KU LEUVEN

FACULTEIT GENEESKUNDE
BIOMEDISCHE WETENSCHAPPEN

Het effect van gerichte geheugen reactivatie tijdens slaap op de consolidatie van het motorisch geheugen bij ouderen en mensen met de ziekte van Parkinson

The effect of targeted memory reactivation during sleep on motor memory consolidation in older adults and people with Parkinson's disease

Masterproef voorgedragen tot het behalen van de graad van Master in de biomedische wetenschappen door

Charlotte MORIS

Promotor en begeleider: Prof Moran GILAT
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Preface

This thesis was written as a part of a five-year course in biomedical sciences at KU Leuven. From September 2021 to May 2022 I became part of a research team that studied the effect of sleep on motor memory consolidation in older adults and people with Parkinson's disease (PD).
Since Parkinson's disease is the second most common neurodegenerative disorder and usually considered a motor disorder, it was interesting to discover that PD has a lot of non-motor symptoms too. Sleep problems are part of those non-motor symptoms. It was very interesting to learn more about the biological processes involved in sleep and that these could possibly contribute to rehabilitation. The importance of sleep and motor memory consolidation can make a big difference for people with Parkinson's disease and this without additional pharmacological treatments.

This master thesis, along with the research was a very interesting and instructive journey in which I was able to develop new skills and knowledge such as scoring the different phases of sleep and to implement acquired knowledge and skills into practice. I would therefore like to use this opportunity to thank a number of people who have helped me throughout this process.

I would first like to thank my supervisor Professor Moran Gilat for the opportunity to work on this project and for the guidance and support during the research and writing of this master's thesis. I am also grateful to my co-supervisor, Professor Alice Nieuwboer, for reading and providing feedback.

In addition, I would also like to thank the participants during the study. Without their involvement and commitment, this research and therefore this thesis would not have been possible.

Finally, special thanks go out to my family and friends and especially my parents and sister for the support over the past years during my studies and especially while working on this master thesis.

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List of abbreviations

AHI	apnea hypopnea index
ATH	absolute threshold of hearing
BG	basal ganglia
CI	confidence interval
COMT	catechol-O-methyl transferase
DBS	deep brain stimulation
DLPF	dorsolateral prefrontal cortex
DT	dual task
ECG	electrocardiography
EEG	electroencephalogram
EMG	electromyography
EOG	electrooculography
fMRI	functional magnetic resonance imaging
h	hours
HADS-A	hospital anxiety and depression scale - anxiety
HADS-D	hospital anxiety and depression scale - depression
HC	healthy controls
HY	Hoehn and Yahr scale
LEDD	levodopa equivalent daily dose
M1	primary motor cortex
mg	milligram
min	minutes
MMSE	mini mental state eximination
MOA-B	monoamine oxidase aldehyde dehydrogenase B
MoCA	montreal cognitve assessment
MSL	motor sequence learning
N1	non rapid eye movement sleep phase 1
N2	non rapid eye movement sleep phase 2
N3	non rapid eye movement sleep phase 3
NREM	non rapid eye movement
NonReactSeq	non-reactivated sequence
PD	parkinson's disease
PMA	premotor area

PMC	premotor cortex
post	post-nap measurement
PPC	posterior parietal cortex
pre	pre-nap measurement
PSG	polysomnography
PVT	psychomotor vigilance test
RBD	rapid eye movement behaviour disorder
REM	rapid eye movement
ret	retention measurement, 24h later
ReactSeq	reactivated sequence
RT	reaction time
SCOPA-D	scales of outcomes in PD - day
SCOPA-N	scales of outcomes in PD - night
SD	standard deviation
SE	standard error
SMA	supplementary motor area
SNpc	substantia nigra pars compacta
SO	slow oscillation
SRTT	serial reaction time task
STN	subthalamic nucleus
SWS	slow wave sleep
TMR	targeted memory reactivation
UPDRS	unified Parkinson's disease rating scale
VAS	visual analogue scale

Abstract

People with Parkinson's disease (PD) have difficulty with consolidating new motor skills due to dysfunction in the basal ganglia, with impact on rehabilitation. Sleep is thought to facilitate consolidation, but PD tend to experience poor sleep. In healthy young people, targeted memory reactivation (TMR) applied during napping improves consolidation, but no study has investigated the effect of post-learning napping with TMR on motor learning in healthy older controls (HC) or PD. The aim of this thesis project was to investigate whether post-learning napping with TMR could improve consolidation of a motor sequence learning task in a pilot sample of five PD and five HC. Speed and accuracy on a serial reaction time task (SRTT) were compared between two different sequences before and after a 2-hour nap coupled with auditory replay of one of the sequences via TMR. A retention measurement of the SRTT was also performed 24 hours later. The primary outcomes, speed (RT) and accuracy, were compared before and immediately after the nap with TMR as the primary endpoint of the study. Secondary endpoints were speed and accuracy after a period of 24 hours and during a dual task (DT) in order to assess long-term retention and automatization effect, respectively. Results of this randomized control pilot study showed no significant three-way interaction effect for time by sequence by group ($F(1,8)=0.04$; $p=0.848$). The two-way interactions were also non-significant. Post-hoc testing showed stabilized performance for both groups on both sequences after the 2-hour nap with TMR (all $p>0.05$). This stabilization persisted at retention in the PD group. In HC, however, the additional night resulted in a significantly slower RT for the reactivated sequence (ReactSeq) ($p=0.016$) and a stabilization of RT for the non-reactivated sequence (NonReactSeq) ($p=0.135$), while accuracy remained unchanged. A positive effect was seen in PD, however, whereby the ReactSeq became faster ($p=0.010$) and accuracy seemed to improve ($p=0.350$), with further practice at retention, while the NonReactSeq stabilized. Moreover, the DT improved for the ReactSeq in the PD group ($p=0.025$), though this seemed to come at a cost of worse accuracy ($p=0.044$). In the HC, both sequences became significantly faster ($p<0.004$) with similar accuracies with further practice at retention and showed no DT effects (all $p>0.05$). The results of this thesis showed no effect on the primary endpoint, but do provide some support for better consolidation in PD after napping with TMR, so that they could improve RT with further practice at retention and achieve better automatization, although possibly at a cost of worst accuracy. Further studies with larger samples are now needed to verify these results and ascertain the true effects of post-learning napping with TMR on motor memory consolidation in HC and PD.

1 INTRODUCTORY LITERATURE OVERVIEW

1.1 Parkinson's disease

Epidemiology

Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting about 1-2% of the population aged 60 years or above (1). PD is the fastest growing neurodegenerative disorder in the world. The number of people with PD is expected to double to over 12 million worldwide by 2040 (2). Possible reasons for this so-called 'Parkinson pandemic' may be improved diagnostic accuracy, but also the increase in size and longevity of the general population, and by-products of industrialization, such as the use of pesticides, solvents, and heavy metals (2).

Pathology

The most important pathological feature of PD is the degeneration of dopaminergic neurons in the pars compacta of the substantia nigra ($\mathrm{SN_{pc}}$). The most affected area of the $\mathrm{SN_{pc}}$ contains neurons that project to the dorsal putamen of the striatum (3). This neurodegeneration leads to reduced facilitation of movements (1). Importantly, other neurotransmitters are also affected in PD, including the cholinergic, serotonergic and noradrenergic systems (4). The degeneration in PD is the result of Lewy pathology. Specifically, misfolded α-synuclein becomes insoluble and aggregates to form Lewy neurites or Lewy bodies within the cell body (1,3).

The α-synuclein aggregation is not limited to the $\mathrm{SN_{pc}}$, but eventually becomes widespread throughout the brain as the disease inevitably progresses (1,3). The resulting neurodegeneration can be divided into 6 stages according to the Braak hypothesis (**Figure 1**) (5,6). Stage 1 represents the onset of PD pathology when Lewy bodies start to affect nuclei in the lower brainstem. In this stage the nervus vagus, medulla oblongata and the nucleus olfactorius anterior become particularly affected (6). In stage 2, Lewy bodies progress and start to appear in the caudal raphe nuclei, the reticular formation and the locus coeruleus (5,6). As the disease slowly progresses with time, more and more Lewy bodies appear in different areas. During stage 3, the pathology reaches the $\mathrm{SN_{pc}}$ and distributes to the nucleus basalis of Meynert. Severe degeneration of dopaminergic neurons of the $\mathrm{SN_{pc}}$ occurs during stage 4 (5,6). Next, the mesocortex, allocortex, amygdala and parts of the thalamus are additionally

affected. Eventually, during stage 5 the Lewy bodies become widespread and start to affect the neocortical temporal, parietal and frontal lobes of the brain. In the final stage 6, the entire neocortex becomes affected. The different stages are associated with the development and worsening of motor and non-motor symptoms in PD, as described below (5,6).

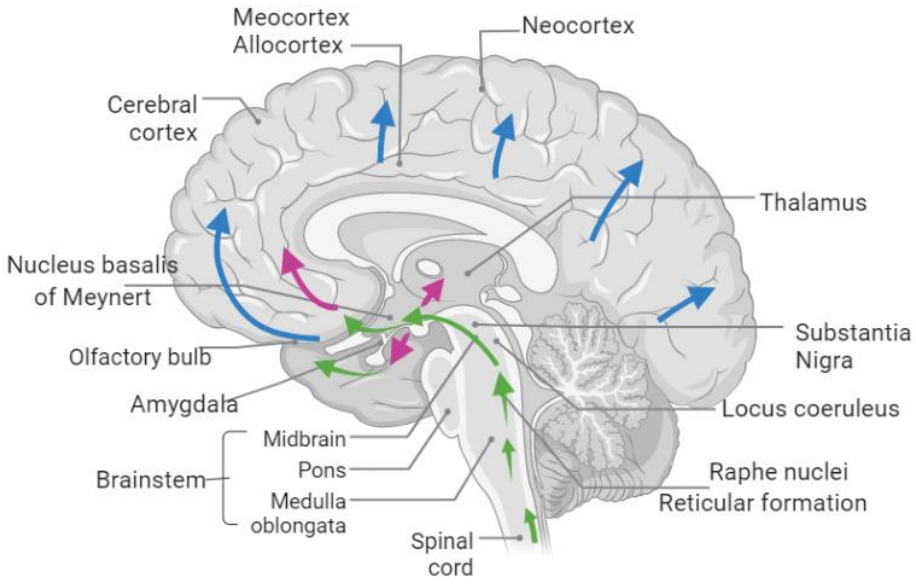


Figure 1: Distribution of α-synuclein pathology throughout the brain of Parkinson's disease according to the Braak hypothesis. Pathology starts in the lower brainstem and then spreads further along the upper brainstem to the substantia nigra in the midbrain, and eventually affects the entire cerebral cortex.

NOTE: Green arrows denote Braak stages 1 and 2; purple arrows denote stage 3 and 4; blue arrows denote stages 5 and 6.

Figure adapted from Braak et al. (5) *with BioRender.com*

Symptoms

People with PD develop a diversity of motor and non-motor symptoms. The cardinal motor symptoms of PD are bradykinesia, rest tremor (mostly with a frequency of 3 to 5 Hz (7)), muscular rigidity that can initially present asymmetrically at disease onset but will soon affect the body bilaterally. These classical symptoms define the clinical diagnosis of PD. Later in the disease course, patients typically also develop more complex motor problems, including speech dysfunction, postural instability and gait impairment with possible freezing. Dysphagia can also occur in the later stages, forming the greatest risk for mortality following aspiration pneumonia (3). The motor symptoms of PD occur only after already 40-60% dopaminergic neurons in the $\mathrm{SN_{pc}}$ are lost (8). Non-motor symptoms are also highly prevalence, and include cognitive impairment, olfactory dysfunction, pain, psychiatric symptoms, autonomic dysfunction (e.g. constipation, urinary urgency and frequency, orthostatic hypotension), fatigue

and sleep disturbances (3,7). These non-motor symptoms are similarly with greatly reduced health-related quality of life (3).

The presentation of clinical symptoms is linked to the heterogeneous distribution of α-synuclein (6). Stages 1 and 2 of the Braak hypothesis are considered prodromal or presymptomatic, given the lack of motor features, though early non-motor symptoms, such as autonomic, olfactory, and sleep dysfunctions, can already be evident. The appearance of motor symptoms is linked to stages 3 and 4 of the Braak model. The last two stages are commonly associated with cognitive impairment and more complex and debilitating motor symptoms, such as freezing of gait, falls and dysphagia (6).

Treatment of PD

Currently, there are no approved treatments that can slow down the disease progression. Treatment of PD is therefore purely symptomatic. The mainstay treatment for PD is pharmacological management, and in particular dopamine-replacement therapy that aims to increase dopamine concentrations or stimulate dopamine receptors (3,9). The most commonly used drug is Levodopa, a precursor to dopamine, which can cross the blood-brain barrier, where it is transformed to dopamine. Levodopa is usually paired with carbidopa to reduce peripheral uptake of Levodopa (9). Dopamine agonists stimulate dopaminergic receptors in the central nervous system (9). Other common drugs include catechol-O-methyl transferase (COMT) inhibitors and monoamine oxidase aldehyde dehydrogenase B (MOA-B) inhibitors, which inhibit the enzymes involved in the post-synaptic breakdown of dopamine, thereby prolonging its effect (9). However, these types of drugs can induce acute adverse reactions, such as nausea, daytime sleepiness, hallucinations and impulse control disorders. Moreover, long-term use of levodopa is linked to worsening motor fluctuations and debilitating dyskinesias (3).

Deep brain stimulation (DBS) is another possible treatment when levodopa proves effective, but at the cost of motor fluctuations or dyskinesias, or when severe tremor is not adequately suppressed by medication (3,10). The main target for DBS in PD is the subthalamic nucleus (STN), which is generally effective for reducing cardinal motor symptoms in moderate-to-severe PD (10). Symptoms resistant to dopaminergic medication, such as freezing of gait, postural instability or dysarthrophonia, similarly do not show much improvement following DBS (10). Moreover, patients with significant cognitive decline, depression, psychotic disorders or personality disorders are contraindicated for DBS because it could aggravate these symptoms (10). Therefore, DBS treatment is not applicable to all patients. DBS surgery is also highly

invasive with risk for serious adverse outcomes, such as bleeding and post-surgery infection. In addition, there can be device-related complications such as lead breakage or malfunctioning of the pulse generator (10). Lastly, there are some possible stimulus-induced side effects that can be caused by stimulating neighbouring structures and fibres. These side effects depend on the anatomical location of the DBS and can be variable, including speech problems, muscle contractions, nausea, and dyskinesia (10).

Rehabilitation has therefore emerged as an important therapeutic strategy for managing the more complex motor symptoms in PD (11,12). Rehabilitation in PD entails physical activity for promoting general health (13), exercises whereby particular outcomes are achieved with the goal of improving certain symptoms, such as treadmill training to improve gait speed (14), and motor skill learning, see below (12). Motor rehabilitation in PD benefits from adding external sensory information during learning, such as via auditory or visual cueing. These cues are processed via compensatory circuits that largely bypass the defective basal ganglia (BG) (11,13).

1.2 Motor skill learning

Motor skill learning is an important aspect of neurorehabilitation because learning or re-learning motor skills is important for continuing to function as independently as possible (11). Examples of motor skill learning are (re)learning to make complex sequential movements, such as getting in and out of bed, as well as fine motor skills such as writing and typing. However, people with PD have difficulty with the consolidation of motor skills and this seriously reduces the long-term benefits of rehabilitation (14).

Motor learning can be described as the process by which a new set of inter-related movements are acquired, optimised and retained following repeated practice and consolidation (15). The final goal of motor learning is the optimisation, long-term retention, transfer and automatization of newly learned or re-learned skills (14).

Motor learning can be considered to involve three stages. Each stage involves different parts of the brain. In healthy adults, there is a shift from cortical to subcortical neural activity and a general decrease in brain activity throughout the learning process (11). The first stage of motor learning is initial learning (11), during which the performer uses attentional resources to figure out the task demands, mostly through the interpretation of verbal instructions and feedback (11). Many mistakes are made at this stage and performance is often slow at first, but shows rapid improvements with repeated practice (11,14). The initial learning phase involves the

dorsolateral prefrontal cortex (DLPF) and the posterior parietal cortex (PPC), which are connected to the anterior striatum (**Figure 2**) (16). During early learning there is a functional interaction between the prefrontal cortex and both the hippocampus and striatum. This forms the associative corticostriatal loop (16). The hippocampus and prefrontal cortex control the spatial-sequential component, creating a spatial map of the motor task (16,17). The striatum is particularly involved in the procedural component of the motor task (16,17). The involvement of both structures in the different memory processes points to possible interactions between those brain structures (17). There is thought to be a competitive interaction between the hippocampus and striatum and this competition seems to decrease as performance of the learned task improves with practice and consolidation (17).

Consolidation is the second stage of motor learning, during which the newly acquired information is optimised and reorganised for long-term neocortical storage without additional practice (i.e. 'offline') (14,18). Offline consolidation makes the initially labile memory trace more stable over time and robust to interference (18). It also facilitates transfer of the learned task to similar, yet untrained tasks (15,19). The consolidation process is strongly determined by the dorsal striatum's capacity to chunk individual motor sequences into motor engrams, which are more efficient to store and easier to recall (16). Spatial and sensory information is stored in motor maps in the promoter cortex (PMC) and the posterior parietal cortex (PPC) is involved in the integration of sensory information into the motor program (**Figure 2**) (16). The pre-supplementary motor area (SMA) is important for the integration of spatial information with sensorimotor processing (16).

The final autonomous learning stage follows after further practice and consolidation and allows performance to become automatized, which means that performers need less attentional resources to perform the skill, ultimately allowing them to simultaneously perform another consecutive task with minimal interference on the primary motor task (i.e. dual-tasking) (11).

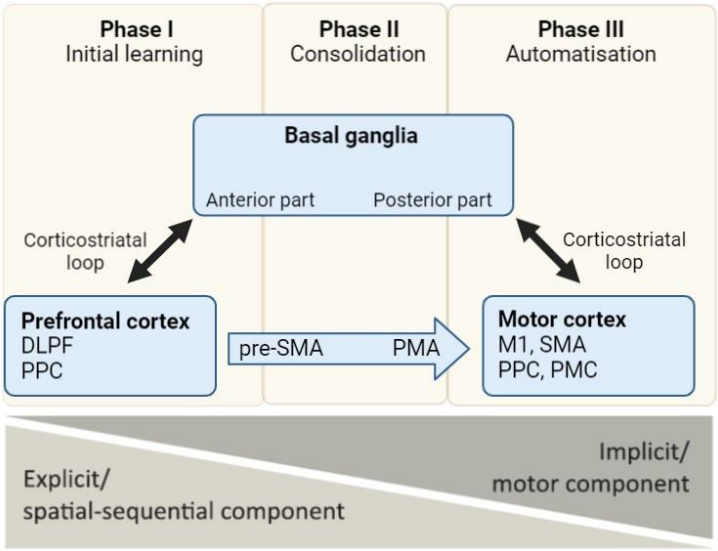


Figure 2: Schematic representation of the brain areas involved in the three stages of motor learning.

NOTE: DLPF, dorsolateral prefrontal cortex; PPC, posterior parietal cortex; PMA, premotor area; PMA, premotor area; M1, primary motor cortex; SMA, supplementary motor area; PMC, premotor cortex. Figure adapted from Dahms et al., 2020 (16) *with BioRender.com*

People with PD frequently show reduced consolidation of the newly learned skill and therefore have impaired retention and automatization (11). Consolidation deficits in PD are in large part due to their basal ganglia (BG) dysfunctions, given that the BG, and in particular the striatum are important for motor learning (18). In healthy adults, activation shifts from the anterior striatum during initial learning to the posterior striatum with repeated practice and consolidation (15). The posterior striatum is particularly important for chunking the motor sequences and transforming them for long-term storage (20). People with PD, however, have particular dysfunctions in the posterior striatum due to dopamine deficiency (15). The anterior striatum, on the other hand, is relatively spared in the early stages of the disease (15). This may explain why initial learning is largely preserved in PD, while there are difficulties with consolidation and automatization.

Motor learning and consolidation effects can be determined by performance on a motor sequence learning (MSL) task. The serial reaction time task (SRTT) is a frequently used MSL task, during which participants learn one or more motor sequences by pressing corresponding keys as quickly as possible with one of their fingers in response to visual cues presented in sequential order on a screen (16). Learning on the SRTT can be defined by faster reaction times, and improved accuracy in terms of reduced erroneous responses (16).

The SRTT consists of both implicit and explicit learning components. Implicit or procedural learning refers to improvements in performance that occur in a largely unconscious manner, and include automatization and habit formation (14). This memory system is associated with activity in the posterior striatum (17). Pure implicit motor learning occurs in the absence of prior knowledge of the task to be learned. Explicit or declarative motor learning refers to the ability to verbalise learning processes and outcomes and requires a high degree of awareness (11). Explicit learning often requires rules to be spoken and demands cognitive input. This memory system is thought to rely on the anterior striatum, as well as the medial temporal lobe and especially the hippocampus (17).

However, in practical terms, the distinction between pure implicit and explicit learning components is difficult to make (11). Both processes are often required for optimal learning. Explicit processes are more required during initial learning and implicit processes occur in particular at the consolidation and automatization stages (16). Instructions in motor tasks are explicit, but incidental learning can occur because subjects are unaware of the learning process (14). Moreover, participants often have at least partial declarative recollection of a sequence that was expected to be implicitly learned (14). Previous SRTT studies have shown that overall learning in PD is slower and more dependent on cognitive strategies, i.e. explicit learning (14).

1.3 Consolidation during sleep

Motor memory consolidation is thus an essential step in the motor learning process. The reactivation theory of consolidation states that during initial learning, the hippocampus integrates information from different cortical parts to form a coherent memory engram (17). Reactivation of these engrams after practice, i.e. during consolidation, is thought to strengthen synaptic connectivity, allowing memories to eventually become ‘stored’ in the neocortex and become hippocampus independent (17). As it turns out, sleep appears a particularly favourable state for consolidation to occur (19,21).

Young adults show an improvement in performance through practice (22). This improved performance is preserved and will even be further enhanced offline by a period of sleep (22). Both a night of sleep and a daytime nap lead to an improvement in offline learning and an increase in resistance to interference compared to an equivalent period of wakefulness (22,23). Older adults also show improvement in performance through exercise demonstrating that they are also able to learn new motor sequences (22). Sleep facilitates the stabilisation of

performance in older adults, but an off-line improvement after sleep, as is the case in healthy young people, is not observed (22). It has been suggested that sleep-dependent consolidation of motor memory would decrease with age due to changes in sleep quantity or quality or reduced integrity of neural networks as a result of age-dependent cortical and subcortical atrophy (18).

According to the active system consolidation model, the newly acquired memories are continuously reactivated by the hippocampus during post-learning sleep leading to reorganisation and storage of memory in cortical networks (21). The transfer of information from the hippocampus to the neocortex appears to be particularly regulated during non-rapid eye movement (NREM) sleep and has been related to neurophysiological features of NREM sleep, including sleep spindles and slow oscillation (SO) (24). The synaptic homeostasis model proposes another theory describing motor consolidation during sleep (19). According to this model, encoding information during wakefulness would cause progressive synaptic strengthening resulting in saturation of the ability to learn (19). Subsequent NREM sleep then restores the ability to learn new information by down-scaling of synaptic strength (19,24). Recently, it was proposed that both models work in synergy (19), so that local memory reactivation and global downscaling during sleep are both required to optimise the memory process (19).

Sleep can be studied in a sleep laboratory using polysomnography (PSG), which provides measures of electroencephalography (EEG), electromyography (EMG), electrooculography (EOG), as well as breathing and autonomic functioning (25). Sleep consists of different stages, each with distinctive characteristics on the PSG (25). During quiescent wakefulness with the eyes closed a sinusoidal alpha rhythm (8-13 Hz) is seen on the EEG of the occipital cortex (26). NREM sleep stage 1 (N1) is often the first stage of sleep associated with the presence of low-amplitude, mixed-frequency activity on EEG (4-7 Hz) and slow rolling eye movements on the EOG (26). NREM sleep, stage 2 (N2), is characterised by sleep spindles and K-complexes (27). Sleep spindles are short bursts of high-frequency sigma waves (11-16 Hz) that last for 0.5 seconds or longer and are usually most prominent in the central areas of the brain (26). K-complexes are biphasic slow-waves with amplitudes above 75 µV and a duration that is greater than or equal to 0.5 seconds, K-complexes are most prominent in frontal areas (26). During NREM stage 3 (N3) sleep, there is abundant slow wave activity (0.5 – 2 Hz) in the frontal regions of the brain (24). N3 sleep is therefore also called slow-wave sleep (SWS). Rapid eye movement (REM) sleep, typically occurs after NREM sleep and becomes more frequent towards the end of the night (26,27). REM sleep is characterized by low-amplitude mixed-

frequency oscillations on EEG and complete muscle atonia on the EMG (26,27). Rapid eye movements are also frequently present on the EOG (26).

1.4 Sleep and Parkinson

Besides their BG dysfunctions, people with PD are also frequently troubled by sleep problems, which are likely to impact on motor memory consolidation (28). Indeed, sleep fragmentation is the most common complaint in PD resulting in sleep maintenance insomnia with frequent awaking and overall reduced amounts of NREM sleep throughout the night (28).

According to Terpening et al. (2013), there is no difference in learning capacity within a session of a motor skill task between a group of healthy older adults and PD patients and sleep stabilized motor memory in both groups. There was, however, a difference in performance after a period of sleep. Healthy older adults showed improvement with further practice during a second retest, a period after sleeping, while the group with PD did not show this improvement (21). Thus, consolidation during sleep may indeed be impaired in people with PD compared to healthy adults, although in this study there was no wake control group to compare the difference between sleeping and staying awake (21).

Interestingly, in healthy adults, short periods of post-learning napping have been shown to protect the newly learned skill from subsequent sleep deprivation at night (29). During NREM sleep, the motor-learning related neural circuits are less challenged by interference from simultaneous tasks and primed for memory consolidation via sleep spindles and SO (30). Moreover, non-invasive techniques have been developed that can further strengthen the consolidation process during sleep (19). This offers the exciting possibility to combine post-learning napping with such interventions to simultaneously boost motor memory consolidation in PD, especially in the early stages of the disease.

1.5 Targeted Memory Reactivation

One such technique is targeted memory reactivation (TMR), during which learning-related sensory stimuli (e.g. auditory cues) are replayed during subsequent NREM sleep (19). TMR induced hippocampal replays during sleep are thought to further strengthen recently formed neural connections and accelerate the redistribution of memory traces to the neocortex (31). In healthy adults, TMR during post-learning sleep indeed provided better consolidation effects

than what was seen following sleep without TMR (32,33). Importantly, shorter periods of sleep with TMR reached similar effects on motor performance than longer periods of regular sleep without TMR (31). This offers new possibilities to similarly improve motor performance in PD who typically have poor sleep at night. Despite their sleep problems, TMR can still be used as there is enough N2 and N3 sleep left with spindles and SWS of sufficient frequency and amplitude (18). To date, no study has investigated the effect of TMR during sleep on motor memory consolidation in PD.

2 OBJECTIVE AND HYPOTHESES

The primary objective of the present study is to investigate whether post-learning sleep coupled with auditory TMR improves the consolidation (pre-nap vs. post-nap), 24h retention and automatization of a motor sequence learning task in a pilot sample of five people with PD and five healthy older adults by comparing performance on two learned motor sequences before and after a 2-hour nap, during which one of the two sequences is replayed using TMR. The secondary objective is to examine the impact of PD on the response of TMR by comparing their performance to that of healthy older adults.

The primary hypothesis, is that performance on the SRTT, as measured by the difference in speed and accuracy between pre- and post-nap measurements, of the sequence reactivated with TMR will improve compared to the non-reactivated sequence within both the PD group and the healthy controls. The second main hypothesis is that the magnitude of improvement following TMR on the SRTT between pre- and post-nap will be lower in the PD group relative to the healthy controls, given the difficulties PD patients have with motor memory consolidation.

In addition, we expect better retention effects (post-nap vs. retention) on both primary outcomes for the reactivated sequence relative to the non-reactivated sequence for both the PD and healthy control group. Lastly, we hypothesize that performance on both outcomes for the dual task will improve more for the reactivated sequence than for the non-reactivated sequence at retention in both PD and healthy controls.

3 MATERIAL AND METHODS

3.1 Participants

The present thesis was part of a larger European project ("TARGET-SLEEP" – NCT04144283). For the full study, 20 healthy controls and 20 individuals with a clinical diagnosis of idiopathic PD will be recruited. For this thesis, a convenience sample of five people with PD and five controls who were already recruited for the larger projects were included in the present TMR experiment (**Figure 3**). All participants provided written informed consent prior to enrolment. Participants could be included if they were aged 40 years or older and right-handed. PD were included if they had a clinical diagnosis of idiopathic PD made by a Neurologist according to the latest diagnostic criteria of the International Movement Disorder Society. The following exclusion criteria were applied; i) severe untreated sleep apnea as detected during an overnight screening PSG and defined by an apnea hypopnea index (AHI) > 30; ii) comorbidities that could interference with SRTT performance; iii) severe cognitive impairment that could question the voluntary informed consent, as determined by a Mini Mental State Examination (MMSE) score < 24; iv) freezing of gait, as determined by a positive answer on item 1 of the New Freezing of Gait Questionnaire; v) DBS, and vi) actively enrolled in another therapeutic trial. Healthy older adults were screened according to exclusion criteria i-iii. The study was approved by the local medical ethical committee of the University Hospital Leuven (S61792).

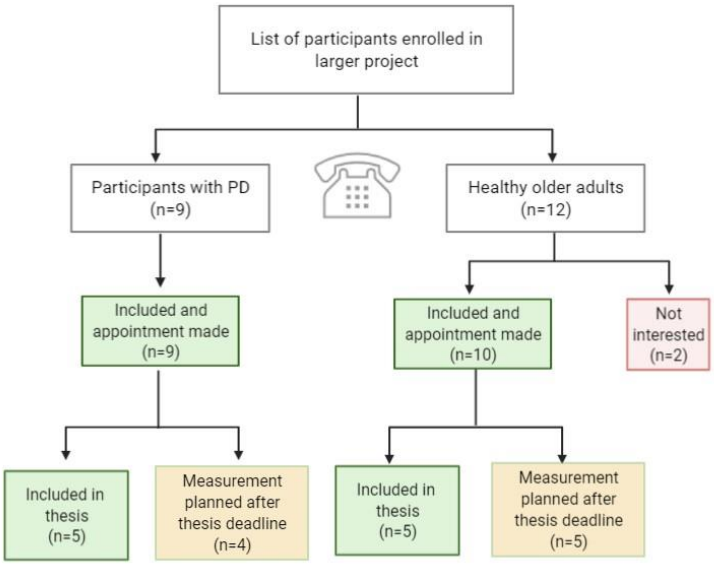


Figure 3: Recruitment schema of current thesis project. In total, nine PD and 12 older adults were contacted of whom five PD and five older adults were able and willing to participate in the thesis period. *Created with BioRender.com*

3.2 Study design

This interventional, randomised controlled trial consisted of three study visits (**Figure 4**). During the first screening visit, a clinical characterization was done for all participants to verify the inclusion and exclusion criteria. Demographic data such as age, gender and years of education were also collected. The following neuropsychological tests were done: the MMSE, montreal cognitive assessment (MoCA), Logical memory I and II, Ray Auditory Verbal Learning, Digit Span, Clox test, Trail making test A and B, Verbal fluency (letter and animals), Rey Osterrieth Complex Figure Copy, Benton line orientation, Boston naming and the Purdue pegboard test to check hand skills. Questionnaires were administered to check sleep quality (Scales for outcomes in PD day and night), anxiety and depression (hospital anxiety and depression scale) and apathy (Lille apathy scale). For the participants with PD, the severity of the disease was assessed with the Unified Parkinson's Disease Rating Scale (UPDRS) and the Hoehn and Yahr (HY) rating scale. Disease duration was documented and medication intake was recorded on standardized forms so that the Levodopa equivalent daily dose (LEDD) could be determined. At the end of visit 1, participants stayed for an overnight PSG at the Centre for Sleep Monitoring of the University Hospital Leuven to determine possible sleep symptoms and to serve as habituation, making participants used to sleeping in a laboratory setting prior to the experimental nap.

Individuals who were eligible to participate in the study returned for the experimental visit 2, which was conducted at the BrainsHub sleep laboratory at the Department of Rehabilitation Sciences of KU Leuven. Participants were asked to keep a sleep diary one week prior to experimental visit 2 to provide insight into sleep quality and duration and they were asked to maintain a consistent sleep rhythm for three nights prior the experiment. During visit 2, sensors, namely O1/O2, Pz, Cz, C3/C4, Fz, F3/F4, references (A1/A2), EOG and EMG were applied to monitor the electrical activity of the brain. Participants then performed the SRTT learning two different sequences that were visually and auditory cued. After learning, they were offered a 2-hour nap opportunity, during which one of the two sequences was replayed using auditory TMR during N2 and N3 sleep. Sleep staging was determined by live visual examination of the PSG signals for characteristics of these stages such as spindles and slow waves by the researchers. Which sequence was replayed via TMR and which sequence was learned first was determined by concealed randomization conducted via an online application by an independent researcher. After the nap, participants were given at least 30 minutes to wake up and counteract the effect of sleep inertia before retesting them on the SRTT (retest 1, post-nap). At the end of visit 2, participants were given an Actigraph (Phillips Respironics Actiwatch II, Netherlands) to wear for 24 hours in combination with a sleep diary. The actigraphy was

used to measure activity patterns and document the night of sleep at home that took place between visits 2 and 3.

During visit 3, which took place 24-hours later, the same SRTT was again performed for both sequences (retest 2, retention). An additional dual-task condition of the SRTT was performed at the end of retest 1 and retest 2. Prior to the start of each SRTT, participant's vigilance was measured with a 10-minute psychomotor vigilance test (PVT). Participants were instructed not to consume alcohol and caffeine on test days. PD patients were allowed to continue to take their medications as usual and all measurements were done on-medication. The LEDD was calculated and a visual analogue scale (VAS) was used for the PD patients to determine the effectiveness of the medication (i.e. "How on do you feel right now?") prior to each SRTT and just before the nap period during visit 2.

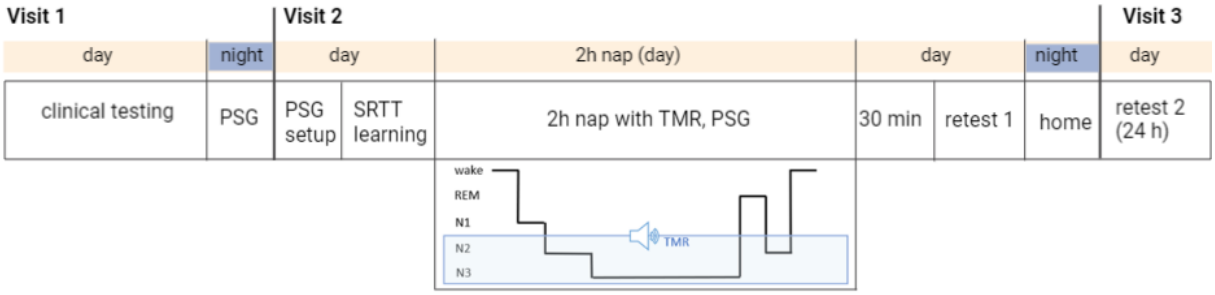


Figure 4: Study timeline including the three visits and experiments performed.

NOTE: PSG, polysomnography; SRTT, serial reaction time task; REM, rapid eye movement sleep; N1, 2, 3: non rapid eye movement sleep stage 1, 2, 3; TMR, targeted memory reactivation.

Figure adapted from Creery et al. (34) *with BioRender.com*

3.3 Polysomnography

During the screening night, in the Centre for Sleep Monitoring of the University Hospital Leuven, participants were screened by video-PSG for severe apnea (AHI>30) as verified by a somnologist. To monitor sleep and detect any sleep symptoms, the following sensors were applied: Electrocardiography (ECG) to measure heart rate, EOGs to monitor eye movements, and EMG of the submentalis muscles to measure tongue muscle tension and movements. A thermistor, nasal flow and two respiratory belts (thorax/abdomen) were used to monitor breathing and a small microphone was fitted to measure snoring or vocalization during sleep. The O1/O2, C3/C4, F3/F4 electroencephalography (EEG) leads were applied to measure cortical activity of the brain using the international standard 10/20 system.

At visit 2, PSG was also applied during the 2-hour nap to identify the different sleep stages. In total, 17 sensors were applied using the international standard 10/20 system, namely O1/O2, Pz, Cz, C3/C4, Fz, F3/F4, reference (A1 and A2), bilateral EOG, EMG of the submentalis muscle's and two respiratory belts (thorax/abdomen). Video with sound was also captured during the nap to observe any movements or snoring and for safety. Sleep staging of the nap was scored offline by a researcher using the FASST toolbox in Matlab (Mathworks, USA).

3.4 Absolute hearing threshold

Each sequence consisted of eight different tones and for each tone the absolute hearing threshold (ATH) was determined at the beginning of visit 2. The ATH was defined as the minimum volume that the participant could hear each tone during wakefulness without interference from another sound being present. While seated, participants were asked to give a nodding sign each time they heard a tone presented through specialised earplugs (Etymotic ER2 in-ear plugs). The volume of each tone was played lower and lower until the participant could no longer hear it and then the volume was increased steadily until the minimal auditory threshold was determined. Determining the ATH accurately for each participant, for each tone separately, ensured that participants could hear all tones equally well, regardless of any hearing difficulties. During the SRTT, the tones were played through the earplugs at a volume of 1000% of the ATH. During the 2-hour nap with TMR, each tone was played at 140% of the ATH, so that participants could hear these tones, but were not awakened by them during stimulation with TMR during N2 and N3 of sleep.

3.5 Serial reaction time task (SRTT)

During the SRTT (**Figure 5**), two motor sequences were learned by finger tapping in response to visual stimuli (35). Participants performed the SRTT with both hands while seated in front of a computer screen, on which eight squares of equal size were presented horizontally. Responses could be given by depressing one of eight keys (one for each index finger, since the thumbs were not used) on an extra-large keyboard (Monster 2, Accuratus, UK) containing only those keys. Responses were cued by filling the squares with a green colour one by one in either a sequential or random order, depending on the task condition. Participants had to respond to the cues by pressing the corresponding key as quickly and accurately as possible. Upon response to the cue presentation, they received an auditory cue through the earplugs, with each key being linked to a particular musical note (A4-G4). Upon each key press, the next

square was filled with zero milliseconds delay. The two sequences used were; sequence A: 4-7-3-8-6-2-5-1 and sequence B: 7-2-8-4-1-6-3-5 and these numbers correspond to one finger each from both hands, from left to right, as shown in **Figure 5c**. Each task condition consisted of alternating rest and SRTT blocks. The rest blocks were represented by a red outline of the squares on the screen and lasted 20 seconds. Before each task block, the outline of the squares turned green giving participants four seconds to put their hands back before the first visual cue (a full green coloured block) was given. Each task block lasted until 48 keys were depressed, ideally forming six correct sequences.

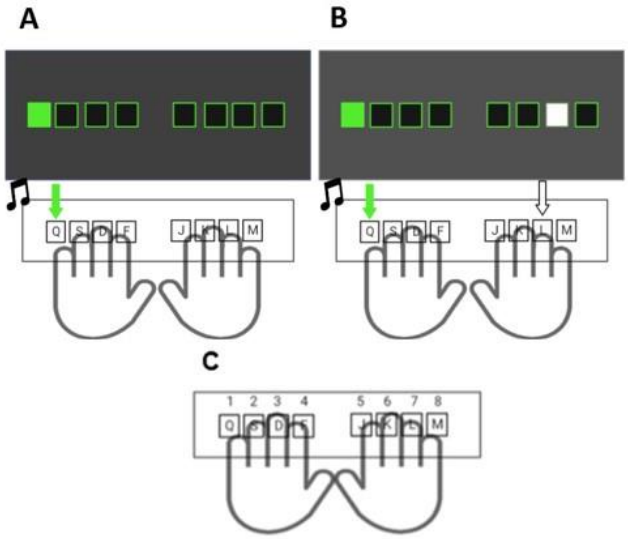


Figure 5: Schematic representation of the serial reaction time task and dual task for two sequences. A) During the SRTT, one of the squares on the screen turns green and the participants were instructed to press the corresponding key on the keyboard as quickly and accurately as possible. They received an auditory cue as feedback after each keypress; B) During the dual task, participants performed the same SRTT as in panel A, but were asked to simultaneously count the number of white squares occasionally presented; C) Representation of which number from the sequence corresponds to which key on the keyboard. *SRTT: serial reaction time task, Figure made with BioRender.com.*

The SRTT had different conditions, namely learning, post-test, dual task, and random. (**Figure 6**). The learning condition lasted for 20 blocks. Per block, one of the two sequences was cued in alternating order (10 blocks seqA, 10 blocks seqB). Which block was practiced first was randomized across participants. Prior to the task, participants were made explicitly aware by verbal instruction that there were two different sequences but they were asked not to focus on this. After 20 blocks, a 2-minute break was provided to counter fatigue on the final four blocks of interest that followed (post-test). At the start of learning before the nap and at the end of each retest session after the nap participants also performed a random SRTT in which 48 keys were cued at random for 4 blocks. Participants were made aware that the cues were presented

at random. At the end of retest 1 and retest 2 (24h later) an extra dual task (DT) condition was performed with 48 keys for 4 blocks (**Figure 5b**). During the DT, squares occasionally filled in white, instead of green, colour in a pseudo random order for 5-8 times per block. Similar to the learning blocks, participants were again required to respond to the cued sequences as fast and accurate as possible, while at the same time count the white squares. After each DT block, the researcher would verbally ask how many white squares were counted. After completing the SRTT during initial learning before the nap, participants were given a 30-minute lunch break before attempting to nap for two hours during which TMR was provided in N2 and N3 sleep. After the nap, a minimum of 30 minutes elapsed prior to retest 1.

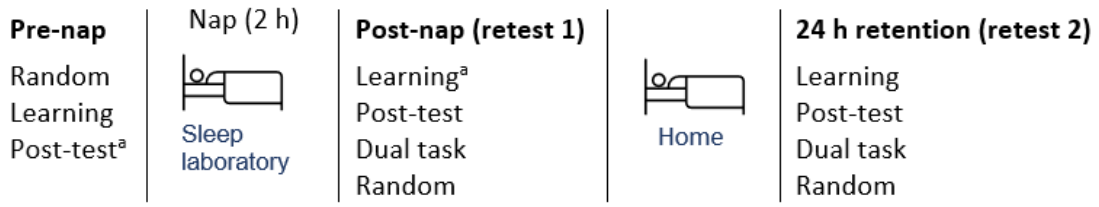


Figure 6: Order of different task conditions of the SRTT for each time point. Learning consisted of 20 blocks in which the two sequences were alternated per block. Post-test, random and dual task each consisted of four blocks. The 'a' defines the primary endpoint, whereby the last four blocks of learning (post-test) pre-nap were compared to the first four blocks of learning post-nap.

Importantly, in the present thesis the primary endpoint of consolidation refers to the period between the end of learning pre-nap and the start of learning post-nap. During this period there was no further practice and any behavioural effect can thus be considered the result of 'pure' consolidation. The retention measurement 24h later was considered a secondary endpoint, as besides consolidation, task performance was also influenced by further practice during the post-nap assessment.

Before the experiment, a behavioural pilot study was conducted in 13 healthy older adults who each performed the SRTT once. Based on this pilot, the number of blocks for learning, the order of the two sequences, and the number of repetitions of the sequences per block for the SRTT were determined for the present experiment as based on the learning curves observed.

3.6 Sleep and Targeted Memory Reactivation

The inter-tone-interval for TMR was determined by the average reaction time for correct responses during the last four blocks of learning (post-test) before the nap. TMR was provided

for one of the sequences during sleep stages N2 and N3. Participants were made aware that sounds would be played during the nap, but that they may not hear them. They were instructed to ignore the sound and continue to sleep in the event that they should hear them. The TMR protocol alternated between periods of active stimulation, during which the sequence was repeated eight times inter-leaved by short one-second rest intervals, and rest blocks of one minute without TMR. The cues were presented 'open-loop' during N2 and N3, regardless of the phase of the SO (36,37). There was one participant in de PD group (Participant 5 in Supplementary Figure S6) who slept well during the nap but who could not hear all the stimulation because the computer sound was switched off, due to human error during data acquisition. The PC sound was turned on about halfway through the nap and a total of 428 stimulations could still be provided with the appropriate volume (140% ATH).

3.7 Statistical analysis

Statistical analysis was performed using SPSS version 28.0. Comparisons between PD group and the healthy control (HC) group for demographics and clinical characterisation were performed using independent sample t-test when data was normally distributed (as verified with the Shapiro-Wilk test) or with the nonparametric Mann-Whitney-U test for non-normally distributed data. A chi-square test was used to compare categorical variables such as gender between groups.

The primary outcomes were speed and accuracy, in which speed was defined as the averaged reaction time (RT) for correct responses per block and accuracy as the number of correct responses per block. Three different endpoints were defined. The primary endpoint of offline consolidation, was defined as the difference in performance (speed and accuracy) between the first four blocks of retest 1 after the 2-hour nap and the last four blocks of learning just before the nap. A time (pre-nap, post-nap, retention) by intervention (reactivated sequence, non-reactivated sequence) by group (PD, HC) mixed model ANOVA was used to test for interaction effects on the primary outcomes. The secondary endpoint of retention, was defined by the difference in performance on the primary outcomes for both sequences during the post-test at retest 1 versus the first four blocks of learning at retest 2. The secondary endpoint of dual-tasking was defined by the difference in performance on the primary outcomes for both sequences during the four blocks of dual-tasking between retest 1 and retest 2. The secondary endpoints were similarly tested with 2x2x2 mixed model ANOVA's. Prior to applying mixed design ANOVA in this thesis, the normality of the variables, sphericity and variance homogeneity were first assumed.

As an exploratory analysis, sleep staging during the naps was compared between PD and control groups using independent t-tests or Mann-Whitney U tests to investigate whether there was a difference in the amount of N2 and N3 sleep during which TMR could be applied. In addition, the number of cues presented during N2 and N3 with TMR between the PD and HC groups and total sleep time during overnight sleep at home were compared between groups. A correlation between the number of cues during N2 and N3 and the difference in speed between the reactivated (ReactSeq) and non-reactivated sequence (NonReactSeq) was examined by Pearson correlation for normally distributed data. Furthermore, a correlation between the number of sleep during the night at home, before the retention measurement, and difference in speed between the sequences post-nap and at retention was examined with a Pearson or non-parametric Spearman (for non-normally distributed data) correlation coefficient.

Moreover, the following control analyses were conducted to support the interpretation of the primary analyses. Within the PD group, medication status was compared between test moments by using a VAS. A mixed design ANOVA was used to compare the PVT outcomes as well as speed and accuracy for the random blocks between the groups across the three different time points. For all SRTT analysis, any outliers in reaction time which were greater than the mean + 2*standard deviation for that block were removed from the dataset, as per prior work (38). Alpha was set at 0.05 for all analyses and correction for multiple testing in post hoc tests was performed using Bonferroni correction.

4 RESULTS

4.1 Outliers

For all data analyses, RT that were greater than the mean + 2*standard deviations within that block were considered outliers and removed from the data set. Per block there were between 0 and 4 outliers out of a total of 48 keys per block, corresponding to less than 10% of all cues per block. Moreover, there was no significant difference in the number of outliers between the last two blocks before the nap and the first two blocks after the nap for both the ReactSeq (t= -1.71; p=0.121) and the NonReactSeq (t= -1.00; p=0.434). Nor was there a significant difference in the number of outliers between PD and HC for both sequences for these time points (ReactSeq pre-nap: t=-0.60; p=0.565; ReactSeq post-nap: t=-1.55; p=0.160; NonReactSeq pre-nap: t=0.00; p=1.000; NonReactSeq post-nap: t=1.28; p=0.237).

4.2 Participant characteristics

For this thesis, a total of five people with PD and five healthy older people were included. Demographic and clinical characteristics are shown in **Table 1**. A significant difference was found in years of education between PD and HC whereby PD had fewer years of education than HC. Moreover, the incidence of rapid eye movement behaviour disorder (RBD) was higher for PD than HC, which was expected given that RBD is a symptom of PD. No significant difference was found between groups for age, sex, AHI, SCOPA-night, SCOPA-day, HADS-A and HADS-D, Lille apathy score, MoCA and Purdue pegboard scores.

Table 1: Participants demographic statistics

Variables	PD (n=5)	HC (n=5)	test-value	p-value
Age (years)	60.0 (3.8)	69.4 (2.0)	-2.20	0.059
Sex (Man/Female)[a]	2/3	3/2	0.40	0.527
Education (years)	14.9 (0.4)	17.0 (0.7)	-2.59	**0.032**
AHI	6.58 (2.8)	9.45 (1.0)	-0.97	0.360
SCOPA-night[b]	6.60 (2.2)	2.00 (1.5 - 8.0)	8.00	0.421
SCOPA-day	5.60 (1.9)	2.20 (1.1)	1.52	0.167
HADS-A[b]	2.00 (2.0 - 3.0)	2.80 (0.9)	12.00	1.000
HADS-D	2.60 (0.5)	2.40 (1.4)	0.14	0.896
Lille apathy total score	-30.20 (1.50)	-31.8 (1.4)	0.78	0.456
MoCA	26.6 (0.9)	28.0 (0.8)	-1.16	0.280
Purdue-right	11.8 (1.5)	14.2 (0.7)	-1.47	0.181

Purdue-left	12.6 (1.5)	12.8 (0.7)	-1.22	0.906
RBD (pos/neg)[a]	3/2	0/5	4.29	**0.038**
UPDRS I	8.20 (0.6)	-	-	-
UPDRS II	8.40 (1.8)	-	-	-
UPDRS III	22.4 (4.3)	-	-	-
UPDRS IV	0.00 (0.0 - 3.5)	-	-	-
UPDRS total	40.4 (5.2)	-	-	-
HY[a]	2.50 (2.0 - 2.5)	-	-	-
LEDD (mg)	668.4 (229)	-	-	-
Disease duration (years)	4.80 (0.9)	-	-	-

NOTE: A t-test was used to compare the groups with mean (standard error) reported, unless noted otherwise. a=Chi-square test was performed for categorical data and frequencies reported; b=Data not normally distributed and a Mann-Whitney U test was performed with the median (25th and 75th percentiles) reported. P-values shown in bold indicate a significant p value (p<0.05). Lille apathy scale has a scoring range from -36 to +36. Abbreviations: AHI=Apnea Hypopnea Index, SCOPA=Scales for Outcomes in Parkinson's Disease night and day symptom subscales, HADS-A=Hospital anxiety and Depression Scale – Anxiety subscale with scoring range from 0-21, HADS-D=Hospital anxiety and depression scale – Depression subscale with scoring range from 0-21, MoCA= Montreal Cognitive Assessment with scoring range 0-30, RBD=Rapid eye movement Behaviour Disorder, UDPRS= unified Parkinson's disease rating scale sections I-IV and total score with each question having a score range of 0-4, HY=Hoehn and Yahr scale with scoring range 0-5, LEDD=levodopa equivalent daily dose.

4.3 Serial reaction time task (SRTT)

Average RT for each block during each time point is presented in **Figure 7** for PD and **Figure 8** for HC. The numbers and dotted lines indicate the average of the first two blocks of learning and the two blocks of post-test per sequence during the different time points and the average of the dual task blocks. Accuracy for each block during each time point is presented in **Supplementary Figure S4**. These overview figures show a clear learning curve during each practice session and a main effect over the three time points for both groups. These figures also indicate that participants could effectively learn both sequences, as over time they outperformed themselves during the single-task sequence learning when compared to the random blocks.

Statistical p-values for interaction effects and main effects for speed and accuracy for the different SRTT conditions are shown in **Supplementary Table S1 and S2**.

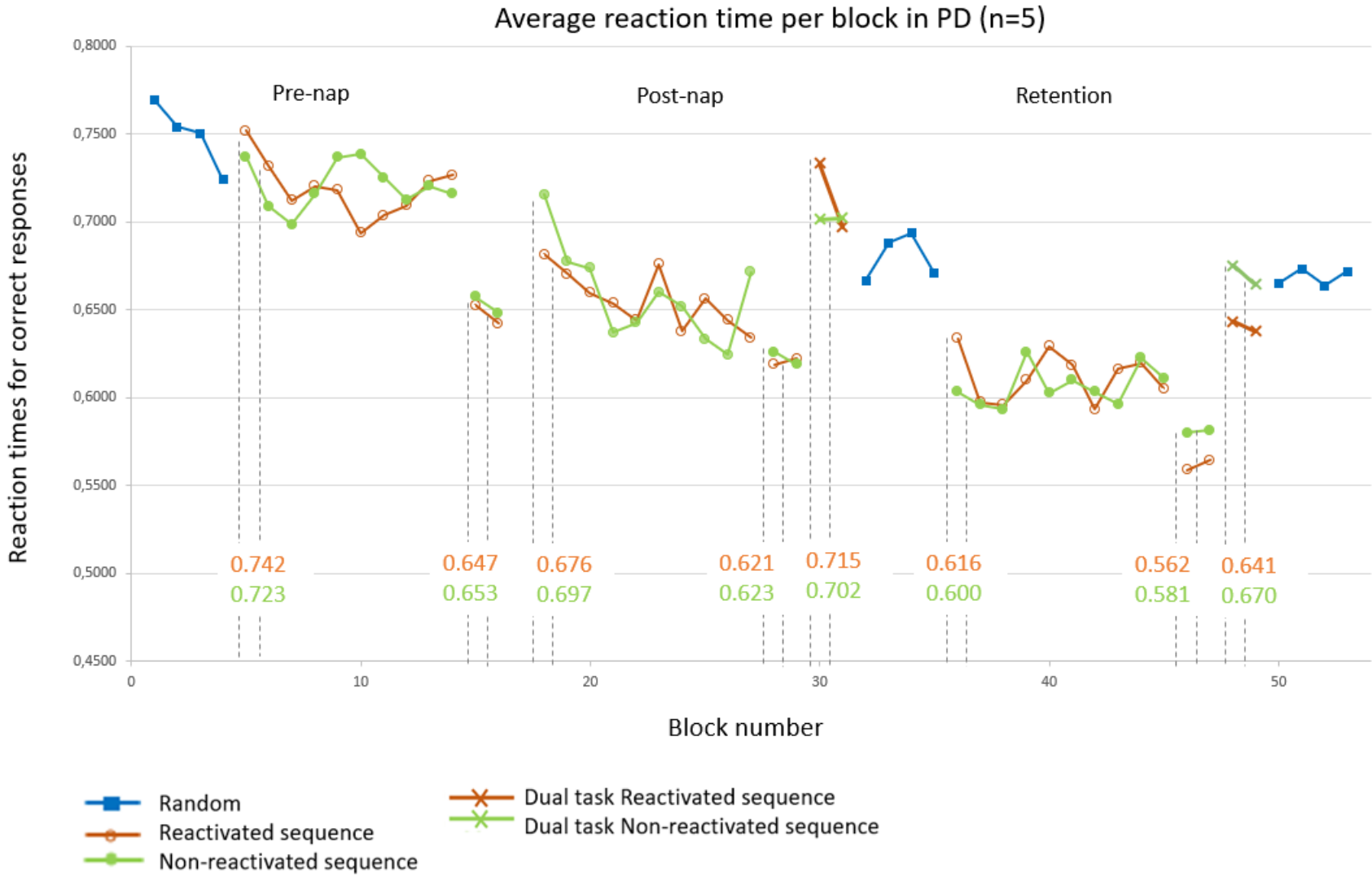


Figure 7: Average reaction time for correct responses per block in people with Parkinson's Disease for pre-nap (block 1-16), post-nap (block 17-35), retention (block 36-53); *reaction times are presented in seconds; the numbers in the graph represent the average of the first two and last blocks of learning and of the dual task.*

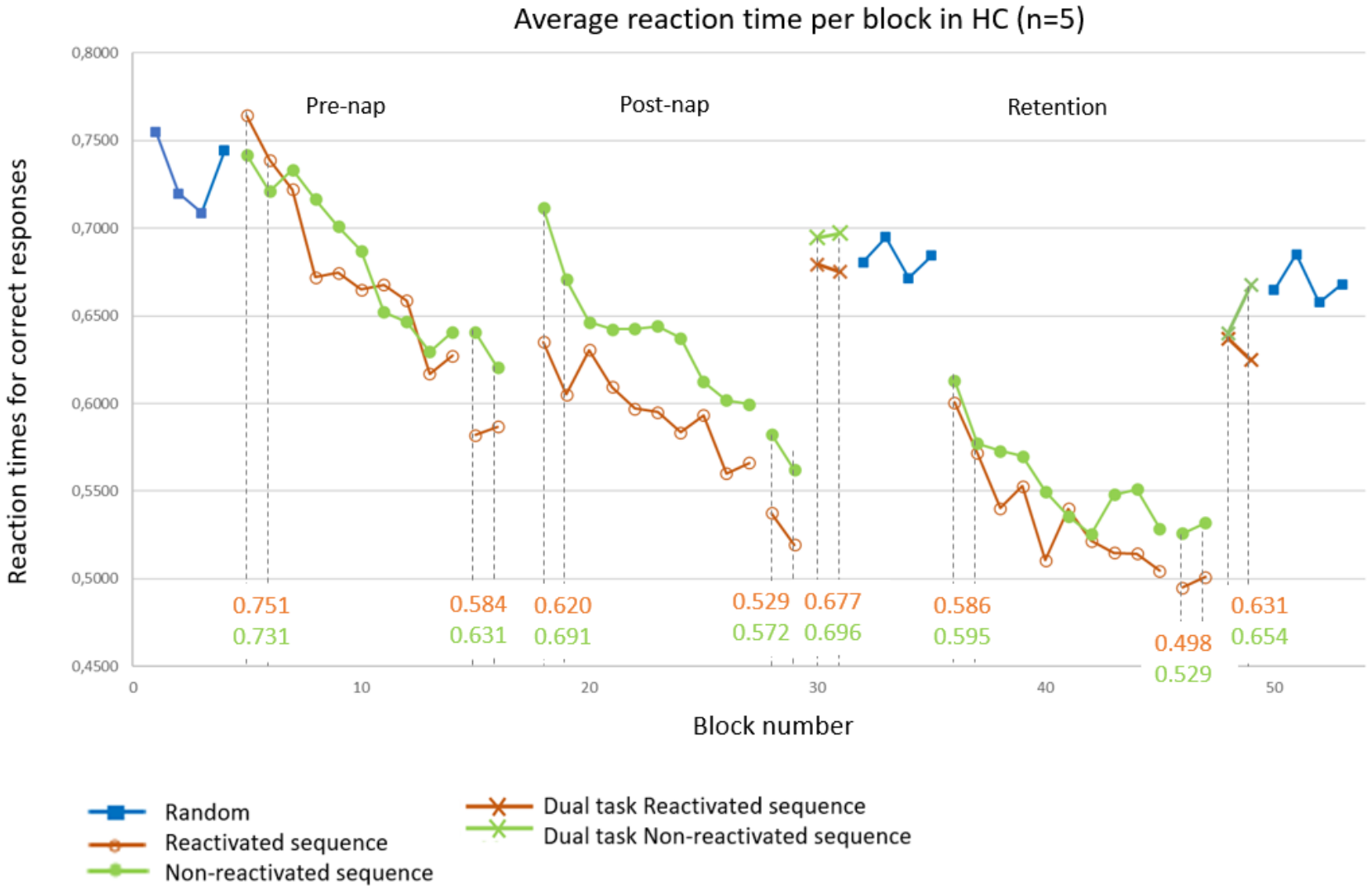


Figure 8: Average reaction time for correct responses per block in Healthy Controls for pre-nap (block 1-16), post-nap (block 17-35), retention (block 36-53); *reaction times are presented in seconds; the numbers in the graph represent the average of the first two and last blocks of learning and of the dual task.*

Primary endpoint: offline consolidation

Speed

There was no significant three-way interaction effect for time (pre, post) by sequence (ReactSeq, NonReactSeq) by group (PD, CT) (F(1,8)=0.04; p=0.848), nor were there significant two-way interaction effects (all p>0.05). However, there was a main effect of time (F(1,8)=9.03; d=0.53; p=0.017) and main effect of sequence (F(1,8)=6.48; d=0.45; p=0.034) (**Supplementary Table S1**). Post-hoc tests showed the main effect of time was driven by a difference between pre and post in the HC group (p=0.041), but this difference was no longer found to be significant after correcting for multiple comparisons (α=0.025). The main effect of sequence was driven by a significant difference (also after adjustment for multiple comparisons) between ReactSeq and the NonReactSeq in the HC group (p=0.019) at post-nap (p=0.007), but not in de PD group (p=0.530). **Figure 9** shows a slight increase in RT from pre to post, but this increase seemed less pronounced for the ReactSeq in both groups. The ReactSeq seemed to have faster RT at post-nap than the NonReactSeq, but this difference was only significant for the HC (**Figure 9B**) and not for the PD (**Figure 9A**).

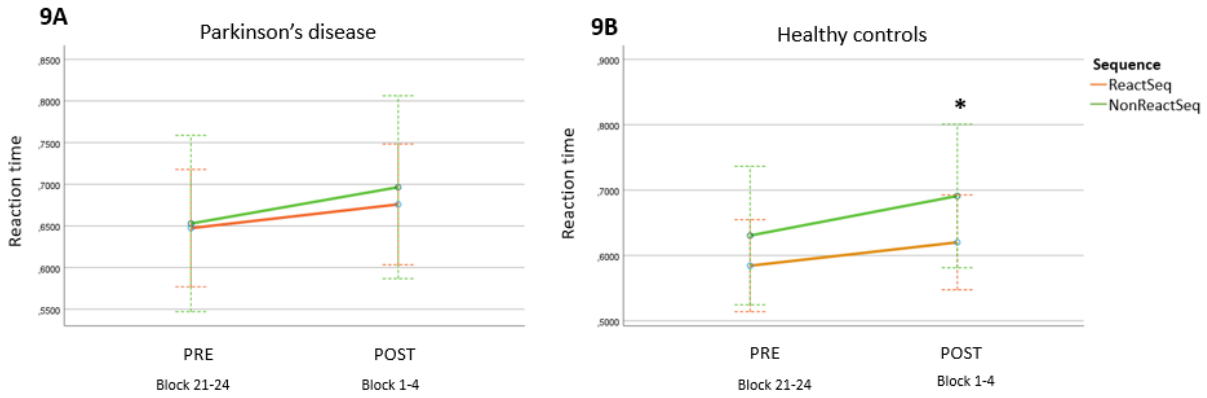


Figure 9: Reaction time (in seconds) during the last four blocks of training pre-nap and first four blocks of training post-nap in PD (A) and HC (B).

*NOTE: error bars show the 95% CI, *=significant difference, green=non-reactivated sequence (NonReactSeq) and orange=reactivated sequence (ReactSeq)*

Accuracy

There was also no significant three-way interaction effect for time (pre, post) by sequence (ReactSeq, NonReactSeq) by group (PD, HC) (F(1,8)=3.53; p=0.097), nor were there significant two-way interaction effects or main effects (all p>0.05) (**Supplementary Table S2**). Exploratory post-hoc testing revealed that the degradation in accuracy for the ReactSeq in PD (**Figure 10A**) was in fact significant (p=0.039), but not after correcting for multiple comparisons

(p=0.039, α=0.025), indicating that PD made more errors on the ReactSeq, while they seemed to improve accuracy for the NonReactSeq, albeit non-significantly (p=0.433). For the HC (**Figure 10B**), we see an opposite trend in the expected direction, namely whereby accuracy seemed to improve for the ReactSeq and worsen for the NonReactSeq from pre to post. However, these differences were not statistically significant (ReactSeq: p=0.521; NonReactSeq: p=0.528).

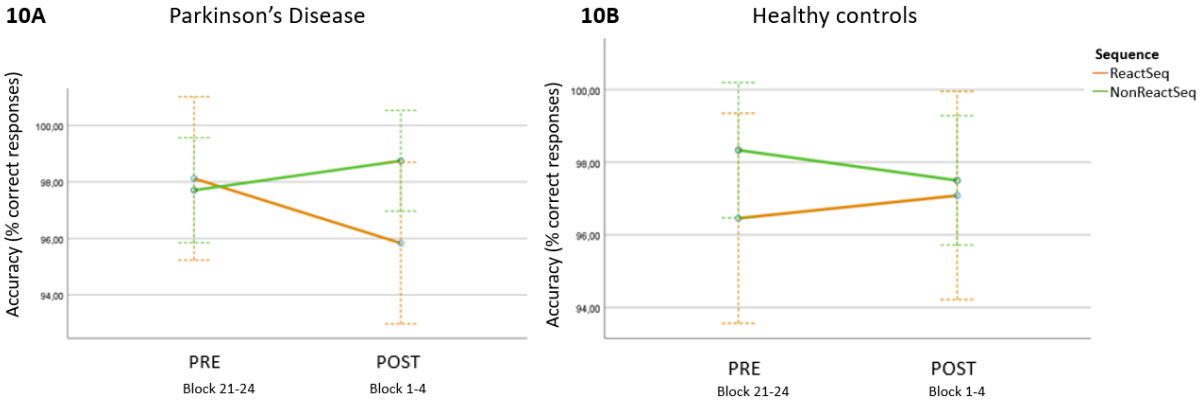


Figure 10: Accuracy during the last four blocks of training pre-nap and first four first blocks of training post-nap in PD (A) and HC (B).

NOTE: error bars show the 95% CI, green=non-reactivated sequence (NonReactSeq) and orange=reactivated sequence (ReactSeq)

Secondary endpoint 1: Retention

Speed

There was no significant three-way interaction effect for time (post, ret) by sequence (ReactSeq, NonReactSeq) by group (PD, CT) (F(1,8)=0.24; p=0.635) (**Supplementary Table S1**), indicating that the two-way interaction effects were not significantly different between PD and HC. The time by group interaction effect was significant (F(1,8)=11.57; d=0.59; p=0.009). The other two-way interaction effects (for sequence by time or sequence by group) were not significant (all p>0.05). Simple main effect analysis showed that the time by group interaction was driven by a significant difference in RT between post and retention for the HC group (mean difference (post-ret)= -0.40; SE=0.01; F(1,8)=12.77; p=0.007) (**Figure 11B**), but not for the PD group (mean difference (post-ret)=0.01; SE=0.01; F(1,8)=1.53; p=0.251) (**Figure 11A**). The significant increase in RT between post and retention for HC group seems to be driven by the ReactSeq (mean difference (post-ret)= -0.06; SE=0.02; p=0.016 and not by the NonReactSeq (mean difference (post-ret)= -0.02; SE=0.01; p=0.135).

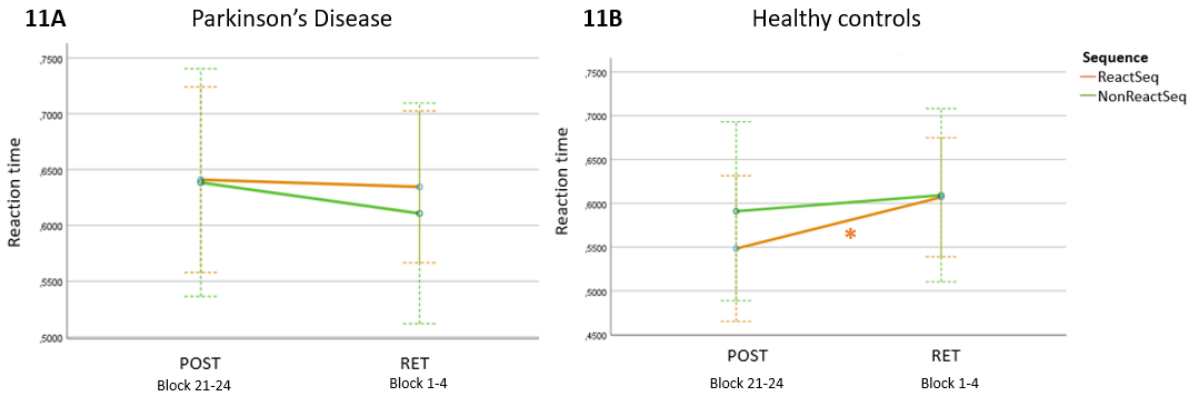


Figure 11: Reaction time (in seconds) during the last four blocks of training post-nap and the first four blocks of training at retention in PD (A) and HC (B).

*NOTE: error bars show the 95% CI, *=significant difference, green=non-reactivated sequence (NonReactSeq) and orange=reactivated sequence (ReactSeq)*

Accuracy

There was no significant three-way interaction effect between time (post, ret) by sequence (ReactSeq, NonReactSeq) by group (PD, HC) ($F(1,8)=1.98$; $p=0.197$), meaning that there was no difference in accuracy between the groups on the two-way interactions, which were also non-significant (all $p>0.05$) (**Supplementary Table S2**). Nor were there main effects for accuracy (all $p>0.05$). **Figure 12A** suggests that accuracy for the NonReactSeq becomes better in the PD group at retention ($p=0.622$) and the ReactSeq becomes worse ($p=0.633$), though both comparisons were non-significant. HC, made slightly more errors for both sequences (**Figure 12B**), but these differences were also not significant (all $p>0.05$).

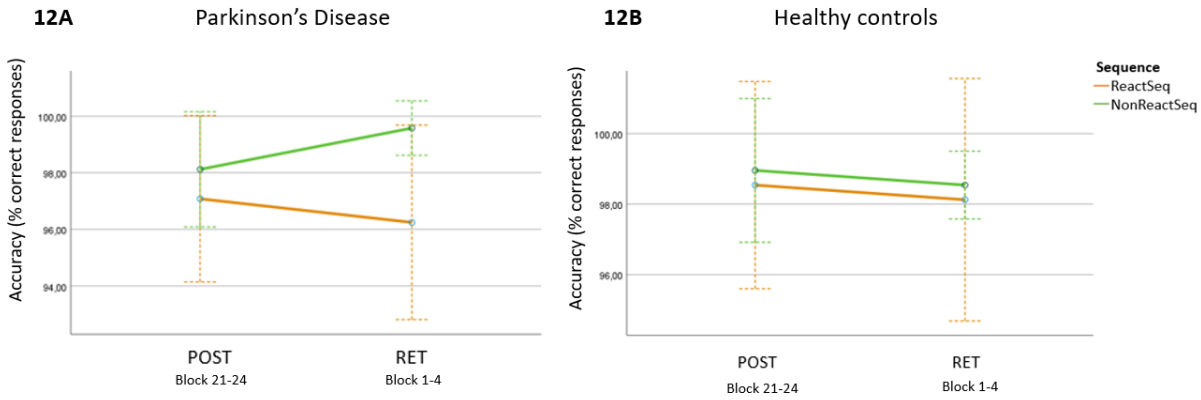


Figure 12: Accuracy during the last four blocks of training post-nap and the first four blocks of training at retention in PD (A) and HC (B).

NOTE: error bars show the 95% CI, green=non-reactivated sequence (NonReactSeq) and orange=reactivated sequence (ReactSeq)

Further practice at retention

Speed

As an exploratory analysis, we analysed the practice effect at retention for both groups. There was no three-way interaction effect for time (ret_start, ret_end) by sequence (ReactSeq, NonReactSeq) by group (PD, HC) ($F(1,8)=0.30$; $p=0.604$). However, a significant interaction between sequence (ReactSeq, NonReactSeq) and time (ret_start, ret_end) was found ($F(1,8)=5.43$; $d=0.40$; $p=0.048$) (**Supplementary Table S1**). Simple main effect analysis showed that the interaction was driven by a significant difference in PD between the start of retention and the end of retention for the ReactSeq (mean difference (start-end)=0.05; SE=0.02; $p=0.010$), but not for the NonReactSeq (mean difference (start-end)=0.02; SE=0.02; $p=0.288$) (**Figure 13A**). In the HC group, there was a significant difference for both the ReactSeq (mean difference (start-end)=0.09; SE=0.02, $p<0.001$) and the NonReactSeq (mean difference (start-end)=0.07; SE=0.02; $p=0.004$) in RT between the start and the end of retention (**Figure 13B**).

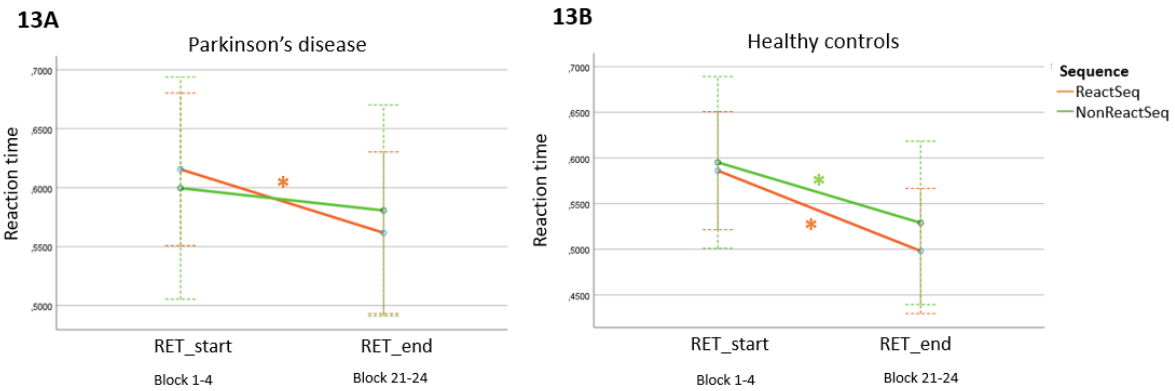


Figure 13: Reaction time (in seconds) during the first four blocks and the last four blocks at retention in PD (A) and HC (B).

*NOTE: error bars show the 95% CI, *=significant difference, green=non-reactivated sequence (NonReactSeq) and orange=reactivated sequence (ReactSeq)*

Accuracy

There was no three-way interaction effect for time (ret_start, ret_end) by sequence (ReactSeq, NonReactSeq) by group (PD, HC) for accuracy, nor were the two-way interaction effects or main effects significant (all $p>0.05$) (**Supplementary Table S2**). Visual exploration of **Figure 14A** shows a seemingly improved accuracy for the ReactSeq from retention_start to retention_end in the PD group, while the NonReactSeq showed a decrease in accuracy. However, these post-hoc comparisons were not significant (all $p>0.05$). In the HC (**Figure 14B**) a slight and non-significant decrease in accuracy was seen for both sequences (both $p>0.05$).

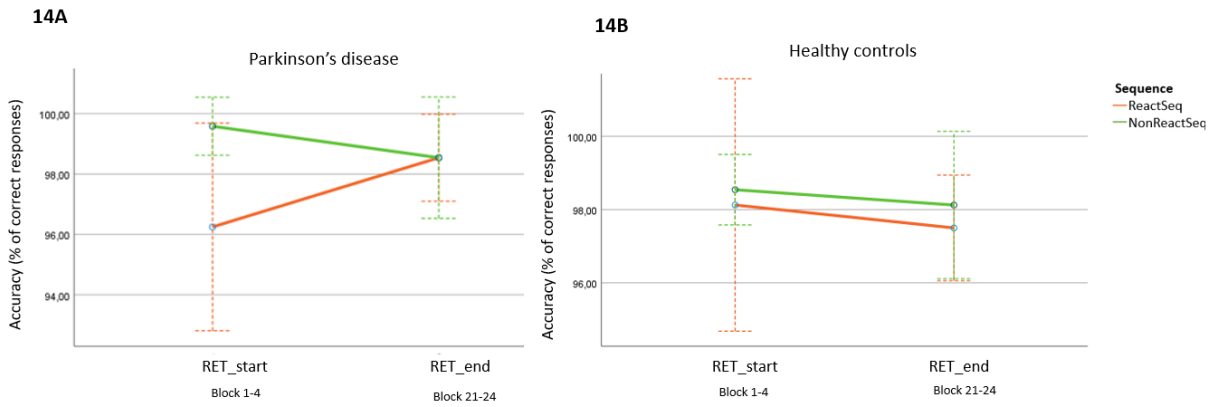


Figure 14: Accuracy during the first four blocks and the last four blocks at retention in PD (A) and HC (B).

NOTE: error bars show the 95% CI, green=non-reactivated sequence (NonReactSeq) and orange=reactivated sequence (ReactSeq)

RT changes over all time points

Speed

There was no significant three-way interaction effect for time (pre_start, pre_end, post_start, post_end, ret_start, ret_end) by sequence (ReactSeq, NonReactSeq) by group (PD versus HC) (F(5,4)=3.17; p=0.143) (**Supplementary Table S1**). There was, however, a significant two-way interaction effect for sequence (ReactSeq, NonReactSeq) by time (pre_start, pre_end, post_start, post_end, ret_start, ret_end) (F(5,4)=17.98, d=0.96, p=0.008). For PD, simple main effect analysis revealed a significant improvement in RT during learning pre-nap for both sequences (ReactSeq: mean difference (pre_start-pre_end)= 0.09; SE=0.02; p=0.003 and NonReactSeq: mean difference (pre_start-pre_end)=0.07; SE=0.03; p=0.049) and a significant improvement in RT for the ReactSeq during further practice at post-nap (mean difference (post_start-post_end)=0.05; SE=0.02; p=0.020) and at retention (mean difference (ret_start-ret_end)=0.05; SE=0.02; p=0.010) (**Figure 15A**). Simple effect analysis in HC revealed a significant improvement for both sequences during learning pre-nap and with practice at the other two time points (all p<0.05) and a significant increase in RT for the ReactSeq from post_end to retention_start depicting further consolidation effects (mean difference (post_end-ret_start)= -0.06; Se=0.02; p=0.016), that were not seen for the NonReactSeq (mean difference (post_end-ret_start)= -0.02; SE=0.01; p=0.135).

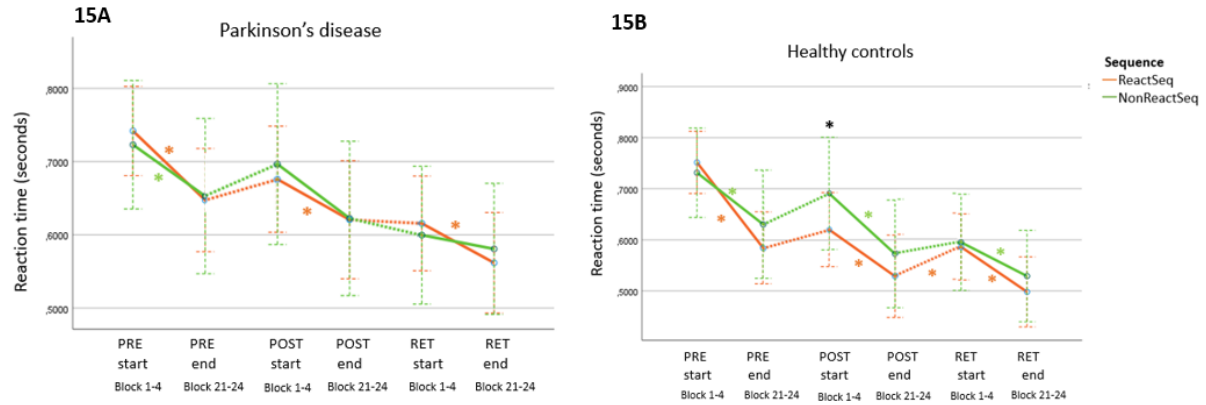


Figure 15: Reaction time across all time points in PD (A) and HC (B).

*NOTE: error bars show the 95% CI, green=non-reactivated sequence (NonReactSeq) and orange= reactivated sequence (ReactSeq), Significant differences in RT for each sequence are indicated with green or orange *, significant differences between both sequences are indicated with a black *, consolidation effects between different time points are indicated in a dotted line*

Secondary endpoint 2: Dual tasking

Speed

No significant three-way interaction effect (F(1,8)=1.65; p=0.235) was found for time (post, ret), by sequence (ReactSeq, NonReactSeq), by group (PD, HC) for the RT on the dual task (**Supplementary Table S1**). There were also no significant two-way interaction effects (p>0.05), though there was a main effect of time (F(1,8)=8.60; d=0.52; p=0.019) regardless of group or sequence. Post-hoc testing revealed an improvement in RT on the DT for both sequences, but only for the ReactSeq was this improvement significant in the PD group (**Figure 16A**) between post and retention (p=0.025, also significant after adjustment with Bonferroni, α=0.025). No differences in RT were found for HC (all p>0.05) (**Figure 16B**).

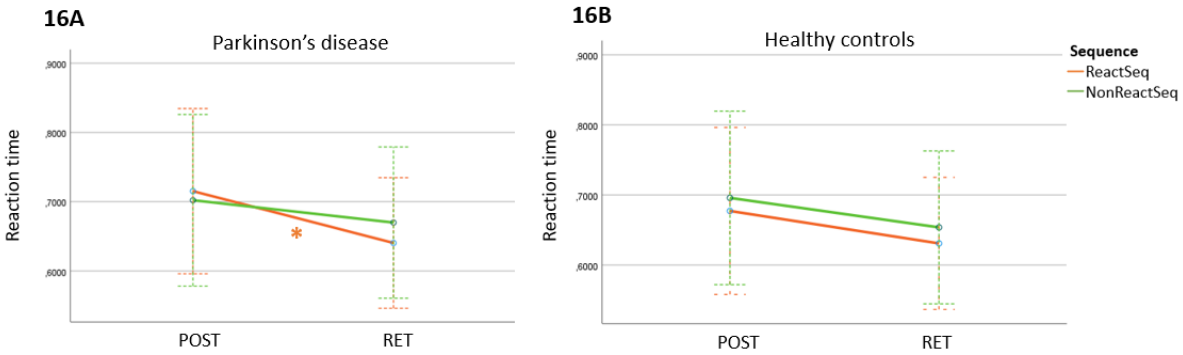


Figure 16: Reaction time (in seconds) of the dual task during post and retention time points in PD (A) and HC (B).

*NOTE: error bars show the 95% CI, *=significant difference, green=non-reactivated sequence (NonReactSeq) and orange=reactivated sequence (ReactSeq)*

Accuracy

There were no three-way interaction effects for time (post,ret) by sequence (ReactSeq, NonReactSeq) by group (PD, HC) ($F(1,8)=0.92$; $p=0.367$) nor were there two-way interaction effects or main effects (all $p>0.05$) (**Supplementary Table S2**). Visual exploration of **Figure 17A** shows a reduction in accuracy for both sequences from post to retention in PD, with a seemingly stronger decline in accuracy for the ReactSeq, albeit not significant after Bonferroni correction ($p=0.044$). In HC (**Figure 17B**) there also appears to be a reduction in accuracy for both sequences from post to retention, though exploratory post-hoc testing revealed none of these differences in either PD or HC reached statistical significance (all $p>0.05$). As an exploratory analysis for the DT, the dual task cost for both sequences was examined in both groups (**Supplementary Figure S5**).

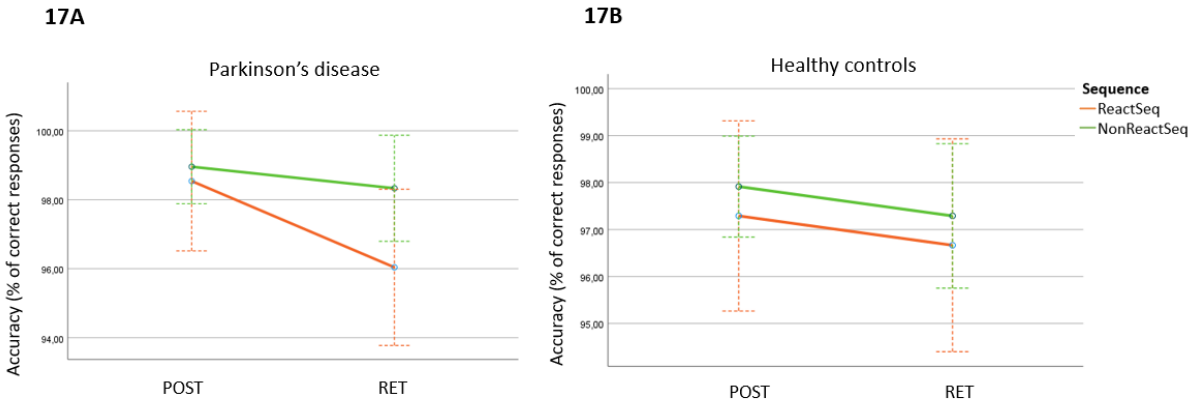


Figure 17: Accuracy of the dual task during post and retention time points in PD (A) and HC (B). *NOTE: error bars show the 95% CI, green=non-reactivated sequence (NonReactSeq) and orange=reactivated sequence (ReactSeq)*

Control analyses

Psychomotor vigilance test (PVT)

No time (pre, post, ret) by group (PD, HC) interaction effect was found ($F(2,7)=1.18$; $p=0.361$), which indicates that there was no difference in vigilance prior to conducting the SRTT between the three time points for both groups and no difference in vigilance between groups (**Supplementary Figure S1**).

Medication Visual Analogue Scale (VAS)

All participants with PD gave an eight or higher on a 10-point VAS relating to subjective on- or off-medication status for all three time points (pre-nap, post-nap, retention) indicating that they were consistently on-medication when performing the tests and during the nap.

Sequences at baseline (blocks 21-24 before the nap)

Speed

First, we tested whether there was a difference in performance for both sequences in the two last blocks per sequence before the nap to ensure that both sequences were learned equally well in both groups. Indeed, no two-way interaction effect was found for sequence (ReactSeq, NonReactSeq) by group (PD, HC) ($F(1,8)=0.83$; $p=0.390$) indicating that there was no difference in RT for either sequence between the groups (**Supplementary Table S1**). There was also no main effect of sequence ($F(1,8)=1.34$; $p=0.281$) (**Supplementary Figure S2A**).

Accuracy

There was similarly no significant two-way interaction effect of sequence (ReactSeq, NonReactSeq) by group (PD, HC) ($F(1,8)=1.18$; $p=0.309$) nor a significant main effect for sequence ($F(1,8)=0.48$; $p=0.509$) (**Supplementary Figure S2B and Table S2**).

Random SRT blocks

Speed

For random blocks, the mixed model ANOVA showed no time (pre, post, ret) by group (PD, HC) interaction effect ($F(2,7)=0.98$; $p=0.422$). There was a main effect of time ($F(2,7)=83.01$; $p<0.001$) (**Supplementary Table S1**) indicating that speed improved over time for both PD and HC. Indeed, both groups improved pre- to post-nap and from pre-nap to retention. No differences in RT were found from post-nap to retention in either group (PD: $p=0.205$, and HC: $p=0.126$) (**Supplementary Figure S3A**). There was no main effect of group ($p=0.924$).

Accuracy

There was no significance time by group interaction effect for the accuracy of random blocks ($F(2,7)=1.13$, $p=0.375$). Also no main time effect ($F(2,7)=2.69$, $p=0.136$) or main group effect ($p=0.254$) was found for accuracy on the random blocks (**Supplementary Figure S3B and Table S2**).

4.4 Exploratory analyses

NAP outcomes

Individual hypnograms are shown in **Supplementary Figure S6**. The different stages of sleep during the 2-hour nap and the number of stimulations given with TMR during each sleep stage are shown in **Table 2**. The results indicate that most participants slept well during the 2-hour

nap opportunity with an average of 109 min total sleep time in PD group and 94.4 min in HC group. Importantly, many stimulations could be given with an average of 1886 TMR cues during N2 and N3 for the PD group and 1699 TMR cues for the HC. There was no group differences on any of the sleep outcomes (all p>0.05, see Table 2). Of note is that one HC (participant 7 in supplementary Figure S6) could not achieve adequate N2 and N3 sleep during the nap (only 4.5 minutes N2, no N3), and in consequence no cues were provided. This HC reported that difficulty falling asleep was a frequent problem, and unrelated to the experimental setup or environment.

Table 2: PSG outcomes and number of TMR cues provided during the 2-hour nap.

	PD (n=5)	**HC (n=5)**	**Test value**	**p-value**
Time in bed (min) [b]	128.12 (126.4-134.9)	127.50 (1.0)	15.00	0.690
Total sleep time (min) [b]	109.00 (7.1)	110.50 (67.3-113.5)	18.00	0.310
Total wake time (min)	18.20 (6.5)	26.90 (10.9)	-0.682	0.515
Sleep onset latency (min)	7.30 (1.3)	9.00 (0.6)	-1.169	0.276
Total N1 (min)	14.4 (2.9)	27.40 (9.5)	-1.310	0.227
Total N2 (min)	40.4 (7.7)	34.70 (11.7)	0.408	0.694
Total N3 (min)	49.30 (16.3)	27.8 (13.1)	1.030	0.333
Total N2 + N3 (min)	94.60 (8.8)	67.00 (19.0)	1.318	0.224
Total REM (min) [b]	0.00 (0.0-12.3)	4.50 (2.0)	9.00	0.548
Total number of TMR cues	2114.4 (445.3)	2058.40 (546.7)	0.079	0.939
Total of TMR cues during wake	111.40 (56.0)	148.60 (73.5)	-0.402	0.698
Total TMR cues during N1[b]	5.00 (1.5-274.5)	71.80 (24.8)	8.50	0.421
Total TMR cues during N2	664.60 (137.6)	811.20 (261.8)	-0.496	0.633
Total TMR cues during N3	1212.60 (381.4)	888.60 (361.9)	0.616	0.555
Total TMR cues during N2 and N3	1886.60 (402.8)	1699.80 (508.9)	0.288	0.781
Total TMR cues during REM[b]	0.00 (0.0-23.5)	0.00 (0.0-177.0)	10.00	0.690

NOTE: For normally distributed data, a t-test was used to compare the groups with mean (standard error) reported. A Mann-Whitney U test was performed when data was not normally distributed and then the median (25th and 75th percentiles) were reported. A Chi-square test was done for categorical data with reporting of frequencies. No significant p-values shown (all p>0.05). b=Mann-Whitney U test was used for analysis.

TMR – motor associations

To test whether the number of TMR cues provided during N2 and N3 correlated with the difference in RT between the ReactSeq and NonReactSeq in the first four blocks post-nap, first the difference in RT for both sequences was determined (NonReactSeq –ReactSeq). In PD, this difference was normally distributed with mean (standard deviation) = 0.071 (0.19) and in the HC group this was not normally distributed with median (25th and 75th percentile) = 0.034 (-0.01-0.05). No significant correlation was found between the number of TMR cues provided during N2 and N3 and the change in RT for PD (r=0.57, p=0.320) nor HC (rho=-0.02, p=0.747) (**Figure 18**). From **Figure 18**, however, it does seem that the number of TMR cues correlated positively with an improvement on the RT for the ReactSeq in PD. For HC, no such association was apparent, though the correlation may be strongly influenced by the HC who achieved little sleep.

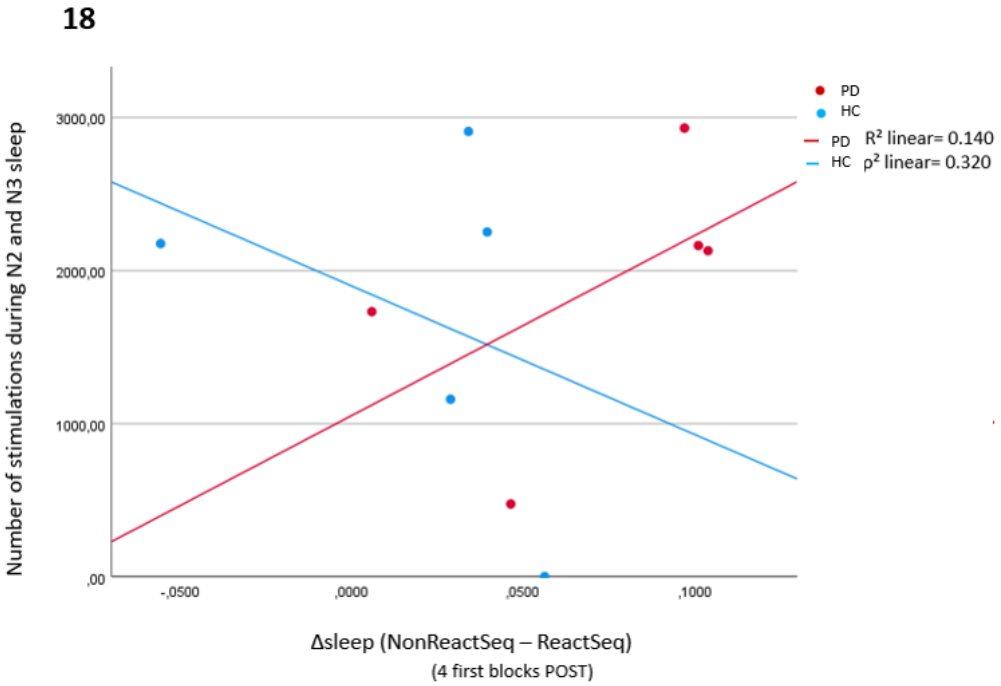


Figure 18: Correlation between the change in RT between the ReactSeq and the NonReactSeq for the first four blocks post-nap and the number of TMR cues provided during N2 and N3 sleep during the 2h-nap in PD (red line) and HC (blue line)

Actigraphy

Participants wore an actigraph to monitor their sleep at home in the night after the nap experiment and before the retention assessment (**Table 3**). Importantly, although PD seemed to sleep less hours in total compared to HC, this difference was not significant. The actigraph data (shown in **Supplementary Figure S7**) was then used to test if total sleep time during the night at home following the nap experiment was associated to the change in performance on

the SRTT at retention (RT retention_start – RT post-nap_end). No significant correlations were found for the ReactSeq (**Figure 19A**) in either group (all p>0.05), but **Figure 19B** does indicate that more total sleep time was associated to slower RT at retention for PD (r=0.66; p=0.226) and with faster RT at retention for HC (r=-0.28; p=0.644). The accuracy (% correct responses) in **Figure 20A** shows that for the ReactSeq total sleep time was not related to performance (all p>0.05). For the NonReactSeq (**Figure 20B**) more total sleep time seems to be associated with better accuracy for both PD (r=0.66; p=0.226) and HC (rho=-0.50; p=0.391), albeit non-significantly.

Table 3: actigraphy: sleep during night between post-nap and retention

	PD (n=5)	**HC (n=5)**	**Test value**	**p-value**
Total sleep during night (hours)	5:29 (0:34)	6:45 (0:12)	-2.09	0.091
Total sleep during day (hours)[b]	0:00 (0:00-0:15)	0:00 (0:00-0:10)	13.00	1.000
Total sleep time night + day (hours)	5:35 (0:35)	6:49 (0:12)	-1.98	0.106
Sleep efficiency night (%)	79.80 (2.9)	81.36 (3.3)	-0.36	0.73
WASO (min)	53.70 (16.3)	83.90 (13.9)	-1.41	0.196
Δ Speed of ReactSeq	0.058 (0.02)	-0.005 (0.01)	2.34	**0.047**
Δ Speed of NonReactSeq	0.023 (0.02)	-0.023 (0.01)	2.36	**0.046**
Δ accuracy of ReactSeq	-0.42 (0.9)	-0.83 (0.8)	0.35	0.735
Δ accuracy of NonReactSeq	-0.4127 (0.5)	0.00 (0.0 – 3.6)	-1.63	0.142

NOTE: For normally distributed data, a t-test was used to compare the groups with mean (standard error) reported. A Mann-Whitney U test was performed when data was not normally distributed and then the median (25th and 75th percentiles) were reported. Significant p-values shown in bold, α=0.05. b=Mann-Whitney U test was used for analysis. Abbreviations: WASO=Wake time After Sleep Onset, Δ=difference in reaction time post end – retention start.

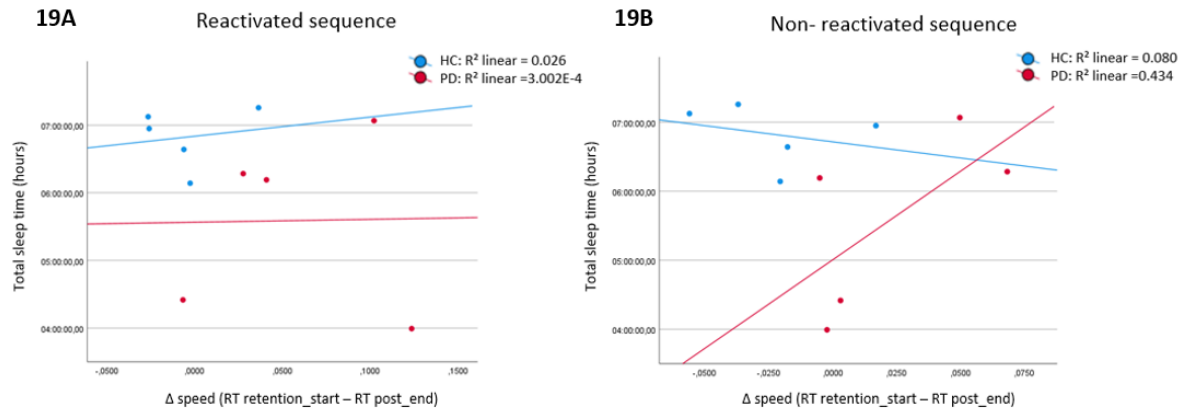


Figure 19: Correlation between the difference in RT between the first four blocks of retention and the last four blocks post for the ReactSeq (A) and the NonReactSeq (B) and total sleep time in hours during the overnight sleep at home. For PD patients (red line), the HC (blue line).

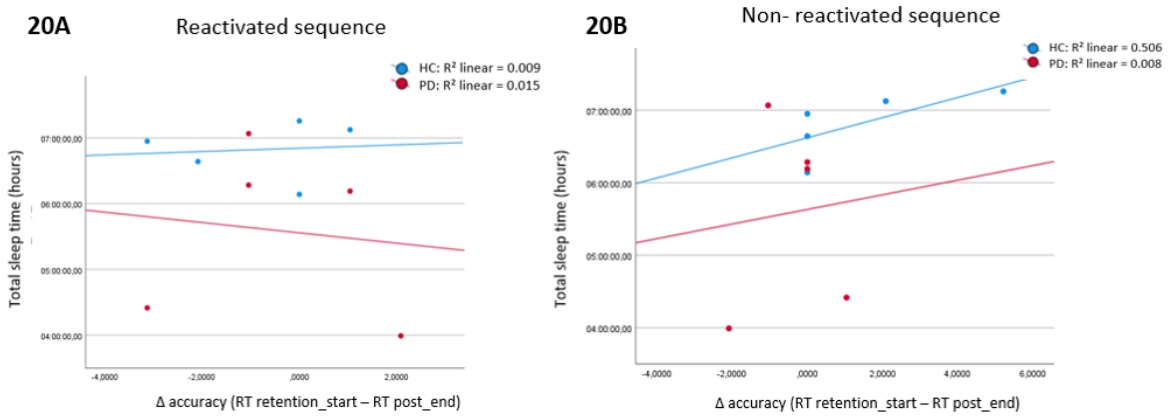


Figure 20: Correlation between the difference in accuracy (% correct responses) between the first four blocks of retention and the last four blocks post for the ReactSeq (A) and the NonReactSeq (B) and total sleep time in hours during the overnight sleep at home. For PD patients (red line), the HC (blue line).

5 DISCUSSION

5.1 Main findings

The aim of this thesis was to investigate the effect of TMR during post learning sleep on offline motor memory consolidation of a SRTT in people with PD and healthy older adults. The primary analysis showed no significant three-way interaction effect for time by sequence by group. As well, there was no difference between the groups on the two-way interactions, which were also non-significant. Interestingly, and in line with the hypothesis, a main effect of sequence was found that was driven mainly by faster RT on the ReactSeq compared to slower RT on the NonReactSeq post-nap within the HC group. In contrast, there was a main effect of time (pre,post) that was driven by slower performance after the nap in both groups, which is not in line with the hypothesis. The decrease in RT was associated with slightly better accuracy for the ReactSeq, as compared to reduced accuracy for the NonReactSeq in HC. An opposite effect was present in PD, whereby both speed and accuracy deteriorated for the ReactSeq from pre- to post-nap, while accuracy slightly improved for the NonReactSeq (**Figures 9 and 10**). However, these differences were statistically non-significant. Taken together, statically, both groups stabilized performance on both sequences after a 2-hour nap with TMR. This stabilization further persisted after a full night of sleep in the PD group. In the HC, however, the additional night of sleep resulted in a significantly slower RT for the ReactSeq and a stabilization for RT of the NonReactSeq, while accuracy on both sequences remained unchanged. Interestingly, PD did seem to benefit from TMR for the DT, showing significantly reduced RT during DT from post-nap to retention of the ReactSeq compared to the NonReactSeq that survived Bonferroni correction, though this improvement seemed to come at a cost of worse accuracy. In HC, no significant effects on the DT were found, with the RT during DT improving slightly on speed and becoming slightly worse on accuracy for both sequences equally.

5.2 Effect of TMR on offline consolidation

No study to date has investigated the effect of post-learning napping with TMR on memory consolidation in older HC, or PD. The primary outcomes, RT and accuracy, did not improve in the present study for the ReactSeq as was expected after a 2-hour nap with TMR in PD. Instead of an improvement, both sequences seemed to stabilize in RT and the ReactSeq even seemed to decrease a bit in accuracy (though this decrease was no longer significant after

Bonferroni correction). This possible decrease in accuracy could perhaps be explained by a speed-accuracy trade-off effect in PD (39), in which the participants might have focused more on pressing the keys as fast as possible, causing them to make more errors. In the HC, a stabilization was observed after the nap in both RT and accuracy for both sequences.

At retention, which followed consolidation and further practice, a stabilization effect was still observed for both RT and accuracy in PD. In HC, the additional night of sleep resulted in a stabilization in accuracy and RT of the NonReactSeq, while the RT on the ReactSeq became significantly slower. This is not in line with the hypothesis that there would be better retention effects for the ReactSeq relative to the NonReactSeq. One possible explanation for this increase in RT for the ReactSeq could be that the ReactSeq was consolidated sufficiently during the nap through TMR, causing the hippocampus to replay the alternative sequence during the subsequent night so that both sequences achieved equal performance levels the next day (17). This interpretation, however, remains speculative at present.

The stabilization following sleep seen in the present study regardless of TMR is in line with previous research. Terpening et al. (2013) showed that both PD, and older HC achieved overnight stabilization on performance of a motor sequence learning task (21,40). King et al. (2013) corroborated these findings and showed that stabilization can be achieved following a 90-minute post-learning nap in older HC (40,41). This stabilization, rather than improvement as seen in young HC (40), could be explained by the reduction of sleep-dependent consolidation effects combined with a reduction in sleep quality that accompanies ageing (21,40). However, given the consistent added benefits achieved with TMR in young HC (19,31), we had still hypothesized that an improvement would be possible after a 2-hour nap coupled with TMR in older HC and PD. However, our results turn out to be less promising given that both sequences showed stabilization in both groups just after the nap regardless of TMR. Thus, TMR does not seem to have a significant additional effect over regular sleep without TMR on the immediate consolidation of the SRTT in older HC and PD.

Possibly, the effect of TMR may become more apparent with additional time and practice. Indeed, King et al. (2017) showed that in older HC the benefits of post learning sleep did not emerge until later time points (41). The same could be true for the TMR effect, and the present data indeed point in that direction, as after the full night of sleep a significant improvement in RT and a non-significant improvement in accuracy was observed for the ReactSeq in PD with further practice and this improvement was not observed for the NonReactSeq. This indicates that TMR nevertheless can have a positive effect on the consolidation of the ReactSeq in the longer term, possibly by protecting the sequence against subsequent sleep deprivation during

the night so that a significant improvement can still be obtained with further practice on the next day. This result adds to the study by Terpening et al. (2013), which suggests that there are no additional learning benefits for people with PD after a full night of sleep without TMR (21). Thus, this thesis could be the first to tentatively suggest a positive effect of TMR applied during a post-learning nap on the long-term consolidation of a motor task in people with PD.

DT interference can be used as a representation for task automatization (11,17). Reduced motor automaticity is a hallmark problem in PD due to their dysfunction in the dorsal striatum (11,12,42). Interestingly, the present DT results showed that there was a significant improvement in RT for the ReactSeq in the PD group, while the NonReactSeq showed no improvement in RT, indicating improved automatization of the motor task for the ReactSeq. However, PD showed a speed-accuracy trade-off as the ReactSeq deteriorated in accuracy during the DT from post to retention, though this was not statistically significant. This suggests that TMR induced a significant improvement in RT for the DT in PD after a full night of sleep and single task practice. TMR may thus support automatization in people with PD. Similarly, for dual task cost (see Supplements), a non-significant decrease for the ReactSeq and a non-significant increase for the NonReactSeq, was found in the PD group. This further indicates that automaticity for the ReactSeq seems to be improving following TMR in PD. In the HC group, there was a non-significant decrease in RT and accuracy for both sequences on the DT and the dual task cost seemed to improve, albeit not significantly, for the ReactSeq and stabilized for the NonReactSeq. A possible explanation could be that automatization in HC is more intact so that TMR does not appear to have any additional effect (43).
Important to note is that the DT was always taken at the end of practice for both the post-nap and retention measurements to avoid interference with the primary endpoint of offline consolidation. However, the amount of practice was kept consistent between both sequences, so any effect on performance during the DT can be explained by TMR.

5.3 Correlations between sleep and differences in reaction time

Reactivation with TMR should have the strongest effect when coupled to slow waves and sleep spindles, which are related to offline memory consolidation (19,32,40,44). These physiological features occur during stages N2 and N3 and the amount of N2 and N3 sleep has previously been related to improved performance on a motor sequence learning task after sleep in healthy people (19,32,40). Moreover, prior studies found a correlation between the strength of motor memory consolidation and the number of reactivations during N2 and N3 sleep (31,44). Therefore, TMR in the present study was provided during these stages of sleep. To explore

whether the number of TMR stimulations provided affected post-sleep motor performance, a correlation analysis was performed between the number of TMR cues given during N2 and N3 sleep and the change in performance for the ReactSeq in PD and in HC separately. No significant correlations were found. However, visual examination of **Figure 18** did indicate a possibly greater difference in RT between the NonReactSeq and the ReactSeq with more stimulations during N2 and N3 in the PD group, suggesting that as expected more TMR cues provided greater improvement in RT of the ReactSeq. This trend was not seen in the HC group. In the HC, there was a non-significant negative correlation that, if proven true, would indicate that more cues would disrupt consolidation of the ReactSeq, which is in contrast to the expected (31). However, this effect seemed to be largely driven by the one HC who achieved little sleep, and as a result did not received any cues with TMR during stages N2 and N3. In addition, a correlation was performed between total sleep time during the full night's sleep without TMR and the difference in RT and accuracy between retention and the end of the post-nap test. No significant correlations were found for both sequences. Visual examination of **Figure 19**, indeed shows that it made no difference how much a participant slept for the ReactSeq in the PD group, suggesting that a nap with TMR seemed sufficient to achieve stabilization and that the extra night of sleep had no added value. Surprisingly, for the NonReactSeq, the graph showed an inverse correlation in the PD group, which would suggest that the NonReactSeq improved more with less sleep during the subsequent night. In the HC group, more overnight sleep seemed to benefit the NonReactSeq, while it showed an inverse trend for the ReactSeq. With regards to accuracy (**Figure 20**), there seemed to be no effect of total sleep time on the ReactSeq for PD and HC. For the NonReactSeq, there seems to be an improvement in accuracy when one slept longer in both PD and HC, as would be expected. Admittedly, however, these correlations are probably spurious due to the limited sample size and because there was actually no improvement, but a stabilization for the NonReactSeq so there may not have been enough variation in the data to show a correlation.

5.4 Strengths and limitations

This is the first study to directly compare the effect of TMR during sleep on motor memory consolidation in older HC and PD. The current study has several other strengths. RT and accuracy were both selected as primary outcomes rather than considering RT alone as in some other studies (21,38). This is necessary in order to identify potential speed-accuracy

trade-off effects. Indeed, a possible trade-off effect was found in the present study with a decrease in accuracy accompanying the improvement in RT in PD. However, the true impact of this trade-off on performance is relative, since the accuracy in PD always remained above 95%.

Moreover, formal screening with PSG in a sleep lab was done prior to inclusion into the present study to test for severe apnea (AHI>30), which has an effect on sleep quality (45), and this overnight sleep also served as a habituation to sleep in a sleep lab with PSG applied prior to the experimental nap and indeed all but one HC slept sufficiently during the nap. Previous studies in the young used a 90-minute nap (38,40), but the present study concerned older adults and people with PD who often have sleep fragmentation (38,40). As such, a 2-hour nap was offered to allow for more sleep and all participants but for one HC indeed had sufficient sleep during these two hours of TMR to be provided. In this study, possible circadian effects were controlled for by the experimental design where participants performed the SRTT at the same time during the day between post-nap and during retention 24h later. Napping was also done in part to counteract circadian effects and, participants were asked to maintain a consistent sleep pattern prior to the study, which was monitored by a sleep diary. Importantly, TMR during the nap did not wake up the participants and the nap did not cause any adverse effects nor did it seem to influence sleep on the subsequent night, as verified by questioning the subjective sleep quality using a VAS.

Addition of a DT is also a strength because this allows for the assessment of possible automatization. In addition, the ATH was determined for each tone and for each participant separately, allowing all participants to hear the different tones equally well, independent of any hearing difficulties. Finally, the control analyses verified that the PVT and medication status were not different between groups or between measurements and that both sequences reached similar performances just before the nap, indicating that the results were not influenced by differences in vigilance or medication status or by differences in difficulty of the two sequences.

There are also limitations to this study that should be considered when interpreting the outcomes. First and foremost, that this is a pilot study with only five participants included in each group. This limited sample size does not ensure sufficient power in statistical analysis and this may not be a representation of the real population. The full study will eventually include 20 participants per group as based on a-priori power calculation. Second, the present study had no wake control group to compare against the TMR effects during sleep. However, the present study applied a within-subject design that did not require a wake control group to study whether TMR is effective during sleep. TMR might work during waking periods, but previous research strongly suggests consolidation effects are optimal during N2 and N3 sleep (19,31).

In addition, there might have been an effect of education since the demographic analysis showed a difference in years of education between the groups, with an average of 14.9 years of education in PD and 17.0 years in HC. This might have had an effect on the learning process during the task, with more education possibly providing better learning effects. Alternatively, it is possible that those with more years of education were more likely to have an office job that required them to type on a keyboard more often, which may be an advantage in the SRTT. This could be taken into account by examining how often people typed or worked with a computer in the past or present. We could have corrected for the difference in years of education by including education in the analysis as a covariate. However, the difference in education had little influence on interpretation of the TMR effects since the study had a within subject design.

Finally, one of the HC (Participant 7 in Supplementary Figure S6) could not sleep sufficiently during the 2-hour nap, so no TMR could be provided, which may have clouded the study outcomes for the HC group. In addition, in the PD group there was one participant (Participant 5 in Supplementary Figure S6) who received less cues than intended (428 instead of 1270), due to human error during data acquisition. However, it remains unknown how many cues are needed to achieve effects on behaviours, so possibly 428 was still sufficient.

5.5 Recommendation for further research

A larger study is needed (and ongoing) that will be able to determine with more certainty whether a post learning nap with TMR improves motor memory consolidation and retention in PD and HC. Further research is also needed to verify the specificity of the cues given with TMR, as it may be that the same effects could be obtained with learning-independent sounds, especially since no significant differences were found between ReactSeq and NonReactSeq in this pilot study (19). This can be verified by including a group that receives a ‘sham sound’ that is unrelated to the learning (19,38). As a control for the effect of sleep, another group could be included that undergoes a nap without TMR. The downside of having three groups in the design is that many more participants are needed to obtain sufficient statistical power for the analysis. In addition, the within group setup of the present study should theoretically be sufficient to answer the question of whether learning-specific TMR can improve consolidation since we used two sequences and thus any effect of the ReactSeq can be related to TMR.

In the present study an ‘open-loop’ form of TMR was applied, regardless of the precise timing of slow waves and or spindles. Future research could consider to apply a ‘closed-loop’ TMR intervention instead (37). Closed-loop TMR allows computer algorithms to stimulate at

indicated times and thus it would also be possible to stimulate specifically during the up-state of the slow wave, which may provide the strongest effect (37). In addition, further studies could examine the effect of TMR during multiple naps or nights with longer learning periods since rehabilitation often requires weeks of training and TMR could possibly have a positive added effect on such long-term training schemes.

The PD patients could be tested off-medication to determine whether medication has an effect on the outcomes. For example, the use of dopaminergic medication would affect the trade-off by causing more errors to be made at greater speed since the medication causes patients to prefer speed over accuracy in instructions that ask them to respond as quickly and accurately as possible (39). In addition, testing off-medication ensures that pure PD can be studied rather than together with the influence of the medication. Moreover, not all PD patients take the same medication which could give a slight variation in the analysis. Finally, if TMR would be applied during a full night's sleep, it is important to also test for TMR effects off-medication, since not all patients take dopaminergic medication during the night and the TMR effect may hence not be equal to a nap on-medication. However, testing patients off would not be comfortable for the patients and has little ecological validity. The goal of the present study was indeed to eventually apply TMR during a nap in rehabilitation practice.

The present study included patients in HY 1-3 without freezing of gait. If proven effective in these relatively less advanced cohorts, than later research should also include PD patients in HY stage 4 and those with freezing who are known to have even poorer consolidation and more sleep problems (6,15). However, these patients may have an even greater need for improvement with the use of TMR in rehabilitation to improve their quality of life (11,15).

The positive effect of using cues (without replaying during sleep) during rehabilitation has already been demonstrated in late-stage PD, since the cues provide bypassing of the affected areas in PD (11). More specifically, external cues guide goal directed behaviour, avoiding the dorsal striatum that is involved in automatized motor control (11,46,47). TMR might work via similar mechanisms, allowing consolidation via these spared goal-directed motor routes.

With regards to rehabilitation, the application of napping after learning might have an added effect as one study suggested that learning just before sleep improved sleep quality itself, which is impaired in PD (48).

Finally, studies can be conducted using neuroimaging such as functional magnetic resonance imaging (fMRI) during the SRTT to study the effects of TMR on brain activity and connectivity in regions that are associated with sleep-dependent motor memory consolidation and automatization in PD, such as the hippocampus and striatum (32). In addition, the EEG obtained during the nap with TMR could be used to investigate possible differences in

physiological characteristics of sleep upon stimulation, such as changes in spindles and slow waves (32).

5.6 Conclusion

This thesis presents the first pilot study showing a possible positive trend of TMR on improving the consolidation of a SRTT in HC and people with PD, albeit non-significantly. Both sequences stabilized after the nap with TMR in both groups, and this stabilization could be maintained at 24h retention. A positive effect, however, was seen in PD with further practice at retention whereby the ReactSeq became significantly faster and accuracy improved, while the NonReactSeq remained stable in RT with more errors. Moreover, RT for the dual task improved significantly for the ReactSeq from post to retention in the PD group and the dual task cost decreased, albeit not significantly. In the HC, both sequences became significantly faster with similar accuracies at the end of retention and showed no significant dual task effects. Surprisingly, no correlations were found between the number of TMR cues provided or total sleep time during the subsequent night and the difference in RT for both groups, though these associations are probably hampered by the small sample size used in this thesis. Taken together, TMR does not seem to affect immediate consolidation, but does seem to support motor learning in PD so that they could improve with further practice after 24 hours and achieve better automatization. Future research is needed to verify these pilot outcomes. If proven true, than TMR should be translated to more day-to-day activities, such as gait and balance tasks to increase clinical translation and be applied during rehabilitation for PD so they can benefit from training effects in the long term (32,38).

6 DUTCH SUMMARY

6.1 Literatuuroverzicht

De ziekte van Parkinson (PD) is de tweede meest voorkomende en de snelst groeiende neurodegeneratieve ziekte in de wereld (1). PD wordt vooral gekenmerkt door de degeneratie van dopaminerge neuronen in de pars compacta van de substantia nigra ($\mathrm{SN_{PC}}$) door de accumulatie van het verkeerd gevouwen α-synucleïne eiwit dat Lewy neurieten en lichamen vormt (3). Hierdoor hebben mensen met PD verminderde dopamine in het striatum en als gevolg last van motorische symptomen zoals rusttremor, bradykinesie, spierstijfheid en posturale instabiliteit, maar ook van niet-motorische symptomen (3,8). Door de verminderde werking van het striatum hebben mensen met PD ook problemen met het proces van geheugenconsolidatie (18). Door dit proces worden aangeleerde motorische bewegingen normaliter opgeslagen in het lange termijn geheugen (18), iets wat verstoord is bij PD (3,14). De problemen met geheugenconsolidatie hebben een invloed op de revalidatie en dat leidt tot een verminderde levenskwaliteit. Mensen met PD zullen zo bijvoorbeeld moeilijkheden ondervinden bij het onthouden van (op)nieuw aangeleerde bewegingen tijdens de revalidatie welke juist heel belangrijk zijn om zo lang mogelijk onafhankelijk te kunnen blijven in het dagelijks leven (14).

Door te oefenen leert men een nieuwe taak steeds beter uit te voeren en deze verbetering zou het best behouden blijven na een periode van slaap (22). Meer nog, bij gezonde jongvolwassenen blijkt verdere verbetering mogelijk te zijn door een periode van slaap aan te bieden na het aanleren van de nieuwe motorische beweging (22,29). Bij gezonde ouderen wordt er een stabilisatie van de herinnering waargenomen na een periode van slaap die het vergeten tegen gaat (22). Volgens Terpening et al. (2013) zouden mensen met PD dezelfde leercapaciteit hebben dan gezonde ouderen en zou de slaap in beide groepen kunnen zorgen voor stabilisatie van de herinnering, maar wel minder effectief blijkt in PD (21). Inderdaad, naast problemen met geheugenconsolidatie hebben mensen met PD ook vaak slaapproblemen die op hun beurt de consolidatie in de weg kunnen staan. Slaapfragmentatie is een van de meest voorkomende klachten bij PD wat resulteert in vaker wakker worden tijdens de nacht en een algemeen verminderde hoeveelheid van diepe niet-REM (NREM) slaap tijdens de nacht (28). De vraag is daarom of er manieren zijn om het effect van slaap op de geheugenconsolidatie te kunnen versterken bij PD.

Recente studies tonen inderdaad aan dat er niet-invasieve technieken bestaan die het consolidatieproces tijdens de slaap verder kunnen versterken (19). Zo een veilige, niet-invasieve interventie is gerichte geheugen reactivatie (TMR). Hierbij worden er leer-gerelateerde stimuli (bijvoorbeeld auditieve signalen) herhaald tijdens de NREM slaap volgend op het leerproces (19). Bij gezonde jongvolwassenen werd al aangetoond dat TMR tijdens slaap na het leren kan zorgen voor betere consolidatie effecten dan bij slaap alleen (32,33). Daarnaast hebben kortere perioden van slaap mét TMR vergelijkbare effecten op de motorische prestaties bij perioden van langere reguliere slaap (31). Dit geeft hoop voor mensen met PD om slapen na het leren te combineren met TMR om zo op een vergelijkbare wijze de consolidatie van het motorisch geheugen te stimuleren, vooral in de vroege stadia van de ziekte wanneer er nog voldoende leercapaciteit is.

6.2 Doel en hypothesen

Het doel van dit project is om te onderzoeken of TMR tijdens de slaap de consolidatie van motorisch geheugen verbetert bij PD en gezonde ouderen. Daarvoor werden er twee verschillende sequenties aangeleerd in een serial reaction time task (SRTT) waarna een 2-uur durend dutje volgde met TMR waarbij één van die twee geleerde sequenties opnieuw werd afgespeeld. Prestaties van de twee sequenties werden vergeleken na het dutje. Dit werd gedaan bij een groep van mensen met PD en bij een groep met gezonde ouderen om beide groepen te kunnen vergelijken.

De hypothese was dat reactietijd en nauwkeurigheid van de met TMR gereactiveerde sequentie zou verbeteren na het dutje ten opzicht van de niet-gereactiveerde sequentie in beide groepen van mensen met PD en gezonde ouderen. Gezien de moeilijkheden met geheugenconsolidatie bij PD werd er verwacht dat de mate van verbetering in de PD groep verminderd zou zijn ten opzichte van de controle groep. Daarnaast werd er een betere retentie en automatisatie verwacht voor de gereactiveerde sequentie in beide groepen, zoals gemeten door een herhalende test na 24 uur, alsook een dubbeltaak.

6.3 Materiaal en methoden

Inclusiecriteria van deze studie waren; i) 40 jaar of ouder; ii) rechtshandig volgens de 'Edinburgh Handedness scale'; iii) mensen met PD werden geïncludeerd indien zij een klinische diagnose van idiopathische PD hadden, gesteld door een neuroloog volgens de

meest recente diagnostische criteria van de International Movement Disorder Society. De volgende exclusiecriteria werden toegepast; i) ernstige slaapapneu zoals vastgesteld tijdens een nachtelijke screening polysomnografie (PSG) door een slaaparts en gedefinieerd door een apneu hypopneu index (AHI) > 30; ii) comorbiditeiten die de SRTT prestaties zouden kunnen hinderen; iii) ernstige cognitieve stoornissen die de vrijwillige geïnformeerde toestemming in twijfel zouden kunnen trekken, zoals bepaald door een Mini Mental State Examination (MMSE)-score < 24; iv) freezing of gait, zoals bepaald door een positief antwoord op item 1 van de New Freezing of Gait Questionnaire; v) diepe hersenstimulatie, en vi) actief ingeschreven in een andere therapeutische trial. Gezonde oudere volwassenen werden gescreend volgens exclusiecriteria i-iii. Deelnemers gaven schriftelijke geïnformeerde toestemming voor start van de metingen en goedkeuring werd verkregen door het lokaal medisch ethisch comité van het universitair ziekenhuis Leuven (S61792).

Dit was een interventionele, gerandomiseerde controle studie bestaande uit 3 visites (**Figuur 4**). Tijdens een eerste visite werden alle deelnemers gescreend aan de hand van neuropsychologische testen en een overnachting met PSG in het slaaplabo van UZ Leuven om eventuele ademhalings- en slaapproblemen na te gaan. De deelnemers met PD werden gescreend voor de ernst van hun motorische en niet-motorische symptomen. Deelnemers die voldeden aan de inclusiecriteria en exclusiecriteria werden uitgenodigd voor visite 2 in het Brainshub slaaplabo van KU Leuven. Tijdens visite 2 voerden de deelnemers een SRTT uit en leerden twee verschillende sequenties die beide visueel en auditief werden gecued. Deelnemers moesten zo snel en accuraat mogelijk de overeenkomstige toets indrukken wanneer ze een groen ingekleurd vierkant te zien kregen op het computerscherm (**Figuur 5**). Elke toets werd gevolgd door een specifieke auditieve muzikale toon. Na het leren van de twee sequenties kregen alle deelnemers de mogelijkheid tot een 2-uur durend dutje met PSG waarbij één van beide sequenties via auditieve TMR opnieuw werd afgespeeld tijdens fase N2 en N3 van de slaap. Dit gebeurde via oortjes op 140% van het eerder bepaalde minimaal hoorbaar volume tijdens waak. Randomisatie bepaalde welke sequentie gereactiveerd en eerst aangeleerd werd. Na het dutje kregen alle deelnemers minstens 30 minuten de tijd om wakker te worden voordat ze de SRTT opnieuw uitvoerden voor beide sequenties. Een dubbeltaak werd extra afgenomen om automatisatie na te gaan. Om retentie effecten na te gaan, herhaalden alle deelnemers de SRTT opnieuw 24 uur later, tijdens visite 3. Deelnemers werden gevraagd geen alcohol en koffie te drinken op de testdagen. Medicatie kon worden ingenomen zoals gewoonlijk.

De primaire uitkomsten waren snelheid en nauwkeurigheid waarbij snelheid gedefinieerd is als de gemiddelde reactietijd voor correcte reacties per blok en nauwkeurigheid als het aantal

correcte reacties per blok. Drie verschillende eindpunten werden gedefinieerd. Het primaire eindpunt van onmiddellijke en pure offline consolidatie werd gedefinieerd als het verschil in prestatie tussen de eerste vier blokken van hertest 1 na het 2-uur durende dutje en de laatste vier blokken van leren net voor het dutje. Een mixed model ANOVA werd gebruikt om de interactie tussen tijd (pre-nap, post-nap, retentie), bij interventie (gereactiveerd, niet-gereactiveerd), bij groep (PD of controle) na te gaan. Het eerste secundaire eindpunt van retentie werd gedefinieerd door het verschil in prestatie op de primaire uitkomsten voor beide reeksen tijdens de vier laatste blokken op de hertest na het dutje versus de eerste vier blokken van leren op de hertest na 24u. Het tweede secundaire eindpunt van dual-tasking werd gedefinieerd door het verschil in prestatie op de primaire uitkomsten voor beide reeksen tijdens de vier blokken van dual-tasking tussen hertest 1 (post) en hertest 2 (retentie). Als verkennende analyse werden de slaapfasen tijdens de dutjes vergeleken tussen de PD- en controlegroepen met behulp van onafhankelijke t-tests of non-parametrische Mann-Withney U test om te onderzoeken of er een verschil was in de hoeveelheid N2- en N3-slaap tijdens welke TMR kon worden toegepast. Daarnaast werd er onderzocht of er een verschil was in het aantal cues dat tijdens N2 en N3 met TMR werd gepresenteerd tussen de PD- en controlegroepen. Correlaties werden nagegaan tussen het aantal stimulaties tijdens N2 en N3 van het dutje en verschil in snelheid tussen de gereactiveerde en de niet-gereactiveerde sequentie bij de post-test. Daarnaast werden correlaties bestudeerd tussen het aantal slaap tijdens de nacht na de postmeting en het verschil in snelheid voor beide sequenties tussen post en retentie. Deze verschillende correlaties werden gedaan met behulp van de Pearson of non-parametrische Spearman correlatie coëfficiënt.

6.4 Resultaten

Het primaire eindpunt, offline consolidatie, toonde een stabilisatie in de reactiesnelheid van beide sequenties in de PD groep en HC groep na het 2-uur durende dutje met TMR. Na het dutje bleek de PD groep meer fouten te maken voor de gereactiveerde sequentie ($p=0.039$, maar niet meer significant na correctie met Bonferroni) wat zou kunnen wijzen op een trade-off effect waarbij er meer fouten worden gemaakt als er meer gefocust wordt op snelheid (39). In de HC zagen we een lichte verbetering in accuraatheid voor de gereactiveerde sequentie en een lichte daling voor de niet-gereactiveerde sequentie, maar deze verschillen waren niet significant ($p>0.05$). Analyse van het secundaire eindpunt retentie liet, in tegenstelling tot de hypothese, geen significante verbetering zien voor de gereactiveerde sequentie in beide groepen. In de PD groep leken beide sequenties te stabiliseren na nog een extra nacht slaap en in de HC groep bleek de gereactiveerde sequentie een hogere reactietijd te hebben na de

extra nacht slaap dan ervoor (p=0.016), de niet-gereactiveerde sequentie stabiliseerde. Verdere analyse liet zien dat er wel een significante verbetering is in reactietijd voor de gereactiveerde sequentie in de PD groep (p=0.010) na verder oefenen na een nacht slaap terwijl deze significante verbetering er niet was voor de niet-gereactiveerde sequentie (p=0.288). In de HC groep bleken de beide sequenties een significante verbetering te tonen in reactietijd bij verder oefenen na een volledige nacht slaap. Het tweede secundaire eindpunt voor dubbeltaak toonde een significante verbetering voor de gereactiveerde sequentie (p=0.025) tijdens de dubbeltaak van post naar retentie in de PD groep terwijl de niet-gereactiveerde sequentie stabiliseerde (p=0.225). In de HC groep stabiliseerde de reactietijd van de dubbeltaak voor beide sequenties (p>0.05). Verdere analyse van de dubbeltaakkost (het verschil in snelheid tussen het uitvoeren van een dubbele taak en het uitvoeren van een enkele taak) toonde visueel aan dat de dubbeltaakkost niet-significant kleiner werd voor de gereactiveerde sequentie (p=0.580) van post naar retentie in de PD groep terwijl de niet-gereactiveerde sequentie steeg in dubbeltaakkost (p=0.729). In de HC groep was hetzelfde effect te zien met en verbetering in dubbeltaakkost voor de gereactiveerde sequentie (p=0.586) en een stabilisatie voor de niet-gereactiveerde sequentie (p=0.958).

6.5 Conclusie

Deze masterproef met piloot studie gaat over het effect van gerichte geheugenreactivatie (Targeted Memory Reactivation) tijdens een dutje na leren van een motorische taak op de offline consolidatie in mensen met de ziekte van Parkinson en gezonde ouderen. Door de kleine sample size konden er amper statistisch significante verschillen worden aangetoond. Toch geeft deze piloot studie een hoopvol inzicht dat TMR een positief effect kan hebben op het leereffect op langere termijn (na 24 uur retentie) voor mensen met PD. TMR beschermd het geheugen tegen vergeten tijdens offline consolidatie en laat nog extra verbetering toe bij extra oefenen, wat niet eerder gezien werd bij mensen met PD (21). Daarnaast kan er ook voorzichtig gesteld worden dat TMR een verbetering kan bezorgen in automatisatie bij mensen met PD aangezien er een verbetering werd gevonden in uitvoering van een dubbeltaak voor de gereactiveerde sequentie. Automatisatie werd ook bereikt voor de gezonde ouderen bij het uitvoeren van de dubbeltaak. Een toekomstige studie met groter aantal deelnemers moet deze effecten echter nog verder aantonen en bevestigen. Verder onderzoek kan de specificiteit van de gegeven stimulaties nagaan om te verifiëren of het exact de geluiden zijn van tijdens het leerproces die zorgen voor het effect, of dat onafhankelijke geluiden ook een positief effect zouden kunnen hebben. Mensen in een verder stadium van PD kunnen in verder onderzoek ook geïncludeerd worden om het effect van TMR in latere stadia van de ziekte te bestuderen

en kwaliteit van leven te verbeteren voor deze populatie patiënten. Tot slot kan er nog onderzoek gedaan worden met neurale beeldvorming om de hersengebieden die betrokken zijn bij leren, offline consolidatie en TMR te bestuderen. De resultaten van dit onderzoek geven hoop op betere leereffecten en een verbeterde automatisatie bij gebruik van TMR. Een dutje met TMR zou daarom geïmplementeerd kunnen worden bij de revalidatie therapieën om verbetering te behalen bij (op)nieuw aanleren van meer dagdagelijkse taken zoals typen, balans oefeningen, schrijven of lopen bij mensen met PD en gezonde ouderen.

7 SUPPLEMENTARY MATERIALS

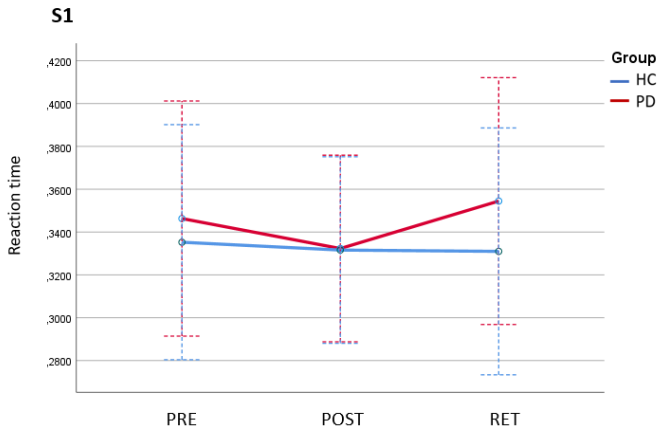


Figure S1: Reaction time (in seconds) on the Psychomotor Vigilance Test (PVT) during the three different time points in PD (red) and HC (blue)

NOTE: error bars show the 95% CI

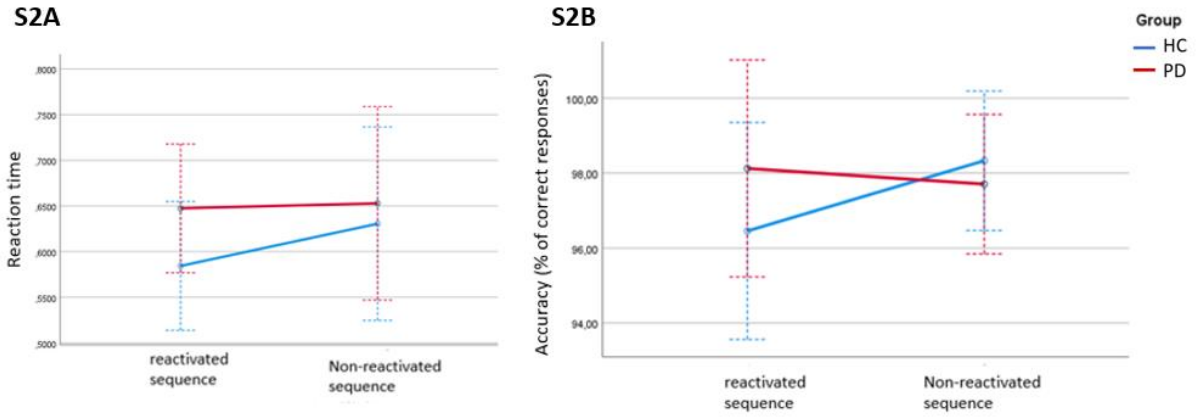


Figure S2: Reaction time (in seconds) (A) and percentage of correct responses (B) during the end of learning before the nap for the Reactivated sequence and Non-reactivated sequence in both PD (red) and HC (blue).

NOTE: error bars show the 95% CI

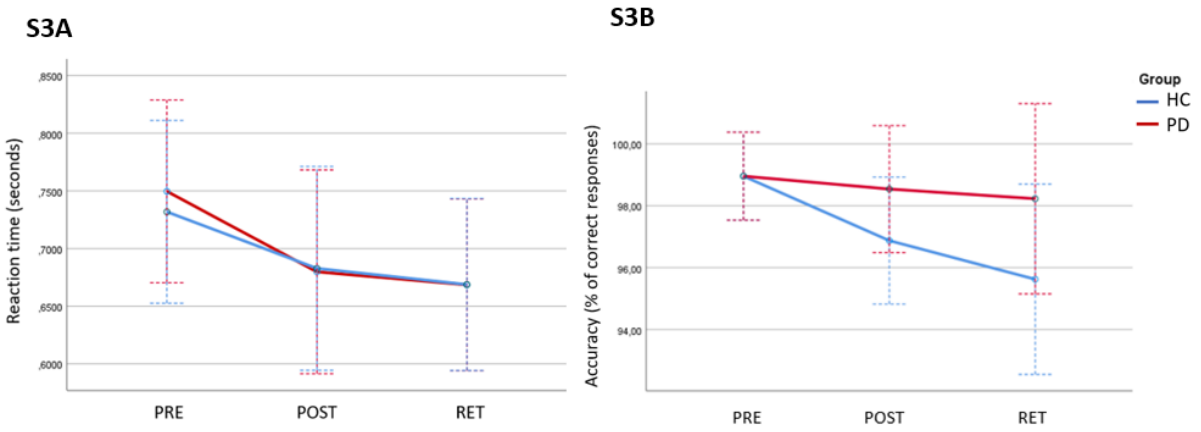


Figure S3: Reaction time (in seconds) (A) and percentage of correct responses (B) during the three time points in PD (red) and HC (blue) participants for the random blocks.

NOTE: error bars show the 95% CI

Supplementary Table S1: Reaction times (in seconds) for all different SRTT

	Tests SRTT	PD (n=5)	HC (n=5)	Time x sequence x group	Time x group	Time x sequence	Sequence x group	Main time	Main sequence
	Random_PRE	0.750 (0.04)	0.732 (0.03)						
	Random_POST	0.680 (0.04)	0.683 (0.03)		P=0.422			**P<0.001**	
	Random_RET	0.669 (0.03)	0.669 (0.03)						
	Learning_PRE_start_RS	0.742 (0.02)	0.751 (0.03)						
	Learning_PRE_start_NRS	0.723 (0.03)	0.731 (0.04)						
Baseline	Learning_PRE_end_RS	0.647 (0.09)	0.584 (0.03)						
Baseline	Learning_PRE_end_NRS	0.653 (0.08)	0.631 (0.12)				P=0.390		P=0.281
	Learning_PRE_end_RS	0.647 (0.09)	0.584 (0.03)						
Primary endpoint	Learning_PRE_end_NRS	0.653 (0.08)	0.631 (0.12)	P=0.848	P=0.679	P=0.435	P=0.145	**P=0.017**	**P=0.034**
Primary endpoint	Learning_POST_start_RS	0.676 (0.07)	0.620 (0.07)						
Primary endpoint	Learning_POST_start_NRS	0.697 (0.11)	0.691 (0.10)						
	Learning_POST_end_RS	0.621 (0.10)	0.529 (0.04)						
Secondary endpoint 1	Learning_POST_end_NRS	0.622 (0.10)	0.572 (0.10)	P=0.635	**P=0.009**	P=0.161	P=0.310	P=0.137	P=0.547
Secondary endpoint 1	Learning_RET_start_RS	0.616 (0.08)	0.586 (0.04)						
Secondary endpoint 1	Learning_RET_start_NRS	0.599 (0.10)	0.595 (0.08)						
	Learning_RET_start_RS	0.616 (0.08)	0.586 (0.04)						

Exploratory analysis 1	Learning_RET_start_NRS	0.599 (0.10)	0.595 (0.08)	P=0.604	P=0.070	**P=0.048**	P=0.507	**P<0.001**	P=0.442
	Learning_RET_end_RS	0.562 (0.04)	0.498 (0.01)						
	Learning_RET_end_NRS	0.581 (0.04)	0.529 (0.03)						
Secondary endpoint 2	DT_POST_RS	0.725 (0.14)	0.677 (0.06)	P=0.235	P=0.787	P=0.155	P=0.558	**P=0.019**	P=0.197
	DT_POST_NRS	0.702 (0.14)	0.696 (0.09)						
	DT_RET_RS	0.640 (0.11)	0.631 (0.06)						
	DT_RET_NRS	0.670 (0.12)	0.654 (0.09)						
Exploratory analysis 2	Dual task cost_POST_RS	0.095 (0.06)	0.149 (0.06)	P=0.847	P=0.907	P=0.334	P=0.599	P=0.754	P=0.491
	Dual task cost_POST_NRS	0.080 (0.06)	0.123 (0.06)						
	Dual task cost_RET_RS	0.079 (0.06)	0.133 (0.06)						
	Dual task cost_RET_NRS	0.089 (0.07)	0.125 (0.04)						
Overview of all time points	LE_PRE_start_RS and NRS LE_PRE_end_RS and NRS LE_POST_start_RS and NRS LE_POST_end_RS and NRS LE_RET_start_RS and NRS LE_RET_end_RS and NRS			P=0.143	P=0.282	**P=0.008**	P=0.310	**P=0.006**	P=0.249

NOTE: mixed design ANOVA, results were reported as mean (standard deviation) when normally distributed as verified by Shapiro-Wilk test
Abbreviations; PRE=pre-nap, POST=post-nap, RET=retention, RS=Reactivated Sequence, NRS=Non-reactivated Sequence, DT=dual task, LE=learning

Supplementary Table S2: Accuracy, percentage of correct responses for all different SRTT

	Tests SRTT	PD (n=5)	HC (n=5)	Time x sequence x group	Time x group	Time x sequence	Sequence x group	Main time	Main sequence
Baseline	Random_PRE	100.00 (97.4-100.0)	98.96 (0.3)		P=0.375			P=0.136	
	Random_POST	100.00 (96.4-100.0)	96.88 (0.9)						
	Random_RET	98.23 (1.1)	95.63 (1.6)						
Primary endpoint	Learning_PRS_start_RS	95.42 (6.7)	97.92 (1.6)						
	Learning_PRE_start_NRS	89.96 (96.9-99.5)	97.71 (0.9)						
	Learning_PRE_end_RS	98.13 (1.2)	96.46 (1.3)				P=0.309		P=0.509
	Learning_PRE_end_NRS	97.71 (1.0)	98.33 (0.5)						
	Learning_PRE_end_RS	97.29 (2.8)	98.54 (1.7)	P=0.097	P=0.584	P=0.483	P=0.954	P=0.448	P=0.207
	Learning_PRE_end_NRS	98.54 (1.7)	96.25 (2.5)						
	Learning_POST_start_RS	96.88 (94.3-96.9)	97.08 (1.4)						
	Learning_POST_start_NRS	98.75 (0.4)	97.50 (1.0)						
	Learning_POST_end_RS	97.08 (1.7)	98.54 (0.5)	P=0.197	P=0.411	P=0.197	P=0.437	P=0.904	P=0.263
	Learning_POST_end_NRS	98.13 (1.2)	98.96 (0.5)						

Secondary endpoint 1	Learning_RET_start_RS	96.25 (2.0)	98.96 (96.9-98.9)							
	Learning_RET_start_NRS	100.00 (98.9-100.0)	98.54 (0.5)							
Exploratory analysis 1	Learning_RET_start_RS	96.25 (2.0)	98.96 (96.9-98.9)							
	Learning_RET_start_NRS	100.00 (98.9-100.0)	98.54 (0.5)	P=0.177	P=0.356	P=0.228	P=0.460	P=0.931	P=0.177	
	Learning_RET_end_RS	98.54 (0.5)	97.50 (0.7)							
	Learning_RET_end_NRS	100.00 (96.3-100.0)	98.13 (0.7)							
Secondary endpoint 2	DT_POST_RS	98.5 (0.8)	97.29 (1.0)	P=0.367	P=0.339	P=0.367	P=0.537	**P=0.045**	P=0.118	
	DT_POST_NRS	98.96 (0.3)	97.92 (0.6)							
	DT_RET_RS	96.04 (0.8)	96.67 (1.2)							
	DT_RET_NRS	98.33 (0.8)	97.29 (0.5)							
Overview of all time points	LE_PRE_start_RS and NRS LE_PRE_end_RS and NRS LE_POST_start_RS and NRS LE_POST_end_RS and NRS LE_RET_start_RS and NRS LE_RET_end_RS and NRS			P=0.225	P=0.753	P=0.477	P=0.570	P=0.586	P=0.242	

NOTE: mixed design ANOVA, results were reported as mean (standard deviation) when normally distributed as verified by Shapiro-Wilk test, when not normally distributed then reported as median (25th percentile – 75th percentile)

Abbreviations; PRE=pre-nap, POST=post-nap, RET=retention, RS=Reactivated Sequence, NRS=Non-reactivated Sequence, DT=dual task, LE=learning

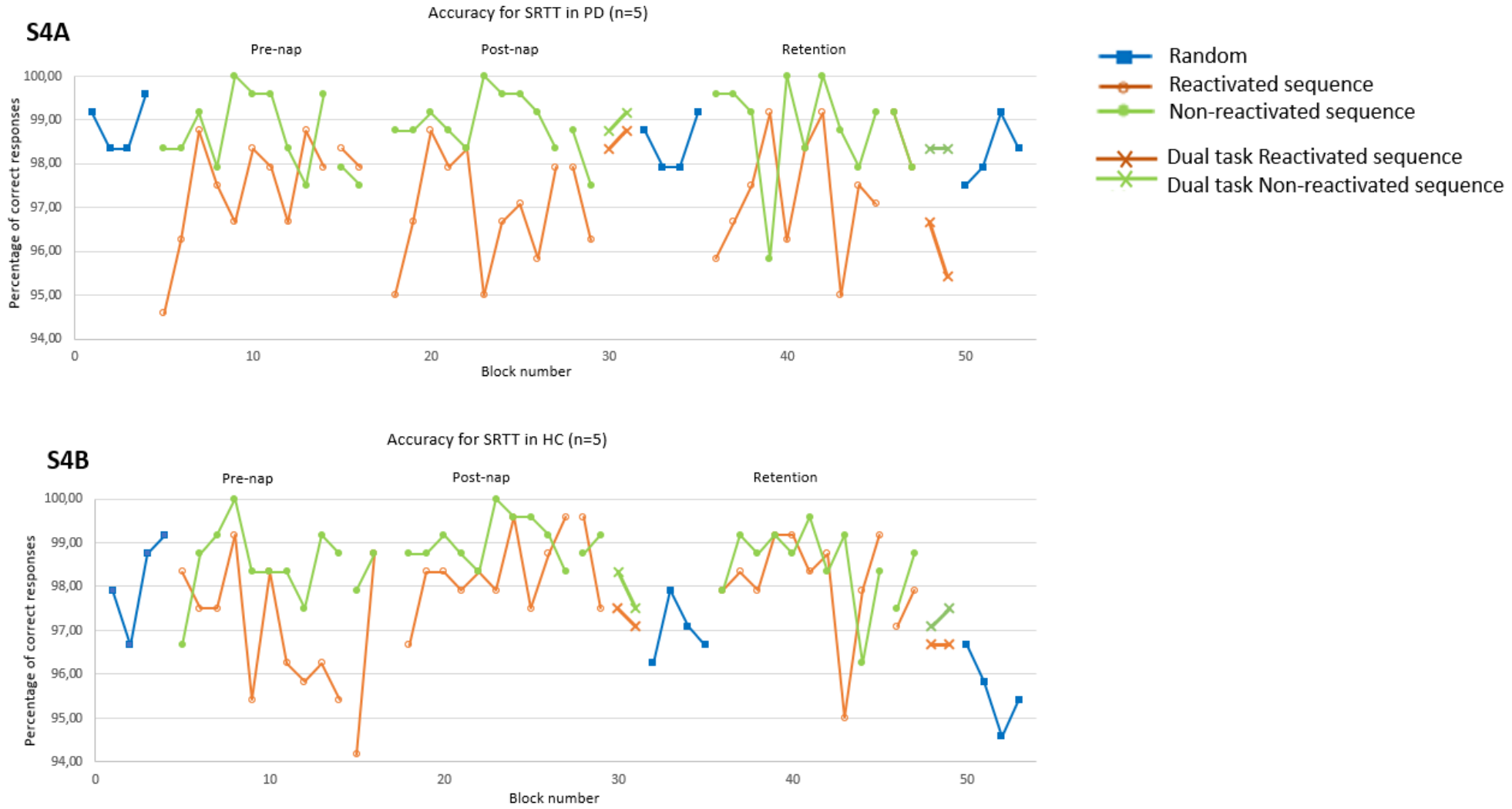
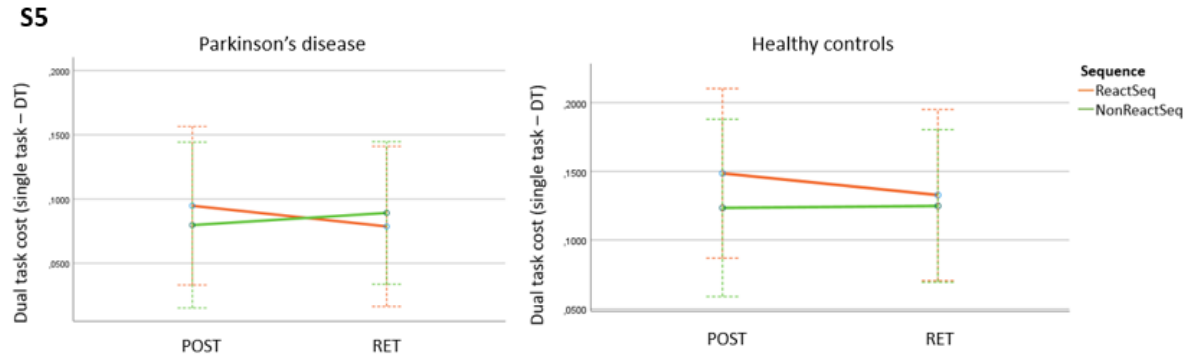


Figure S4: Accuracy per block in people with Parkinson's disease (A) and healthy controls (B) for pre-nap (blocks 1-16), post-nap (block 17-35), retention (blocks 36-53)

Figure S5: dual task cost (reaction time during dual task - reaction time during end of single task) at post and retention in PD (A) and HC (B) participants.

As an exploratory analysis for the DT, we also examined the dual task cost for both sequences in both groups. There was no three-way interaction effect for time (post, ret) by sequence (ReactSeq, NonReactSeq) by group (PD, HC) ($F(1,8)=0.04$); $p=0.847$), nor any two-way interaction effects or main effects (all $p>0.05$). Visual exploration of **Figure S5** shows a decrease in dual task cost from post to retention for the ReactSeq in both groups, meaning that the difference in RT between the single task and dual task became smaller. The NonReactSeq showed a slight increase in dual task cost for both groups. None of these comparisons were statistically significant (all $p>0.05$).

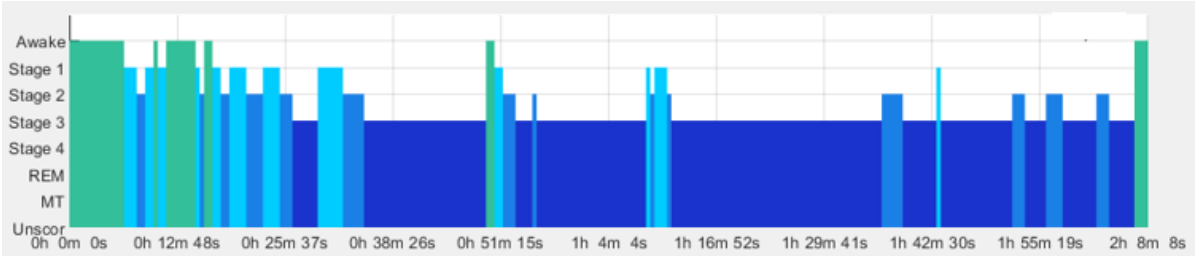


NOTE: error bars show the 95% CI, green=non-reactivated sequence (NonReactSeq) and orange=reactivated sequence (ReactSeq)

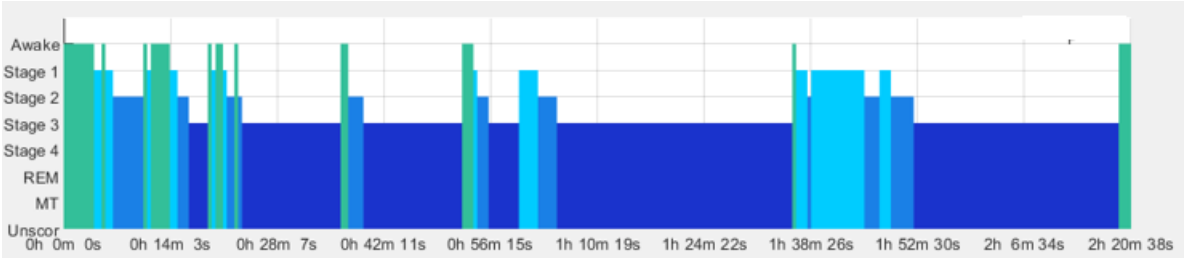
Figure S6: individual hypnograms of the 2-hour nap.

Green=wake; light blue=N1; blue=N2, dark blue=N3, purple=REM

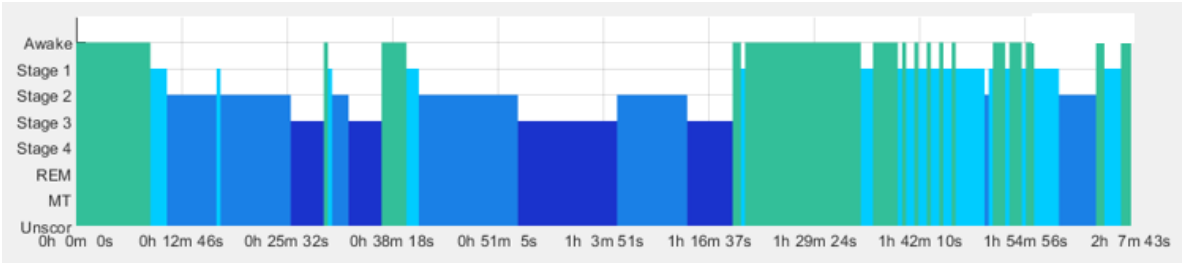
Participant 1 (PD)



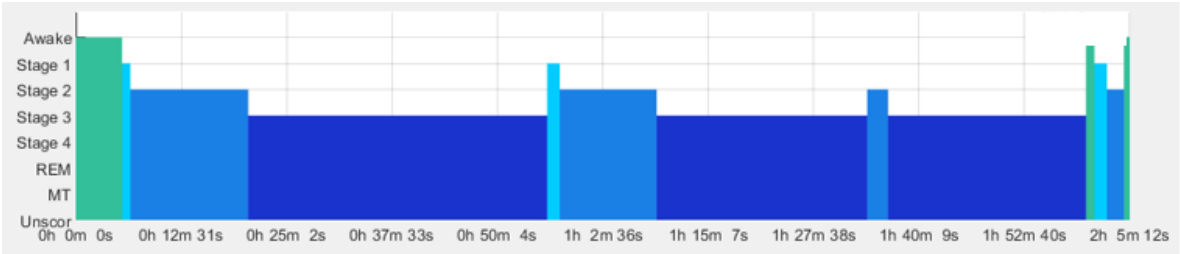
Participant 2 (PD)



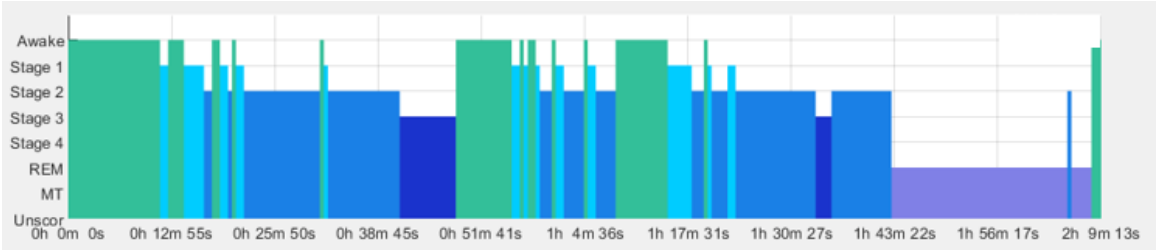
Participant 3 (PD)



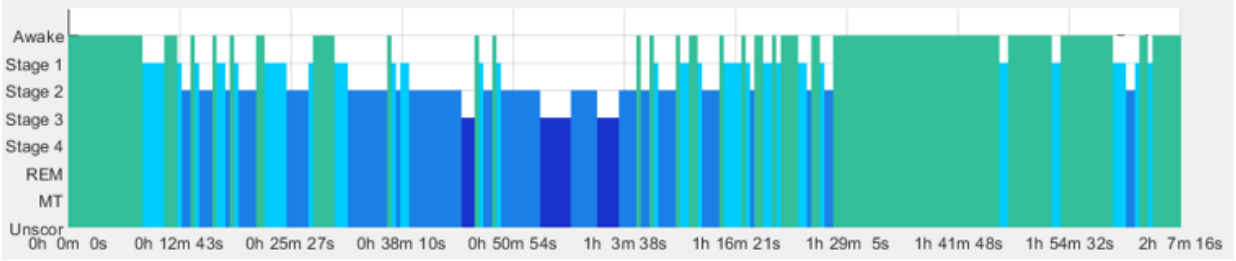
Participant 4 (PD)



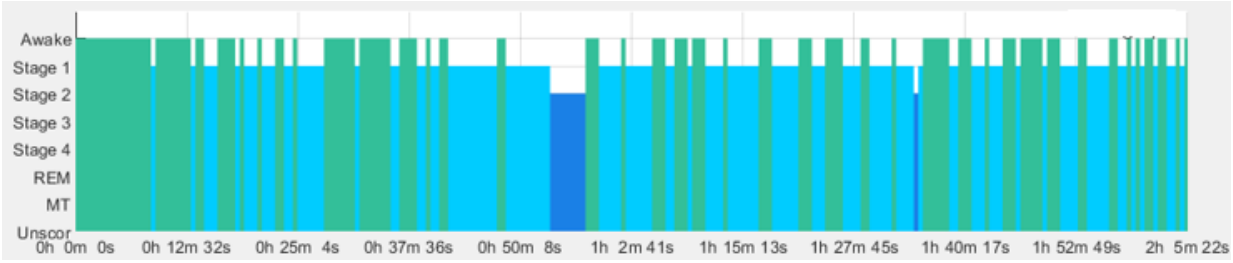
Participant 5 (PD)



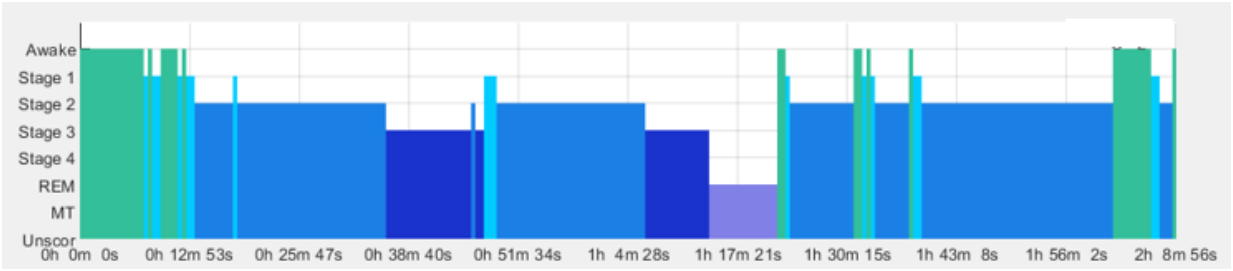
Participant 6 (HC)



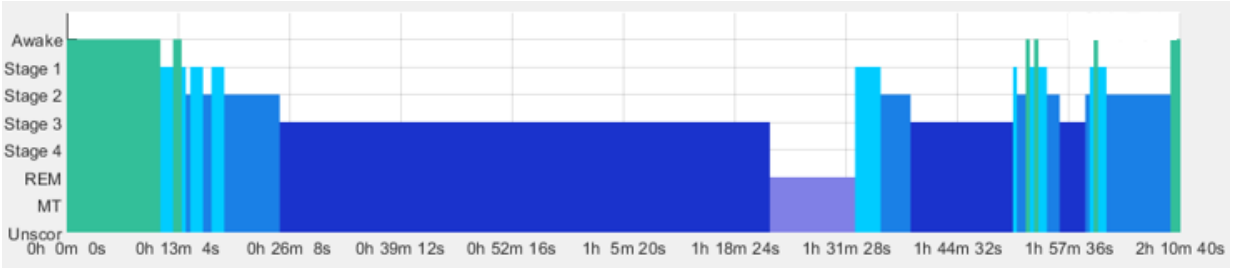
Participant 7 (HC)



Participant 8 (HC)



Participant 9 (HC)



Participant 10 (HC)

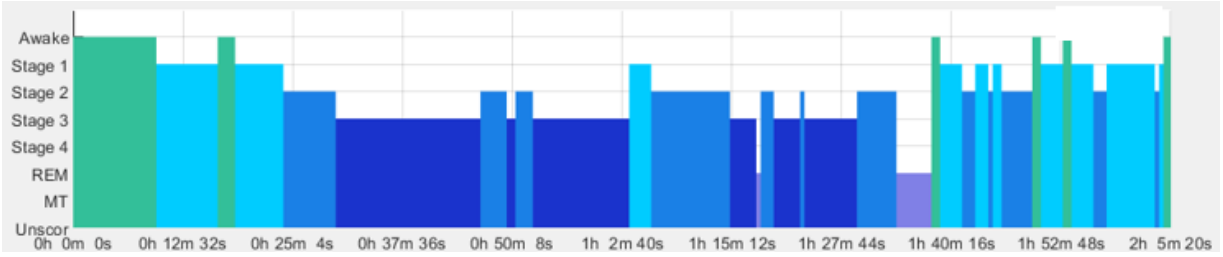
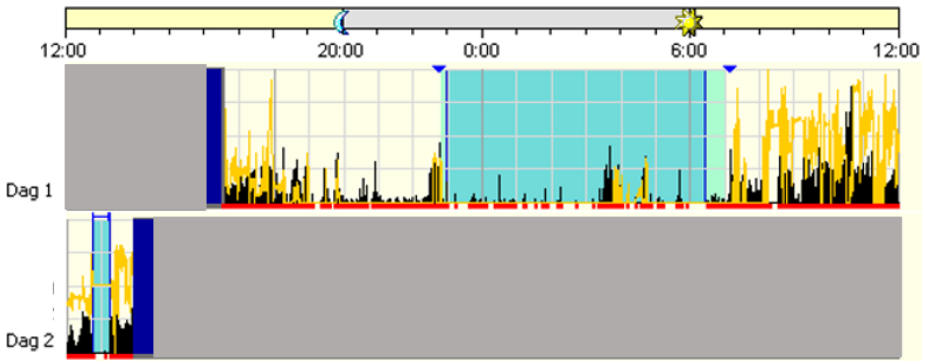
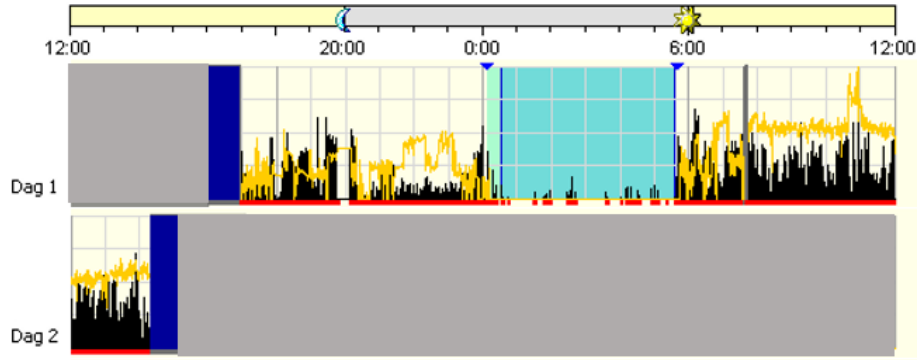


Figure S7: Individual actigraphs of the 24 hours after the post-nap SRTT

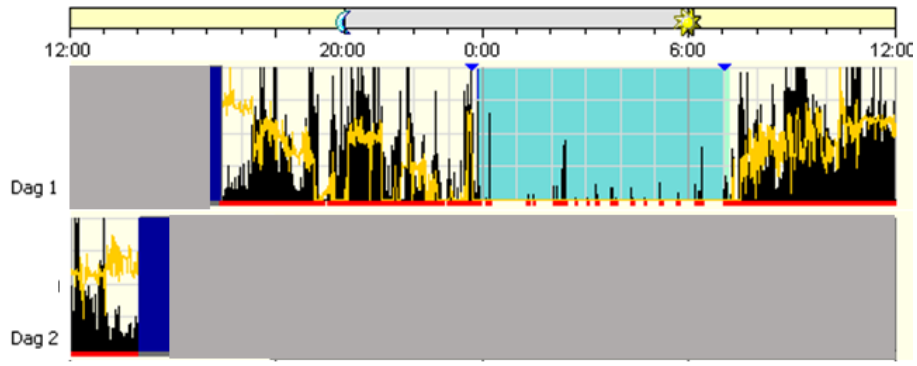
Participant 1 (PD)



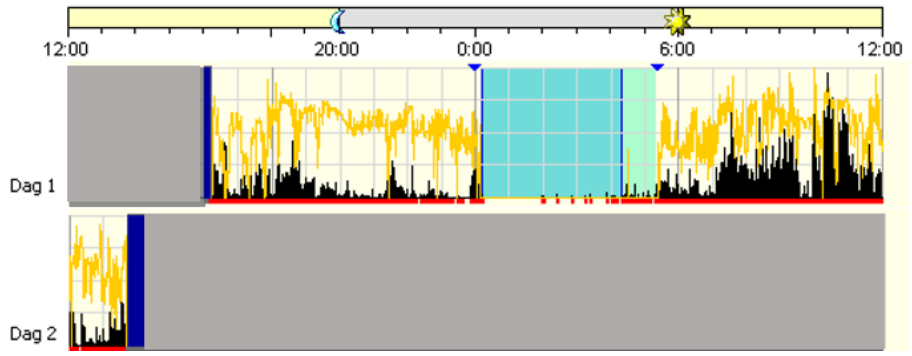
Participant 2 (PD)



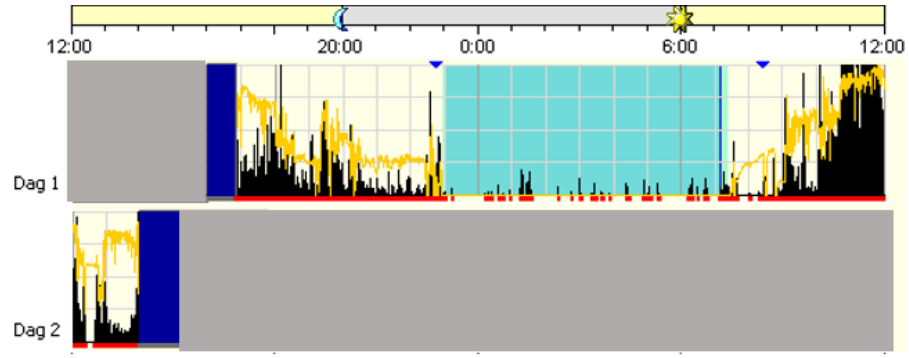
Participant 3 (PD)



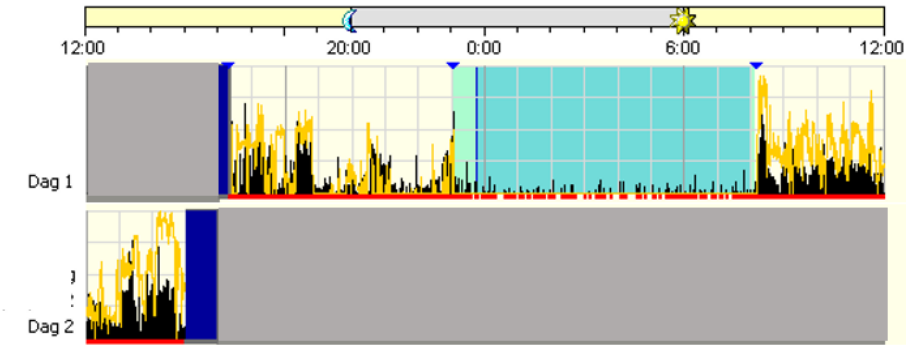
Participant 4 (PD)



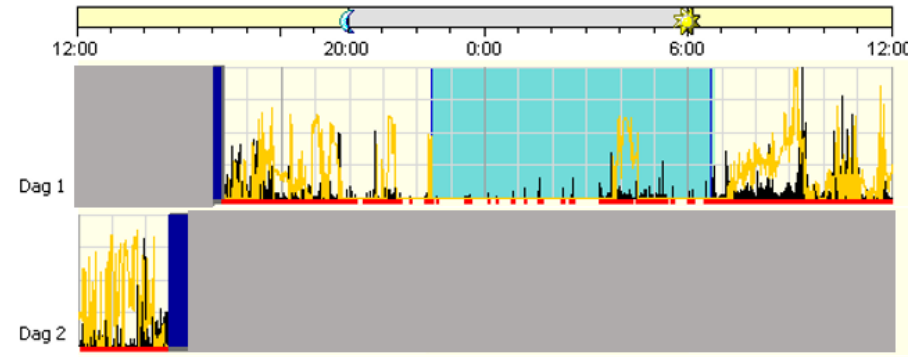
Participant 5 (PD)



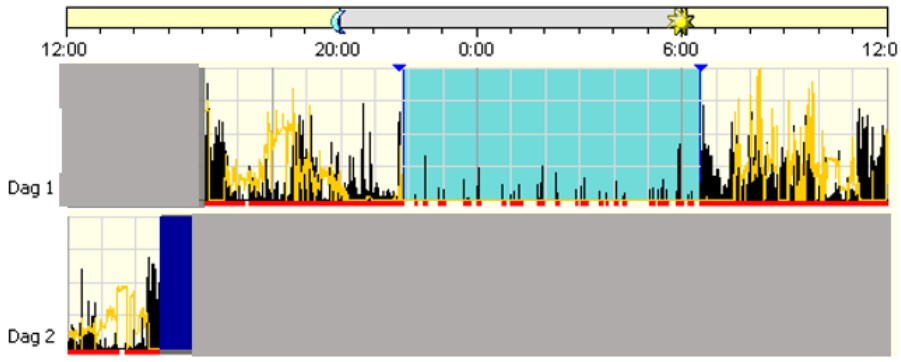
Participant 6 (HC)



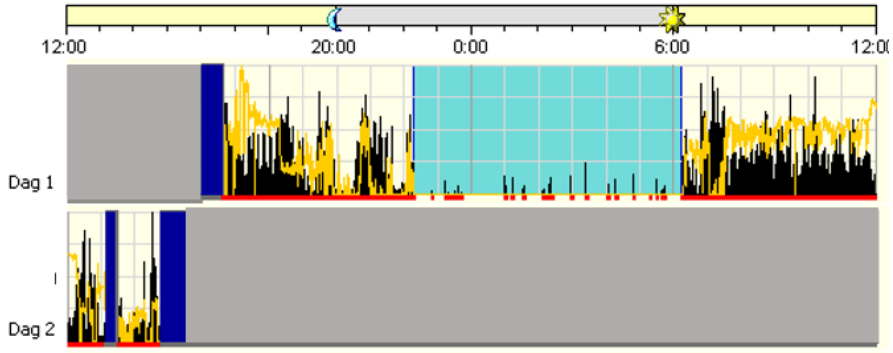
Participant 7 (HC)



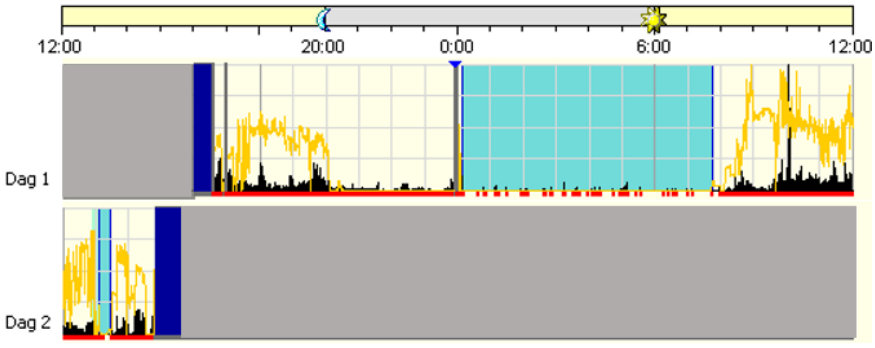
Participant 8 (HC)



Participant 9 (HC)



Participant 10 (HC)



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