 3.9	41.0 ± 4.2 (4)	62.3 ± 4.6 (4)	11.4 ± 2.3 (10)	7.5 ± 1.7	16.8 ± 9.1 (10)
qmAD no benefit group	(n=7)	19.3 ± 3.4 (6)	34.0 ± 9.6 (4)	53.8 ± 12.0 (4)	11.9 ± 2.7	7.7 ± 2.0 (6)	20.7 ± 8.0 (6)
qmAD sleep problems	(n=6)	18.3 ± 3.2	37.4 ± 8.8 (3)	57.4 ± 11.5 (3)	11.2 ± 2.3 (5)	7.2 ± 1.9 (5)	15.4 ± 10.4 (5)
qmAD no sleep problems	(n=12)	18.3 ± 4.8 (11)	37.7 ± 7.6 (5)	59.0 ± 7.2 (5)	11.8 ± 2.5	7.7 ± 1.7	19.5 ± 7.9 (11)
qmAD maintained on donepezil	(n=20)	18.0 ± 3.7	35.0 ± 10.6 (11)	54.6 ± 13.5 (11)	11.8 ± 2.1	7.7 ± 1.6	18.3 ± 9.6 (19)
qmAD changed to other ChEI	(n=4)	21.3 ± 0.6 (3)	37.7 ± 7.6 (3)	59.0 ± 7.2 (3)	11.3 ± 2.6	6.3 ± 2.1 (3)	20.5 ± 5.1

Parameter		ADCS-ADL Basic ADLs Component score	ADCS-ADL Instrumental ADLs Component score	ADCS-ADL Total score	SFS Withdrawal Component score	SFS Interpersonal Communication component score	SFS Independence Performance component score
qmAD maintained 10mg OD donepezil	(n=17)	18.2 ± 3.3 (16)	32.2 ± 9.6 (9)	51.3 ± 12.7 (9)	11.9 ± 2.3 (16)	7.5 ± 1.7	17.3 ± 7.7 (16)
qmAD reverted back to 5mg OD donepezil	(n=4)	17.3 ± 5.9	47.5 ± 0.7 (2)	69.5 ± 0.7 (2)	11.3 ± 1.7	8.3 ± 0.6 (3)	23.3 ± 18.5 (3)
Control vs. qmAD test statistic (p-value)			F=26.1 (0.000)*	F=20.7 (0.000)*		U=58.5 (0.016)	F=16.0 (0.000)*
SMC vs. qmAD test statistic (p-value)							F=9.7 (0.004)*
aMCI vs. qmAD test statistic (p-value)			F=16.3 (0.001)*	F=12.7 (0.002)*	F=5.7 (0.023)	F=4.9 (0.033)*	F=26.8 (0.000)*
Overtime SMC vs. aMCI test statistic (p-value)							F=7.6 (0.028)

Parameter		SFS Recreation component score	SFS Prosocial component score	SFS Independence Competence component score	SFS Total score
Control first session	(n=9)	30.1 ± 5.8 (8)	29.5 ± 13.8 (8)	35.7 ± 1.0	145.7 ± 23.0 (6)
Control second session	(n=8)	27.4 ± 6.9 (7)	27.9 ± 13.3 (7)	36.0 ± 0.0	141.5 ± 16.8 (6)
SMC first session	(n=5)	28.6 ± 8.1	33.4 ± 5.9	34.6 ± 1.5	150.5 ± 20.5 (4)
SMC second session	(n=4)	28.3 ± 5.9	29.3 ± 6.2	35.3 ± 1.2(3)	139.3 ± 13.6 (3)
aMCI first session	(n=9)	29.9 ± 5.2 (7)	32.0 ± 15.3 (6)	35.3 ± 1.4 (8)	151.2 ± 20.9 (6)
aMCI second session	(n=7)	28.9 ± 4.9	34.8 ± 14.3 (5)	36.0 ± 0.0	151.3 ± 22.2(4)
Mild AD first session	(n=3)	22.0 ± 2.6	19.5 ± 9.2 (2)	26.3 ± 7.5	94.0 (1)
Mild AD second session	(n=2)	25.5 ± 6.4	25.0 (1)	29.5 ± 9.2	

Parameter	SFS Recreation component score	SFS Prosocial component score	SFS Independence Competence component score	SFS Total score
qmAD ChEI-naïve (n=27)	14.0 ± 6.2 (26)	17.6 ± 8.8 (22)	25.4 ± 6.3 (22)	93.8 ± 24.7 (14)
qmAD 9wks post ChEI initiation (n=22)	15.1 ± 5.4 (18)	16.0 ± 9.1 (22)	25.4 ± 6.1 (20)	91.3 ± 26.3 (13)
Parameter	SFS Recreation component score	SFS Prosocial component score	SFS Independence Competence component score	SFS Total score
qmAD benefit group (n=11)	13.3 ± 7.1	16.6 ± 7.4 (8)	24.1 ± 6.1 (9)	91.5 (6)
qmAD no benefit group (n=7)	15.2 ± 4.3 (5)	18.4 ± 9.8 (5)	27.7 ± 5.0 (6)	100.3 (3)
qmAD sleep problems (n=5)	12.0 ± 8.0 (4)	14.5 ± 5.9 (4)	24.8 ± 6.4 (4)	87.0 (1)
qmAD no sleep problems (n=12)	14.5 ± 5.9	18.7 ± 4.9 (9)	25.8 ± 5.8 (11)	95.4 ± 18.3 (8)
qmAD maintained on donepezil (n=20)	14.4 ± 6.4 (19)	19.4 ± 7.6 (15)	25.7 ± 6.9 (16)	96.5 ± 25.4 (12)
qmAD changed to other ChEI (n=4)	16.0 ± 5.1 (3)	18.3 ± 12.7 (3)	26.5 ± 3.5	87.0 (1)
qmAD maintained 10mg OD donepezil (n=17)	14.6 ± 6.3	19.4 ± 7.6 (15)	24.3 ± 6.2 (14)	96.5 ± 25.4 (12)
qmAD reverted back to 5mg OD donepezil (n=2)	12.5 ± 10.6		35.5 ± 0.7	
Control vs. qmAD test statistic (p-value)	F=42.4 (0.000)*		U=11.0 (0.000)*	F=19.3 (0.000)*
SMC vs. qmAD test statistic (p-value)	F=21.0 (0.000)*	F=14.4 (0.001)*	F=10.2 (0.004)*	F=17.4 (0.001)*
aMCI vs. qmAD test statistic (p-value)	F=38.1 (0.000)*	F=9.0 (0.006)*	F=18.7 (0.000)*	F=24.6 (0.000)*
Overtime SMC vs. qmAD test statistic (p-value)		F=5.8 (0.029)		
qmAD 10mg vs. 5mg donepezil baseline test statistic (p-value)			F=6.2 (0.026)	

All data are presented as mean $\pm$ SD unless otherwise stated. Only significant comparisons are shown, results are test statistic (p-value). If a result was significant after Dunn-Šidák correction for multiple comparisons (see calculations below) the result is marked with *.

1.3. Functioning Dunn-Šidák correction for multiple comparisons calculations

ADCS-ADL variables, 3 variables, corrected significance level of 0.017.

SFS variables, 7 variables, corrected significance level of 0.007.

1.4. Circadian Assessment Data

Parameter	Average activity count	Inter-daily stability (IS)	Intra-daily variability (IV)	Least active five hours (L5)	L5-onset phase (hh:mm)	Most active ten hours (M10)
Control first session	(n=9) 195.9 ± 92.6	0.6 ± 0.2	0.8 ± 0.3	11.5 ± 4.4	00:42 ± 00:58	338.9 ± 161.1
Control second session	(n=7) 186.7 ± 72.8	0.5 ± 0.1	0.8 ± 0.2	13.1 ± 7.3	00:26 ± 00:58	314.3 ± 112.6
SMC first session	(n=5) 207.6 ± 48.3	0.6 ± 0.1	0.7 ± 0.0	15.9 ± 5.5	00:46 ± 00:36	356.0 ± 70.8
SMC second session	(n=4) 203.4 ± 26.4	0.6 ± 0.1	0.8 ± 0.1	17.8 ± 3.5	00:58 ± 00:35	336.8 ± 52.8
aMCI first session	(n=9) 170.8 ± 37.7	0.6 ± 0.6	0.9 ± 0.1	13.0 ± 6.8	00:32 ± 01:05	302.7 ± 65.2
aMCI second session	(n=7) 176.5 ± 28.2	0.6 ± 0.1	0.9 ± 0.2	11.9 ± 7.1	00:42 ± 00:50	314.6 ± 55.5
Mild AD first session	(n=3) 232.7 ± 98.1	0.5 ± 0.2	0.9 ± 0.2	16.9 ± 4.7	00:14 ± 00:56	409.7 ± 167.1
Mild AD second session	(n=2) 274.2 ± 0.3	0.6 ± 0.1	0.9 ± 0.2	17.2 ± 6.2	00:22 ± 02:26	487.3 ± 55.9
mAD ChEI-naïve	(n=5) 94.9 ± 53.5	0.5 ± 0.2	1.2 ± 0.4	14.2 ± 2.9	00:11 ± 01:18	159.4 ± 91.8
mAD 9wks post ChEI initiation	(n=6) 113.3 ± 76.6	0.5 ± 0.2	1.1 ± 0.2	11.7 ± 5.0	22:49 ± 00:55	199.0 ± 138.6
mAD benefit group	(n=3) 118.0 ± 49.3	0.6 ± 0.1	0.9 ± 0.1	15.3 ± 2.7	00:15 ± 01:31	198.3 ± 84.1
mAD no benefit group	(n=1) 96.2	0.6	1.4	14.8	01:08	165.0
Control vs. mAD test statistic (p-value)	F=4.9 (0.046)					F=5.1 (0.043)
SMC vs. aMCI test statistic (p-value)			F=8.2 (0.015)			
SMC vs. mAD test statistic (p-value)	F=12.2 (0.008)		F=9.5 (0.015)			F=14.4 (0.005)*
aMCI vs. mAD test statistic (p-value)	F=9.7 (0.009)		F=5.7 (0.035)			F=11.7 (0.005)*
Overtime control vs. SMC test statistic (p-value)						F=6.0 (0.037)

Parameter	Average activity count	Inter-daily stability (IS)	Intra-daily variability (IV)	Least active five hours (L5)	L5-onset phase (hh:mm)	Most active ten hours (M10)
Overtime SMC vs. aMCI test statistic (p-value)	F=5.3 (0.050)					F=6.3 (0.036)
Overtime SMC vs. mAD test statistic (p-value)	F=6.0 (0.044)		F=6.1 (0.042)			F=7.4 (0.030)
Overtime aMCI vs. mAD test statistic (p-value)		F=7.3 (0.031)				
Parameter	M10-onset phase (hh:mm)	Amplitude of activity	Relative amplitude	Lomb-Scargle period	Sokolove-Bushell period	Hour 1 activity count
Control first session (n=9)	08:16 ± 00:52	327.3 ± 158.1	0.9 ± 0.0	23.98 ± 0.04	23.96 ± 0.07	47.2 ± 64.8
Control second session (n=7)	08:13 ± 00:52	301.2 ± 107.1	0.9 ± 0.0	24.02 ± 0.03	24.02 ± 0.05	33.8 ± 36.7
SMC first session (n=5)	08:29 ± 00:21	340.1 ± 66.8	0.9 ± 0.0	24.01 ± 0.05	23.99 ± 0.05	28.0 ± 24.9
SMC second session (n=4)	08:29 ± 00:19	319.0 ± 56.5	0.9 ± 0.0	24.01 ± 0.02	23.93 ± 0.11	33.1 ± 10.5
aMCI first session (n=9)	07:52 ± 00:40	289.6 ± 64.9	0.9 ± 0.0	24.00 ± 0.02	23.98 ± 0.05	17.1 ± 11.9
aMCI second session (n=7)	07:42 ± 00:34	302.8 ± 55.2	0.9 ± 0.0	24.01 ± 0.04	24.01 ± 0.11	25.9 ± 24.8
Mild AD first session (n=3)	09:01 ± 02:43	392.8 ± 162.3	0.9 ± 0.0	24.02 ± 0.04	24.04 ± 0.03	45.4 ± 21.1
Mild AD second session (n=2)	08:19 ± 00:53	470.3 ± 62.0	0.9 ± 0.0	24.01 ± 0.04	24.01 ± 0.01	15.3 ± 9.0
mAD ChEI-naïve (n=5)	07:52 ± 00:54	145.3 ± 89.3	0.8 ± 0.1	24.00 ± 0.02	23.93 ± 0.08	16.4 ± 5.7
mAD 9wks post ChEI initiation (n=6)	07:22 ± 00:31	187.2 ± 134.8	0.9 ± 0.1	24.03 ± 0.11	24.03 ± 0.06	11.0 ± 6.0
mAD benefit group (n=3)	07:13 ± 00:03	183.1 ± 82.3	0.8 ± 0.1	23.98 ± 0.04	24.00 ± 0.02	17.4 ± 7.0
mAD no benefit group (n=1)	08:50	150.3	0.8	23.82	24.02	18.7
Control vs. mAD test statistic (p-value)		F=5.5 (0.037)	F=9.7 (0.009)			
SMC vs. mAD test statistic (p-value)		F=15.3 (0.005)*				

Parameter	M10-onset phase (hh:mm)	Amplitude of activity	Relative amplitude	Lomb-Scargle period	Sokolove-Bushell period	Hour 1 activity count
aMCI vs. mAD test statistic (p-value)		F=12.3 (0.004)*	F=7.6 (0.017)			
Overtime control vs. SMC test statistic (p-value)		F=6.5 (0.031)				
Overtime control vs. mAD ChEI-naïve to 9wks post initiation of ChEI test statistic (p-value)						F=5.1 (0.048)
Overtime SMC vs. aMCI test statistic (p-value)		F=6.8 (0.031)				
Overtime SMC vs. mAD test statistic (p-value)		F=8.4 (0.023)				

Parameter	Hour 2 activity count	Hour 3 activity count	Hour 4 activity count	Hour 5 activity count	Hour 6 activity count	Hour 7 activity count
Control first session (n=9)	18.3 ± 14.3	10.7 ± 4.5	11.5 ± 7.6	12.0 ± 5.8	14.8 ± 7.8	63.1 ± 71.9
Control second session (n=7)	18.7 ± 12.2	11.9 ± 6.5	10.7 ± 5.8	15.0 ± 5.7	19.4 ± 11.7	72.4 ± 73.1
SMC first session (n=5)	38.3 ± 52.4	18.7 ± 9.7	14.8 ± 11.1	13.1 ± 5.6	12.7 ± 5.3	45.8 ± 45.5
SMC second session (n=4)	19.3 ± 5.8	21.0 ± 4.5	17.7 ± 5.9	16.8 ± 6.8	18.4 ± 5.0	63.1 ± 24.8
aMCI first session (n=9)	15.1 ± 7.4	13.6 ± 7.0	14.9 ± 12.7	15.1 ± 8.3	21.8 ± 19.2	48.7 ± 30.4
aMCI second session (n=8)	12.0 ± 7.0	12.9 ± 9.2	12.4 ± 8.7	10.5 ± 7.6	19.4 ± 14.7	58.5 ± 71.2
Mild AD first session (n=3)	18.0 ± 9.3	13.8 ± 7.4	19.6 ± 8.7	17.3 ± 9.2	24.9 ± 12.4	66.1 ± 28.5
Mild AD second session (n=2)	22.0 ± 9.8	18.1 ± 10.9	19.4 ± 12.6	22.5 ± 14.6	23.5 ± 13.9	74.9 ± 28.8
mAD ChEI-naïve (n=5)	14.4 ± 5.6	17.4 ± 6.4	14.9 ± 5.9	17.2 ± 8.6	21.4 ± 14.7	30.0 ± 27.5
mAD 9wks post ChEI initiation (n=6)	9.9 ± 5.9	11.5 ± 5.6	16.0 ± 7.3	18.5 ± 6.1	29.2 ± 85.7	85.7 ± 112.3
mAD benefit group (n=3)	14.7 ± 6.4	15.9 ± 5.5	14.5 ± 5.5	22.1 ± 7.3	28.4 ± 15.6	40.2 ± 32.6
mAD no benefit group (n=1)	18.5	26.3	21.9	7.1	8.2	22.6

Parameter	Hour 2 activity count	Hour 3 activity count	Hour 4 activity count	Hour 5 activity count	Hour 6 activity count	Hour 7 activity count
Control vs. mAD test statistic (p-value)	F=5.0 (0.048)					
Overtime control vs. SMC test statistic (p-value)	F=10.3 (0.011) F=6.0 (0.037)					
Overtime control vs. aMCI test statistic (p-value)	F=11.6 (0.005)					
Parameter	Hour 8 activity count	Hour 9 activity count	Hour 10 activity count	Hour 11 activity count	Hour 12 activity count	Hour 13 activity count
Control first session (n=9)	165.1 ± 156.8	257.9 ± 104.7	377.8 ± 169.7	387.4 ± 167.7	377.7 ± 153.0	370.2 ± 159.9
Control second session (n=7)	209.2 ± 164.9	290.4 ± 130.9	370.8 ± 138.7	352.0 ± 127.9	342.3 ± 114.0	342.5 ± 160.7
SMC first session (n=5)	151.2 ± 81.8	331.7 ± 83.0	385.3 ± 100.1	366.4 ± 76.3	359.8 ± 81.0	344.7 ± 120.1
SMC second session (n=4)	141.7 ± 33.6	297.9 ± 46.8	390.2 ± 80.5	368.6 ± 76.2	329.0 ± 43.1	350.4 ± 71.0
aMCI first session (n=9)	157.2 ± 91.1	327.8 ± 94.9	360.0 ± 98.8	324.1 ± 82.7	338.1 ± 71.9	325.4 ± 84.5
aMCI second session (n=8)	165.7 ± 103.4	355.9 ± 120.2	340.6 ± 68.3	352.7 ± 6.4	356.9 ± 62.9	312.7 ± 74.5
Mild AD first session (n=3)	212.1 ± 113.2	249.5 ± 28.5	338.8 ± 85.2	407.4 ± 43.5	429.7 ± 32.6	412.5 ± 40.9
Mild AD second session (n=2)	285.8 ± 126.3	356.7 ± 73.5	517.9 ± 73.6	605.0 ± 66.2	533.6 ± 78.5	473.2 ± 77.0
mAD ChEI-naïve (n=5)	150.0 ± 180.3	172.9 ± 130.2	222.3 ± 138.0	187.2 ± 99.3	163.4 ± 88.5	142.4 ± 79.9
mAD 9wks post ChEI initiation (n=6)	207.3 ± 260.5	245.9 ± 197.2	262.3 ± 165.2	221.5 ± 109.1	212.5 ± 107.4	214.7 ± 114.7
mAD benefit group (n=3)	233.8 ± 196.3	239.7 ± 116.8	245.8 ± 116.3	240.3 ± 50.3	208.1 ± 64.4	159.9 ± 67.7
mAD no benefit group (n=1)	38.3	131.9	337.0	188.9	160.0	200.1
Control vs. mAD test statistic (p-value)	F=6.6 (0.026) F=7.2 (0.021)					
SMC vs. mAD test statistic (p-value)	F=10.2 (0.013) F=13.4 (0.006) F=9.8 (0.014)					

Parameter	Hour 8 activity count	Hour 9 activity count	Hour 10 activity count	Hour 11 activity count	Hour 12 activity count	Hour 13 activity count
aMCI vs. mAD test statistic (p-value)		F=6.6 (0.024)		F=7.7 (0.017)	F=16.2 (0.002)*	F=15.6 (0.002)*
Overtime SMC vs. aMCI test statistic (p-value)					F=5.5 (0.047)	
Parameter	Hour 14 activity count	Hour 15 activity count	Hour 16 activity count	Hour 17 activity count	Hour 18 activity count	Hour 19 activity count
Control first session (n=9)	295.8 ± 153.5	300.8 ± 168.8	333.6 ± 224.4	311.8 ± 220.1	249.4 ± 175.3	253.2 ± 144.2
Control second session (n=7)	283.4 ± 163.0	271.1 ± 135.3	282.0 ± 92.2	275.1 ± 100.8	229.8 ± 95.3	249.5 ± 103.5
SMC first session (n=5)	330.9 ± 88.9	280.4 ± 46.0	359.5 ± 41.0	319.4 ± 72.0	317.4 ± 99.6	273.7 ± 97.9
SMC second session (n=4)	291.4 ± 41.1	317.3 ± 68.8	350.2 ± 101.4	331.2 ± 74.7	310.1 ± 90.5	291.8 ± 29.2
aMCI first session (n=9)	279.2 ± 104.4	263.8 ± 96.0	263.2 ± 74.1	251.9 ± 60.5	245.3 ± 93.4	187.4 ± 80.0
aMCI second session (n=8)	281.2 ± 45.1	279.8 ± 77.2	288.4 ± 86.9	277.4 ± 77.5	263.3 ± 78.0	172.8 ± 69.7
Mild AD first session (n=3)	498.7 ± 48.9	489.5 ± 46.6	436.9 ± 40.4	364.4 ± 38.0	289.5 ± 33.9	322.3 ± 38.6
Mild AD second session (n=2)	580.9 ± 64.0	432.0 ± 97.5	485.8 ± 58.9	478.4 ± 88.1	389.5 ± 43.8	309.7 ± 50.8
mAD ChEI-naïve (n=5)	152.5 ± 68.9	140.6 ± 108.8	118.1 ± 64.2	107.2 ± 69.7	124.1 ± 82.0	138.8 ± 91.0
mAD 9wks post ChEI initiation (n=6)	208.7 ± 124.5	173.2 ± 103.6	130.7 ± 64.1	133.1 ± 81.3	175.0 ± 167.0	159.2 ± 113.8
mAD benefit group (n=3)	179.4 ± 63.8	194.6 ± 106.4	145.5 ± 72.7	134.4 ± 76.9	155.9 ± 91.2	159.2 ± 87.0
mAD no benefit group (n=1)	164.4	97.4	88.8	98.2	113.1	194.6
SMC vs. mAD test statistic (p-value)	F=12.6 (0.008)	F=7.0 (0.029)	F=50.2 (0.000)*	F=22.4 (0.001)*	F=11.2 (0.010)	
aMCI vs. mAD test statistic (p-value)	F=5.8 (0.033)	F=4.8 (0.048)	F=13.4 (0.003)	F=16.6 (0.002)*	F=5.9 (0.032)	
Overtime SMC vs. aMCI test statistic (p-value)			F=5.9 (0.041)			

Parameter	Hour 20 activity count	Hour 21 activity count	Hour 22 activity count	Hour 23 activity count	Hour 24 activity count
Control first session (n=9)	238.0 ± 145.0	173.7 ± 101.6	161.6 ± 73.4	168.9 ± 100.1	95.0 ± 119.3
Control second session (n=7)	217.7 ± 108.7	180.9 ± 115.7	175.1 ± 104.2	160.9 ± 118.0	57.6 ± 47.4
SMC first session (n=5)	252.1 ± 96.6	166.5 ± 57.0	143.9 ± 62.8	169.6 ± 103.4	74.2 ± 23.5
SMC second session (n=4)	263.8 ± 49.7	183.8 ± 14.9	175.4 ± 30.6	199.0 ± 55.7	95.5 ± 18.7
aMCI first session (n=9)	157.3 ± 60.9	121.9 ± 56.7	103.9 ± 59.2	175.7 ± 84.3	69.8 ± 51.7
aMCI second session (n=8)	160.7 ± 60.2	107.6 ± 31.0	110.6 ± 53.8	182.0 ± 93.8	71.3 ± 42.8
Mild AD first session (n=3)	284.7 ± 47.1	240.2 ± 25.8	243.0 ± 100.2	105.9 ± 51.3	54.4 ± 14.8
Mild AD second session (n=2)	322.0 ± 58.9	200.3 ± 34.2	315.4 ± 164.5	67.9 ± 44.4	18.7 ± 12.9
mAD ChEI-naïve (n=5)	83.2 ± 67.7	82.7 ± 63.4	74.7 ± 65.6	75.3 ± 68.5	24.1 ± 19.4
mAD 9wks post ChEI initiation (n=6)	82.0 ± 36.3	62.2 ± 34.7	81.7 ± 66.9	113.4 ± 80.8	47.0 ± 67.9
mAD benefit group (n=3)	111.0 ± 75.8	99.6 ± 77.5	97.2 ± 80.9	73.4 ± 81.5	18.6 ± 8.8
mAD no benefit group (n=1)	6.3	88.3	53.9	130.4	56.1
Control vs. mAD test statistic (p-value)	F=5.9 (0.034)				
SMC vs. mAD test statistic (p-value)	F=10.2 (0.013)				F=13.5 (0.006)
aMCI vs. mAD test statistic (p-value)	F=5.1 (0.043)				

All data are presented as mean $\pm$ SD unless otherwise stated. Only significant comparisons are shown, results are test statistic (p-value). If a result was significant after Dunn-Šidák correction for multiple comparisons (see calculations below) the result is marked with *.

1.5. Circadian Dunn-Šidák correction for multiple comparisons calculations

NPCRA variables, 10 variables, corrected significance level of 0.005.

Periodogram variables, 2 variables, corrected significance level of 0.025.

Averaged data per hour, 24 variables, corrected significance level of 0.002.

1.6. Sleep Assessment Data

Parameter		PSQI							PSQI Global score
		Component 1 score	Component 2 score	Component 3 score	Component 4 score	Component 5 score	Component 6 score	Component 7 score	
Control first session	(n=9)	0.3 ± 0.5	0.2 ± 0.7	0.4 ± 1.0	0.6 ± 1.0	1.2 ± 0.4	0.0 ± 0.0	0.2 ± 0.4	3.0 ± 2.4
Control second session	(n=8)	0.5 ± 0.5	0.5 ± 1.1	0.1 ± 0.4	0.1 ± 0.4	1.0 ± 0.5	0.0 ± 0.0	0.5 ± 0.5	2.8 ± 2.3
SMC first session	(n=5)	1.0 ± 1.2	0.4 ± 0.5	0.6 ± 1.3	1.2 ± 0.8	1.2 ± 0.4	0.0 ± 0.0	0.2 ± 0.4	4.6 ± 3.0
SMC second session	(n=4)	0.8 ± 1.0	0.3 ± 0.5	1.0 ± 0.8	1.0 ± 0.0 (3)	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	4.7 ± 2.1 (3)
aMCI first session	(n=9)	1.0 ± 0.5	1.2 ± 1.3	0.8 ± 1.1	1.2 ± 1.3	1.2 ± 0.4	0.0 ± 0.0	0.6 ± 0.5	6.0 ± 3.8
aMCI second session	(n=8)	0.8 ± 0.7	0.8 ± 1.2	1.3 ± 1.0	1.5 ± 1.2	1.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.5	5.8 ± 3.7
Mild AD first session	(n=3)	0.3 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.6	2.0 ± 1.0
Mild AD second session	(n=2)	0.5 ± 0.7	0.5 ± 0.7	0.0 ± 0.0	0.5 ± 0.7	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.5 ± 2.1
qmAD ChEI-naïve	(n=29)	0.5 ± 0.7	0.8 ± 1.1	0.2 ± 0.6	0.5 ± 0.9	1.1 ± 0.4	0.2 ± 0.8	0.7 ± 0.7	4.0 ± 3.2
qmAD 9wks post ChEI initiation	(n=22)	0.6 ± 0.6	0.8 ± 1.2	0.5 ± 1.0	0.7 ± 1.1	1.2 ± 0.5	0.0 ± 0.0	0.5 ± 0.6	4.3 ± 3.1
qmAD benefit group	(n=11)	0.4 ± 0.5	0.5 ± 0.9	0.1 ± 0.3	0.3 ± 0.6	1.1 ± 0.3	0.5 ± 1.2	0.5 ± 0.7	3.5 ± 2.1
qmAD no benefit group	(n=7)	0.7 ± 1.1	1.4 ± 1.4	0.1 ± 0.4	0.9 ± 1.1	1.1 ± 0.4	0.0 ± 0.0	0.6 ± 0.5	4.9 ± 3.2
qmAD sleep problems	(n=6)	0.3 ± 0.5	1.3 ± 1.2	0.0 ± 0.0	0.3 ± 0.5	1.2 ± 0.4	0.0 ± 0.0	0.8 ± 0.8	4.0 ± 1.8
qmAD no sleep problems	(n=12)	0.6 ± 0.9	0.7 ± 1.2	0.2 ± 0.4	0.6 ± 1.0	1.1 ± 0.3	0.5 ± 1.2	0.4 ± 0.5	4.0 ± 3.0
qmAD maintained on donepezil	(n=21)	0.5 ± 0.6	0.7 ± 1.1	0.2 ± 0.7	0.4 ± 0.8	1.1 ± 0.4	0.3 ± 0.9	0.8 ± 0.8	4.0 ± 3.2
qmAD changed to other ChEI	(n=4)	0.8 ± 1.5	1.8 ± 1.3	0.3 ± 0.5	1.3 ± 1.3	1.3 ± 0.5	0.0 ± 0.0	0.3 ± 0.5	5.5 ± 4.1

Parameter	PSQI Component 1 score	PSQI Component t 2 score	PSQI Component t 3 score	PSQI Component t 4 score	PSQI Component t 5 score	PSQI Component t 6 score	PSQI Component t 7 score	PSQI Global score
qmAD maintained 10mg OD donepezil (n=17)	0.5 ± 0.6	0.7 ± 1.0	0.3 ± 0.8	0.5 ± 0.9	1.2 ± 0.4	0.4 ± 1.0	0.8 ± 0.8	4.4 ± 3.3
qmAD reverted back to 5mg OD donepezil (n=4)	0.5 ± 0.6	0.8 ± 1.5	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.5	0.0 ± 0.0	0.8 ± 1.0	2.8 ± 2.8
Control vs. aMCI test statistic (p-value)	F=8.0 (0.012)							
SMC vs. qmAD test statistic (p-value)				U=36.0 (0.041)				
aMCI vs. qmAD test statistic (p-value)	U=71.0 (0.023)		U=89.0 (0.045)					
Overtime control vs. aMCI test statistic (p-value)			F=5.5 (0.035)					
Overtime control vs. qmAD ChEI-naïve to 9wks post initiation of ChEI test statistic (p-value)			U=48.0 (0.016)	U=47.0 (0.026)				

Parameter	JSQ Fall asleep time weekdays	JSQ Fall asleep time weekends	JSQ Earliest bedtime	JSQ Latest bedtime	JSQ Awakening time	JSQ Earliest awakening	JSQ Latest Awakening
Control first session (n=9)	22:53 ± 00:37	22:53 ± 00:37	22:06 ± 01:01	00:16 ± 01:43	06:51 ± 00:44	06:05 ± 00:50	08:05 ± 01:19
Control second session (n=8)	22:58 ± 00:44	23:05 ± 00:34	22:26 ± 00:44	23:45 ± 00:56	06:58 ± 00:48	06:22 ± 00:45	08:03 ± 01:07
SMC first session (n=5)	22:49 ± 00:13	22:49 ± 00:13	22:15 ± 00:42	23:54 ± 00:23	06:50 ± 00:32	06:19 ± 00:34	07:32 ± 00:53
SMC second session (n=4)	22:52 ± 00:29	22:52 ± 00:29	21:56 ± 01:18	00:22 ± 00:38	06:33 ± 00:08	06:07 ± 00:29	07:40 ± 00:53
aMCI first session (n=9)	22:31 ± 00:29	22:31 ± 00:29	21:23 ± 00:47	23:41 ± 00:44	06:38 ± 01:02	05:51 ± 01:29	07:43 ± 01:06

Parameter	JSQ Fall asleep time weekdays	JSQ Fall asleep time weekends	JSQ Earliest bedtime	JSQ Latest bedtime	JSQ Awakening time	JSQ Earliest awakening	JSQ Latest Awakening
aMCI second session (n=8)	22:43 ± 01:06	22:46 ± 01:08	21:29 ± 01:09	23:55 ± 01:00	06:03 ± 01:11	05:39 ± 01:32	06:52 ± 01:11
Mild AD first session (n=3)	22:00 ± 00:30	22:00 ± 00:30	21:40 ± 00:46	23:30 ± 00:30	07:05 ± 00:53	06:13 ± 00:42	07:35 ± 01:23
Mild AD second session (n=2)	21:52 ± 00:11	21:52 ± 00:11	21:30 ± 00:00	22:45 ± 00:21	06:30 ± 00:42	06:15 ± 01:04	06:52 ± 00:11
qmAD ChEI-naïve (n=29)	22:09 ± 00:56	22:18 ± 00:55	21:29 ± 01:23	23:16 ± 01:15	07:20 ± 00:52	07:01 ± 00:52	08:24 ± 01:27
qmAD 9wks post ChEI initiation (n=22)	22:10 ± 01:19	23:20 ± 05:20	21:25 ± 01:12	23:19 ± 01:17	07:12 ± 01:04	06:57 ± 01:18	07:51 ± 01:34
mAD benefit group (n=11)	22:00 ± 01:18	22:13 ± 01:13	21:05 ± 01:20	22:51 ± 01:10	07:40 ± 00:52	07:21 ± 00:57	09:09 ± 01:45
mAD no benefit group (n=7)	22:17 ± 00:16	22:17 ± 00:16	22:08 ± 01:38	23:34 ± 01:46	07:17 ± 00:54	06:53 ± 00:53	08:06 ± 01:10
mAD sleep problems (n=6)	22:25 ± 00:12	22:25 ± 00:12	21:35 ± 01:10	23:05 ± 00:12	07:30 ± 01:11	06:55 ± 01:21	08:40 ± 02:05
mAD no sleep problems (n=12)	21:57 ± 01:14	22:10 ± 01:09	21:27 ± 01:42	23:10 ± 01:46	07:32 ± 00:45	07:18 ± 00:40	08:56 ± 01:27
mAD maintained on donepezil (n=21)	22:02 ± 01:04	22:14 ± 01:01	21:18 ± 01:16	23:05 ± 01:06	07:20 ± 00:50	07:02 ± 00:48	08:24 ± 01:21
mAD changed to other ChEI (n=4)	22:15 ± 00:17	22:15 ± 00:17	22:15 ± 02:15	00:15 ± 02:11	07:07 ± 01:02	06:33 ± 01:00	07:56 ± 01:34
mAD maintained 10mg OD donepezil (n=17)	22:01 ± 01:10	22:15 ± 01:07	21:17 ± 01:20	23:07 ± 01:14	07:15 ± 00:52	07:07 ± 00:47	08:26 ± 01:29
mAD reverted back to 5mg OD donepezil (n=4)	22:07 ± 00:29	22:07 ± 00:29	21:22 ± 01:07	23:00 ± 00:00	07:37 ± 00:38	06:45 ± 00:57	08:15 ± 00:39
Control vs. qmAD test statistic (p-value)	F=4.8 (0.035)						
SMC vs. aMCI test statistic (p-value)	F=4.4 (0.038)						

Parameter	JSQ Fall asleep time weekdays	JSQ Fall asleep time weekends	JSQ Earliest bedtime	JSQ Latest bedtime	JSQ Awakening time	JSQ Earliest awakening	JSQ Latest Awakening
SMC vs. qmAD test statistic (p-value)	U=27.0 (0.025)						
aMCI vs. qmAD test statistic (p-value)	F=4.2 (0.049) F=8.7 (0.006)						
Parameter	JSQ Hours of sleep per night	JSQ Min. amount of sleep per night	JSQ Max. amount of sleep per night	JSQ No. of times woke in night	JSQ Longest period of wakefulness per night	JSQ Total wake duration per night	
Control first session	(n=9)	07:09 ± 01:45	04:59 ± 02:35	09:09 ± 02:06	0.9 ± 0.4	00:37 ± 01:17	00:38 ± 01:17
Control second session	(n=8)	07:36 ± 00:29	06:14 ± 01:19	09:14 ± 01:06	1.1 ± 0.6	00:13 ± 00:14	00:13 ± 00:08
SMC first session	(n=5)	07:01 ± 01:15	05:25 ± 00:54	08:17 ± 01:49	1.9 ± 0.9	00:40 ± 00:50	00:44 ± 00:47
SMC second session	(n=4)	06:46 ± 00:51 (3)	04:50 ± 00:48	08:49 ± 02:02	2.3 ± 1.0	00:35 ± 00:56	00:40 ± 00:54
aMCI first session	(n=9)	07:10 ± 01:28	05:14 ± 01:58	09:24 ± 02:00	1.9 ± 0.9	00:20 ± 00:38	00:25 ± 00:36
aMCI second session	(n=8)	06:26 ± 00:51	04:50 ± 02:25	08:28 ± 01:32	1.4 ± 1.1	00:10 ± 00:12	00:27 ± 00:39
Mild AD first session	(n=3)	08:47 ± 00:32	06:25 ± 00:43	09:37 ± 00:48	1.8 ± 0.8	00:05 ± 00:00	00:09 ± 00:03
Mild AD second session	(n=2)	08:22 ± 00:45	07:15 ± 00:56	09:07 ± 00:24	1.0 ± 0.0	00:05 ± 00:00	00:05 ± 00:00
qmAD ChEI-naïve	(n=29)	08:47 ± 00:32	07:09 ± 01:52	10:19 ± 02:16	1.6 ± 1.1	00:07 ± 00:16	00:14 ± 00:40
qmAD 9wks post ChEI initiation	(n=22)	08:13 ± 02:02	06:51 ± 01:52	09:38 ± 01:56	1.4 ± 1.3	00:06 ± 00:05	00:08 ± 00:09
mAD benefit group	(n=11)	09:20 ± 01:45	08:09 ± 01:31	11:53 ± 01:25	2.0 ± 1.5	00:04 ± 00:02	00:08 ± 00:06
mAD no benefit group	(n=7)	08:11 ± 00:49	06:30 ± 02:32	09:09 ± 02:27	1.1 ± 0.4	00:05 ± 00:02	00:06 ± 00:02
mAD sleep problems	(n=6)	08:35 ± 00:58	07:20 ± 01:20	10:35 ± 02:19	1.3 ± 0.8	00:05 ± 00:02	00:07 ± 00:04

Parameter	JSQ Hours of sleep per night	JSQ Min. amount of sleep per night	JSQ Max. amount of sleep per night	JSQ No. of times woke in night	JSQ Longest period of wakefulness per night	JSQ Total wake duration per night
mAD no sleep problems (n=12)	09:02 ± 01:47	07:36 ± 02:25	10:56 ± 02:21	1.8 ± 1.4	00:04 ± 00:02	00:07 ± 00:05
mAD maintained on donepezil (n=21)	08:43 ± 01:55	07:23 ± 01:36	10:32 ± 01:58	1.7 ± 1.2	00:08 ± 00:18	00:18 ± 00:47
mAD changed to other ChEI (n=4)	07:47 ± 00:52	05:13 ± 02:46	08:35 ± 03:19	1.0 ± 0.0	00:04 ± 00:01	00:04 ± 00:01
mAD maintained 10mg OD donepezil (n=17)	08:38 ± 02:06	07:24 ± 01:46	10:33 ± 02:06	1.9 ± 1.2	00:09 ± 00:20	00:20 ± 00:52
mAD reverted back to 5mg OD donepezil (n=4)	09:06 ± 00:43	07:21 ± 00:40	10:28 ± 01:27	1.0 ± 1.4	00:03 ± 00:04	00:06 ± 00:07
Control vs. SMC test statistic (p-value)				F=8.0 (0.015)		
Control vs. aMCI test statistic (p-value)				F=7.9 (0.012)		
Control vs. qmAD test statistic (p-value)	F=4.8 (0.034)	F=7.7 (0.009)				
SMC vs. qmAD test statistic (p-value)					U=30.0 (0.013)	U=25.0 (0.019)
aMCI vs. qmAD test statistic (p-value)	F=5.0 (0.031)	F=7.1 (0.011)				
Overtime control vs. qmAD ChEI-naïve to 9wks post initiation of ChEI test statistic (p-value)	F=6.7 (0.015)	F=5.3 (0.028)				
mAD Benefit vs. non-benefit baseline test statistic (p-value)			F=9.0 (0.008)			
mAD donepezil vs. other ChEI baseline test statistic (p-value)		F=4.9 (0.037)				

Parameter	JSQ Snore on most nights n (%)	JSQ Snoring being loud n (%)	JSQ Stop breathing n (%)	JSQ Doze or fall asleep during day n (%)	JSQ Afraid won't fall back to sleep n (%)	JSQ Restless or disturbed sleep n (%)	JSQ Snore, snort or gasp loudly n (%)	JSQ Heart pounding in night n (%)
Control first session	(n=9) 5 (55.6)	1 (11.1)	0 (0.0)	5 (55.6)	0 (0.0)	0 (0.0)	1 (11.1)	0 (0.0)
Control second session	(n=8) 1 (12.5)	0 (0.0)	1 (12.5)	2 (25.0)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)
SMC first session	(n=5) 1 (20.0)	0 (0.0)	1 (20.0)	2 (40.0)	0 (0.0)	1 (20.0)	0 (0.0)	0 (0.0)
SMC second session	(n=4) 1 (25.0)	0 (0.0)	1 (25.0)	2 (50.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)
aMCI first session	(n=9) 4 (44.4)	2 (22.2)	0 (0.0)	8 (88.9)	0 (0.0)	3 (33.3)	0 (0.0)	0 (0.0)
aMCI second session	(n=8) 5 (62.5)	0 (0.0)	0 (0.0)	6 (75.0)	0 (0.0)	2 (25.0)	0 (0.0)	1 (12.5)
Mild AD first session	(n=3) 2 (66.7)	1 (33.3)	1 (33.3)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mild AD second session	(n=2) 1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
mAD ChEI-naïve	(n=29) 14 (48.3)	3 (10.3)	2 (6.9)	20 (69.0)	0 (0.0)	6 (20.7)	3 (10.3)	0 (0.0)
mAD 9wks post ChEI initiation	(n=20) 12 (50.0)	3 (12.5)	1 (4.3)	20 (83.3)	0 (0.0)	3 (12.5)	0 (0.0)	0 (0.0)

Parameter	JSQ Wake up screaming, violent or confused n (%)	JSQ Walk in sleep n (%)	JSQ Wet the bed n (%)	JSQ Grind teeth in the night n (%)	JSQ Wake up to urinate n (%)	JSQ Wake up with chest pain n (%)	JSQ Wake up due to hunger, or thirst n (%)	JSQ Wake up from bad dreams n (%)
Control first session	(n=9) 0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	7 (77.8)	0 (0.0)	1 (11.1)	1 (11.1)
Control second session	(n=8) 0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (50.0)	0 (0.0)	0 (0.0)	3 (37.5)
SMC first session	(n=5) 0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (80.0)	0 (0.0)	0 (0.0)	2 (40.0)

Parameter	JSQ Wake up screaming, violent or confused n (%)	JSQ Walk in sleep n (%)	JSQ Wet the bed n (%)	JSQ Grind teeth in the night n (%)	JSQ Wake up to urinate n (%)	JSQ Wake up with chest pain n (%)	JSQ Wake up due to hunger, or thirst n (%)	JSQ Wake up from bad dreams n (%)
SMC second session	(n=4)	0 (0.0)	0 (0.0)	0 (0.0)	4 (100)	0 (0.0)	0 (0.0)	3 (75.0)
aMCI first session	(n=9)	0 (0.0)	0 (0.0)	0 (0.0)	6 (66.7)	0 (0.0)	0 (0.0)	5 (55.6)
aMCI second session	(n=8)	0 (0.0)	0 (0.0)	0 (0.0)	6 (75.0)	1 (12.5)	1 (12.5)	1 (12.5)
Mild AD first session	(n=3)	0 (0.0)	0 (0.0)	0 (0.0)	3 (100)	0 (0.0)	0 (0.0)	0 (0.0)
Mild AD second session	(n=2)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	1 (50.0)
mAD ChEI-naïve	(n=29)	0 (0.0)	4 (13.8)	1 (3.4)	23 (79.3)	0 (0.0)	6 (20.7)	3 (10.3)
mAD 9wks post ChEI initiation	(n=20)	0 (0.0)	1 (4.2)	1 (4.2)	19 (95.0)	0 (0.0)	1 (4.2)	6 (30.0)

Parameter	JSQ Wake up from noise or partner n (%)	JSQ Sleep with someone else in bed n (%)	JSQ Get up due to children, pets and family members n (%)	JSQ Stop breathing n (%)	JSQ Sweat a lot during the night n (%)	JSQ Fall out of bed whilst asleep n (%)	JSQ Unusual movements whilst asleep n (%)	JSQ Dream n (%)
Control first session	(n=9)	3 (33.3)	6 (66.7)	0 (0.0)	2 (22.2)	0 (0.0)	0 (0.0)	7 (77.8)
Control second session	(n=8)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (37.5)
SMC first session	(n=5)	0 (0.0)	4 (80.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (100)
SMC second session	(n=4)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (75.0)

Parameter	JSQ Get up							JSQ Unusual movements whilst asleep n (%)	JSQ Dream n (%)
	JSQ Wake up from noise or partner n (%)	JSQ Sleep with someone else in bed n (%)	JSQ Get up due to children, pets and family members n (%)	JSQ Stop breathing n (%)	JSQ Sweat a lot during the night n (%)	JSQ Fall out of bed whilst asleep n (%)			
aMCI first session	(n=9) 3 (33.3)	8 (88.9)	2 (22.2)	0 (0.0)	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)	6 (66.7)
aMCI second session	(n=8) 2 (25.0)	5 (62.5)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	4 (50.0)
Mild AD first session	(n=3) 1 (33.3)	3 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (66.7)
Mild AD second session	(n=2) 0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)
mAD ChEI-naïve	(n=29) 5 (17.2)	16 (55.2)	2 (6.9)	1 (3.4)	1 (3.4)	1 (3.4)	1 (3.4)	3 (10.3)	15 (51.7)
mAD 9wks post ChEI initiation	(n=20) 4 (16.7)	12 (50.0)	2 (8.3)	0 (0.0)	2 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	14 (58.3)

Parameter	JSQ Wake up due to heartburn or reflux n (%)	JSQ Wake up with restless creepy leg cramps n (%)	JSQ Wake up with shortness of breath or asthma n (%)	JSQ Wake up to heat, cold or noise n (%)	JSQ Wake up from too much light in bedroom n (%)	JSQ Time stay in bed after waking n (%)	JSQ Depend on alarm to wake up n (%)	JSQ Sleep in >1 hr past usual wake time n (%)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Control first session	(n=9) 0 (0.0)	0 (0.0)	0 (0.0)	2 (22.2)	1 (11.1)	00:34 ± 00:34	2 (22.2)	1 (11.1)
Control second session	(n=8) 0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	00:23 ± 00:20	1 (12.5)	0 (0.0)
SMC first session	(n=5) 0 (0.0)	1 (20.0)	0 (0.0)	3 (60.0)	3 (60.0)	00:45 ± 00:49	1 (20.0)	0 (0.0)
SMC second session	(n=4) 0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	00:42 ± 00:39	1 (25.0)	0 (0.0)

Parameter	JSQ Wake up due to heartburn or reflux n (%)	JSQ Wake up with restless creepy leg cramps n (%)	JSQ Wake up with shortness of breath or asthma n (%)	JSQ Wake up to heat, cold or noise n (%)	JSQ Wake up from too much light in bedroom n (%)	JSQ Time stay in bed after waking	JSQ Depend on alarm to wake up n (%)	JSQ Sleep in >1 hr past usual wake time n (%)
aMCI first session (n=9)	0 (0.0)	1 (11.1)	0 (0.0)	5 (55.6)	0 (0.0)	01:04 ± 00:39	0 (0.0)	0 (0.0)
aMCI second session (n=8)	1 (12.5)	1 (12.5)	0 (0.0)	1 (12.5)	1 (12.5)	00:51 ± 00:34	0 (0.0)	0 (0.0)
Mild AD first session (n=3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	00:36 ± 00:47	0 (0.0)	0 (0.0)
Mild AD second session (n=2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	01:22 ± 00:31	0 (0.0)	0 (0.0)
qmAD ChEI-naïve (n=29)	0 (0.0)	2 (6.9)	0 (0.0)	12 (41.4)	5 (17.2)	00:43 ± 00:39	2 (6.9)	3 (10.3)
qmAD 9wks post ChEI initiation (n=20)	0 (0.0)	0 (0.0)	0 (0.0)	6 (25.0)	3 (12.5)	01:02 ± 01:04	0 (0.0)	0 (0.0)

Parameter	JSQ Vivid dream-like images when waking n (%)	JSQ Wake up with a headache n (%)	JSQ Wake up with a dry mouth n (%)	JSQ Have a hard time waking up n (%)	JSQ Feel unable to move (paralysed) n (%)	JSQ Wake up disoriented or confused n (%)	JSQ Wake up sick to stomach n (%)	JSQ Wake up 1-2hrs earlier than wanted n (%)
Control first session (n=9)	0 (0.0)	0 (0.0)	4 (44.4)	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (11.1)
Control second session (n=8)	1 (12.5)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
SMC first session (n=5)	1 (20.0)	1 (20.0)	1 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (20.0)
SMC second session (n=4)	1 (25.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Parameter	JSQ Vivid dream-like images when waking n (%)	JSQ Wake up with a headache n (%)	JSQ Wake up with a dry mouth n (%)	JSQ Have a hard time waking up n (%)	JSQ Feel unable to move (paralysed) n (%)	JSQ Wake up disoriented or confused n (%)	JSQ Wake up sick to stomach n (%)	JSQ Wake up 1-2hrs earlier than wanted n (%)
aMCI first session	(n=9) 1 (11.1)	2 (22.2)	7 (77.8)	0 (0.0)	0 (0.0)	3 (33.3)	0 (0.0)	0 (0.0)
aMCI second session	(n=8) 1 (12.5)	1 (12.5)	6 (75.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (25.0)
Mild AD first session	(n=3) 0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mild AD second session	(n=2) 0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
mAD ChEI-naïve	(n=29) 1 (3.4)	6 (20.7)	14 (48.3)	0 (0.0)	0 (0.0)	7 (24.1)	0 (0.0)	2 (6.9)
mAD 9wks post ChEI initiation	(n=20) 5 (20.8)	1 (4.2)	9 (37.5)	1 (4.2)	0 (0.0)	3 (12.5)	0 (0.0)	0 (0.0)

Parameter	JSQ No. of naps per week	JSQ Naps refreshing and restore alertness n (%)	JSQ Feel sleepy during the day n (%)	JSQ Fall asleep whilst driving n (%)	JSQ Feel weak or fall down if surprised, angry or excited n (%)	JSQ Worry about things (anxiety) n (%)	JSQ Feel muscular tension or stress n (%)	JSQ Fall asleep at work or at social events n (%)
Control first session	(n=9) 2.7 ± 3.0	5 (100) #5	2 (22.2)	0 (0.0)	0 (0.0)	1 (11.1)	0 (0.0)	0 (0.0)
Control second session	(n=8) 4.0 ± 4.4 (7)	3 (75.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
SMC first session	(n=5) 2.5 ± 2.5 (3)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
SMC second session	(n=4) 1.3 ± 2.3 (3)	1 (100) #1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Parameter	JSQ No. of naps per week	JSQ Naps refreshing and restore alertness n (%)*	JSQ Feel sleepy during the day n (%)	JSQ Fall asleep whilst driving n (%)	JSQ Feel weak or fall down if surprised, angry or excited n (%)	JSQ Worry about things (anxiety) n (%)	JSQ Feel muscular tension or stress n (%)	JSQ Fall asleep at work or at social events n (%)
aMCI first session	(n=9)	4.1 ± 3.4	5 (62.5) #8	2 (22.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
aMCI second session	(n=8)	3.0 ± 2.9	4 (66.7)	0 (0.0)	0 (0.0)	1 (12.5)	1 (12.5)	0 (0.0)
Mild AD first session	(n=3)	3.5 ± 5.0	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mild AD second session	(n=2)			0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
qmAD ChEI-naïve	(n=29)	9.4 ± 8.2 (27)	14 (58.3) #25	14 (48.3)	0 (0.0)	3 (10.3)	1 (3.4)	2 (6.9)
qmAD 9wks post ChEI initiation	(n=22)	9.0 ± 8.7	12 (57.1) #21	10 (41.7)	0 (0.0)	1 (4.2)	2 (8.3)	0 (0.0)

Parameter	Bed time (hh:m)	Get up time (hh:m)	Time in bed (hh:mm)	Sleep start time (hh:mm)	Sleep end time (hh:mm)	Assumed sleep duration (hh:mm)
Control first session	(n=9)	23:35 ± 00:50	07:14 ± 00:38	07:39 ± 00:37	23:40 ± 00:49	07:14 ± 00:38 07:33 ± 00:36
Control second session	(n=7)	23:11 ± 00:56	07:14 ± 00:47	07:51 ± 00:28	23:25 ± 00:46	07:13 ± 00:47 07:48 ± 00:28
SMC first session	(n=5)	23:26 ± 00:20	06:48 ± 00:10	07:21 ± 00:15	23:33 ± 00:22	06:47 ± 00:09 07:14 ± 00:18
SMC second session	(n=4)	23:29 ± 00:15	06:55 ± 00:23	07:26 ± 00:31	23:34 ± 00:16	06:55 ± 00:23 07:20 ± 00:32
aMCI first session	(n=9)	23:12 ± 00:37	07:03 ± 00:36	07:50 ± 00:40	23:16 ± 00:38	07:03 ± 00:36 07:46 ± 00:39
aMCI second session	(n=7)	23:25 ± 00:34	06:57 ± 00:42	07:31 ± 00:45	23:29 ± 00:35	06:57 ± 00:42 07:27 ± 00:45
Mild AD first session	(n=3)	23:10 ± 00:51	07:13 ± 01:17	08:03 ± 00:50	23:16 ± 00:48	07:13 ± 01:17 07:56 ± 00:48

Parameter	Bed time (hh:m)	Get up time (hh:m)	Time in bed (hh:mm)	Sleep start time (hh:mm)	Sleep end time (hh:mm)	Assumed sleep duration (hh:mm)
Mild AD second session	(n=2) 23:13 ± 00:31	06:51 ± 00:37	08:38 ± 00:07	22:20 ± 00:32	06:51 ± 00:38	08:30 ± 00:05
mAD ChEI-naïve	(n=5) 22:14 ± 00:52	07:48 ± 01:51	09:34 ± 02:12	22:24 ± 00:49	07:48 ± 01:52	09:23 ± 02:05
mAD 9wks post ChEI initiation	(n=5) 22:10 ± 01:02	07:30 ± 01:06	09:19 ± 01:38	22:16 ± 01:01	07:29 ± 01:05	09:12 ± 01:36
mAD benefit group	(n=3) 22:01 ± 01:06	06:47 ± 01:01	08:46 ± 02:02	22:09 ± 01:02	06:47 ± 01:01	08:38 ± 01:58
mAD no benefit group	(n=1) 22:56	07:54	08:58	23:00	07:54	08:54
Control vs. mAD test statistic (p-value)	F=8.2 (0.014)		F=6.2 (0.028)	F=7.7 (0.017)		F=6.3 (0.027)
SMC vs. mAD test statistic (p-value)	F=8.4 (0.020)			F=8.1 (0.022)		
aMCI vs. mAD test statistic (p-value)	F=6.0 (0.030)		F=4.9 (0.046)	F=4.9 (0.047)		F=4.8 (0.049)

Parameter	Actual sleep duration (hh:mm)	Actual sleep duration (%)	Actual wake duration (hh:mm)	Actual wake duration (%)	Sleep efficiency (%)	Sleep latency time (hh:mm)
Control first session	(n=9) 06:52 ± 00:35	91.1 ± 4.1	00:40 ± 00:19	8.9 ± 4.1	90.0 ± 4.3	00:05 ± 00:04
Control second session	(n=7) 06:57 ± 00:29	89.4 ± 5.3	00:50 ± 00:26	10.6 ± 5.3	88.8 ± 5.4	00:02 ± 00:01
SMC first session	(n=5) 06:22 ± 00:27	88.2 ± 3.4	00:51 ± 00:13	11.8 ± 3.4	86.7 ± 3.6	00:06 ± 00:03
SMC second session	(n=4) 06:20 ± 00:24	86.5 ± 4.0	00:59 ± 00:20	13.5 ± 4.1	85.4 ± 4.3	00:05 ± 00:04
aMCI first session	(n=9) 07:02 ± 00:30	90.8 ± 3.3	00:43 ± 00:18	9.2 ± 3.3	90.0 ± 3.4	00:03 ± 00:01
aMCI second session	(n=7) 06:46 ± 00:29	91.3 ± 4.7	00:39 ± 00:25	8.7 ± 4.7	90.5 ± 4.8	00:03 ± 00:01
Mild AD first session	(n=3) 06:54 ± 00:50	87.1 ± 4.0	01:01 ± 00:20	12.9 ± 4.0	86.0 ± 4.4	00:06 ± 00:03
Mild AD second session	(n=2) 07:17 ± 00:24	85.7 ± 3.6	01:12 ± 00:17	14.3 ± 3.6	84.5 ± 3.4	00:07 ± 00:00

Parameter		Actual sleep duration (hh:mm)	Actual sleep duration (%)	Actual wake duration (hh:mm)	Actual wake duration (%)	Sleep efficiency (%)	Sleep latency time (hh:mm)
mAD ChEI-naïve	(n=5)	08:24 ± 01:58	89.3 ± 1.6	00:59 ± 00:09	10.7 ± 1.6	87.8 ± 1.4	00:09 ± 00:08
mAD 9wks post ChEI initiation	(n=5)	08:10 ± 01:25	89.0 ± 3.7	01:01 ± 00:27	11.0 ± 3.7	87.8 ± 3.7	00:06 ± 00:02
mAD benefit group	(n=3)	07:38 ± 01:49	88.3 ± 1.1	00:59 ± 00:10	11.7 ± 1.1	87.0 ± 1.0	00:07 ± 00:03
mAD no benefit group	(n=1)	08:03	90.4	00:51	9.6	89.8	00:03
Control vs. mAD test statistic (p-value)		F=4.9 (0.048)					
SMC vs. aMCI test statistic (p-value)		F=5.4 (0.040)					
aMCI vs. mAD test statistic (p-value)		F=5.2 (0.042)					

Parameter		No. of sleep bouts	No. of wake bouts	Mean sleep bout duration (hh:mm)	Mean wake bout time (hh:mm)	No. of immobile mins	Immobile mins (%)
Control first session	(n=9)	21.0 ± 9.4	20.7 ± 9.4	00:24 ± 00:10	00:02 ± 00:00	398.7 ± 35.6	87.9 ± 5.0
Control second session	(n=7)	23.6 ± 10.5	23.1 ± 10.5	00:22 ± 00:09	00:02 ± 00:00	404.8 ± 26.4	86.5 ± 3.4
SMC first session	(n=5)	24.4 ± 4.1	24.0 ± 4.1	00:17 ± 00:04	00:02 ± 00:00	361.5 ± 28.5	83.0 ± 4.7
SMC second session	(n=4)	25.4 ± 7.4	25.0 ± 7.6	00:17 ± 00:05	00:02 ± 00:00	359.4 ± 34.0	81.6 ± 7.3
aMCI first session	(n=9)	19.2 ± 5.9	18.8 ± 5.9	00:26 ± 00:08	00:02 ± 00:01	399.6 ± 26.0	86.0 ± 4.6
aMCI second session	(n=7)	18.9 ± 8.7	18.6 ± 8.8	00:30 ± 00:20	00:02 ± 00:00	390.0 ± 32.7	87.4 ± 5.4
Mild AD first session	(n=3)	27.6 ± 7.4	27.5 ± 7.7	00:16 ± 00:05	00:02 ± 00:00	379.0 ± 31.4	79.6 ± 3.5
Mild AD second session	(n=2)	31.6 ± 2.1	31.4 ± 1.9	00:14 ± 00:02	00:02 ± 00:00	410.8 ± 4.9	80.6 ± 0.1
mAD ChEI-naïve	(n=5)	24.4 ± 5.0	24.2 ± 5.1	00:22 ± 00:03	00:03 ± 00:00	469.2 ± 89.9	83.6 ± 3.9

Parameter	No. of sleep bouts	No. of wake bouts	Mean sleep bout duration (hh:mm)	Mean wake bout time (hh:mm)	No. of immobile mins	Immobile mins (%)
mAD 9wks post ChEI initiation	(n=5)	24.5 ± 12.2	24.5 ± 12.2	00:03 ± 00:10	456.3 ± 63.5	83.3 ± 6.4
mAD benefit group	(n=3)	24.9 ± 6.1	24.6 ± 6.4	00:02 ± 00:01	435.1 ± 96.2	84.1 ± 1.9
mAD no benefit group	(n=1)	20.2	20.2	00:03	470.2	88.0
SMC vs. aMCI test statistic (p-value)					F=7.6 (0.019)	
SMC vs. mAD test statistic (p-value)					F=6.5 (0.034)	
aMCI vs. mAD test statistic (p-value)					F=5.0 (0.046)	

Parameter	No. of moving mins	Moving mins (%)	No. of immobile phases	Mean length of immobilities	No. of one minute immobilities	One minute immobility (%)
Control first session	(n=9)	55.4 ± 23.6	11.9 ± 5.0	37.1 ± 12.7	12.3 ± 4.1	13.6 ± 4.8
Control second session	(n=7)	63.6 ± 17.3	13.3 ± 3.4	40.8 ± 11.1	10.9 ± 3.1	15.0 ± 4.3
SMC first session	(n=5)	73.6 ± 20.2	17.0 ± 4.9	45.0 ± 10.8	8.9 ± 2.6	21.6 ± 6.3
SMC second session	(n=4)	81.6 ± 34.0	18.6 ± 7.2	45.8 ± 16.0	9.2 ± 4.2	22.1 ± 7.4
aMCI first session	(n=9)	67.1 ± 26.3	14.0 ± 4.6	38.5 ± 10.5	11.5 ± 3.1	17.9 ± 5.5
aMCI second session	(n=7)	57.9 ± 29.3	12.6 ± 5.4	35.8 ± 14.3	13.1 ± 5.8	15.9 ± 5.9
Mild AD first session	(n=3)	97.7 ± 24.9	20.4 ± 3.5	52.7 ± 3.3	7.4 ± 0.4	26.0 ± 7.2
Mild AD second session	(n=2)	99.7 ± 1.4	19.4 ± 0.1	56.4 ± 6.1	7.5 ± 0.8	24.5 ± 1.5
mAD ChEI-naïve	(n=5)	95.0 ± 41.5	16.4 ± 3.9	51.9 ± 19.6	10.1 ± 2.6	21.1 ± 4.9
mAD 9wks post ChEI initiation	(n=5)	96.0 ± 50.8	16.7 ± 6.4	49.4 ± 25.0	10.9 ± 3.3	23.8 ± 6.0

Parameter	No. of moving mins	Moving mins (%)	No. of immobile phases	Mean length of immobilities	No. of one minute immobilities	One minute immobility (%)
mAD benefit group	(n=3)	83.4 ± 25.3	15.9 ± 1.9	48.6 ± 18.0	10.0 ± 4.9	19.6 ± 1.5
mAD no benefit group	(n=1)	64.6	12.0	36.8	6.6	17.2
Control vs. SMC test statistic (p-value)						F=7.2 (0.020)
Control vs. mAD test statistic (p-value)		F=5.3 (0.039)			F=4.9 (0.046)	F=7.6 (0.017)

Parameter	Total activity score	Mean activity score	Mean score in active periods	Fragmentation index	Average wake movement activity count	
Control first session	(n=9)	4904.1 ± 2218.9	10.5 ± 4.6	88.5 ± 34.3	25.8 ± 9.6	279.2 ± 134.6
Control second session	(n=7)	6181.5 ± 3179.6	12.8 ± 6.4	90.2 ± 23.5	28.6 ± 7.6	268.9 ± 106.6
SMC first session	(n=5)	6427.9 ± 1996.8	14.7 ± 4.9	88.0 ± 28.6	38.6 ± 10.9	285.7 ± 63.9
SMC second session	(n=4)	7731.4 ± 2237.5	17.4 ± 4.2	99.4 ± 17.4	40.6 ± 14.6	280.2 ± 42.0
aMCI first session	(n=9)	5818.2 ± 2904.4	12.1 ± 5.3	85.6 ± 29.7	31.9 ± 9.8	241.8 ± 51.8
aMCI second session	(n=7)	5080.0 ± 3397.1	10.9 ± 6.2	84.5 ± 31.9	28.5 ± 11.1	246.9 ± 40.6
Mild AD first session	(n=3)	7252.9 ± 1776.9	15.3 ± 4.7	77.4 ± 30.2	46.3 ± 10.7	328.3 ± 129.9
Mild AD second session	(n=2)	9624.5 ± 4919.8	18.7 ± 9.7	96.4 ± 49.9	43.9 ± 1.6	410.3 ± 40.0
mAD ChEI-naïve	(n=5)	7749.5 ± 1232.6	14.1 ± 3.4	94.8 ± 40.8	37.5 ± 8.7	136.8 ± 72.7
mAD 9wks post ChEI initiation	(n=5)	7594.5 ± 3200.8	13.6 ± 4.8	85.4 ± 31.9	40.5 ± 12.1	165.0 ± 100.7
mAD benefit group	(n=3)	7714.8 ± 1240.0	15.0 ± 2.1	95.6 ± 22.2	35.5 ± 3.4	171.3 ± 63.2
mAD no benefit group	(n=1)	9025.4	16.7	146.7	29.2	130.7

Parameter	Total activity score	Mean activity score	Mean score in active periods	Fragmentation index	Average wake movement activity count
Control vs. SMC test statistic (p-value)				F=5.1 (0.043)	
Control vs. mAD test statistic (p-value)	F=6.9 (0.022)			F=5.0 (0.045)	
SMC vs. mAD test statistic (p-value)					F=11.8 (0.009)
aMCI vs. mAD test statistic (p-value)					F=10.0 (0.008)

Parameter	EEG Time in bed duration (hh:mm)	EEG Sleep duration (hh:mm)	EEG Wake duration (hh:mm)	EEG Wake duration (%)	EEG REM sleep duration (hh:mm)	EEG REM sleep (%)
Control first session (n=7)	08:04 ± 00:37	07:44 ± 00:43	00:12 ± 00:07	2.4 ± 1.4	01:45 ± 00:21	21.7 ± 4.3
Control second session (n=5)	08:08 ± 00:23	07:39 ± 00:23	00:23 ± 00:09	4.6 ± 1.9	01:36 ± 00:32	19.5 ± 6.1
SMC first session (n=3)	07:18 ± 00:13	06:57 ± 00:07	00:15 ± 00:07	3.4 ± 1.6	01:38 ± 00:12	22.5 ± 3.0
SMC second session (n=3)	07:56 ± 00:14	06:56 ± 01:25	00:56 ± 01:09	12.1 ± 15.1	01:24 ± 00:48	17.3 ± 9.7
aMCI first session (n=6)	07:58 ± 01:17	06:34 ± 00:57	01:24 ± 00:36	17.2 ± 6.2	01:05 ± 00:16	14.0 ± 4.9
aMCI second session (n=5)	08:24 ± 01:05	07:00 ± 01:09	01:24 ± 00:50	16.7 ± 9.4	01:19 ± 00:25	15.5 ± 4.0
Mild AD first session (n=3)	07:49 ± 00:24	06:43 ± 00:24	01:05 ± 00:44	13.6 ± 8.4	01:07 ± 00:49	14.2 ± 10.5
Mild AD second session (n=2)	08:58 ± 00:45	07:35 ± 00:38	01:22 ± 01:22	14.6 ± 14.0	01:31 ± 00:10	17.0 ± 3.2
mAD ChEI-naïve (n=1)	09:24	08:38	00:45	8.0	01:24	14.9
mAD 9wks post ChEI initiation (n=2)	08:59 ± 00:05	07:20 ± 01:16	01:37 ± 01:08	18.0 ± 12.8	01:05 ± 00:19	12.0 ± 3.5
Control vs. aMCI test statistic (p-value)		F=6.5 (0.027)	F=27.6 (0.000)*	F=38.1 (0.000)*	F=14.4 (0.003)	F=9.2 (0.011)

Parameter	EEG NREM sleep duration (hh:mm)	EEG NREM sleep (%)	EEG REM latency (hh:mm)	EEG NREM latency (hh:mm)	EEG sleep efficiency (%)	EEG Stage 1 sleep duration (hh:mm)
Control first session (n=7)	05:59 ± 00:45	74.1 ± 5.3	01:10 ± 01:01	00:03 ± 00:02	95.9 ± 1.8	01:12 ± 00:23
Control second session (n=5)	06:03 ± 00:25	74.5 ± 5.3	01:28 ± 01:06	00:08 ± 00:11	94.0 ± 2.0	01:20 ± 00:33
SMC first session (n=3)	05:19 ± 00:18	72.9 ± 3.5	00:59 ± 00:04	00:05 ± 00:03	95.3 ± 2.1	00:41 ± 00:41
SMC second session (n=3)	05:33 ± 00:41	69.7 ± 6.8	00:52 ± 00:28	00:04 ± 00:00	87.1 ± 15.4	00:49 ± 00:33
aMCI first session (n=6)	05:29 ± 01:03	68.6 ± 3.9	01:08 ± 00:42	00:06 ± 00:04	82.6 ± 6.0	00:44 ± 00:17
aMCI second session (n=5)	05:41 ± 01:02	67.7 ± 9.8	01:56 ± 01:10	00:04 ± 00:04	83.3 ± 9.4	00:38 ± 00:07
Mild AD first session (n=3)	05:36 ± 00:43	71.9 ± 12.3	01:49 ± 01:02	00:04 ± 00:05	86.1 ± 8.3	01:14 ± 00:53
Mild AD second session (n=2)	06:04 ± 00:29	68.2 ± 11.0	01:33 ± 00:16	00:04 ± 00:02	85.2 ± 14.2	00:52 ± 00:32
mAD ChEI-naïve (n=1)	07:14	76.9	01:23	00:08	91.8	01:42
mAD 9wks post ChEI initiation (n=2)	06:15 ± 00:56	69.5 ± 9.8	02:13 ± 01:21	00:03 ± 00:03	81.5 ± 13.3	01:55 ± 00:03
Control vs. aMCI test statistic (p-value)					F=30.7 (0.000)*	F=6.4 (0.028)

Parameter	EEG Stage 1 sleep (%)	EEG Stage 2 sleep duration (hh:mm)	EEG Stage 2 sleep (%)	EEG Stage 3 sleep duration (hh:mm)	EEG Stage 3 sleep (%)	EEG Stage 4 sleep duration (hh:mm)
Control first session (n=7)	14.8 ± 4.2	03:13 ± 00:50	39.8 ± 9.0	01:26 ± 00:46	17.8 ± 9.2	00:08 ± 00:11
Control second session (n=5)	16.5 ± 7.2	03:12 ± 01:16	38.9 ± 13.8	01:19 ± 00:32	16.3 ± 7.0	00:13 ± 00:18
SMC first session (n=3)	9.2 ± 9.1	02:41 ± 00:31	36.8 ± 6.6	01:42 ± 00:40	23.4 ± 9.8	00:15 ± 00:08
SMC second session (n=3)	10.3 ± 6.8	03:17 ± 00:52	41.2 ± 10.0	01:12 ± 00:41	15.2 ± 8.5	00:14 ± 00:18
aMCI first session (n=6)	9.2 ± 3.9	03:14 ± 00:30	41.6 ± 9.5	00:58 ± 00:29	11.8 ± 5.0	00:34 ± 00:48

Parameter	EEG Stage 1 sleep (%)	EEG Stage 2 sleep duration (hh:mm)	EEG Stage 2 sleep (%)	EEG Stage 3 sleep duration (hh:mm)	EEG Stage 3 sleep (%)	EEG Stage 4 sleep duration (hh:mm)
aMCI second session (n=5)	7.7 ± 2.3	03:25 ± 00:49	40.7 ± 9.2	01:12 ± 00:47	14.2 ± 8.7	00:26 ± 00:26
Mild AD first session (n=3)	16.0 ± 12.0	03:25 ± 00:29	43.8 ± 7.2	00:53 ± 00:12	11.3 ± 2.4	00:04 ± 00:04
Mild AD second session (n=2)	9.4 ± 5.2	03:45 ± 01:41	42.8 ± 22.4	01:12 ± 00:20	13.2 ± 2.6	00:16 ± 00:20
mAD ChEI-naïve (n=1)	18.1	02:46	29.4	02:27	26.1	00:19
mAD 9wks post ChEI initiation (n=2)	21.3 ± 0.4	02:41 ± 00:24	29.8 ± 4.2	01:26 ± 00:28	15.9 ± 5.0	00:13 ± 00:01
Control vs. aMCI test statistic (p-value)	F=6.1 (0.031)					
Overtime control vs. aMCI test statistic (p-value)						

Parameter	EEG Stage 4 sleep (%)	EEG Deep sleep latency (hh:mm)	EEG Movement duration (hh:mm)	EEG Movement (%)	EEG Wake after sleep onset (hh:mm)	EEG Area 1 Sleep duration (hh:mm)
Control first session (n=7)	1.7 ± 2.4	00:18 ± 00:10	00:08 ± 00:03	1.7 ± 0.9	00:08 ± 00:05	02:33 ± 00:15
Control second session (n=5)	2.8 ± 4.0	00:24 ± 00:13	00:07 ± 00:03	1.2 ± 0.8	00:15 ± 00:12	02:26 ± 00:10
SMC first session (n=3)	3.5 ± 1.8	00:11 ± 00:06	00:06 ± 00:04	1.2 ± 0.8	00:10 ± 00:04	02:18 ± 00:04
SMC second session (n=3)	3.1 ± 3.8	00:16 ± 00:15	00:04 ± 00:02	0.8 ± 0.3	00:52 ± 01:09	02:31 ± 00:06
aMCI first session (n=6)	6.0 ± 8.4	00:40 ± 00:35	00:01 ± 00:02	0.2 ± 0.3	01:15 ± 00:38	02:08 ± 00:20
aMCI second session (n=5)	5.1 ± 4.9	00:54 ± 00:57	00:00 ± 00:00	0.1 ± 0.2	01:20 ± 00:48	02:10 ± 00:44
Mild AD first session (n=3)	0.9 ± 0.8	00:24 ± 00:11	00:01 ± 00:02	0.3 ± 0.5	00:58 ± 00:33	02:13 ± 00:14
Mild AD second session (n=2)	2.7 ± 3.5	00:31 ± 00:05	00:01 ± 00:01	0.2 ± 0.2	01:16 ± 01:18	02:35 ± 00:04
mAD ChEI-naïve (n=1)	3.4	00:20	00:01	0.2	00:36	02:45

Parameter	EEG Stage 4 sleep (%)	EEG Deep sleep latency (hh:mm)	EEG Movement duration (hh:mm)	EEG Movement (%)	EEG Wake after sleep onset (hh:mm)	EEG Area 1 Sleep duration (hh:mm)
mAD 9wks post ChEI initiation	(n=2) 2.5 ± 0.2	00:27 ± 00:19	00:03 ± 00:03	0.6 ± 0.5	01:30 ± 01:08	01:56 ± 01:07
Control vs. aMCI test statistic (p-value)			F=22.5 (0.001)*	F=16.4 (0.002)*	F=21.9 (0.001)*	F=6.8 (0.024)

Parameter	EEG Area 1 Wake duration (hh:mm)	EEG Area 1 Movement duration (hh:mm)	EEG Area 1 REM sleep duration (hh:mm)	EEG Area 1 Stage 1 sleep duration (hh:mm)	EEG Area 1 Stage 2 sleep duration (hh:mm)	EEG Area 1 Stage 3 sleep duration (hh:mm)
Control first session	(n=7) 00:06 ± 00:04	00:03 ± 00:02	00:28 ± 00:17	00:17 ± 00:09	00:56 ± 00:23	00:46 ± 00:25
Control second session	(n=5) 00:12 ± 00:10	00:02 ± 00:02	00:21 ± 00:18	00:19 ± 00:14	00:52 ± 00:24	00:48 ± 00:16
SMC first session	(n=3) 00:06 ± 00:04	00:02 ± 00:02	00:27 ± 00:14	00:10 ± 00:11	00:38 ± 00:15	00:49 ± 00:10
SMC second session	(n=3) 00:06 ± 00:02	00:02 ± 00:01	00:28 ± 00:06	00:15 ± 00:09	00:49 ± 00:26	00:46 ± 00:26
aMCI first session	(n=6) 00:32 ± 00:18	00:00 ± 00:00	00:14 ± 00:09	00:12 ± 00:05	01:01 ± 00:12	00:25 ± 00:07
aMCI second session	(n=5) 00:38 ± 00:51	00:00 ± 00:00	00:16 ± 00:13	00:10 ± 00:01	00:49 ± 00:24	00:36 ± 00:20
Mild AD first session	(n=3) 00:23 ± 00:19	00:00 ± 00:00	00:08 ± 00:11	00:17 ± 00:10	01:08 ± 00:29	00:39 ± 00:08
Mild AD second session	(n=2) 00:24 ± 00:18	00:00 ± 00:01	00:16 ± 00:01	00:14 ± 00:06	01:12 ± 00:21	00:40 ± 00:05
mAD ChEI-naïve	(n=1) 00:23	00:00 ± 00:00	00:11	00:33	00:49	00:59
mAD 9wks post ChEI initiation	(n=2) 01:04 ± 01:06	00:00 ± 00:00	00:15 ± 00:21	00:28 ± 00:05	00:37 ± 00:08	00:30 ± 00:25
Control vs. aMCI test statistic (p-value)	F=13.7 (0.003)					

Parameter	EEG Area 1 Stage 4 sleep duration (hh:mm)	EEG Area 1 Sleep efficiency (%)	EEG Area 1 No. of stage changes	EEG Area 1 REM sleep latency (hh:mm)	EEG Area 2 Sleep duration (hh:mm)	EEG Area 2 Wake duration (hh:mm)
Control first session	(n=7) 00:06 ± 00:11	94.6 ± 3.1	76.6 ± 13.2	00:43 ± 00:35	02:36 ± 00:15	00:03 ± 00:03
Control second session	(n=5) 00:06 ± 00:05	91.1 ± 6.27	71.2 ± 6.3	00:54 ± 00:54	02:34 ± 00:11	00:08 ± 00:07
SMC first session	(n=3) 00:13 ± 00:09	94.5 ± 3.4	65.7 ± 19.6	00:59 ± 00:04	02:19 ± 00:03	00:06 ± 00:05
SMC second session	(n=3) 00:13 ± 00:16	95.1 ± 1.2	77.3 ± 13.7	00:52 ± 00:28	02:10 ± 00:38	00:28 ± 00:34
aMCI first session	(n=6) 00:15 ± 00:26	80.4 ± 9.5	95.5 ± 24.4	01:08 ± 00:42	02:06 ± 00:26	00:33 ± 00:25
aMCI second session	(n=5) 00:20 ± 00:21	78.7 ± 26.4	91.6 ± 35.6	01:17 ± 01:10	02:34 ± 00:22	00:15 ± 00:11
Mild AD first session	(n=3) 00:03 ± 00:03	85.6 ± 11.7	75.0 ± 19.5	00:49 ± 00:43	02:15 ± 00:20	00:21 ± 00:28
Mild AD second session	(n=2) 00:13 ± 00:17	86.7 ± 9.5	88.5 ± 14.8	01:33 ± 00:16	02:44 ± 00:06	00:15 ± 00:08
mAD ChEI-naïve	(n=1) 00:14	87.6	115	01:23	02:59	00:08
mAD 9wks post ChEI initiation	(n=2) 00:06 ± 00:07	64.1 ± 36.9	79.5 ± 41.7	00:38 ± 00:53	02:55 ± 00:03	00:03 ± 00:01
Control vs. aMCI test statistic (p-value)	F=13.9 (0.003)				F=6.9 (0.023)	F=9.9 (0.009)

Parameter	EEG Area 2 Movement duration (hh:mm)	EEG Area 2 REM sleep duration (hh:mm)	EEG Area 2 Stage 1 sleep duration (hh:mm)	EEG Area 2 Stage 2 sleep duration (hh:mm)	EEG Area 2 Stage 3 sleep duration (hh:mm)	EEG Area 2 Stage 4 sleep duration (hh:mm)
Control first session	(n=7) 00:02 ± 00:01	00:31 ± 00:15	00:20 ± 00:13	01:10 ± 00:28	00:35 ± 00:26	00:01 ± 00:01
Control second session	(n=5) 00:03 ± 00:01	00:34 ± 00:09	00:31 ± 00:28	01:12 ± 00:35	00:17 ± 00:15	00:01 ± 00:01
SMC first session	(n=3) 00:02 ± 00:01	00:32 ± 00:17	00:12 ± 00:13	00:52 ± 00:06	00:41 ± 00:25	00:02 ± 00:01
SMC second session	(n=3) 00:01 ± 00:01	00:26 ± 00:23	00:15 ± 00:14	01:09 ± 00:11	00:18 ± 00:10	00:01 ± 00:02

Parameter	EEG Area 2		EEG Area 2		EEG Area 2		EEG Area 2		EEG Area 2	
	Movement duration (hh:mm)	REM sleep duration (hh:mm)	Stage 1 sleep duration (hh:mm)	Stage 2 sleep duration (hh:mm)	Stage 3 sleep duration (hh:mm)	Stage 4 sleep duration (hh:mm)	Stage 1 sleep duration (hh:mm)	Stage 2 sleep duration (hh:mm)	Stage 3 sleep duration (hh:mm)	Stage 4 sleep duration (hh:mm)
aMCI first session	(n=6)	00:00 ± 00:00	00:21 ± 00:16	00:15 ± 00:08	01:13 ± 00:15	00:15 ± 00:14	00:13 ± 00:21			
aMCI second session	(n=5)	00:00 ± 00:00	00:31 ± 00:18	00:10 ± 00:04	01:20 ± 00:21	00:27 ± 00:21	00:05 ± 00:03			
Mild AD first session	(n=3)	00:00 ± 00:01	00:29 ± 00:28	00:22 ± 00:24	01:15 ± 00:07	00:09 ± 00:03	00:00 ± 00:00			
Mild AD second session	(n=2)	00:01 ± 00:01	00:41 ± 00:13	00:10 ± 00:04	01:22 ± 00:36	00:27 ± 00:23	00:03 ± 00:03			
mAD ChEI-naïve	(n=1)	00:00	00:35	00:14	01:01	01:05	00:05			
mAD 9wks post ChEI initiation	(n=2)	00:03 ± 00:02	00:32 ± 00:15	00:25 ± 00:03	01:14 ± 00:16	00:42 ± 00:06	00:02 ± 00:02			
Control vs. aMCI test statistic (p-value)	F=24.0 (0.000)*									
Overtime control vs. aMCI test statistic (p-value)	F=6.1 (0.038) F=5.7 (0.044)									

Parameter	EEG Area 2 Sleep efficiency (%)	EEG Area 2 No. of stage changes	EEG Area 2 REM sleep latency (hh:mm)	EEG Area 3 Sleep duration (hh:mm)	EEG Area 3 Wake duration (hh:mm)	EEG Area 3 Movement duration (hh:mm)
Control first session (n=7)	96.8 ± 2.5	70.3 ± 32.9	00:05 ± 00:13	02:25 ± 00:39	00:03 ± 00:03	00:03 ± 00:01
Control second session (n=5)	93.5 ± 3.6	53.4 ± 15.3	00:00 ± 00:00	02:43 ± 00:08	00:02 ± 00:03	00:02 ± 00:01
SMC first session (n=3)	95.2 ± 3.1	72.3 ± 52.2	00:11 ± 00:18	02:26 ± 00:04	00:04 ± 00:02	00:02 ± 00:01
SMC second session (n=3)	81.2 ± 21.8	62.3 ± 40.2	00:14 ± 00:24	02:39 ± 00:04	00:22 ± 00:35	00:01 ± 00:01
aMCI first session (n=6)	79.7 ± 13.5	82.2 ± 24.3	00:05 ± 00:13	02:39 ± 00:26	00:19 ± 00:13	00:01 ± 00:01
aMCI second session (n=5)	91.5 ± 6.3	80.8 ± 34.4	00:00 ± 00:00	02:48 ± 00:22	00:32 ± 00:28	00:00 ± 00:00

Parameter		EEG Area 2			EEG Area 3		
		EEG Area 2 Sleep efficiency (%)	EEG Area 2 No. of stage changes	EEG Area 2 REM sleep latency (hh:mm)	EEG Area 3 Sleep duration (hh:mm)	EEG Area 3 Wake duration (hh:mm)	EEG Area 3 Movement duration (hh:mm)
Mild AD first session	(n=3)	86.7 ± 16.9	55.3 ± 10.1	00:30 ± 00:52	02:36 ± 00:08	00:21 ± 00:07	00:01 ± 00:01
Mild AD second session	(n=2)	91.5 ± 4.0	64.5 ± 19.1	00:00 ± 00:00	02:59 ± 00:15	00:43 ± 00:56	00:00 ± 00:00
mAD ChEI-naïve	(n=1)	95.5	94.0	0.0	03:08	00:14	00:01
mAD 9wks post ChEI initiation	(n=2)	97.1 ± 0.6	114.5 ± 14.8	00:09 ± 00:12	03:00 ± 00:02	00:30 ± 00:04	00:00 ± 00:00
Control vs. aMCI test statistic (p-value)		F=10.9 (0.007)			F=10.4 (0.008)		
		F=7.8 (0.017)					
Parameter		EEG Area 3			EEG Area 3		
		EEG Area 3 REM sleep duration (hh:mm)	EEG Area 3 Stage 1 sleep duration (hh:mm)	EEG Area 3 Stage 2 sleep duration (hh:mm)	EEG Area 3 Stage 3 sleep duration (hh:mm)	EEG Area 3 Stage 4 sleep duration (hh:mm)	EEG Area 3 Sleep efficiency (%)
Control first session	(n=7)	00:38 ± 00:23	00:33 ± 00:17	01:02 ± 00:24	00:06 ± 00:08	00:01 ± 00:02	95.2 ± 3.4
Control second session	(n=5)	00:41 ± 00:06	00:30 ± 00:19	01:08 ± 00:28	00:14 ± 00:14	00:06 ± 00:13	97.6 ± 2.0
SMC first session	(n=3)	00:39 ± 00:18	00:19 ± 00:18	01:11 ± 00:18	00:11 ± 00:09	00:00 ± 00:00	96.3 ± 2.2
SMC second session	(n=3)	00:30 ± 00:27	00:19 ± 00:11	01:18 ± 00:26	00:08 ± 00:06	00:00 ± 00:00	84.9 ± 23.6
aMCI first session	(n=6)	00:29 ± 00:14	00:17 ± 00:09	01:10 ± 00:16	00:18 ± 00:16	00:06 ± 00:06	87.7 ± 8.1
aMCI second session	(n=5)	00:32 ± 00:14	00:18 ± 00:07	01:16 ± 00:28	00:10 ± 00:10	00:01 ± 00:03	79.6 ± 19.9
Mild AD first session	(n=3)	00:30 ± 00:20	00:36 ± 00:21	01:02 ± 00:08	00:06 ± 00:09	00:02 ± 00:03	86.1 ± 6.4
Mild AD second session	(n=2)	00:33 ± 00:21	00:29 ± 00:22	01:11 ± 00:44	00:04 ± 00:02	00:00 ± 00:00	77.4 ± 29.1
mAD ChEI-naïve	(n=1)	00:38	00:56	00:57	00:23	00:00	92.3

Parameter		EEG Area 3 REM sleep duration (hh:mm)	EEG Area 3 Stage 1 sleep duration (hh:mm)	EEG Area 3 Stage 2 sleep duration (hh:mm)	EEG Area 3 Stage 3 sleep duration (hh:mm)	EEG Area 3 Stage 4 sleep duration (hh:mm)	EEG Area 3 Sleep efficiency (%)
mAD 9wks post ChEI initiation	(n=2)	00:18 ± 00:13	01:02 ± 00:01	00:50 ± 00:00	00:15 ± 00:04	00:05 ± 00:05	83.2 ± 2.3
Control vs. aMCI test statistic (p-value)		F=5.1 (0.046)					

Parameter		EEG Area 3 No. of stage changes	EEG Area 3 REM sleep latency (hh:mm)
Control first session	(n=7)	41.3 ± 22.2	00:00 ± 00:00
Control second session	(n=5)	55.6 ± 22.5	00:16 ± 00:23
SMC first session	(n=3)	52.3 ± 9.7	00:00 ± 00:00
SMC second session	(n=3)	34.3 ± 7.6	00:11 ± 00:18
aMCI first session	(n=6)	89.7 ± 30.3	00:15 ± 00:18
aMCI second session	(n=5)	76.0 ± 15.6	00:00 ± 00:00
Mild AD first session	(n=3)	56.7 ± 20.7	00:11 ± 00:19
Mild AD second session	(n=2)	45.5 ± 9.2	00:00 ± 00:00
mAD ChEI-naïve	(n=1)	77.0	00:00
mAD 9wks post ChEI initiation	(n=2)	65.5 ± 3.5	00:00 ± 00:00
Control vs. aMCI test statistic (p-value)		F=11.0 (0.007)	

All data are presented as mean $\pm$ SD unless otherwise stated. Only significant comparisons are shown, results are test statistic (p-value). If a result was significant after Dunn-Šidák correction for multiple comparisons (see calculations below) the result is marked with *. # n represents sample size if altered from that stated.

1.7. Sleep Dunn-Šidák correction for multiple comparisons calculations

PSQI variables, 8 variables, corrected significance level of 0.006.

JSQ variables, 75 variables, corrected significance level of 0.001.

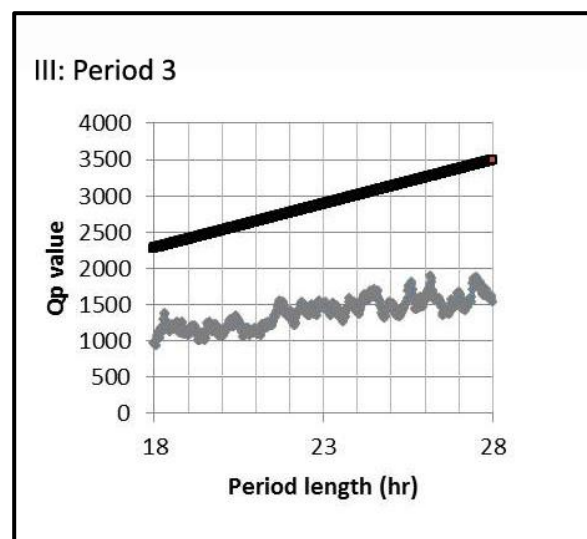
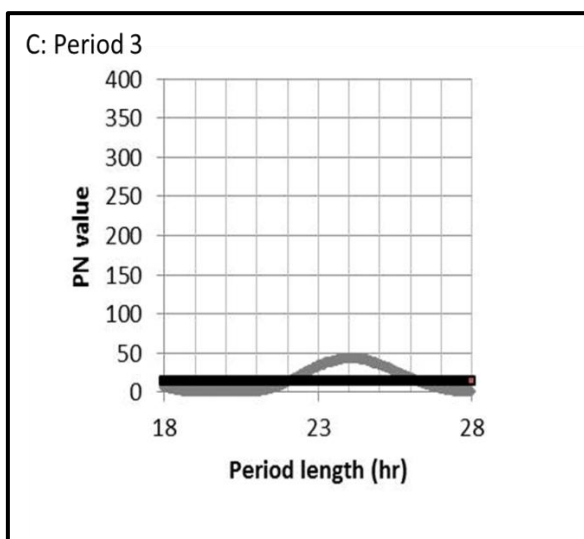
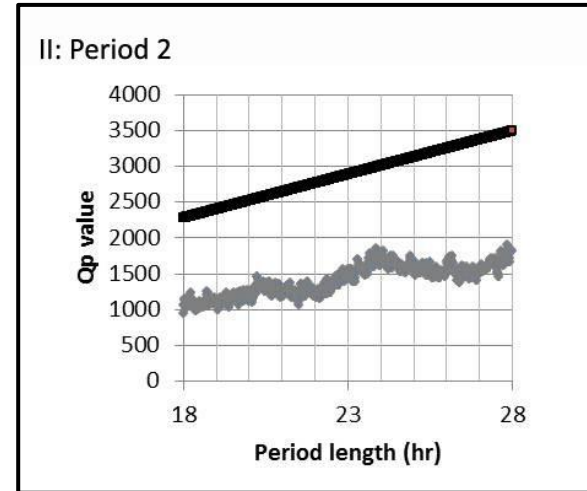
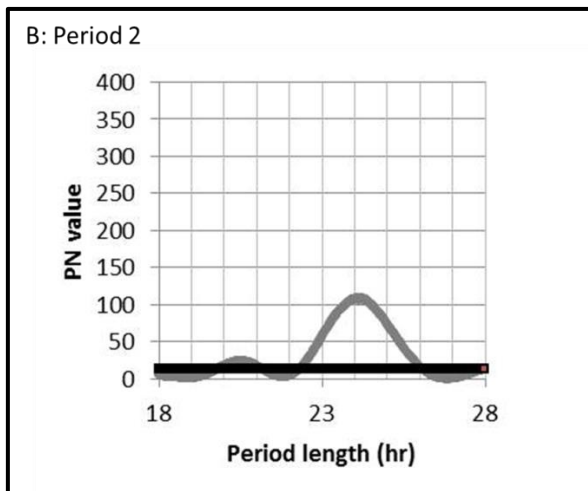
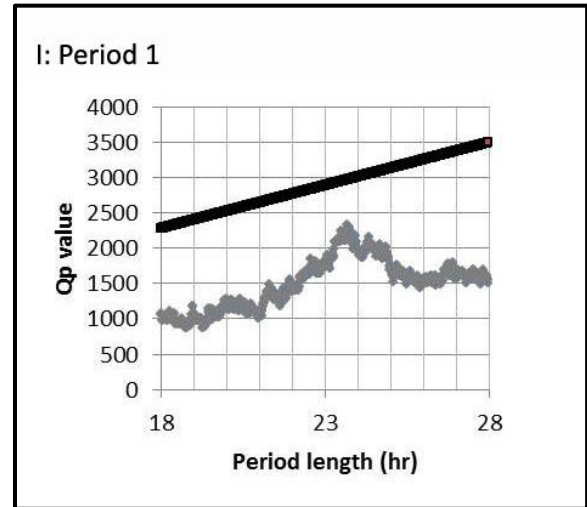
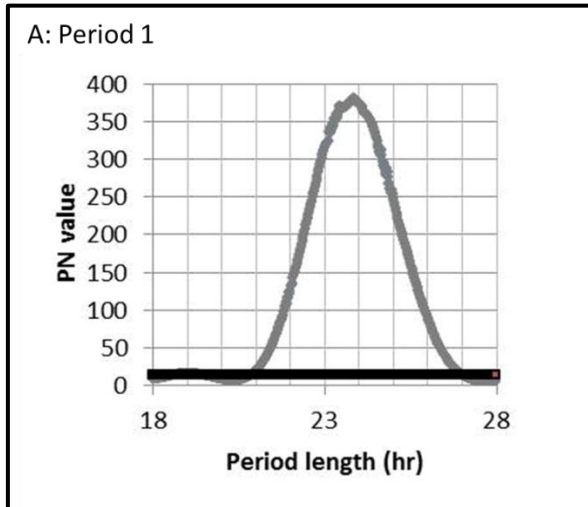
Actigraphy sleep analysis, 29 variables, corrected significance level of 0.002.

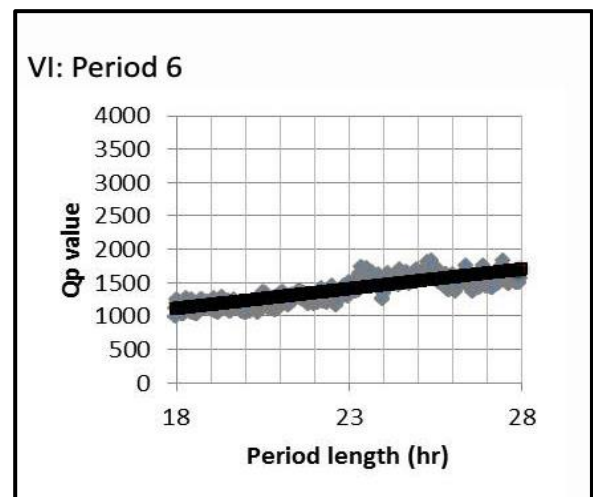
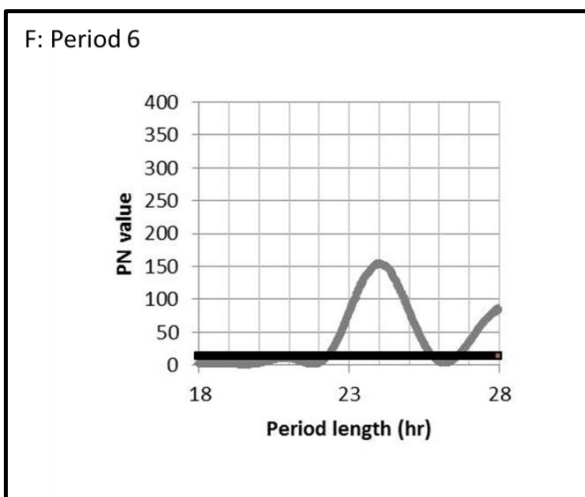
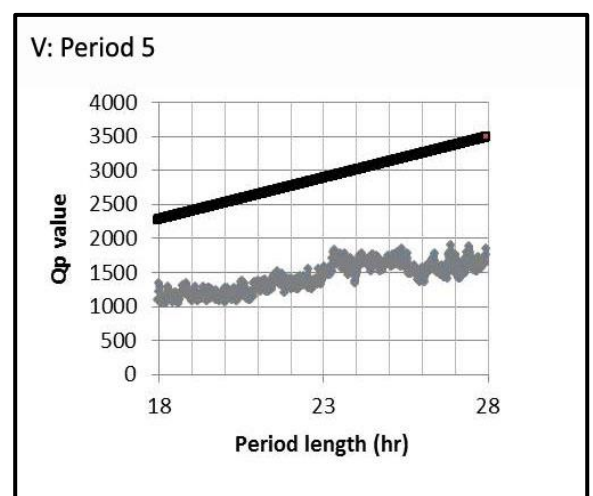
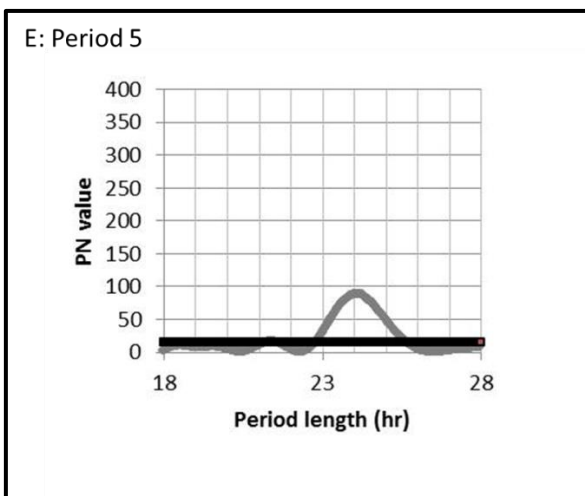
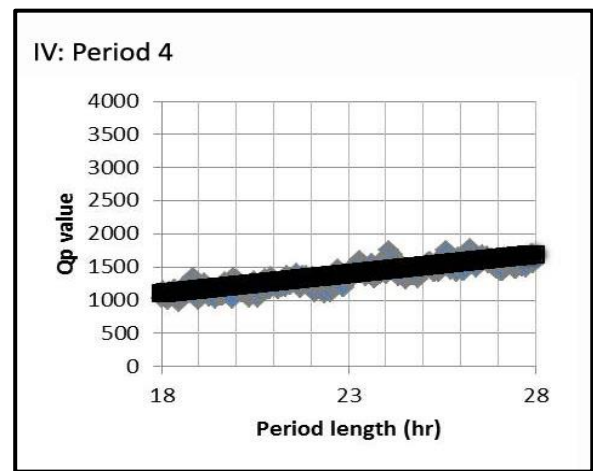
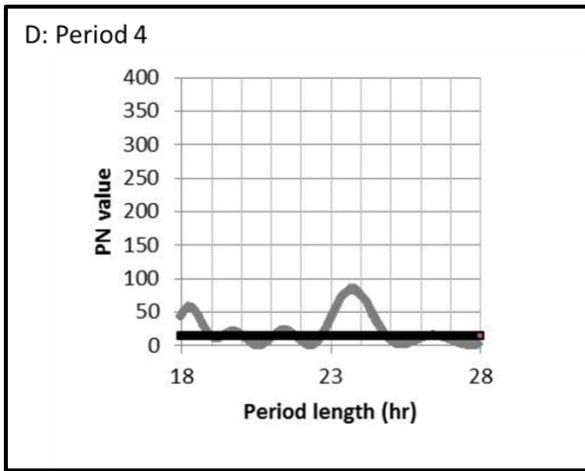
EEG scoring whole night, 23 variables, corrected significance level of 0.002.

EEG scoring thirds of night (areas), 39 variables, corrected significance level of 0.001.

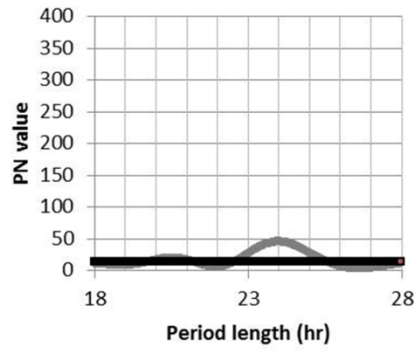
2. Study 2

2.1. Comparison of Periodogram analyses

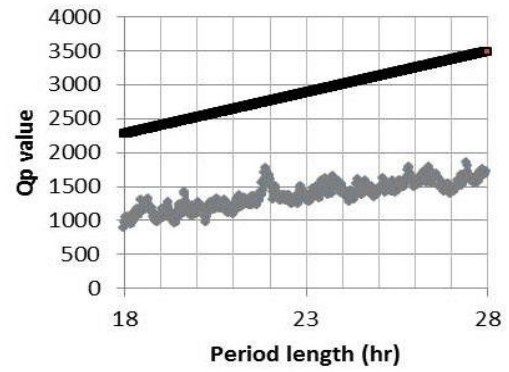




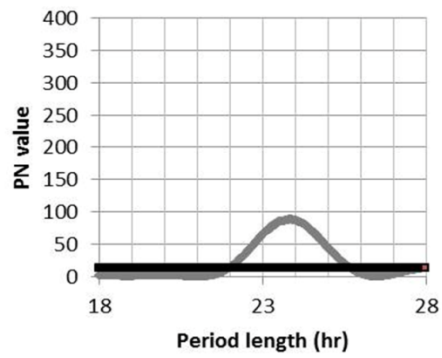
G: Period 7



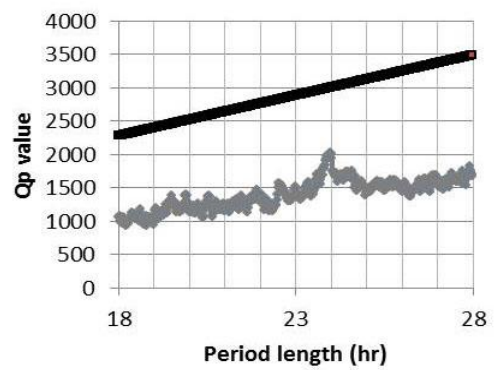
VII: Period 7



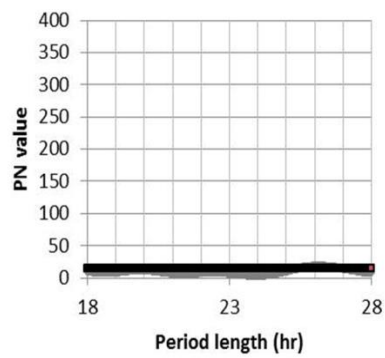
H: Period 8



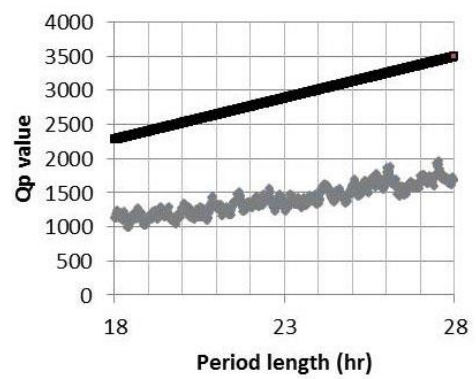
VIII: PERIOD 8



I: Period 9



IX: Period 9



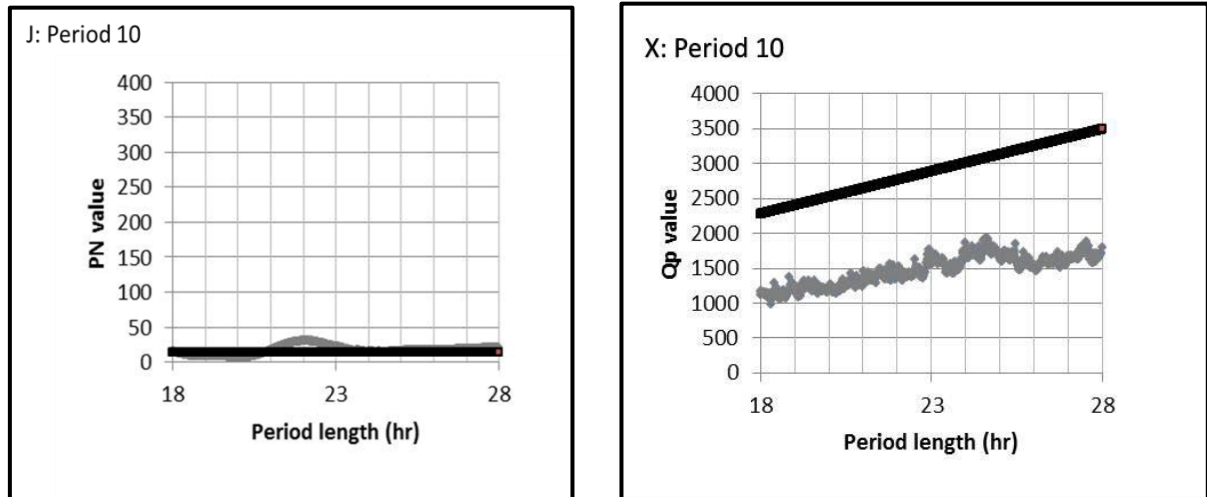


Figure 32. Lomb & Scargle method and Sokolove-Bushell method Periodogram analysis conducted on each period of actigraphy data of the brain cancer study (Study 2). The graphs (A-J) show Lomb & Scargle periodogram analyses. Black data points indicate PN (normalised power) values with grey lines showing significance level. All periodogram analyses were conducted at $p=0.001$. Graphs are as follows (h): A: Period 1 (period=23.85h) B: Period 2 (period=24.07h), C: Period 3 (period=24.10h), D: Period 4 (p=23.70h), E: Period 5 (period=24.17h), F: Period 6 (period=24.07h), G: Period 7 (period=23.90h), H: Period 8 (period=23.87h), I: Period 9 with caregiver-induced activity removed (period=26.12h) and J: Period 10 with caregiver-induced activity removed (period=22.05h). All period lengths were significant.

The graphs (I-X) show Sokolove-Bushell periodogram analyses. Black lines indicate significance level and grey values indicate Qp value. All periodogram analyses were conducted at $p=0.001$. Graphs are as follows: I: Period 1, II: Period 2, III: Period 3, IV: Period 4, V: Period 5, VI: Period 6, VII: Period 7, VIII: Period 8, IX: Period 9 with caregiver-induced activity removed and X: Period 10 with caregiver-induced activity removed. Only periods 4 and 6 were significant with periods of 24.0h and 23.6h respectively.

3. Validation of Methods

3.1. Social Functioning Scale (SFS) validation

<u>Controls</u>	Withdrawal	Inter. Comm.	Indep. Perf.	Recreation	Prosocial	Indep. Comp.
Interpersonal Communication (9)	0.89					
Independence Performance (7)	0.64	0.69				
Recreation (8)	0.79	0.78	0.42			
Prosocial (8)	0.47	0.77	0.21	0.87		
Independence Competence (9)	0.92	0.88	0.69	0.78	0.54	
Total (6)	0.94	0.95	0.51	0.95	0.94	0.95

Table shows internal consistency Pearson correlations of SFS component scores for the control group. Chronbach's alpha for the whole scale was 0.70. Sample size in parentheses. Inter. Comm.: Interpersonal communication, Indep. Perf.: Independence Performance, Indep. Comp.: Independence Competence, With.: Withdrawal.

<u>Moderate AD</u>	Withdrawal	Inter. Comm.	Indep. Perf.	Recreation	Prosocial	Indep. Comp.
Interpersonal Communication (25)	0.34					
Independence Performance (24)	0.30	0.32				
Recreation (24)	0.37	0.31	0.74			
Prosocial (20)	0.54	0.25	0.30	0.57		
Independence Competence (21)	0.21	0.36	0.91	0.57	0.26	
Total (14)	0.52	0.63	0.80	0.87	0.66	0.81

Table shows internal consistency Pearson correlations of SFS component scores for the qmAD group. Chronbach alpha for the whole scale was 0.77. Sample size in parentheses.

ADCS-ADL and SFS components	With.	Inter. Comm.	Indep. Perf.	Recreation	Prosocial	Indep. Comp.	Total score
Basic ADLs	-0.03	-0.03	0.71 (p=0.000)	0.50 (p=0.013)	0.07	0.66 (p=0.001)	0.53
Instrumental ADLs	0.28	0.29	0.93 (p=0.000)	0.84 (p=0.000)	0.59	0.75 (p=0.002)	0.84 (p=0.009)
Total score	0.21	0.26	0.95 (p=0.000)	0.83 (p=0.000)	0.55	0.80 (p=0.001)	0.82 (p=0.012)

Table shows Pearson correlation of SFS and ADCS-ADL component scores for the qmAD group. All values are presented as R statistic (p-value, if significant). Variables had sample sizes between 8 and 26.

3.2. Actigraphy validation for sleep assessment

Amnesic Mild Cognitive Impairment

Agreement rate Sadeh <i>et al.</i> method (%)	Agreement rate Cole <i>et al.</i> method (%)	Agreement of sleep epochs Sadeh <i>et al.</i> method (%)	Agreement of wake epochs Sadeh <i>et al.</i> method (%)	Agreement of sleep epochs Cole <i>et al.</i> method (%)	Agreement of wake epochs Cole <i>et al.</i> method (%)
76.9 ± 5.7	79.5 ± 9.0	83.6 ± 8.7	57.8 ± 20.6	100.0 ± 0.0	0.1 ± 0.2

Agreement rates between EEG and actigraphy for sleep assessment. Six aMCI individuals were included. All methods are found in Chapter 2 (p.54) and discussed in chapter 7 (p. 270). Data are presented as mean ± SD.

3.3. EEG concordance

Participant number and assessment	Concordance rate (%)
aMCI 1 (first)	86.25
aMCI 1 (second)	84.93
aMCI 2 (first)	74.60
aMCI 2 (second)	80.60
aMCI 3 (first)	82.85
aMCI 3 (second)	88.47
aMCI 4 (first)	69.72
aMCI 4 (second)	82.22
aMCI 5 (first)	77.71
aMCI 5 (second)	81.94
aMCI 6 (first)	70.97
aMCI 6 (second)	71.11
aMCI 7 (first)	86.04
aMCI 7 (second)	91.45
aMCI 8 (first)	71.94

Table shows epoch-by-epoch concordance rates of my EEG stage scoring in comparison to that of Dr. Lazar (University of Cambridge). Average concordance of stage scoring was 80.05%.

3.4. Code for Binomial regression analysis in R

Comparison between groups at one assessment

```
# jupiter <- read.csv("/Data/Raw data.csv", header = T)
# maxval <- read.csv("/Data/ maxvals.csv", header = T)
# copy this file, paste into R, hit return and the macro is loaded
# Example: jupitersleep(jupiter,maxval,"GHQTotal",c("sex","Age"))

jupitersleep <- function(datafile,maxv,resname,covname = NULL){
  y <- datafile[,resname]
  ny <- maxv[resname]
  ny <- ny[[1]]*(y*0 +1) - y
  group <- datafile$Group - 1
  l <- length(covname)
  if(l==0) out <- glm(cbind(y,ny) ~ group, na.action = na.omit, family = binomial, data = datafile)
  if(l==1) out <- glm(cbind(y,ny) ~ group + datafile[,covname], na.action = na.omit, family = binomial,
  data = datafile)
  if(l==2) out <- glm(cbind(y,ny) ~ group + datafile[,covname[1]] + datafile[,covname[2]], na.action =
  na.omit, family = binomial, data = datafile)
  if(l==3) out <- glm(cbind(y,ny) ~ group + datafile[,covname[1]] + datafile[,covname[2]] +
  datafile[,covname[3]], na.action = na.omit, family = binomial, data = datafile)
  if(l==4) out <- glm(cbind(y,ny) ~ group + datafile[,covname[1]] + datafile[,covname[2]] +
  datafile[,covname[3]] + datafile[,covname[4]]
  , na.action = na.omit, family = binomial, data = datafile)
  summary(out)
}
```

Comparison between assessments

```
> jupiter <- read.csv("/Data/whole experiment timecourse.csv", header = T)
> attach(jupiter)
> factor(Group)
Levels: 1 2
> disoriented<- jupiter[,4]
> out.disoriented<-lmer(2*cbind(jupiter,disoriented)~Time*Group + (1|ID), family = binomial, data =
jupiter)
> summary(out.disoriented)
```

4. Questionnaires

4.1. Jupiter Sleep Questionnaire



JUPITER
MEDICAL
CENTER

Jupiter Medical Center - Sleep Questionnaire

1025 Military Trail, Suite 210, Jupiter FL 33458

(561) 744-4478 Fax (561) 748-4114

Email: Sleep@jupitermed.com

Participant number.....

Gender of participant : Male Female

Date of birth of participant:

Date completed.....

Please complete all questions. All questions are about the participant's sleep over the last two weeks unless otherwise stated.

Falling asleep

1. What time do they usually fall asleep on a week night? am/pm
weekend night? am/pm
2. How much does this time vary during a typical month? (earliest).....am/pm
to am/pm (latest)
3. How long does it usually take to fall asleep? minutes
4. How many nights a week does it take longer than 30 minutes to fall asleep?
None 1-2 3-4 5 or more
5. How many nights a week does it take longer than 60 minutes to fall asleep?
None 1-2 3-4 5 or more
6. When falling asleep, or trying to sleep, are they frequently bothered by:
 - () Thoughts racing through their mind?
 - () Feel muscular tension?
 - () Feel afraid of not being able to sleep?
 - () Creepy, crawly, achy, or twitchy feeling in legs?
 - () Have any kind of pain or discomfort?
 - () Suddenly become awake or alert?
 - () Feeling sad or depressed?
 - () Have anxiety or worry about things?
 - () Feel unable to move?
 - () Have vivid, dream-like images or scenes?
 - () Feel afraid of the dark or anything else?

About their sleep

7. How many hours of sleep do they usually get each night?.....hours
8. Does their nightly amount of sleep vary? From.....to.....hours
9. How many times do they awaken each night?

21. When waking up, do they often?

- () Depend on an alarm to wake up?
- () Sleep in more than 1 hour past usual wake time?
- () Have vivid, dream like images when waking?
- () Wake up with a headache?
- () Wake up with a dry mouth?
- () Have a hard time waking up?
- () Feel unable to move (paralysed)?
- () Wake up disoriented or confused?
- () Wake up sick to their stomach?
- () Wake up 1-2 hours earlier than they want to?

About daytime activities & alertness

22. How many naps do they take in a typical week?

If they do take naps, How long are their naps?

23. Are the naps refreshing and do they restore alertness? Yes No

24. During the day, or their normal time awake, do they often:

- () Feel sleepy during the day, where they could easily sleep?
- () Actually fall asleep while driving or stopped at a light?
- () Feel weak or fall down if surprised, angry, or excited?
- () Worry about things (anxiety)?
- () Feel muscular tension or stress?
- () Fall asleep at work or at social events

4.2. MMSE (Mini-Mental State Examination)

Participant Number..... Date.....

ORIENTATION

What day of the week is it?	1/0
What is the date today?	
Day	1/0
Month	1/0
Year	1/0
What is the season?	1/0
Can you tell me where we are now? For instance what county are we in?	1/0
What are the names of two main streets/landmarks/nearby villages?	1/0
What is the name of this city?	1/0
What floor of the building are we on?	1/0
What is the name of this place/type of building/address and postcode?	1/0

LANGUAGE/NAMING/REPEATING

What is this called? (pencil)	1/0
What is this called? (watch)	1/0
I am going to say something and I would like you to repeat it after me. 'No ifs ands or buts'	1/0

REGISTRATION

Now I am going to name three objects. After I have finished saying all three, I want you to repeat them. Remember what they are because I am going to ask you to name them again in a few minutes.

APPLE	TABLE	PENNY	no. of repeats=	Total=	/3
or TREE	SPOON	BAG			

ATTENTION/CONCENTRATION/CALCULATION

Now I would like you to take seven away from 100. Now take seven away from the number you got and keep on subtracting until I ask you to stop.

93	86	79	72	65	Total=	/5
Please spell the word WORLD and now backwards.					or	
D	L	R	O	W	Total=	/5

RECALL

What were the three objects I asked you to repeat a little while ago?

APPLE	TABLE	PENNY	or TREE	SPOON	BAG	Total=	/3

READING COMPREHENSION

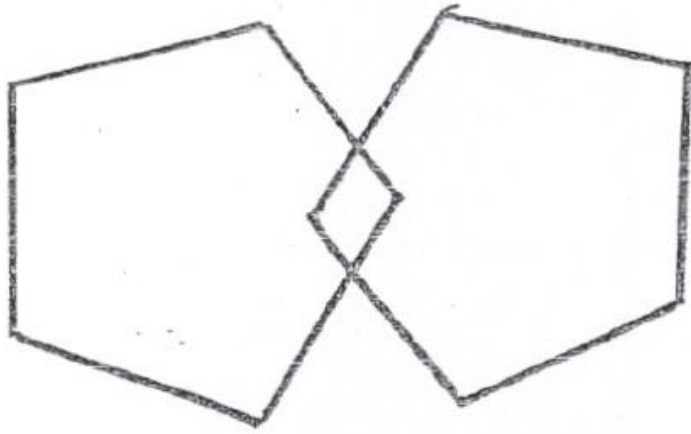
Read this page and then do what it says. Show 'CLOSE YOUR EYES'. 1/0

PRAXIS

I am going to give you a piece of paper. When I do, I want you to take the paper in your right hand (or dominant hand), fold it in half with both hands and put it in your lap.

RIGHT HAND	FOLDS	LAP	Total=	/3
Please write a complete sentence on this piece of paper.				1/0
Copy this design (pentagons).				1/0

TEST TOTAL	/30



CLOSE YOUR EYES

4.3. Morningness-Eveningness (Lark/Owl) Questionnaire

Participant number: _____ Date completed: _____

1.1.1.1. INSTRUCTIONS

- A. Please read each question very carefully before answering.
- B. Answer all questions.
- C. Answer questions in numerical order.
- D. Each question should be answered independently of others. Do **NOT** go back and check your answers.
- E. For some questions, you are required to respond by placing a cross alongside your answer. In such cases, select **ONE** answer only.
- F. Please answer each question as honestly as possible.
Both your answers and results will be kept in strict confidence.

Question 1

Considering your own feelings, at what time would you get up if you were entirely free to plan your day?

	or earlier	05:00 – 06:30	
		06:31 – 07:45	
Time:		07:46 – 09:45	
		09:46 – 11:00	
		11:01 – 12:00 or later	

Question 2

Considering only your own feelings, at what time would you go to bed if you were entirely free to plan your day?

	20:00 – 21:00	
	21:01 – 22:15	
	22:16 – 00:30	
Time:	00:31 – 01:45	
	01:46 – 03:00 or later	

Question 3

If there is a specific time for you at which you have to get up in the morning, to what extent are you dependent on being woken up by an alarm clock?

a.	Not at all dependent	[	]
b.	Slightly dependent	[	]
c.	Fairly dependent	[	]
d.	Very dependent	[	]

Question 4

Assuming adequate environmental conditions, how easy do you find getting up in the morning?

a.	Not at all easy	[	]
b.	Slightly easy	[	]
c.	Fairly easy	[	]
d.	Very easy	[	]

Question 5

How alert do you feel during the first half hour after having woken in the morning?

a.	Not at all alert	[	]

b.	Slightly alert	[	]
c.	Fairly alert	[	]
d.	Very alert	[	]

Question 6

How is your appetite during the first half hour after having woken in the morning?

a.	Not at all good	[	]
b.	Slightly good	[	]
c.	Fairly good	[	]
d.	Very good	[	]

Question 7

During the first half hour after having woken in the morning, how tired do you feel?

a.	Very tired	[	]
b.	Slightly tired	[	]
c.	Fairly refreshed	[	]
d.	Very refreshed	[	]

Question 8

When you have no commitments the next day, at what time do you go to bed compared to your usual bedtime?

a.	Seldom or never later	[	]
b.	Less than one hour later	[	]
c.	1-2 hours later	[	]
d.	More than 2 hours later	[	]

Question 9

You have decided to engage in some physical exercise. A friend suggests that you do this one hour twice a week and the best time for him is between 07:00 and 08:00h. Bearing in mind nothing else but your own inclinations, how do you think you would perform?

a.	Would be on good form	[	]
b.	Would be on reasonable form	[	]
c.	Would find it difficult	[	]
d.	Would find it very difficult	[	]

Question 10

At what time in the evening do you feel tired and as a result need to sleep?

Time:

Question 11

You wish to be at your peak for a test which you know is going to be mentally exhausting and lasting for two hours. You are entirely free to plan your day, and considering only your own "feeling best" - which ONE of the four testing times would you choose?

a.	08:00 – 10:00	[	]
b.	11:00 – 13:00	[	]
c.	15:00 – 17:00	[	]
d.	19:00 – 21:00	[	]

Question 12

If you went to bed at 23:00h at what level of tiredness would you be?

a.	Not at all tired	[	]
b.	A little tired	[	]
c.	Fairly tired	[	]
d.	Very tired	[	]

Question 13

For some reason you have gone to bed several hours later than usual, but there is no need to get up at any particular time the next morning. Which ONE of the following events are you most likely to experience?

a.	Will wake up at the usual time and will NOT fall asleep again	[	]
b.	Will wake up at the usual time and will doze thereafter	[	]
c.	Will wake up at the usual time but will fall asleep again	[	]
d.	Will NOT wake up until later than usual	[	]

Question 14

One night you have to remain awake between 04:00 and 06:00h in order to carry out a night watch. You have no commitments the next day. Which ONE of the following alternatives will suit you best?

a.	Will NOT go to bed until watch was over	[	]
b.	Would take a nap before the watch and sleep after	[	]
c.	Would take a good sleep before the watch and nap after	[	]
d.	Would take ALL sleep before the night watch	[	]

Question 15

You have to do two hours of hard physical work. You are entirely free to plan your day and considering only your own “feeling best” not your physical abilities, which ONE of the following times would you choose?

a.	08:00 – 10:00	[	]
b.	11:00 – 13:00	[	]
c.	15:00 – 17:00	[	]
d.	19:00 – 21:00	[	]

Question 16

You have decided to engage in some physical exercise. A friend suggests that you do this for one hour twice a week and the best time for him is between 22:00 and 23:00h. Bearing in mind nothing

else but your own “feeling best” not your physical abilities - how well do you think you would perform:

a.	Would be on good form	[	]
b.	Would be on reasonable form	[	]
c.	Would find it difficult	[	]
d.	Would find it very difficult	[	]

Question 17

Suppose that you can choose your own work hours. Assume that you had to work a FIVE-hour day (including breaks) and that your job was interesting and paid by results. Which FIVE CONSECUTIVE HOURS would you choose:

Note hours here:

Question 18

At what time of day do you think that you reach your “feeling best” peak?

Note Time here:

Question 19

One hears of “morning” and “evening” types. Which ONE of these types do you consider yourself to be?

a.	definitely a “morning type”	[	]
b.	Rather more a “morning” than an “evening” type	[	]
c.	Rather more an “evening” than a “morning” type	[	]
d.	definitely an “evening” type	[	]

SCORING for non-student population (> 21 years)

Definitely morning type	70-86
Moderately morning type	64-69
Neither type	53-63
Moderately evening type	31-52
Definitely evening type	16-30

[Taillard J, Philip p, Chastang JF, Bidelac B (2004), J Biol Rhythms, 19(1), 76-86.]

4.4. Pittsburgh Sleep Quality Index (adapted version)

Participant number: _____

Date completed: _____

For each of the questions, check the one best response. Please answer all questions.

1) During the past month, how often have they had trouble sleeping because they.....

a) Cannot get to sleep within 30 minutes

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a
			week _____

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

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a
			week _____

c) Have to get up to use the bathroom

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a
			week _____

d) Cannot breathe comfortably

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a
			week _____

e) Cough or snore loudly

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a
			week _____

f) Feel too cold

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a
			week _____

g) Feel too hot

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a
			week _____

h) Had bad dreams

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a
			week _____

i) Have pain

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a
			week _____

j) Other reason(s), please describe

How often during the past month have they 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 their sleep quality overall?

Very good _____
 Fairly good _____
 Fairly bad _____
 Very bad _____

7) During the past month, how often have they taken medicine (prescribed or “over the counter”) to help them 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 they 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 them 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 they 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 _____

11. How often in the past month have they 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 they're sleep

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a
			week _____

d) Episodes of disorientation or confusion during sleep?

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a
			week _____

e) Other restlessness while they sleep; please describe

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a
			week _____

4.5. Pittsburgh Sleep Quality Index (original version)

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.

- 2) During the past month, when have you usually gone to bed at night?
Usual bed time
- 3) During the past month, how long (in minutes) has it usually take you to fall asleep each night?
Number of minutes
- 4) During the past month, when have you usually got up in the morning?
Usual getting up time
- 5) 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, check the one best response. Please answer all questions.

6) During the past month, how often have they had trouble sleeping because they.....

a) Cannot get to sleep within 30 minutes

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a
			week _____

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

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a
			week _____

c) Have to get up to use the bathroom

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a
			week _____

d) Cannot breathe comfortably

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a
			week _____

e) Cough or snore loudly

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a
			week _____

f) Feel too cold

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a
			week _____

g) Feel too hot

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a

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 they 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 _____

7) During the past month, how would you rate their sleep quality overall?

Very good _____
Fairly good _____
Fairly bad _____
Very bad _____

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

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 often have they 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 _____

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

No problem at all _____
Only a very slight problem _____
Somewhat of a problem _____
A very big problem _____

11) Do they 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 _____

12. How often in the past month have they had...

a) Loud snoring

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a
			week _____

b) Long pauses between breaths while asleep

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a
			week _____

c) Legs twitching or jerking while they're sleep

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a
			week _____

d) Episodes of disorientation or confusion during sleep?

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a
			week _____

e) Other restlessness while they sleep; please describe

Not during the	Less than	Once or twice	Three or
past month _____	once a week _____	a week _____	more
			times a
			week _____

4.6. HAD Scale

Participant number..... Date completed.....

This questionnaire is designed to find out how you often feel. Read each item and place a firm tick in the box opposite the reply which comes closest to how you have been feeling in the past week. Don't take too long over your replies: your immediate reaction to each item will probably be more accurate than a long thought out response. *Tick only one box in each section.*

A. You feel tense or 'wound up':	Very often	() 2
Most of the time	Sometimes	() 1
A lot of the time	Not at all	() 0
Time to time, occasionally		
Not at all		
-
D. You still enjoy the things you used to enjoy:	A. You get a sort of frightened feeling like 'butterflies' in your stomach:	
Definitely as much	Not at all	() 0
Not quite as much	Occasionally	() 1
Only a little	Quite often	() 2
Hardly at all	Very often	() 3
-
A. You get a sort of frightened feeling as if something awful is about to happen:	D. You have lost interest in your appearance:	
Very definitely and quite badly	Definitely	() 3
Yes, but not too badly	You don't take so much care as you should	() 2
A little, but it doesn't worry them	You may not take quite as much care	() 1
Not at all	You take just as much care as ever	() 0
-
D. You can laugh and see the funny side of things:	A. You feel restless as if you have to be on the move:	
As much as you always could	Very much indeed	() 3
Not quite so much now	Quite a lot	() 2
Definitely not so much now	Not very much	() 1
Not at all	Not at all	() 0
-
A. Worrying thoughts go through your mind:	D. You look forward with enjoyment to things:	
A great deal of the time	As much as ever you did	() 0
A lot of the time	Rather less than you used to	() 1
From time to time but not too often	Definitely less than you used to	() 2
Only occasionally	Hardly at all	() 3
-
D. You feel cheerful:	A. You get sudden feelings of panic:	
Not at all	Very often indeed	() 3
Not often	Quite often	() 2
Sometimes	Not very often	() 1
Most of the time	Not at all	() 0
-
A. You can sit at ease and feel relaxed:	
Definitely	() 0
Usually	() 1
Not often	() 2
Not at all	() 3
-
D. You feel as if you are slowed down:	
Nearly all the time	() 3

- D. They can enjoy a good book or radio or TV programme:
- Often () 0
- Sometimes () 1
- Not often () 2
- Very seldom () 3

D=Depression (8-10).....
A=Anxiety (8-10).....

4.7. Restless Legs Syndrome (Patient Self-Report Version)

Participant Number:.....

Date completed:.....

Please answer all questions by ticking the relevant answer. Please only tick one answer per question.

1. Have you ever had 'growing pains'?

Occasionally (less than 1 time a month)	Sometimes (1-2 times a month)	Frequently (1-2 times a week to daily)	Only in the past Never

2. Do you have uncomfortable or funny feelings (creeping, crawling, tingling) in your legs?

Occasionally (less than 1 time a month)	Sometimes (1-2 times a month)	Frequently (1-2 times a week to daily)	Only in the past Never

3. Do you ever:

Yes No Don't Know

A. Notice funny feelings in your legs (or do they seem worse) when lying down or sitting?

B. Have partial relief with movement (wiggling feet, toes, or walking)?

C. Notice that the feeling is worse at night?

D. Have a lot of fidgeting or wiggling of your feet or toes when sitting or lying down?

E. Have repeated jerking movements in toes or legs or the whole body while sleeping?

4.8. The Social Functioning Scale

Participant number..... Date completed.....

Please tick one box for each question unless stated.

1. How many hours of the day do they spend alone? E.g. alone in a room, walking out alone, listening to radio or watching TV alone etc.

Hours spent alone per day

- 0-3 Very little time spent alone ()
 3-6 Some of the time ()
 6-9 Quite a lot of the time ()
 9-12 A great deal of time ()
 12 Practically all the time ()

2. How often will they start a conversation at home?

- Almost never ()
 Rarely ()
 Sometimes ()
 Often ()

3. How often do they leave the house (for any reason)?

- Almost never ()
 Rarely ()
 Sometimes ()
 Often ()

4. How do they react to the presence of strangers?

- Avoid them ()
 Feel nervous ()
 Accept them ()
 Like them ()

5. How many friends do they have at the moment? (people who they see regularly, do activities with, etc.).....

6. Do they have a partner?

Yes No

7. How often are they able to carry out a sensible or rational conversation?

- Almost never ()
 Rarely ()
 Sometimes ()
 Often ()

8. How easy or difficult do they find it talking to people at the moment?

- Very easy ()
 Quite easy ()
 Average ()
 Quite difficult ()
 Very difficult ()

Please place a tick against each item to show how often they have done the following over the past 3 months. Please only tick one column per activity.

	Never	Rarely	Sometimes	Often
Buying items from shops (without help)	()	()	()	()
Washing pots, tidying up etc.	()	()	()	()

Regular washing, bathing etc.	()	()	()	()
Washing own clothes	()	()	()	()
Looking for a job	()	()	()	()
Doing the food shopping	()	()	()	()
Prepare and cook a meal	()	()	()	()
Leaving the house alone	()	()	()	()
Using buses, trains etc.	()	()	()	()
Using money	()	()	()	()
Budgeting	()	()	()	()
Choosing and buying clothes for self	()	()	()	()
Take care of personal appearance	()	()	()	()

Please place a tick in the appropriate column to indicate how often they have done any of the following activities over the past 3 months. Please only tick one column per activity.

	Never	Rarely	Sometimes	Often
Playing musical instruments	()	()	()	()
Sewing, knitting	()	()	()	()
Gardening	()	()	()	()
Reading things	()	()	()	()
Watching television	()	()	()	()
Listening to records or radio	()	()	()	()
Cooking	()	()	()	()
D.I.Y. activities	()	()	()	()
Fixing things (car, bike, household etc.)	()	()	()	()
Walking, rambling	()	()	()	()
Driving/cycling (for recreation)	()	()	()	()
Swimming	()	()	()	()
Hobby (e.g. collecting things)	()	()	()	()
Shopping	()	()	()	()
Artistic activity (painting, crafts etc.)	()	()	()	()

Please place a tick in the appropriate column to show how often they have participated in any of the following activities over the past 3 months. Please only tick one column per activity.

	Never	Rarely	Sometimes	Often
Cinema	()	()	()	()
Theatre/concert	()	()	()	()
Watching an indoor sport (squash, table-tennis)	()	()	()	()
Watching an outdoor sport (football, rugby)	()	()	()	()
Art gallery/museum	()	()	()	()
Exhibition	()	()	()	()
Visiting places of interest	()	()	()	()
Meetings, talks etc.	()	()	()	()

	Never	Rarely	Sometimes	Often
Evening classes	()	()	()	()
Visiting relatives in their homes	()	()	()	()

Being visited by relatives	()	()	()	()
Visiting friends (including partner)	()	()	()	()
Parties	()	()	()	()
Formal occasions	()	()	()	()
Disco etc.	()	()	()	()
Night club/social club	()	()	()	()
Playing an indoor sport	()	()	()	()
Playing an outdoor sport	()	()	()	()
Club/society	()	()	()	()
Pub	()	()	()	()
Eating out	()	()	()	()
Church activity	()	()	()	()

Please place a tick against each item to show how able they are at doing or using the following.
Please only tick one column per row.

	Adequately	Need help	Unable	Not known
Public transport	()	()	()	()
Handling money	()	()	()	()
Budgeting	()	()	()	()
Cooking for self	()	()	()	()
Weekly shopping	()	()	()	()
Looking for a job	()	()	()	()
Washing own clothes	()	()	()	()
Personal Hygiene	()	()	()	()
Washing, tidying etc.	()	()	()	()
Purchasing from shops	()	()	()	()
Leaving the house alone	()	()	()	()
Choosing and buying clothes	()	()	()	()
Caring for personal appearance	()	()	()	()

4.9. ADCS-Activities of Daily Living (ADL) Inventory

VALIDATION OF A CORE COGNITION BATTERY OF TESTS

ADCS-ADL-1-7

Protocol	Study Site	Visit	Subject's ID
OPTIMA 2	4008.005		

ADCS – ACTIVITIES OF DAILY LIVING		51242
Was evaluation performed? No ☐ If yes, complete form below.		
Evaluation performed on visit date ☐ or specify date: _____ DO NOT WRITE		
Before beginning, provide and review questionnaire guidelines with the informant/caregiver.		
EVALUATION	RESULT	
1. Regarding eating , which best describes patient's usual performance during the past 4 weeks:		
ate without physical help, and used a knife	☐ 3	
used a fork or spoon, but not a knife, to eat	☐ 2	
used fingers to eat	☐ 1	
patient usually or always was fed by someone else	☐ 0	
2. Regarding walking (or getting around in a wheelchair), in the past 4 weeks, which best describes his/her optimal performance:		
mobile outside of home without physical help	☐ 3	
mobile across a room without physical help	☐ 2	
transferred from bed to chair without help	☐ 1	
required physical help to walk or transfer	☐ 0	
3. Regarding bowel and bladder function at the toilet , which best describes his/her usual performance in the past 4 weeks:		
did everything necessary without supervision or help	☐ 3	
needed supervision, but no physical help	☐ 2	
needed physical help, and was usually continent	☐ 1	
needed physical help, and was usually incontinent	☐ 0	
4. Regarding bathing , in the past 4 weeks, which best describes his/her usual performance:		
bathed without reminding or physical help	☐ 3	
no physical help, but needed supervision/reminders to bathe completely	☐ 2	
needed minor physical help (e.g., with washing hair) to bathe completely	☐ 1	
needed to be bathed completely	☐ 0	

Investigator's Name:	Staff's initials:	Date:
(St. June 2002) (revised) Restricted Confidential - Merck & Co., Inc., Whitehouse Station, New Jersey, USA		
Printed in USA		

VALIDATION OF A CORE COGNITION BATTERY OF TESTS

ADCS-ADL-2-7

Protocol	Study Site	Visit	Patient / Subject's ID
OPTIMA 2	4008.005		

ADCS – ACTIVITIES OF DAILY LIVING (Continued)		51242
EVALUATION	RESULT	
5. Regarding grooming , in the past 4 weeks, which best describes his/her optimal performance:		
cleaned and cut fingernails without physical help	☐ 3	
brushed or combed hair without physical help	☐ 2	
kept face and hands clean without physical help	☐ 1	
needed help for grooming of hair, face, hands, and fingernails	☐ 0	
6. Regarding dressing , in the past 4 weeks, did patient select his/her first set of clothes for the day?	YES NO DONT KNOW ☐ ☐ ☐ (If yes, ask A and B; else ask B only)	
A) Which best describes his/her usual performance ?		
without supervision or help	☐ 3	
with supervision	☐ 2	
with physical help	☐ 1	
B) Regarding physically getting dressed , which best describes his/her usual performance in the past 4 weeks:		
dressed completely without supervision or physical help	☐ 4	
dressed completely with supervision, but without help	☐ 3	
needed physical help only for buttons, clasps, or shoelaces	☐ 2	
dressed without help if clothes needed no fastening or buttoning	☐ 1	
always needed help, regardless of the type of clothing	☐ 0	
7. In the past 4 weeks, did patient use a telephone ?	YES NO DONT KNOW ☐ ☐ ☐ (If yes, ask A)	
A) Which best describes his/her highest level of performance :		
made calls after looking up numbers in white or yellow pages, or by dialing directory assistance	☐ 5	
made calls only to well-known numbers, without referring to a directory or list	☐ 4	
made calls only to well-known numbers, by using a directory or list	☐ 3	
answered the phone; did not make calls	☐ 2	
did not answer the phone, but spoke when put on the line	☐ 1	

Investigator's Name:	Staff's initials:	Date:
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Printed in USA		

VALIDATION OF A CORE COGNITION BATTERY OF TESTS
ADCS-ADL3-7

Protocol	Study Site	Visit	Patient / Subject's ID
OPTIMA 2	4008.005		

ADCS - ACTIVITIES OF DAILY LIVING (Continued)			51242
EVALUATION		RESULT	
8. In the past 4 weeks, did patient watch television ?			
Did patient:		YES ☐ NO ☐ DONT KNOW ☐	(If yes, ask A, B and C)
A) usually select or ask for different programs or his/her favorite show?		☐ 1	☐ 0 ☐ 0
B) usually talk about the content of a program while watching it?		☐ 1	☐ 0 ☐ 0
C) talk about the content of a program within a day (24 hours) after watching it ?		☐ 1	☐ 0 ☐ 0
9. In the past 4 weeks, did patient ever appear to pay attention to conversation or small talk for at least 5 minutes? <i>Note: Patient did not need to initiate the conversation.</i>			
A) Which best describes his/her usual degree of participation :		YES ☐ NO ☐ DONT KNOW ☐	(If yes, ask A)
usually said things that were related to the topic			☐ 3
usually said things that were not related to the topic			☐ 2
rarely or never spoke			☐ 1
10. In the past 4 weeks, did patient clear the dishes from the table after a meal or snack?			
A) Which best describes how he/she usually performed :		YES ☐ NO ☐ DONT KNOW ☐	(If yes, ask A)
without supervision or help			☐ 3
with supervision			☐ 2
with physical help			☐ 1
11. In the past 4 weeks, did patient usually manage to find his/her personal belongings at home?			
A) Which best describes how he/she usually performed :		YES ☐ NO ☐ DONT KNOW ☐	(If yes, ask A)
without supervision or help			☐ 3
with supervision			☐ 2
with physical help			☐ 1

Investigator's Name:	Staff's initials:	Date:
Restricted Confidential - Merck & Co., Inc., Whitehouse Station, New Jersey, USA		

VALIDATION OF A CORE COGNITION BATTERY OF TESTS
ADCS-ADL4-7

Protocol	Study Site	Visit	Patient / Subject's ID
OPTIMA 2	4008.005		

ADCS - ACTIVITIES OF DAILY LIVING (Continued)			51242
EVALUATION		RESULT	
12. In the past 4 weeks, did patient obtain a hot or cold beverage for him/herself? (A cold beverage includes a glass of water.)			
A) Which best describes how he/she usually performed :		YES ☐ NO ☐ DONT KNOW ☐	(If yes, ask A)
made a hot beverage, usually without physical help			☐ 3
made a hot beverage, usually if someone else heated the water			☐ 2
obtained a cold beverage, usually without physical help			☐ 1
13. In the past 4 weeks, did patient make him/herself a meal or snack at home ?			
A) Which best describes his/her highest level of food preparation:		YES ☐ NO ☐ DONT KNOW ☐	(If yes, ask A)
cooked or microwaved food, with little or no help			☐ 4
cooked or microwaved food, with extensive help			☐ 3
mixed or combined food items for a meal or snack, without cooking or microwaving (e.g., made a sandwich)			☐ 2
obtained food on his/her own, without mixing or cooking it			☐ 1
14. In the past 4 weeks, did patient dispose of garbage or litter in an appropriate place or container at home?			
A) Which best describes how he/she usually performed :		YES ☐ NO ☐ DONT KNOW ☐	(If yes, ask A)
without supervision or help			☐ 3
with supervision			☐ 2
with physical help			☐ 1
15. In the past 4 weeks, did patient get around (or travel) outside of his/her home :			
A) Which best describes his/her optimal performance :		YES ☐ NO ☐ DONT KNOW ☐	(If yes, ask A)
alone, went at least 1 mile away from home			☐ 4
alone, but remained within 1 mile of home			☐ 3
only when accompanied and supervised, regardless of the trip			☐ 2
only with physical help, regardless of the trip			☐ 1

Investigator's Name:	Staff's initials:	Date:
Restricted Confidential - Merck & Co., Inc., Whitehouse Station, New Jersey, USA		

Protocol	Study Site	Visit	Patient / Subject's ID
OPTIMA 2	4008.005		

ADCS - ACTIVITIES OF DAILY LIVING (Continued)		51242
EVALUATION		RESULT
16. In the past 4 weeks, did patient ever go shopping?		YES NO DON'T KNOW ☐ ☐ ☐ (If yes, ask A and B)
A) Which one best describes how patient usually selects items: without supervision or physical help with some supervision or physical help not at all, or selected mainly random or inappropriate items		☐ 3 ☐ 2 ☐ 1
AND		YES NO DON'T KNOW ☐ 1 ☐ 0 ☐ 0
B) Did patient usually pay for items without supervision or physical help?		YES NO DON'T KNOW ☐ ☐ ☐ (If yes, ask A)
17. In the past 4 weeks, did patient keep appointments or meetings with other people, such as relatives, a doctor, the hairdresser, etc.?		YES NO DON'T KNOW ☐ ☐ ☐ (If yes, ask A)
A) Which best describes his/her awareness of the meeting ahead of time: usually remembered, may have needed written reminders e.g. notes, a diary, or calendar only remembered the appointment after verbal reminders on the day usually did not remember, in spite of verbal reminders on the day		☐ 3 ☐ 2 ☐ 1
18. In the past 4 weeks, was patient ever left on his/her own?		YES NO DON'T KNOW ☐ ☐ ☐ (If yes, ask A, B and C)
Was patient left: A) away from home, for 15 minutes or longer, during the day? B) at home, for an hour or longer, during the day? C) at home, for less than 1 hour, during the day?		YES NO DON'T KNOW ☐ 1 ☐ 0 ☐ 0 ☐ 1 ☐ 0 ☐ 0 ☐ 1 ☐ 0 ☐ 0
19. In the past 4 weeks, did patient talk about current events? (This means events or incidents that occurred during the past month.)		YES NO DON'T KNOW ☐ ☐ ☐ (If yes, ask A, B and C)
Did patient talk about events that ...: A) he/she heard or read about or saw on TV but did not take part in? B) patient took part in outside home involving family, friends, or neighbors? C) occurred at home that he/she took part in or watched?		YES NO DON'T KNOW ☐ 1 ☐ 0 ☐ 0 ☐ 1 ☐ 0 ☐ 0 ☐ 1 ☐ 0 ☐ 0

Investigator's Name:	Staff's initials:	Date:

Protocol	Study Site	Visit	Patient / Subject's ID
OPTIMA 2	4008.005		

ADCS - ACTIVITIES OF DAILY LIVING (Continued)		51242
EVALUATION		RESULT
20. In the past 4 weeks, did patient read a magazine, newspaper or book for more than 5 minutes at a time?		YES NO DON'T KNOW ☐ ☐ ☐ (If yes, ask A and B)
Did patient usually: A) talk about details of what he/she read while or shortly (<than 1 hour) after reading? B) talk about what he/she read 1 hour or longer after reading?		YES NO DON'T KNOW ☐ 1 ☐ 0 ☐ 0 ☐ 1 ☐ 0 ☐ 0
21. In the past 4 weeks, did patient ever write things down? Note: If patient wrote things only after encouragement or with help, the response should still be 'yes.'		YES NO DON'T KNOW ☐ ☐ ☐ (If yes, ask A)
A) Which best describes the most complicated things that he/she wrote: letters or long notes that other people understood short notes or messages that other people understood his/her signature or name		☐ 3 ☐ 2 ☐ 1
22. In the past 4 weeks, did patient perform a pastime, hobby or game?		YES NO DON'T KNOW ☐ ☐ ☐ (If yes, ask A, B and C)
A) Which pastimes did he/she perform? Ask about all of the following, check all that apply: Card or board game (including bridge, chess, checkers) ☐ Bingo ☐ Crosswords ☐ Art ☐ Musical instrument ☐ Knitting ☐ Sewing ☐ Reading ☐ Gardening ☐ Golf ☐ Tennis ☐ Workshop ☐ Fishing ☐ Other ☐ Specify: _____ Note: Walking does NOT count as a hobby/pastime for this scale. If patient performs hobbies/pastimes only at day care, check here ☐		
B) How did patient usually perform his/her most common pastimes: without supervision or help with supervision with help		☐ 3 ☐ 2 ☐ 1

Investigator's Name:	Staff's initials:	Date:

VALIDATION OF A CORE COGNITION BATTERY OF TESTS

ADCS-ADL 7-7

Protocol OPTIMA 2	Study Site 4008.005	Visit	Patient / Subject's ID

ADCS - ACTIVITIES OF DAILY LIVING (Continued)		51242
EVALUATION		RESULT
23. In the past 4 weeks, did patient use a household appliance to do chores?		YES ☐ NO ☐ DON'T KNOW ☐ (If yes, ask A and B)
A) Ask about all of the following, check those that were used: Washer ☐ Dryer ☐ Vacuum ☐ Dishwasher ☐ Toaster ☐ Toaster oven ☐ Range ☐ Microwave ☐ Food processor ☐ Other ☐ Specify: _____		
B) For the most commonly used appliances, which best describes how patient usually used them: without help, operating more than on-off controls if needed ☐ 4 without help, but operated only on/off controls ☐ 3 with supervision, but no physical help ☐ 2 with physical help ☐ 1		

Investigator's Name:	Staff's initials:	Date:

4.10. Health Questionnaire

Participant number:.....

Date completed:.....

General Part

Please complete all questions about the participant

1. Date of birth of participant.....
2. Age of participant.....
3. Weight of participant.....
4. Height of participant.....
5. Sex of participant: Male/Female
6. Collar size: Male: Less than 17 inches ()
 17 inches or greater ()
 Female: Less than 16 inches ()
 16 inches or greater ()
7. Current status: Please circle which describes them best:
Single (never married) Married Partnership
In a relationship Divorced Widowed
Separated

Children: Yes/No

8. Current living situation: Please circle which best describes their living situation:
Alone With Partner With Friends
With Family With Parents
9. What type of accommodation do they live in:
House Nursing Home Supported Housing
Flat Shared Accommodation Hospital

10. What is the highest level of education they have ever completed: Please circle one.
Secondary school Polytechnic
Apprenticeship Bachelor degree (BSc/BA)
Grammar school Masters degree
Doctoral degree

Other, please describe:.....

Medical Part

11. Have they ever been told that they have high blood pressure?
Yes No

If Yes, please give details

.....

12. Have they had any history of heart trouble?
Yes No

If Yes, please give details

.....

13. Have they a family history of heart disease/stroke?

Yes No

If Yes, please give details

.....

14. Have they ever been told by a doctor that they have asthma?

Yes No

15. Do they suffer from a wheezy chest?

Yes No

16. Do they ever have pains in their heart and chest?

Yes No

17. Do they ever feel faint or have spells of dizziness?

Yes No

18. Has a doctor ever told them that they have a bone or joint problem which could be made worse by exercise?

Yes No

19. Have they been in hospital at all in the last two years?

Yes No

If yes, please give details

.....

20. Have they had any operations or major illness in the last 6 months?

Yes No

If yes, please give details

.....

21. Are they undergoing treatment or having any regular medication at the moment?

Yes No

If Yes, please give details

.....

22. Are they taking any pills or any other medicines, including inhalers, for any of the following:

Heart trouble/angina? Yes/No

Chest pains or blood pressure? Yes/No

Asthma or other chest diseases? Yes/No

Or for anything else? Yes/No

If Yes, please give full details:.....

23. Do they have any physical disabilities of any kind?

Yes No

If Yes, please give details

.....

24. Have they ever suffered any complications from previous trials or treatments?

Yes No

If Yes, please give details

.....

25. Do they suffer from any allergies?

Yes No

If Yes, please give details

.....

26. Have they you ever had an adverse reaction to a medicine or food?

Yes No

If Yes, please give details

.....

27. What is their average weekly intake of alcohol?

.....units per week

(One unit is half a pint of beer/one glass of wine/one measure of spirits)

28. Has there been a time when they regularly consumed more than 14 units of alcohol per week?

Yes No

If Yes, please give details

.....

29. How many cigarettes, cigars and/or tobacco do they smoke:

None 1-5 a day 5-10 a day 10-20 a day more than 20 per day
Less than 10 a month

Other, please specify:

.....

30. Have they, or a member of their family, ever been told by a doctor that they had epilepsy problems?

Yes No

If Yes, please give details

.....

31. Have they recently been checked for glaucoma by an optician?

Yes No

If Yes, have they been diagnosed with glaucoma?

Yes No

32. Have they ever been diagnosed with a mental problem such as depression?

Yes No

33. Have they done any shift work in the last year?

Yes No

34. Have they travelled across more than one time zone in the past 3 months?

Yes No

35. How often, on average, do they consume caffeine containing beverages or food? (coffee, tea, cola beverages, chocolate etc.)

Never	()
Rarely (less than 10 times a month)	()
Occasionally (less than 1 time per day)	()
Sometimes (1-3 times per day)	()
Frequently (3-7 times per day)	()
Often (more than 7 times per day)	()

36. Does the person in your care have any visual problems?

Yes No

If Yes, please give details

.....

4.11. CANTABeclipse Test Descriptions

Screening Test

Big/Little Circle (BLC)

A series of pairs of circles, one large and one small, are presented to the participant. The participant is instructed first to point to the smaller of the two circles, on the touch screen, and then, after 20 trials to point to the larger circle. This is a visual choice test to train a subject to: follow an explicit instructional rule; and reverse a rule. This will take approximately 3 minutes.

Visuo-Spatial Memory Test

Paired Associates Learning (PAL)

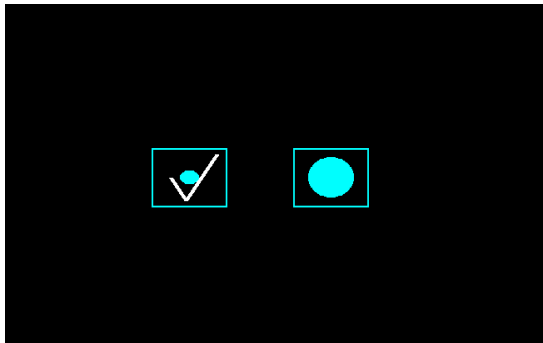
Six boxes are displayed on the screen. All are opened in a randomised order. One or more of them will contain a pattern. The participant is required to remember patterns associated with different locations on the screen, and during the test phase, as each pattern is presented, point to the appropriate location on the touch screen. The test starts at a very simple level and gradual increases in difficulty.

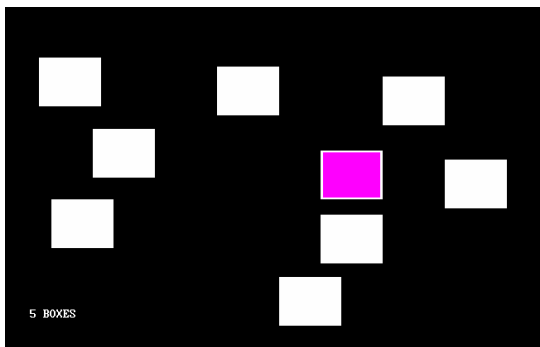
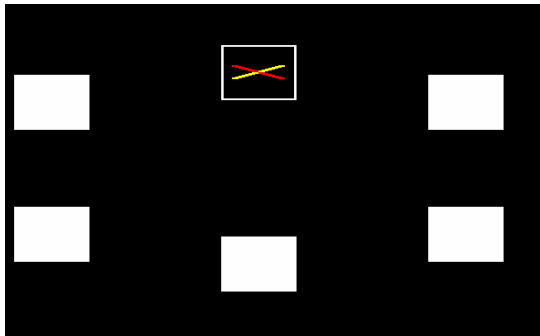
This test is a form of delayed response procedure, which tests two different aspects of the ability to form visuo-spatial associations: the number of patterns placed correctly on the first presentation of each trial gives an index of 'list memory'; and the number gives an index of 'list memory'; and the number of repeat, reminder presentations needed for the subject to learn all the associations provides a measure of 'list learning'. This test should take approximately 10 minutes.

Executive Function, Working Memory and Planning Test

Spatial Span (SSP)

A set of white squares is shown on the screen. Some of the squares change in colour, one by one, in a variable sequence. At the end of each sequence a tone indicates that the participant should touch each of the boxes coloured by the computer in the same order as they were originally presented. The number of squares ranges from 2 to 9 squares.





This test gives a measure of the participant's spatial memory span. This test should take approximately 5 minutes.

Attention Test

Choice Reaction Time (CRT)

This 2-choice reaction time test has stimulus and response uncertainty, with two possible stimuli (left and right) and two possible outcomes (left and right pad buttons). The participant must press the left hand button on the press pad if the stimulus is displayed on the left hand side of the screen, and the right hand button on the press pad if the stimulus is displayed on the right hand side of the screen. This test should take approximately 7 minutes.

Verbal Memory Test

Graded Naming Test (GNT)

The test consists of thirty black and white line drawings, ordered with increasing difficulty. Reduced efficiency in retrieving the name of an object can be the first and only indication of impaired language functioning. This test assesses object-naming ability, but is in addition graded in difficulty to allow for individual differences. This means that it may be able to detect any word-finding difficulty even those with an extensive naming vocabulary. This test should take approximately 10 minutes.