The Objective Measurement of Sleep-Wake Disturbance in Parkinson's Disease

A thesis in fulfilment of the requirements for the degree of Doctor of Philosophy

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Statement of Authentication

This thesis is submitted to the University of Sydney in fulfilment of the requirement is for the Degree Doctor of Philosophy.

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

Signature: Date:

Published Papers Associated with this Thesis

Bolitho SJ, Naismith SL, Rajaratnam SMW, Grunstein RR, Hodges JR, Terpening Z, Rogers NL, Lewis SJG. Disturbances in melatonin secretion and circadian sleep-wake regulation in Parkinson's disease. Sleep Medicine. 2014; 15(3):342-7

- This paper is presented as Chapter 2 of this thesis

Bolitho SJ, Naismith SL, Salahuddin P, Terpening Z, Grunstein RR, Lewis SJG. Objective measurement of daytime napping, cognitive dysfunction and subjective sleepiness in Parkinson's disease. PLoS ONE. 2013; 8(11):e81233

- This paper is presented as Chapter 3 of this thesis

Bolitho SJ, Lewis SJG. Improving the electrophysiological measurement of REM without atonia in the diagnosis of REM sleep behaviour disorder. Advances in Clinical Neuroscience and Rehabilitation 2014; 13(7)

- This paper is presented as Chapter 4 of this thesis

Bolitho SJ, Naismith SL, Terpening Z, Grunstein RR, Melehan K, Yee B, Coeytaux A, Gilat M, Lewis SJG. Investigating REM without atonia in Parkinson's disease using the REM sleep behaviour disorder screening questionnaire. Movement Disorders 2014; 29(6):736-42

- This paper is presented as Chapter 5 of this thesis
- Editorial of Chapter 5 is included as Appendix A of this thesis

    Postuma RB, Diagnosing REM Sleep Behaviour Disorder in Parkinson's Disease Can We Avoid the Polysomnography. Movement Disorders 2014; 29(6):713-714

Bolitho SJ, Naismith SL, Terpening Z, Grunstein RR, Melehan K, Yee B, Coeytaux A, Lewis SJG. Investigating the night to night variability of REM without atonia in Parkinson's disease. Sleep Medicine 2014; 16(1):190-3

- This paper is presented as Chapter 6 of this thesis

Bolitho SJ, Grunstein RR, Naismith SL, Mehelam K, Lewis SJG. Unified objective techniques are needed to standardise the diagnosis of REM sleep behaviour disorder. Movement Disorders 2014; 29(14):1836

- This paper is presented as Appendix B of this thesis

Other peer reviewed publications

A substantial contribution was also made to the following papers as part of complementary research to this thesis. Techniques utilised and outcomes of these empiric studies assisted in the study design and interpretation of data from this thesis. Each manuscript has been included in Appendix C

Gunn DG, Naismith SL, **Bolitho SJ**, Lewis SJG. Actigraphically-defined sleep disturbance in Parkinson's disease is associated with differential aspects of cognitive functioning. Journal of Clinical Neuroscience 2014; 21(7):1112-1115

Naismith SL, Hickie IB, Terpening Z, Rajaratnam SMW, Hodges J, **Bolitho S**, Rogers NL, Lewis SJG. Circadian misalignment and sleep disruption in Mild Cognitive Impairment. Journal of Alzheimers Disease 2014; 38(4): 857-866

Naismith SL, Hermens DF, Ip TKC, **Bolitho SJ**, Scott E, Rogers NL, Hickie IB, Circadian profiles in young people during the early stages of affective disorder. Translational Psychiatry, 2012; 2: e123.

Terpening Z, Naismith SL, Melehan K, Gittins C, **Bolitho SJ**, Lewis SJG. The contribution of nocturnal sleep to the consolidation of motor skill learning in healthy ageing and Parkinson's disease. Journal of Sleep Research. 2013; 22(4): 398-405.

Shine JM, Moore ST, **Bolitho SJ**, Morris TR, Dilda V, Naismith SL, Lewis SJG. Assessing the utility of Freezing of Gait Questionnaires in Parkinson's Disease. Parkinsonism and Related Disorders. 2011; 18: 25-9.

Co-Author Declaration

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Abstract

Parkinson's disease (PD) is an increasingly prevalent neurodegenerative disease affecting older adults. Motor symptoms, including tremor, rigidity and tremor were classically predominant. However, troublesome non-motor symptomatology are known to impair quality of life for patients with PD and there carers.

Sleep-wake disturbances are gaining increased attention in PD encompassing disturbances of the circadian, homeostatic and ultradian sleep systems. Such symptoms are observed in over two thirds of patients manifesting with a range of features including insomnia, rapid eye movement (REM) sleep behaviour disorder (RBD), and excessive daytime somnolence. As well as impacting on quality of life for patients and their carers, these symptoms have been linked to the troublesome problems of cognitive deficits, mood disturbance and visual hallucinations.

Mechanisms exploring the interaction of sleep-wake disturbance, other non-motor symptoms and cardinal motor symptoms including tremor, rigidity and tremor and non-motor symptoms in PD are not well understood but dopaminergic and non-dopaminergic pathology across the brainstem, basal forebrain, hypothalamus and frontostriatal pathways are likely to be implicated. Bidirectional causality between sleep wake disturbance and concomitant symptoms in PD provide insights into common chemical and neural mechanisms which prior to the development of therapy, must be understood. Furthermore, sleep-wake disorders in PD at present provide a maker of early diagnosis for which future disease modifying treatment can be targeted. However objective and reliable measurement techniques are yet to be devised in this field.

This thesis aims to utilise the objective measurement of sleep-wake disturbances across the circadian, homeostatic and ultradian sleep systems in PD through four empiric experiments to help inform our understanding of these critical symptoms in PD. While the usefulness of self-report data is not doubted as a means of engaging the patient and hearing their voice they cannot serve the same identification and measurement uses of objective data. Thus these objective techniques could be used to assess the validity of the questionnaires which have been devised to measure the same entities and to add a further dimension of patient input into the treatment. Improved objective, accurate and reliable measurement techniques will help reduce any potential bias in data.

Chapter 1 - Introduction

1.1 - Sleep-Wake Disorders and Parkinson's Disease

Parkinson's Disease (PD) is a multisystem neurodegenerative disorder that increases in prevalence with ageing. Although the cardinal features of PD were proposed as the motor problems of tremor, rigidity and bradykinesia, non-motor symptoms such as cognitive decline, mood disturbance and autonomic dysfunction frequently contribute to reduced quality of life for patients and their carers (1). To date much of the research on PD has focused on the movement oriented symptoms and their progression. Less emphasis has been directed towards the non-motor symptoms that have profound impacts on the holistic well-being of the patient.

Sleep-Wake disturbances represent some of the most important non-motor symptoms in PD and are coexistent with each other as well as other physical and important neuropsychiatric features including cognitive decline and mood disturbance. However, the mechanisms underlying the interaction of sleep-wake disturbances, other non-motor and motor symptoms in PD are not well understood.

1.1.1 - The Aims of this Thesis

This thesis aims to highlight the effects of sleep-wake disturbance in PD patients using novel objective measurement approaches. It reports on sleep-wake disturbances across the circadian, homeostatic and ultradian sleep systems as well as their interaction with other disease specific variables. It then aims to interrogate and compare self-report measures with novel objective measures to improve our understanding of sleep-wake disturbances and their effects on PD patients.

1.1.2 - Epidemiological and Economic Analysis of Parkinson's disease

The importance of research into troublesome sleep-wake symptoms such as those manifesting with circadian sleep phase abnormalities, excessive daytime sleepiness (EDS) and REM sleep behaviour disorder (RBD) are emphasised by an epidemiological and economic analysis of PD. It is has been estimated that around 10 million PD patients are found worldwide and that greater than 65,000 Australians are currently diagnosed with PD, which equates to approximately 1 in every 350 people in Australia. Published cohorts report greater than 1% of the population over the age of sixty are diagnosed with PD, with a prevalence of approximately 0.3% of the total population (2, 3).

Of concern to patients, carers and the community is the increasing prevalence of PD noted over the last ten years, which is at least in part attributable to the aging population. Age is reported to be the most important risk factor for developing PD, with the incidence peaking in the eighth decade. The median age at diagnosis is reported to be sixty and the average disease duration at death is fifteen years (4). In Australia, more than fourteen thousand more new diagnoses of PD were noted in 2014 compared to 2005 (5).

The prevalence of PD in Australia is higher than many conditions considered National Health Priority areas including breast cancer, lymphoma and leukaemia. Furthermore, this prevalence is projected to increase by 79% over the next thirty years(5). The burden of disease is considered high, with an annual cost for PD in Australia being greater than $10 billion (5). At its intermediate stages, PD is considered to be more burdensome than primary progressive multiple sclerosis and

in the advanced stages is on par with the terminal stages of cancer or severe dementia. Advances in our intervention strategies to improve quality of life for patients and their carers are therefore imperative. However, accurate, reliable and objective measurement techniques for the specific disease parameters in PD are limited. Precise quantitative measurement is likely to underpin future progress in PD research.

1.1.3 - Clinicopathological Progression in Parkinson's disease

Pathologically, the hallmark of PD is the Lewy body, which are aggregates of misfolded alpha-synuclein protein found within dying neurons. Other proteolytic stress proteins, including ubiquitin and phosphorylated neurofilaments, have also been identified in the pathology of PD (6). It has been proposed that there is a caudo-rostral spread of this Lewy Body pathology in PD and this would be in keeping with the predominance of sleep-wake disturbance in preclinical and early PD (7). For example, the ascending reticular activating system, which is responsible for wakefulness lies in the medulla and pons. Similarly, the regulation of the stages of sleep is also believed to rely on pontine nuclei, further illustrating why sleep-wake disturbances are often an early, pre-motor feature of PD.

The continuing deposition of Lewy bodies ascends through the brainstem and in the advanced stages is disseminated throughout neocortical structures. The Braak pathological staging model describes the progression of PD in six stages beginning in the lower brainstem, which then ascends over time. Initially it was proposed that as the deposition of Lewy bodies ascends, so too does the burden of Lewy body pathology in already affected structures (8). However, exceptions to this model exist.

For example the pathology within the dorsal motor nucleus of the vagus nerve, which is affected early in the pathological process does not correlate with cortical Lewy body burden (9).

Recently, a prion-like hypothesis for disease spread has been proposed, suggesting that neurons with Lewy bodies within them may affect other neighbouring neurons (10). Pathology outside of the central nervous system also exists including recent evidence confirming phosphorylated alpha-synuclein deposits in dermal nerve fibres in patients with PD (11). Alpha synuclein containing inclusions have also been found in colonic mucosa, submandibular salivary glands in addition to solid tissue samples of the enteric and autonomic nervous system, adding further weight to the possibility of a transfective process in the pathology of PD (12).

Stages 1 and 2 of the Braak model are considered pre-clinical as involvement of the substantia nigra and the corresponding motor deficits are yet to occur. Within stages 1 and 2, structures including the dorsal motor nucleus of the vagus nerve and the anterior olfactory structures are proposed to be first affected coinciding with the pre-clinical symptoms of anosmia and autonomic dysfunction. Importantly, sleep-wake disturbances in PD can occur in this pre-clinical phase, which is presumably related to local pathological processes.

Lewy body deposition and consequent dysfunction of structures critical to sleep-wake regulation occurs in the lower brainstem in stage 1 and 2 coinciding with emerging evidence supporting excessive daytime sleepiness and REM sleep behaviour disorder as preclinical problems in PD (13) . Specifically, structures in the

reticular activating system such as the dorsal raphe nucleus, locus coeruleus and pedunculopontine nucleus are affected prior to the emergence of motor symptoms. In addition to regulating sleep-wake systems, these structures are also critical for the regulation of mood. Thus the coincidence of mood dysfunction and sleep-wake dysregulation at diagnosis, raises questions of bidirectional causality.

Cardinal features of PD, including tremor, rigidity and bradykinesia occur at stage 3 corresponding to dopaminergic cell loss within the substantia nigra. Stage 4 includes limited cortical involvement confined to the temporal mesocortex and allocortex. Advanced stages 5 and 6, result in further cognitive decline as there is a pathological spread to include the neocortex, often with a formal diagnosis of dementia and the development of visual hallucinations.

1.1.4 - Sleep-wake Disturbances and their association with Parkinson's Disease

Sleep-wake disturbances are a frequently observed non-motor problem, gaining increased attention in Parkinson's disease (PD) (14, 15). Sleep-wake disturbance in PD encompasses disturbance of the all systems of sleep architecture including disorders of circadian, homeostatic and ultradian sleep systems.

The circadian sleep system contributes to the initiation of sleep as well as entraining sleep to the twenty four hour day/night cycle. This is controlled by the retino-hypothalamic pathway and pineal gland, which is responsible for the secretion of the chronobiotic hormone, melatonin (16). The homeostatic sleep system is proposed to control energy restoration through the gradual increase in the need to sleep (sleep

pressure) with the consequent increase in activity dependent metabolites that gradually down regulate the activating reticular system (17). Finally the ultradian sleep system controls the switching between the stages of sleep - Non Rapid Eye Movement (NREM) and REM sleep (18).

Such symptoms are observed in over two thirds of patients (19) manifesting with a range of sleep-wake symptoms (20, 21) including circadian phase disturbances and REM sleep behavior disorder. Insomnia secondary to Parkinson's disease, defined as a complaint of sleep onset, sleep maintenance, waking too early or lack of restorative sleep is reported in more than 50% of patients with PD (22, 23). In addition to disorders of nocturnal sleep, excessive daytime sleepiness (EDS) is observed (24), commonly manifesting as daytime napping (25). Empiric experiments are essential to help inform our understanding of these critical symptoms in PD.

As stated above, non-motor symptoms confer reduced quality of life for patients and their carers (14, 26). The potential link between sleep-wake disorders and mood dysfunction, although frequently identified in Parkinson's disease is not well understood. Mood disorders and sleep-wake disorders, such as insomnia are intricately related in studies of cohorts free of neurodegenerative disease (27, 28). Whilst studies based on self-report measures have confirmed patients with PD and depression exhibit more sleep disturbance, the responsible causal mechanisms have not been established (29). Sleep-wake disturbance in PD has been linked to cognitive decline including reduced processing speed, working memory and verbal fluency (30-32) . Recent studies have demonstrated that working memory in PD can be modified by sleep only after restoration of the normal sleep architecture (33).

Mechanisms exploring the interaction between sleep-wake disturbances and other non-motor and motor symptoms in PD are not well understood. Dopaminergic and non-dopaminergic pathology across the brainstem, basal forebrain, hypothalamus and frontostriatal pathways are likely to be implicated (13, 18, 22, 24).

Obstructive sleep apnea is another potential cause of EDS in PD (34), although evidence suggesting an increased incidence of obstructive sleep apnea in PD compared to aged match controls is not conclusive (35-37). Restless legs syndrome, in which patients report an unpleasant urge to move their limbs during periods often at night, are observed in PD cohorts (38). The incidence of restless leg syndrome in PD cohorts is approximately 12% and results in difficulty initiating sleep with consequent altered sleep architecture (39). Periodic limb movements of sleep, involving repetitive and stereotyped limb movements in sleep, are frequently observed in restless leg syndrome but can occur independently. Periodic limb movements of sleep (PLMs) have also been implicated in nocturnal sleep disturbance both with and without restless legs syndrome in 30% of patients with PD (40). The mechanisms underlying RLS and PLMs within PD cohorts are not well understood, however disruption of dopaminergic circuits have been implicated. This proposal is further supported through there symptomatic benefit derived from dopaminergic therapy (41).

1.1.5 - Medication Effects on Sleep-wake Disturbance in Parkinson's Disease
1.1.5.1 - Dopaminergic Medication
In addition to the chemical and cellular changes caused by the progression of Lewy body pathology in PD, the medications used most frequently to ameliorate symptoms

can also alter sleep-wake regulation through the manipulation of neurotransmitter systems. The dopamine agonist class of PD medication has most frequently been linked to increasing daytime somnolence in PD (42). Such an effect could be being facilitated through the differential activation of the multiple dopamine receptors in the basal ganglia (43).

Two groups of dopamine receptors have been identified including D1 like receptors (D1 and D5) and D2 like receptors (D2, D3 and D4) (see Butini et all for review (44)). D1 like receptors are principally expressed in the striatum, substantia nigra amygdala and multiple cortical regions. D1 like receptors are implicated in locomotion, reward and memory circuits (44). D2 like receptors are found in the multiple regions including the substantia nigra, hypothalamus, amygdala, hippocampus and retina with diverse functions. D1 receptors are important to the circadian sleep systems through their expression in the retina and suprachiasmatic nucleus. However, these receptors are also important for homeostatic and ultradian sleep through their presence in the striatum and other parts of the reticular activating system. D1 receptor agonists have been shown to exert wake promoting effects. However, D2 related activation results in differential effects with agonists and antagonists exhibiting opposite effects dependent on their individual dose. Similar results have been reported with manipulation of the D3 receptors. Low dose dopamine agonists are thought to preferentially activate D2 receptors promoting sleep. Conversely higher concentrations activate D1 receptors resulting in wakefulness and increased locomotor activity. Finally, sudden onset episodes of REM sleep have been linked to the use of dopamine agonists (34).

1.1.5.2 - Melatonin

In addition to the important role that melatonin plays in affecting the light/dark regulation of circadian rhythms, the sleep promoting effect of melatonin has been subject to debate (45). Initial studies using exogenous melatonin failed to show a sleep promoting effect, although this now appears to be due to the short half-life of the melatonin preparation used along with inadequate dosing (46). Subsequent studies have provided compelling evidence that melatonin does have a sleep promoting effect via direct neuronal suppression in patients with insomnia and delayed sleep phase syndrome (46-49). Furthermore, a recent consensus statement from the British Association for Psychopharmacology has proposed melatonin as first line therapy for insomnia in older adults (50). However there is insufficient evidence to support melatonin as an effective treatment for insomnia in patients with PD and further studies are required to explore this hypothesis (51).

Melatonin has also been reported to improve REM without atonia, the electrophysiological hallmark of RBD, in addition to improved symptomatic improvements (52-54). However these studies had minimal subjects with Parkinson's disease and did not control for potential confounds such as concomitant use of antidepressant medication. Prospective randomised controlled studies are needed to determine the efficacy in cohorts of patients with PD (55) .

1.1.5.3 – Antidepressants, Anxiolytics and Sedatives

Selective serotonin reuptake inhibitors (SSRI), nonspecific serotonin and noradrenaline reuptake inhibitors (SNRI) and tricyclic antidepressants that are

commonly used in PD to improve mood, also alter the ratio of cholinergic to aminergic tone, which is critical to switching between NREM and REM sleep (18, 56, 57). These medications all suppress REM sleep. Therefore, using these medications to suppress REM is a therapeutic modality in addition to wake promoting medications, in the treatment of narcolepsy. The consequence of suppressing REM is not fully understood. Medications such as SSRIs and SNRIs are also implicated in causing secondary REM sleep behaviour disorder, although the mechanism for this causation is not known (58).

Clonazepam a benzodiazepine used to treat RBD and insomnia, is reported to disrupt sleep architecture. Specifically these medications reduce the quantity of slow wave sleep, critical to mood and cognitive function (59). Other sedative medications such as GABA A receptor agonists (e.g. eszopiclone) are reported to improve insomnia in patients with PD (60). Of note, zolpidem a similar frequently used sedative medication has not been studied for insomnia in PD cohorts but has been reported to improve motor symptoms (61). Cognitive behavioural therapy has also been reported to improve insomnia in patients with PD (62).

1.1.5.4 - Wake Promoting Medication

Psychomotor stimulants and related agents such as modafinil and sodium oxybate are proposed to enhance the activity of dopamine, facilitating increased activity and promoting wakefulness. However studies in cohorts of patients with PD where excessive daytime sleepiness was identified on self-report measures, did not report improved sleepiness (63, 64). Combining the data from five randomised controlled trials of modafinil used in EDS cohorts of PD, Trotti et al 2014 reported a reduced

score on the ESS (65). This study suggests modafinil may improve subjective but not objective measures of EDS in PD (65). These counterintuitive results may be due to inherent deficiencies in self-report measures in patients with known cognitive and mood related co-morbidities. Therefore, studies using objectively identified EDS are required.

1.2 - Neurobiology of Sleep-wake regulation and Parkinson's Disease

1.2.1 - Circadian Sleep Systems

The circadian system regulates a range of inter-related physiological systems including feeding, thermoregulation and critically the sleep-wake cycle. The main driver of the circadian cycle is the level of daylight and this operates through the retino-hypothalamic pathway where the suprachiasmatic nucleus (SCN) ultimately controls the secretion of melatonin from the pineal gland (17). Whilst circadian disturbance is well recognised in Alzheimer's disease (66), specific contributions from structures such as the SCN or pineal gland have not been established in PD. In addition, it has been recognised that the anterior hypothalamus sends monosynaptic outputs to the lateral hypothalamus, overlapping with the wake promoting orexin neurons (67). As the synchrony between sleep and the circadian system is dependent on the dorsolateral hypothalamic nuclei (67), it has been proposed that increased orexin, through abnormal signaling from the SCN, is a potential mechanism for de-regulation of this interaction (68).

As light levels fall in the day, melatonin secretion is increased and acts as a major sleep promoter through its actions in the wake promoting and sleep promoting nuclei of the brainstem. Serial plasma melatonin measurement and evaluation of the

corresponding dim light melatonin onset (DLMO) (figure 1) have been used

previously to assess circadian phase (68).
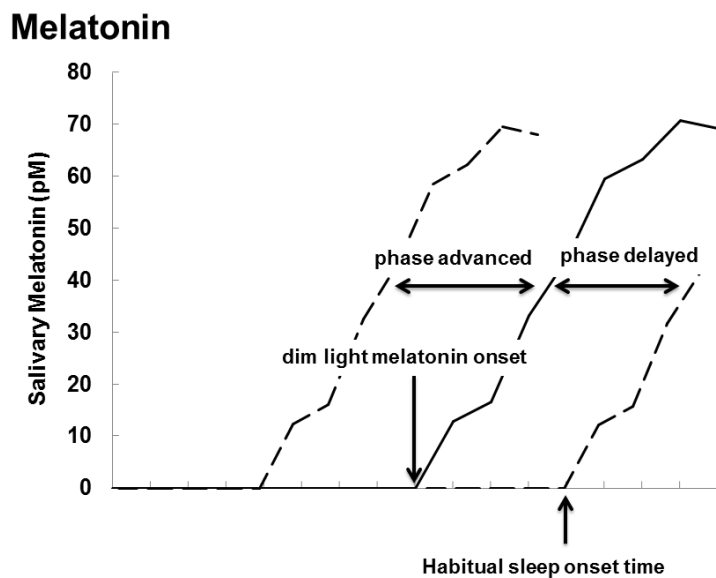
Melatonin

Figure 1 – A graph depicting the increase in melatonin secretion, the dim light

melatonin onset prior to sleep onset and possible abnormal circadian phase advance

or delay.

Studies in non-PD cohorts have successfully utilized melatonin measurements

derived from serial salivary sampling (69, 70). The DLMO and estimates of the area

under the melatonin curve can be calculated from these readings. Furthermore, by

subtracting the DLMO from the habitual sleep onset (HSO) the entrainment phase

angle can be calculated (see figure 2) and this has been used as a marker of internal

circadian dysynchrony (68). To date, these measures have not been applied to a PD

cohort.
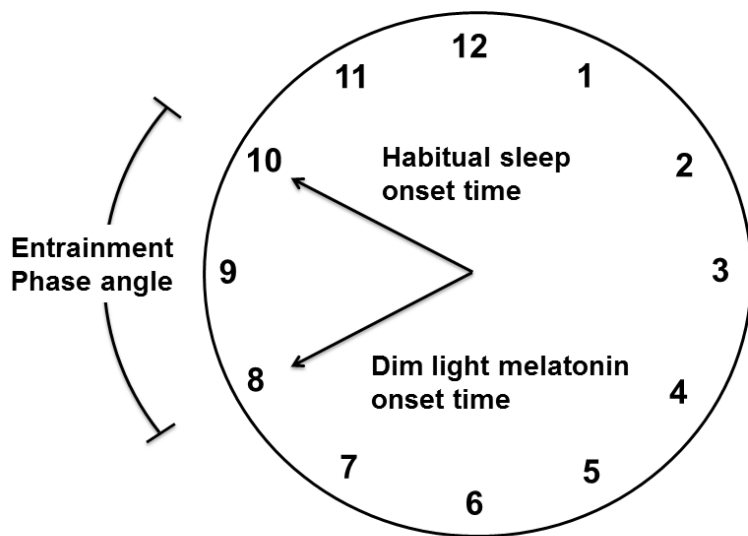
Figure 2 – A diagram depicting the entrainment phase angle representing the time after activation of the circadian sleep system in which habitual sleep onset takes place.

1.2.1.1 - Circadian Disturbance and Parkinson's Disease

Abnormal circadian rhythmicity has been associated with reduced nocturnal sleep quality and daytime somnolence (71, 72) and aging alone, has been identified in the mechanism of these problems (73). However, despite the frequently observed sleep-wake disturbance in PD, the precise abnormalities of circadian rhythms are not well understood (42). Previous studies investigating circadian disturbance in PD have reported a circadian phase advance in those treated with dopaminergic medication but not in untreated PD or aged matched healthy controls (74, 75). In addition, Bordet et al 2003 also showed an altered melatonin secretion pattern in PD patients with motor complications on L dopa treatment (76). Reduced melatonin secretion without circadian phase shift was also reported in a study of patients with early PD

(77). More recently, Videnovic et al 2014 reported attenuated circadian rhythm of melatonin secretion in PD compared to controls without circadian phase difference (78). All of these studies employed invasive 24 hour plasma sampling.

1.2.2 - Homeostatic Sleep Systems

The evolving pressure to sleep that grows throughout the waking day correlates with an increase in the activity dependent metabolites such as adenosine, gamma amino butyric acid (GABA), prostaglandin D2 (PGD2), interleukin–1A (Il-1A) and tumour necrosis factor (TNFα). The accumulation of these activity dependent metabolites precedes the transition from wake to NREM sleep (18) . In mammals the synthesis and accumulation of brain metabolites has been shown to be directly proportional to the intensity and duration wakefulness. The accumulation of these metabolites subsequently inhibits these wake promoting structures (18). Thus the inability to clear activity dependent metabolites, through the restorative function of sleep would result in excessive daytime sleepiness.

The transition from wake to NREM sleep involves activity dependent metabolites gradually reversing the tonic inhibition from the thalamic reticular nucleus on the thalamocortical neurons, resulting in gradual hyperpolarisation (17). Hyperpolarisation of thalamocortical neurons allows the activation of low threshold ionic calcium (Ca^{2+}) spikes, initiated in the reticular nucleus and mirrored in the thalamocortical neurons (79). When the hyperpolarisation reaches a certain threshold, spindles are replaced with delta waves representing the interplay between hyperpolarisation cation current and transient low threshold ionic calcium (Ca^{2+}) current resulting in delta waves (79). Rhythmic and synchronised spike bursts from

thalamic neurons are associated with Ca^{2+} entry. This may activate Ca^{2+} calmodulin dependent protein kinase implicated in synaptic plasticity required for memory, learning and dream mentation (80). It is interesting to note that alpha Ca2+-calmodulin dependent kinase II plays a causal role in cognitive and motor deficits in animal models of PD and thus could represent a common pathology between EDS and cognitive deficits in PD (81) (82) (83).

1.2.2.1 - Excessive daytime sleepiness and the Homeostatic Sleep System in Parkinson's Disease

Excessive daytime sleepiness (EDS) is observed in over half of all PD patients, where it commonly manifests as daytime napping (21, 84). The development of daytime somnolence in PD has been associated with increasing age, disease duration, disease progression, postural instability, depression and the use of dopamine agonists (85-87). However, much like the emergence of idiopathic RBD in later life (88), EDS can also represent a pre-motor feature heralding the development of PD (13, 24, 25). The presence of daytime somnolence has been linked to executive dysfunction in PD implying a link between EDS and cognition in this condition (13, 89, 90). Furthermore, impairments in frontostriatal neural circuitry have been implicated in the reduced arousal, attentional modulation and working memory seen in PD (90-92).

Previous studies in non-PD samples have shown that older individuals with EDS are more likely to nap during the day (93, 94). The presence of daytime napping has also been proposed as a robust manifestation of EDS in PD (25) and has been used in this cohort as a measure of EDS (24, 95). More extensive work has been conducted

on napping in healthy older adults where it appears to be associated with increased morbidity (96) and mortality (97). In these older cohorts increased napping has been associated with decreased global cognitive performance (93, 98-100) and in particular deficits in executive function (99). Of note, studies that have utilized 'prescribed' napping to restore the effects of sleep deprivation in healthy cohorts, have demonstrated improved executive performance on tasks such as reaction time and symbol digit substitution (101-103). These combined observations highlight the possibility that the increased frequency of napping seen in older adults and patients with PD might represent a compensatory neurobiological strategy to a primary neuropathological insult.

The dopaminergic SNc/VTA and peri-aqueductal grey matter have been established as having a wake promoting effect (17). It follows that EDS in PD has been linked to dopaminergic cell loss in these regions (24). However EDS is proposed as a possible pre-motor problem, which implies that the chemical and neural correlates of EDS will also involve non-dopaminergic neurons in the lower brainstem (13). Cell death in structures such as the basalis nucleus (BN), pedunculopontine nucleus (PPN), locus coeruleus (LC), dorsal raphe (DR), substantia nigra/ventral tegmental area (SNc/VTA), tuberomamillary nucleus (TMN), mesencephalic reticular formation (MRF) could all facilitate EDS (17). In addition, depletion of the neurotransmitters activating these nuclei, such as acetylcholine (ACh), noradrenaline (NA), serotonin (5-HT), dopamine (DA), Histamine (Hist) and glutamate (Glut) may also play a role (18).

Disruption of energy restoration through excessive accumulation and failure to clear activity dependent metabolites with consequent failure of homeostatic circuitry is probable in multiple secondary mechanisms of sleep wake disturbance in PD. Abnormal sleep architecture is also seen as a consequence of sleep disordered breathing, restless legs syndrome and the periodic limb movements of sleep. Nocturnal disturbance is also frequently noted secondary to physical symptoms of PD including pain, stiffness, nocturia and 'akinesia where there is a failure to roll over effectively (17, 104).

1.2.3 - REM Sleep Circuitry and the Ultradian Sleep System

After the initiation of sleep, including the gradual transition to slow wave sleep via the lighter stages of NREM sleep (stage 1 and stage 2 sleep), there is a switch to REM sleep (for review see Swick et al 2005 (105)). NREM and REM alternate in four to five cycles in a typical nocturnal sleep cycle, with longer periods of REM sleep in the second half of nocturnal sleep (106). The cycling of NREM and REM sleep is controlled by the ultradian sleep system with complex neural and chemical control systems involving in the reticular activating system, basal forebrain and hypothalamus (16, 17, 107). Understanding REM sleep circuitry is important, given that REM sleep behaviour disorder is proposed as the most reliable marker of the future emergence of Parkinson's disease (108).

REM sleep is characterised polysomnographically by a desynchronised cortical EEG, rapid eye movements and muscle atonia (17). Each of these REM characteristics are controlled by brain stem structures, switched on and off depending on the relative availability of cholinergic and aminergic neurotransmitters

(16-18). It is believed that the transition to REM sleep is facilitated by the reversal of gamma amino butyric acid (GABA) inhibition on the pedunculopontine nucleus, which predominates in NREM sleep. Concurrently GABA inhibits cells in the LC and DR (18). Changes in the ratio of cholinergic to aminergic tone facilitates the switching of REM including the REM sign of muscle atonia (17) . Thus the switching from NREM to REM is controlled via cholinergic PPN neurons, with signal transmission from glutamate and GABA, via kainite, NMDA and GABA-B receptors (18).

Animal models have demonstrated RWA through the placement of lesions in the α locus coeruleus suggesting this structure is critical to the normal muscle atonia seen in REM sleep. Pharmacological models have also shown reductions in the length or complete absence of REM in subjects taking medications known to increase cholinergic (109, 110) or aminergic tone, such as selective serotonin re-uptake inhibitors or non-specific noradrenaline and serotonin re-uptake inhibitors (111). Thus it appears that increasing acetylcholine, serotonin or noradrenaline and thereby altering the ratio of cholinergic to aminergic tone, can inhibit the switching to REM sleep.

1.2.3.1 - REM Sleep Behaviour Disorder and Parkinson's disease

Studies have emerged investigating the link between Parkinson's disease and the presence REM sleep behaviour disorder (RBD) (112-115). RBD is noted to occur in 40-60% of patients where it manifests as dream enactment in the REM phase of sleep when the skeletal muscles should ordinarily be electrically silent (19). In addition to conferring comorbidity to both patients and their bed partners, RBD has

also been linked to multiple troublesome symptoms including autonomic dysfunction, decreased olfaction and colour discrimination (116). As well, visual hallucinations are increasingly being linked to RBD in PD (117, 118). These symptoms often coexist with cognitive deficits (20, 30, 90) and dementia (116), which in turn represent independent predictors for nursing home admission, conferring increased burden on the community (26). As such, accurate screening and diagnosis is essential for managing the comorbidity associated with RBD in PD.

RBD has been recognised as the most significant premotor feature of PD occurring up to 15 years prior to the diagnosis. The likelihood ratio of RBD as a prodromal marker of PD was recently identified as 130, higher than all other markers (119), Given that the emergence of idiopathic RBD in later life can herald the development of PD (115), this observation might offer some clues toward the underlying neuropathology. It has been reported that the vast majority of patients with idiopathic RBD will transition to an alpha synucleinopathy such as PD (along with Lewy Body Dementia or Multiple System Atrophy) with the symptoms commencing many years prior to the clinical diagnosis of PD (115). It follows that the location of pathology in RBD will involve brain stem structures consistent with the pre-clinical stages of the Braak pathological staging system for PD (for review see (120)). These structures will likely be caudal to the substantia nigra (SNc)/ventral lateral tegmentum (VTA) [10] and evidence from animal models suggests that pathology in the alpha locus coeruleus region of the pontine tegmentum, results in REM atonia [11]. However, the precise circuitry responsible for RBD in humans has not been identified (121).

The hallmark of RBD diagnosis is the demonstration of REM without atonia (RWA) (see figure 3) in the surface EMG leads from nocturnal polysomnography (ICSD-2) (23). Identifying RWA, has become critical to the diagnosis of RBD (122). Furthermore, quantifying the severity of RWA is of particular interest as it appears to predict the transition from idiopathic RBD to PD (123).
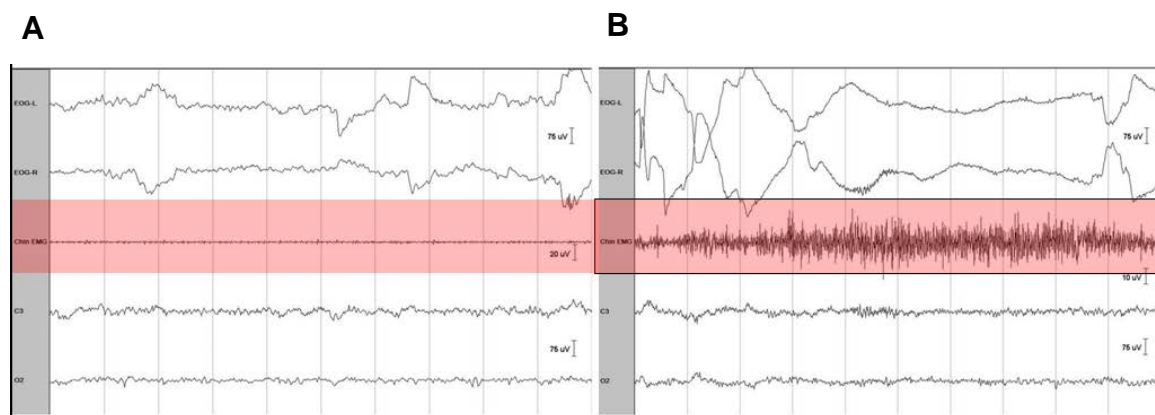
Figure 3 – An excerpt from a nocturnal polysomnogram showing EEG, EOG and surface EMG activity. Panel A reports REM with atonia. Panel B reports REM without atonia.

1.3 - Advancing the Understanding of Sleep-Wake Disturbance in Parkinson's disease using Self-report Measures

1.3.1 Sleep-Diaries and Circadian Phase in Parkinson's Disease

Self-report measures have frequently been used in the assessment of sleep-wake disturbance in PD (124, 125). For example, sleep diaries have commonly been used to assess sleep duration, nocturnal arousals and circadian phase. However, inaccuracy of these measures in normal subjects and other cohorts free from a neurodegenerative condition has been reported (126-128). There is minimal data

using sleep diaries to assess circadian phase in PD cohorts. Inherent in these measures are issues of reliability and validity, which are compounded in patients with neurodegeneration.

1.3.2 Self-Report Measures of Excessive Daytime Sleepiness in Parkinson's Disease

Self-report measures designed specifically to evaluate PD cohorts for sleep-wake disturbances have been developed and include the Scale for Outcomes in Parkinson's disease - Sleep (SCOPA-S) and the Parkinson's disease Sleep scale (PDSS) (124, 125). The PDSS or the SCOPA-S were both reported to identify sleep-wake disturbance in PD (124, 125). Studies using the SCOPA-S have been compared to other versions of the SCOPA that exist for cognition, psychiatric complications and autonomic problems (129). Kurtis et al 2013 reports depression, fatigue, cognitive impairment and autonomic symptoms such as urinary and thermoregulatory problems may contribute to sleep-wake disturbance in PD (129). Similarly the PDSS has been reported to identify reduced quality of life and mood disturbance (130). It is concerning that neither the SCOPA-S, or PDSS were validated in conjunction with an objective measure of sleep. Similarly the Epworth Sleepiness Scale (ESS) (131) has also been used in PD cohorts to identify cognitive deficits (13, 89, 90). Of note both the SCOPA-S and PDSS used the ESS as part of their validation (124, 125) despite the fact that this instrument had not actually been validated itself in PD.

1.3.3 REM Sleep Behaviour Disorder and Self-Report Measures in Parkinson's Disease

Multiple self-report measures have been developed for the identification of REM sleep behaviour disorders including the REM Sleep Behaviour Disorder Screening Questionnaire (RBDSQ) (132), the Single Screening question (RBD1Q) (133) and parts of the Mayo Sleep Questionnaire (MSQ) (134).

Studies using the RBDSQ have identified a link between visual hallucinations and RBD in PD (117, 118). The RBDSQ has also been used to identify cognitive decline such as reduced processing speed and verbal fluency (20, 30, 90). The RBDSQ also identified increased wake bouts during nocturnal sleep (31).

1.3.4 Limitations with Self-Report Measures in Parkinson's Disease

Symptoms of mood disturbance and cognitive deficit often coincide with sleep-wake problems and may bias self-report measures in cohorts of PD. Impaired recall has been shown to impair the use of self-report measures in non PD cohorts (135). Such problems have already been identified in other non-motor symptoms that often occur with sleep-wake disturbance in patients with PD, including freezing of gait and visual hallucinations (136, 137).

1.3.5 Novel Objective Measurement in Sleep-Wake Disturbance in Parkinson's Disease

1.3.5.1 Serial Salivary Melatonin in the Measurement of Circadian Phase in Parkinson's Disease

1.2.1.1 refers to circadian sleep systems that can be assessed through melatonin curves. Through these curves, the activation of the circadian system can be calculated from the dim light melatonin onset time. The quantity of melatonin secreted can be estimated from the area under the curve. Finally, synchrony between the sleep and the circadian system can be assessed through the entrainment phase angle (see figure 2) (68).

Circadian rhythm disturbances have previously been investigated in patients with PD utilising invasive serial measurement of plasma melatonin. Inconsistent results were identified along with methodological problems such as not controlling for the acute inhibitory effect of light exposure on melatonin synthesis (74-76, 138). Serial salivary melatonin measurement as shown in Figure 4, provide an alternative, non-invasive technique, that has been successfully demonstrated in non-PD cohorts (69, 139).

The unique application of this method to a PD cohort, collected in an environment controlled for light levels, temperature, posture and eating would provide a novel objective assessment of circadian phase.

For these in-laboratory circadian phase assessment, participants need to be maintained in a seated posture for at least 30 minutes before samples are collected. Sampling occurs at 30 minute intervals from 6 hours before habitual sleep onset (HSO), until 2 hours after HSO (68, 69). During this type of assessment, participants have to be physiologically and behaviorally monitored under controlled conditions with fixed light levels (less than 30 lux) and a controlled ambient temperature (24±1°C). Participants maintained a seated posture for at least 20 minutes before each sample collection. Patients are also asked to abstain from substances believed to affect melatonin and/or sleep (e.g. caffeine, turkey, bananas, tomatoes). The subsequent measurement of salivary Melatonin is then achieved by double antibody radioimmunoassay (Cat no. RK-DSM2; Buhlmann Laboratories AG, Schonenbuch, Switzerland).
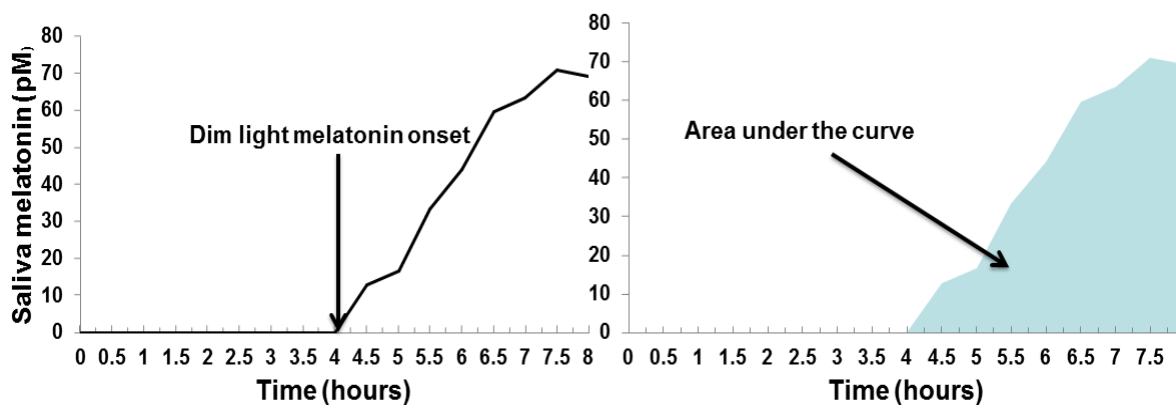
Figure 4 – A graph depicting the serial salivary melatonin measurement. The activation of the circadian phase is indicated by the dim light melatonin onset. The area under the curve indicates the quantity of melatonin secreted.

1.3.5.2 The Utility of Actigraphy to measure Excessive Daytime Sleepiness in Parkinson's Disease

As highlighted above (Section 1.2.2.1), previous studies in PD have reported a link between self-reported daytime sleepiness and deficits in cognition (13, 89, 90). However, these relationships have yet to be investigated using an objective measure. Studies in non-PD cohorts have successfully utilised actigraphy as a non-invasive measure of daytime napping (140). To date daytime actigraphy has not been applied in PD. This measurement tool is commonly worn as a watch like device on the wrist less affected by tremor (see figure 5). An actigraph combines movement data from a three axis accelerometer with light levels from a spectrometer to determine rest intervals based on software and manual scoring. Multiple variables are derived from the actigram including total nap time, the number of nap bouts per day, total nocturnal sleep time (TST), wake after sleep onset (WASO) and sleep efficiency ((TST – WASO)/TST) sleep. Actigraphy previously been reported as a robust estimate of polysomnography defined sleep (141). The use of actigraphy to explore sleep disturbance in PD was first demonstrated by Naismith et al 2011 to explore RBD (31). Subsequently, multiple studies have further validated the use of actigraphy to assess nocturnal sleep disturbance in PD (20, 142, 143).

The implementation of this technology in a PD cohort, would be first to use actigraphy, a validated objective, non-invasive and inexpensive measure of daytime sleep in PD. By comparing excessive napping to other non-motor, motor and disease specific problems in PD, the correlates of excessive napping would be determined.
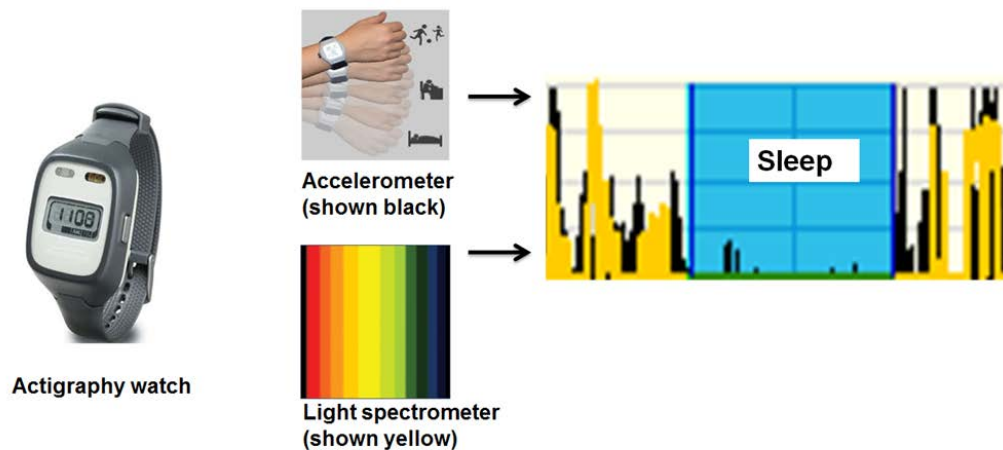
Figure 5 – An actigraphy watch, with inbuilt three axis accelerometer, light spectrometer and actigram. Multiple variables are derived from the actigram including total nap time, the number of nap bouts per day, total nocturnal sleep time (TST), wake after sleep onset (WASO) and sleep efficiency ((TST – WASO)/TST) sleep.

1.3.5.3 The REM Atonia Index to measure REM Without Atonia in Parkinson's Disease

The demonstration of REM without atonia (RWA) in muscles that should be electrically silent during REM sleep is critical to identifying RBD (144). Given the potential for RBD to herald the development of PD, multiple techniques have been developed to improve the measurement of RWA and subsequent electrophysiological diagnosis of RBD (for review see (145)). The importance of measuring RWA as a continuous variable has been demonstrated in a recent study that reported that the severity of RWA predicted the development of PD in cases of idiopathic RBD (123).

The REM atonia index (see figure 6) was developed as an automated signal processing algorithm to assess the surface EMG from nocturnal polysomnography for RWA (146-149). The REM atonia index averages the EMG signal in each 1 second epoch of REM sleep and grades the epoch as normal or abnormal based on a voltage threshold (< 1 µV = normal, 1-2 µV = indeterminate, > 2 µV = abnormal). The REM atonia index is the ratio of normal to abnormal epochs of REM. A REM atonia index cut-off score is used to indicate significant RWA and could be compared and contrasted with self-report measures used in RBD as an objective measure of RBD (146)

Panel - A
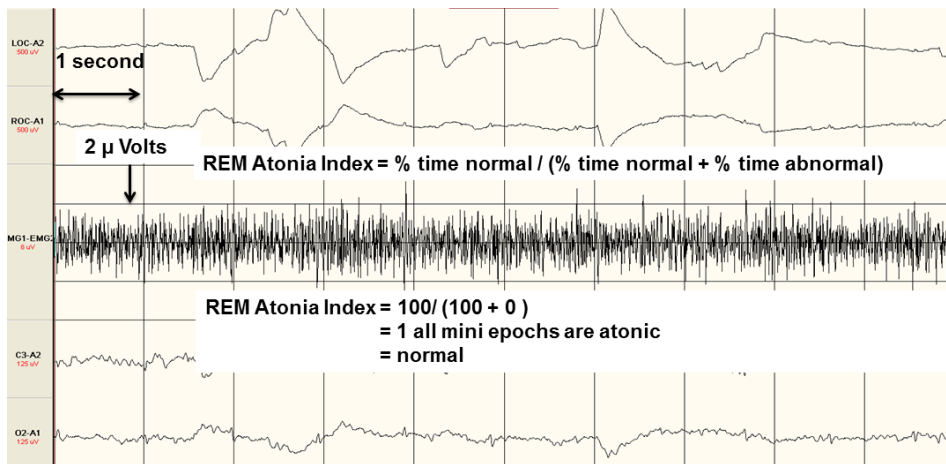
Panel - B
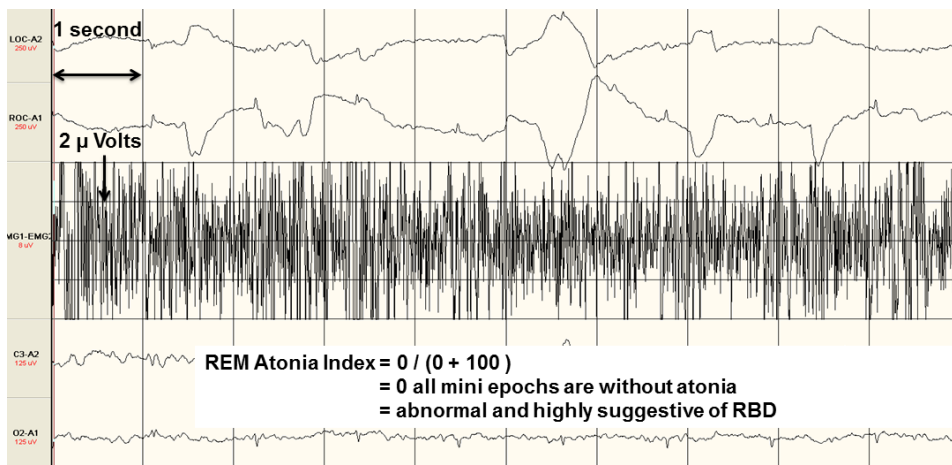
Figure 6 – An excerpt from a nocturnal polysomnogram. The top two channels in each panel show the eye movements (EOG), the middle channel of each panel shows the surface EMG from the mentalis muscle and the bottom two channels from each panel show an excerpt from the cortical EEG.

1.4 - Concluding remarks

This thesis aims to utilise the objective measurement of sleep-wake disturbances across the circadian, homeostatic and ultradian sleep systems in PD through four empiric experiments to help inform our understanding of these critical symptoms in PD. While the utility of self-report data is not doubted as a means of engaging the patient and 'hearing their voice', there is a clear need for objective data that can quantify measurements. Thus, these objective techniques could be used to assess the validity of the questionnaires, which have been devised to measure the same entities and to add a further dimension of patient input into the treatment. Improved objective accurate and reliable measurement techniques will help reduce any potential bias in data.

Circadian rhythm disturbances have previously been investigated in patients with PD. However, these studies did not control for the acute inhibitory effect of light exposure on melatonin synthesis, included small numbers of participants and took no account of the effects of age, disease duration, disease stage, or mood disturbances. Furthermore, the relationship between circadian phase and habitual sleep-onset time used as a measure of synchrony between the circadian system and the sleep–wake cycle has not been explored within cohorts of patients with PD. Chapter Two of this thesis investigates (*Disturbances in melatonin secretion and circadian sleep-wake regulation in Parkinson's disease, Sleep Medicine 2014*) circadian disturbance in PD. It is proposed that perturbations of melatonin secretion altered by neuropathological changes or by dopaminergic replacement therapy may be contributing to the sleep-wake disturbance seen in PD. This study is the first to combine serial salivary melatonin measurement, polysomnography (PSG) and wrist

actigraphy to identify whether PD patients demonstrate circadian disturbance compared to age matched healthy controls. Furthermore, this study was conducted to elucidate whether disturbances in melatonin secretion are associated with PD pathology or could be attributed to the use of dopaminergic therapy. A greater understanding of these processes will aid the design of future treatment strategies.

In addition to nocturnal sleep disturbance, excessive daytime sleepiness (EDS), a disorder of the homeostatic sleep system, is frequently observed in patients with PD. EDS confers significant morbidity to patients with PD and their carers, particularly in view of the concomitant cognitive and mood disturbance. Studies in non-PD cohorts have successfully utilised daytime actigraphy as a non-invasive measure of daytime napping. However, previous studies in PD have utilised self-report questionnaires such as the Epworth Sleepiness Scale, Scale for Outcomes in Parkinson's disease - Sleep (SCOPA-S) and Parkinson's disease Sleep Scale to measure EDS, which may be prone to bias in cohorts with cognitive and mood dysfunction. Chapter Three of this thesis (*Objective measurement of daytime napping, cognitive dysfunction and subjective sleepiness in Parkinson's disease. PLoS ONE. 2013*) explores objective and subjective measurement of daytime sleep disturbance in a sample of PD patients and a group of age matched healthy controls that had all undergone neuropsychological testing. This is the first study to compare the ESS, a widely used self-report questionnaire that rates the probability of napping, with an objective measure of napping. This thesis hypothesised that the duration of daytime napping would be greater in PD patients as compared to controls and that excessive napping would be associated with impaired cognitive performance, specifically within domains mediated by fronto-subcortical circuitry. Furthermore, it was proposed that

as the ESS rates the probability of napping in several situations, patients with excessive daytime sleepiness as determined by the ESS, should also exhibit greater levels of napping as identified objectively by actigraphy. Finally, this study suggests that the objective measurement of daytime napping will more accurately identify those patients who may benefit from pharmacologic and behavioural interventions to improve these symptoms.

Rapid eye movement (REM) sleep behaviour disorder (RBD) is observed in over half of all PD patients and is linked to significant troublesome symptoms such as visual hallucinations, cognitive decline and mood disturbance. As such, accurate screening and diagnosis is essential for managing the comorbidity associated with RBD in PD Furthermore, the emergence of idiopathic RBD in later life can represent a pre-motor feature heralding the development of PD and thus allowing earlier diagnosis, which may offer a window for more effective intervention. Chapter Four of this thesis (*Improving the electrophysiological measurement of REM without atonia in the diagnosis of REM sleep behaviour disorder. Advances in Clinical Neuroscience and Rehabilitation 2014*) reviews methods of measurement of RWA, the electrophysiological hallmark of RBD. Identifying RWA, in the surface EMG leads from nocturnal polysomnography (PSG), has become central to the diagnosis of RBD and may become the most reliable biomarker predicting the future development of PD in at risk populations.

Quantifying RWA could provide a measurement to grade response to treatment in addition to providing further insights into the pathophysiological mechanisms underlying RBD. However, although there are putative benefits, the utility of measuring RWA as a continuous variable is yet to be determined. Chapter Five (*Investigating REM without atonia in Parkinson's disease using the REM sleep behaviour disorder screening questionnaire. Movement Disorders 2014*) investigates the reliability self-report questionnaires specific to dream enactment behaviour to correctly identify RWA in patients with PD. Furthermore, the diagnostic utility of visually and automatically measured RWA as a continuous variable and night to night variability of RWA was interrogated in Chapter Six of this thesis (*Investigating the night to night variability of REM without atonia in Parkinson's disease. Sleep Medicine 2014*). It is proposed that precise measurement of RWA, the electrical hallmark of RBD will improve the diagnosis of RBD in patients with PD.

Findings from this thesis will recommend improved measurement techniques specific to disorders within the circadian, homeostatic and ultradian sleep systems in patients with Parkinson's disease. Understanding bidirectional causality in between sleep-wake disturbances and concomitant symptomatology in PD will hopefully provide insights into the neural and chemical mechanisms behind these poorly understood problems. Improvements in this area will then target existing and new therapies to improve quality of life for patients with PD and their carers. Furthermore, the early identification of sleep-wake disorders in PD will predict the future development of PD in at risk populations to minimise and prevent irreversible structural brain damage.

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Chapter 2

Disturbances in melatonin secretion and circadian sleep-wake regulation in

Parkinson's disease.

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Original Article

Disturbances in melatonin secretion and circadian sleep–wake regulation in Parkinson disease

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ABSTRACT

Objective: Using salivary dim light melatonin onset (DLMO) and actigraphy, our study sought to determine if Parkinson disease (PD) patients demonstrate circadian disturbance compared to healthy controls. Additionally, our study investigated if circadian disturbances represent a disease-related process or may be attributed to dopaminergic therapy.

Methods: Twenty-nine patients with PD were divided into unmedicated and medicated groups and were compared to 27 healthy controls. All participants underwent neurologic assessment and 14 days of actigraphy to establish habitual sleep-onset time (HSO). DLMO time and area under the melatonin curve (AUC) were calculated from salivary melatonin sampling. The phase angle of entrainment was calculated by subtracting DLMO from HSO. Overnight polysomnography (PSG) was performed to determine sleep architecture.

Results: DLMO and HSO were not different across the groups. However, the phase angle of entrainment was more than twice as long in the medicated PD group compared to the unmedicated PD group ($U = 35.5$; $P = .002$) and was more than 50% longer than controls ($U = 130.0$; $P = .021$). The medicated PD group showed more than double the melatonin AUC compared to the unmedicated group ($U = 31$; $P = 0.001$) and controls ($U = 87$; $P = .001$). There was no difference in these measures comparing unmedicated PD and controls.

Conclusions: In PD dopaminergic treatment profoundly increases the secretion of melatonin. Our study reported no difference in circadian phase and HSO between groups. However, PD patients treated with dopaminergic therapy unexpectedly showed a delayed sleep onset relative to DLMO, suggesting dopaminergic therapy in PD results in an uncoupling of circadian and sleep regulation.

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1. Introduction

Sleep–wake disturbances are gaining increased attention in patients with Parkinson disease (PD). Such symptoms are observed in over two-thirds of patients [1] manifesting with a range of features, including insomnia, rapid eye movement (REM) sleep behavior disorder, and excessive daytime somnolence [1]. In addition to impact on quality of life for patients and their caretakers [2], these symptoms have been linked to cognitive deficits [3,4] and the development of PD dementia [5].

Sleep–wake cycles are regulated by the circadian system, mainly from the hypothalamic suprachiasmatic nuclei (SCN), which controls the rhythm of melatonin synthesis in the pineal gland. Although circadian disturbance is well-recognized in Alzheimer disease [6], specific contributions from structures such as the SCN or pineal gland have not been established in PD. In PD there is widespread neuronal loss with neurotransmitter deficits across dopaminergic and nondopaminergic systems throughout the brainstem, basal forebrain, hypothalamus, and frontostriatal pathways [7–9]. In addition, it has been recognized that the anterior hypothalamus sends monosynaptic outputs to the lateral hypothalamus, overlapping wake-promoting orexin neurons [10]. Because the synchrony between sleep and the circadian system is

dependent on the dorsolateral hypothalamic nuclei [10], it has been proposed that increased orexin through abnormal signaling from the SCN is a potential mechanism for the deregulation of this interaction [11].

Circadian rhythm disturbances have previously been investigated in patients with PD, utilizing the serial measurement of plasma melatonin to determine the onset in the rise of melatonin levels. These studies have reported a circadian phase advance with an earlier onset time of melatonin secretion in patients treated with dopaminergic medication compared to untreated patients and age-matched healthy controls [12,13]. Furthermore increased melatonin secretion has been reported in PD patients who have developed levodopa (L-dopa)-related motor complications compared to patients without these complications and newly diagnosed untreated PD [14]. However, these studies did not control for the acute inhibitory effect of light exposure on melatonin synthesis [15]. Furthermore, these studies included small numbers of participants and took no account of the effects of age, disease duration, disease stage, or mood disturbances.

Thus it is clear that existing studies have employed invasive 24-h plasma sampling with some methodologic deficiencies. Work in non-PD cohorts has successfully utilized melatonin measurements derived from a noninvasive serial salivary sampling [16,17]. This approach has allowed the time of melatonin onset under dim light conditions, referred to as dim light melatonin onset (DLMO) to be determined as a measure of circadian phase and evening melatonin output level. The relationship between DLMO and habitual sleep-onset time (HSO) can be used as a measure of synchrony between the circadian system and the sleep–wake cycle [11].

In addition to the important role melatonin plays in affecting the light–dark regulation circadian rhythms, the sleep-promoting effect of melatonin has been subject to debate [18]. Initial studies using exogenous melatonin failed to show a sleep-promoting effect. However, this lack of effect now appears to be due to the short half-life of the melatonin preparation used and inadequate dosing [19]. Subsequent studies provide compelling evidence that melatonin does have a sleep-promoting effect via direct neuronal suppression (for review see [20]). Furthermore, a recent consensus statement from the British Association for Psychopharmacology has proposed melatonin as first-line therapy for insomnia in older adults [21]. Therefore, if melatonin secretion was altered by neuropathologic changes or by dopaminergic replacement therapy it may be contributing to the sleep–wake disturbance seen in PD.

To our knowledge, our study is the first to combine salivary DLMO, polysomnography (PSG), and wrist actigraphy to identify if PD patients demonstrate circadian disturbance compared to age-matched healthy controls. Furthermore, our study was conducted to elucidate if disturbances in melatonin secretion are associated with PD pathology or if they could be attributed to the use of dopaminergic therapy. We suggest that a greater understanding of these processes will aid the design of future treatment strategies.

2. Methods

2.1. Participants

Twenty-nine patients with PD and 28 age-matched controls were recruited from the Brain and Mind Research Institute PD Research Clinic, University of Sydney, Australia. Patients with a history of obstructive sleep apnea were excluded. All patients satisfied the UK PD Society Brain Bank criteria [22]. The patient group comprised 13 patients who were unmedicated and 16 who were treated with dopaminergic medication. Of these, 11 patients were on L-dopa monotherapy, three were on dopamine agonist

monotherapy, and two were on L-dopa plus a dopamine agonist. Three of the unmedicated patients were taking an antidepressant agent (amitriptyline, venlafaxine, mirtazapine), and three of the patients medicated with dopaminergic replacement therapy were taking an antidepressant agent (mirtazapine, amitriptyline, duloxetine). One of the age-matched healthy controls was taking paroxetine.

2.2. Clinical assessment

Patients were assessed in their "on" state and L-dopa dose equivalents were calculated for dopaminergic medication [23]. Disease stage was rated on the Hoehn and Yahr scale [24] and motor severity was scored on section III of the Unified PD Rating Scale [25]. Disease duration was calculated from time since disease diagnosis and was matched between patient groups. No patients were demented as assessed by the Movement Disorders Society PD Dementia criteria [26] and no participants had a history of major depression. The Mini-Mental State Examination (MMSE) was recorded as a global measure of cognition [27] and depressive symptoms were self-rated using the Beck Depression Inventory-II (BDI-II) Scores of 0–13 were indicative of minimal depressive symptoms [28].

2.3. Sleep and circadian assessment

Participants completed sleep diaries and were required to wear a wrist actiwatch (Minimitter Actiwatch Spectrum) on the wrist less affected by tremor every day for 14 days prior to in-laboratory DLMO assessment. Actigraphy sleep-rest intervals and determination of the HSO were calculated using Actiware 5.0 software (Minimitter-Respironics Inc, Bend, Oregon) and Actiwatch Firmware, version 01.01.0007 (Minimitter-Respironics Inc, Bend, Oregon), in conjunction with manual scoring by an experienced sleep technician [29,30]. HSO was determined calculating the mean of sleep-onset times derived from actigraphy data over the 14-day sampling period and was corroborated by the sleep diary data. Participants then attended the chronobiology and sleep laboratory at the Brain and Mind Research Institute for overnight PSG followed by circadian phase assessment.

Nocturnal PSG recordings were performed in the laboratory 1–2 weeks prior to the circadian phase assessment. Nocturnal PSG recordings were collected on an ambulatory recording system (Compumedics Siesta, Melbourne, Vic, Australia) using the following electroencephalographic montage (C3–M2, O2–M1, Fz–M1, Pz–M2): two electrooculographic channels (left and right outer canthi) and electromyogram (submentalis). Electroencephalographic data were sampled at 250 Hz. Sleep stages were visually scored by an experienced sleep technician using standardized criteria [31]. While in the laboratory, participants were physiologically and behaviorally monitored under controlled conditions with fixed light levels (<50 one time during waking and <1 one time during scheduled sleep periods) and ambient temperature (24 ± 1 °C). The following sleep variables were calculated: total sleep time (minutes), percentage of time in REM, percentage of time in slow-wave sleep, sleep-onset latency (SOL) (minutes), latency to REM sleep (minutes), and wake after sleep onset (minutes).

For the in-laboratory circadian phase assessment, participants were asked to arrive 7 h prior to their HSO, to familiarize themselves with the laboratory setting and to ensure they were in a controlled posture for at least 30 min before the first sample was collected. Saliva samples were collected at 30-min intervals (Salivette, Sarstedt, Germany) from 6 h before HSO until 2 h after HSO, per previously published protocols [11,17]. During this assessment, participants were physiologically and behaviorally

monitored under controlled conditions with fixed light levels that were confirmed with measurement to be less than 30 lux and ambient temperature (24 ± 1 °C). Participants maintained a seated posture for at least 20 min before each sample collection. On the day of melatonin measurement while in the sleep laboratory, patients were asked to abstain from substances believed to affect melatonin or sleep (e.g., caffeine, turkey, bananas, tomatoes). To minimize the effect of eating food, dinner was provided in two halves which could be consumed during the interval between two nonconsecutive melatonin sample collections.

Melatonin was assayed in 200 μL of saliva by double-antibody radioimmunoassay according to the manufacturer's instructions (Cat No. RK-DSM2; Buhlmann Laboratories AG, Schonenbuch, Switzerland). The lowest detectable level of melatonin was 4.3 pM. The intra-assay coefficient of variation was <10% across the range of the standard curve. The interassay coefficient of variation was 15% at 19.5 pM and 12.3% at 177 pM.

The area under the melatonin curve (AUC) was calculated using the trapezoidal method for each participant over the entire 8-h sampling period [11]. To ensure that any potential difference in AUC was not due timing of the melatonin sampling, the AUC in the first hour post-DLMO and the average AUC post-DLMO was calculated for all participants. A threshold for melatonin was calculated as the mean of the first three readings plus two standard deviations (SD). The DLMO was identified as the point when the saliva melatonin reached this threshold and remained elevated for at least the next sampling time in accordance with previously published criteria [11,17]. The phase angle of entrainment (measured in minutes) was calculated by subtracting the DLMO time from the HSO time.

2.4. Standard protocols approvals, registrations, and patient consent

Approval for the study was obtained from the University of Sydney Human Research Ethics Committee (HREC 08-2008/11105) and all patients gave written informed consent.

2.5. Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS version 20, for IBM. Age was compared between the groups using a one-way analysis of variance. Gender was compared using a χ^2 test. Subsequent variables violated assumptions of normality. Nonparametric data were first analyzed using the independent samples Kruskal–Wallis analysis of variance to determine if group differences existed using an α level of .05. Subsequent post hoc comparisons between groups were assessed using the Mann–Whitney U test. Bonferroni correction was used for multiple comparisons.

3. Results

Of the 78 participants in our study, 16 had a sporadic melatonin profile without an apparent rise in melatonin (4 unmedicated PD, 7 medicated PD, and 5 controls). A further five did not register any melatonin concentrations over the 8-h sampling period (1 unmedicated PD, 2 medicated PD, and 2 controls). DLMO, phase angle of entrainment, and AUC could not be evaluated for these participants.

Demographic, clinical, and circadian data are presented in Table 1. Age, depressive symptoms, and MMSE scores did not differ across the three groups. On average there were minimal depressive symptoms and high MMSE scores. There was no significant difference between the number of participants taking antidepressant medication across the three groups ($\chi^2 = 4.0$; $P = .135$). The medicated and unmedicated patient groups were matched for disease duration and did not differ on measures of disease stage (Hoehn and Yahr) or motor severity (section III of the Unified PD Rating Scale).

DLMO and HSO were not different across the three groups. However, there was a significant difference across groups in the phase angle of entrainment ($\chi^2 = 10.6$; $P = .005$) (Table 1). As shown in Fig. 1, the medicated group had a longer phase angle compared to the unmedicated groups ($U = 35.5$; $P = .002$). The medicated group also had a longer phase angle compared to the healthy control group ($U = 130.0$; $P = .021$). There was no difference in this measure between the unmedicated group and controls.

As shown in Table 1, the melatonin AUC was significantly different across the three groups ($\chi^2 = 14.0$; $P = .001$). The medicated patient group had more than double the AUC compared to unmedicated PD ($U = 31$; $P = .001$) and controls ($U = 87$; $P = .001$), respectively (Figs. 2 and 3). However, there was no difference when comparing unmedicated patients and controls.

Table 1
Descriptive, neurology, sleep, and circadian rhythm data for patients and controls.

	Unmedicated PD mean ± SD $n = 13$	Medicated PD mean ± SD $n = 16$	Controls mean ± SD $n = 28$	Statistic	P value
Age (y)	64.8 ± 6.0	63.6 ± 9.8	68.3 ± 9.0	$F = 1.7$	.195
Beck Depression Inventory-II	8.2 ± 6.1	6.9 ± 3.1	5.1 ± 4.0	$\chi^2 = 3.9$	.142
MMSE	28.1 ± 2.1	28.8 ± 1.4	29.1 ± 1.2	$\chi^2 = 2.1$	.359
UPDRS-III	27.0 ± 13.9	28.5 ± 14.4	–	$U = 98.0$	.792
Hoehn and Yahr	2.0 ± 0.5	1.9 ± 0.5	–	$U = 97.5$	.742
Disease duration (y)	1.0 ± 0.8	1.8 ± 1.3	–	$U = 63.0$	.072
ʟ-dopa dose equivalent (mg)	–	420.3 ± 195.4	–		–
Total sleep time (min)	406.3 ± 38.7	393.9 ± 60.6	392.0 ± 50.7	$\chi^2 = 0.4$	.435
Slow-wave sleep (%)	16.9 ± 13.2	18.8 ± 12.0	16.4 ± 10.1	$\chi^2 = 0.6$	.735
REM sleep (%)	20.7 ± 5.9	21.6 ± 5.6	20.6 ± 5.3	$\chi^2 = 0.2$	.929
Sleep-onset latency	30.2 ± 22.1	14.8 ± 12.7	15.8 ± 14.0	$\chi^2 = 6.4$	.041
REM latency (min)	94.1 ± 71.0	78.8 ± 42.7	68.0 ± 23.4	$\chi^2 = 0.1$	.970
WASO (min)	86.00 ± 84.6	59.8 ± 51.2	96.8 ± 96.8	$\chi^2 = 1.1$	.580
DLMO (h:min)	20:58 ± 00:76	20:08 ± 00:78	20:58 ± 00:86	$\chi^2 = 3.1$	.210
HSO (h:min)	22:02 ± 00:56	22:46 ± 00:53	22:41 ± 00:51	$\chi^2 = 5.1$	.079
Entrainment phase angle (min)	65 ± 68	159 ± 72	103 ± 74	$\chi^2 = 10.6$	.005
AUC (pM)	124.9 ± 82.0	317.0 ± 175.2	146.7 ± 112.7	$\chi^2 = 14.0$	.001
AUC 1-h post-DLMO (pM)	26.4 ± 11.6	39.2 ± 15.7	24.9 ± 12.8	$\chi^2 = 10.6$	.005
AUC post-DLMO (pM/sample)	20.3 ± 12.3	32.7 ± 15.8	19.2 ± 12.6	$\chi^2 = 8.8$	.012

Abbreviations: PD, Parkinson disease; SD, standard deviation; y, years; MMSE, Mini-Mental State Examination; UPDRS-III, Unified Parkinson Disease Rating Scale Section III; ʟ-dopa, levodopa; min, minutes; REM, rapid eye movement; WASO, wake after sleep onset; h:min, hours and minutes; DLMO, dim light melatonin onset; HSO, habitual sleep-onset time; AUC, area under the melatonin curve.
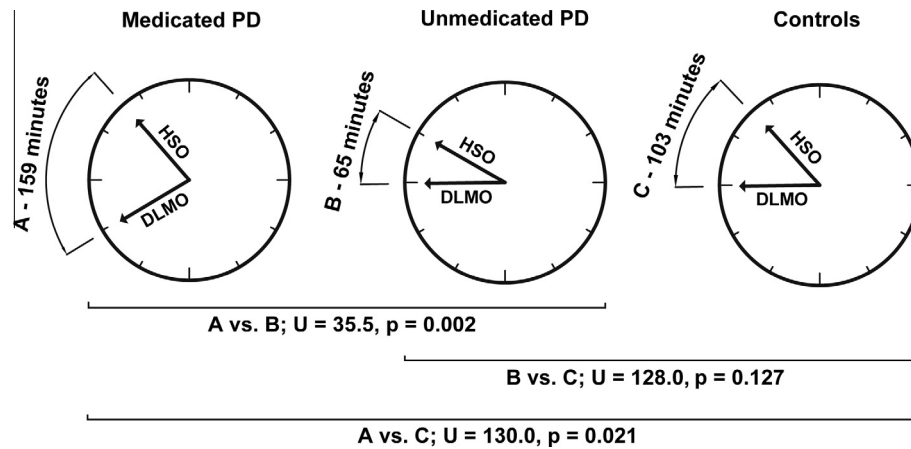
Fig. 1. Entrainment phase angle, habitual sleep-onset time (HSO), and dim light melatonin onset (DLMO). A graph demonstrating the longer entrainment phase angle (minutes) reported in patients with Parkinson disease (PD) who were medicated with dopaminergic replacement therapy compared to unmedicated patients with PD and healthy age-matched controls, respectively. The entrainment phase angle is calculated by subtracting the DLMO from the HSO measured in minutes.
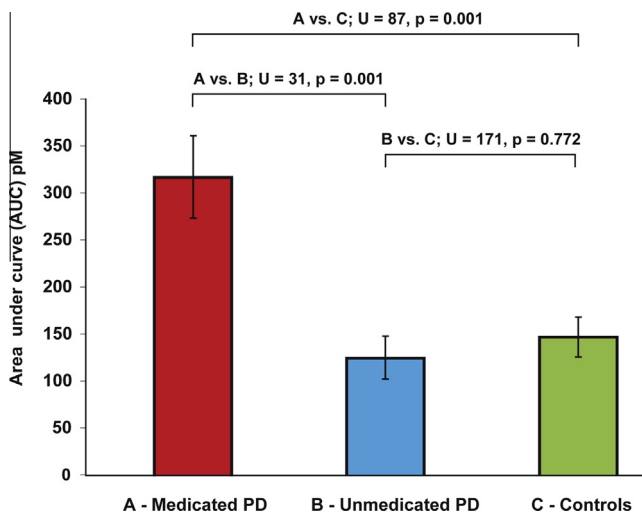

Fig. 2. Area under the melatonin curve (AUC) (pM). A chart depicting the increased AUC (mean ± standard error) reported in patients with Parkinson disease (PD) who were medicated with dopaminergic replacement therapy compared to unmedicated patients with PD and healthy age-matched controls, respectively. The melatonin curve was created for each participant by collecting melatonin levels every 30 min over the 8-h sampling period. The AUC was calculated using the trapezoidal rule.

To ensure that the increased AUC noted in the medicated group compared to unmedicated PD and controls, respectively, was not due to an error in the window of the melatonin curve sampled, melatonin data were plotted for all participants over the 8-h sampling period (Fig. 3; panel A). Furthermore, the average AUC 1 h post-DLMO and the average AUC post-DLMO was calculated. For the medicated PD group, the AUC 1 h post-DLMO was significantly higher than both unmedicated PD groups (39.2 [SD, 15.7] vs 26.4 [SD, 11.6]; $U = 54$; $P = .028$) and controls (39.2 [SD, 15.7] vs 24.9 [SD, 12.8]; $U = 109$; $P = .005$) (Fig. 3; panel B). Similarly the average AUC post-DLMO was significantly higher in the medicated PD group compared to both the unmedicated PD group (32.7 [SD, 15.8] vs 20.3 [SD, 12.3]; $U = 50$; $P = .018$) and controls (32.7 [SD, 15.8] vs 19.2 [SD, 12.6]; $P = .002$).

SOL was noted to be different between the groups ($\chi^2 = 6.4$; $P = .041$), with the medicated PD group reporting the lowest value of 14.8 min (SD, 12.7). However, this result was not sustained in a post hoc analysis comparing unmedicated PD to medicated PD

(30.2 [SD, 22.1] vs 14.8 [SD, 12.7]; $U = 41.5$; $P = .022$), medicated PD to controls (14.8 [SD, 12.7] vs 15.8 [SD, 14.0]; $U = 196$; $P = .915$), and unmedicated PD to controls (30.2 [SD, 22.1] vs 15.8 [SD, 14.0]; $U = 71.5$; $P = .023$) when correcting for multiple comparisons. Other sleep variables assessed using PSG revealed no differences among the three groups (see Table 1).

4. Discussion

Our study demonstrated that dopaminergic treatment in PD profoundly increases the secretion of melatonin. Moreover, although no differences in circadian phase (DLMO) or sleep timing (HSO) were found in PD compared to age-matched healthy controls, patients treated with dopaminergic therapy unexpectedly showed a delayed sleep onset relative to their DLMO. This finding suggests that dopaminergic therapy in PD results in uncoupling of circadian and sleep–wake regulation. This finding questions previous work showing a circadian phase advance in PD by recording plasma melatonin levels [12–14].

We observed differences between medicated PD and unmedicated PD patients on both the phase angle of entrainment and AUC despite these groups being matched for disease duration, stage, and motor severity. Specifically medicated PD patients had a significantly longer phase angle and greater melatonin output than the unmedicated PD group. Interestingly the unmedicated PD patients demonstrated similar results compared to controls on both of these measures, suggesting that the disease process itself may not be responsible for these changes. Furthermore, there was no difference in age, global cognition, depression, or use of antidepressant medications that could have formed an alternate explanation of these results.

The increased phase angle reported in the medicated PD group was not accompanied by evidence of insomnia. The medicated PD group reported the shortest SOL of the three groups. Other sleep variables collected during PSG indicated that the medication-related changes in the phase angle of entrainment and melatonin secretion were not accompanied by changes in sleep architecture.

The difference in phase angle of entrainment cannot be readily explained by a difference in DLMO or HSO times between the groups. Indeed the increased difference in HSO relative to DLMO in medicated patients, as indicated by the longer phase angle of entrainment, suggests that there may be an uncoupling or alterations in the internal phase relationships between the circadian rhythm of melatonin synthesis and the sleep–wake cycle. It is
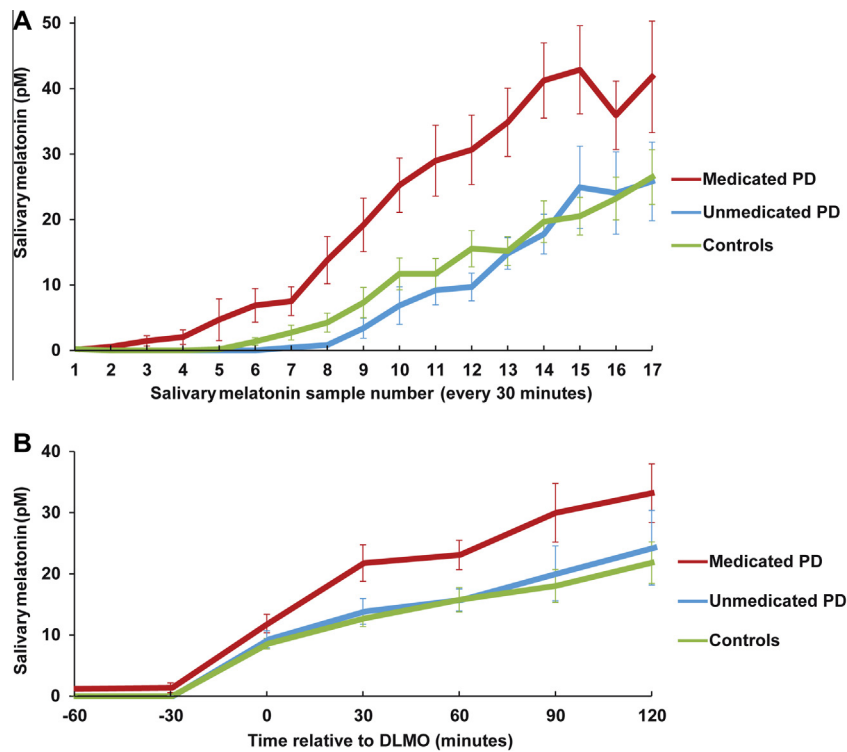
Fig. 3. Melatonin curve. A graph plotting the salivary melatonin levels (pM) (mean ± standard error) collected during the 8-h sampling period for patients with Parkinson disease (PD) who were medicated with dopaminergic replacement therapy, unmedicated patients with PD, and healthy age-matched controls. Panel A reports the increased area under the melatonin curve (AUC) in the medicated PD group compared to unmedicated PD and healthy age-matched controls, respectively. Panel B indicates that the increased AUC seen in the medicated PD group compared to unmedicated PD and controls, respectively, remained when the melatonin levels were plotted relative to their dim light melatonin onset time (DLMO).

possible that such alterations could account for some of the sleep disturbances noted in treated PD patients. For example, insomnia is more prevalent in patients with longer disease duration who are more likely to be taking doses of dopaminergic therapy [32].

Our results indicate that dopaminergic treatment rather than the neuropathology underlying PD is responsible for both the prolonged phase angle of entrainment and increased melatonin output level. However, despite matching for disease duration, stage, and motor severity it is possible that the assessment of medicated patients in their "on" state might have masked underlying neuropathologic deficits. This increased melatonin secretion in response to dopaminergic therapy may be related to recent findings linking dopamine to the regulation of the pineal gland. Animal models have identified the D4 dopamine receptor on the pineal gland [33]. Furthermore, the release of serotonin and melatonin from the pineal gland is reported to be controlled by circadian-related heterodimerization of adrenergic and dopamine D4 receptors [34].

Given the putative sleep-promoting properties of melatonin, the finding of an increased phase angle of entrainment in the presence of increased melatonin secretion would seem paradoxical [35]. Although establishing the neurochemical basis of these findings was beyond the scope of our study, our observation suggests that there may be some form of melatonin resistance among patients during the activation of their circadian systems and could account for the limited success of this therapy in PD patients with insomnia [36,37]. Alternatively it is possible that melatonin function follows an inverse U-shaped relationship similar to dopamine and serotonin, in which high levels can bring about paradoxical function. Further studies are needed to identify the mechanism affecting these phenomena.

Although melatonin levels were measured during the evening, these results raise the question of dopaminergic replacement

therapy interfering with melatonin secretion during the day through similar pineal gland receptor-based mechanisms. Future studies using daytime melatonin sampling may be able to determine if the putative hypnotic properties of melatonin are implicated in the excessive daytime sleepiness seen in PD, which has previously been attributed to dopaminergic medication [32].

A relatively high number of participants in our study were excluded due to a sporadic melatonin profile from which DLMO could not be derived. The effects of sialorrhoea in PD combined with the reduced melatonin secretion accompanying aging could have contributed to this limitation [38]. Although 24-h melatonin sampling is not required to calculate the DLMO [39], an estimate of melatonin secretion over the entire circadian cycle would be more precise and should be considered in future work.

5. Conclusion

Our study suggests that, although there is no evidence of circadian phase change in PD, dopaminergic treatment profoundly affects the secretion of melatonin and the regulation of circadian phase and sleep timing. Studies are now needed to determine if these results contribute to specific sleep–wake disturbance in PD and to determine if these changes can be corrected with pharmacologic and nonpharmacologic approaches to help improve sleep in this common neurodegenerative disease.

Author contributions

Drafting/revising the manuscript for content, including medical writing for content; S.J. Bolitho, S.L. Naismith, S.M.W. Rajaratnam, R.R. Grunstein, J.R. Hodges, Z. Terpening, S.J.G. Lewis.

Conflict of interest

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: http://dx.doi.org/10.1016/j.sleep.2013.10.016.

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Chapter 3

Objective measurement of daytime napping, cognitive dysfunction and subjective

sleepiness in Parkinson's disease.

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Objective Measurement of Daytime Napping, Cognitive Dysfunction and Subjective Sleepiness in Parkinson's Disease

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Abstract

Introduction: Sleep-wake disturbances and concomitant cognitive dysfunction in Parkinson's disease (PD) contribute significantly to morbidity in patients and their carers. Subjectively reported daytime sleep disturbance is observed in over half of all patients with PD and has been linked to executive cognitive dysfunction. The current study used daytime actigraphy, a novel objective measure of napping and related this to neuropsychological performance in a sample of PD patients and healthy, age and gender-matched controls. Furthermore this study aimed to identify patients with PD who may benefit from pharmacologic and behavioural intervention to improve these symptoms.

Methods: Eighty-five PD patients and 21 healthy, age-matched controls completed 14 days of wrist actigraphy within two weeks of neuropsychological testing. Objective napping measures were derived from actigraphy using a standardised protocol and subjective daytime sleepiness was recorded by the previously validated Epworth Sleepiness Scale.

Results: Patients with PD had a 225% increase in the mean nap time per day (minutes) as recorded by actigraphy compared to age matched controls (39.2 ± 35.2 vs. 11.5 ± 11.0 minutes respectively, p < 0.001). Significantly, differences in napping duration between patients, as recorded by actigraphy were not distinguished by their ratings on the subjective measurement of excessive daytime sleepiness. Finally, those patients with excessive daytime napping showed greater cognitive deficits in the domains of attention, semantic verbal fluency and processing speed.

Conclusion: This study confirms increased levels of napping in PD, a finding that is concordant with subjective reports. However, subjective self-report measures of excessive daytime sleepiness do not robustly identify excessive napping in PD. Fronto-subcortical cognitive dysfunction was observed in those patients who napped excessively. Furthermore, this study suggests that daytime actigraphy, a non-invasive and inexpensive objective measure of daytime sleep, can identify patients with PD who may benefit from pharmacologic and behavioural interventions to improve these symptoms.

Introduction

Sleep-wake disturbance is gaining increased attention in Parkinson's disease (PD). Such problems are observed in over two thirds of patients [1] manifesting with a range of sleep symptoms [2]. In addition to nocturnal sleep disturbance, daytime sleep disturbance is defined as encompassing both

excessive daytime sleepiness (EDS) and excessive daytime napping, which are frequently observed in patients with PD [3,4]. Whilst EDS and excessive daytime napping are separate constructs within sleep medicine, the distinction of these phenomena within PD cohorts remains less clear. Previous studies have considered these symptoms both as separate entities [5,6] and also as being measures along the same

continuum within PD cohorts [3,4,7-9] . It is possible that the overlap of these phenomena in part represents a common neural and chemical mechanism involving the reticular activating system of the brainstem, basal forebrain and hypothalamus through activation of the homeostatic sleep system [10,11]. Thus it is difficult to make a clear distinction between excessive daytime sleepiness and excessive daytime napping in PD.

In addition to daytime sleep disturbance, fatigue is frequently observed within PD cohorts. It is important to distinguish fatigue, an overwhelming lack of energy [12] from excessive daytime sleepiness and excessive daytime napping, which exist with impairment of normal arousal mechanisms [12]. Previous studies in PD have identified a link between the self-reported tendency to doze or nap in contrast to fatigue , measured with the Epworth Sleepiness Scale [13] and cognitive deficits [14-16]. Non-motor symptomatology in PD, including cognitive dysfunction and daytime somnolence contribute significantly to poor quality of life for patients and their carers [17]. Cognitive deficits are an independent predictor of admission to a nursing home, conferring a negative impact of burden of health to the community [18]. However these relationships in daytime somnolence have yet to be investigated using an objective measure.

The development of daytime somnolence in PD has been associated with increasing age, disease duration, disease progression, postural instability, depression and the use of dopamine agonists [8,19,20]. However, much like the emergence of idiopathic REM sleep behavior disorder (RBD) in later life [21], daytime sleep disturbance can also represent a pre-motor feature heralding the development of PD [3,4,14]. Daytime sleep disturbance has been linked to executive dysfunction in PD [16] and impairments in frontostriatal neural circuitry have been implicated in the reduced arousal, attentional modulation and general working memory seen in PD [16,22,23]. These observations may imply a link between excessive napping and reduced cognition in PD [14-16].

The pattern of neuronal loss and neurotransmitter deficits giving rise to daytime sleep disturbance in PD are not well understood but dopaminergic and non-dopaminergic pathology across the brainstem, basal forebrain, hypothalamus and frontostriatal pathways have been suggested [3,7,10,14]. Such pathology may impair wake promoting structures [11,24,25] and/or possibly disrupt the proposed sleep homeostat [25]. The control of this sleep homeostat is not well defined, but some have suggested it operates via the accumulation of activity dependent metabolites that promote sleep throughout the day (including adenosine, gamma amino butyric acid (GABA), prostaglandin D2 (PGD2), interlukin–1A (ll-1A) and tumour necrosis factor-alpha (TNFα)) [10].

Previous studies in non-PD samples have shown that older individuals with EDS are more likely to nap during the day [26,27]. More extensive work has been conducted on napping in healthy, older adults where it appears to be associated with increased morbidity [28] and mortality [29]. In these older cohorts increased napping has been associated with decreased global cognition [26,30-32] and in particular deficits in executive function [31]. Interestingly, studies that have

utilised 'prescribed' napping to restore the effects of sleep deprivation in healthy cohorts, have demonstrated improved executive performance on tasks such as reaction time and symbol digit substitution [33-35]. These combined observations highlight the possibility that the increased frequency of napping seen in older adults and patients with PD might represent a compensatory neurobiological strategy to a primary neuropathological insult (rather than playing a causative role in cognitive deficits).

Studies in non-PD cohorts have successfully utilised daytime actigraphy as a non-invasive measure of daytime napping [32,36-38]. Furthermore, the use of actigraphy to explore sleep disturbance has been well validated in nocturnal sleep disturbance in PD [39,40]. The current study sought to compare objective and subjective measurement of daytime sleep disturbance in a sample of PD patients and a group of age matched healthy controls that had all undergone neuropsychological testing.

This is the first study to compare the ESS, a widely used self-report questionnaire that rates the probability of napping, with an objective measure of napping. We hypothesised that the duration of daytime napping would be greater in PD patients as compared to controls and that excessive napping would be associated with impaired cognitive performance, specifically within domains mediated by fronto-subcortical circuitry. Furthermore we propose that as the ESS rates the probability of napping in several situations, patients with excessive daytime sleepiness as determined by the ESS, should also exhibit greater levels of napping as identified objectively by actigraphy Finally, we suggest that the objective measurement of daytime napping will more accurately identify those patients who may benefit from pharmacologic and behavioural interventions to improve these symptoms.

Methods

Ethics statement

Permission for the study was obtained from the University of Sydney Human Research Ethics Committee (HREC 02-2008/11105). All patients gave written informed consent.

Participants

Eighty five patients and 21 age matched healthy controls were recruited from the Brain & Mind Research Institute (BMRI) PD Research Clinic, University of Sydney. All participants with a known or suspected diagnosis of obstructive sleep apnea were excluded, including any participant who had previously had CPAP prescribed or who had greater than mild OSA on a diagnostic sleep study [16]. Patients were then asked three screening questions to identify snoring, nocturnal snorting or gasping or a history of nocturnal apneas and were excluded if these were present. No patients were demented as assessed by the Movement Disorders Society criteria [41] and participants with a diagnosis of major depression were excluded. Five patients were unmedicated, thirty patients were on levodopa monotherapy, six were on dopamine agonist monotherapy, forty were on levodopa plus an adjuvant agent (e.g. dopamine agonist, COMT inhibitor, MAO inhibitor), three

were on a dopamine agonist plus amantadine and one was on a dopamine agonist plus Rasagiline. Thirteen patients with PD were taking medications to aid sleep. Twelve of these were taking a benzodiazepine and one was taking melatonin. None of the controls were taking sleeping medications. Five patients had deep brain stimulators in situ.

Clinical assessment

All neurological and neuropsychological assessments were conducted within one session to confirm study eligibility. Patients were assessed in their 'on' state and levodopa dose equivalents were calculated for dopaminergic medication [42]. Disease stage was rated on the Hoehn and Yahr (H&Y) scale [43], disease duration was calculated from time since disease diagnosis, and depressive symptoms were self-rated using the Beck Depression Inventory–II (BDI-II, scores of 0-13 indicative of minimal depressive symptoms) [44].

Neuropsychological functioning was assessed within the PD cohort using standardised tests and appropriate normative data (with corrections for age and level of education). These variables were included in the healthy control group for descriptive purposes only. Language generativity was assessed with semantic verbal fluency via the Controlled Oral Word Associated Test (COWAT animals; z-score) [15,45]. Set-shifting was measured using the Trailmaking Test, Part B (TMT-B; z-score) [46,47]. Processing speed was assessed using the choice reaction time test from the Cambridge Neuropsychological Test Automated Battery (CANTAB; z-score) [16,48]. The Mini Mental State Examination (MMSE) [49] was administered for reporting purposes. Similarly the ability to retain learned verbal memory was assessed using the Logical Memory (percentage retention) subtest from the Wechsler Memory Scale - III [50] and working memory, assessed using the Digit Span backwards subtest of the Wechsler Adult Intelligence Scale – III (raw score) [51] were included for reporting purposes.

Actigraphic assessment

The use of actigraphy to assess daytime sleep has been validated previously in healthy subjects in both the laboratory and community setting [37,38] and the measurement of daytime sleep-wake disturbance in this study was conducted according to previously established protocols [46,52]. Following clinical assessment, participants were required to wear a wrist actiwatch (Minimitter Actiwatch Spectrum) on the wrist less affected by tremor every day for fourteen days. Actigraphy rest intervals were calculated using Actiware 5.0 software (Minimitter-Respironics Inc, Bend, Oregon) in conjunction with manual scoring by an experienced sleep technician. An episode of daytime sleep was defined as resting with no movement on actigraphy during the day for a minimum duration of thirty minutes. The primary measure of daytime sleep was the nap time per day (minutes) which was calculated by summing all napping each day and averaging this over the 14 day measurement period. The number of nap bouts per day were also reported. Total nocturnal sleep time (TST), wake after sleep onset (WASO) and sleep efficiency ((TST – WASO)/ TST) were also derived from the actiwatch as per previously

established protocols [39,46]. Patients were defined as exhibiting 'excessive daytime napping' if their nap time per day was greater than a threshold derived from the control data. This threshold was defined as the average nap time per day (duration) + 1.5 standard deviations (minutes).

Subjective Assessment of Daytime Sleep Disturbance

Patients were asked to complete the Epworth Sleepiness Scale (ESS) (score ≥ to 10 indicative of a high probability of daytime sleep) [13], within two weeks of completing the actigraphy.

Statistical analysis

Statistical analysis was conducted on PASW Statistics Version 20 for Windows. Age was compared between the groups using a t-test. Gender was compared using a chi-square test. Subsequent variables violated assumptions of normality and non-parametric Mann-Whitney U test were used for these comparisons. All tests were two-tailed with an α value of 0.05. The three cognitive variables were compared between groups utilising a Bonferroni correction for multiple comparisons.

Results

Patients vs. Controls

As shown in Table 1, there was no significant difference in age or gender between the PD group and control groups. The groups were not different with regard to global cognition (i.e. MMSE). However, the patient group had higher ESS scores (p=0.001). As measured by the BDI-II, depressive symptoms were significantly higher in the PD group by an average of five points (p<0.001). However, the average BDI-II in the PD group was only 9.2 (SD 6.7), suggestive of only minimal depression.

Napping data shown in Figure 1 reports that patients in this study had a 225% increase in the mean nap time per day compared to age matched controls (39.2 ± 35.2 vs. 11.5 ±11.0 minutes respectively, p < 0.001). Similarly there was a 244% increase in median nap time per day in the (p=0.003) and significantly more nap bouts per day when comparing the patient group to controls (0.6 ± 0.5 vs. 0.2 ± 0.3 respectively, p < 0.001). To ensure that the increased napping noted in the PD group was not due to sleep deprivation, the average total nocturnal sleep time, derived from actigraphy over the fourteen day sampling period, was compared to controls. There was no difference in this measure between the two groups to suggest the patients with PD had a sleep debt (p=0.303). Furthermore, there was no difference in sleep efficiency (p = 0.602) or wake after sleep onset (p = 0.329) comparing the PD group to controls.

As mood disturbance has been linked previously to daytime somnolence [53], an analysis of co-variance (ANCOVA) was performed to assess the contribution of mood disturbance (BDI-II) to the measure of nap time per day between PD and control groups. This result affirmed the increased nap time per day seen in the PD group compared to controls and remained significantly increased when mood disturbance (BDI-II) was

Table 1. Descriptive, neurologic, sleep and cognitive data for patients and controls.

	Controls	Parkinson's Disease	Statistic	p-value
	Mean ± SD (n = 21)	Mean ± SD (n = 85)		
Age (years)	63.4 ± 9.5	64.8 ± 7.4	t = -0.6	0.537
Sex, Male: Female	12:9	53:32	χ^2 = 0.2	0.661
Hoehn and Yahr		2.0 ± 0.7		
Disease duration (years)		5.9 ± 5.2		
Levodopa dose equivalent (mg)		641.9 ± 466.3		
Participants taking sleeping tablets	0	13		
Participants with DBS in situ		5		
Average nap time per day (min)	11.5 ± 11.0	39.2 ± 35.2	U = 345.0	< 0.001
Median average nap time per day (min) (IQR)	7.7 ± 16.7	26.5 ± 34.6	U = 345.0	< 0.001
Average naps per day	0.2 ± 0.3	0.6 ± 0.5	U = 375.0	< 0.001
Total nocturnal sleep time (min)	438.0 ± 39.2	453.0 ± 67.1	U = 762.5	0.303
Sleep efficiency (%)	91.1 ± 3.0	90.2 ± 4.7	U = 767.5	0.602
Wake after sleep onset (min)	35.3 ± 8.4	34.3 ± 12.0	U = 713.0	0.329
Epworth Sleepiness Scale	4.6 ± 3.6	8.1 ± 4.3	U = 483.5	0.001
Number of participants with ESS ≥ 10	3	33	χ^2 = 4.521	0.033
Beck Depression Inventory-II	3.1 ± 3.9	9.2 ± 6.7	U = 338.0	< 0.001
Mini-Mental State Examination	28.4 ± 1.6	28.4 ± 1.8	U = 851.0	0.732
Logical Memory retention (% retention)	10.9 ± 3.3	11.0 ± 3.4	U = 890.0	0.984
Digit span backwards raw score	7.1 ± 2.3	6.8 ± 1.8	U = 885.0	0.952
Verbal Fluency animals z-score	0.5 ± 1.6	0.1 ± 1.3	U = 827.5	0.606
Trailmaking Test, Part B z-score	-0.1 ± 1.6	-0.9 ± 1.8	U = 627.5	0.016
Choice reaction time z-score	-0.02 ± 1.2	-0.1 ± 1.4	U = 884.0	0.946

IQR, interquartile range.

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used as a covariate (F = 9.6, p =0.003). This result was further corroborated by the finding that BDI-II did not correlate with nap time per day in the PD group (Spearman rho = 0.095, p = 0.390).

Patients with Excessive Daytime Napping vs. Patients without Excessive Daytime Napping

Table 2 shows the comparison of patients with (n=41) and without (n=44) excessive daytime napping. These groups showed no differences in their disease duration, disease stage or levodopa dose equivalent. Furthermore, patients on sleeping tablets were not over represented in either the excessive or normal napping group (χ^2 = 0.194, p = 0.660). Similarly, global cognition (MMSE), mood disturbance (BDI-II), retention of learned verbal memory (Logical Memory percentage retention), and working memory (Digit Span backwards) were not different between the groups. As age was noted to be different between the two sub-groups of PD patients, age-adjusted normative z-scores were used. As shown in Figure 2, patients who exhibited excessive daytime napping had significantly poorer mental flexibility and set-shifting (TMT-B z-score, p=0.016), and semantic verbal fluency (COWAT animals z-score, p=0.004). Though the processing speed was also slower in those with excessive napping this did not meet the correction for multiple comparisons and represents a trend (choice reaction time z-score, p=0.022). Within the PD cohort, there was no evidence of a deficit in nocturnal sleep (total nocturnal sleep time p = 0.356), sleep efficiency (p = 0.800) or wake after sleep onset (p = 0.544), that could explain the excessive daytime napping and cognitive deficit seen in these results. Furthermore, the patients with DBS were not over represented in either excessive or normal nappers (χ^2 = 0.294, p = 0.587).

By contrast, differences in napping duration between patients with and without excessive daytime napping as recorded by actigraphy were not distinguished by their ratings on the ESS. Table 3 shows results comparing patients with PD, divided into those with a tendency to nap during the day based on an ESS ≥ 10. Those who were positive on the ESS also had poorer set-shifting (TMT-B z-score p = 0.005) and a trend to reduced processing speed when adjusting for multiple comparisons (choice reaction time z-score, p = 0.047). Although not the primary focus of this study, those who were positive on the ESS also had a trend towards poorer working memory (digit span backwards raw score, p = 0.020). However, unlike when the group was divided by excessive napping identified with actigraphy, Figure 3 reports that patients identified to be positive on the ESS, had significantly greater mood deficit (p = 0.006), disease stage (p = 0.006) and levodopa dose equivalent (p < 0.001).

Discussion

This study is the first to use actigraphy, a previously validated objective measure of daytime sleep, to record the duration and correlates of excessive daytime napping in PD. Patients with PD, reported significantly greater number of nap bouts as well as time spent napping in the day, compared to healthy age matched controls. Patients with PD who exhibited excessive napping through the day had poorer performance on neuropsychological tests probing fronto-subcortical functions including set-shifting, semantic verbal fluency and processing speed. This result is in keeping with previous findings evaluating excessive daytime somnolence [16]. However, other
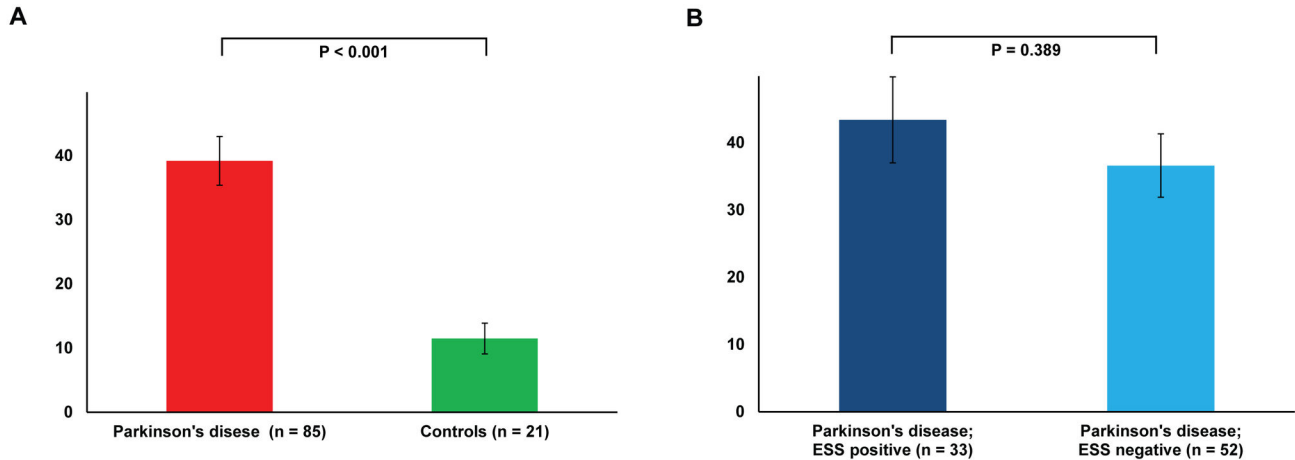
Figure 1. Average nap time per day (minutes). A chart depicting the average nap time per day (± standard error) calculated by summing the daytime napping periods identified by actigraphy and averaging this over the 14 day measurement period. Panel A - Parkinson's disease vs. Controls. Panel B - Parkinson's disease patients divided into those who are Epworth Sleepiness Scale positive (score ≥ to 10 indicative of sleepiness) vs. Parkinson's Disease patients who are Epworth Sleepiness Scale negative.
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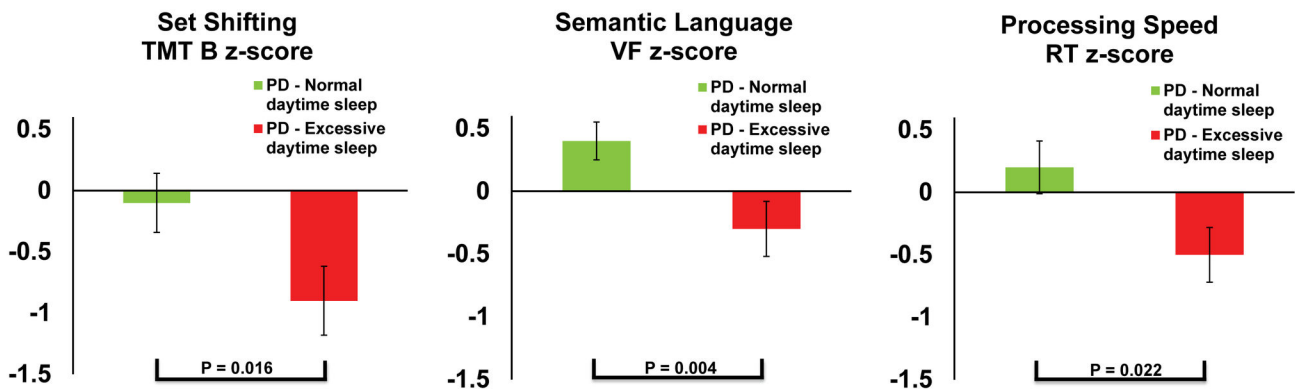
Figure 2. Cognitive performance of excessive nappers within the Parkinson's disease cohort. A chart comparing the cognitive performance (mean ± standard error) of patients with Parkinson's disease (PD) divided into those with excessive daytime napping vs. those with normal daytime napping. Set shifting was measured with the Trailmaking task part B (TMT B; z score). Semantic verbal fluency (VF) was tested via the Controlled Oral Word Associated Test (COWAT animals; z score) and processing speed was measured with the choice reaction time (RT) test from the Cambridge Neuropsychological Test Automated Battery (CANTAB; z score).
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studies have suggested prescribed napping can improve cognition. This paradox may imply that excessive napping is a compensatory process to preexisting cognitive deficit. Alternatively these results may imply the neural and chemical processes that bring about excessive or uncontrolled napping, rather than intended or prescribed napping are linked to the cognitive dysfunction seen in this study.

These results were not accounted for by age, mood disturbance, dementia, disease duration, disease stage or levodopa dose equivalent. As patients with obstructive apnea were excluded from the trial, this common cause of daytime sleepiness could not explain the excessive daytime napping

seen in the PD group. Furthermore there was no evidence of sleep deprivation or poor sleep quality in patients compared to controls that may be an alternative cause of the increased daytime napping seen. Of note there was no difference in total sleep time, sleep efficiency or wake after sleep onset between patients with PD and controls. This finding is consistent with previous studies in this area although mixed results have been reported, which may reflect issues of sample size (for review see 54). Within the PD group, those with excessive daytime napping did not exhibit less nocturnal sleep time, sleep efficiency or wake after sleep onset time.

Table 2. Descriptive, neurologic, sleep and cognitive data in Parkinson's disease patients: excessive vs. normal daytime sleep.

	Normal daytime sleep	Excessive daytime sleep	Statistic	P -value
	Mean ± SD (n=44)	Mean ± SD (n = 41)		
Age (years)	62.4 ± 7.1	67.3 ± 7.0	t = -3.3	0.001
Hoehn and Yahr	2.0 ± 0.6	2.1 ± 0.8	U = 828.0	0.485
Disease duration (years)	5.8 ± 4.7	5.8 ± 5.6	U = 825.5	0.501
Levodopa dose equivalent (mg)	675.7 ± 516.2	605.6 ± 409.3	U = 855.0	0.679
Participants taking sleeping tablets	5	7	χ^2 = 0.194	0.660
Participants with DBS in situ	2	3	χ^2 = 0.294	0.587
Average nap time per day (min)	14.9 ± 7.6	65.3 ± 34.6	U = 0.0	<0.001
Average naps per day	0.3 ± 0.2	1.0 ± 0.5	U = 56.0	<0.001
Total nocturnal sleep time (min)	446.0 ± 55.5	460.7 ± 77.1	U = 797.0	0.356
Sleep efficiency (%)	90.0 ± 5.1	91.0 ± 4.2	U = 831.5	0.800
Wake after sleep onset (min)	35.7 ± 13.3	32.9 ± 10.4	U = 793.5	0.544
Epworth Sleepiness Scale	7.8 ± 4.7	8.6 ± 3.9	U = 760.0	0.210
Beck depression inventory-II	8.8 ± 7.3	9.6 ± 6.1	U = 780.5	0.365
Mini-Mental State Examination	28.5 ± 1.8	28.4 ± 1.8	U = 877.0	0.819
Logical Memory retention (% retention)	11.0 ± 3.6	11.0 ± 3.4	U = 900.5	0.989
Digit span backwards raw score	6.9 ± 1.8	6.8 ± 1.9	U = 868.0	0.761
Verbal Fluency animals z-score	0.4 ± 1.0	-0.3 ± 1.4	U = 576.0	0.004
Trailmaking Test, Part B z-score	-0.1 ± 1.6	-0.9 ± 1.8	U = 627.5	0.016
Choice reaction time z-score	0.2 ± 1.4	-0.5 ± 1.4	U = 641.0	0.022

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Table 3. Parkinson's disease patients: Epworth sleepiness scale (ESS) positive v Epworth sleepiness scale (ESS) negative.

	ESS positive	ESS Negative	Statistic	P -value
	Mean ± SD (n=33)	Mean ± SD (n = 52)		
Age (years)	64.5 ± 6.6	65.0 ± 8.0	t = 0.3	0.783
Hoehn and Yahr	2.2 ± 0.7	1.9 ± 0.6	U = 573.0	0.006
Disease duration (years)	6.9 ± 5.1	5.3 ± 5.2	U = 667.0	0.085
Levodopa dose equivalent (mg)	858.9 ± 434.4	500.0 ± 439.1	U = 458.0	< 0.001
Participants taking sleeping tablets	5	8	χ^2 = 0.001	0.977
Participants with DBS in situ	1	4	χ^2 = 0.793	0.373
Average nap time per day (min)	43.4 ± 37.0	36.6 ± 34.1	U = 762.5	0.389
Average naps per day	0.6 ± 0.5	0.7 ± 0.5	U = 783.5	0.500
Epworth Sleepiness Scale	12.5 ± 2.6	5.3 ± 2.6	U = 0.0	< 0.001
Beck depression inventory-II	12.3 ± 7.5	7.3 ± 5.4	U = 508.5	0.002
Mini-Mental State Examination	28.2 ± 1.9	28.6 ± 1.8	U = 772.0	0.418
Logical Memory retention (% retention)	11.0 ± 3.6	11.0 ± 3.4	U = 900.5	0.358
Digit span backwards raw score	6.2 ± 1.9	7.3 ± 1.8	U = 868.0	0.020
Verbal Fluency animals z-score	-0.04 ± 1.0	0.2 ± 1.4	U = 768.0	0.417
Trailmaking Test, Part B z-score	-1.2 ± 2.0	-0.06 ± 1.4	U = 544.0	0.005
Choice reaction time z-score	-0.6 ± 1.6	0.2 ± 1.2	U = 638.0	0.047

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Within this study, the ESS could not discriminate between patients with and without excessive daytime napping that was identified with actigraphy. This result may reflect the fact that EDS and excessive daytime napping are actually separate constructs. However, several earlier studies have suggested an overlap between EDS and excessive daytime napping exists within PD cohorts [3,4,7-9]. Thus the results presented here suggest that the ESS may not be an ideal measure of all elements of daytime sleep disturbance within PD patients.

This study suggests that interventions aimed at reducing daytime sleep disturbance in PD may have additional benefits on cognition. Previously, the psychomotor stimulant modafinil has been investigated for the treatment of EDS in PD with mixed results [55-57]. Modafinil is believed to promote wakefulness by inhibiting a dopamine re-uptake and may also affect noradrenergic reuptake. Other wake promoting agents such as sodium oxybate and caffeine may act to reduce the effects of activity dependent metabolites that promote the global dampening of wake promoting structures and corresponding neurotransmitters release [58] have also been trialed in PD [59]. However, these studies did not identify patients with daytime sleep disturbance using an objective measure such as actigraphy. Rather, they used the ESS, which in this study did not identify excessive daytime napping. Therefore, future studies utilising this objective measurement may identify a target cohort of PD in which to better assess the efficacy of pharmacologic and behavioural interventions for these symptoms.

A similar pattern of cognitive dysfunction was also noted when dividing the group into those with daytime sleep disturbance on either the ESS or actigraphy despite the ESS positive group not identifying higher amounts of napping. This
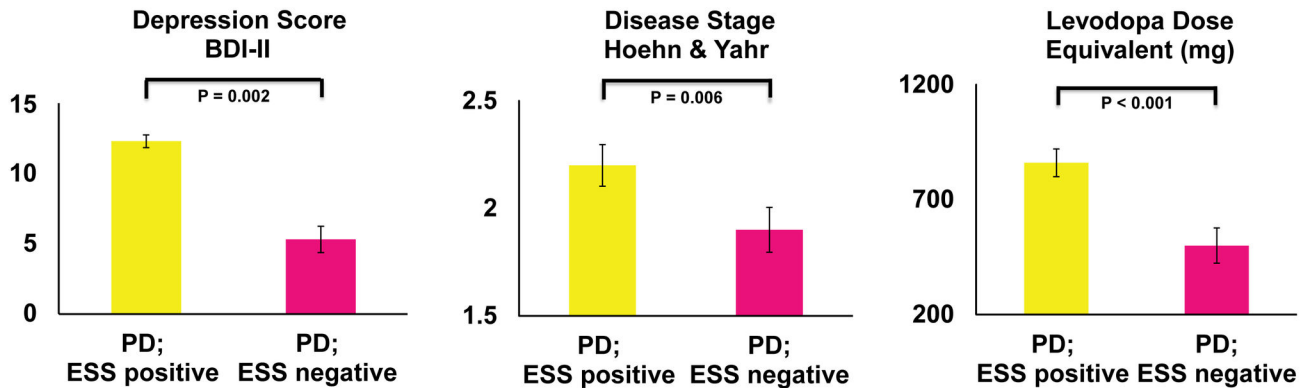
Figure 3. Mood and disease specific variables within the Parkinson's disease cohort based on subjective sleepiness scores. A chart that reports depression scores, disease stage and Levodopa dose equivalents (mean ± standard error) when patients with Parkinson's Disease are divided into those who are Epworth Sleepiness Scale positive (score ≥ to 10 indicative of a positive tendency to sleep during the day) vs. those who are Epworth Sleepiness Scale negative.
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result implies that both measurements are tapping into the same subset of patients with PD. However, patients who were positive on the ESS also had higher levels of depression and more advanced disease both of which are known to be independent predictors of cognitive dysfunction. These confounds were not identified when dividing the group based on actigraphy implying that the ESS may be affected by other non-sleep related symptoms. Establishing the reason why the ESS was not able to identify excessive napping was beyond the scope of this study. However, it may be that the ESS is tapping into the akinetic rigid phenotype of PD within which EDS, excessive daytime napping, depression and cognitive dysfunctions exist in varying combinations. Further studies are needed to confirm if scores on the ESS confounded by concomitant problems associated with the sleep disturbance in PD such as depression and more advanced disease.

Results from this study suggest a common pathology linking excessive daytime napping and specific domains of cognitive function in PD. However, putative mechanisms explaining this link have not been elucidated. Previous studies have suggested that daytime sleep disturbance might arise from damage to wake promoting structures in the brain stem, basal forebrain and hypothalamus or corresponding deficit in wake promoting neurotransmitters. It is difficult to infer that the executive cognitive deficit seen in this study may result directly from these changes. However, pathology across thalamocortical, hypothalamocortico and basalocorticoal circuitry could explain the cognitive dysfunction observed. Although there was no evidence of sleep debt that could explain the link between cognition dysfunction and excessive napping seen in patients with PD, this study was not able to exclude poorly consolidated sleep as a cause for these results. Further studies using power spectral analysis of polysomnography will help determine if the cognitive deficit seen in these results correlates with a specific deficit of sleep microarchitecture.

In animal models, adenosine and other activity dependent metabolites have been shown to facilitate both global dampening of cortical activity in addition to directly inhibiting wake promoting structures such as the cholinergic pedunculopontine nucleus [11]. Given metabolic byproducts could be increased in an oxidative stress model of PD [60], it is possible that this neurochemical process may be contributing to the link between excessive daytime napping and cognition observed in this study. Calmodulin dependent kinase II has been shown to play a causal role in cognitive and motor deficits in animal models of PD. This chemical is critical to establishing NREM sleep architecture and is also linked to synaptic plasticity and learning [61]. Thus, alterations in calmodulin dependent kinase II levels may provide a novel explanation of these results representing a common pathological mechanism between daytime sleep disturbance and cognitive deficit in PD [62-64].

The difference in means for these cognitive variables across the two groups ranged from 0.7 to 0.8 standard deviations and may be interpreted as modest. However, this study was not powered sufficiently to determine the effect size of the impaired cognition linked to excessive napping and this represents a limitation of the study. Further studies measuring excessive napping measured prospectively are needed to determine this effect size and the impact the cognitive deficit has on functional status and quality of life.

The ESS does not, by its intended design have a definitive time scale over which the daytime sleep disturbance is assessed. Instead it asks participants to rate their probability of napping "in recent times". This represents a limitation when comparing the ESS with actigraphy. To minimise this limitation the ESS was administered within two weeks of completing the actigraphy. Reassuringly, studies in non PD cohorts have shown the ESS to have minimal variability over periods longer than this window [65,66]. Furthermore, although actigraphy is a validated measure of sleep, it cannot confirm the cortical EEG correlates of sleep architecture. The lack of polysomnography

also represents a limitation in this study. Finally, it is possible that actigraphy may under or over classify daytime sleep based on extra movements or a lack of movement respectively. Further studies characterising this limitation in PD cohorts are needed. Studies using daytime sleep diaries rather than the ESS may also provide better subjective measurement of daytime napping for future comparison with actigraphy.

In summary, using an objective measurement this study has confirmed that patients with PD exhibit excessive napping compared to healthy age matched controls. Conflicting results between self-report questionnaires (namely the widely used ESS) and wrist actigraphy confirm the need for more objective measurement of daytime sleep-wake disturbance. Further, those patients with PD who nap excessively during the day have greater cognitive deficits in the domains of attention, semantic verbal fluency and processing speed. These results highlight a possible interrelationship between sleep and

cognitive circuitry in PD that may represent common pathology. Further studies are now needed to evaluate the effect of prescribed napping, targeted at those with excessive daytime napping. Furthermore, given the potential for pharmacological and behavioural interventions to reduce excessive napping, trials are needed to investigate if these treatments can improve focal cognitive deficits in PD. This would have far reaching benefit to the quality of life of patients and their carers, in addition to reducing the burden of illness in the community.

Author Contributions

Conceived and designed the experiments: SJB SLN ZT RRG SJGL. Performed the experiments: SJB ZT PS SJGL. Analyzed the data: SJB SLN ZT SJGL. Contributed reagents/materials/analysis tools: ZT PS. Wrote the manuscript: SJB.

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Chapter 4

Improving the electrophysiological measurement of REM without atonia in the

diagnosis of REM sleep behaviour disorder.

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Chapter 5

Investigating REM without atonia in Parkinson's disease using the REM sleep

behaviour disorder screening questionnaire.

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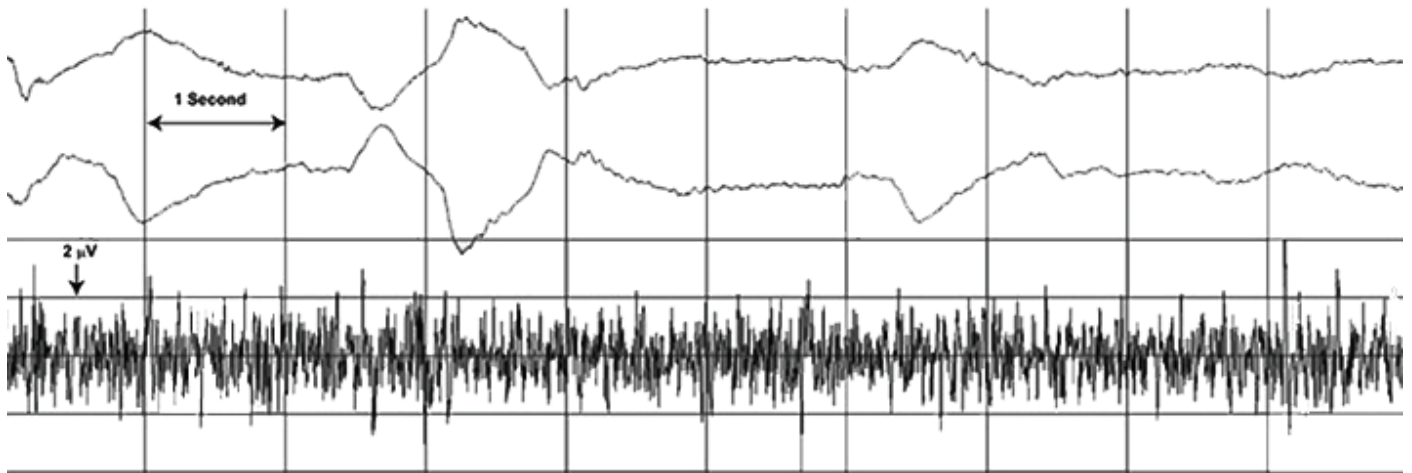
Improving the electrophysiological measurement of REM without atonia in the diagnosis of REM sleep behaviour disorder

Posted in Clinical Review Article on 20th Jan 2014

Summary points

- REM sleep behaviour disorder is frequently observed in the synucleinopathies (Parkinson's disease, Lewy Body Dementia and Multiple System Atrophy).

- Recent evidence concludes that REM sleep behaviour disorder is a biomarker heralding the future development of an alpha synucleinopathy.

- Normally, muscles should demonstrate electrical silence (atonia) during REM sleep. The demonstration of REM without atonia is critical to the diagnosis of RBD represents the electrophysiological hallmark of RBD.

- Multiple physiological tools, including visual scoring systems and automated signal processing algorithms have been developed to improve the objectivity of the measurement of REM without atonia.

- The emergence of techniques to measure REM without atonia has raised several technical questions regarding the data collection and the method in which it is analysed.

Introduction

REM sleep behaviour disorder (RBD) is frequently observed in the synucleinopathies (Parkinson's disease, Lewy Body Dementia and Multiple System Atrophy). Within Parkinson's disease cohorts RBD is observed in more than half of all patients with PD[1] and has been linked with the akinetic rigid phenotype of PD[2], visual hallucinations[3], selective cognitive deficits[4] and dementia[2]. As such accurate screening and diagnosis is essential for managing the comorbidity associated with RBD in PD[5]. In addition, the emergence of RBD in later life can represent a pre-motor feature heralding the development of synucleinopathy and may thus have utility as a future biomarker[6]. Recent studies have suggested that almost all patients with idiopathic RBD will develop a neurodegenerative disorder, most probably an α synucleinopathy, if they live long enough[7, 8]. The predominance of synucleinopathies was also reported by Boeve et al 2013 in a clinicopathological study of 172 cases of RBD[9].

The diagnosis of RBD is based on patient history in conjunction with the demonstration REM without atonia noted in the surface EMG of the mentalis muscle during polysomnography[10]. Given the importance in accurately identifying RBD, multiple physiological tools have been developed to improve objective diagnosis. Specifically these tools have been focused on the measurement of REM sleep without atonia (RWA). Normally, muscles should demonstrate electrical silence (atonia) during REM sleep, thus RWA represents the electrophysiological hallmark of RBD. In addition to visual scoring systems to quantify RWA, automated signal processing algorithms have been developed to improve the objectivity of this measurement[11, 12]. This review aims to investigate the use of RWA derived from surface EMG collected during polysomnography to identify RBD in PD.

REM Without Atonia in RBD

The identification of RWA has become critical to the diagnosis of RBD. However, the precise chemical and neural mechanisms of RWA are yet to be determined. Evidence from animal models suggest a structure in the pons referred to as the subcoeruleus or lateral dorsal tegmentum is responsible for normal atonia expected in the REM phase of sleep[13]. REM atonia is proposed to be controlled through the ratio of cholinergic to aminergic tone, differentially activating kainite receptors in the midbrain[14]. Given that RBD can pre date the motor diagnosis of PD by up to 15 years[15], the regions suggested in these animal models are in agreement with structures expected to be damaged through the deposition of alpha synuclein in the pre-clinical stages of the Braak pathological staging system[16]. However, attributing RWA to a structural deficit in the brainstem raises several questions pertaining to the varying clinical phenotypes of RBD. Principally RBD

appears as paroxysmal nocturnal episodes with varying frequency and severity among patients[17]. There is some evidence that the night to night variability of RWA is relatively constant in idiopathic RBD[18], however this is yet to be confirmed within PD cohorts. Furthermore, varying severities of RWA with concomitant RBD have been reported in the literature[17]. Studies proposing cut scores for the amount of RWA consistent with RBD suggest that despite reaching an electrophysiological diagnosis of RBD, the majority of the REM sleep remains normal (atonic)[12, 19]. It may also be the case that sub-clinical RBD exists[20], in which RWA is present on polysomnography, however dream enactment behaviour is not sufficiently prominent as to impair the sleep quality of patients and their bed partners .

The heterogeneous phenotype of RBD suggests that RWA is likely to result from both structural lesions in the pons in addition to abnormalities of the cholinergic and aminergic chemicals that control REM atonia. Given these unanswered questions, it is imperative to improving the understanding of RBD that accurate objective techniques are developed to measure RWA as a continuous variable. In addition to gauging the effect of treatment, continuously variable RWA will allow accurate diagnosis of RBD necessary in the prediction of consequent neurodegenerative disorders.

Quantifying REM Without Atonia

The first method proposed to quantify RWA as a continuous variable was developed by Lapierre and Montplaisier in 1992 and was validated in a cohort of idiopathic RBD patients[12, 21]. This method evaluated tonic or baseline RWA based on abnormally high EMG signal (defined as signal greater than 2 times the baseline or greater than an absolute voltage of 10 microvolts) being present for more than 50% of each epoch of REM sleep. The EMG tonic density was calculated as the percentage of epochs of REM demonstrating tonic RWA. If the tonic EMG density was greater than 30% this was deemed suggestive of a diagnosis of RBD and resulted in a sensitivity and specificity of 73.8% and 90.0% respectively when compared to the ICSD-2 diagnostic guidelines[22]. Furthermore, a second measurement of phasic EMG density was derived based on the percentage of 2 second mini epochs containing a phasic element of REM such as rapid eye movements that concomitantly reported abnormally high EMG activity (defined as greater than 4 times the baseline signal). Phasic EMG density greater than 15% was deemed suggestive of RBD and comparing this diagnostic tool with the current guideline reported a sensitivity and specificity of 88.9% and 82.5% respectively.

The method proposed by Lapierre and Montplaisier provides an accurate tool to measure RWA as a continuous variable and diagnose RBD. However, the visual scoring system is labour intensive and still has

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a subjective element conferring possible bias. To improve this, an automated computer based algorithm was developed by Ferri et al 2008[11]. This algorithm generates a REM atonia index that grades RWA and has been validated in a mixed cohort of RBD[11] and was recently validated in PD[23]. The REM atonia index averages the EMG signal in each 1 second epoch of REM sleep and grades the epoch as normal (figure 1 panel A) or abnormal (figure 1 panel B) based on a voltage threshold (< 1 μV = normal, 1-2 μV = indeterminate, > 2 μV = abnormal). The REM atonia index is the ratio of normal to abnormal epochs of REM. This index was found to correlate closely with the visual scoring system developed by Montplaiser *et al* (2010). The REM atonia index has been further improved with a noise reduction algorithm[24] and represents an objective computational measurement of continuously variable RWA and thus a purely objective diagnosis of RBD. As such the REM atonia index represents an instrument that could be applied to at risk populations such as idiopathic RBD or those with mild cognitive impairment, to determine who will transition to PD or another synucleinopathy. This method does not divide the EMG into tonic and phasic elements and given the high accuracy reported and the close correlation with the previously described visual scoring system, questions the need to make this division.

Both of the methods described so far rely on surface EMG data from the mentalis muscle collected during polysomnography. However, it is possible that by restricting the assessment of RWA to the mentalis muscle, some patients with RWA in other muscles, specifically in the upper and lower limbs may not be detected by this approach. To counter this problem Frauscher et al 2012 evaluated tonic and phasic RWA, using criteria very similar to those proposed by Montplaisier et al 2010, in multiple muscles in the upper and lower limbs and over the sternocleidomastoid muscle[19]. This study concludes that the optimal assessment of RWA should include the measurement of tonic or phasic activity within the mentalis muscle in addition to phasic activity within the left and right flexor digitorum brevis muscle[19].
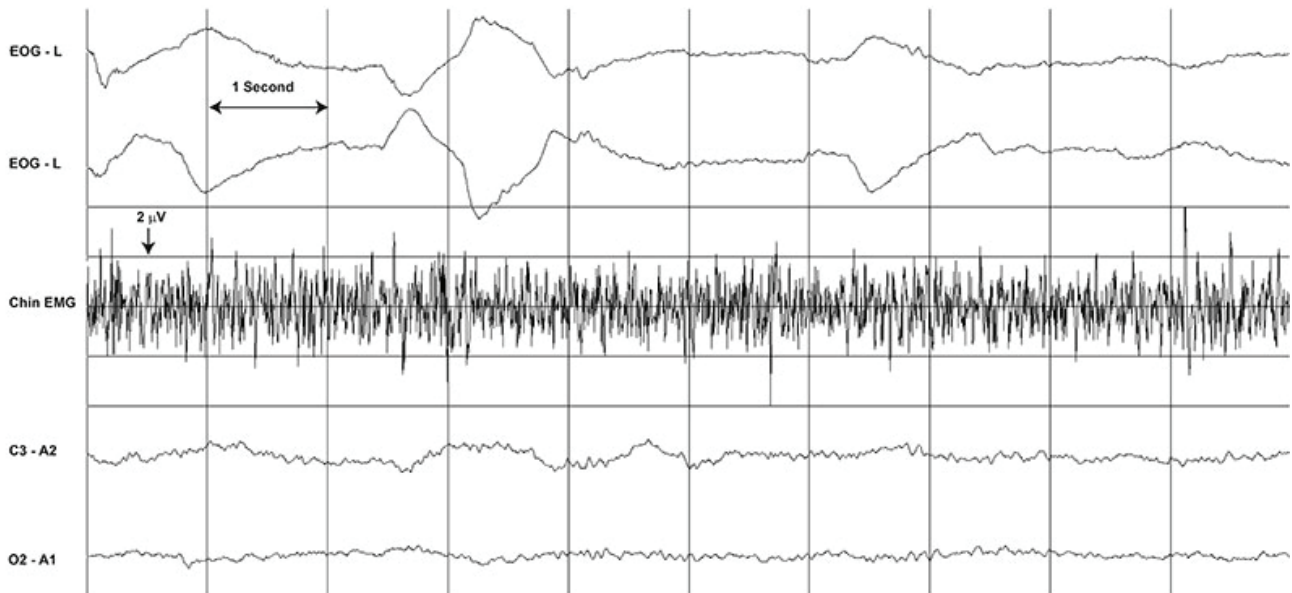
Technical difficulties in Acquiring and Measuring REM Without Atonia

Measurement techniques developed to quantify RWA have raised multiple technical questions regarding the optimal acquisition of surface EMG data, used to assess RWA. One problem raised by these techniques is the surface EMG signal is a low voltage signal susceptible to interference from snoring, breathing and other electrical noise. Furthermore, there is no agreement as to whether this signal should be assessed relative to the patient's own baseline EMG signal or whether arbitrary voltage thresholds should be applied to all participants. Studies have also deemed a variety of voltage thresholds, below which the signal is consistent with atonia. The visual scoring method proposed by Montplaiser et al 2010 determined baseline surface EMG as between 3-7 microvolts, however the method used to derive this value was not described. Conversely the automated REM atonia index described by Ferri et al 2008 reported an average EMG signal during REM less than 1 microvolt to be the threshold of normal atonia. Similarly a variety of epoch lengths have been described to determine RWA ranging from 1 to 30 seconds and there is conflicting evidence as to whether to divide the surface EMG into tonic and phasic components or to assess the signal as one. Finally, there is a lack of agreement regarding which muscle to measure the signal. All of methods described rely on the accurate scoring of REM, which is difficult in patients within PD cohorts that experience frequent arousals with high rates of obstructive sleep apnea. In order to utilise the potential of RWA both in the diagnosis of RBD and in the prediction of neurodegenerative disease, studies are needed to answer these technical questions.

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Figure 1 – An excerpt from polysomnography from which the REM atonia index can be calculated. C3-M2 and O2-M1 (electroencephalographic montage), EOG–L, EOG–R (left and right electrooculographic channels), chin EMG.
A chart depicting an excerpt from nocturnal polysomnography for 2 patients with Parkinson's disease from which the REM atonia index and RBD diagnosis can be derived. The REM atonia index averages the EMG signal in each 1 second epoch of REM sleep and grades the epoch as normal or abnormal based on a voltage threshold (< 1 µV = normal, 1-2 µV = indeterminate, > 2 µV = abnormal). The REM atonia index = % time normal/(% time normal + % time abnormal). Panel A shows a patient in which all the mini epochs have an average EMG signal < 1 µ. The REM atonia index for this patients = 100/(100+0) = 0 which is normal. Panel B show a patient where all the mini epochs have an average EMG > 2 µV. The REM atonia index for this patient = 0/(0 + 100) = 0. This is abnormal and highly suggestive of RBD.

Conclusion

The accurate screening and diagnosis of RBD is essential for reducing comorbidity in PD. Furthermore, recent evidence concludes that REM sleep behaviour disorder is a biomarker heralding the development of an alpha synucleinopathy and provides up to 15 year window in which it might be possible to intervene and prevent or at least minimise the consequences of these syndromes. The emergence of techniques to measure RWA as a continuous variable have raised several technical questions regarding the data collection and the method in which it is analysed. Developing a unified approach to the objective quantification RWA, the electrophysiological hallmark of RBD, is critical to both the diagnosis of RBD as well as the future prediction of the neurodegenerative disorders preceded by RBD. It is hoped that the more accurate determination and quantification of RWA in RBD may improve the management of patients with PD in the future.

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Conflicts of interest statement

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Chapter 5

Investigating REM without atonia in Parkinson's disease using the REM sleep

behaviour disorder screening questionnaire.

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Investigating Rapid Eye Movement Sleep Without Atonia in Parkinson's Disease Using the Rapid Eye Movement Sleep Behavior Disorder Screening Questionnaire

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ABSTRACT: Rapid eye movement (REM) sleep behavior disorder (RBD) is frequently observed in patients with Parkinson's disease (PD). Accurate diagnosis is essential for managing this condition. Furthermore, the emergence of idiopathic RBD in later life can represent a premotor feature, heralding the development of PD. Reliable, accurate methods for identifying RBD may offer a window for early intervention. This study sought to identify whether the RBD screening questionnaire (RBDSQ) and three questionnaires focused on dream enactment were able to correctly identify patients with REM without atonia (RWA), the neurophysiological hallmark of RBD. Forty-six patients with PD underwent neurological and sleep assessment in addition to completing the RBDSQ, the RBD single question (RBD1Q), and the Mayo Sleep Questionnaire (MSQ). The REM atonia index was derived for all participants as an objective measure of RWA. Patients identified to be RBD positive on the RBDSQ did not show increased RWA on polysomnography (80% sensitivity and 55% specificity). However, patients positive for RBD on questionnaires specific to dream enactment correctly identified higher degrees of RWA and improved the diagnostic accuracy of these questionnaires. This study suggests that the RBDSQ does not accurately identify RWA, essential for diagnosing RBD in PD. Furthermore, the results suggest that self-report measures of RBD need to focus questions on dream enactment behavior to better identify RWA and RBD. Further studies are needed to develop accurate determination and quantification of RWA in RBD to improve management of patients with PD in the future. © 2014 International Parkinson and Movement Disorder Society

Key Words: Parkinson's disease; REM sleep behavior disorder; REM without atonia; REM atonia index; REM sleep behavior screening questionnaire

Rapid eye movement (REM) sleep behavior disorder (RBD) is observed in over half of all Parkinson's disease (PD) patients.[1] The development of RBD has

been associated with visual hallucinations[2,3] and cognitive deficit,[4-7] which represent independent predictors for nursing home admission. As such, accurate screening and diagnosis is essential for managing the comorbidity associated with RBD in PD.[8] Furthermore, because the emergence of idiopathic RBD (iRBD) in later life[9] can represent a premotor feature heralding the development of PD,[9,10] early diagnosis may offer a window for early intervention.

Identifying REM without atonia (RWA), in the surface electromyography (EMG) leads from nocturnal PSG, has become critical to the diagnosis of RBD.[11,12] However, at present, the guidelines only confirm or exclude RWA without reference to severity, although cutoff scores have been suggested.[11,13] A recent study

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quantifying the severity of RWA is of interest because the severity of RWA appears to predict the transition from iRBD to PD.[14] Quantifying RWA could provide a measurement to grade response to treatment in addition to providing further insights into the pathophysiological mechanisms underlying RBD. However, although there are putative benefits, the utility of measuring RWA as a continuous variable is yet to be determined.

Multiple scoring systems have also been developed to improve quantification of RWA.[11,13] One such method, developed by Ferri et al., used a quantitative statistical analysis of the chin EMG during sleep to develop the REM atonia index.[15,16] This index was found to correlate closely with a scoring system developed by Montplaiser et al.[13] The REM atonia index represents a computational measurement of continuously variable RWA and an objective diagnosis of RBD. As such, the REM atonia index represents an instrument that could be applied to at-risk populations, such as iRBD, to determine who will transition to PD or another synucleinopathy.

Another instrument used to evaluate RBD is the REM Sleep Behavior Disorder Screening Questionnaire (RBDSQ).[17] This questionnaire was found to be accurate when compared with the *International Classification of Sleep Disorders*, 2nd edition (ICSD-2), guidelines.[12] An initial study in a mixed cohort found the RBDSQ to be sensitive (96%), but with poor specificity (56%).[17] High sensitivity (96%) was also reported in a cohort of PD patients.[18] However, in each of these studies, measurement of RWA as a continuous variable was not calculated. Furthermore, the initial validation study concluded that the RBDSQ was not able to identify subclinical iRBD in a cohort at risk of developing synucleinopathy.[17]

Some of the limited utility of the RBDSQ questionnaire may relate to the broad range of questions, which cover a number of frequently observed non-RBD sleep disturbances, including sleep quality, dream content, and abnormal movements. Whereas these problems may form part of the RBD phenotype in PD, they may be present in the absence of RBD. As such, these questions may have limited utility in PD cohorts or in identifying iRBD that represents preclinical PD.

To enhance the utility of questionnaires to identify RBD, a "single" screening question (RBD1Q) has been proposed.[19] A similar single question is also proposed by the RBD section of the Mayo Sleep Questionnaire (MSQ).[20] This question covers four of the individual items included in the RBDSQ, namely, items 3, 6.1, 6.2, and 6.3, all of which ask questions probing dream enactment behavior.

The current study investigated the reliability of the RBDSQ and three questionnaires specific to dream enactment behavior to correctly identify RWA in patients with PD, using the objective REM atonia index. Furthermore, using established cut-off scores, diagnostic utility was investigated. It was hypothesized that the RBDSQ would offer limited utility in identifying those patients who demonstrate pathological RWA by virtue of the high incidence of concomitant sleep disorders captured by the current questionnaire. However, it was predicted that by limiting the RBDSQ to the items specific to dream enactment behavior, the accuracy for identifying RWA would be improved.

Patients and Methods

Participants

Forty-six patients with PD were recruited from the Brain and Mind Research Institute, Parkinson's Disease Research Clinic, University of Sydney (Camperdown, NSW, Australia). All patients satisfied the UK Parkinson's Disease Society Brain Bank criteria.[21] Participants with a known or suspected diagnosis of obstructive sleep apnea (OSA) were excluded. Patients were also excluded if they had DBS.

No patient had dementia.[22] One patient was taking amitriptyline, 2 were taking mirtazapine, and 1 was taking venlafaxine. Two patients were taking clonazepam as treatment for RBD. Permission was obtained from the local research ethics committee, and all patients gave written informed consent.

Clinical Assessment

Patients were assessed in their "on" state, and levodopa dose equivalents were calculated.[23] Disease stage was rated with the H & Y scale,[24] and motor severity was scored on section III of the UPDRS (UPDRS-III).[25] Disease duration was calculated from the date of diagnosis.

Sleep Assessment

Nocturnal polysomnography (PSG) recordings were collected on two consecutive nights using a sleep-lab–based ambulatory recording system (Compumedics Siesta, Melbourne, Victoria, Australia). Night 1 was considered an adaptation night. PSG data were collected using the following montage: electroencephalographic (EEG; C3-A2, O2-M1, Fz-M1, and Pz-A2); two electrooculographic channels (left and right outer canthi) and EMG (submentalis). EEG and EMG data were sampled at 256 Hz. Sleep architecture was visually scored by an experienced sleep technician using standardized criteria.[26] The following variables were collected for descriptive purposes: total sleep duration; percentage of time in REM; percentage of time in slow wave sleep (SWS); latency to REM sleep; and wake after sleep onset (WASO).

TABLE 1. Patient demographics, disease-related variables, self-report results, and REM atonia index for 46 patients with idiopathic PD

	Mean ± SD (n = 46)
Age, years	64.6 ± 7.6
Male/female	35:11
UPDRS-III	27.3 ± 13.4
H & Y	1.9 ± 0.5
Disease duration, years	3.0 ± 3.4
Levodopa dose equivalent (equivalent units)	355.37 ± 323.9
Mini–Mental State Examination	28.5 ± 1.7
RBDSQ	6.0 ± 3.7
REM atonia index	0.90 ± 0.12
REM EMG density, %	35.5 ± 32.3
Total sleep time, minutes	399.3 ± 61.2
REM, %	20.2 ± 6.2
SWS, %	19.2 ± 12.6
REM latency, minutes	88.4 ± 51.8
WASO, minutes	83.9 ± 49.8

SD, standard deviation.

RWA

The primary measure of RWA was the REM atonia index.[15,16,27,28] A secondary analysis using visually scored RWA was also included to derive the REM EMG density as per established protocols.[13,29] Data acquired from the chin-surface EMG leads during overnight PSG was digitally filtered using a digital band-pass filter (10–100 Hz) and a notch filter at 50 Hz.[28] After application of a noise reduction algorithm,[28] the REM atonia index was calculated as per established protocols.[15] An REM atonia index cutoff score of less than 0.9 was used to indicate a diagnosis of RBD in PD.[16] REM density was based on any RWA (either tonic or phasic RWA more than 2 times the baseline), and a cutoff score of 18% was used to indicate a diagnosis of RBD.[29] All sleep studies were inspected for nonphysiological noise, and participants were excluded

if this was present. A global impression was included to approximate the ISCD-2 guideline, which consisted of those patients positive for RBD on the REM atonia index who also reported a clinical history of dream enactment.

RBDSQs

All patients completed the RBDSQ, and, in accord with previous studies, a cutoff score of 6 was used to indicate a diagnosis of RBD in PD.[18] Four questions from the RBDSQ (items 3, 6.1, 6.2, and 6.3) were identified to ask a question similar to the single RBD screening question proposed recently by Postuma et al. (RBD1Q) and Boeve et al. (MSQ).[19,20] These four questions were extracted from the full questionnaire and combined to create a RBDSQ subscore. Patients were deemed to be positive on the subscore if they answered yes to any one of these four questions (RBDSQ subscore ≥1 = RBD positive). The RBDQ1 was administered to patients with assistance from bed partners, and the MSQ was administered to bed partners.

Statistical Analyses

Statistical analysis was conducted on PASW Statistics (Version 20 for Windows). Categorical variables were compared using chi-square tests. Because other variables violated assumptions of normality, Mann-Whitney's nonparametric U tests were used. An α-value of 0.05 was used for all tests.

Results

Neurological and sleep data for patients are shown in Table 1. A comparison of the total RBDSQ and REM atonia index is shown in Table 2 (panel A). This analysis showed that the RBDSQ had a sensitivity of

TABLE 2. Questionnaires versus REM atonia index

	REM Atonia Index Positive	REM Atonia Index Negative	
A. RBD Diagnosis: REM Atonia Index Versus RBDSQ Total (n = 46)			
RBDSQ positive	12	14	PPV = 46%
RBDSQ negative	3	17	NPV = 85%
	Sensitivity = 80%	Specificity = 55%	
B. RBD Diagnosis: REM Atonia Index Versus RBDSQ Subscore (n = 46)			
RBDSQ subscore positive	14	17	PPV = 47%
RBDSQ subscore negative	1	14	NPV = 93%
	Sensitivity = 93%	Specificity = 45%	
C. RBD Diagnosis: REM Atonia Index Versus RBD1Q (n = 46)			
RBD1Q positive	15	16	PPV = 48%
RBD1Q negative	0	15	NPV = 100%
	Sensitivity = 100%	Specificity = 48%	
D. RBD Diagnosis: REM Atonia Index Versus MSQ (n = 31)			
MSQ positive	10	13	PPV = 43%
MSQ negative	0	8	NPV = 100%
	Sensitivity = 100%	Specificity = 36%	

TABLE 3. Questionnaires versus REM EMG density

	REM EMG Density	REM EMG Density	
A. RBD Diagnosis: REM EMG Density Versus RBDSQ Total (n = 46)			
RBDSQ positive	20	6	PPV = 77%
RBDSQ negative	7	13	NPV = 65%
	Sensitivity = 74%	Specificity = 68%	
B. RBD Diagnosis: REM EMG Density Versus RBDSQ Subscore (n = 46)			
RBDSQ subscore positive	23	8	PPV = 74%
RBDSQ subscore negative	4	11	NPV = 73%
	Sensitivity = 85%	Specificity = 58%	
C. RBD Diagnosis: REM EMG Density Versus RBD1Q (n = 46)			
RBD1Q positive	25	6	PPV = 81%
RBD1Q negative	2	13	NPV = 87%
	Sensitivity = 93%	Specificity = 68%	
D. RBD Diagnosis: REM EMG Density Versus MSQ (n = 31)			
MSQ positive	19	4	PPV = 83%
MSQ negative	1	7	NPV = 88%
	Sensitivity = 95%	Specificity = 64%	

80% and specificity of 55% for detecting RWA. Whereas the REM atonia index identified 15 of 18 patients with RWA, 3 of these screened negative on the RBDSQ, thus representing false negatives. Of the 31 patients that scored negative for RWA on the REM atonia index, 14 screened positive on the RBDSQ, representing false positives.

Table 2 (panel B) reports the comparison of the RBDSQ subscore with the REM atonia index. Sensitivity and specificity of the RBDSQ subscore to identify a positive REM atonia index were 93% and 45%, respectively. In this analysis, 1 of the 15 RWA-positive patients was a false negative and 17 of the 31 RWA-negative patients were false positives. When patients were asked the RBDQ1, results were identical to that of the RBDSQ subscore (Table 2, panel C). However, when the MSQ was asked to bed partners (Table 2, panel D), sensitivity increased to 100%.

The diagnosis of RBD, based on the REM atonia index and REM EMG density, was in agreement (chi square = 15.7; $P < 0.001$). Two-by-two tables comparing the RBD diagnosis from questionnaires and the visually derived REM EMG density are shown in Table 3. This analysis showed that the RBDSQ had a sensitivity of 74% and specificity 68%. Sensitivity increased to 85% when using the RBDSQ subscore and further to 93% and 95% when using the RBD1Q and MSQ, respectively.

Because all patients who were positive for RBD on the REM atonia index also reported a positive clinical history of dream enactment, the results found by dividing the group based on the global impression were no different to those found when dividing the group based on the REM atonia index alone (shown in Table 2). However, when using the visually derived REM EMG density, 1 false positive was identified.

To investigate the diagnostic discrepancy between the RBDSQ and REM atonia index, neurological and sleep variables were divided into RBD positive and RBD negative based on the RBDSQ (Supporting Table 1).

Dividing the PD cohort into RBD positive and RBD negative based on the RBDSQ did not identify a difference in RWA, as identified by the atonia index (0.94 ± 0.09 vs. 0.87 ± 0.13; U= 176; $P = 0.063$). When using the REM EMG density, the RBDSQ did identify higher amounts of RWA ($21.6\% \pm 29.1\%$ vs. $46.2\% \pm 30.9\%$; U = 126; $P = 0.003$).

When dividing the patients with PD based on the RBDSQ subscore into RBD and positive and negative groups, these groups also showed no statistical differences in demographics or disease-related variables (Supporting Table 2). However, compared with those screening negative on the RBDSQ subscore, those patients who were positive on the RBDSQ subscore exhibited a significantly greater REM atonia index (0.94 ± 0.10 vs. 0.85 ± 0.019; U = 169; $P = 0.032$). A similar result was noted when dividing the REM atonia index based on either the RBD1Q (0.97 ± 0.02 vs. 0.87 ± 0.013; U = 103; $P = 0.002$) and the MSQ (0.98 ± 0.02 vs. 0.90 ± 0.1; U = 36; $P = 0.010$). Similarly, there was statistical agreement when comparing the RBD diagnosis based on the REM atonia index, compared to dream enactment status from the RBDSQ subscore (chi square = 6.816; $P = 0.009$). To better understand the distribution of the RWA across those with and without a history of dream enactment, the REM atonia index was compared to the RBDSQ subscore in a scatter plot (see Fig. 1).

Discussion

This study is the first to compare the RBDSQ in patients with PD to an objective measure of RWA: the REM atonia index. Significantly, 46% of patients with
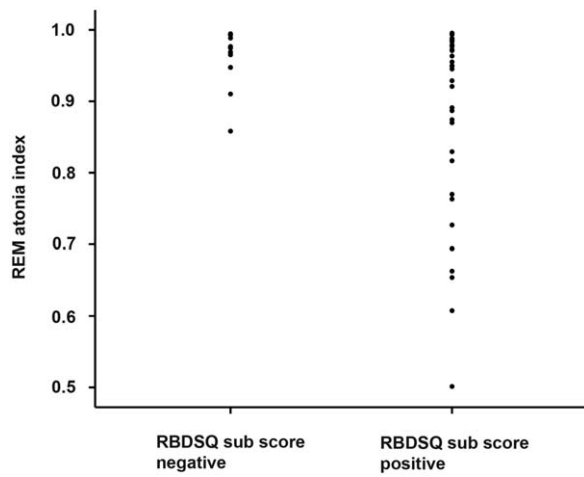
FIG. 1. REM atonia index versus RBDSQ subscore. A scatter plot depicting the REM atonia index in patients with PD who were positive for RBD on the RBDSQ subscore, compared with those who were negative on the RBDSQ subscore.

PD who were positive for RBD on the RBDSQ did not exhibit RWA, as identified by the REM atonia index. Furthermore, sensitivity and specificity of the RBDSQ, when compared to RWA, as identified by the REM atonia index, was lower than had been reported in previous studies.[17,18,30] This result highlights differences between the current diagnostic guidelines and the REM atonia index. The RBDSQ also reported excessive false-positive and -negative rates. Given that emerging evidence suggests that severity of RWA predicts development of PD, these results raise concerns about the RBDSQ's utility in predicting preclinical PD.

The low accuracy of the RBDSQ to identify RWA may be a result of patients who are asymptomatic or unaware of symptoms. Alternatively, it may be a result of problems with the REM atonia index, although the REM atonia index is simply a ratio of normal/abnormal REM atonia, and, when compared to a diagnosis made by visually scoring the surface EMG, the two methods were in agreement. However, subtle differences were identified. A higher proportion of those identified to be positive on the RBDSQ were deemed positive on the REM EMG density, albeit with lower sensitivity. Of note, both the REM atonia index and the visually derived REM EMG density have been recommended by the International REM Sleep Behavior Disorder Study Group (IRBD-SG).[31] Although beyond the scope of this study, these results suggest that direct comparisons of these two techniques is needed.

In addition to differences between the measurement techniques for RWA, these results suggest that inadequacy of the RBDSQ is, at least in part, a result of questions in the RBDSQ that tap into other problems associated with the akinetic rigid phenotype that links PD to RBD. This study found greater sleep disturbance (WASO) in those who screened positive for RBD on both the RBDSQ and the RBDSQ subscore. Several questions in the RBDSQ target general sleep disturbance, yet sleep disturbance is frequently observed in PD and not exclusively observed in those with RBD. Similarly, the RBDSQ asks a question specific to vivid dream imaging, which has been linked to visual hallucinations,[32,33] and to nonspecific nocturnal leg movements, both of which could potentially exist without RBD in PD. By removing these questions from the RBDSQ and creating a subscore based on questions specific to dream enactment, the RBDSQ subscore was able to identify differences in RWA. A higher degree of RWA was also identified in those patients who endorsed questions focused on dream enactment in the RBD1Q and the MSQ. Thus, it appears that heterogeneity within this phenotype may be cofounding the utility of the RBDSQ in the accurate identification of RWA in PD.

The importance of screening questions focused on dream enactment was also evident in the higher sensitivity when using either the RBDSQ subscore or the RBD1Q. A further increase in sensitivity to 100% was noted when the MSQ questionnaire was administered to bed partners exclusively, confirming the importance of corroborative history from bed partners to identify a clinical history of RBD. Despite the improved identification of higher RWA when the RBDSQ was limited to questions aimed purely at dream enactment, similar low specificity was noted across the questionnaires. This result highlights limitations of self-report measures in PD.[34,35] Caution must be used when interpreting the low specificity for the MSQ in view of the 15 participants who did not have bed partners available.

All patients positive for RBD on the REM atonia index also described a history of dream enactment, and thus recent work identifying asymptomatic RWA could not be evaluated in this cohort.[36] Adding the clinical history of dream enactment to the REM atonia index did not alter the diagnosis, in comparison to using the REM atonia index alone. This supports recent studies that have suggested that, at a certain severity of RWA, a purely electrophysiological diagnosis of RBD is sufficient.[11,13,15,16,28]

All the questionnaires used in this study reported high false-positive rates, confirming that a clinical history alone is not sufficient to confirm a diagnosis of RBD. However, it is possible that RWA is a paroxysmal phenomenon and that multiple nights are needed to identify RBD. A recent study in iRBD showed that the night-to-night variability of the REM atonia index measured over a period of 2.5 years was less than 20%.[27] Variability of the REM atonia index within PD patients over time has not yet been established and will form the basis of future studies. It is also possible that the cutoff score for the REM atonia index is too low. When analyzing distribution of the RWA across those with and without a history of dream enactment (Fig. 1), if the cutoff

score for the REM atonia index was increased, more subjects with a positive dream enactment history would be deemed positive on the REM atonia index. However, this would come at the expense of false negatives. It is beyond of the scope of this study to suggest modifications to improve the REM atonia index. Further multicenter studies are needed to confirm the optimal cutoff score to apply to the measurement of RWA as a continuous variable.

These results have identified varying levels of RWA with varying severity of dream enactment. Although animal models have demonstrated RWA with a single brainstem lesion,[37] other mechanisms must exist to vary both RWA and concomitant dream enactment. Quantification of RWA in PD patients may improve our understanding of the mechanisms underlying RBD and may prove beneficial in evaluation of treatments for RBD.

Patients with a history of OSA or snoring were excluded from this study to minimize false-positive RWA. Studies are needed to determine the effect of OSA and snoring artefact on the REM atonia index to understand this limitation. Furthermore, because the REM atonia index is designed to detect phasic and tonic chin EMG activity together, it is not able to detect phasic activity identified in other limbs[11] and represents a limitation of this method. Another potential limitation of the REM atonia index is interference from electrical noise in the surface EMG leads. Although a noise reduction algorithm was employed to improve signal quality, 3 participants were excluded from the study based on clearly nonphysiological noise present in the EMG trace. Studies are needed to describe the optimal data acquisition parameters and the threshold of noise, over which the REM atonia index is unacceptably affected.

In conclusion, this study suggests that the RBDSQ cannot be relied upon to accurately identify RWA in PD. This lack of accuracy may relate to some of the nonspecific questions in the RBDSQ that tap into concomitant problems in PD. By limiting this questionnaire to questions specific to dream enactment, or by using similar focused questions in the RBD1Q or MSQ, the accuracy of these questionnaires to identify RWA and RBD was improved. However, all the questionnaires investigated in this study reported alarming false-positive rates, which reiterate ongoing problems with the self-report measure in PD. It is hoped that the more accurate determination and quantification of RWA in RBD may improve management of patients with PD in the future. ∎

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Chapter 6

Investigating the night to night variability of REM without atonia in Parkinson's

Disease.

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Brief Communication

Investigating the night-to-night variability of REM without atonia in Parkinson's disease

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ABSTRACT

Objectives: Rapid eye movement (REM) sleep behaviour disorder is frequently observed in Parkinson's disease and is characterized electrophysiologically by the absence of atonia during REM sleep. However, the night-to-night variability of REM sleep without atonia is yet to be determined in Parkinson's disease.
Methods: Using polysomnography, this study measured the variability of REM sleep without atonia across two consecutive nights, using the REM atonia index in 38 patients with Parkinson's disease.
Results: The intraclass correlation coefficient between the REM sleep atonia index across two nights was 0.816 ($F = 9.795$, $p < 0.001$) and the difference between the two nights was 4.7% (standard deviation (SD) 8.2).
Conclusion: The REM atonia index demonstrated low variability across two consecutive nights of PSG. Furthermore, the diagnosis of REM sleep behaviour disorder based on this electrophysiological marker and other clinical variables was in agreement across the two nights.

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1. Introduction

Rapid eye movement (REM) sleep behaviour disorder (RBD) is gaining attention in Parkinson's disease (PD) cohorts both as a co-morbidity [1] and as a potential biomarker predicting the development of PD in at-risk populations [2–4]. The demonstration of REM without atonia (RWA), where muscles should be electrically silent, is essential for the diagnosis of RBD [5,6]. Furthermore, the importance of measuring RWA as a continuous variable has been demonstrated in a recent study, which reported that the severity of this objective measure predicted the development of PD in cases of idiopathic RBD [7].

The International Classification of Sleep Disorders (ISCD)-2 guideline for the diagnosis of RBD requires the presence of RWA in addition to either 'Sleep related injurious or potentially injurious disruptive behaviours by history' and/or 'Abnormal behaviors during REM sleep documented on polysomnogram (PSG)' [5]. Two different techniques, the automated REM atonia index [8] and the visually scored REM electromyograph (EMG) density [9,10], have

been proposed by the International REM Sleep Behaviour Disorder Study Group (IRBD-SG) as alternatives to satisfy the RWA requirement in this guideline [6]. Previously, these two methods were noted to be closely related [11]. Using the REM atonia index, the night-to-night variability of RWA in idiopathic RBD was reported to be relatively low [12]. However, the degree of night-to-night RWA variability in PD has yet to be confirmed. This study aimed to measure the variability of RWA across two consecutive nights of polysomnography (PSG) in 38 patients with PD. It is believed that establishing this variability will improve the understanding of the electrophysiological contribution to the diagnosis of RBD.

2. Methods

2.1. Participants

Thirty-eight patients with PD were recruited from the Brain & Mind Research Institute (BMRI), Parkinson's Disease Research Clinic, The University of Sydney. All patients satisfied the UK PD Society Brain Bank criteria [13]. All participants with a known or suspected diagnosis of obstructive sleep apnoea (OSA) were excluded, including any participant who had previously had continuous positive airway pressure (CPAP) prescribed or who had greater than mild OSA on a previous diagnostic sleep study. Patients were then asked

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three screening questions to identify snoring, nocturnal snorting or gasping or a history of nocturnal apnoea and were excluded if these were present. Patients with deep brain stimulation were also excluded.

No patients were demented [14] or had a diagnosis of major depression. Nine patients were unmedicated, 11 were on levodopa monotherapy, six were on dopamine agonist monotherapy and 12 were on levodopa plus an adjuvant (dopamine agonist, catechol O-methyltransferase [COMT] inhibitor or monoamine oxidase [MAO] inhibitor). Six patients without RBD were taking antidepressants (one amitriptyline, one mirtazapine, one venlafaxine, one duloxetine, one fluoxetine and one citalopram). One patient diagnosed with RBD was taking an antidepressant (citalopram). One patient was taking clonazepam as treatment for RBD and one patient was taking temazepam to aid sleep. Permission was obtained from the local research ethics committee and all patients gave written informed consent.

2.2. Clinical assessment

Patients were assessed in their 'on' state and levodopa dose equivalents were calculated [15]. The disease stage was rated on the Hoehn and Yahr (H&Y) scale [16] and motor severity was scored on section III of the Unified Parkinson's Disease Rating Scale (UPDRS-III) [17]. The disease duration was calculated from the time since disease diagnosis.

2.3. Sleep assessment

Nocturnal video PSG recordings were collected on two consecutive nights using a sleep-laboratory-based recording system (Compumedics Siesta, Melbourne, VIC, Australia). The PSG data were collected using the following montage: electroencephalographic (C3-A2, O2-M1, Fz-M1 and Pz-A2), two electrooculographic channels (left and right outer canthi) and electromyogram (sub-mentalis). Electroencephalographic (EEG) data were sampled at 250 Hz and EMG data were sampled at 256 Hz. Sleep architecture was visually scored by an experienced sleep technician using standardized criteria [18]. As the attenuation of the EMG signal could not be used to corroborate REM, REM was scored using the cortical EEG, electrooculograph (EOG), 'saw tooth' waves and an absence of vertex sharp waves, spindles, and K complexes. Total sleep time (TST), REM percent, slow wave sleep (SWS) percent, latency to REM and SWS, and wake after sleep onset (WASO) were reported.

2.4. REM atonia index

Data acquired from the chin-surface EMG leads during overnight PSG were digitally filtered (band pass 10–100 Hz, notch 50 Hz) [19]. The REM atonia index averages the EMG signal in each 1-s epoch of REM sleep and grades the epoch as normal or abnormal based on a voltage threshold (<1 μV = normal, 1–2 μV = indeterminate and >2 μV = abnormal). The REM atonia index is the proportion of normal and abnormal mini-epochs that are deemed normal [11]. A noise reduction algorithm was applied to the REM atonia index [19]. For each mini-epoch of REM, this algorithm determines the mini-epoch with the lowest average voltage in a 60-s moving window around the mini-epoch of REM and estimates this is a measure of noise. The minimum value in this moving window is subtracted from each mini-epoch of REM before it is graded as normal, abnormal or indeterminate [19]. An REM atonia index cut-off of <0.9 was used to indicate RWA in PD [8]. The ICSD-2 guideline was used to confirm the diagnosis of RBD, which included fulfilling the RWA threshold recommended in the IRBD-SG consensus statement [5,6]. The effect of variability in RWA on the diagnosis of RBD, according to the IRBD-SG, was evaluated between night 1 and night 2 [6]. The variability

between night 1 and night 2 was calculated as the percentage difference of the mean values from the two nights.

2.5. Statistical analyses

Statistical analysis was conducted on SPSS Statistics Version 21 for Windows. Categorical variables were compared using a chi-squared test. Non-parametric Wilcoxon signed-rank tests were used for the comparison of related variables across the two nights. An intraclass correlation coefficient (ICC) was calculated for comparison of the REM atonia index between night 1 and night 2. An α value of 0.05 was used for all tests.

3. Results

Of the 38 patients with PD in this study, 10 patients fulfilled the diagnostic criteria for RBD [5]. The average age was 63.4 years (standard deviation (SD) 7.6) and there was no evidence of global cognitive deficit with an average mini–mental state examination (MMSE) score of 28.5 (SD 1.6). The average disease duration was 2.8 years (SD 3.2), and the disease stage (H&Y) and motor severity (UPDRS III) were 1.9 (SD 0.5) and 25.8 (SD 12.5), respectively. The mean levodopa dose equivalency was 334.9 mg (SD 311.7).

The comparison of the REM atonia index between night 1 and night 2 is shown in Fig. 1. The ICC between these two nights was 0.816 ($F = 9.795$, $p < 0.001$), and the ICC for participants with and without RBD was 0.619 ($F = 4.001$, $p = 0.025$) and 0.617 ($F = 4.331$, $p < 0.001$), respectively. The difference in the REM atonia index between the two nights was 4.7% (SD 8.2). The classification of RWA between the nights according to the IRBD-SG recommendations was in agreement ($\chi^2 = 8.919$, $p = 0.003$) (Table 1) [6]. A total of 10 participants were identified to have an abnormal REM atonia index on either night. Four of these participants had an abnormal REM atonia index on both nights, two were abnormal only on night 1, and four were abnormal only on night 2. The REM atonia index for all participants is shown in Fig. 1. All of the 10 patients who met the threshold for RWA recommended in the IRBD-SG consensus statement on either night also fulfilled the diagnostic criteria for RBD set out in the ICSD-2 [5].

The first night of PSG reported a shorter TST (night 1 369.5 ± 79.0 vs. night 2 403.3 ± 61.3, $Z = -2.785$, $p = 0.005$) and longer REM latency (night 1 98.9 ± 56.7 vs. night 2 81.8 ± 45.4, $Z = -2.647$, $p = 0.008$). Other sleep variables were not different.

4. Discussion

This is the first study to demonstrate the night-to-night variability of RWA in PD. Our results demonstrate that the REM atonia index was highly correlated between the two nights of PSG (ICC 0.816, $p < 0.001$). Furthermore, the percentage variation in the REM atonia index was low (4.7%).

The low RWA variability indicated by our results suggests that observed first-night effects (shorter TST and longer REM latency) had a minimal impact on the measurement of RWA. Repeating this

Table 1
REM without atonia threshold: night 1 vs. night 2 ($\chi^2 = 8.919$, $p = 0.003$). A two-by-two table comparing the classification of REM without atonia (RWA) across the two nights according to the REM atonia index threshold of <0.9 being consistent with significant RWA.

	Night 2 REM AI positive	Night 2 REM AI negative
Night 1 REM AI positive	4	2
Night 1 REM AI negative	4	28

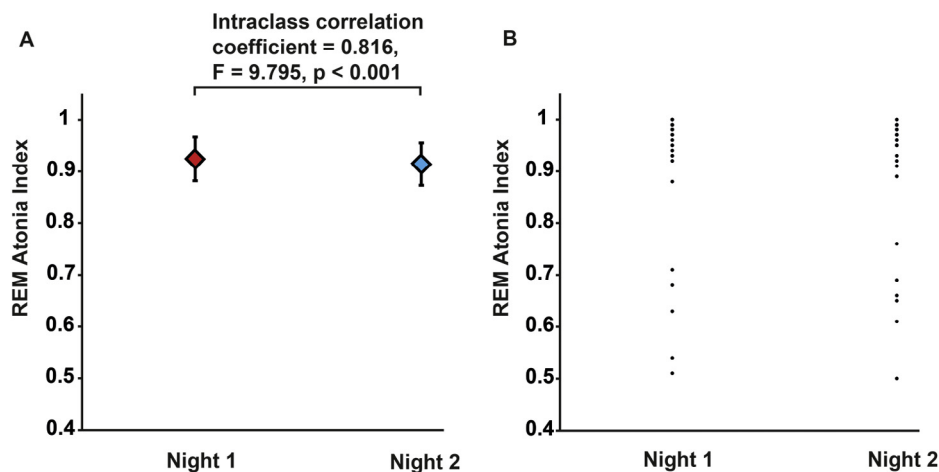*Abbreviation:* REM AI, Rapid eye movement atonia index.

Fig. 1. Panel A – a graph comparing the REM atonia index derived from the first and second night of PSG in 38 patients with PD (mean ± 95% confidence interval). Panel B – a chart depicting the REM atonia index for all individuals calculated for night 1 and night 2.

study with an adaption night could further characterize the observed first-night effects. These results suggest that objectively quantifying RWA electrophysiologically may provide a more convenient and robust marker of RBD than capturing a paroxysmal episode of florid dream enactment during video PSG.

In addition, the low variability of the REM atonia index implies that at least some degree of RWA is irreversible. This finding may support the existence of a pathological defect in the region of the pons, which has been proposed in animal models postulated to explain REM atonia. However, the percentage change observed in RWA between the two nights implies that mechanisms do exist to vary the severity of RWA.

When applying cut-off scores to the REM atonia index, the variation in RWA identified did not statistically alter the classification of RWA according to the IRBD-SG consensus statement across the two nights [6]. However, for six participants, meeting the threshold for RWA changed between the two nights. As a small variation in REM atonia index could alter fulfilling the IRBD-SG consensus statement recommendations for significant RWA [6], further studies are needed to better understand if this change relates to genuinely variable degrees of RWA or if this discrepancy relates to errors in data acquisition. These results suggest that the proposed REM atonia index cut-off value used in this study to establish abnormal RWA is not always reliable. Future studies are needed to define the optimal cut-off score for the REM atonia index in PD.

Repeating PSG is likely to increase the identification of RWA and subsequent diagnosis of RBD, in view of patients who record insufficient REM sleep on one night of PSG. Further studies in larger cohorts could also quantify the effects of antidepressant medications on RWA, given these medications are implicated in altering sleep architecture and causing RBD [20]. Another significant issue for pursuing this diagnostic approach is interference from electrical noise in the electrically susceptible surface EMG leads used in recording RWA. Although a noise reduction algorithm was employed to improve signal quality, studies are needed to describe the threshold of noise over which the REM atonia index is unacceptably affected. Furthermore, although this study excluded established or suspected OSA based on clinical history, the PSG montage did not include sufficient respiratory parameters to excluded subclinical OSA, which is a limitation of the study.

In conclusion, the REM atonia index demonstrated low variability across two consecutive nights of PSG in PD. These findings imply that the objective REM atonia index may be a useful biomarker on which to base a diagnosis of RBD and to monitor future treatment responses. Furthermore, by applying the established cut-off score to the REM atonia index to establish a significant threshold of RWA, this electrophysiological marker was in agreement across the two nights. However, the infrequent variability was evident. Further studies are needed to better understand if this relates to paroxysmal degrees of RWA, errors in data acquisition, or in the definition of an optimal cut-off score for the REM atonia index in PD.

Conflict of interest

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: http://dx.doi.org/10.1016/j.sleep.2014.08.007.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.sleep.2014.08.007.

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Chapter 7 - Discussion

Discussion

This thesis presents four empiric experiments, which execute objective measurement techniques in the exploration of sleep-wake disturbance in Parkinson's disease (PD). Innovative methods were utilised for the first time in patients with PD, including serial salivary melatonin levels, wrist actigraphy to assess daytime napping and signal quantification from nocturnal polysomnography. These novel techniques confirmed the presence of significant sleep-wake disturbances in PD operating across the circadian, homeostatic and ultradian sleep systems. In each case, the limitations of self-report measures were reported. Beyond the premise that improved objective measurements will minimise bias, these studies were also able to confirm the coexistence of sleep-wake disturbance with troublesome non-motor symptoms, such as cognitive decline and mood disturbance. The demonstration that coincident mood disturbance and cognitive deficits appear to frequently coexist with disorders of sleep-wake regulation provides novel insights into potentially common mechanisms that may underpin bidirectional causality. However, the precise nature of these neural and chemical correlates will require further investigation utilising the techniques demonstrated in this thesis. Finally, this thesis demonstrates a number of possible approaches that could be applied to at risk cohorts to predict the emergence of PD in pre-motor stages of the disease, identifying those patients who might benefit most from future disease modifying therapies.

Serial salivary melatonin measurement combined with actigraphy, as demonstrated in Chapter Two, was confirmed as a robust, non-invasive circadian marker. In this study, dopaminergic-treatment profoundly increased the secretion of melatonin in patients with PD. Circadian phase abnormalities, identified in previous studies, were

not replicated. The work presented here demonstrated a novel finding, namely that patients treated with dopaminergic therapy have an uncoupling of the circadian system and sleep regulation. This uncoupling suggests a possible link between dopamine and the regulation of the pineal gland. Indeed, previous animal studies have reported dopamine receptors in this region (1) but other work has suggested that the release of serotonin and melatonin from the pineal gland is controlled by a newly identified circadian receptor (2). This receptor is made up of parts of an adrenergic and dopamine D4 receptor combined together to form a new hybrid receptor (2). Further studies are required to explore this novel receptor and its role in the circadian system. Currently, the pattern of neuronal loss and neurotransmitter deficits giving rise to reported circadian sleep disturbance in PD are not well understood. Dopaminergic and non-dopaminergic pathology across the brainstem, basal forebrain, hypothalamus and frontostriatal pathways are likely to be implicated. Further contributions from circadian structures include the suprachiasmatic nucleus (SCN) in the hypothalamus and melatonin secretion from the pineal gland. Future studies that utilise objective circadian measurements like the ones presented in this thesis, could be combined with dopaminergic manipulation (e.g. On vs. Off treatment studies) to more directly record the impact of this neurotransmitter. Similarly, manipulation of other neurotransmitter systems (e.g. serotonergic via dietary restriction protocols (3)) could also probe these influences. Finally, prospective clinicopathological studies could also reveal critical insights by exploring patterns of cell death and receptor change.

Sleep disturbances generally increase over the duration of disease, which of course is often mirrored by increasing doses of dopaminergic therapy. Therefore, the finding of circadian decoupling in patients on treatment may underpin disturbances in sleep-wake regulation operating through alterations in melatonin secretion. For example, insomnia is more prevalent in patients with a longer disease duration who are more likely to be taking higher doses of dopaminergic therapy. An over stimulated melatonergic system from dopamine replacement therapy as suggested by this thesis, may bring about a similar result to patients taken off dopamine, treated with endogenous melatonin. A comparative study based on chapter two, comparing patients on and off dopamine and melatonin therapies, would explore this hypothesis. This would further elucidate the uncoupling of the circadian system and sleep regulation observed in this thesis.

Future studies using daytime melatonin sampling could help determine if the putative hypnotic properties of melatonin are implicated in the excessive daytime sleepiness seen in PD, which has previously been attributed to a more direct effect of dopaminergic medication acting on the wake and sleep promoting centres. Clock genes are implicated in the pathogenesis of circadian disruption in PD (4). Using methodology demonstrated in Chapter Two in combination with measurement of the products of clock genes from buccal mucosal swabs would explore this hypothesis (5). A greater understanding of these mechanisms will inform future pharmacological and non-pharmacological approaches, such as bright light therapy and exercise, combined with objective circadian measurement to help improve quality of life for patients and their carers.

Chapter Three of this thesis presented results from wrist actigraphy, a previously validated objective and inexpensive measure of daytime sleep, applied for the first time to record the duration and correlations of excessive daytime napping in PD. In doing so, these results provide a robust non-invasive measure, that could be implemented in the community as part of a future research effort to identify the associations of this troublesome problem. Patients with PD reported a significantly greater number of nap bouts, as well as time spent napping in the day, compared to healthy age-matched controls. Patients with PD who exhibited excessive napping through the day had poorer performance on neuropsychological tests probing fronto-subcortical function, including set-shifting, semantic verbal fluency and processing speed. Furthermore, the results from this study highlight that the commonly used self-report Epworth sleepiness Scale (ESS), which has been used extensively in previous studies of EDS in PD, did not accurately identify objective napping in PD. Of particular concern and importance for designing future studies were the results from the present study that suggested the ESS inadvertently identified cognitive decline and mood disturbance rather than the intended assessment of EDS as manifested by napping. Given the high frequency in which these symptoms exist in patient with PD, this result raises questions as to the accuracy of self-report measures in PD cohorts in general. Novel techniques demonstrated throughout this thesis would therefore seem needed to compliment self-report measures.

The results discussed in Chapter Three suggest that interventions aimed at reducing daytime sleep disturbance in PD, may have additional benefits on cognition. However, previous studies of wake-promoting drugs in patients with PD and EDS, have reported disappointing results (6-8). However, these previous studies utilised

the ESS. Future studies utilising actigraphy may offer a more reliable way of assessing the efficacy of pharmacological and behavioural interventions for this symptom.

The reputed link between reduced cognitive performance and excessive napping contrasts with studies that have utilised 'prescribed' napping to restore executive performance on tasks such as reaction time and symbol digit substitution. These combined observations highlight the possibility that increased napping might represent a compensatory neurobiological strategy to a primary neuropathological insult, rather than playing a causative role in cognitive deficits.

Disruption of brainstem and basal forebrain structures is believed to underpin many of the sleep-wake disorders in PD and may also represent the pathophysiological link to mood dysfunction and cognitive decline. Structures affected early in PD such as the dorsal raphe nucleus, locus coeruleus and pedunculopontine nucleus have known functions across these domains (9). Furthermore, these circuitry disruptions may be compounded by impaired sleep architecture where there is an inability to clear activity dependent metabolites (10). This could lead to a 'dampening' of cortical activity with direct inhibition of wake promoting structures (11). This would be further amplified by the increased oxidative stress processes that have been proposed in PD (12, 13). These insights do potentially allow for targeted interventions. For example, the commonly prescribed anti-glycaemic medication metformin, may reduce oxidative stress in PD (14) whereas specific nocturnal dopaminergic dosing may reduce cardinal symptoms known to disrupt sleep (15, 16). Medications which inhibit wake promoting chemicals include istradefylline (17, 18), an adenosine

antagonist similar to caffeine (19), or flumazenil a GABA antagonist (20). Studies evaluating these medications are needed in cohorts of PD with actigraphically confirmed EDS as demonstrated in this thesis. Ultimately, a more precise understanding of the neural and chemical correlates of sleep-wake disorders in PD would potentially allow new approaches for restoration of the normal sleep architecture. Clinical studies using the techniques identified in this thesis will better inform this knowledge, such as prescribed daytime napping monitored by wrist actigraphy to aid the clearance of wake dependent metabolites.

The emergence of REM sleep behaviour disorder in later life is the most reliable prodromal marker for the future development of PD and other synucleinopathies. The objective REM without atonia (RWA) EMG biomarker is the electrophysiological hallmark critical to the diagnosis. Chapters Four and Five reported two studies that utilised the REM atonia index, an automated signal processing technique, to quantify RWA in a cohort of patients with PD. The REM atonia index has the additional benefit of being a continuous variable, wherein it allows the severity grading of RWA, which offers prognostic qualities. The studies presented in this thesis were the first to apply this algorithm outside of the group that developed it, and in doing so, validated this technique through comparison with accepted visual scoring methods. At present, the diagnosis of RBD requires both clinical and electrophysiological criteria. Further studies are required to determine if there are thresholds for RWA over which a purely electrophysiological based diagnosis RBD could be made. With improved home based sleep studies this could provide an automated screening process in at risk individuals. Furthermore, the significance of asymptomatic RWA remains uncertain. Whether this phenomenon is a normal variant or a prodromal marker of the future

development of a neurodegenerative disorder is yet to be determined. This could be evaluated with large cohort studies of older adults, using the REM atonia index.

Chapter Four of this thesis was the first study to compare the widely used REM sleep behaviour disorder screening questionnaire (RBDQ) in patients with PD, to the REM sleep without atonia (RWA) index. Similar results were noted to those in Chapter Three, with discrepancies between the subjective self-report questionnaire and the objective biomarker. The inadequacy of the RBDSQ is at least in part due to questions that tap into other symptoms, such as visual hallucinations. The questionnaires assessed in this study reported high false-positive rates, confirming that a clinical history alone is insufficient to confirm a diagnosis of RBD, whereas a history focused purely on dream enactment offers a higher diagnostic utility.

Until the publication of the study reported in Chapter Five, the night-to-night variability of RWA as a continuous variable was unknown in PD. This study concluded that the night- to-night variability is minimal, suggesting that RWA is a robust electrophysiological variable in the diagnosis of RBD. However, for multiple participants in this study, meeting the threshold for RWA changed between the two nights. Thus the study reported diagnostic uncertainty using the different guidelines currently used for the diagnosis of RBD. Future studies are needed to form a consensus on the ideal diagnostic threshold for RWA in the diagnosis of RBD. The application of an automated RWA, as demonstrated in these two studies, would offer potential for future studies confirming idiopathic RBD as a pre-motor feature heralding the development of PD as well as being an objective measure for symptomatic trials. Reliable and accurate methods for identifying RBD may thus offer

a window for early intervention in disease modifying trials and a robust measure to determine symptomatic improvement rather than relying on subjective questionnaires that can fail to quantify RBD.

This thesis applies the objective measurement of sleep-wake disturbances, through a series of empiric experiments to help inform our understanding of these critical symptoms in PD. These innovative methods and devices were applied for the first time in cohorts of patients with PD, compared against previously validated self-report questionnaires. The work presented here confirms the significance of sleep-wake disturbances across the circadian, homeostatic and ultradian sleep systems in PD. The interaction of sleep-wake disturbance, other non-motor symptoms and cardinal motor symptoms was also explored. In each case, significant limitations of self-report questionnaires were confirmed. This thesis therefore proposes that objective measurements will be critical in future studies to explore the neurobiology of sleep-wake disturbances and to better identify any bidirectional causality linking frequently coincident mood disturbance and cognitive deficits. The application of the techniques demonstrated in this thesis would allow more accurate outcome measures for trials of symptomatic therapy. Finally, this thesis demonstrates non-invasive methods and devices, which could be applied to at risk cohorts to predict the emergence of PD and to monitor therapeutic response in future disease modifying therapies.

Given our ageing population, the need for diagnostic, predictive and sensitive monitoring biomarkers in Parkinson's disease has never been greater. Objective, accurate and reliable measurement techniques, as demonstrated in this thesis, underpins further research in this field.

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Appendix A – Editorial of Chapter 5

Diagnosing REM Sleep Behaviour Disorder in Parkinson's Disease Can We Avoid

the Polysomnography.

Postuma RB

Movement Disorders 2014

EDITORIAL

Diagnosing REM Sleep Behavior Disorder in Parkinson's Disease—Can We Avoid the Polysomnogram?

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Rapid eye movement (REM) sleep behavior disorder (RBD) is emerging as a key non-motor manifestation of Parkinson's disease (PD). Persons with RBD talk, cry out, punch, or kick in response to the content of their dreams.[1] In addition to potential injury and sleep disruption (especially to spouses), RBD has other implications in PD. It is the strongest clinical predictor (or prodromal marker) of future PD and other synucleinopathies. This implies that RBD patients are the ideal candidate population for neuroprotective trials; treatment can start early enough to meaningfully intervene and before symptomatic PD medications confound assessments. Moreover, once a patient has PD, RBD may identify a disease subtype that is characterized by more severe autonomic abnormalities, gait dysfunction, and dementia.[2-4] So, diagnosing RBD in PD has both treatment and prognostic implications.

The gold standard of diagnosis of RBD is an overnight polysomnogram, particularly to document loss of REM atonia (the necessary substrate of dream enactment behavior). Polysomnography also rules out mimics such as obstructive sleep apnea and non-REM parasomnia. Overnight polysomnography, however, is time consuming, expensive, and often resisted by patients, particularly those with concomitant anxiety or insomnia. If diagnosis could be made on history alone, this would have major practical advantages.

To enable history-based diagnosis, numerous questionnaires have been developed to screen for RBD. They range in complexity from single questions, to 13-item severity screening scales.[5-9] Although generally designed as screens, they could potentially make the diagnosis if sufficiently reliable; so, how good are they?

To answer this question, Bolitho et al[8] explored diagnosis of RBD within PD by comparing four different screening questionnaires with two different overnight polysomnographic REM atonia measures. The four questionnaires were the 13-item RBD screening questionnaire (RBDSQ)[9]; a four-item dream enactment subscore of the RBDSQ; the single-question, caregiver-administered Mayo Sleep questionnaire[11]; and the single-question, patient-administered RBD1Q.[7] This is an important contribution, because this is the first study to compare screening methods head to head. The two REM atonia quantification techniques were visual scoring (labor intensive, but generally considered the gold standard) and automatic software detection (much less labor intensive, but with reliability less established). Their findings suggested that caution is needed in RBD diagnosis.

Compared with the gold-standard (visually scored electromyography), sensitivities of the four questionnaires ranged from 74% to 95% (the lowest was for the RBDSQ), and specificities ranged from 58% to 68% (the lowest was for the RBDSQ four-item subscore). The RBD1Q and Mayo Sleep Questionnaire, had the best sensitivity/specificity combinations (93%/68% and 95%/64%, respectively). Thus, the Mayo and RBD1Q questionnaires both performed better and were simpler; so they emerged, at least from this study, as the screening procedures of choice. It would be of interest to see whether other screens, such as the 13-item RBD-HK[6] or the five-item Innsbruck REM Sleep Behavior Disorder Inventory[9] can perform better than the single-question screens in head-to-head comparison.

Regardless of the questionnaire used, there appears to be a specificity problem with screening for RBD. Does this imply that clinical history is inaccurate? Before drawing this conclusion, one must look more closely.

To start, can one question the gold standard? There is night-to-night variability in polysomnography, and sensitivity can vary according to which electromyography leads are assessed (chin vs. chin and limb combined). Consequently, studies in idiopathic RBD

suggest that gold-standard, visually scored polysomnography can miss up to 20% of true RBD cases.[12] This may be worse in PD if the polysomnographic measures validated in idiopathic RBD translate imperfectly to PD. So, some *false positives* in this study may actually have had true RBD. Of note, the specificities in this study were all low, but sensitivities were generally high. The first response to such a finding should be to check the cutoff for the gold-standard scales; if the cutoff is made stricter, then sensitivity decreases but specificity increases, and perhaps a better balance may be achieved. Of note, specificity was much worse when data were compared with the automatically determined atonia index (as low as 36% for the Mayo Sleep Questionnaire). Other alternate cutoffs may be possible,[13] and setting the cutoff higher may improve specificity without overly compromising sensitivity.

However, if the findings from this study are correct and RBD screens are nonspecific, then what are the implications? First, there are research considerations. As mentioned above, numerous studies suggest that RBD is an important marker of disease subtype.[2] However, this is not found in all studies. In general, studies that used polysomnographic confirmation of diagnosis have found more differences between groups than those that relied upon history alone. Perhaps non-specificity of questionnaires with resulting misclassification bias explains this variation; therefore, studies investigating associations between disease subtypes and RBD should not rely upon questionnaires and should use polysomnographic confirmation if possible.

Second, this finding introduces a note of caution into our clinics when diagnosing RBD; must we perform a polysomnogram in all PD patients with suspect RBD? If so, then the implications for patient burden and health care resources are considerable; PD is the second most common neurodegenerative disorder, and between one-third and one-half of patients with PD have RBD. However, there are reasons to think that careful clinical history can suffice in most cases. First, additional questioning by an experienced clinician should be able to outperform questionnaire screens; for example (unlike non-REM parasomnia), during an episode, RBD patients will not walk, respond to bed-partner intervention, or interact with their non-immediate environment (eyes are typically closed). Moreover, RBD episodes predominate in the latter part of the night. Absence of snoring or arrests in respiration also provides evidence against apnea (although only weakly so). Second, unlike idiopathic RBD with its 1% to 2% prevalence, RBD within established PD is common—so moderate specificity still translates into a reasonably likely correct diagnosis. Third, whereas a diagnosis of idiopathic RBD has very high stakes (ie, a new diagnosis of probable prodromal PD), the stakes are lower in already established PD. Many patients have mild symptoms of dream enactment and may not need treatment; observation with simple bed safety measures may suffice. Treatment response is usually robust and can also help confirm the diagnosis (note, however, that clonazepam treats both RBD and non-REM parasomnia). Therefore, outside of research settings, it may be reasonable to diagnose RBD in PD empirically and to investigate further only if treatment response is atypical.

To conclude, what can we take home from this study? First, simple, single-question screens seem to work as well as longer ones. Second, no screening questionnaire can be fully trusted; further clinical history for positive screen results plus polysomnographic confirmation in some may be needed. Third, it is clear that the ideal way to clinically diagnose RBD is not yet defined; there is plenty of room to improve. ∎

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Appendix B

Rapid Eye Movement Atonia Is Note Rapid Eye Movement Sleep Behavior Disorder

Fantini ML, Figorilli M, Ferri R.

Movement Disorders 2014

Reply: Unified objective techniques are needed to standardise the diagnosis of REM sleep behaviour disorder.

Bolitho SJ, Grunstein RR, Naismith SL, Mehelam K, Lewis SJG.

Movement Disorders 2014

Rapid Eye Movement Atonia Is Not Rapid Eye Movement Sleep Behavior Disorder

We have read with interest the paper by Bolitho et al.[1] recently published in *Movement* Disorders, assessing the ability of four screening questionnaires to correctly identify rapid eye movement (REM) sleep without atonia (RSWA) in patients with Parkinson disease (PD). We also agree with Dr. Postuma's editorial[2] about the importance of comparing different screening tools "head to head."

However, we believe that the validity of the study results might be significantly limited by some problematic aspects. RSWA is a crucial feature of REM sleep behavior disorder (RBD); however, it only represents one of the diagnostic criteria,[3] and its exact extent has not yet been defined, especially in patients with RBD and PD. Nevertheless, authors implicitly assume equivalence between RSWA and RBD, and surprisingly enough, do not provide any data about the diagnosis of RBD according to standard criteria (eg, International Classification of Sleep Disorders, 2nd edition[4] at the time of the study). Given these premises, measures of sensitivity and specificity of the different screening questionnaires could not be obtained.

But even assuming "equivalence" between RSWA and RBD, the study found an unexpectedly high rate of false-positive RBD diagnosed by questionnaire (eg, subjects who scored positively on the questionnaire but did not have RSWA), and a virtual absence of false-negative (subjects who are unaware of their RBD but who exhibit RWSA). This is very surprising, in light of studies showing that 18% of PD patients without a history of dream-enacting behaviors actually have RSWA or video-behavioral RBD manifestations[5] or reporting a sensitivity of only 33% for the clinical interview for RBD in PD patients.[6] According to our and others' experience, detection of RSWA in unaware PD patients, especially those without a bed partner, is not uncommon. This raises some concerns about the methodology used to assess sleep in these patients. First, Bolitho et al.[1] do not mention how they scored REM sleep stage in this population (eg, allowing the presence of muscle tone during REM sleep in all subjects). Second, but even more

critical, they provide no data about video-recorded behaviors during nocturnal polysomnography, which are an essential part of the diagnostic criteria according to the ICSD-2.[4] Actually, the authors stated that sleep was assessed with ambulatory polysomnography without mention of concomitant video-recording. If this is true, the calculation of RSWA appears to be at least problematic. Indeed, authors need to explain how they could correctly assess REM sleep epochs in RBD patients, and especially how they differentiated EMG changes related to RBD episodes from those attributable to normal arousals during REM sleep, body position changes, cough, wakefulness, and so forth. To what extent was EMG activity related to RBD episodes included or excluded from their calculation of RSWA? Conversely, if video-recording was performed and carefully inspected, the authors should give details of RBD episodes observed in questionnaire-positive and -negative RBD patients.

In conclusion, we believe that the mere comparison of results of two sets of parameters (questionnaires and RSWA), both of which are not perfect indicators of a disorder (RBD), cannot be performed without a clear and sound clinical diagnosis of the disorder itself, following established standard criteria. The evident lack of information and the probable impossibility of establishing such a firm diagnosis (with the data available in the paper) make these results not conclusive. ∎

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Reply: Unified Techniques Are Needed to Diagnose REM Sleep Behavior Disorder

We are grateful for the opportunity to respond to the letter from Fantini and colleagues regarding our recent paper.[1] The objective of our study was to identify *"...if the RBD screening questionnaire and 3 questionnaires focused on dream enactment were able to correctly identify patients with REM without atonia [RWA]."* The suggestion that we *"implicitly assume the equivalence between RSWA and RBD"* fails to acknowledge this objective. Electrophysiological and clinical data were combined to establish the diagnosis of REM sleep behavior disorder (RBD).[2] At no point in our manuscript is an electrophysiological diagnosis of RBD suggested to be the gold standard, and as such we find ourselves aligned with the sentiments of Fantini and colleagues.

Fantini and colleagues raised a concern regarding the lack of asymptomatic RWA identified. However, establishing this incidence was not our objective. This would require a cohort powered to represent the phenotypes of Parkinson's disease (PD). Establishing the video correlates of RWA was also not an objective. Video polysomnography was undertaken. However, contrary to what is stated in the letter of Fantini and colleagues, video-recorded behaviors are not an essential part of the International Classification of Sleep Disorders – 2nd edition (ICSD-2) RBD diagnostic criteria. Rather, patients need to exhibit "Sleep related injurious or potentially injurious disruptive behaviors by history" or "Abnormal R [REM] behaviors documented on polysomnogram."[3] Furthermore, the International REM Sleep Behavior Disorder Study Group (IRBD-SG) recommends quantification of RWA.[2] All participants who met the threshold for RWA exhibited "Sleep related injurious or potentially injurious disruptive behaviours by history" and as such video correlates would not contribute further to their diagnosis.

Rapid eye movement atonia cannot be used to identify REM sleep in patients with RBD. However, REM sleep can be identified by other characteristics.[4] Arousals were excluded visually when deriving the REM electromyogram density and automatically using the REM atonia index. Possibly REM periods would have been longer in our study because of the inclusion of periods before the first rapid eye movement and less prescriptive REM off criteria.[5] If, as suggested, this resulted in REM being scored incorrectly in periods of non-REM sleep, this would result in more patients with asymptomatic RWA. However, no asymptomatic patients met the RWA threshold. Identifying REM remains a source of error specific to manually scored sleep.

In conclusion, our study independently recreated techniques designed to measure RWA. These techniques were used to assess whether self-report measures accurately identify RWA. Using criteria set out in the IRBD-SG consensus statement, the accuracy of these self-report measures was estimated. All participants in this study had their polysomnogram PSG and quantification of RWA conducted as per published standards. We suggest that any potential discrepancies raised by our results are entirely consistent with the need for an improved, unified objective diagnosis of RBD. Our position echoes the final statement in the recent editorial by Postuma stating that "it is clear that the ideal way to clinically diagnose RBD is not yet defined; there is plenty of room to improve."[6] ∎

Samuel J. Bolitho, MBBS,[1] Ron R. Grunstein, PhD,[3] Sharon L. Naismith, DPsych,[2] Kerri Melehan, MRespSc,[3,4] and Simon J.G. Lewis, MD[1]

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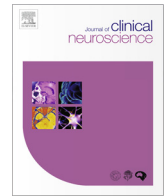
Appendix C

Gunn DG, Naismith SL, **Bolitho SJ**, Lewis SJG. Actigraphically-defined sleep disturbance in Parkinson's disease is associated with differential aspects of cognitive functioning. Journal of Clinical Neuroscience 2014; 21(7):1112-1115

Naismith SL, Hickie IB, Terpening Z, Rajaratnam SMW, Hodges J, **Bolitho S**, Rogers NL, Lewis SJG. Circadian misalignment and sleep disruption in Mild Cognitive Impairment. Journal of Alzheimers Disease 2014; 38(4): 857-866

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Clinical Study

Actigraphically-defined sleep disturbance in Parkinson's disease is associated with differential aspects of cognitive functioning

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A B S T R A C T

The frequency of sleep disturbance and cognitive impairment in Parkinson's disease has led to the suggestion that these processes might share common neural circuitry. This study aimed to identify the relationships between measures of cognitive functioning and an objective measure of sleep disturbance. Ninety-five patients with idiopathic Parkinson's disease and 48 healthy controls underwent neurological and neuropsychological examination. They wore an actigraphy watch for 2 weeks, from which a measure of nocturnal sleep efficiency was calculated. Multiple regression models showed that working memory and verbal memory consolidation were significantly associated with sleep efficiency, as well as education and age. By contrast, verbal fluency and attentional set-shifting were not associated with sleep efficiency, after accounting for age and education. These findings reveal that nocturnal sleep disturbance in Parkinson's disease is associated with specific cognitive difficulties, rather than a global pattern of cognitive dysfunction. This may in part reflect common neural underpinnings.

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1. Introduction

Advances in our understanding of sleep have demonstrated that this behaviour is critical for memory consolidation [1] and optimal neuropsychological functioning [2]. The frequent combination of cognitive impairment and sleep disturbance that is seen across many neurodegenerative diseases has led to the suggestion that these processes might be underpinned by disruptions in common neural circuitry [3].

Sleep-wake disturbance is particularly common in Parkinson's disease (PD) with approximately two-thirds of PD patients reporting this feature [4]. There is a considerable range of sleep-wake disturbances in PD, which can manifest as rapid eye movement (REM) sleep behaviour disorder (RBD), hypersomnolence, sleep-disordered breathing and insomnia (for a review see Gunn et al. [5]). Of significance, sleep-wake disturbance in PD has been linked to a range of cognitive and psychiatric complaints [6–8], increased carer burden [9] and reduced quality of life [10], warranting efforts to delineate its pathogenesis.

While few studies have examined the relationship between sleep and cognition in detail, our research group recently conducted a study using self-report questionnaires of sleep-wake disturbance in PD and noted differential patterns of deficit [11]. Specifically, greater nocturnal sleep disturbance was associated with impaired working memory and memory consolidation. In

contrast, excessive daytime somnolence was associated with slowed processing speed and reduced attentional set-shifting, and RBD symptoms were correlated with both working memory and verbal fluency. These differential patterns suggest that anatomically distinct pathophysiological changes may underpin specific profiles of sleep and cognitive complaints. Thus, further elucidating these relationships may help to identify the associated pathological substrates and allow for development of more targeted pharmaceutical interventions or individually tailored cognitive training programs [5,12].

Building on this notion, only one previous study has used actigraphy to explore the relationships between sleep disturbance and cognition in PD to our knowledge. In a sample of 35 PD patients this study found that sleep efficiency (described as total sleep time minus wake time divided by the time in bed multiplied by 100) was associated with motor symptom severity, increased dopaminergic mediation and male sex [13]. A further analysis of this sample found that poorer sleep efficiency was associated with reduced attention/executive functioning but not memory or psychomotor speed [14]. Several authors have highlighted the considerable phenotypic variation across variables such as age of onset, motor symptom severity, cognition and mood disturbance that exist within PD [5,15,16].

Disease heterogeneity in PD is likely to impact on sleep-wake disturbances and influence sleep efficiency. Therefore, the present study sought to evaluate whether actigraphically-defined sleep efficiency is associated with specific patterns of neuropsychological functioning in PD accounting for a range of potentially confounding demographic and disease variables.

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2. Methods

2.1. Participants

Ninety-five patients (58 men, 37 women) were recruited from the Brain & Mind Research Institute PD Research Clinic, University of Sydney, Australia. All patients satisfied the United Kingdom Parkinson's Disease Society Brain Bank criteria and were deemed unlikely to have dementia [17] or major depression according to the Diagnostic and Statistical Manual version IV (DSM-IV) [18] criteria by consensus rating of a neurologist (S.J.G.L.) and a neuropsychologist (S.L.N.). Exclusion criteria included dementia diagnosis; neurological disease other than PD (for example epilepsy); psychosis; prior stroke or head injury (with loss of consciousness >30 minutes); diagnosis of obstructive sleep apnoea; or inadequate English for neuropsychological assessment. Thirty-four patients were on levodopa monotherapy, nine were on dopamine agonist monotherapy, and 52 were on levodopa plus an adjuvant agent (such as dopamine agonist, catechol-O-methyl transferase [COMT] inhibitor or monoamine oxidase [MAO] inhibitor). Twenty-two patients were taking antidepressants and 13 were taking benzodiazepines. A further 48 (27 men, 21 women) age-matched volunteers were recruited as healthy control subjects after being screened for neurological and psychiatric disease. All participants scored $\geqslant$24 on the Mini Mental State Examination (MMSE) [19]. Permission for the study was obtained from the University of Sydney Human Research Ethics Committee and all patients gave written informed consent.

2.2. Clinical assessment

All neurological and neuropsychological assessments were conducted within one session to confirm study eligibility. Patients were assessed in their "on" state with the motor section of the Unified Parkinson's Disease Rating Scale (UPDRS-III) [17] and levodopa dose equivalents were calculated for dopaminergic medication [20]. Disease stage was rated on the Hoehn and Yahr scale [21], disease duration was calculated as the time (years) since disease diagnosis, and depressive symptoms were self-rated using the Beck Depression Inventory-II (BDI-II) [22]. The REM Sleep Behavior Disorder Screening Questionnaire (RSBDSQ) [23] was used to assess for the presence of RBD with a cut-off score of 6 or greater suggestive of RBD [24]. Question six of the clinician rated Non-Motor Symptom Scale [25] was used to assess PD patients' severity and frequency of symptoms of Restless Legs Syndrome (RLS).

Neuropsychological functioning was assessed by a neuropsychologist using standardised tests. The MMSE was administered for reporting purposes. Working memory was assessed using the Digit Span Backwards subtest (raw score) of the Wechsler Adult Intelligence Scale-III (WAIS-III) [26]. The Logical Memory subtest of the WAIS-III was used to assess the consolidation (Logical Memory % Retention) of verbal material [27]. Verbal Fluency was assessed via the Controlled Oral Word Association Test (letters F, A, S) [28]. Executive functioning was examined using the Trailmaking Test. Part A was subtracted from Part B so that the score represented the pure attentional set-shifting component of Part B (TMTB–A, seconds) as has previously been reported [11].

2.3. Actigraphic assessment

Wrist-worn actigraphy has been demonstrated to be a reliable method to assess sleep disturbance in PD patient samples [29]. Measurement of sleep-wake disturbance was conducted according to previously established protocols [30]. Following clinical assessment, participants were required to complete a sleep diary and wear a wrist actiwatch (MiniMitter Actiwatch Spectrum, Koninklijke Philips Electronics, Amsterdam, Netherlands) on their least severe disease side arm for 14 consecutive days. Actigraphy rest intervals were calculated using Actiware 5.0 software (MiniMitter-Respironics, Bend, OR, USA) in conjunction with manual scoring. One rest interval per 24 hour period was scored (total rest time). The primary measure of sleep disturbance was "sleep efficiency", which reflected the percentage of total time spent "asleep" during the rest interval, calculated as [(total rest time - — wake after sleep onset)/(total rest time)] × 100]. Actigraphy also recorded the average variability in sleep onset and offset, which was used to measure the robustness of the sleep-wake cycle over the 14 trial days.

2.4. Statistical analyses

Statistical analysis was conducted on using the Statistical Package for the Social Sciences version 20 (SPSS, Chicago, IL, USA) for Windows (Microsoft, Redmond, WA, USA). Between-group comparisons used independent samples t-tests. Univariate correlations were conducted using Pearson correlation coefficients. To determine the relative contribution of sleep efficiency to neuropsychological functioning, multiple regression was used. Four backward elimination regression models (method = backward) were constructed with Digit Span Backwards, Logical Memory % Retention, Verbal Fluency and TMTB–A as dependent variables. In addition to sleep efficiency, age, education, BDI-II depression score, disease duration, UPDRS-III and dopamine dose equivalent were included as independent variables in the model to account for the potential impact that these variables may have on cognition. Semi-partial correlations were used to determine the "unique" (that is, the variance that was attributed only to that predictor, and not shared) variance of significant predictors.

3. Results

Demographic, cognitive and sleep disturbance variables are presented in Table 1. The PD group and the healthy controls did not differ with respect to age, sex, education or MMSE score. With respect to the cognitive variables, the PD group performed significantly worse on measures of verbal memory consolidation (Logical Memory % Retention, $t = -2.0$, $p = .047$) and set-shifting (TMTB–A, $t = 4.3$, $p < .001$). There were no differences between patients and controls on any of the actigraphy variables or self-report measures of sleep, other than the PD group reporting more symptoms of RBD (RSBDSQ total, $t = 5.9$, $p < .001$).

3.1. Analysis of sleep disturbance and neuropsychological functioning

Healthy controls showed a significant inverse relationship between age and sleep efficiency ($r = -.390$, $p = .007$) but no correlations were observed between sleep efficiency and any other demographic, cognitive or sleep variable.

By contrast, for the PD group, univariate correlations identified that actigraphically-defined sleep efficiency was significantly correlated with Digit Span Backwards ($r = .279$, $p = .006$) and Logical Memory % Retention ($r = .234$, $p = .023$) but not with Verbal Fluency ($r = .103$, $p = .318$) or TMTB–A ($r = -.132$, $p = .206$). In addition, sleep efficiency in the PD group was significantly correlated with disease duration ($r = -.258$, $p = .012$) and dopamine dose equivalent ($r = -.436$, $p < .001$). Table 2 shows the relationships between the cognitive variables and the potential confounding variables age, education, BDI-II score, disease duration, UPDRS score and levodopa dose equivalent.

Table 1
Demographic, neuropsychological and sleep disturbance data of Parkinson's disease patients

	Mean (SD)		t/χ²
	PD (n = 95)	Healthy controls (n = 48)	
Age, years	64.6 (7.8)	65.1 (9.5)	−.3
Sex, male:female	58:37	27:21	.4[a]
Education, years	13.9 (3.1)	14.1 (3.1)	−.5
Hoehn and Yahr, stage	2.0 (0.7)	–	–
Disease duration, years	5.3 (5.5)	–	–
UPDRS motor score	23.8 (11.4)	–	–
Levodopa dose equivalent, mg	594.5 (489.4)	–	–
RSBDSQ total score	5.47 (3.6)	2.0 (1.5)	5.9**
NMSS restless legs total	1.5 (2.7)	–	–
Mini Mental State Exam	28.6 (1.5)	28.8	−.6
Beck Depression Inventory-II	9.4 (6.6)	4.62 (4.9)	4.0**
Neuropsychological data			
Digit Span Backwards, raw score	6.81 (1.9)	7.3 (2.5)	−1.1
Logical Memory% Retention, raw score	75.9 (19.9)	81.7 (14.3)	−2.0*
Letter Fluency (FAS), raw score	42.2 (16.8)	42.0 (12.2)	.1
TMTB–A, seconds	66.3 (53.4)	36.9 (20.7)	4.3**
Actigraphy variables			
Sleep efficiency, %	90.2 (5.1)	91.2 (3.4)	−1.4
Sleep onset, hh:mm	20:21 (6:4)	20:26 (6:4)	−.1
Sleep onset variability, minutes	65.8 (63.9)	64.0 (79.5)	.1
Sleep offset, hh:mm	7:00 (0:55)	7:10 (0:54)	−1.0
Sleep offset variability, minutes	54.0 (40.9)	53.2 (48.9)	.9

[a] χ² analysis.
* p < .05.
** p < .01.
FAS = verbal fluency test for words starting with the letters "F", "A" and "S", hh:mm = hours:minutes, NMSS = Non-Motor Symptoms Scale, PD = Parkinson's disease, RSBDSQ = REM Sleep Behavior Disorder Screening Questionnaire, SD = standard deviation, TMTB–A = Trailmaking Test Part B – Part A, UPDRS = Unified Parkinson's Disease Rating Scale.

Table 2
Correlations between neuropsychological measures and demographic variables for the Parkinson's disease group

	Digit Span Backwards	Logical Memory % Retention	Letter fluency (FAS)	TMTB–A
Age	−.134	−.215*	−.302**	.384**
Education	.295**	.221*	.329**	−.266**
Beck Depression Inventory-II	.015	−.070	−.287**	.063
Disease duration	−.032	−.082	−.232*	.181
UPDRS motor score	−.178	−.215*	−.299**	.265**
Levodopa dose equivalent	−.159	−.206*	−.143	.170

* p < .05.
** p < .01.
FAS = verbal fluency test for words starting with the letters "F", "A" and "S", TMTB–A = Trailmaking Test Part B – Part A, UPDRS = Unified Parkinson's Disease Rating Scale.

3.2. The effect of RBD, RLS and sleep-wake cycle

Forty-seven people in the PD group met criteria for RBD on the RSBDSQ using a cut-off score of 6. Comparing this group against those patients without self-reported symptoms of RBD (n = 48) revealed no significant differences on the measure of sleep efficiency (t = −.2, p = .875). Similarly, when comparing those with (n = 33) and without symptoms of RLS (n = 62), there were no significant differences in sleep efficiency (t = 1.9, p = .065). Finally, to investigate whether variability in the sleep-wake cycle of patients impacted upon sleep efficiency, comparisons were made between those patients in the upper and lower quartiles of both sleep onset and offset variability. No significant differences of sleep efficiency were found between those patients in the upper (n = 30) and lower quartiles (n = 24) of sleep onset (t = .5, p = .628) or offset (t = .1, p = .902) variability.

3.3. Relationships between sleep efficiency and cognitive variables

To determine the veracity of the relationship between sleep efficiency and cognition in PD, multiple regression analyses were conducted and included the potential confounding variables of age, education, BDI-II depression score, disease duration, UPDRS-III and dopamine dose equivalent. Note that all potential confounds were entered, even if they were not associated with sleep efficiency, since most are known correlates of cognition.

The first backward regression analysis exploring working memory revealed that only sleep efficiency (B = .10, sleep efficiency [SE] B = .04, p = .007) and education (B = .16, SE B = .06, p = .006) were significant predictors of Digit Span Backwards, overall accounting for 15.1% of variance in working memory performance (F = 8.21, p = .001). Sleep efficiency and education uniquely predicted 7.0% and 7.3% of the variance, respectively.

The second regression analysis investigating memory consolidation revealed that sleep efficiency (B = .94, SE B = .39, p = .019) and age (B = −.60, SE B = .25, p = .020) remained the only significant predictors of Logical Memory % Retention. Together, they accounted for 11.0% of variance (F = 5.61, p = .005) and uniquely predicted 5.6% and 5.5% of the variance in memory consolidation, respectively.

4. Discussion

The current study is the largest study to our knowledge to explore the association between objectively-measured sleep disturbance and neuropsychological functioning in PD. The findings both support and extend our prior work, which demonstrated differential patterns of self-reported sleep dysfunction in association with neuropsychological functioning [11]. In the former study, patients endorsing higher levels of nocturnal sleep disturbance demonstrated poorer performance on Digit Span Backwards and Logical Memory. In keeping with this finding, the current study found that an objective measure of nocturnal sleep disturbance, namely actigraphically-defined sleep efficiency, was also a significant predictor of working memory (Digit Span Backwards) and memory consolidation (Logical Memory). Importantly, these findings remained significant even when controlling for likely confounds such as age, depressive symptoms, education, levodopa dose and disease severity. By contrast, sleep efficiency did not appear to be pertinent to performance on tasks of verbal fluency and attentional set-shifting, a finding which is consistent with our prior research suggesting that dysfunction in these cognitive domains is preferentially related to daytime somnolence and symptoms of RBD, rather than nocturnal sleep disturbance.

There is a range of other factors that could potentially impact upon sleep efficiency and cognitive performance, such as level of exercise, dopaminergic and non-dopaminergic treatment, medication side effects, pain and additional underlying brain pathology such as Alzheimer's disease. However, this study explored a number of likely confounds and did not demonstrate any difference in sleep efficiency measures between those patients with and without RBD, RLS or a disrupted pattern of sleep onset and offset.

Interestingly, in the present study the PD group did not differ from the controls in terms of their average sleep efficiency. Despite this, within the PD group there existed a relationship between sleep efficiency and aspects of cognition that were not present in the control group. This finding potentially highlights the complex-

ity of sleep disturbance in PD, with particular aspects of sleep disturbance making a greater contribution to cognitive impairment in certain subsets of PD patients. Future research should attempt to delineate the unique contribution of these factors to sleep disturbance and cognition in PD.

The finding that actigraphically-defined sleep disturbance plays a role in working memory and the recall of verbal information is consistent with past research concerning sleep-dependent memory consolidation [1]. It is also in broad agreement with a recent study that correlated actigraphically-defined sleep efficiency with working memory as assessed by Digit Span [14]. The present study expands upon these observations in a larger sample of PD patients and importantly revealed that sleep efficiency in PD is associated with memory consolidation.

Overall, the results of this study suggest that sleep efficiency in PD is associated with specific patterns of cognitive dysfunction rather than a generalised reduction in cognitive functioning. Thus nocturnal sleep disturbance in PD may be impacting on cognition through dysfunction in underlying neural substrates predominantly within frontal and temporal networks. The cause of this disruption is not well understood but may include underlying pathology in the cortical projections of the suprachiasmatic nucleus–dorsomedial nucleus pathway modulating sleep-wake behaviour [5], dopaminergic [13] and non-dopaminergic treatment, nocturia and pain.

Whilst polysomnography represents the gold standard for the assessment of sleep disturbance, wrist-worn actigraphy performed over multiple nights has been confirmed as a reliable methodology that has been previously validated in PD patient samples [29]. In the absence of accompanying polysomnography we are unable to make direct comment regarding how the contribution of specific sleep staging might impact upon cognition. Examination of these relationships is clearly worthy of further research, particularly in light of data suggesting that discrete nocturnal neurophysiological events, such as sleep spindles and slow oscillations, are critical to overnight memory consolidation (see reviews by Naismith et al. [3] and Stickgold [1]). A greater understanding of the interaction between sleep and cognitive networks in PD, as well as the impact of sleep apnoea, RBD and hypersomnolence, will hopefully allow for more targeted interventions in the future.

Conflicts of Interest/Disclosures

Acknowledgements

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Circadian profiles in young people during the early stages of affective disorder

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Although disturbances of the circadian system are strongly linked to affective disorders, no known studies have examined melatonin profiles in young people in early stages of illness. In this study, 44 patients with an affective disorder underwent clinical and neuropsychological assessments. They were then rated by a psychiatrist according to a clinical staging model and were categorized as having an 'attenuated syndrome' or an 'established disorder'. During the evening, salivary melatonin was sampled under dim light conditions over an 8-h interval and for each patient, the time of melatonin onset, total area under the curve and phase angle (difference between time of melatonin onset and time of habitual sleep onset) were computed. Results showed that there was no difference in the timing of melatonin onset across illness stages. However, area under the curve analyses showed that those patients with 'established disorders' had markedly reduced levels of melatonin secretion, and shorter phase angles, relative to those with 'attenuated syndromes'. These lower levels, in turn, were related to lower subjective sleepiness, and poorer performance on neuropsychological tests of verbal memory. Overall, these results suggest that for patients with established illness, dysfunction of the circadian system relates clearly to functional features and markers of underlying neurobiological change. Although the interpretation of these results would be greatly enhanced by control data, this work has important implications for the early delivery of chronobiological interventions in young people with affective disorders.

Introduction

Over the last decade, there has been increasing interest in the relationship between the circadian system and affective disorders. Various disruptions of circadian rhythms have been described in depressive disorders, including shifts in the onset and offset of the sleep phase relative to environmental time (phase-advance or delay), as well as disruption to the endogenous release of key hormones such as melatonin and cortisol and changes in core body temperature rhythm.[1] Disruption to the rhythmicity of the systems under the control of the circadian system is likely to represent a fundamental disturbance of centrally regulated neurohormonal function, and may well underpin many of the somatic symptoms so often reported by patients with common mental disorders.[2]

There is evidence to suggest that sleep and/or circadian disturbance may be causally linked to both the emergence and persistence of affective disorders (see review by Harvey et al.[3] and Wulff et al.[4]). Indeed, this notion is well supported by studies conducted in patients with seasonal affective disorder, where circadian misalignment has been linked with the onset, extent and resolution of depressive symptoms.[5] Additionally, sleep–wake disturbance has been noted to be a prodromal, inter-episodic and prognostic feature of bipolar disorder (see review by Harvey et al.[3]). Misalignment of the circadian system relative to environmental time cues has profound affects not only on mood, but also on cognition, and

a range of other physiological systems under circadian control, including thermoregulatory, endocrinological, immunological, cardiovascular and metabolic systems.[4,6,7] Together, these data suggest that changes in the circadian system may not only represent a potential biomarker for illness onset and progression but may also be associated with adverse health and psychosocial outcomes.

The pattern of somatic features, sleep disturbance, daytime fatigue and related anxiety and depressive symptoms that often emerges throughout the adolescent period indicates the need to focus more closely on the underlying physiology of the developing circadian system.[8] Developmental changes in the sleep–wake and circadian systems are common in adolescents and young adults, with delayed sleep phase syndrome a common feature of adolescence (see review by Crowley et al.[9]). It has been postulated that changes to both circadian timing and period are explained largely by intrinsic biological drives, rather than extrinsic environmental or psychosocial factors.[9]

As altered sleep patterns may precede the onset and persistence of psychological distress in young people,[10] better characterization of these features may lead to identification of vulnerability markers that can then underpin better targeting of early interventions.[4,11] To date, there has been little attempt to characterize these features in those at high risk or during the onset phases of affective disorders.[4] Such objectives can, however, be accomplished by utilizing novel clinical staging

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paradigms seeking to identify young people in early stages of major mental disorders.[11,12] Within this framework, young people presenting for care with admixtures of anxiety and depressive symptoms are typically categorized as being within early or 'attenuated syndrome' phases as compared with those with 'established disorders' (that is, first episode of major illness or later relapsing or persisting phases).[12,13] Concurrently with the longitudinal evaluation of this model, we are testing whether there are distinct biomarkers evident at the different phases of affective illness.

The aim of the present study was to examine circadian parameters, notably the timing, secretion and synchrony of melatonin, according to the clinical stage of affective illness.[12] Specifically, we aimed to examine melatonin onset and secretion patterns in those with early 'attenuated syndromes' as compared with those with 'established disorders'. It was hypothesized that those in later stages of illness would exhibit evidence of altered circadian functioning, in comparison with those in early illness phases.

Subjects and methods

Participants. A total of 44 young individuals were recruited from services that offer specialized assessment and early intervention of mental health problems in young people (Youth Mental Health Clinic (YMHC) at the Brain & Mind Research Institute (BMRI); and *headspace*, Campbelltown, Sydney, Australia[13,14]). Inclusion criteria for this substudy were: (1) individuals aged 12–30 years seeking professional help primarily for significant anxiety or depressive symptoms; and (2) willingness to participate in overnight assessments of circadian rhythms (salivary melatonin) and sleep. Participants were excluded if they did not have sufficient English-language skills. The assessment protocol was approved by the University of Sydney Human Research Ethics Committee. Participants gave written informed consent before participation in the study.

Clinical assessments. As described elsewhere,[12,13] patients entering the mental health services were assessed and managed by medically and/or psychologically trained health professionals. In this study, an independent psychiatrist or trained research psychologist conducted a standardized clinical interview, focussing on ratings of depressive symptom severity (*Hamilton Depression Rating Scale* (HDRS))[15] as well as assessment of the detailed criteria developed for formal application of our clinical staging framework.[12] The clinical stage of affective disorder was rated by two psychiatrists with extensive clinical and research expertise in affective disorders and staging paradigms (ES and IH). Patients were rated as having either an 'attenuated syndrome' (stage 1b) or an 'established disorder' (stage 2 and above). Within this staging system, patients may also be classified as being in 'stage 1a'. However, the stage 1a group is much more heterogeneous; although they are help seeking, with mild symptoms, there is subsequently less confidence that such subjects would transition to full-threshold disorders (see Hickie *et al.*[12]). Classification at stage 2 or above depends on the recognition of depressive syndromes with more severe

features (for example, agitation, psychomotor retardation, psychotic features and additional intermittent hypomanic features). Such disorders may traditionally be classified as meeting full-threshold criteria for major depression according to a DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition) or ICD-10 (International Statistical Classification of Diseases and Related Health Problems, 10th Revision) diagnosis, and later stages are characterized predominantly by illness persistence or recurrence. Consequently, 52 and 56% of participants in our study who were classified as being in stage 1b and stage 2+, respectively, were taking antidepressant medications ($\chi^2 = 0.08$, not significant (NS)). In comparison, 15% of those in stage 1b were taking atypical antipsychotic medications as compared with 63% of those in stage 2+ ($\chi^2 = 10.4$, $P < 0.01$).

Sleep and circadian assessment. As described previously,[16] participants completed diaries and/or actigraphy for 7 continuous days and nights before circadian assessment. Habitual sleep onset (HSO) was derived on a daily basis from a combination of light and activity data, which was supplemented by diary information, and averaged across the recording period. Participants then attended the *Chronobiology and Sleep Laboratory* at the BMRI for overnight circadian assessment. Participants were asked to arrive 7 h before their HSO, to familiarize with the laboratory settings, and to ensure they were in a maintained posture for at least 30 min before the first sample being collected. As per standard dim light melatonin onset (DLMO) protocols, saliva samples were collected at 30-min intervals using Salivettes (Sarstedt, Germany) from 6 h before HSO until 2 h after HSO. That is, participants were kept awake 2 h past their HSO. At all times, while in the laboratory, participants were physiologically and behaviourally monitored under controlled conditions, with fixed light levels (<50 lx) and ambient temperature ($24 \pm 1\,^\circ$C). Participants maintained a seated posture for at least 20 min before each sample collection.

Melatonin was assayed in 200 µl saliva by double antibody RIA (Cat no. RK-DSM2; Buhlmann Laboratories AG, Schönenbuch, Switzerland) according to the manufacturer's instructions. The lowest detectable level of melatonin was 4.3 PM. The intra-assay coefficient of variation was $<10\%$ across the range of the standard curve. The inter-assay coefficient of variation was 15% at 19.5 PM and 12.3% at 177 PM. The total area under the curve (AUC) was calculated using the trapezoid method, for each participant over the entire 8-h sampling period. To determine the DLMO, the average melatonin levels of the first three sampling times was calculated and a threshold of two standard deviations greater than this value was established for each subject. The DLMO was defined as the time when the saliva melatonin level first exceeded this threshold and remained elevated for at least the next sampling time. The phase angle of entrainment was calculated by subtracting the time of DLMO from HSO (measured in min).

Self-report data. Patients were asked to complete the *Beck Depression Inventory-II* for depressive symptom severity.[17] In order to assess daytime sleepiness in everyday or social

situations, patients completed the *Epworth Sleepiness Scale.*[18] Sleepiness during the evening of DLMO assessment was rated using the *Karolinska Sleepiness Scale*[19] (range = 1 'very alert' to 9 'extremely sleepy-fighting sleep'). Ratings were obtained 3 h before HSO (KSS−3), and 1.5 h after HSO (KSS + 1.5).

Neuropsychological assessment. Within 2 weeks of laboratory assessment, a neuropsychologist administered the *Trailmaking Test Part A & Part B*[20] to assess psychomotor speed and set-shifting, respectively. Performance on this test was converted to a z-score according to age matched normative data.[21] The Rey Auditory Verbal Learning Test (RAVLT)[22] was administered to measure unstructured verbal learning. Total learning over the five trials was used (RAVLT-encoding, maximum = 75) and scores were converted to z-scores according to age- and education-adjusted normative data.[23] We specifically chose to examine these two measures as our prior work[24,25] has shown them to be sensitive to underlying neurobiological changes in depressive disorders, even in young people at early stages of illness.[26]

Statistical analysis. Data were analysed using the Statistical Package for Social Sciences (SPSS version 19, IBM, Chicago, IL, USA). For continuous data, analyses employed Pearson's or Spearman's correlations where appropriate. For analyses between stage 1b and stage 2 +, all categorical data were checked for distribution and normality. Student's t-tests were employed to analyse these data utilizing assumptions of equal or unequal variance where appropriate. All analyses were two-tailed and used an α level of 0.05.

Results

For three patients (n = 2, stage 1b and n = 1, stage 2 +), melatonin data were not observed (within the sensitivity of the assay) over the 8 h sampling period. For another three patients (n = 1, stage 1b and n = 2, stage 2 +), melatonin was detected but did not reach the threshold as required by the algorithm. Thus, DLMO and phase angle data were not available on a total of six patients. Demographic, clinical and DLMO data for the sample are presented in Table 1. There was no difference in the time of DLMO between the clinical stage groups. The stage 1b and stage 2 + groups did not differ statistically in terms of their level of subjective sleepiness 3 h before HSO and 1.5 h after HSO (KSS−3 and KSS + 1.5), nor in their levels of daytime sleepiness, as assessed by the Epworth Sleepiness Scale. As expected, patients in earlier clinical stages were significantly younger; however, there was no difference in gender distribution. There was no significant difference in HSO in the week before assessment. However, stage 1b patients had significantly greater phase angles than stage 2 + patients when their DLMO was compared with their HSO times.

Analysis of salivary melatonin AUC data for patients in stage 1b vs stage 2 + showed that those in earlier stages had almost double the melatonin concentration of those in later stages (Table 1 and Figure 1). This was apparent for the entire sample as well as for melatonin concentration 2 h before and 2 h after HSO. Additionally, after controlling for age and depression

Table 1 Demographic, psychiatric and circadian data for patients with stage 1b (n = 28) and 2+ (n = 16) affective disorders

	Stage 1b, mean (s.d.)	Stage 2+, mean (s.d.)	t-value
Age, years	20.5 (4.3)	23.2 (4.7)	−2.2*
Sex, male/female[a]	15/13	8/8	0.2
Hamilton Depression Rating scale	12.9 (6.5)	14.1 (8.3)	−0.5
Education, years	12.6 (2.7)	12.6 (2.8)	−0.4
Beck Depression Inventory[b]	18.0 (8.6)	24.8 (11.1)	−2.1*
Trailmaking Part A, z-score[b]	−0.0 (1.2)	0.2 (0.7)	−0.7
Trailmaking Part B, z-score[b]	−0.3 (1.9)	−0.4 (1.2)	0.3
RAVLT-encoding	0.27 (1.3)	−0.48 (1.4)	1.7
Epworth Sleepiness scale	6.4 (3.7)	7.1 (4.1)	−0.6
Habitual sleep onset, time	00:34 (01:27)	23:56 (01:12)	1.4
AUC, total sampled[b]	142.3 (85.7)	69.3 (60.4)	2.7**
AUC, 2 h before HSO[b]	51.5 (38.8)	20.3 (22.0)	3.3**
AUC, 2 h after HSO[b]	85.2 (62.4)	42.1 (36.5)	2.8*
DLMO, time	21:43 (01:41)	22:23 (01:37)	−1.2
Phase angle, min	169.08 (98.0)	90.0 (94.2)	2.4*
KSS−3	5.6 (2.1)	6.8 (1.6)	−1.9
KSS+1.5	7.8 (1.9)	8.0 (1.4)	−0.4

Abbreviations: AUC, area under the curve; DLMO, dim light melatonin onset; KSS, Karolinska Sleepiness Scale; RAVLT, Rey Auditory Verbal Learning Test. *P < 0.05, **P < 0.01. All test statistics are Student's t-test unless otherwise specified; [a]The χ^2 test; [b]Student's t-test with unequal variances assumed. AUC for melatonin over the sampling period (total sample), and for 2 h before and after habitual sleep onset; KSS 3 h before habitual sleep onset (KSS−3), and 1.5 h after habitual sleep onset (KSS+1.5). Note that DLMO data are missing for three patients in stage 1b and three patients in stage 2+ because of inability to detect melatonin within the sensitivity of the assay or not reaching the threshold as required by the algorithm.

severity, these analyses remained significant (total DLMO: $F_{3,36} = 4.9$, $P < 0.05$; DLMO 2 h before HSO: $F_{3,36} = 5.6$, $P < 0.05$; and DLMO 2 h after HBO: $F_{3,36} = 4.6$, $P < 0.05$).

Correlations with depressive symptoms. There was no significant association between salivary melatonin AUC and either self-reported or clinician-rated depressive symptoms for patients with stage 1b (Beck Depression Inventory (BDI), $r = −0.15$, NS; HDRS, $r = −0.15$, NS) or stage 2 + (BDI, $r = 0.12$, NS; HDRS, $r = 0.37$, NS) affective disorders.

Correlation with subjective sleepiness. For both staging groups, there was no association between subjective sleepiness 3 h before HSO, and total salivary melatonin AUC in the 3 h before HSO (KSS−3: $r = −0.17$ and $r = 0.28$ for stage 1b and 2 +, respectively). However, lower levels of salivary melatonin AUC 2 h after HSO were related to decreased levels of subjective sleepiness for those in stage 2 + only (KSS + 1.5: $r = 0.5$, $P < 0.05$ and $r = 0.29$, NS for stage 2 + and 1b, respectively).

Correlation with cognition. For those in stage 1b, there were no significant relationships between total salivary melatonin AUC and performance on tasks of psychomotor speed (*Trailmaking Part A*, $r = 0.12$, NS), new learning (RAVLT-encoding, $r = −0.04$, NS) or set-shifting (*Trailmaking Part B*, $r = −0.06$, NS). For those in stage 2 +, lower levels of salivary melatonin AUC were not associated with processing speed or set-shifting (*Trailmaking Part A*, $r = 0.46$, NS; *Trailmaking Part B*, $r = 0.32$, NS). However, as shown in Figure 2, lower melatonin levels were associated with poorer performance on a task of verbal memory encoding (*RAVLT-encoding*, $r = 0.58$, $P < 0.05$).
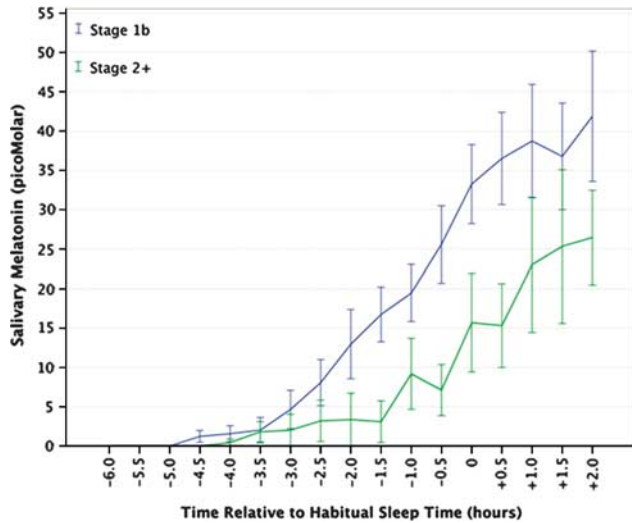
Figure 1 Graph demonstrating reduced salivary melatonin data (mean ± s.e.m.) for patients with stage 2 + affective disorders, relative to stage 1b. According to prior actigraphy monitoring, habitual sleep onset would normally occur at sample 0.
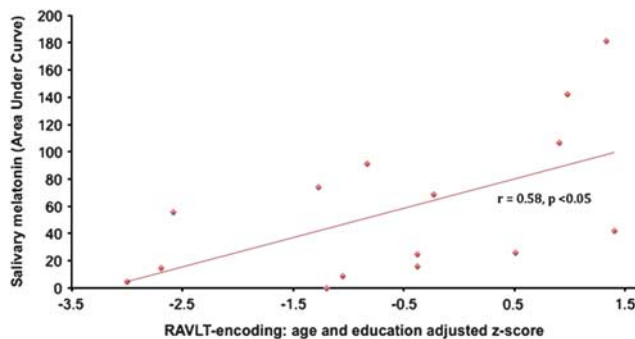

Figure 2 Scatterplot demonstrating the relationship between decreased salivary melatonin (area under curve) and memory performance (Rey Auditory Verbal Learning Test (RAVLT)-encoding trials) for patients with stage 2 + (*n* = 15) affective disorders. Note that neuropsychological assessment occurred within a 2-week timeframe and did not occur in the evening of dim light melatonin onset (DLMO) assessment.

Discussion

The present study has demonstrated that young people in later stages (that is, 'established disorders') of major affective disorders have significantly lower salivary melatonin levels assessed during the first part of the melatonin secretory period than those who are still at earlier stages of illness (that is, 'attenuated syndromes'). In turn, lower salivary melatonin levels appeared to have functional correlates as they were associated with decreased ratings of sleepiness. This finding is consistent with previous studies describing the relationship between melatonin and sleepiness levels.[27,28] The fact that this was more apparent in the time after HSO is expected, as this is when melatonin secretion would be elevated, although it is important to note that there may be more variability in melatonin levels during this time. In addition, the present study has shown that differences in melatonin secretion profiles are related to other markers of underlying neurobiology, namely neuropsychological functioning. Specifically, lower melatonin

levels during the first part of the night were related to poorer performance on the encoding component of the RAVLT. Performance on this task of unstructured verbal learning (that is, word-list learning) is commonly compromised in patients with affective disorders,[25,29] and in this sample, over two-thirds of participants performed below average. As prior work has demonstrated that poor performance may reflect dysfunction in frontotemporal, including hippocampal circuitry, these preliminary data may suggest that disturbances of the circadian system and neuropsychological dysfunction may reflect abnormalities in common underlying neural circuitry. From these data, we cannot ascertain the temporal relationship between dysfunction in the sleep and cognitive systems, as neuropsychological testing did not occur at the same time. Future studies may focus on delineating these relationships further.

Regarding circadian timing, differences were not apparent between the clinical staging groups, with DLMO occurring on average at 2130 and 2200 h for those with 'attenuated syndromes' and 'established disorders', respectively. Interestingly, for those in later clinical stages, the timing of melatonin onset and habitual sleep onset occurred within the expected 120-min period, whereas for those with 'attenuated syndromes', the difference in the timing of these two systems was ~3 h. Although it is difficult to interpret these findings in the absence of a control group and full melatonin profile, these data may suggest that the circadian pacemaker is phase-shifted (advanced) relative to timing of the sleep–wake cycle. Alternatively, the sleep–wake timing may be delayed, although it is noted that sleep onset is only weakly influenced by the circadian system, and delays in sleep may be due to a number of confounders. As we did not measure peak melatonin amplitude in our current protocol, we cannot ascertain whether there was attenuation in circadian amplitude in this group. However, this seems unlikely as those with 'attenuated syndromes' did not have reduced melatonin secretion (that is, the absolute amount of melatonin secretion was higher) relative to those with 'established disorders'. Conversely, another possible explanation for these findings could be that patients with 'established disorders' have a shortened phase angle or even a phase delay, relative to those at early stages of illness. As stated above, in the absence of a control group and full melatonin profile, further studies are now required to delineate these possibilities. It is also worth noting that a greater proportion of those with 'established disorders' were taking antipsychotic medications. Although little is known about the effect of atypical antipsychotics on circadian parameters, a preliminary study has suggested that the older 'typical' antipsychotics have disruptive effects on circadian rhythms.[30] Thus, we cannot rule out the possible contribution of antipsychotic medication to the disturbances in circadian parameters observed within this study.

In terms of underlying circadian regulation of the sleep–wake cycle, it is clear that the circadian clock in the anterior hypothalamus is critical for establishing the circadian rhythm of sleep–wake behaviour.[31] However, the suprachiasmatic nucleus itself has only minimal monosynaptic outputs to sleep–regulation centres such as the ventrolateral preoptic nucleus and the lateral hypothalamus and has no outputs to

brainstem arousal sites.[31] Thus, the weakened relationship between the sleep and circadian systems in early-stage patients (that is, as suggested by increased phase angles) may reflect alterations in a multiple range of divergent pathways. Indeed, recent models regarding synchrony of the sleep and circadian systems highlight the critical role of the dorsomedial hypothalamic nucleus. The dorsomedial hypothalamic nucleus sends a glutamatergic projection to the lateral hypothalamus (overlapping with the field of orexin-containing neurons) as well as a GABAergic projection to the ventrolateral preoptic nucleus.[31] Thus, desynchronization between the sleep and circadian system may be because of increased wake promotion via orexin neurons (resulting in a delayed sleep phase despite homeostatic pressure) or a reduction in amplitude of the circadian signal reaching the ventrolateral preoptic nucleus.[32] As the dorsomedial hypothalamic nucleus is very sensitive to self-imposed schedules, and integrates circadian signals with environmental and social factors, behavioural feedback is recognized to be a critical influence in sleep–wake patterns particularly to light exposure and meal times. In this study, we cannot attribute the findings regarding melatonin secretion and sleep timing in patients with 'attenuated syndromes' to any particular process; therefore, further empirical studies specifically examining the synchrony between these systems are now warranted.

Overall, these data suggest that with the emergence of major mood disorders in young people, fundamental changes in the sleep and circadian systems are occurring that are not merely because of pubertal development, as commonly seen in 'healthy' adolescents. Importantly, they highlight that interventions targeting the circadian system are warranted even in early phases of illness where there appears to be some degree of misalignment of the sleep and circadian systems. As more marked dysfunction in the circadian system was observed in those with 'established disorders', these data suggest that persistence of depressive symptoms may perpetuate disruptions within the circadian system. Conversely, persistent sleep–wake disturbance in young people may contribute to ongoing psychological distress, an observation that has been reported recently from epidemiological data.[10] In either case, these data indicate that differential interventions may be required for those presenting at different clinical stages. Specifically, behavioural interventions targeting depressive symptoms and sleep–wake functioning concurrently appear warranted in those with 'attenuated syndromes' and sleep–wake disturbance. In 'established disorders', clinical assessment should incorporate circadian markers (for example, melatonin, core body temperature) where possible and interventions may be much more targeted in order to address the lowered levels of melatonin likely to be observed in this group. Such agents may incorporate the use of pharmacological compounds such as melatonin, melatonin analogues or the newer antidepressants targeting both mood and sleep symptoms (see Hickie and Rogers[1] for a review). Although at this stage it is unclear whether circadian misalignment is causally linked to cognitive dysfunction, or merely co-occurs with neurobiological changes observed with the disease, it is possible that improvements in both the mood and sleep–wake systems will have broader benefits for both cognition and functional outcomes. In this regard, it is worth noting that the

decreased melatonin observed in this group was only weakly associated with depressive symptom severity, suggesting that interventions aiming to improve functioning should target more than depressive symptoms alone.

Although the current study represents the first to examine circadian rhythms in young people with emerging affective disorders, some limitations exist. First, as expected, those in later stages had more severe disorders and, consequently, greater numbers of patients had psychotic or hypomanic features, and psychotropic use was more common in this group. Second, although in the present study we measured melatonin secretion under appropriate dim light conditions, studies have shown that the timing of melatonin onset can be influenced by light exposure the prior day.[33] Thus, some variability between groups in terms of light exposure may have influenced these findings. Third, as this was not a longitudinal study, we are uncertain whether patients move from a weakened sleep–wake and circadian relationship early in the course of disorder to a more compensated shift in sleep–wake cycle (that is, with later sleep-onset (phase-delay) and resynchronizing with the circadian rhythm).

In conclusion, this study presents the first preliminary findings suggesting that melatonin may be a viable marker of affective disease progression and may assist with personalized treatment planning. Future research may extend these findings by examining the predictive utility of melatonin as a biomarker for disease progression. It may also examine whether individually tailored chronobiological interventions provide more optimal treatment outcomes for young people with affective disorders than conventional treatment approaches.

Conflict of interest

IBH has led projects for health professionals and the community supported by governmental, community agency, and drug industry partners (Wyeth, Eli Lily, Servier, Pfizer, Astra Zeneca) for the identification and management of depression and anxiety. He has served on advisory boards convened by the drug industry in relation to specific antidepressants, including nefazodone, duloxetine, and desvenlafaxine, and has participated in a multicenter clinical trial of agomelatine effects on sleep architecture in depression. He has participated in Servler-sponsored educational programs related to circadian-based therapies.

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Circadian Misalignment and Sleep Disruption in Mild Cognitive Impairment

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Abstract.

Background: While it is evident that Alzheimer's disease is associated with disturbed sleep and circadian rhythms, the extent to which such changes are evident in older people 'at risk' of developing dementia is unknown.

Objective: In this study, we aimed to determine whether patients with mild cognitive impairment (MCI) demonstrated significant alterations in the timing of melatonin secretion onset and amount, as well as sleep architecture.

Methods: Thirty patients with MCI and 28 age-matched controls underwent psychiatric, medical, and neuropsychological assessment, followed by overnight polysomnography and dim light melatonin onset assessment. Participants also performed an episodic memory task while in the laboratory. Dim light melatonin onset was computed using a standardized algorithm, and area under the curve was computed for melatonin secretion. Sleep architecture measures including wake after sleep onset and latency to rapid eye movement sleep were derived.

Results: Patients with MCI had advanced timing of their melatonin secretion onset relative to controls, but the levels of melatonin secreted did not differ between groups. The MCI group also had greater wake after sleep onset and increased rapid eye movement sleep latency. There were differential associations between dim light melatonin onset and cognition between the two groups, with earlier dim light melatonin onset being associated with poorer memory performance in MCI patients.

Conclusion: Circadian misalignment and sleep disruption is evident in patients with MCI, and is consistent with changes observed in Alzheimer's disease. Such findings could be a marker for disease trajectory, and may even be implicated in disease pathogenesis.

Keywords: Circadian, melatonin, mild cognitive impairment, salivary, sleep

INTRODUCTION

With advancing age, changes can be observed in sleep latency, quality, and consolidation along with increased insomnia, nocturnal wakefulness, and less time spent in slow wave and rapid eye movement sleep. Older individuals may also experience reduced circadian amplitude and period length in both body temperature and melatonin rhythms [1–5]. Over and above these common alterations observed as part of the 'normal' aging process, at least 40% of patients with Alzheimer's disease (AD) exhibit severe dysfunction of sleep-wake and circadian systems, manifesting clinically as sundowning, excessive daytime sleepiness, nocturnal wandering, agitation, and day-night reversal [6, 7].

Accordingly, a corpus of research over the last decade has sought to elucidate the pathophysiology of circadian rhythm alterations in patients with AD.

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Detailed examination has revealed that such patients have poor consolidation of their rest-activity rhythms, reduced circadian amplitudes, and misalignment of key circadian markers such as core body temperature and cortisol [8]. These observations have occurred in conjunction with data showing that in AD, there is neuronal degeneration of the hypothalamic suprachiasmatic nuclei (SCN) [3], the site of the major circadian pacemaker. In keeping with these observations, melatonin, the most robust marker of the circadian system, has received considerable attention (see reviews, [9, 10]). It has also been postulated that this neurohormone, a potent antioxidant that is produced predominantly in the pineal gland (as well as various peripheral nervous system sites), may play a significant neuroprotective role in the inhibition of oxidative and amyloid pathology [11–13], thus warranting efforts to identify its role at various stages of the disease. Neuropathological studies in AD have shown decreased melatonin levels in the cerebrospinal fluid, even in preclinical stages [14, 15]. Furthermore, decreased melatonin MT1 receptor expression [16] and reduced neurons in the SCN [17] have been reported, which may be related to the circadian rhythm abnormalities observed in AD.

Despite it being recognized that endogenous melatonin levels are reduced in AD, studies in which melatonin was administered to patients with AD have produced inconsistent findings. While a number of open-label and double-blind melatonin trials have shown improvements in sleep quality, nocturnal arousals, cognition, and sundowning, others have shown negative or inconclusive effects of melatonin on sleep and agitation (see reviews, [9, 12]). Such conflicting findings could be explained by factors such as the timing and dose of melatonin administration as well as by key clinical factors such as disease heterogeneity, stage and severity of disease, and by whether on an individual level, patients actually had any melatonin deficiency [18], or change in SCN melatonin receptors [16].

Relative to the number of studies examining circadian and sleep-wake disturbance in AD, there has been a dearth of such research in individuals with mild cognitive impairment (MCI), an 'at risk' or potential prodromal stage of dementia, whereby individuals' manifest cognitive decline in the context of largely preserved functioning [19, 20]. The MCI syndrome has traditionally been characterized by predominant memory deficits (amnestic MCI, aMCI), where conversion rates to AD are almost 50% over a five-year period. However, a non-amnestic subtype (naMCI) is now recognized and may be associated with various etiological underpinnings and disease trajectories [19]. While detailed data regarding sleep-wake disturbance in the subtypes of MCI is not yet available, caregiver or clinician ratings suggest that sleep-wake disturbance is evident in up to 60% of patients with MCI [21]. Importantly, sleep disturbance appears to be associated with neuropsychological dysfunction, suggesting that these features may share common neurobiological underpinnings [22]. Melatonin administration to MCI patients appears to have positive effects on sleep, neuropsychological functioning, and mood (e.g., [23, 24]). Thus, it is possible that chronobiotic interventions such as melatonin and light therapies may be most beneficial if delivered early in the disease course.

Overall, available data suggests that sleep-wake and circadian changes are evident in those with AD. By contrast, such features have not been adequately characterized in patients with MCI. We aimed to assess concurrently aspects of the circadian and sleep-wake system in older patients with MCI, relative to age-matched controls. Specifically, we aimed to determine whether patients with MCI differed from controls in terms of the timing and amount of melatonin secretion as well as some specific components of sleep architecture. We further sought to examine associations between such changes and performance on tasks of memory consolidation.

MATERIALS AND METHODS

Participants

Thirty patients aged 50 years and over and meeting criteria for MCI [20] were recruited from a specialist 'Healthy Brain Ageing' Clinic, at the Brain & Mind Research Institute, Sydney, Australia. Patients were specifically seeking assessment and intervention for their cognitive problems. Twenty-eight age- and education-matched healthy volunteers were recruited from the community via local advertisements.

Exclusion criteria were: history of stroke; neurological disorder; head injury with loss of consciousness ≥30-minutes; current major depression; at least mild depressive symptoms as evidenced by a Hamilton Depression Rating Scale score ≥8; history of psychosis or bipolar disorder; Mini Mental State Examination Score (MMSE) <24 [25] and/or diagnosis of dementia; shiftworkers; transmeridian travel within the prior 60-days; use of cholinesterase inhibitors; use of medication known to affect sleep and/or melatonin secretion including beta-blockers, lithium, thyroid replacement

therapy, or benzodiazepines. Patients taking sedative hypnotics were requested to abstain for two-weeks prior to sleep assessment. For ethical reasons, however, we did not ask patients to abstain from taking antidepressant medication. A full medical history was conducted and patients with diagnosed or suspected sleep apnea were excluded, as well as those with known eye disease (e.g., cataracts, retinopathy). Additionally, controls were screened extensively and were excluded for lifetime history of major depression and/or prior antidepressant use. All participants were required to have adequate English for neuropsychological assessment, be willing to wear an actigraphy watch and complete sleep diaries for two weeks, in addition to undergoing overnight sleep assessments. This research was approved by the Human Research Ethics Committee of The University of Sydney. Written informed consent was obtained from all participants.

Procedure

After telephone screening, all participants underwent clinical assessments to confirm study eligibility. Participants were then issued with actigraphs (Actiwatch Spectrum, Philips Respironics) and sleep diaries to complete for two-weeks, prior to undergoing the three-night circadian and sleep protocol.

Clinical assessments

All participants received a medical assessment by an Old Age Psychiatrist including risk for major sleep disorders, measurement of body mass index, and medication use. Psychiatric history was assessed using the Structured Clinical Interview for DSM-IV disorders [26]. As detailed elsewhere [22], a standardized neuropsychological assessment was conducted by a Clinical Neuropsychologist incorporating the assessment of: attention/working memory; processing speed; verbal and visual learning and memory; language; visuospatial function; and, executive function. The MMSE was administered for descriptive purposes. All assessors were blinded to sleep measurements. A clinical diagnosis of MCI was obtained using Petersen's criteria requiring cognitive decline of at least 1.5 standard deviations on at least one neuropsychological test, relative to age- and education-adjusted normative data [20]. Per criteria, each participant was required to have subjective and objective cognitive decline, but with the general preservation of function. MCI diagnoses were consensus rated by an Old Age Psychiatrist and two Neuropsychologists, based on clinical profile and neuropsychological assessment, and with reference to structural MRI scans. The broad clinical definition of MCI was further categorized into amnestic and non-amnestic subtypes [20]. In order to be categorized as aMCI, participants were required to demonstrate clear evidence of deficits in memory consolidation, which were not merely due to poor encoding.

Circadian and sleep assessment

Participants' sleep-wake behavior for the 14-nights prior to commencing the in-laboratory portion of the protocol was assessed using actigraphy (Actiwatch Spectrum, Minimitter, Philips Respironics, OR) and sleep diaries in accordance with previously published protocols [27]. Actigraphy was scored by an experienced sleep technician blinded to participant diagnosis. From this assessment, habitual sleep onset (HSO) was derived from the mean of the sleep onset times over the 14-day period. The HSO was then used to inform timing of the circadian assessment protocol. Participants attended the Chronobiology and Sleep Laboratory at the Brain & Mind Research Institute. At all times while in the laboratory, participants were physiologically and behaviorally monitored under controlled conditions, with fixed light levels (<50 lx during waking; <30 lx during saliva sampling; <1 lx during scheduled sleep periods) and ambient temperature (24 ± 1°C), and were asked to abstain from substances believed to effect melatonin and/or sleep. Assessment of circadian function (melatonin rhythm timing and levels) and sleep architecture were examined using melatonin and polysomnographic (PSG) assessments, on separate nights. Since the sampling of melatonin required participants to stay awake two-hours past their HSO, this assessment was conducted on the final night, so as to avoid residual effects of the sleep restriction on PSG-measured sleep.

Melatonin sampling

Salivary melatonin was assessed to determine dim light melatonin onset (DLMO), a reliable and valid marker of the circadian pacemaker in relation to plasma melatonin [10, 28]. Participants arrived seven-hours prior to their HSO, to familiarize with the laboratory settings, and to ensure they were in a controlled (seated) posture for at least 30-minutes prior to the sample collection. Saliva samples (1.5 ml) (Salivette, Sarstedt, Germany) were collected at 30-minute intervals from six-hours prior to HSO until two-hours after HSO (i.e., participants were kept awake two-hours past

their HSO). Participants maintained a seated posture for at least 20-minutes prior to each sample collection. Samples were immediately frozen at −20°C.

As detailed previously [29], melatonin was assayed in 200 μl saliva by double antibody RIA (Cat# RK-DSM2; Buhlmann Laboratories AG, Schönenbuch, Switzerland). The lowest detectable level of melatonin was 4.3 pM. The total area under the curve was calculated using the trapezoid method, for each participant over the entire eight-hour sampling period and separately for the two-hour interval before HSO and the two-hour interval after HSO. To determine the DLMO [28], the average melatonin levels of the first three sampling times was calculated and a threshold of two standard deviations greater than this value was established for each subject. The DLMO was defined as the time when the saliva melatonin level first exceeded this threshold and remained elevated for at least the next sampling time. The relationship between the time of onset of melatonin secretion and the HSO time, referred to as the phase angle of entrainment, was calculated by subtracting DLMO time from HSO time (minutes).

Polysomnography

Nocturnal PSG recordings were collected for two consecutive nights on an ambulatory recording system (Compumedics Siesta, Melbourne, Vic, Australia) using a six-channel electroencephalographic (EEG) montage (C3-M2, O2-M1, Fz-M1, Pz-M2); two electrooculographic channels (left and right outer canthi); and electromyogram (sub-mentalis). EEG data were sampled at 250 Hz. Night one was considered an adaptation night. Sleep architecture on night two was visually scored on a computer by an experienced sleep technician using standardized criteria [30], with modifications for older participants [31]. For descriptive purposes, time of sleep onset (24-hour clock time), time of sleep offset (24-hour clock time), total sleep duration (minutes), and time spent in slow wave sleep (SWS) and rapid eye movement (REM) sleep were calculated. Due to data showing altered REM latency in AD [32], and increased wake after sleep onset (WASO) in MCI [22], we chose to examine these two measures as key sleep outcome variables (minutes).

Evening neuropsychological assessment

On the evening of the second PSG, a subset of participants ($n = 43$) completed the Rey Auditory Verbal Learning Test (RAVLT) [33], and were again asked to recall the same material the following day (i.e., after a period of sleep). Key measures were memory consolidation during the evening (i.e., % retention trial 7/trial 5; RAVLT%) as well as the number of words recalled the following day (i.e., RAVLT7-am, maximum = 15).

Self-report data

For descriptive purposes, participants were asked to complete the Horne-Östberg Morningness-Eveningness Questionnaire (MEQ) [34] to assess chronotype. The Epworth Sleepiness Scale was included as a measure of daytime sleepiness [35].

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS version 20, IBM Corp.). For continuous data, analyses employed Pearson's or Spearman's correlations. Chi-square analyses were used for categorical data. For normally distributed data, analyses between groups utilized student's t-tests. For data where assumptions of normality were violated, Mann-Whitney U-tests were employed. All analyses were two-tailed and used an alpha level of 0.05, with the exception of the Fishers r-to-z test, which used a one-tailed test.

RESULTS

Out of the 58 participants, melatonin data did not reach the threshold level- within the sensitivity of the assay for six participants: two controls and four patients with MCI. Thus, melatonin data are reported on 26 patients with MCI and 26 controls. Of these, DLMO data could not be computed for a further three controls and three patients due to either an inability to detect melatonin within the sensitivity of the assay or data not reaching the threshold as required by the algorithm.

Demographic, clinical, and self-report data for the sample are presented in Table 1. Of the 26 patients with MCI, 11 had aMCI and 15 had naMCI. The patient and control groups did not significantly differ in terms of age, gender, education, body mass index, or severity of depressive symptoms. However, there were a significantly greater proportion of patients with MCI taking antidepressant medication (14 versus 3, $\chi^2 = 7.1$, $p = 0.007$). As expected, those with MCI performed slightly lower on the MMSE compared to controls. Self-reported chronotype was not significantly different between the groups, however,

Table 1
Descriptive data for healthy controls and patients with mild cognitive impairment (MCI)

	Control mean (SD) $n = 26$	MCI mean (SD) $n = 26$	Test statistic	p-value
Age, years	65.9 (9.8)	70.1 (9.9)	−1.5	0.128
Gender, male:female[#]	12:14	17:9	1.9	0.163
Education, years	13.8 (3.1)	13.6 (4.0)	0.2	0.848
Body Mass Index	26.2 (4.0)	25.6 (4.8)	0.5	0.645
Hamilton Depression Rating	2.0 (2.1)	3.2 (2.5)	−1.8	0.077
MMSE score	29.2 (1.1)	27.5 (2.1)	3.6	0.000
PSQI total score	4.7 (3.0)	6.0 (2.9)	−1.5	0.130
Morningness-Eveningness Questionnaire	63.9 (7.8)	62.0 (6.1)	1.0	0.330
Epworth Sleepiness Scale	5.1 (3.0)	7.8 (4.4)	−2.1	0.045
RAVLT%	72.4 (28.5)	65.6 (38.3)	0.7	0.513
RAVLT7-am	8.2 (3.5)	6.0 (2.6)	2.2	0.037

All test statistics are students t-test unless otherwise specified; [#]Chi-square. PSQI, Pittsburgh Sleep Quality Index; MMSE, Mini Mental State Examination; RAVLT%, Rey Auditory Verbal Learning Test, evening performance, trial 7/trial 5*100; RAVLT7-am, Rey Auditory Verbal Learning Test, morning recall of 15 words.

Table 2
Actigraphic, circadian and polysomnographic sleep data for healthy controls and patients with mild cognitive impairment (MCI)

	Control mean (SD) $n = 26$	MCI mean (SD) $n = 26$	Test statistic	p-value
Circadian				
Habitual sleep onset, time[‡]	23:04 (1:03)	22:18 (0:41)	−2.7	0.007
AUC, total sampled[‡]	91.6 (61.9)	158.7 (170.7)	−1.2	0.207
AUC, 2-hours pre-HSO[‡]	31.7 (27.3)	43.4 (45.1)	−0.6	0.530
AUC, 2-hours post-HSO[‡]	48.3 (32.7)	82.7 (74.1)	−1.5	0.124
DLMO, time[1]	20:55 (1:09)	19:44 (1:02)	3.7	0.001
Phase angle, minutes	124.1 (59.0)	156.3 (67.7)	−1.7	0.096
Polysomnography[2]				
Sleep onset	22:54 (0:55)	22:27 (0:41)	1.7	0.080
Sleep offset	06:39 (0:57)	06:42 (0:49)	−0.2	0.848
TST, minutes[‡]	398.5 (51.1)	381.5 (82.1)	−0.9	0.395
REM latency[‡]	69.4 (38.7)	121.4 (89.9)	−2.6	0.012
WASO, minutes[‡]	75.1 (44.6)	124.6 (62.3)	−2.7	0.007
SWS, minutes	54.4 (31.3)	58.8 (49.7)	−0.3	0.729
REM, minutes	91.2 (25.0)	81.5 (36.0)	1.0	0.309

All test statistics are students t-test unless otherwise specified; [‡]Mann-Whitney U test Z-statistic. AUC, Area under the curve for melatonin over the sampling period (total sample), and for two-hours prior to and after habitual sleep onset; DLMO, dim light melatonin onset. [1]Melatonin data is available for 26 controls and 26 patients with MCI, and of these a further 3 controls and 3 patients do not have DLMO data due to inability to detect melatonin within the sensitivity of the assay or not reaching the threshold as required by the algorithm. Note that for those in whom values of melatonin returned scores below the assay sensitivity, values entered into AUC values were zero. [2]PSG data was available for 22 controls and 21 patients with MCI. TST, total sleep time; WASO, wake after sleep onset; REM, rapid eye movement sleep; SWS, slow wave sleep.

those with MCI reported greater levels of daytime sleepiness.

Table 2 shows circadian and sleep-wake data. There were no significant differences between aMCI and naMCI subtypes in terms of actigraphy-defined HSO or DLMO timing ($t = 0.08$, $p = 0.940$; $t = -0.41$, $p = 0.968$, respectively). There were no significant differences in the amount of melatonin secretion between the control and patient groups over the sampling period and of note, the patient group demonstrated large variability here. Nor were there group differences in the relationship between melatonin onset and HSO times (i.e., phase angle of entrainment).

Patients with MCI had significantly earlier HSO times, as shown by actigraphy, and significantly earlier melatonin onset (Fig. 1), in comparison to controls. In order to ensure that the earlier HSO and DLMO times were not confounded by antidepressant medication, these analyses were repeated with those ($n = 14$) taking antidepressants excluded. The resultant analyses remained significant ($t = 2.5$, $p = 0.016$; $t = 2.2$, $p = 0.032$ for HSO and DLMO respectively).

With regard to sleep architecture, there were no differences between groups in terms of sleep onset, offset or total sleep time, or the duration of SWS and REM sleep. However, patients with MCI had increased latency to REM sleep and greater nocturnal wakefulness (WASO) relative to controls. There were no differences between amnestic and non-amnestic subtypes with respect to these significant findings
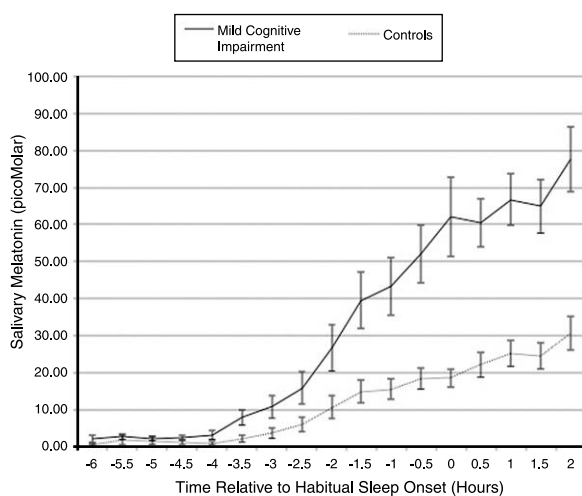
Fig. 1. Salivary melatonin concentration (picoMolar) displayed as a function of relative habitual sleep onset time. Patients with mild cognitive impairment recorded earlier melatonin onset in comparison to age-matched healthy control subjects. However, non-parametric analyses showed that there was no difference between groups in terms of the amount of melatonin secretion. Data represents mean salivary melatonin concentration with standard error for each time point.

(REM latency, $t = -0.17$, $p = 0.868$; WASO, $t = 0.58$, $p = 0.573$). When these analyses were repeated after excluding the sub-sample taking antidepressant medication, the group difference in WASO remained significant ($Z = -2.8$, $p = 0.004$). However, the difference in REM latency was no longer significant ($Z = -1.3$, $p = 0.187$).

Correlations with memory

Associations between memory performance and DLMO were examined for each group at two times of the day: evening and morning. In terms of evening memory performance, no significant association was found between DLMO and evening memory consolidation for healthy controls (RAVLT%: $r = -0.15$, $p = 0.535$). For patients with MCI, however, earlier DLMO onset was moderately associated with poorer consolidation of verbal material in the evening ($r = 0.50$, $p = 0.036$). The difference between these two correlation coefficients was statistically significant (*Fisher's r to z = −1.91, p = 0.028*). By contrast, when memory for the same material was examined the next morning (RAVLT7-am), for healthy controls, earlier DLMO onset was moderately associated with better recall of verbal material ($r = -0.58$, $p = 0.012$). However, no significant association was found for patients with MCI ($r = -0.10$, $p = 0.741$). The dif-

ference between these two correlation coefficients was statistically significant (*Fisher's r to z = −1.93, p = 0.026*).

DISCUSSION

This study represents the first to examine concurrently salivary melatonin and polysomnographic sleep in patients with MCI. We found that the onset of melatonin secretion occurs earlier in MCI patients than in age-matched control subjects, despite the levels of melatonin being similar across the two groups. Of significance, earlier melatonin onset in MCI was also related to poorer memory consolidation during the evening. By contrast, in the control group, DLMO appears to associate with retention of that same material the following day. Overall, these data suggest that for controls and patients with MCI, there are differential relationships between circadian timing and patterns of memory consolidation. In our three-night protocol, this study also incorporated measures of sleep architecture. In accordance with our prior work using actigraphy [22], this current data showed that patients with MCI had significantly greater nocturnal wakefulness (WASO). Additionally, even though sleep onset times did not differ between groups, patients with MCI took significantly longer to enter REM sleep.

Overall, our findings suggest that both circadian and sleep-wake systems are disturbed in MCI. The earlier circadian phase found in those with MCI is consistent with data reported previously in AD [6, 7, 14, 15]. While it is noted that the levels of salivary melatonin did not differ from controls, we cannot, however, make a direct comparison to the investigations conducted in AD, since those studies utilized neuropathological tissue. While such studies have shown that levels of cerebrospinal fluid melatonin are correlated with pineal melatonin [36], salivary melatonin in AD has not been examined.

It has been noted that disruption in circadian rhythms and sleep that occur with aging are paralleled by alterations in the neural and temporal organization of the hypothalamic SCN, as well as altered neuropeptide synthesis and a decreased photic input to the circadian pacemaker (for review, see [3]). Our results support the notion that the circadian regulation of melatonin is disturbed in MCI, a finding which is not merely attributable to aging. While it is unclear from our data whether the earlier timing of melatonin onset reflects dysfunction of the SCN or from the pineal production of melatonin, we note that melatonin levels *per se*,

were not altered in our sample (within the sampling period), suggesting that the latter explanation is less likely. From our data, we cannot infer whether melatonin synthesis is impaired. That is, it is possible that patients with MCI have normal melatonin synthesis but alterations in the timing of release.

Consistent with our prior study using actigraphy [22], we found that patients with MCI had almost an hour (50-minutes) more of nocturnal wakefulness compared to controls. Latency to REM was also delayed in MCI, with the first REM cycle occurring on average 50-minutes later than that of controls. Such findings are aligned with those reported in patients with AD over two decades ago, where it was suggested that REM latency may have diagnostic utility [32]. While the mechanism underpinning the increased REM latency is unclear, it is noted that differences in other aspects of sleep architecture (e.g., time spent in SWS and REM) and total sleep time were not apparent.

While the pathophysiological mechanisms underpinning the circadian and sleep-wake changes observed in MCI cannot be ascertained from this study, it is possible that there is disruption to common neurobiological circuits subserving both sleep and cognition. Of significance, we found that circadian timing in MCI was also related to reduced ability to form new episodic memories during the evening, and that there were differential relationships between patients and controls in terms of both evening memory acquisition and overnight memory retention. This is perhaps not surprising given the increasing data showing how circadian rhythms contribute to memory formation [37]. Currently, there is no clear specificity regarding the neural circuitry likely to underpin memory formation. However, it appears that neurophysiological events occurring during non-REM sleep play a large role [38].

In terms of neurotransmitter systems, it is possible that the noradrenergic system is involved, particularly given its role in pineal melatonin synthesis [36]. The cholinergic system, which projects from the basal forebrain and brainstem to the hypothalamus, has also received much attention [39]. This cholinergic circuitry has been shown to be critical for memory, REM sleep onset, and the regulation of wakefulness and arousal [39, 40]. Atrophy of the basal forebrain has been documented in MCI as well as in AD [41], and the integrity of associated fiber tracts from this region may indeed be compromised [42]. While our data do not provide the capacity to examine these systems, the finding of delayed REM sleep onset in MCI may at least partly reflect alterations to the circadian pacemaker [43] and to cholinergic systems which are known to

be implicated in AD [44]. Interestingly, the use of cholinesterase inhibitors do show positive effects on sleep and circadian rhythms but detailed data are lacking regarding whether such changes may counteract memory dysfunction (see [2] for a review), or other aspects of neuropsychological dysfunction.

With respect to etiological mechanisms, the physiological basis of the disturbances in the sleep-wake, circadian, and cognitive systems are unknown. Indeed, MCI is a heterogeneous 'at risk' syndrome encompassing people with multiple etiologies and trajectories. While aMCI appears to yield the highest longitudinal transition to AD, other pathophysiological mechanisms (e.g., vascular, Lewy bodies) are likely contributors, and may be particularly prevalent in non-amnestic subtypes [19]. Notably, in this study, the observed earlier circadian phase did not differ between amnestic and non-amnestic subtypes. Thus, it is possible that the disruption to circadian rhythms does not reflect AD pathology *per se*, but could reflect common alterations to neural circuitry subserving sleep-wake, circadian, and cognitive systems. Unfortunately, in this study, we did not have an AD group, which would have enabled us to examine whether the patterns observed here are consistent with those seen in later disease stages. Additionally, incorporation of biomarkers for AD (i.e., cerebrospinal fluid or amyloid imaging) would have enhanced our capacity to attribute our findings to emerging AD. Future studies incorporating biomarkers would thus be helpful to determine if these observed sleep-wake changes reflect AD pathology specifically or are non-specific markers.

Clearly, the clinical utility of the findings of this study would become apparent if they were shown to have predictive capacity for disease trajectory or if they were able to be incorporated into personalized treatment strategies targeting either the sleep, circadian, and/or cognitive systems. For example, chronotherapies utilizing light [45] or mimicking the effects of light (e.g., cholinergic agonists with higher affinity for mAChRs than nAChRs) may help to realign (i.e., phase delay) circadian rhythms, while therapies such as physical exercise [46] and melatonin (see [47] for a review) that reduce nocturnal awakenings or latency to REM may improve sleep. Similarly, administration of melatonin in the late night or early morning may delay melatonin onset [48] although the soporific effects of melatonin administered during the daytime need to be considered. While prior studies in MCI have certainly shown improvements in sleep with melatonin administration (see [12] for a review), melatonin is generally given at or before bedtime, and effects on circadian

phase were not established. Ultimately, the significance of such interventions would be assessed not only by their effects on sleep and circadian timing, but also by their capacity to enhance broader aspects of functioning including cognition. Indeed, some data in MCI do support the capacity of melatonin to enhance memory [23], an outcome which is perhaps unsurprising given the role of the circadian system in memory formation [39]. Since some side-effects of melatonin do exist (e.g., sleepiness, dizziness, headaches) and there is potential for interaction with other medications, further controlled trial data is now required to evaluate the efficacy of this hormone in this 'at risk' clinical group.

While the current study represents the first to examine circadian rhythms in MCI using salivary melatonin secretion, some limitations exist. Firstly, antidepressant medication use was more common in those with MCI. When those taking antidepressants were excluded, the earlier HSO and DLMO times and increased nocturnal wakefulness remained evident. However, the finding of decreased REM latency was no longer significant. This finding is aligned with prior reports [49] and suggests that antidepressants may mediate REM latency in MCI. The higher use of antidepressants in the MCI group could also reflect vulnerability to depression in this help-seeking sample (they were used for symptoms reported commonly by persons with MCI including sleep disturbance, depressive symptomatology, and anxiety). Secondly, while we did screen for known eye disease, changes to the retina may have influenced photic input to the SCN, and in this regard, future studies examining the retina are required in order to rule out this potential confound [50]. Third, while the use of laboratory-based analysis of melatonin and PSG in this study is a strength as it ensures a controlled scientific environment, the measurements obtained within the laboratory may differ from those obtained in the home setting. However, it is noted that the HSO obtained from two-weeks of actigraphy was concordant with that obtained in the laboratory setting, thus affirming that the melatonin analysis is likely to reflect that observed out of the laboratory environment. Finally, to comprehensively assess the melatonin rhythm, sampling throughout the night is required.

In conclusion, this study is the first to show that the melatonin rhythm is significantly advanced in MCI, and the degree of advance is associated with poorer memory consolidation. Advanced melatonin rhythm was observed concomitantly with disturbed sleep including more nocturnal awakenings and increased REM latency. From our data, it is unclear whether circadian misalignment and sleep disruption are etiologically linked to neural compromise and resultant neuropsychological dysfunction in MCI. Longitudinal studies are now required to determine if sleep-wake changes observed in MCI are merely a biomarker, or whether they may actually contribute to or mediate disease course and trajectories. Future studies that provide a physiological basis for melatonin and other chronobiotic therapies in MCI will also enhance our knowledge in this area, providing for personalized treatment planning.

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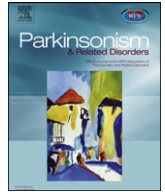
Assessing the utility of Freezing of Gait Questionnaires in Parkinson's Disease

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ABSTRACT

There are currently two validated questionnaires, the Freezing of Gait Questionnaire and the New Freezing of Gait Questionnaire, that are intended to assess the degree of freezing of gait in patients with Parkinson's disease. However, to date no study has attempted to determine whether ratings on these questionnaires accurately reflect the severity (frequency and duration) of actual freezing episodes experienced by patients. We studied twenty-four patients with Parkinson's disease who self-reported significant freezing while in their practically-defined 'off' state. Prior to clinical assessment they completed both freezing of gait questionnaires before being video-recorded while performing a series of timed up-and-go tasks, which incorporated turning, rotating and passing through narrow gaps. The rating of video recordings by two independent observers identified a total of 530 freezing events. The frequency and duration of freezing episodes for each patient were calculated and correlated with questionnaire ratings. Scores on either questionnaire did not correlate with either the frequency or duration of freezing episodes experienced by patients during objective assessment. These results suggest the need to re-evaluate the utility of questionnaires in the assessment of freezing of gait. Furthermore, these results highlight the need for accurate objective methods of identifying freezing events when assessing future clinical interventions aimed at reducing this potentially disabling symptom of Parkinson's disease.

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1. Introduction

Freezing of Gait (FOG) is a paroxysmal disabling symptom that commonly affects patients with Parkinson's Disease (PD), particularly in the later stages [1,2]. Patients typically experience abrupt episodes where they are unable to move their feet, leading to an increased incidence of falls and subsequent nursing home placement [3,4]. The pathophysiological mechanisms underlying FOG remain poorly understood (for review see [5]) and response to current treatments is at best limited.

The assessment of FOG is difficult given the paroxysmal nature of this phenomenon. Indeed, patients may appear free of this symptom in the clinical setting, although evaluation during the 'off' state can increase the likelihood of recording freezing episodes [6]. In order to allow a more practical symptom appraisal, researchers have previously sought to design questionnaires capable of better characterizing and quantifying FOG.

The first such questionnaire (FOG-Q) comprised six questions (maximum score 24 points) that sought to assess both freezing of gait, as well as global gait disturbance [7]. This tool was validated in a large cohort of advanced PD patients who were participating in 'LARGO', a multi-center double-blind, placebo-controlled trial comparing the effects of Rasagiline and Entacapone [8]. The validation study found that a single item on the FOG-Q (question 3 − *Do you feel that your feet get glued to the floor while walking, making a turn or when trying to initiate walking (freezing)?*) was able to identify 'freezers' at least as well as the specific freezing item on the Unified Parkinson's Disease Rating Scale (UPDRS question 14), which was previously viewed as the most reliable questionnaire measure of FOG [9]. It was concluded that the FOG-Q was useful both as a screening tool and also as an assessment of treatment intervention given the symptomatic benefits reported in the LARGO trial [8]. The authors of this validation study acknowledged the lack of specificity inherent in the FOG-Q as a subset of questions that were primarily concerned with overall gait dysfunction rather than FOG *per se*.

To address these concerns, a new questionnaire was developed which sought to introduce questions that were specific to FOG in PD [10]. The New Freezing of Gait Questionnaire (NFOG-Q) is a clinician-administered tool that aims to assess both the clinical aspects of FOG as well as its subsequent impairments on quality of life. In order to increase the likelihood of accurate self-assessment by

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patients, the NFOG-Q incorporates a short focused video that shows a number of FOG examples. The addition of this video appeared to increase the ratings of severity of the condition, however it did not add to the sensitivity or specificity of the tool with regards to identifying FOG. To account for this, the NFOG-Q allocates a single question to act as a screening tool for the presence or absence of FOG. Given the lack of standardized and effective community-based identification of FOG, the NFOG-Q has become a valuable tool for the assessment of freezing.

Clearly, the ability to accurately monitor FOG episodes is of great importance, especially in the evaluation of future therapeutic interventions. For example, the response of FOG symptoms to deep brain stimulation in novel target regions, such as the pedunculo-pontine nuclei [11], will require sensitive and specific outcome measures to determine benefits. However, to date no studies have sought to demonstrate the ability of the NFOG-Q (or the FOG-Q) to reflect actual FOG episodes experienced by patients. This study sought to determine whether scores on these questionnaires correlated with the frequency and/or duration of freezing episodes measured objectively in PD patients reporting FOG.

2. Methods

2.1. Recruitment

Twenty-four patients who were attending the Parkinson's Disease Research Clinic at the Brain and Mind Research Institute, University of Sydney were identified for this study by severe self-reported freezing behavior. All patients satisfied UKPDS Brain Bank criteria [12], had a Mini-Mental State Examination (MMSE) [13] score of ≥24 and were deemed unlikely to have dementia or major depression according to DSM-IV criteria by consensus rating of a Neurologist (SJGL) and a Neuropsychologist (SLN). Clinical details are presented in Table 1. The study was approved by The University of Sydney Human Research and Ethics Committee and written informed consent was obtained.

2.2. Clinical evaluation and questionnaires

Patients were assessed in the practically-defined 'off' state following overnight withdrawal of dopaminergic therapy. Six patients also had Deep Brain Stimulation (five Subthalamic Nuclei and one Pedunculopontine Nuclei), which were turned off for 1 h prior to assessment. They were evaluated on the Movement Disorder Society Unified Parkinson's Disease Rating Scale – Section III (MDS-UPDRS-III) [14] and Hoehn and Yahr stage score [15]. None of the patients described any increase in freezing behavior following the administration of their usual dopaminergic therapy. Upon arrival at the clinic, patients were administered the FOG-Q and the NFOG-Q. The NFOG-Q was subsequently separated into two parts, Section 1 for screening and Sections 2 and 3 were taken to represent the severity and frequency of FOG [10].

2.3. Timed up-and-go (TUG) tasks

Patients performed a series of timed up-and-go tasks on a standardized course (Fig. 1A) to provoke FOG. All TUG tasks started from a sitting position, from which patients walked along the center of a large open corridor. Five meters (5 m) from the chair was a 0.6 m × 0.6 m target box marked on the floor with yellow tape, in which turning movements were performed. The standard TUG required a 180° turn within the box and a return to the starting chair. Three enhanced TUG assessments
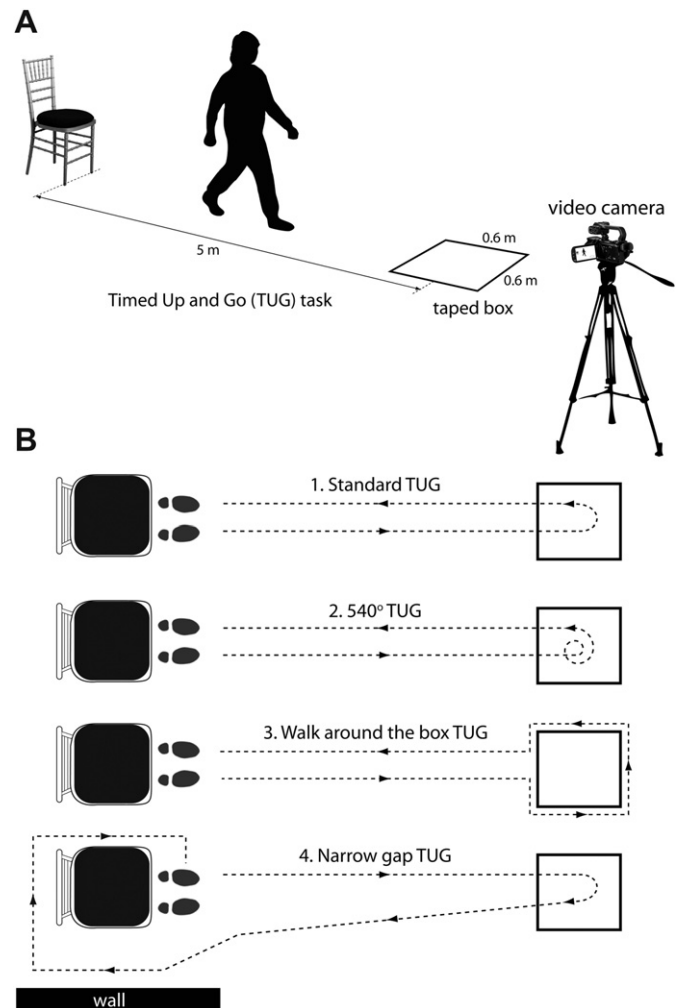

Fig. 1. (A) The Timed Up and Go (TUG) task utilized for FOG assessment. Each TUG trial started with the patient seated in a chair, which was placed 5 m from a 0.6 × 0.6 square target defined by a taped box on the floor. A video camera was placed on a tripod and situated 3 m from the end of the taped box at an angle offset approximately 20° from the runway. (B) Clinical assessment trials. (1) Standard TUG trial with a 180° turn inside the taped box then return to the chair; (2) a 540° turn inside the taped box; (3) walking around the outside of the box making tight turns without touching the tape; (4) negotiation of a narrow gap (<1 m) on the return portion of the trial. All tasks were performed with turns to the patient's right and left.

were also carried out (Fig. 1B); '540°' TUG in which patients performed two revolutions within the box; 'walk-around the box' TUG in which patients were instructed to walk around the outside of the box making tight turns without touching the tape; and 'narrow gap' TUG, entailing lateral movement of the chair at the start position (after the patient had begun the TUG) to create a <1 m gap with the wall, alternately to the left or right side, that the patients were required to negotiate on the return journey. All TUG tasks were performed with turns to the patient's left and right. In addition, dual-tasking (vocalizing the months of the year forwards and backwards whilst walking) was utilized on two trials per patient. The requirements for each TUG task were explained just prior to the trial. If a patient had failed to fully comprehend the requirements of a specific trial it was abandoned and performed again from the start. The beginning of each TUG trial was signaled by a request from the investigator to begin and was completed on return to the seated position.

2.4. Video assessment

All trials were video recorded from a consistent vantage point 3 m from the 'taped-box' (STM, TRM and VD; see Fig. 1A). All videos were independently reviewed by two clinicians with an interest in Movement Disorders (JMS and SJGL) for freezing episodes, which were defined as the paroxysmal cessation of a patient's normal progress through a specific routine, as described elsewhere [16]. The end of each

Table 1
Demographic, neurological, cognitive and freezing characteristics of the sample.

	Range	Mean	SD
N = 24			
Age, years	56–84	69.00	8.41
Hoehn and Yahr	2–4	2.66	0.53
UPDRS III	19–65	40.24	11.06
Mini-Mental State Examination	24–30	28.57	1.61
Freezing questionnaires			
FOG-Q total	10–22	14.96	3.58
NFOG-Q: 2 and 3	0–25	17.72	5.61
Clinical Assessment			
Frequency of freezing episodes	1–63	21.71	17.61
Percentage of time freezing	0.3–75.7	23.70	23.02

FOG episode was defined as the ability of the patient to perform an effective step with gait-parameters similar to their normal stride. The presence of 'trembling in place' was not used as an identification criteria. Freezing episodes were logged according to their time of onset and offset. The frequency and duration of episodes were determined for each patient utilizing a video FOG tagging program developed by the investigators (TRM and STM). Each video observer was instructed to tag the onset of a freeze by pressing the 'T' key and holding down the key throughout the duration of the event. Video editing tools enabled the observers to drag the ends of a horizontal bar representing the duration of the tagged freeze (which also moved backwards or forwards through the video by a corresponding amount of time) to accurately log the onset and offset of each FOG event. To facilitate accurate assessment of freeze duration, the raters were instructed to repeatedly view the video footage and adjust onset/offset points until entirely satisfied. An intra-class correlation analysis (ICC) established the reliability (ICC = 0.9) of the video FOG scores from the two raters for both frequency and duration of freezing episodes. The total duration of all trials was calculated as the sum total of the time taken from the beginning to the completion of each individual trial. Percentage time spent freezing was defined as the total duration of FOG episodes (regardless of type of freezing) over the total test duration.

In accordance with previous research [16], we also distinguished a number of sub-types of FOG relating to the clinical situation in which they occurred, and calculated the relative frequency of each sub-type.

 (i) *Turn hesitations*, in which one of the patient's legs failed to complete the normal turning circle or a tight turn during any of the routines;
 (ii) *Runway freezes*, which were defined as FOG episodes occurring in the absence of turns, targets or gaps;
 (iii) *Freezing during narrow gaps*, defined as freezing occurring during the navigation of a narrow portion of the routine;
 (iv) *Target hesitations*, which occurred upon the arrival (within 2 m) to a defined target, such as a line of tape on the ground;
 (v) *Start hesitations*, where patients had difficulty initiating gait at the start of a TUG task in any of the routines.

2.5. Statistical analysis

Results from the questionnaires were tabulated and compared statistically with the output measures of the video assessments. The data were analyzed through pairwise parametric correlations using Statistical Package for the Social Sciences software (SPSS Inc., Chicago IL).

3. Results

The TUG tasks were highly successful in eliciting FOG. The total number of FOG events was 530, averaging 21.7 (SD 17.6) per subject (range 1–63). This is consistent with a recent study [17] in which the mean number of FOG events was 23.7 (SD 20.7), ranging from 0 to 66 episodes per subject. The mean percentage of time freezing was 23.7% (SD 23.0), ranging from 0.3 to 75.7%. There was a large variability in the frequency and duration of FOG episodes across subjects, which allowed for a robust correlation analysis with the rating scales.

The proportion of the sub-types of FOG (Fig. 2) was generally similar to those previously reported [16]. Patients were most likely to freeze during turns (299 FOG events; 56.4% of total) and 22 of the 24 patients (92%) suffered a freeze during a turn, making it the most sensitive measure for eliciting a freezing episode. There were a substantial number of runway freezes (141 events; 26.6% of total; 58% of patients), however the vast majority (120 events) occurred in only 4 patients, with the balance (21) occurring infrequently in the rest of the cohort. We also observed freezing during narrow gaps (56 events; 10.6% of total; 42% of patients) as well as target (22 events; 4.2% of total; 38% of patients) and start hesitations (12 events; 2.3% of total: 33% of patients). Marked 'trembling in place' was observed on a number of trials, however the presence or absence of this feature was not analyzed in this study.

The correlations between the percentage of time spent 'frozen' during the TUG tasks and ratings on the FOG-Q ($r = 0.30$, $p = 0.150$) and NFOG-Q ($r = 0.35$, $p = 0.095$) were not significant (Fig. 3A). The correlation was even weaker (Fig. 3B) between the number of FOG
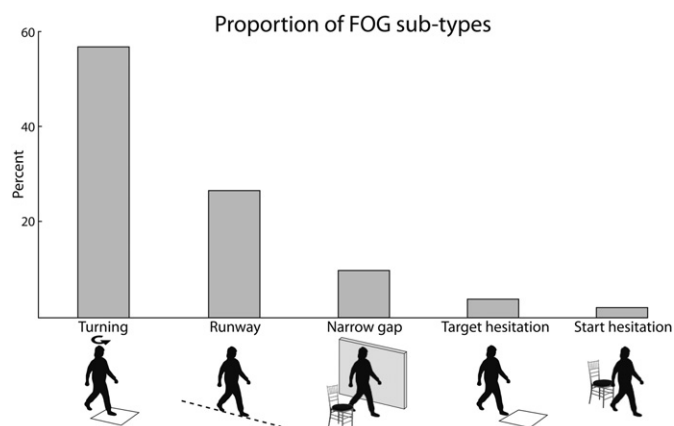

Fig. 2. Relative proportion of FOG sub-types observed during the TUG trials. Patients were more likely to experience FOG during turning, followed by runway freezes, navigation of narrow gaps, target freezes and start hesitations.

events per subject and ratings on the FOG-Q ($r = 0.11$, $p = 0.613$) and NFOG-Q ($r = 0.30$, $p = 0.150$). There was a trend for a single question on the FOG-Q (question 3 – *Do you feel that your feet get glued to the floor while walking, making a turn or when trying to initiate walking (freezing)?*) to be associated with frequency of freezing episodes ($r = 0.40$, $p = 0.052$) but not with the percentage of total time spent frozen ($r = 0.29$, $p = 0.178$). However, this data was poorly distributed for a correlation analysis with only one subject scoring less than 2 on FOG-Q question 3.

4. Discussion

The major finding of this study was that the FOG-Q and the NFOG-Q rating scores did not correlate with actual clinical measures of FOG severity (frequency and duration of freezing episodes) in a cohort of PD patients with established FOG while in the clinical 'off' state. A single item on the FOG-Q (namely, the third question) trended towards significance when correlated with the total frequency of freezing episodes experienced by patients. This study also confirmed the utility of the TUG task as a reliable method for provoking FOG in the clinical environment, particularly in the 'off' state [17,18]. The use of the TUG and associated turning and obstacle avoidance tasks proved useful in demonstrating the different sub-types of FOG, dominated by turning episodes (over half of 530 FOG events), then (in decreasing order of frequency) runway, narrow gaps, target and start hesitations. The similarity in relative proportions of freeze sub-types with those previously reported [16] suggests that the clinical tasks conducted in this experiment were consistent with those utilized to study FOG across different clinical centers.

FOG-Q and NFOG-Q have been validated in large cohorts of PD patients [9,10]. However, this validation did not utilize objective clinimetric tools that can distinguish specific freezing episodes in a clinical environment, but relied purely on subjective patient and carer responses to the questions, as well as self-reporting and clinician-mediated questionnaires for the comparative analysis. The lack of any correlation between FOG-Q and NFOG-Q scores and actual freezing in PD patients suggests that such subjective 'validation' techniques are inadequate and may not in fact validate rating scales in an objective or clinical sense. Although our findings do not undermine the validity of the FOG questionnaires to act as screening tool for the presence of FOG in a sample of patients with PD, our results suggest that a single question (FOG-Q question 3) may be sufficient for this task. The ability to accurately
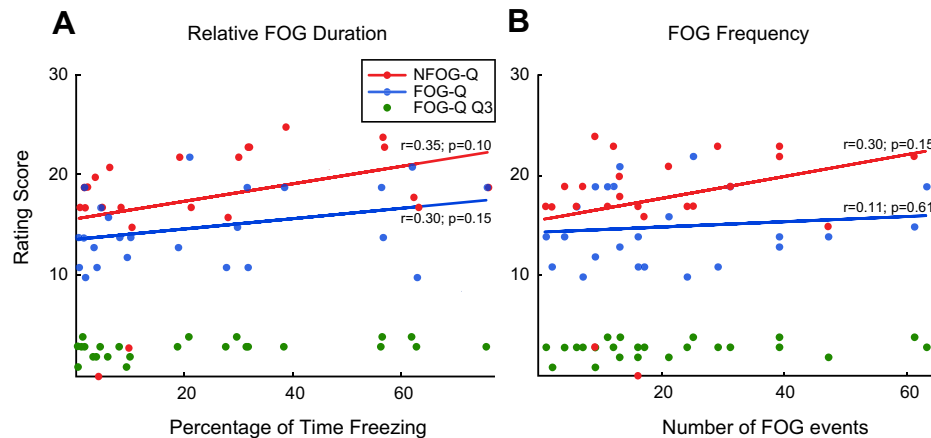
Fig. 3. (A) NFOG-Q and FOG-Q rating scores plotted as a function of percentage of time spent freezing for each subject. (B) NFOG-Q and FOG-Q rating scores plotted as a function of number of FOG events per subject. NFOG-Q and FOG-Q scores did not correlate with the relative duration or frequency of freezing of gait.

assess FOG severity is fundamental to the future evaluation of interventions aimed at decreasing the frequency and duration of FOG events. The results of our study suggest that existing FOG questionnaires are unsuited to this task and may in fact provide an inaccurate estimate of FOG severity, which may be exacerbated in patients with more advanced disease who spend longer periods in the 'off' state [1].

One interpretation of the findings in this study is that the questionnaires are probing a more general freezing phenomenon, rather than freezing confined to the domain of gait [19,20]. In keeping with this viewpoint, a number of studies have shown that scores on these questionnaires correlate with specific impairments in cognition [21,22], particularly under temporal pressure [23,24]. Other research has shown specific links between self-reported freezing and panic attacks in patients with PD [25]. This explanation suggests that the pathophysiological mechanism of FOG may not operate independently in one specific domain, such as motor function. As such, neural regions responsible for domain-general functions, such as the subcortical nuclei and brainstem structures, may be responsible for these clinical correlations. If correct, this would suggest that freezing behavior occurring across walking, handwriting or even thinking may be due to an underlying and unifying mechanism [26].

Given the potential limitations of questionnaire ratings there is a pressing need for the development of novel tools that can be used to objectively assess FOG. Members of our research team have previously developed and validated an ambulatory objective technique for identifying the presence and duration of a FOG event based on a frequency analysis of the vertical acceleration of the leg [27], and other groups have further validated this freeze detection algorithm [28]. FOG is identified with an accuracy of 80—90% based on the appearance of high-frequency 'trembling' in a 3—8 Hz 'freeze' band and a corresponding decrease in power in the loco-motor (0—3 Hz) band [27]. This technique is also capable of iden-tifying FOG sub-types (start hesitation, turning, runway freeze) based on context (i.e., did the FOG event occur after a period of standing or sitting still, in conjunction with angular velocity indi-cating a turn, or whilst walking). The high-frequency lower limb oscillations, known clinically as 'trembling in place', are often (but not always) visible to the naked eye [27]. Ambulatory monitoring of FOG with inertial sensor arrays will likely prove more accurate than clinical observation and allows the possibility to extend objective monitoring from the clinic to the community. However, further work is required to validate objective freeze monitoring, particu-larly in the absence of simultaneous clinical observation.

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The contribution of nocturnal sleep to the consolidation of motor skill learning in healthy ageing and Parkinson's disease

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SUMMARY

The benefits of sleep for the consolidation of procedural motor skills are less robust in older adults, although the precise reasons for this remain unclear. To date, even less is known about these processes in older adults with neurodegenerative diseases, particularly those which impact on motor functioning. While sleep disturbance and motor symptoms are frequent disabling features of Parkinson's disease, no known studies have directly probed sleep-dependent memory consolidation for motor skill learning in Parkinson's disease. Forty patients with idiopathic Parkinson's disease (age = 63.7 years ± 7.7; disease duration 4.1 years ± 4.4) completed a motor skill learning task pre- and post-sleep and were compared to 20 age- and sex-matched controls recruited from the community. Polysomnography was undertaken during the post-training night and measures of sleep architecture were derived. Parkinson's disease patients did not demonstrate any apparent deficits in within-session learning and overnight stabilization compared to controls, with both groups failing to demonstrate offline improvements in performance (i.e. memory consolidation). In controls, longer duration in slow wave sleep was associated with improved next-day session learning ($P = 0.007$). However, in Parkinson's disease, no relationships between sleep parameters and learning measures were found. Slow wave sleep microarchitecture and the use of dopaminergic medications may contribute to impaired sleep-dependent multi-session acquisition of motor skill learning in Parkinson's disease.

INTRODUCTION

There is robust evidence that motor skill learning benefits from sleep (Korman *et al.*, 2007; Maquet, 2001; Maquet *et al.*, 2003; Spencer *et al.*, 2007; Stickgold *et al.*, 2002; Wilson *et al.*, 2012; Walker *et al.*, 2002, 2005). The consolidation of procedural motor skills is thought to comprise an early period of acquisition and rapid learning followed by a period of stabilization, and a late post-training performance enhancement which is apparent in some motor tasks if learning is followed by a period of sleep (Korman *et al.*, 2007; Maquet *et al.*, 2003; Walker, 2005). While this effect has been investigated widely in younger adults (Fischer *et al.*, 2002; Walker *et al.*, 2002, 2003), recent studies have suggested that this effect is less pronounced and frequently absent in older adults over the age of 45 years (Spencer *et al.*, 2007; Wilson *et al.*, 2012).

Further work elucidating the mechanisms by which sleep disturbance in older adults impair motor skill learning is needed. In the literature, particular focus has been placed on the contribution of slow wave sleep (SWS), rapid eye movement (REM) sleep and Stage 2 sleep characterized by the presence of sleep spindles, as mediators of performance enhancement (Karni *et al.*, 1994; Payne, 2011; Stickgold, 2005; Walker *et al.*, 2002). While a longer duration of Stage 2 non-rapid eye movement (NREM) sleep has been found to improve procedural motor skill consolidation in younger adults (Walker *et al.*, 2002, 2003), preliminary studies in older adults (without dementia) have failed to demonstrate any relationships between sleep-dependent

consolidation of a motor skill task and any measure of sleep architecture (Peters *et al.*, 2008; Tucker *et al.*, 2011). While the reasons for this remain unclear, it is hypothesized that with advancing age sleep-dependent memory consolidation is degraded by changes in sleep quantity or quality, declines in the integrity of specific neural networks and/or other sleep or disease related factors (Spencer *et al.*, 2007). More specifically, age-related changes in SWS and a change in cholinergic tone are believed to play a role in differentially impacting memory consolidation (Harand *et al.*, 2012). Further, atrophy of particular brain regions within the neostriatal and cerebellar region, changes in fronto–striatal circuitry and reduced dopaminergic neurotransmission have all been shown to mediate the incremental acquisition of skilled motor behaviours (see review by Hornung *et al.*, 2005).

These inter-relationships between sleep physiology and motor skills learning may, however, be difficult to tease out in healthy older adults who have only minimal disruption to these brain regions and who generally perform the task with a high degree of accuracy. To date, there is a paucity of research exploring these relationships in patients with more severe disruption to these neural networks such as those with neurodegenerative conditions (see review by Naismith *et al.*, 2011a). This is due partly to the fact that knowledge of sleep-related changes in neurodegenerative disorders remains in its infancy, but also because our understanding of lifespan changes in sleep-dependent memory consolidation is still evolving.

One neurodegenerative disease that is known to have deficits in motor skill learning is Parkinson's disease (PD). In PD complaints of both sleep disturbance and cognitive impairment are frequent (Gunn *et al.*, 2010), and strong inter-relationships between markers of sleep disturbance and memory performance have been demonstrated (Naismith *et al.*, 2010; Naismith and Lewis, 2011; Naismith *et al.*, 2011b). Notably, with increased disease duration there appears to be a progressive reduction in SWS and REM sleep (Diederich *et al.*, 2005). Of particular interest is the ability of these patients to learn and consolidate motor skills, as this aspect of functioning is affected strongly by the disorder (Muslimovic *et al.*, 2007), and may relate to disrupted cortico–striato–thalamic circuitry and changes in dopaminegic neurotransmission. In a recent study in PD, Scullin *et al.* (2012) demonstrated that improvements in memory consolidation on a working memory task following a period of sleep were correlated positively with the amount of SWS between training sessions. There are, however, no studies that have directly probed sleep-dependent procedural memory consolidation in PD by evaluating cognitive performance following a period of sleep, with concomitant polysomnographic measures of sleep architecture. By evaluating motor skill learning in PD, we might be better able to characterize age-related decline of sleep-dependent consolidation for this skill, and afford new insights into the determinants of memory consolidation for motor tasks in PD. Furthermore, this may elucidate aspects of sleep that support this cognitive skill in the wider population, as well as providing novel insights into future treatment strategies.

The current study sought to explore whether particular aspects of sleep architecture as measured by polysomnography (PSG) were related to the ability of patients with PD and healthy older adults to learn and consolidate a procedural-based motor skill learning task (sequential finger-tapping). Based on preliminary work in healthy older adults, we predicted that sleep-dependent memory consolidation would be impaired in older healthy adults, leading to no overnight gains in performance, and that this would be even more pronounced in PD patients who have reduced dopaminergic neurotransmission and disruptions to fronto–striatal circuitry implicated in motor skill learning and consolidation. Furthermore, it was hypothesized that features of SWS would mediate this relationship, and that that this effect would be most prominent in healthy older adults who have more preserved neural networks and less prominent sleep disturbance.

METHODS

Participants

Forty participants with idiopathic PD (11 female) and 20 healthy controls (12 female) were recruited from the Brain and Mind Research Institute (BMRI) Parkinson's Disease Research Clinic, University of Sydney. Demographics details are presented in Table 1. All patients satisfied UK Parkinson's Disease Society (UK PDS) Brain Bank criteria (Gibb and Lees, 1988), were non-demented according to myelodysplastic syndrome (MDS) criteria (Dubois *et al.*, 2007) and had, on average, only minimal or mild depressive symptoms [Beck Depression Inventory–II (BDI-II) score of <19; Beck *et al.*, 1996]. Exclusion criteria were: history of stroke; neurological disorder other than PD; head injury with loss of consciousness >30 min; medical conditions known to affect cognition (e.g. cancer); other psychiatric illness; shift-workers; transmeridian travel within the prior 60 days; and use of medications other than those for PD known to affect sleep and/or melatonin secretion including beta-blockers, lithium or benzodiazepines. Permission for the study was obtained from the University of Sydney research ethics committee and all participants provided written informed consent.

Measures

Clinical measures

As described in full previously (Naismith *et al.*, 2011a,b), a neurologist recorded Hoehn & Yahr staging and disease duration (years). L-Dopa equivalent daily dose (DDE) for each patient was calculated using previously published guidelines (Katzenschlager *et al.*, 2008) (Table 1). Within the Parkinson group, 13 patients were drug-naive and 27 were taking one or more dopaminergic agents, including levodopa (*n* = 22), dopamine agonist (*n* = 2) and levodopa and dopamine agonist (*n* = 3). No differences in finger-

Table 1 Clinical measures, sleep parameters and motor skill performance for all participants

Clinical measures	Parkinson's disease n = 40		Control n = 20		t-value/ χ^2
	Mean	SD	Mean	SD	
Age, years	63.6	7.6	66.1	9.5	1.023
Gender (male : female)	29 : 11	–	8 : 12	–	9.157*
Formal education, years	13.6	2.8	13.8	3.3	0.242
Hoehn & Yahr, stage	1.7	0.5	–	–	–
Disease duration, years	4.1	4.4	–	–	–
L-Dopa equivalent daily dose, mg day^{-1}	401.5	451.9	–	–	–
Beck Depression Inventory II	6.9	5.1	6.8	6.0	−0.079
Mini Mental State Examination	28.6	1.6	29.2	0.9	1.172
Sleep architecture					
Total sleep time, min	393.7	70.6	377.6	70.7	−0.836
Stage 2 sleep duration, min	231.1	56.7	219.8	59.5	−0.717
Slow wave sleep duration, min	64.8	48.8	57.6	31.6	−0.685
Rapid eye movement sleep duration, min	82.8	27.2	80.5	28.2	−0.313
Wake after sleep onset	89.1	44.5	91.1	45.3	0.176
Sleep efficiency	77.4	10.2	77.2	2.8	0.192

SD, standard deviation. * indicates the significance $P < 0.05$

tapping performance or any sleep measures were found between those of dopaminergic medication and those who were drug-naive (data not shown). All patients underwent assessment and testing in their 'on' state (showing response to usual medication) and remained on their usual medication for the duration of the study. Basic demographics including age, years of formal education, depressive symptom severity as measured by the BDI-II (Beck *et al.*, 1996) and general cognitive status as measured by the Mini Mental State Examination (MMSE) (Folstein *et al.*, 1975) were recorded for all participants. All participants underwent formal neuropsychological testing undertaken by a trained clinical neuropsychologist to exclude dementia. All participants completed the Horne Östberg Morningness–Eveningness Questionnaire as a measure of circadian preference.

Finger-tapping motor sequencing task (FTT)

A standard FTT task (see Walker *et al.*, 2003) was used as a measure of procedural motor skill learning. Participants attempted the FTT on two consecutive days that took place pre- and post-sleep. The first administration took place 2 h before habitual bedtime (range 19:08–21:39 hours) and required subjects to repeatedly tap a sequence of five numbers (4-1-3-2-4) 'as quickly and as accurately as possible' across 12 intervals of 30 s (trials 1–12), interrupted by a 30-s pause, using their non-dominant hand (see Fig. 1). During tapping trials, the numerical sequence was displayed in red against a white background at the top of the screen to minimize any working memory requirement. Each key press produced a square box concealing the accuracy of the responses, forming a row from left to right. Each interval was scored as either correct or incorrect. Key-press times were not recorded. Changes in motor performance were measured across a six-trial retest session (trials 13–18) following a night of sleep, divided into early retest (trials 13–15) and late retest (trials 16–18). Participants were not told that they would be readministered the FTT the following morning, thereby minimizing deliberate rehearsal of the task. Outcome variables for the FTT included the following:

1. Pre-training learning score (mean number of correctly tapped sequences averaged across the first three trials in the pre-sleep training session) and pre-training error rate (mean number of errors averaged across the first three trials of the pre-sleep training session).
2. Post-training learning score (mean number of correctly tapped sequences averaged across the last three trials of the pre-sleep training session) and post-training error rate (mean number of errors averaged across the last three trials of the pre-sleep training session)
3. Early retest learning score (mean number of correctly tapped sequences averaged across the first three trials of the post-sleep training session) and early retest error rate (mean number of errors averaged across the first three trials of the post-sleep training session)
4. Late retest learning score (mean number of correctly tapped sequences averaged across the last three trials of the post-sleep training session) and late retest error rate (mean number of errors averaged across the last three trials of the post-sleep training session)
5. Overnight early motor skill improvement (the percentage overnight improvement in motor skill defined as the early retest learning score/post-training learning score × 100), also termed offline memory consolidation.
6. Overnight late motor skill improvement (the percentage overnight improvement in motor skill defined as the late retest learning score/post-training learning score × 100) (Fig. 1).

Sleep architecture

Participants underwent two consecutive nights of conventional polysomnography in the Chronobiology and Sleep Laboratory at the Brain and Mind Research Institute. Nocturnal polysomnography (PSG) recordings were collected on an ambulatory
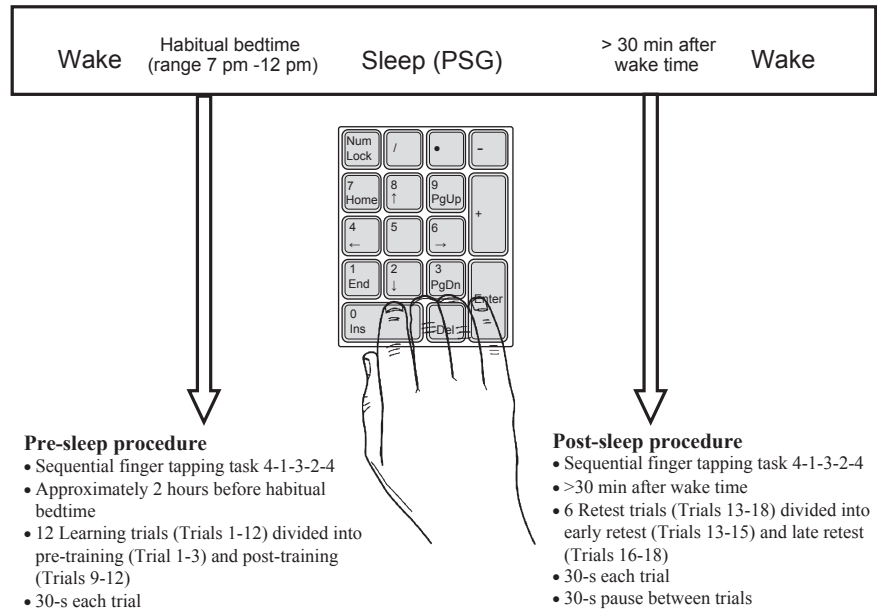
Figure 1. Schematic representation of the motor skill learning protocol.

recording system (Compumedics Siesta, Melbourne, Vic, Australia) using a six-channel electroencephalographic (EEG) montage (C3-M2, O2-M1, Fz-M1, Pz-M2, Fpz and Cz); two electro-oculographic (EOG) channels (left and right outer canthi) and electromyogram (EMG) (submentalis). EEG data were sampled at 250 Hz. Night 1 was considered an adaptation night and included pulse oximetry recordings. Measures of sleep architecture and administration of the FTT took place on night 2, allowing us to examine sleep architecture as well as measures of sleep efficiency and fragmentation. Sleep architecture stages were scored manually in 30-s epochs by an experienced sleep technician using Rechtschaffen and Kales standardized scoring criteria (Rechtschaffen and Kales, 1968), with modifications for older participants (Webb and Dreblow, 1982). While in the laboratory, participants were monitored physiologically and behaviourally at all times under controlled conditions, with fixed light levels (<50 lux during waking; <1 lux during scheduled sleep periods) and ambient temperature (24 ± 1 °C). Patients were required to maintain their usual bedtime and wake-up schedule during the study, and asked to abstain from caffeinated beverages. Outcome variables from the PSG assessment used in analyses included: (i) total sleep time (TST) (min); (ii) Stage 2 sleep duration (min); (iii) SWS duration (min); (iv) REM sleep duration (min); (v) wake after sleep onset (WASO) (min); and (vi) sleep efficiency (TST/time in bed × 100).

Statistical analysis

All analyses were conducted using SPSS version 18.0 (PASW statistics, SPSS Inc., Chicago, IL, USA) for Macintosh. Univariate correlations were used to determine the associations between performance on the motor sequencing task and sleep architecture, utilizing non-parametric analyses as appropriate. Comparative analyses of experimental perfor-

mance were carried out using repeated measures across pre-training, post-training, early and late retest sessions and partial correlations between sleep measures and post-sleep finger-tapping performance after controlling for pre-sleep performance. All analyses were two-tailed and employed an alpha level of 0.05, with a Bonferroni correction applied for multiple comparisons.

RESULTS

Basic demographics, disease characteristics and measures of sleep architecture averaged across each group are presented in Table 1. PD participants were, on average, 4.1 years post-diagnosis, with a mean Hoehn & Yahr score of 1.7 [standard deviation (SD) = 0.5]; 88% of PD participants had disease durations of fewer than 3 years, while only five patients had much longer disease durations of 6, 7, 9, 13 and 20 years, respectively. There were no significant differences between patient and control participants in terms of age, years of formal education, general cognitive status, circadian preference (data not shown) and depressive symptoms (Table 1), but there was a greater proportion of males in the PD group compared to controls ($P = 0.027$). The two groups did not differ on any sleep parameter, including TST (Table 1). Across both groups, no significant differences were observed in sleep parameters as a function of gender.

Initial learning

As illustrated in Fig. 2, both controls and PD patients performed the motor skill task well in the initial trials, with their average pre-training error rate (i.e. the first three trials in the testing session) low, at 1.9 and 2.1%, respectively. The error rate did not differ between the groups in the either the pre-training ($t_{58} = 0.56$, $P = 0.58$) or post-training ($t_{58} = -1.28$,
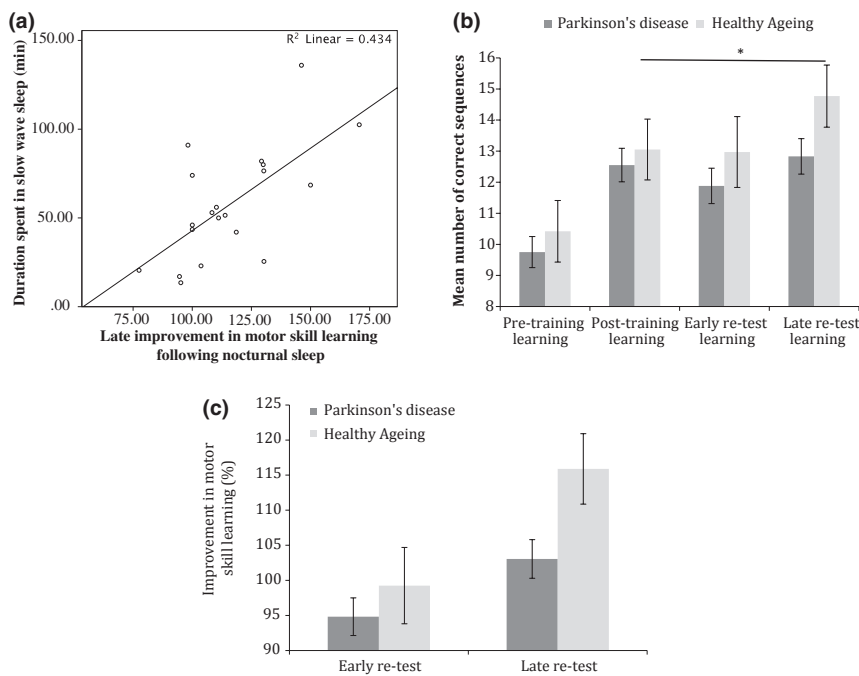
Figure 2. (a) Scatterplot illustrating the relationship between late improvement in motor skill learning following nocturnal sleep and slow wave sleep duration in healthy ageing. (b) Change in motor skill performance across pre-training, post-training, early retest early and late retest trials across groups. Error bars represent standard errors. (c) Change in motor skill performance following nocturnal sleep across groups. Error bars represent standard errors.

$P = 0.21$) sessions. In terms of motor skill performance, mean pre-training and post-training learning did not differ between PD patients and controls (pre-training: $t_{58} = 0.67$, $P = 0.51$; post-training: $t_{58} = 0.49$, $P = 0.63$). Overall, these findings suggest that performance of the motor skill learning task was at a group level comparable between PD and controls.

Motor skill learning over time and following a period of sleep

Repeated-measures analysis of variance (ANOVA) compared learning and error rates across the four different time-points: pre-training, post-training, early and late retests for PD patients and controls. In terms of learning, the main effect of session type was significant ($F_{3,174} = 49.70$, $P < 0.001$). Mean learning was highest in the late retest session compared to the early retest session and pre- and post-training sessions (see Fig. 2). The main effect of group was not significant ($F_{1, 58} = 1.13$, $P = 0.29$), nor was the interaction ($F_{3,174} = 2.09$, $P = 0.11$), suggesting that PD patients and controls did not differ in their learning performance (see also Fig. 1). In terms of error rates, the main effects of session and group were both non-significant.

Exploring the within-group relationships further, controls failed to demonstrate an improvement in overnight motor skill performance in the early retest session compared to their post-training baseline performance ($t_{19} = 0.13$, $P = 0.90$). Conversely, however, controls performed significantly better in the late retest session compared to their post-training

baseline performance ($t_{19} = 3.14$, $P = 0.005$). In PD patients, no significant improvement in performance was found in either the early or late retest sessions compared to their post-training baseline performance ($P > 0.05$).

Offline consolidation of the task was measured by comparing the relative performance on the motor skill learning task before (post-training learning) and after (early retest) a period of sleep. This measure takes into consideration the individual's baseline level of performance, which can vary on account of age or other factors (see Spencer *et al.*, 2007 for a discussion of this issue). No significant difference in terms of offline consolidation was observed in either group.

In controls, however, there was evidence of within-session improvement, with enhanced performance in the late retest condition compared to PD patients ($t_{58} = 2.45$, $P = 0.018$). Inspection of the data indicated that in the early retest phase controls and PD patients were recalling 99 and 95%, respectively, of what they had encoded in the post-training session prior to sleep, and that these values increased to 115 and 103%, respectively, by the late retest session.

Sleep-dependent motor skill learning and its relationship to sleep architecture

The relationship between motor skill learning and measures of sleep architecture for all participants is presented in Table 2. No significant relationships were found between early improvement in performance following sleep (i.e. offline consolidation) and any sleep measure in either the control or PD group.

Table 2 Correlations between polysomnographic sleep measures and motor skill performance for patients with Parkinson's disease (n = 40) and healthy control subjects (n = 20)

Sleep variables	Parkinson's disease				Healthy ageing			
	Early retest (average of first three blocks following sleep)	Late retest (average of last three blocks following sleep)	Early improvement in motor skill learning following sleep	Late improvement in motor skill learning following sleep	Early retest early (average of first three blocks following sleep)	Late retest (average of last three blocks following sleep)	Early improvement in motor skill learning following sleep	Late improvement in motor skill learning following sleep
Total sleep time (TST) min	−0.040	0.102	0.073	0.261	0.101	0.098	0.247	0.294
Stage 2 sleep duration, min	−0.076	−0.033	0.120	0.162	−0.050	−0.161	0.093	−0.067
Slow wave sleep duration, min	0.044	0.115	0.260	0.058	0.015	0.084	0.245	0.586**
Rapid eye movement (REM) sleep duration, min	0.264	0.246	0.223	0.139	0.449	0.421	0.301	0.142
Wake after sleep onset (WASO), min	−0.270	−0.327	−0.107	−0.122	−0.246	−0.454	−0.123	−0.352
Sleep efficiency	0.270	0.394	0.198	0.313	0.179	0.292	0.150	0.290

**$P < 0.001$.

In control participants, late improvement in performance following sleep was correlated positively with duration spent in SWS ($P = 0.007$) (Fig. 2). Partial correlational analyses revealed that this relationship remained significant even when controlling for age ($r = -0.64$, $P = 0.003$). Interestingly, in the PD group the amount of time spent in SWS was not correlated with any late improvement in the motor skill task following a period of sleep. In order to compare the difference in correlations between the groups, a Fisher $r–z$ transformation was undertaken, demonstrating a significant difference between the groups ($z = -2.09$, $P = 0.037$).

Furthermore, the amount of time spent in SWS did not differ between controls and PD patients, nor did time spent in SWS correlate with any disease variable in the PD group. There was, however, a significant positive correlation between late improvement in the motor skill task relative to baseline performance with disease duration ($r = -0.38$, $P = 0.031$) and DDE ($r = -0.37$, $P = 0.023$). Multiple regression analyses exploring the relative contribution of both disease duration and DDE to offline consolidation in PD found DDE to be the only significant independent predictor ($P = 0.039$).

After correcting for multiple comparisons, no other significant correlations between motor skills learning and sleep architecture measures were observed in either the PD or control groups.

DISCUSSION

The current study is the first to explore the specific contributions of sleep architecture to motor skill learning and consolidation in PD, comparing this directly to performance in a healthy ageing sample.

The main finding of this study is that PD patients do not demonstrate any apparent deficit in within-session learning and overnight stabilization of a motor skill task compared to healthy older adults, as evidenced by the non-significant difference between post-training and early retest performances in both groups. While both groups failed to demonstrate offline improvements in performance, neither showed a significant decline in performance, suggesting that both groups had stabilization of the memory trace following sleep. If patients with PD had impaired memory stabilization processes, then we would have predicted

performance deficits in the early retest condition, which were not found.

The second important finding of the study is that while neither group demonstrated enhanced performance immediately following sleep, healthy older adults performed better in the second half of the post-sleep retest session (termed late retest learning) compared to their post-training learning, an effect that was not observed in the PD group. This effect in healthy older adults was also found to be associated with a longer duration in SWS. No such improvement was observed in the PD group. Our findings support the growing body of evidence linking SWS to sleep-enhanced procedural and declarative learning in the absence of cognitive decline (Payne, 2011; Stickgold, 2005). In contrast to previous studies in younger populations (Walker *et al.*, 2002, 2003) no relationships were found with Stage 2 sleep. Interestingly, while it is well known that the proportion of SWS sleep declines with advancing age (Floyd *et al.*, 2000; Naismith, 2011), these findings demonstrate that in healthy older adults longer duration spent in SWS during the post-training night relates to within-session improvement post-sleep. As others have proposed (e.g. Conte *et al.*, 2012), pre-sleep learning may have a beneficial reorganizing effect on sleep quality in healthy older adults which, in turn, is beneficial for performance on the next-day training session.

Conversely, in patients with PD, the relationship between SWS duration and post-sleep learning performance was absent. While PD patients can learn the task in the same way as normal subjects, with comparable learning and error rates in the pre-sleep learning sessions, they did not demonstrate the within-session improvement observed in healthy older adults following a period of sleep. Our findings contrast with a recent finding by Scullin *et al.* (2012), which demonstrated that the duration spent in nocturnal SWS enhanced a different cognitive skill, working memory training, in Parkinson's disease. There are a number of possible explanations for our findings. It could be that the contribution of SWS to memory consolidation in PD differs according to the cognitive skill being learnt. Future studies could include a broader range of cognitive tasks to determine whether factors such as task design, level of difficulty and test modality play a role. An alternative explanation is that sampling differences, such as disease duration or disease severity, modify these relationships. Our findings, however, do not support this explanation. The total time spent in SWS did not differ between those with and without PD and did not correlate with age, disease severity, dopamine dose equivalence, depressive symptoms or general cognitive functioning.

Another explanation, and the one most suggested by the findings of this study, is that the optimal benefits of these sleep stages on post-sleep motor skill learning in PD relate to specific neurophysiological mechanisms that are predominantly, but not necessarily restricted to, SWS. Interestingly, SWS is greatly reduced with advancing age and in dementia relative to healthy younger adults (Bliwise, 1993), and has been shown to be reduced in PD (Diederich *et al.*, 2005).

Studies employing power spectral analyses have also found a significant decrease in slow wave activity in PD patients compared to healthy controls (Brunner *et al.*, 2002). It is plausible that disruption to cortico–striato–thalamic circuitry and changes in dopaminergic neurotransmission drive changes in SWS in this clinical population. Future studies employing measures of sleep microarchitecture may prove useful to delineate whether specific neurophysiological components of SWS sleep are absent in PD, giving rise to impaired consolidation.

Our findings also suggest that there is a specific interaction between dopaminergic medication use and motor skill learning in PD. Specifically, a greater DDE was associated with improved consolidation on the motor skill task, which supports previous findings that dopaminergic medication has benefits for offline sleep-specific cognitive improvements in a range of cognitive skills (De Lima *et al.*, 2011; Schicknick *et al.*, 2012; Scullin *et al.*, 2012). While all patients were tested in their 'on' state, there may also be an effect of dopaminergic medication at an individual level on motor sequence learning, which was not controlled for in this study. This may have differed as a function of time of day, given that pre-sleep learning took place in the evening and post-sleep learning in the morning. Furthermore, no attempt was made to control for disease lateralization or handedness, with all participants completing the task using their non-dominant hand, which is another possible limitation of this study.

The current findings add to the growing body of studies by our group and others which propose that specific patterns of sleep–wake disruption are linked to specific profiles of cognitive performance in PD (Naismith *et al.*, 2011a,b; Scullin *et al.*, 2012). These findings offer some promise for improved clinical management of PD by offering insights into methods by which motor skill learning can be enhanced in PD. For example, optimal learning of new motor skills in targeted fall prevention programmes may be enhanced by the use of behavioural strategies aimed at increasing components of nocturnal SWS (e.g. minimizing daytime napping, increasing exercise, diet modification and controlling body temperature) which have beneficial effects for learning in healthy ageing. The correction of underlying sleep–wake disturbance may also improve the efficacy of cognitive training programmes targeting specific cognitive deficits such as motor skill learning in PD. As such, the current findings may help to direct novel, more directed approaches that ease the burden of this prevalent disease.

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AUTHOR CONTRIBUTIONS

Zoë Terpening, Sharon L. Naismith, Simon J.G. Lewis carried out the research project, statistical analyses and manuscript. Kerri Melehan, Catherine Gittins, Sam Bolitho carried out the review and critique.

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