BMJ Open

The SCHIP study
Sleep-Cognition-Hypothesis In maritime Pilots - The effect of long-term work-related poor sleep on cognition in healthy middle-aged men
Methodology of a prospective cohort study in maritime pilots

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-026992
Article Type:	Protocol
Date Submitted by the Author:	01-Oct-2018
Complete List of Authors:	Thomas, Jana; Radboudumc, Geriatrics; Radboud Alzheimer Center Ooms, Sharon; Radboudumc, Geriatrics; Radboud Alzheimer Center Verbeek, Marcel; Radboudumc; Radboud Alzheimer Center Booij, Jan; Academic Medical Center, Department of Nuclear Medicine Rijpkema, Mark; Radboudumc, Radiology and nuclear medicine Kessels, Roy; Donders Institute for Brain, Cognition and Behaviour, Radboud university medical center, Medical Psychology; Vincent van Gogh Institute for Psychiatry Overeem, Sebastiaan; Eindhoven Univeristy of Technology, Eindhoven Medtech Innovation Center; Sleep Medicine Center Kempenhaeghe Claassen, Jurgen; Radboudumc, Geriatrics; Radboud Alzheimer Center
Keywords:	Alzheimer's disease, Amyloid accumulation, Neurodegeneration, Cognitive function, Sleep

The SCHIP study

Sleep-Cognition-Hypothesis In maritime Pilots - The effect of long-term work-related poor sleep on cognition in healthy middle-aged men

Methodology of a prospective cohort study in maritime pilots

Jana Thomas, MSc*[1,2,3]; Sharon Ooms, MSc*[1,2,3]; Marcel M. Verbeek, PhD[2,3,6]; Jan Booij, MD, PhD[7,8], Mark Rijpkema, PhD[7]; Roy P.C. Kessels, PhD[2,3,9]; Sebastiaan Overeem, MD, PhD[4,5] and Jurgen A.H.R. Claassen, MD, PhD[1,2,3]

(1) Department of Geriatric Medicine, Radboud University Medical Centre, Nijmegen, The Netherlands; (2) Radboudumc Alzheimer Centre, Nijmegen, The Netherlands; (3) Donders Institute for Brain, Cognition and Behavior, Nijmegen, The Netherlands; (4) Sleep Medicine Centre Kempenhaeghe, Heeze, The Netherlands; (5) Eindhoven Medtech Innovation Center, Eindhoven University of Technology, Eindhoven, The Netherlands; (6) Department of Neurology, Radboud University Medical Centre, Nijmegen, The Netherlands; (7) Department of Radiology and Nuclear Medicine, Radboud University Medical Center, Nijmegen, The Netherlands; (8) Department of Radiology and Nuclear Medicine, Amsterdam University Medical Center, Amsterdam, The Netherlands; (9) Department of Medical Psychology, Radboud University Medical Centre, Nijmegen, The Netherlands

**authors contributed equally to this work*

Corresponding author:
Jana Thomas
Jana.thomas@radboudumc.nl
Reinier Postlaan 4
6526 GC Nijmegen
The Netherlands

Wordcount: 3.339 words (without abstract, figures, references)

ABSTRACT

Introduction: Evidence indicates a bi-directional relationship between poor sleep and Alzheimer's disease (AD). While AD may lead to disruption of normal sleep, poor sleep in itself may play a causal role in the development of AD by influencing the production and/or clearance of the amyloid-beta (Aβ) protein. This led to the hypothesis that extended periods (>10 years) of disturbed sleep could lead to Aβ accumulation with subsequent cognitive AD related decline.

Objective: To describe the methodology of the SCHIP study, a cohort study in maritime pilots that aims at investigating the relationship between chronic sleep disturbances due to an extrinsic cause (work related), cognitive function and amyloid accumulation among healthy middle-aged men to test the hypothesis that prolonged abnormal sleep behavior increases the risk of AD-related cognitive decline.

Methods: Our study sample consists of a group of healthy middle-aged maritime pilots, who have had irregular sleeping patterns for more than 15 years due to irregular work schedules. This chronic sleep deprivation group will be compared to a group of healthy, age and education matched controls with normal sleep. Participants will complete 10 days of actigraphy (Actiwatch 2, Philips Respironics) combined with a sleep-wake diary. They will undergo one night of polysomnography (PSG), followed by comprehensive assessment of cognitive function. Additionally, participants will undergo amyloid PET-CT (Positron Emission Tomography- Computed Tomography) to measure brain amyloid accumulation and MRI to investigate atrophy and vascular changes.

Analysis: All analyses will be performed using IBM SPSS version 20.0 (SPSS Inc, Chicago, USA). We will perform independent samples t-tests to compare all outcome parameters.

Ethics and dissemination: The study protocol was approved by our institutional ethical review board (NL55712.091.16, file number 2016-2337) and will be performed according to good clinical practice (GCP) rules. Data and results will be published in in 2019 and 2020.

Key words: Alzheimer's disease, amyloid accumulation, neurodegeneration, cognitive function, sleep, sleep deprivation, shift work

ARTICLE SUMMARY

Strengths and limitations of the study

- the unique cohort of maritime pilots allows the prospective assessment of long-term externally caused sleep disturbances and their potential consequences for cognitive functioning and amyloid accumulation
- the unique cohort might also be a limitation to the study, because they might not be comparable to the control group to some extent
- in addition to imaging techniques (PET-CT and MRI), we use sensitive and well-validated neuropsychological tests to measure different domains of cognitive function (reaction time, visual memory, executive function, semantic memory and episodic memory)
- we will make use of four different instruments to get a comprehensive measure of sleeping patterns (two subjective and two objective ones)
- our results could give rise to new treatment opportunities, that aim at sleep improvement and management in order to prevent or reduce amyloid accumulation and in turn delay or even prevent the development of AD

1. INTRODUCTION

Alzheimer's disease (AD) is the most prevalent cause of dementia and currently affects approximately 36 million people worldwide (1). Thus far, no successful treatment is available. One of the major contributors to the neurodegeneration seen in the brains of AD patients is amyloid-beta (Aβ) (2). The amyloid-cascade hypothesis characterizes amyloid accumulation as the fundamental initiating pathway for the development of AD (3). While memory impairment and disorientation are hallmarks of the disease, approximately 44% of people living with AD also suffer from some form of sleep disturbance (4). AD patients have more awakenings during the night, present decreased slow wave sleep (SWS), less rapid eye movement (REM) sleep and more frequent daytime napping compared to age-matched controls without dementia (4). Neurodegeneration in the sleep-wake regulating areas of the brain has been identified as a potential cause of the frequent sleep disturbances among AD patients (5).

Recent evidence suggests that sleep and AD have a bi-directional relationship, in which sleep disturbances are not just a symptom of AD, but also contribute to AD pathophysiology (6). Elderly people suffering from insomnia are more likely to develop AD compared to controls without insomnia (7). Furthermore, disrupted circadian rhythm among otherwise healthy individuals (8) and sleep-disordered breathing disorders (9) increase the chance of developing AD later in life. Poor sleep quality specifically has been shown to increase the risk of AD among older individuals (5, 10-13). A recent meta-analysis of epidemiological studies found that poor sleep increased AD risk with a relative risk (RR) of 1.7 and that approximately 15% of cases of AD in the population might be attributable to sleep problems (14).

The effect of acute sleep deprivation on AD has been shown in both rodent and human studies, that investigated the effect of sleep and sleep deprivation on Aβ levels. Rodents and humans show fluctuations in Aβ levels over a 24-hour rhythm, where levels rise during wakefulness and decrease during sleep (15, 16). Following acute sleep deprivation, the drop in Aβ levels that would normally occur after sufficient sleep the following morning, was absent. Based on this evidence, chronic sleep disturbances might lead to a pronounced accumulation of Aβ in the brain, which in turn increases the risk to develop AD.

To date, observational and epidemiological studies have identified a relationship between poor sleep and the risk of developing AD. Experimental research has found that sleep deprivation and Aβ levels are related in both animals and humans. However, the direct effect of chronic sleep deprivation and its consequences on cognitive functioning, particularly the risk to develop AD later in life, has not been studied before.

In this article we describe the methodology and the participant cohort of the SCHIP (Sleep-Cognition Hypothesis In maritime Pilots) study. We designed the SCHIP study in order to investigate the effect of chronic sleep deprivation on cognitive function, structural brain changes, and amyloid accumulation by using a unique cohort of healthy subjects with long-term sleep disruption due to their occupation as maritime pilots. Because of the bi-directional nature of the relationship between sleep and AD, poor sleep and sleep disorders could represent an early symptom that precedes the clinical manifestation of AD. Therefore, it is especially important to investigate the effect of chronic sleep deprivation in a group that experiences disrupted sleep, which is caused by an external factor (work) and not by an intrinsic sleeping disorder (such as insomnia) which could be AD-related. Therefore participants in this study were selected based on their chronic sleep disruptions caused by objectifiable external occupational factors.

Results and insights born from the SCHIP study could shed more light on sleep disturbance as one of the risk factors to develop AD and contribute to new and improved treatment strategies. In the SCHIP study we will investigate whether participants with chronic sleep disturbances perform worse on cognitive assessment in comparison to a healthy control group and whether chronic sleep disturbances are associated with elevated brain amyloid concentrations and structural brain changes.

2. METHODS AND ANALYSIS

2.1. Participants

Considering that this study is a proof of principle study, it is not possible to establish the exact effect size of the change in cognitive function we expect to see between the maritime pilots and the healthy

controls.

To account for possible 10% withdrawal, we chose to have n=20 participants in each arm.

In order to reach enough power to detect clinically relevant differences regarding the results of the PET-CT scan, we performed a sample size power calculation using G Power (version 3.1.9.3) (17). Reported normal values (mean and standard deviation) for this age group in the literature vary between studies but are in the order of a mean standard uptake value (SUV) between 0.9 and 1.1 with standard deviations (SDs) in the range of 0.05 – 0.2. We define a relevant difference between maritime pilots and normal values to be 0.2 or more (an SUV of 1.3 or higher is considered as abnormal in most studies). This results in a large expected effect size (>0.8). The precise number of age- and education matched subjects in the database is not known yet but is estimated to be at least 50 (between 50 and 100). We have applied a one-tailed test (it is not possible to have a SUV lower than normal values). With an alpha of 0.05, with n=20 our power is 0.95 or higher (depending on the number of normal subjects). The lower level for power (0.85) is reached with 13-15 pilots.

Based on these calculations, we will aim to recruit 20 pilots, allowing for drop-out of 5-7 pilots (either due to withdrawal of consent or artefacts in the PET-CT).

Participants will be recruited within the national organization of Dutch Maritime Pilots (Nederlandse Loodswezen). We selected the maritime pilots as a suitable study population because of their unique irregular working schedule. In a usual workweek they have a maximum of two hours of rest in between episodes of two hours (or more) of work, for a period of 24 hours. This rhythm continues, in most cases, for four or five consecutive days a week, followed by a week off. The week off is often characterized by limited sleep as well, because the maritime pilots use that week to catch up with social engagements (friends, family, etc.).

The responsibility of a maritime pilot is to handle large international ships arriving by sea and to maneuver them into their final docking position in one of the Dutch harbors. This profession carries high responsibilities and requires accurate knowledge of the dimensions of the harbor and the ships besides technical knowledge and navigational skills. The 2-by-2-hour schedule is necessary because guiding the ships is a time intensive procedure, that can take hours to complete. Maintaining this work schedule for more than 15 years will result in prolonged exposure to sleep deprivation.

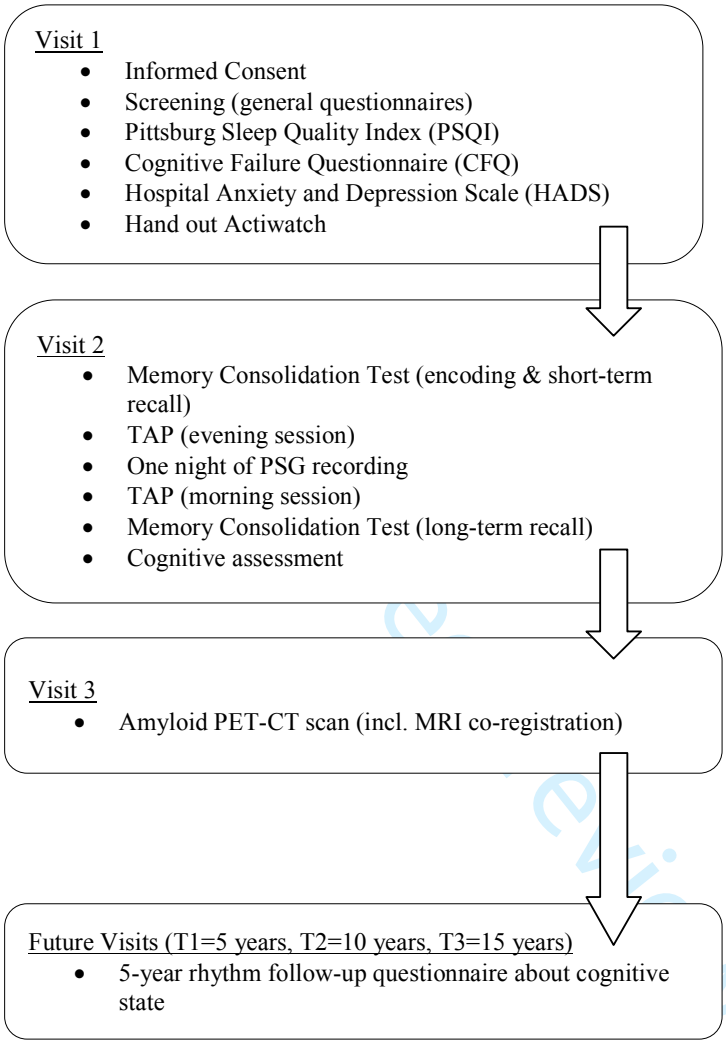
We will reach out to the whole maritime pilot community, in order to recruit pilots who are approximately 50 years old, with at least 15 years of uninterrupted work history as a maritime pilot. Additional inclusion criteria are not using neuro-active medications or psycho-stimulants, consumption of <14 alcoholic beverages per week, a body mass index of 18-30kg/m^2 and no subjective cognitive complaints (Cognitive Failure Questionnaire (CFQ <43). As control group, we will recruit 20 age, sex and education matched healthy adults with normal sleep indicated by a score of >5 on a subjective sleep questionnaire (Pittsburgh Sleep Quality Index, PSQI). The study protocol was approved by our institutional review board (NL55712.091.16, file number 2016-2337) and will be performed according to good clinical practice (GCP) rules.

2.2. Experimental Design

The aim of the SCHIP study is to investigate the relationship between chronic sleep disturbances due to an extrinsic cause (work related), cognitive function and amyloid accumulation among healthy middle-aged men to test the hypothesis that prolonged abnormal sleep behavior increases the risk of AD-related cognitive decline. In order to test this hypothesis, maritime pilots will have three visits and controls will have two visits (figure 1). During the first visit participants will complete general questionnaires about medical history, sleeping habits and cognitive state. Approximately 10 days after the first visit has taken place, participants are invited to the sleep center Kempenhaeghe (Heeze, The Netherlands) for the second visit. They will arrive at the sleep center at 19.00 h and complete a memory consolidation test (modified 'Doors Test') and a test for attentional performance (TAP 2.3) (18), followed by overnight polysomnography (PSG). Participants wake up at their normal wake time and complete a neuropsychological assessment after breakfast around 9.00h.

Visit three (only for the maritime pilots) will be performed at the Radboud university medical center in Nijmegen (The Netherlands), where participants will undergo a standard amyloid PET-CT to measure brain amyloid accumulation and a 3T-MRI for coregistration. Participants are scheduled in their week off in order to prevent short term effects of sleep disruption on amyloid concentrations in the brain (19).

Figure 1: Overview experimental design



2.3. Sleep Measurements

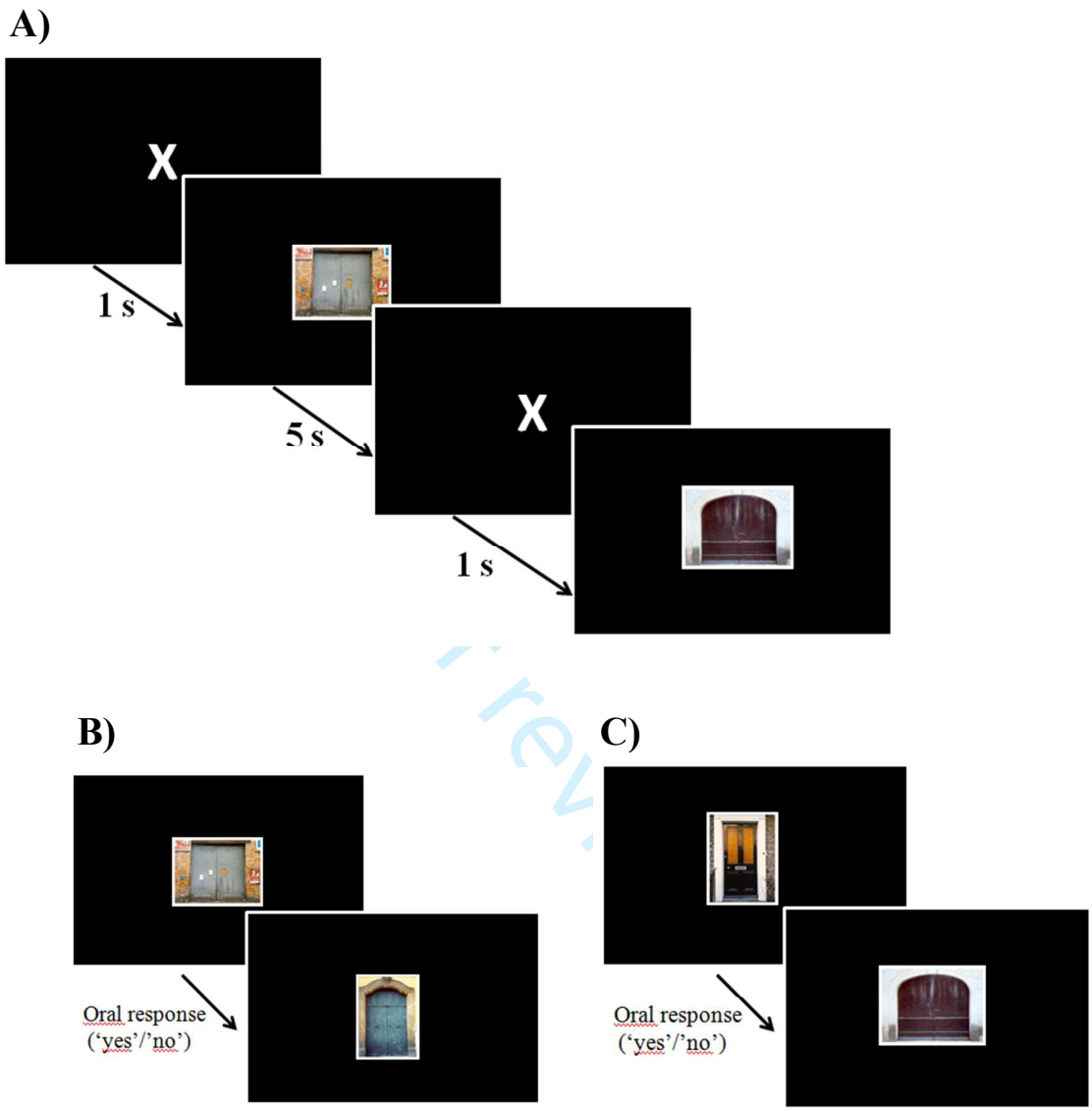
In order to obtain a comprehensive measure of sleeping patterns, we will use the Pittsburg Sleep Quality Index (PSQI) with questions about average sleeping behavior, including the report of bedtime, get-up time, sleep latency, total sleep time, sleep disturbances (pain, breathing etc.) and use of sleep medication. The maritime pilots will be instructed to fill in the questionnaire twice, once with regard to a work week and once with regard to a week off. Additionally all participants are asked to maintain a sleep-wake diary on a daily basis. In this diary they have to keep track of their bed times, the time it took to fall asleep (sleep latency), the number of awakenings and their get up times. Furthermore they will receive an accelerometer, the Actiwatch 2 (Philips Respironics; Eindhoven, The Netherlands), in

order to obtain more objective measurements of sleeping behavior. The Actiwatch is worn around the wrist and measures sleep and wakefulness automatically based on movement. Participants are instructed to fill in the sleep diary and to wear the Actiwatch for 10 days preceding the second visit.

2.4. Cognitive assessment

The neuropsychological assessment has been designed to measure the following cognitive domains, using validated Dutch versions of widely used neuropsychological tests. Episodic memory is assessed using the WMS-IV Logical Memory and the Rey-Auditory Verbal Learning Test (RAVLT). Working memory and executive function are measured by WAIS-IV Digit Span, Trail Making Test (TMT-A, TMT-B) and WAIS-IV Digit Symbol test. Semantic memory and language are assessed by letter fluency (D-A-T), semantic fluency (animal/profession naming) and the Boston Naming Test-Short Form (BNT) (20). Attentional performance is studied using the alertness test of the Test of Attentional Performance (TAP 2.3) (18). This test is conducted twice, once in the evening around 19.00 h and once in the morning around 9.00 h. In order to test overnight memory consolidation, a novel paradigm was developed based on the Doors Test, a visual recognition task developed by Baddely and colleagues (21) and extended using a validated database of 2000 pictures of doors (22). During the encoding trial, we present 120 pictures of doors that participants are instructed to remember. All targets (doors) are presented twice in a different, pseudo-randomized order. Targets are shown for 5 seconds each, separated by a fixation cross presented for 1 second. Approximately 10 minutes after the encoding trial, participants are presented with 30 of the original doors and 15 distracters (new pictures of doors, not presented before) that are randomly mixed. During this short-term recognition test, the task is to indicate whether the door had been presented before (oral response with 'yes') or not (oral response 'no'). For the long-term delayed recall, the same procedure is applied in the morning, using the other 90 original pictures, plus 45 new distracters. Calculating the hits and false alarms, we compute the sensitivity (A′) for short-term and for long-term delayed recall ($A' = 0.5 + ((\text{hit-rate} - \text{false alarm rate}) \times (1 + \text{hit rate} - \text{false alarm rate}))/ (4 \times \text{hit rate} \times (1 - \text{false alarm rate}))$).

Figure 2: Memory Consolidation Task Design



A) Example of Encoding trials – 120 doors separated by fixation cross, B) Example of short-term recognition trials (after approx. 10 min) – oral response (pressing spacebar to move to next stimulus), 30 targets and 15 distracters, C) Example of long-term recognition trials (after one night) - oral response (pressing spacebar to move to next stimulus), 90 targets and 45 distracters

2.5. Amyloid PET-CT scan with co-registered magnetic resonance imaging (MRI)

Brain amyloid PET-CT scan will be performed to measure amyloid load. Participants will be scanned at the Radboudumc department of Radiology and Nuclear medicine on a PET scanner (Biograph mCT, Siemens, Erlangen, Germany). Subjects will receive an intravenous bolus of the well-validated PET tracer ^{18}F-flutemetamol, and static brain images will be acquired from 90-110 min post-injection

(frames of 5 mins), as recommended (see SPC; http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002557/WC500172950.pdf). The individual reconstructed PET images will be co-registered with individual structural T_1 MRI scans.

The PET scans will be rated visually as positive or negative for the presence of amyloid depositions typical of AD, as earlier described (23). For quantitative purposes, grey matter volumes of interest will be defined on the individual MRIs (e.g. frontal brain areas, the precuneus, and hippocampus) as well as for cerebella grey matter (to assess non-specific uptake). The amyloid burden will be quantified using the standardized uptake value ratio, since it has been validated that this analysis method has comparable agreement with full kinetic modeling (24). Brain MRI will be performed on a 3 Tesla system (Magnetom Trio, Siemens, Erlangen, Germany). The structural T_1-weighted images will be used for co-registration purposes, and to define grey matter in the volumes of interest. In addition, these scans will be used to perform volumetric measurements (e.g. of the hippocampus). Also, arterial spin labeling (ASL) will be performed to measure global and regional cerebral blood flow (CBF), since reduced regional CBF is an early marker of AD.

2.6. Future follow-up visits

Because of the insufficient knowledge about the correlation between amyloid accumulation in the brain and the actual development of Alzheimer's disease, we decided to follow the maritime pilots in a 5 year cycle in order to monitor any cognitive changes. We will contact them 3 times in total, which leaves us with a maximum follow-up period of 15 years. At each time point, they will be asked to answer 3 questions online:

1) Did you develop cognitive complaints over the last 5 years?

2) Were you diagnosed with mild cognitive impairment during the last 5 years? If yes, when and what was the precise diagnosis?

3) Were you diagnosed with dementia in the past 5 years? If yes, when and what was the precise diagnosis?

If the answer is 'Yes' to one of these questions, we will reach out to the participants for clarification.

2.7. Statistical analysis

All analyses will be performed using IBM SPSS version 20.0 (SPSS Inc, Chicago, USA). Statistical significance is set at $p < 0.05$, with Bonferroni correction for multiple comparisons when appropriate, combined with reports of effect size and 95% confidence intervals. All continuous variables will be assessed for normal distribution by inspection of histograms and the Kolmogorov-Smirnov test. Levene's test will be used to assess equality of variances. We will perform an independent samples t-test to compare all outcome parameters. The primary outcome for cognitive function will be the score on each test respectively adjusted for age and education. Regarding imaging, the primary outcome measure will be the visual read of the amyloid PET scans (positive or negative PET). Secondary outcome measurements will be quantitative PET (SUV ratios), brain volume (MRI), and CBF measurements (MRI-ASL).

3. ETHICS AND DISSEMINATION

The study protocol was approved by our institutional review board, CMO (Commissie Mensgebonden Onderzoek) (NL55712.091.16, file number 2016-2337) and performed according to good clinical practice (GCP) rules. Written informed consent will be obtained from all participants. We are planning to publish the data and results of the SCHIP study in two or three articles in 2019 and 2020.

4. DISCUSSION

In this article we presented the design and methodology of the SCHIP study. The main aim of the SCHIP study is to investigate the effect of chronic sleep deprivation on cognition and amyloid accumulation in a group of maritime pilots. Since previous studies assessed sleep disruption for only one or a few days, the SCHIP study extends these studies by investigating the effect of long term sleep disturbances on cognitive function and amyloid accumulation. We expect that the maritime pilots have had long-term (>10 years) exposure to fragmented sleep due to their work schedules and that this long-term poor sleep has led to reduction in SWS. Every slow wave observed on an electro-encephalography (EEG) is a pause in synaptic activity (25). Synaptic activity and Aβ levels appear

strongly related, as Aβ levels increase due to synaptic activity (26). More synaptic activity is observed during wakefulness, especially in the default mode network (DMN) or other highly interconnected brain areas (26). During sleep, synaptic activity is reduced, which could result in a decrease in Aβ levels in the brain (26). Therefore, poor sleep (especially poor SWS) over the course of many years could increase Aβ concentrations, which in turn could trigger AD-associated neurodegeneration and loss of cognitive function.

To test this hypothesis, we will perform extensive cognitive testing, using tests that are sensitive to subtle decline in episodic memory, which is affected early in AD. Additionally, amyloid positivity will be measured using amyloid PET-CT scans in order to explore the effect of long-term poor sleep on amyloid concentration in the brain. Cerebral blood flow and cerebrovascular resistance will be investigated, as reductions in blood flow and increases in resistance occur early in the Alzheimer disease process (27). Finally, global grey matter volume and hippocampal volume will be measured. The unique cohort of maritime pilots will allow the prospective assessment of long-term sleep disturbances and its consequences for cognitive functioning and amyloid accumulation. The design of the SCHIP study presents a number of strengths. No other previous investigation has looked into the effect of chronic sleep disturbances on cognition in healthy men to this extent. Since sleep disorders might also be early symptoms of preclinical AD, it is especially important in this age group to investigate the effect of chronic sleep disruption due to an external factor and not due to intrinsic sleep disorders. Furthermore, we measure different domains of cognitive function (reaction time, visual memory, executive function, semantic memory and episodic memory) using sensitive and well-validated neuropsychological tasks. In addition to testing cognitive functions, we will perform brain imaging to detect amyloid accumulation as potential consequence of chronic sleep deprivation. We will make use of four different instruments to get a comprehensive measure of sleeping patterns, two subjective and two objective ones. The subjective measurements consist of the PSQI and the maintenance of the sleep-wake diary. The objective assessments include the data from the Actiwatch 2 (Philips Respironics) in addition to a night of PSG. The data from the Actiwatch can be compared to the sleep-wake diary entries. These data can then be used to verify the sleeping behavior of participants, making sure they maintain regularity and consistency in their sleeping habits.

Results of the SCHIP study will give us more insights into the consequences of long term disrupted sleep on cognitive function and amyloid accumulation in an AD related context. Our results could give rise to new treatment opportunities, that aim at sleep improvement and management in order to prevent or reduce amyloid accumulation and in turn delay or even prevent the development of AD.

Figure 1: Overview experimental design

Figure 2: Memory Consolidation Task Design

A) Example of Encoding trials – 120 doors separated by fixation cross, B) Example of short-term recognition trials (after approx. 10 min) – oral response (pressing spacebar to move to next stimulus), 30 targets and 15 distracters, C) Example of long-term recognition trials (after one night) - oral response (pressing spacebar to move to next stimulus), 90 targets and 45 distracters

5. REFERENCES

1. Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP. The global prevalence of dementia: a systematic review and metaanalysis. Alzheimers Dement. 2013;9(1):63-75. e2.
2. Jagust WJ, Mormino EC. Lifespan brain activity, β-amyloid, and Alzheimer's disease. Trends Cogn Sci. 2011;15(11):520-6.
3. Korczyn AD. The amyloid cascade hypothesis. Alzheimers Dement. 2008;4(3):176-8.
4. Vitiello MV, Borson S. Sleep disturbances in patients with Alzheimer's disease. CNS Drugs. 2001;15(10):777-96.
5. Ju Y-ES, McLeland JS, Toedebusch CD, Xiong C, Fagan AM, Duntley SP, et al. Sleep quality and preclinical Alzheimer disease. JAMA neurology. 2013;70(5):587-93.
6. Ju Y-ES, Lucey BP, Holtzman DM. Sleep and Alzheimer disease pathology [mdash] a bidirectional relationship. Nature reviews Neurology. 2014;10(2):115-9.
7. Osorio RS, Pirraglia E, Agüera-Ortiz LF, During EH, Sacks H, Ayappa I, et al. Greater risk of Alzheimer's disease in older adults with insomnia. J Am Geriatr Soc. 2011;59(3):559-62.
8. Tranah GJ, Blackwell T, Stone KL, Ancoli-Israel S, Paudel ML, Ensrud KE, et al. Circadian activity rhythms and risk of incident dementia and mild cognitive impairment in older women. Ann Neurol. 2011;70(5):722-32.
9. Yaffe K, Laffan AM, Harrison SL, Redline S, Spira AP, Ensrud KE, et al. Sleep-disordered breathing, hypoxia, and risk of mild cognitive impairment and dementia in older women. Jama. 2011;306(6):613-9.
10. Benedict C, Byberg L, Cedernaes J, Hogenkamp PS, Giedratis V, Kilander L, et al. Self-reported sleep disturbance is associated with Alzheimer's disease risk in men. Alzheimers Dement. 2015;11(9):1090-7.
11. Sterniczuk R, Theou O, Rusak B, Rockwood K. Sleep disturbance is associated with incident dementia and mortality. Curr Alzheimer Res. 2013;10(7):767-75.
12. Hahn EA, Wang H-X, Andel R, Fratiglioni L. A change in sleep pattern may predict Alzheimer disease. The American Journal of Geriatric Psychiatry. 2014;22(11):1262-71.
13. Potvin O, Lorrain D, Forget H, Dube M, Grenier S, Preville M, et al. Sleep quality and 1-year incident cognitive impairment in community-dwelling older adults. Sleep. 2012;35(4):491-9.
14. Bubu OM, Brannick M, Mortimer J, Umasabor-Bubu O, Sebastião YV, Wen Y, et al. Sleep, cognitive impairment, and Alzheimer's disease: a systematic review and meta-analysis. Sleep. 2016;40(1):zsw032.
15. Kang J-E, Lim MM, Bateman RJ, Lee JJ, Smyth LP, Cirrito JR, et al. Amyloid-β dynamics are regulated by orexin and the sleep-wake cycle. Science (80-). 2009;326(5955):1005-7.
16. Ooms S, Overeem S, Besse K, Rikkert MO, Verbeek M, Claassen JA. Effect of 1 night of total sleep deprivation on cerebrospinal fluid β-amyloid 42 in healthy middle-aged men: a randomized clinical trial. JAMA neurology. 2014;71(8):971-7.
17. Faul F, Erdfelder E, Lang A-G, Buchner A. G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007;39(2):175-91.
18. Zimmermann P, Fimm B. A test battery for attentional performance. Applied neuropsychology of attention Theory, diagnosis and rehabilitation. 2002:110-51.
19. Shokri-Kojori E, Wang G-J, Wiers CE, Demiral SB, Guo M, Kim SW, et al. β-Amyloid accumulation in the human brain after one night of sleep deprivation. Proceedings of the National Academy of Sciences. 2018;115(17):4483-8.
20. Bigler ED. Symptom validity testing, effort, and neuropsychological assessment. J Int Neuropsychol Soc. 2012;18(4):632-40.
21. Baddeley A, Emslie H, Nimmo-Smith I. Doors and People: A Test of Visual and Verbal Recall and Recognition, Thames Valley Test Company, Bury St. Edmunds, UK. 1994.
22. Baddeley AD, Hitch GJ, Quinlan PT, Bowes L, Stone R. Doors for memory: A searchable database. The Quarterly Journal of Experimental Psychology. 2016;69(11):2111-8.

23. Frings L, Hellwig S, Bormann T, Spehl TS, Buchert R, Meyer PT. Amyloid load but not regional glucose metabolism predicts conversion to Alzheimer's dementia in a memory clinic population. Eur J Nucl Med Mol Imaging. 2018;45(8):1442-8.
24. Cecchin D, Barthel H, Poggiali D, Cagnin A, Tiepolt S, Zucchetta P, et al. A new integrated dual time-point amyloid PET/MRI data analysis method. Eur J Nucl Med Mol Imaging. 2017;44(12):2060-72.
25. Nir Y, Staba RJ, Andrillon T, Vyazovskiy VV, Cirelli C, Fried I, et al. Regional slow waves and spindles in human sleep. Neuron. 2011;70(1):153-69.
26. Kang D, Lim HK, Jung WS, Jeong J-H, Kim TW, Han J-H, et al. Sleep and Alzheimer's Disease. Sleep Medicine Research. 2015;6(1):1-9.
27. de Heus RA, de Jong DL, Sanders ML, van Spijker GJ, Oudegeest-Sander MH, Hopman MT, et al. Dynamic regulation of cerebral blood flow in patients with Alzheimer disease. Hypertension. 2018;72(1):139-50.

6. AUTHORS' CONTRIBUTION

Jana Thomas was involved in setting up the study, recruiting participants, gathering baseline characteristics, analyzing of first data and writing most of the sections in this manuscript.

Sharon Ooms helped with setting up the study, recruitment and design of the project and writing the manuscript.

Marcel Verbeek contributed to the design of the study.

Jan Booij and Mark Rijpkema were major contributors in choosing and designing the right PET-CT/MRI procedure and wrote part of the manuscript.

Roy Kessels contributed to selecting the right neuropsychological tests, helped with the statistical analyzes of the first data and contributed to the revision of this manuscript.

Sebastiaan Overeem was a major contributor in setting up a collaboration with the sleeping center Kempenhaeghe (Heeze, The Netherlands) and helped revising the manuscript.

Jurgen Claassen was a major contributor in obtaining funding, setting up the study, designing the project and was extensively involved in writing and revising the manuscript.

All authors read and approved the final manuscript.

7. FUNDING STATEMENT

The SCHIP study is funded by ISAO (Internationale Stichting Alzheimer Onderzoek) (grant number 15040). Additional financial resources were provided by the Radboud University Medical Center (Nijmegen, The Netherlands).

8. COMPETING INTEREST STATEMENT

The authors declare that they have no competing interest.

Figure 1: Overview experimental design

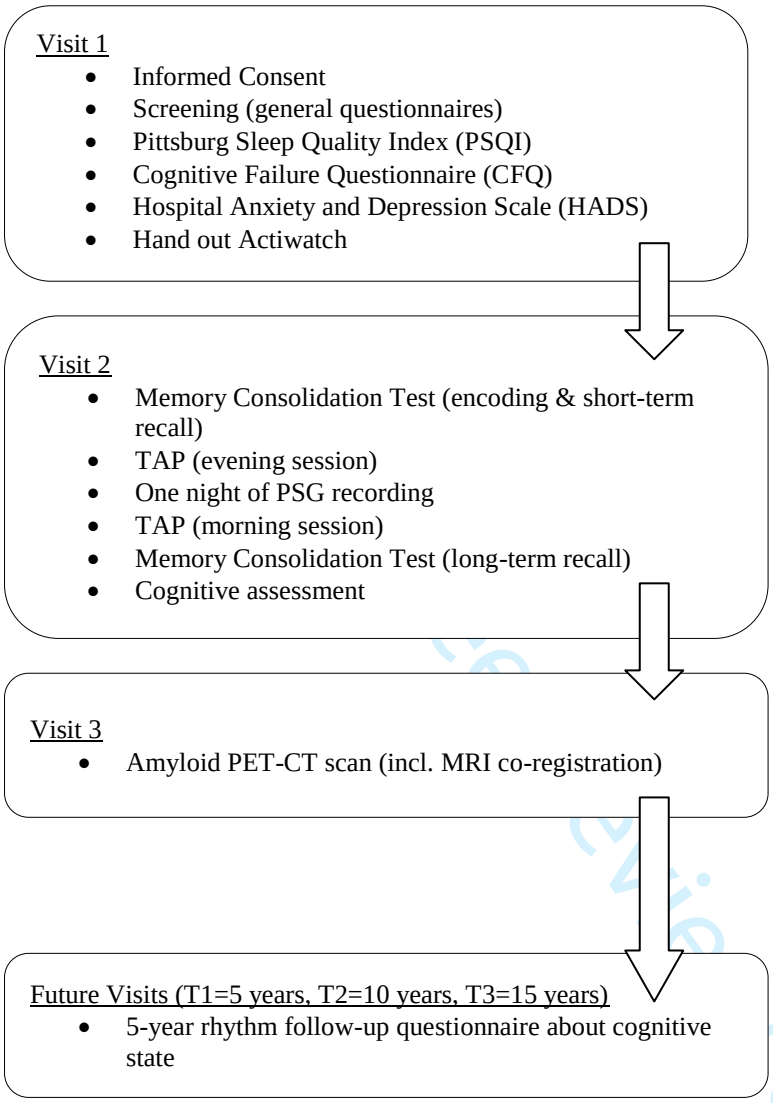
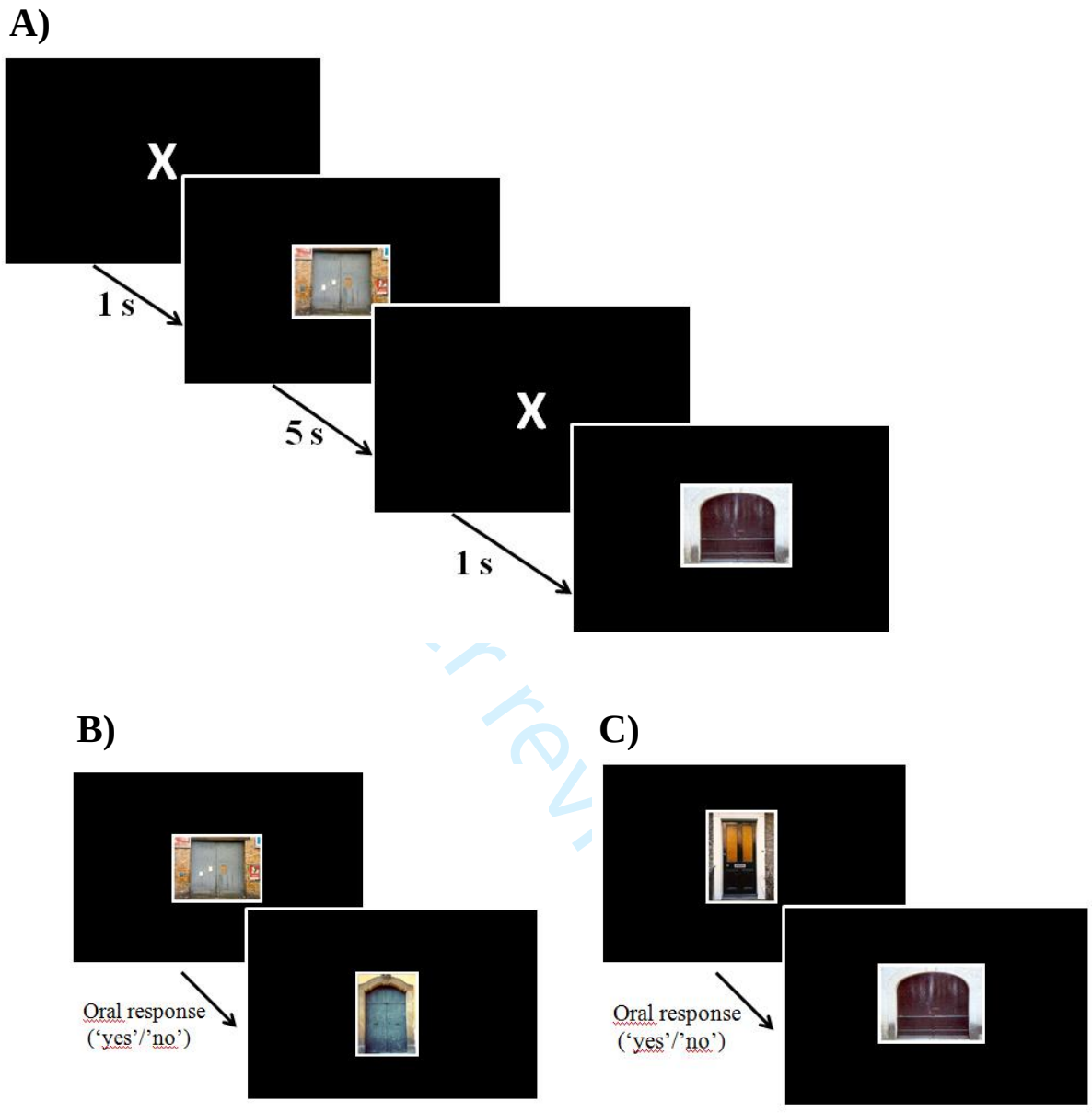


Figure 2: Memory Consolidation Task Design



A) Example of Encoding trials – 120 doors separated by fixation cross, B) Example of short-term recognition trials (after approx. 10 min) – oral response (pressing spacebar to move to next stimulus), 30 targets and 15 distracters, C) Example of long-term recognition trials (after one night) - oral response (pressing spacebar to move to next stimulus), 90 targets and 45 distracters

BMJ Open

Sleep-Cognition-Hypothesis In maritime Pilots - What is the effect of long-term work-related poor sleep on cognition and amyloid accumulation in healthy middle-aged maritime pilots: Methodology of a case-control study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-026992.R1
Article Type:	Protocol
Date Submitted by the Author:	10-Apr-2019
Complete List of Authors:	Thomas, Jana; Radboudumc, Geriatrics; Radboud Alzheimer Center Ooms, Sharon; Radboudumc, Geriatrics; Radboud Alzheimer Center Verbeek, Marcel; Radboudumc; Radboud Alzheimer Center Booij, Jan; Academic Medical Center, Department of Nuclear Medicine Rijpkema, Mark; Radboudumc, Radiology and nuclear medicine Kessels, Roy; Donders Institute for Brain, Cognition and Behaviour, Radboud university medical center, Medical Psychology; Vincent van Gogh Institute for Psychiatry Overeem, Sebastiaan; Eindhoven Univeristy of Technology, Eindhoven Medtech Innovation Center; Sleep Medicine Center Kempenhaeghe Claassen, Jurgen; Radboudumc, Geriatrics; Radboud Alzheimer Center
Primary Subject Heading:	Geriatric medicine
Secondary Subject Heading:	Neurology, Radiology and imaging, Public health
Keywords:	Alzheimer's disease, Amyloid accumulation, Neurodegeneration, Cognitive function, Sleep

Sleep-Cognition-Hypothesis In maritime Pilots – What is the effect of long-term work-related poor sleep on cognition and amyloid accumulation in healthy middle-aged maritime pilots: Methodology of a case-control study

Jana Thomas, MSc*[1,2,3]; Sharon Ooms, MSc*[1,2,3]; Marcel M. Verbeek, PhD[2,3,6]; Jan Booij, MD, PhD[7,8], Mark Rijpkema, PhD[7]; Roy P.C. Kessels, PhD[2,3,9]; Sebastiaan Overeem, MD, PhD[4,5] and Jurgen A.H.R. Claassen, MD, PhD[1,2,3]

(1) Department of Geriatric Medicine, Radboud University Medical Centre, Nijmegen, The Netherlands; (2) Radboudumc Alzheimer Centre, Nijmegen, The Netherlands; (3) Donders Institute for Brain, Cognition and Behavior, Nijmegen, The Netherlands; (4) Sleep Medicine Centre Kempenhaeghe, Heeze, The Netherlands; (5) Eindhoven Medtech Innovation Center, Eindhoven University of Technology, Eindhoven, The Netherlands; (6) Department of Neurology, Radboud University Medical Centre, Nijmegen, The Netherlands; (7) Department of Radiology and Nuclear Medicine, Radboud University Medical Center, Nijmegen, The Netherlands; (8) Department of Radiology and Nuclear Medicine, Amsterdam University Medical Center, Amsterdam, The Netherlands; (9) Department of Medical Psychology, Radboud University Medical Centre, Nijmegen, The Netherlands

**authors contributed equally to this work*

Corresponding author:
Jana Thomas
Jana.thomas@radboudumc.nl
Reinier Postlaan 4
6526 GC Nijmegen
The Netherlands

Word count: 3.539 words (without abstract, figures, references)

ABSTRACT

Introduction: Evidence indicates a bi-directional relationship between poor sleep and Alzheimer's disease (AD). While AD may lead to disruption of normal sleep, poor sleep in itself may play a causal role in the development of AD by influencing the production and/or clearance of the amyloid-beta (Aβ) protein. This led to the hypothesis that extended periods (>10 years) of partial sleep deprivation could lead to Aβ accumulation with subsequent cognitive AD related decline. This manuscript describes the methodology of the SCHIP study, a cohort study in maritime pilots that aims at investigating the relationship between chronic partial sleep deprivation due to an extrinsic cause (work related), cognitive function and amyloid accumulation among healthy middle-aged maritime pilots, to test the hypothesis that prolonged partial sleep deprivation increases the risk of AD-related cognitive decline.

Methods: Our study sample consists of a group of healthy middle-aged maritime pilots (n=20), who have had irregular sleeping patterns for more than 15 years due to irregular work schedules. This chronic partial sleep deprivation group will be compared to a group of healthy, age and education matched controls (n=20) with normal sleep. Participants will complete 10 days of actigraphy (Actiwatch 2, Philips Respironics) combined with a sleep-wake diary. They will undergo one night of polysomnography (PSG), followed by comprehensive assessment of cognitive function. Additionally, participants will undergo amyloid PET-CT (Positron Emission Tomography- Computed Tomography) to measure brain amyloid accumulation and MRI to investigate atrophy and vascular changes.

Analysis: All analyses will be performed using IBM SPSS version 20.0 (SPSS Inc, Chicago, USA). We will perform independent samples t-tests to compare all outcome parameters.

Ethics and dissemination: The study protocol was approved by our institutional ethical review board (NL55712.091.16, file number 2016-2337) and will be performed according to good clinical practice (GCP) rules. Data and results will be published in 2020.

Key words: Alzheimer's disease, amyloid accumulation, neurodegeneration, cognitive function, sleep, sleep deprivation, shift work

ARTICLE SUMMARY

Strengths and limitations of the study

- the unique cohort of maritime pilots allows the prospective assessment of long-term externally caused partial sleep deprivation and their potential consequences for cognitive functioning and amyloid accumulation
- the unique cohort might also be a limitation to the study, because they might not be comparable to the control group to some extent (e.g. IQ, work environment, personality)
- in addition to imaging techniques (PET-CT and MRI), we use sensitive and well-validated neuropsychological tests to measure different domains of cognitive function (reaction time, visual memory, executive function, semantic memory and episodic memory)
- we will make use of four different instruments to get a comprehensive measure of sleeping patterns (two subjective and two objective ones)
- our results could give rise to new treatment opportunities, that aim at sleep improvement and management in order to prevent or reduce amyloid accumulation and in turn delay or even prevent the development of AD

1. INTRODUCTION

Alzheimer's disease (AD) is the most prevalent cause of dementia and currently affects approximately 36 million people worldwide (1). Thus far, no successful treatment is available. One of the major contributors to the neurodegeneration seen in the brains of AD patients is amyloid-beta (Aβ) (2). The amyloid-cascade hypothesis characterizes amyloid accumulation as the fundamental initiating pathway for the development of AD (3). The reason why amyloid accumulates however is not clear yet. Recent evidence suggests that poor sleep might be one of the risk factors for amyloid accumulation and thereby increases the risk of AD development (4). Elderly people suffering from insomnia are more likely to develop AD compared to controls without insomnia (4). Furthermore, disrupted circadian rhythm among otherwise healthy individuals (5) and sleep-disordered breathing disorders (6) increase the chance of developing AD later in life. Poor sleep quality specifically has been shown to increase the risk of AD among older individuals (7-11). A recent meta-analysis of epidemiological studies found that poor sleep increased AD risk and that approximately 15% of cases of AD in the population might be attributable to sleep problems (12).

The effect of acute sleep deprivation on AD has been shown in both rodent and human studies, that investigated the effect of sleep and sleep deprivation on Aβ levels. Rodents and humans show fluctuations in Aβ levels over a 24-hour rhythm, where levels rise during wakefulness and decrease during sleep (13, 14). Following acute sleep deprivation, the drop in Aβ levels that would normally occur after sufficient sleep the following morning, was absent. Based on this evidence, chronic sleep disturbances might lead to a pronounced accumulation of Aβ in the brain, which in turn increases the risk to develop AD.

To date, observational and epidemiological studies have identified a relationship between poor sleep and the risk of developing AD. Experimental research has found that sleep deprivation and Aβ levels are related in both animals and humans. However, the direct effect of chronic partial sleep deprivation and its consequences on cognitive functioning, particularly the risk to develop AD later in life, has not been studied before.

In this article we describe the methodology and the participant cohort of the SCHIP (Sleep-Cognition Hypothesis In maritime Pilots) study. We designed the SCHIP study in order to investigate the effect

of chronic partial sleep deprivation on cognitive function, structural brain changes, and amyloid accumulation by using a unique cohort of healthy subjects with long-term partial sleep deprivation due to their occupation as maritime pilots. Because of the bi-directional nature of the relationship between sleep and AD, in which poor sleep is a symptom of the disease that precedes the clinical manifestation of AD, but might also be a risk factor that potentially contributes to the development of the disease, it is especially important to investigate the effect of chronic sleep deprivation in a group that experiences disrupted sleep, which is caused by an external factor (work) and not by an intrinsic sleeping disorder (such as insomnia) which could be AD-related. Therefore participants in this study were selected based on their chronic partial sleep deprivation caused by objectifiable external occupational factors Results and insights born from the SCHIP study could shed more light on sleep disturbance as one of the risk factors to develop AD and contribute to new and improved treatment strategies. In the SCHIP study we will investigate whether participants with chronic partial sleep deprivation perform worse on cognitive assessment in comparison to a healthy control group and whether chronic partial sleep deprivation is associated with elevated brain amyloid concentrations and structural brain changes.

2. METHODS AND ANALYSIS

2.1. Participants

Considering that this study is a proof of principle study, it is not possible to establish the exact effect size of the change in cognitive function we expect to see between the maritime pilots and the healthy controls.

To account for possible 10% withdrawal, we chose to have n=20 participants in each arm.

In order to reach enough power to detect clinically relevant differences regarding the results of the PET-CT scan, we performed a sample size power calculation using G Power (version 3.1.9.3) (15). Reported normal values (mean and standard deviation) for this age group in the literature vary between studies but are in the order of a mean standard uptake value (SUV) between 0.9 and 1.1 with standard deviations (SDs) in the range of 0.05 – 0.2. We define a relevant difference between maritime pilots and normal values to be 0.2 or more (an SUV of 1.3 or higher is considered as abnormal in most

studies). This results in a large expected effect size (>0.8). The precise number of age- and education matched subjects in the database is not known yet but is estimated to be at least 50 (between 50 and 100). We have applied a one-tailed test (it is not possible to have a SUV lower than normal values). With an alpha of 0.05, with n=20 our power is 0.95 or higher (depending on the number of normal subjects). The lower level for power (0.85) is reached with 13-15 pilots.

Based on these calculations, we will aim to recruit 20 pilots, allowing for drop-out of 5-7 pilots (either due to withdrawal of consent or artefacts in the PET-CT).

Participants will be recruited within the national organization of Dutch Maritime Pilots (Nederlandse Loodswezen). We selected the maritime pilots as a suitable study population because of their unique irregular working schedule. They are called to work depending on the number and kind of ships that arrive. In a typical 7-day work week they have to be available 24 hours per day during which they can be called several times. This results in multiple divided short sleep periods over 24 hours during a work week, this is followed by a week off.

The responsibility of a maritime pilot is to handle large international ships arriving by sea and to maneuver them into their final docking position in one of the Dutch harbors. This profession carries high responsibilities and requires accurate knowledge of the dimensions of the harbor and the ships besides technical knowledge and navigational skills. This results in irregular working schedules because guiding the ships is a time intensive procedure, that can take hours to complete. Maintaining this schedule for more than 15 years will result in prolonged exposure to sleep deprivation.

We will reach out to the whole maritime pilot community, in order to recruit pilots who are approximately 50 to 60 years old, with at least 15 years of uninterrupted work history as a maritime pilot.

Additional inclusion criteria are not using neuro-active medications or psycho-stimulants, consumption of <14 alcoholic beverages per week, a body mass index of 18-30kg/m^2 and no subjective cognitive complaints (Cognitive Failure Questionnaire (CFQ <43). As control group, we will recruit 20 age, sex and education matched healthy adults with normal sleep indicated by a score of >5 on a subjective sleep questionnaire (Pittsburgh Sleep Quality Index, PSQI). The study protocol was approved by our institutional review board (NL55712.091.16, file number 2016-2337) and will be

performed according to good clinical practice (GCP) rules. Inclusion of participants started in August 2018 and we expect to complete the analysis of all data in August 2020.

2.2. Experimental Design

The aim of the SCHIP study is to investigate the relationship between chronic partial sleep deprivation due to an extrinsic cause (work related), cognitive function and amyloid accumulation among healthy middle-aged men to test the hypothesis that prolonged partial sleep deprivation increases the risk of AD-related cognitive decline. In order to test this hypothesis, maritime pilots will have three visits and controls will have two visits (figure 1). During the first visit participants will complete general questionnaires about medical history, sleeping habits and cognitive state. Approximately 10 days after the first visit has taken place, participants are invited to the sleep center Kempenhaeghe (Heeze, The Netherlands) for the second visit. They will arrive at the sleep center at 19.00 h and complete a memory consolidation test (modified 'Doors Test') and a test for attentional performance (TAP 2.3) (16), followed by overnight polysomnography (PSG). Participants wake up at their normal wake time and complete a neuropsychological assessment after breakfast around 9.00h.

Visit three (only for the maritime pilots) will be performed at the Radboud university medical center in Nijmegen (The Netherlands), where participants will undergo a standard amyloid PET-CT to measure brain amyloid accumulation and a 3T-MRI for coregistration. Participants are scheduled in their week off in order to prevent short term effects of sleep disruption on amyloid concentrations in the brain (17). They are instructed not eat or drink anything except from water 3 hours prior to the scan.

Figure 1: Overview experimental design

2.3 Patient and Public Involvement

No patients were involved in this study because this study involves healthy participants.

However, the participants' organization contacted us due to recent evidence from the literature on the relationship between sleep and AD. They expressed their worries about their health and their own risk of developing AD considering their irregular sleep. They reported feeling very tired at the end of a

work week and speculated that within their group of maritime pilots (including already retired pilots), cases of dementia occurred more frequently than expected.

Participants were also tightly involved in the design, realization and feasibility of the study. For example, they were involved in choosing the technique to measure amyloid and expressed the preference for a PET-CT scan over cerebrospinal fluid (CSF) measurements of amyloid.

Dissemination of results to participants:

Results from the PSG measurements will be reported only to participants if we find abnormalities such as apnea or sleeping disorders. This will be done by one of our sleep clinicians via telephone. Results from the PET-CT scan will be disclosed via telephone by one of our clinicians as well. This is according to the protocol that has been approved by the local ethical committee. Any incidental findings on the PET-CT and/or MRI will be disseminated as well.

2.4. Sleep Measurements

In order to obtain a comprehensive measure of sleeping patterns, we will use the Pittsburg Sleep Quality Index (PSQI) with questions about average sleeping behavior, including the report of bedtime, get-up time, sleep latency, total sleep time, sleep disturbances (pain, breathing etc.) and use of sleep medication. The maritime pilots will be instructed to fill in the questionnaire twice, once with regard to a work week and once with regard to a week off. Additionally all participants are asked to maintain a sleep-wake diary on a daily basis. In this diary they have to keep track of their bed times, the time it took to fall asleep (sleep latency), the number of awakenings and their get up times. Furthermore they will receive an accelerometer, the Actiwatch 2 (Philips Respironics; Eindhoven, The Netherlands), in order to obtain more objective measurements of sleeping behavior. The Actiwatch is worn around the wrist and measures total sleep time and number of awakenings during sleep automatically based on movement. Participants are instructed to fill in the sleep diary and to wear the Actiwatch for 10 days preceding the second visit.

2.5. Cognitive assessment

The neuropsychological assessment has been designed to measure the following cognitive domains, using validated Dutch versions of widely used neuropsychological tests. Episodic memory is assessed using the WMS-IV Logical Memory and the Rey-Auditory Verbal Learning Test (RAVLT). Working memory and executive function are measured by WAIS-IV Digit Span, Trail Making Test (TMT-A, TMT-B) and WAIS-IV Digit Symbol test. Semantic memory and language are assessed by letter fluency (D-A-T), semantic fluency (animal/profession naming) and the Boston Naming Test-Short Form (BNT) (18). Attentional performance is studied using the alertness test of the Test of Attentional Performance (TAP 2.3) (16). This test is conducted twice, once in the evening around 19.00 h and once in the morning around 9.00 h. In order to test overnight memory consolidation, a novel paradigm was developed based on the Doors Test, a visual recognition task developed by Baddely and colleagues (19) and extended using a validated database of 2000 pictures of doors (20). During the encoding trial, we present 120 pictures of doors that participants are instructed to remember. All targets (doors) are presented twice in a different, pseudo-randomized order. Targets are shown for 5 seconds each, separated by a fixation cross presented for 1 second (figure 2). Approximately 10 minutes after the encoding trial, participants are presented with 30 of the original doors and 15 distracters (new pictures of doors, not presented before) that are randomly mixed. During this short-term recognition test, the task is to indicate whether the door had been presented before (oral response with 'yes') or not (oral response 'no'). For the long-term delayed recall, the same procedure is applied in the morning, using the other 90 original pictures, plus 45 new distracters (figure 2). Calculating the hits and false alarms, we compute the sensitivity (A′) for short-term and for long-term delayed recall (A′= 0.5 + ((hit-rate – false alarm rate) × (1 + hit rate – false alarm rate))/ (4 × hit rate × (1 – false alarm rate)).

Figure 2: Memory Consolidation Task Design

A) Example of Encoding trials – 120 doors separated by fixation cross, B) Example of short-term recognition trials (after approx. 10 min) – oral response (pressing spacebar to move to next stimulus), 30 targets and 15 distracters, C) Example of long-term recognition trials (after one night) - oral response (pressing spacebar to move to next stimulus), 90 targets and 45 distracters

2.6. Amyloid PET-CT scan with co-registered magnetic resonance imaging (MRI)

Brain amyloid PET-CT scan will be performed to measure amyloid load. Participants will be scanned at the Radboudumc department of Radiology and Nuclear medicine on a PET scanner (Biograph mCT, Siemens, Erlangen, Germany). Subjects will receive an intravenous bolus of the well-validated PET tracer ^{18}F-flutemetamol, and static brain images will be acquired from 90-110 min post-injection (frames of 5 mins), as recommended (see SPC; http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002557/WC500172950.pdf). The individual reconstructed PET images will be co-registered with individual structural T_1 MRI scans.

The PET scans will be rated visually as positive or negative by an experienced nuclear medicine physician for the presence of amyloid depositions typical of AD. Scores will be expressed as a global SUV, which will be compared against population normative values, as earlier described (21). For quantitative purposes, grey matter volumes of interest will be defined on the individual MRIs (e.g. frontal brain areas, the precuneus, and hippocampus) as well as for cerebella grey matter (to assess non-specific uptake). The amyloid burden will be quantified using the standardized uptake value ratio, since it has been validated that this analysis method has comparable agreement with full kinetic modeling (22). Brain MRI will be performed on a 3 Tesla system (Magnetom Trio, Siemens, Erlangen, Germany). The structural T_1-weighted images will be used for co-registration purposes, and to define grey matter in the volumes of interest. In addition, these scans will be used to perform volumetric measurements (e.g. of the hippocampus). Also, arterial spin labeling (ASL) will be performed to measure global and regional cerebral blood flow (CBF), since reduced regional CBF is an early marker of AD. Individual anatomic MRI scans will be co-registered with the individual PET scans using the image processing platform FLS (fsl.fmrib.ox.ac.uk) to calculate standard uptake value ratios. The PET-CT data will be expressed in Centiloid units to evaluate our data with historical control data as was recently validated by Battle and co-workers (23).

2.7. Future follow-up visits

Because of the insufficient knowledge about the correlation between amyloid accumulation in the brain and the actual development of Alzheimer's disease, we decided to follow the maritime pilots in a 5 year cycle in order to monitor any cognitive changes. We will contact them 3 times in total, which leaves us with a maximum follow-up period of 15 years. At each time point, they will be asked to answer 3 questions online:

1) Did you develop cognitive complaints over the last 5 years? This question will be further elaborated on with the Cognitive Failure Questionnaire (CFQ). The CFQ is a validated questionnaire that aims at detecting daily disruptions of cognitive functions. Participants are confronted with 25 statements and have to indicate how often they experience the situation that is described in the statements with a score between 0 (never) and 4 (very often).

2) Were you diagnosed with mild cognitive impairment during the last 5 years? If yes, when and what was the precise diagnosis?

3) Were you diagnosed with dementia in the past 5 years? If yes, when and what was the precise diagnosis?

If the answer is 'Yes' to one of these questions, we will reach out to the participants for clarification.

2.8. Statistical analysis

All analyses will be performed using IBM SPSS version 20.0 (SPSS Inc, Chicago, USA). Statistical significance is set at $p < 0.05$, with Bonferroni correction for multiple comparisons when appropriate, combined with reports of effect size and 95% confidence intervals. All continuous variables will be assessed for normal distribution by inspection of histograms and the Kolmogorov-Smirnov test. Levene's test will be used to assess equality of variances. We will perform an independent samples t-test to compare all outcome parameters. The primary outcome for cognitive function will be the score on each test respectively adjusted for age and education. Regarding imaging, the primary outcome measure will be the visual read of the amyloid PET scans (positive or negative PET). Secondary outcome measurements will be quantitative PET (SUV ratios), brain volume (MRI), and CBF measurements (MRI-ASL).

3. ETHICS AND DISSEMINATION

The study protocol was approved by our institutional review board, CMO (Commissie Mensgebonden Onderzoek) (NL55712.091.16, file number 2016-2337) and performed according to good clinical practice (GCP) rules. Written informed consent will be obtained from all participants. We are planning to publish the data and results of the SCHIP study in two or three articles in 2019 and 2020.

4. DISCUSSION

In this article we presented the design and methodology of the SCHIP study. The main aim of the SCHIP study is to investigate the effect of chronic partial sleep deprivation on cognition and amyloid accumulation in a group of maritime pilots. Since previous studies assessed sleep deprivation for only one or a few days, the SCHIP study extends these studies by investigating the effect of long term partial sleep deprivation on cognitive function and amyloid accumulation. We expect that the maritime pilots have had long-term (>10 years) exposure to fragmented sleep due to their work schedules and that this long-term partial sleep deprivation has led to reduction in SWS. Every slow wave observed on an electro-encephalography (EEG) is a pause in synaptic activity (24). Synaptic activity and Aβ levels appear strongly related, as Aβ levels increase due to synaptic activity (25). More synaptic activity is observed during wakefulness, especially in the default mode network (DMN) or other highly interconnected brain areas (25). During sleep, synaptic activity is reduced, which could result in a decrease in Aβ levels in the brain (25). Therefore, partial sleep deprivation (especially poor SWS) over the course of many years could increase Aβ concentrations, which in turn could trigger AD-associated neurodegeneration and loss of cognitive function.

To test this hypothesis, we will perform extensive cognitive testing, using tests that are sensitive to subtle decline in episodic memory, which is affected early in AD. Additionally, amyloid positivity will be measured using amyloid PET-CT scans in order to explore the effect of long-term partial sleep deprivation on amyloid concentration in the brain. Cerebral blood flow and cerebrovascular resistance will be investigated, as reductions in blood flow and increases in resistance occur early in the

Alzheimer disease process (26). Finally, global grey matter volume and hippocampal volume will be measured.

The unique cohort of maritime pilots will allow the prospective assessment of long-term partial sleep deprivation and its consequences for cognitive functioning and amyloid accumulation. The design of the SCHIP study presents a number of strengths. No other previous investigation has looked into the effect of chronic partial sleep deprivation on cognition in healthy men to this extent. Since sleep disorders might also be early symptoms of preclinical AD, it is especially important in this age group to investigate the effect of chronic sleep disruption due to an external factor and not due to intrinsic sleep disorders. All enrolled maritime pilots did not have any sleeping disorders and did not use sleep medication as confirmed by a general health questionnaire which was filled in upon screening for participation. Furthermore, we measure different domains of cognitive function (reaction time, visual memory, executive function, semantic memory and episodic memory) using sensitive and well-validated neuropsychological tasks. In addition to testing cognitive functions, we will perform brain imaging to detect amyloid accumulation as potential consequence of chronic sleep deprivation. We will make use of four different instruments to get a comprehensive measure of sleeping patterns, two subjective and two objective ones. The subjective measurements consist of the PSQI and the maintenance of the sleep-wake diary. The objective assessments include the data from the Actiwatch 2 (Philips Respironics) in addition to a night of PSG. The data from the Actiwatch can be compared to the sleep-wake diary entries. These data can then be used to verify the sleeping behavior of participants, making sure they maintain regularity and consistency in their sleeping habits.

Results of the SCHIP study will give us more insights into the consequences of long term disrupted sleep on cognitive function and amyloid accumulation in an AD related context. Our results could give rise to new treatment opportunities, that aim at sleep improvement and management in order to prevent or reduce amyloid accumulation and in turn delay or even prevent the development of AD.

5. REFERENCES

1. Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP. The global prevalence of dementia: a systematic review and metaanalysis. Alzheimers Dement. 2013;9(1):63-75. e2.
2. Jagust WJ, Mormino EC. Lifespan brain activity, β-amyloid, and Alzheimer's disease. Trends Cogn Sci. 2011;15(11):520-6.
3. Korczyn AD. The amyloid cascade hypothesis. Alzheimers Dement. 2008;4(3):176-8.
4. Osorio RS, Pirraglia E, Agüera-Ortiz LF, During EH, Sacks H, Ayappa I, et al. Greater risk of Alzheimer's disease in older adults with insomnia. J Am Geriatr Soc. 2011;59(3):559-62.
5. Tranah GJ, Blackwell T, Stone KL, Ancoli-Israel S, Paudel ML, Ensrud KE, et al. Circadian activity rhythms and risk of incident dementia and mild cognitive impairment in older women. Ann Neurol. 2011;70(5):722-32.
6. Yaffe K, Laffan AM, Harrison SL, Redline S, Spira AP, Ensrud KE, et al. Sleep-disordered breathing, hypoxia, and risk of mild cognitive impairment and dementia in older women. Jama. 2011;306(6):613-9.
7. Benedict C, Byberg L, Cedernaes J, Hogenkamp PS, Giedratis V, Kilander L, et al. Self-reported sleep disturbance is associated with Alzheimer's disease risk in men. Alzheimers Dement. 2015;11(9):1090-7.
8. Sterniczuk R, Theou O, Rusak B, Rockwood K. Sleep disturbance is associated with incident dementia and mortality. Curr Alzheimer Res. 2013;10(7):767-75.
9. Hahn EA, Wang H-X, Andel R, Fratiglioni L. A change in sleep pattern may predict Alzheimer disease. The American Journal of Geriatric Psychiatry. 2014;22(11):1262-71.
10. Potvin O, Lorrain D, Forget H, Dube M, Grenier S, Preville M, et al. Sleep quality and 1-year incident cognitive impairment in community-dwelling older adults. Sleep. 2012;35(4):491-9.
11. Ju Y-ES, McLeland JS, Toedebusch CD, Xiong C, Fagan AM, Duntley SP, et al. Sleep quality and preclinical Alzheimer disease. JAMA neurology. 2013;70(5):587-93.
12. Bubu OM, Brannick M, Mortimer J, Umasabor-Bubu O, Sebastião YV, Wen Y, et al. Sleep, cognitive impairment, and Alzheimer's disease: a systematic review and meta-analysis. Sleep. 2016;40(1):zsw032.
13. Kang J-E, Lim MM, Bateman RJ, Lee JJ, Smyth LP, Cirrito JR, et al. Amyloid-β dynamics are regulated by orexin and the sleep-wake cycle. Science (80-). 2009;326(5955):1005-7.
14. Ooms S, Overeem S, Besse K, Rikkert MO, Verbeek M, Claassen JA. Effect of 1 night of total sleep deprivation on cerebrospinal fluid β-amyloid 42 in healthy middle-aged men: a randomized clinical trial. JAMA neurology. 2014;71(8):971-7.
15. Faul F, Erdfelder E, Lang A-G, Buchner A. G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007;39(2):175-91.
16. Zimmermann P, Fimm B. A test battery for attentional performance. Applied neuropsychology of attention Theory, diagnosis and rehabilitation. 2002:110-51.
17. Shokri-Kojori E, Wang G-J, Wiers CE, Demiral SB, Guo M, Kim SW, et al. β-Amyloid accumulation in the human brain after one night of sleep deprivation. Proceedings of the National Academy of Sciences. 2018;115(17):4483-8.
18. Bigler ED. Symptom validity testing, effort, and neuropsychological assessment. J Int Neuropsychol Soc. 2012;18(4):632-40.
19. Baddeley A, Emslie H, Nimmo-Smith I. Doors and People: A Test of Visual and Verbal Recall and Recognition, Thames Valley Test Company, Bury St. Edmunds, UK. 1994.
20. Baddeley AD, Hitch GJ, Quinlan PT, Bowes L, Stone R. Doors for memory: A searchable database. The Quarterly Journal of Experimental Psychology. 2016;69(11):2111-8.
21. Frings L, Hellwig S, Bormann T, Spehl TS, Buchert R, Meyer PT. Amyloid load but not regional glucose metabolism predicts conversion to Alzheimer's dementia in a memory clinic population. Eur J Nucl Med Mol Imaging. 2018;45(8):1442-8.

22. Cecchin D, Barthel H, Poggiali D, Cagnin A, Tiepolt S, Zucchetta P, et al. A new integrated dual time-point amyloid PET/MRI data analysis method. Eur J Nucl Med Mol Imaging. 2017;44(12):2060-72.
23. Battle MR, Pillay LC, Lowe VJ, Knopman D, Kemp B, Rowe CC, et al. Centiloid scaling for quantification of brain amyloid with [18 F] flutemetamol using multiple processing methods. EJNMMI Res. 2018;8(1):107.
24. Nir Y, Staba RJ, Andrillon T, Vyazovskiy VV, Cirelli C, Fried I, et al. Regional slow waves and spindles in human sleep. Neuron. 2011;70(1):153-69.
25. Kang D, Lim HK, Jung WS, Jeong J-H, Kim TW, Han J-H, et al. Sleep and Alzheimer's Disease. Sleep Medicine Research. 2015;6(1):1-9.
26. de Heus RA, de Jong DL, Sanders ML, van Spijker GJ, Oudegeest-Sander MH, Hopman MT, et al. Dynamic regulation of cerebral blood flow in patients with Alzheimer disease. Hypertension. 2018;72(1):139-50.

6. AUTHORS' CONTRIBUTION

Jana Thomas was involved in setting up the study, recruiting participants, gathering baseline characteristics, analyzing of first data and writing most of the sections in this manuscript.

Sharon Ooms helped with setting up the study, recruitment and design of the project and writing the manuscript.

Marcel Verbeek contributed to the design of the study.

Jan Booij and Mark Rijpkema were major contributors in choosing and designing the right PET-CT/MRI procedure and wrote part of the manuscript.

Roy Kessels contributed to selecting the right neuropsychological tests, helped with the statistical analyzes of the first data and contributed to the revision of this manuscript.

Sebastiaan Overeem was a major contributor in setting up a collaboration with the sleeping center Kempenhaeghe (Heeze, The Netherlands) and helped revising the manuscript.

Jurgen Claassen was a major contributor in obtaining funding, setting up the study, designing the project and was extensively involved in writing and revising the manuscript.

All authors read and approved the final manuscript.

7. FUNDING STATEMENT

The SCHIP study is funded by ISAO (Internationale Stichting Alzheimer Onderzoek) (grant number 15040). Additional financial resources were provided by the Radboud University Medical Center (Nijmegen, The Netherlands).

8. COMPETING INTEREST STATEMENT

The authors declare that they have no competing interest.

Figure 1: Overview experimental design

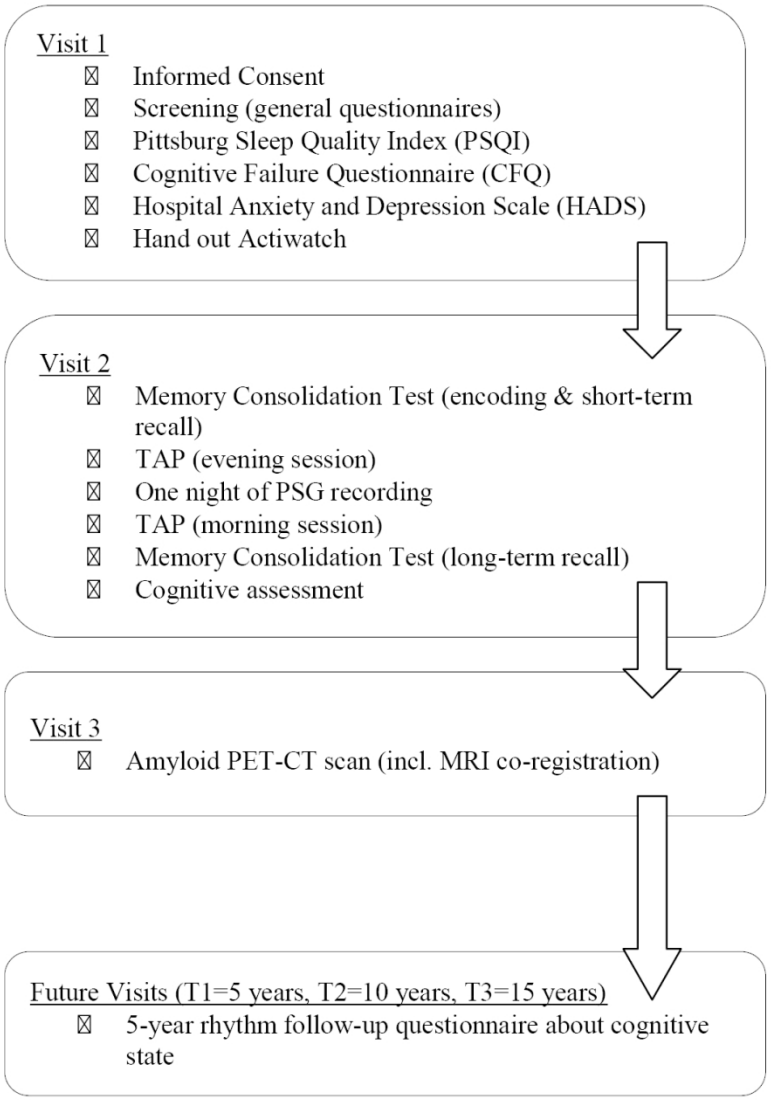


Figure 1: Overview experimental design

90x140mm (300 x 300 DPI)

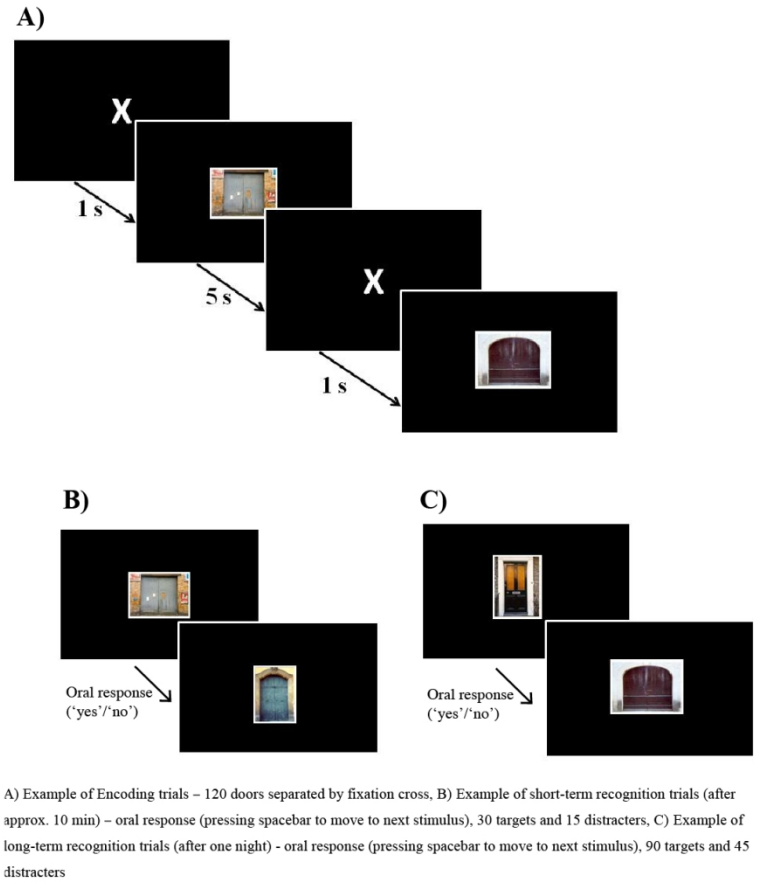


Figure 2: Memory Consolidation Task Design
A) Example of Encoding trials – 120 doors separated by fixation cross, B) Example of short-term recognition trials (after approx. 10 min) – oral response (pressing spacebar to move to next stimulus), 30 targets and 15 distracters, C) Example of long-term recognition trials (after one night) - oral response (pressing spacebar to move to next stimulus), 90 targets and 45 distracters

120x119mm (300 x 300 DPI)

BMJ Open

Sleep-Cognition-Hypothesis In maritime Pilots - What is the effect of long-term work-related poor sleep on cognition and amyloid accumulation in healthy middle-aged maritime pilots: Methodology of a case-control study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-026992.R2
Article Type:	Protocol
Date Submitted by the Author:	21-May-2019
Complete List of Authors:	Thomas, Jana; Radboudumc, Geriatrics; Radboud Alzheimer Center Ooms, Sharon; Radboudumc, Geriatrics; Radboud Alzheimer Center Verbeek, Marcel; Radboudumc; Radboud Alzheimer Center Booij, Jan; Academic Medical Center, Department of Nuclear Medicine Rijpkema, Mark; Radboudumc, Radiology and nuclear medicine Kessels, Roy; Donders Institute for Brain, Cognition and Behaviour, Radboud university medical center, Medical Psychology; Vincent van Gogh Institute for Psychiatry Overeem, Sebastiaan; Eindhoven Univeristy of Technology, Eindhoven Medtech Innovation Center; Sleep Medicine Center Kempenhaeghe Claassen, Jurgen; Radboudumc, Geriatrics; Radboud Alzheimer Center
Primary Subject Heading:	Geriatric medicine
Secondary Subject Heading:	Neurology, Radiology and imaging, Public health
Keywords:	Alzheimer's disease, Amyloid accumulation, Neurodegeneration, Cognitive function, Sleep

Sleep-Cognition-Hypothesis In maritime Pilots – What is the effect of long-term work-related poor sleep on cognition and amyloid accumulation in healthy middle-aged maritime pilots: Methodology of a case-control study

Jana Thomas, MSc[1,2,3]; Sharon Ooms, PhD[1,2,3]; Marcel M. Verbeek, PhD[2,3,6]; Jan Booij, MD, PhD[7,8], Mark Rijpkema, PhD[7]; Roy P.C. Kessels, PhD[2,3,9]; Sebastiaan Overeem, MD, PhD[4,5] and Jurgen A.H.R. Claassen, MD, PhD[1,2,3]

(1) Department of Geriatric Medicine, Radboud University Medical Centre, Nijmegen, The Netherlands; (2) Radboudumc Alzheimer Centre, Nijmegen, The Netherlands; (3) Donders Institute for Brain, Cognition and Behavior, Nijmegen, The Netherlands; (4) Sleep Medicine Centre Kempenhaeghe, Heeze, The Netherlands; (5) Eindhoven Medtech Innovation Center, Eindhoven University of Technology, Eindhoven, The Netherlands; (6) Department of Neurology, Radboud University Medical Centre, Nijmegen, The Netherlands; (7) Department of Radiology and Nuclear Medicine, Radboud University Medical Center, Nijmegen, The Netherlands; (8) Department of Radiology and Nuclear Medicine, Amsterdam University Medical Center, Amsterdam, The Netherlands; (9) Department of Medical Psychology, Radboud University Medical Centre, Nijmegen, The Netherlands

Corresponding author:
Jana Thomas
Jana.thomas@radboudumc.nl
Reinier Postlaan 4
6526 GC Nijmegen
The Netherlands

Word count: 3.793 words (without abstract, figures, references)

ABSTRACT

Introduction: Evidence indicates a bi-directional relationship between poor sleep and Alzheimer's disease (AD). While AD may lead to disruption of normal sleep, poor sleep in itself may play a causal role in the development of AD by influencing the production and/or clearance of the amyloid-beta (Aβ) protein. This led to the hypothesis that extended periods (>10 years) of sleep loss could lead to Aβ accumulation with subsequent cognitive AD related decline. This manuscript describes the methodology of the SCHIP study, a cohort study in maritime pilots that aims at investigating the relationship between prolonged work-related sleep loss, cognitive function and amyloid accumulation among healthy middle-aged maritime pilots, to test the hypothesis that prolonged sleep loss deprivation increases the risk of AD-related cognitive decline.

Methods: Our study sample consists of a group of healthy middle-aged maritime pilots (n=20), who have been exposed to highly irregular work schedules for more than 15 years. The maritime pilots will be compared to a group of healthy, age and education matched controls (n=20) with normal sleep. Participants will complete 10 days of actigraphy (Actiwatch 2, Philips Respironics) combined with a sleep-wake diary. They will undergo one night of polysomnography (PSG), followed by comprehensive assessment of cognitive function. Additionally, participants will undergo amyloid PET-CT (Positron Emission Tomography- Computed Tomography) to measure brain amyloid accumulation and MRI to investigate atrophy and vascular changes.

Analysis: All analyses will be performed using IBM SPSS version 20.0 (SPSS Inc, Chicago, USA). We will perform independent samples t-tests to compare all outcome parameters.

Ethics and dissemination: The study protocol was approved by our institutional ethical review board (NL55712.091.16, file number 2016-2337) and will be performed according to good clinical practice (GCP) rules. Data and results will be published in 2020.

Key words: Alzheimer's disease, amyloid accumulation, neurodegeneration, cognitive function, sleep, sleep deprivation, shift work

ARTICLE SUMMARY

Strengths and limitations of the study

- the unique cohort of maritime pilots allows the prospective assessment of the effect of chronic exposure to irregular maritime work schedules on cognitive functioning and amyloid accumulation; the unique cohort might also be a limitation to the study, because they might not be comparable to the control group to some extent (e.g. IQ, work environment, personality)
- since we include participants up to 60 years old we will have to take possible aging effects on sleep in to account while interpreting our results
- in addition to imaging techniques (PET-CT and MRI), we use sensitive and well-validated neuropsychological tests to measure different domains of cognitive function (reaction time, visual memory, executive function, semantic memory and episodic memory)
- we will make use of four different instruments to obtain a comprehensive measure of sleeping patterns (two subjective and two objective ones)
- our results could give rise to new treatment opportunities, that aim at sleep improvement and management in order to prevent or reduce amyloid accumulation and in turn delay or even prevent the development of AD

1. INTRODUCTION

Alzheimer's disease (AD) is the most prevalent cause of dementia and currently affects approximately 36 million people worldwide (1). Thus far, no successful treatment is available. One of the major contributors to the neurodegeneration seen in the brains of AD patients is amyloid-beta (Aβ) (2). The amyloid-cascade hypothesis characterizes amyloid accumulation as the fundamental initiating pathway for the development of AD (3). The reason why amyloid accumulates however is not clear yet. Recent evidence suggests that poor sleep might be one of the risk factors for amyloid accumulation and thereby increases the risk of AD development (4). Elderly people suffering from insomnia are more likely to develop AD compared to controls without insomnia (4). Furthermore, disrupted circadian rhythm among otherwise healthy individuals (5) and sleep-disordered breathing disorders (6) increase the chance of developing AD later in life. Poor sleep quality specifically has been shown to increase the risk of AD among older individuals (7-11). A recent meta-analysis of epidemiological studies found that poor sleep increased AD risk and that approximately 15% of cases of AD in the population might be attributable to sleep problems (12).

The effect of acute sleep deprivation on AD has been shown in both rodent and human studies, that investigated the effect of sleep and sleep deprivation on Aβ levels. Rodents and humans show fluctuations in Aβ levels over a 24-hour rhythm, where levels rise during wakefulness and decrease during sleep (13, 14). Following acute sleep deprivation, the drop in Aβ levels that would normally occur after sufficient sleep the following morning, was absent. Based on this evidence, chronic sleep disturbances might lead to a pronounced accumulation of Aβ in the brain, which in turn increases the risk to develop AD.

To date, observational and epidemiological studies have identified a relationship between poor sleep and the risk of developing AD. Experimental research has found that sleep deprivation and Aβ levels are related in both animals and humans. However, the direct effect of chronic partial sleep deprivation and its consequences on cognitive functioning, particularly the risk to develop AD later in life, has not been studied before.

In this article we describe the methodology and the participant cohort of the SCHIP (Sleep-Cognition Hypothesis In maritime Pilots) study. We designed the SCHIP study in order to investigate the effect

of long term work-related sleep loss on cognitive function, structural brain changes, and amyloid accumulation by using a unique cohort of healthy subjects with highly irregular shift work as maritime pilots. Because of the bi-directional nature of the relationship between sleep and AD, in which poor sleep is a symptom of the disease that precedes the clinical manifestation of AD, but might also be a risk factor that potentially contributes to the development of the disease, it is especially important to investigate the effects of sleep loss, caused by an external factor (work) and not by an intrinsic sleeping disorder (such as insomnia) that could be AD-related. Therefore participants in this study were selected based on their prolonged exposure to irregular maritime work-schedules. In this maritime pilot group, sleep loss is characterized as a combination of sleep deprivation, sleep restriction and sleep fragmentation or disruption. We will refer to these using the umbrella term 'sleep loss' throughout. An example of an individual working schedule from maritime pilot is shown in figure 1. Results and insights born from the SCHIP study could shed more light on sleep disturbance as one of the risk factors to develop AD and contribute to new and improved treatment strategies. In the SCHIP study we will investigate whether maritime pilots perform worse on cognitive assessment in comparison to a healthy control group and whether they have evidence of elevated brain amyloid concentrations and structural brain changes.

2. METHODS AND ANALYSIS

2.1. Participants

Considering that this study is a proof of principle study, it is not possible to establish the exact effect size of the change in cognitive function we expect to see between the maritime pilots and the healthy controls.

To account for possible 10% withdrawal, we chose to have n=20 participants in each arm.

In order to reach enough power to detect clinically relevant differences regarding the results of the PET-CT scan, we performed a sample size power calculation using G Power (version 3.1.9.3) (15). Reported normal values (mean and standard deviation) for this age group in the literature vary between studies but are in the order of a mean standard uptake value (SUV) between 0.9 and 1.1 with standard

deviations (SDs) in the range of 0.05 – 0.2. We define a relevant difference between maritime pilots and normal values to be 0.2 or more (an SUV of 1.3 or higher is considered as abnormal in most studies). This results in a large expected effect size (>0.8). The precise number of age- and education matched subjects in the database is not known yet but is estimated to be at least 50 (between 50 and 100). We have applied a one-tailed test (it is not possible to have a SUV lower than normal values). With an alpha of 0.05, with n=20 our power is 0.95 or higher (depending on the number of normal subjects). The lower level for power (0.85) is reached with 13-15 pilots.

Based on these calculations, we will aim to recruit 20 pilots, allowing for drop-out of 5-7 pilots (either due to withdrawal of consent or artefacts in the PET-CT).

Participants will be recruited within the national organization of Dutch Maritime Pilots (Nederlandse Loodswezen). We selected the maritime pilots as a suitable study population because of their unique irregular working schedule. They are called to work depending on the number and kind of ships that arrive. In a typical 7-day work week they have to be available 24 hours per day during which they can be called several times. This results in multiple divided short sleep periods over 24 hours during a work week, this is followed by a week off. An example of a workday of a maritime pilot is illustrated in figure 1.

Figure 1: Example maritime pilot working schedule. Working hours indicated in red.

The responsibility of a maritime pilot is to handle large international ships arriving by sea and to maneuver them into their final docking position in one of the Dutch harbors. This profession carries high responsibilities and requires accurate knowledge of the dimensions of the harbor and the ships besides technical knowledge and navigational skills. This results in irregular working schedules because guiding the ships is a time intensive procedure, that can take hours to complete. Maintaining this schedule for more than 15 years will result in chronic exposure to sleep loss, either due to partial sleep deprivation (missing a full night of sleep due to work), sleep restriction (a much shorter night of sleep than normal) or sleep fragmentation or disruption (short periods of sleep interrupted by calls to work).

We will reach out to the whole maritime pilot community, in order to recruit pilots who are approximately 50 to 60 years old, with at least 15 years of uninterrupted work history as a maritime pilot.

Additional inclusion criteria are not using neuro-active medications or psycho-stimulants, consumption of <14 alcoholic beverages per week, a body mass index of 18-30kg/m^2 and no subjective cognitive complaints (Cognitive Failure Questionnaire (CFQ <43). As control group, we will recruit 20 age, sex and education matched healthy adults with normal sleep indicated by a score of >5 on a subjective sleep questionnaire (Pittsburgh Sleep Quality Index, PSQI). The study protocol was approved by our institutional review board (NL55712.091.16, file number 2016-2337) and will be performed according to good clinical practice (GCP) rules. Inclusion of participants started in August 2018 and we expect to complete the analysis of all data in August 2020.

2.2. Experimental Design

The aim of the SCHIP study is to investigate the relationship between long-term work exposure to irregular working schedules, cognitive function and amyloid accumulation among healthy middle-aged men to test the hypothesis that work-related prolonged sleep loss increases the risk of AD-related cognitive decline. In order to test this hypothesis, maritime pilots will have three visits and controls will have two visits (figure 2). During the first visit participants will complete general questionnaires about medical history, sleeping habits and cognitive state. Approximately 10 days after the first visit has taken place, participants are invited to the sleep center Kempenhaeghe (Heeze, The Netherlands) for the second visit. They will arrive at the sleep center at 19.00 h and complete a memory consolidation test (modified 'Doors Test') and a test for attentional performance (TAP 2.3) (16), followed by overnight polysomnography (PSG). Participants wake up at their normal wake time and complete a neuropsychological assessment after breakfast around 9.00h.

Visit three (only for the maritime pilots) will be performed at the Radboud university medical center in Nijmegen (The Netherlands), where participants will undergo a standard amyloid PET-CT to measure brain amyloid accumulation and a 3T-MRI for coregistration. Participants are scheduled in their week

off in order to prevent short term effects of sleep disruption on amyloid concentrations in the brain (17). They are instructed not eat or drink anything except from water 3 hours prior to the scan.

Figure 2: Overview experimental design

2.3 Patient and Public Involvement

No patients were involved in this study because this study involves healthy participants.

However, the participants' organization contacted us due to recent evidence from the literature on the relationship between sleep and AD. They expressed their worries about their health and their own risk of developing AD considering their irregular sleep. They reported feeling very tired at the end of a work week and speculated that within their group of maritime pilots (including already retired pilots), cases of dementia occurred more frequently than expected.

Participants were also tightly involved in the design, realization and feasibility of the study. For example, they were involved in choosing the technique to measure amyloid and expressed the preference for a PET-CT scan over cerebrospinal fluid (CSF) measurements of amyloid.

Dissemination of results to participants:

Results from the PSG measurements will be reported only to participants if we find abnormalities such as apnea or sleeping disorders. This will be done by one of our sleep clinicians via telephone. Results from the PET-CT scan will be disclosed via telephone by one of our clinicians as well. This is according to the protocol that has been approved by the local ethical committee. Any incidental findings on the PET-CT and/or MRI will be disseminated as well.

2.4. Sleep Measurements

In order to obtain a comprehensive measure of sleeping patterns, we will use the Pittsburg Sleep Quality Index (PSQI) with questions about average sleeping behavior, including the report of bedtime, get-up time, sleep latency, total sleep time, sleep disturbances (pain, breathing etc.) and use of sleep medication. The maritime pilots will be instructed to fill in the questionnaire twice, once with regard to a work week and once with regard to a week off. Additionally all participants are asked to maintain a sleep-wake diary on a daily basis. In this diary they have to keep track of their bed times, the time it

took to fall asleep (sleep latency), the number of awakenings and their get up times. Furthermore they will receive an accelerometer, the Actiwatch 2 (Philips Respironics; Eindhoven, The Netherlands), in order to obtain more objective measurements of sleeping behavior. The Actiwatch is worn around the wrist and measures total sleep time and number of awakenings during sleep automatically based on movement. Participants are instructed to fill in the sleep diary and to wear the Actiwatch for 10 days preceding the second visit.

2.5. Cognitive assessment

The neuropsychological assessment has been designed to measure the following cognitive domains, using validated Dutch versions of widely used neuropsychological tests. Episodic memory is assessed using the WMS-IV Logical Memory and the Rey-Auditory Verbal Learning Test (RAVLT). Working memory and executive function are measured by WAIS-IV Digit Span, Trail Making Test (TMT-A, TMT-B) and WAIS-IV Digit Symbol test. Semantic memory and language are assessed by letter fluency (D-A-T), semantic fluency (animal/profession naming) and the Boston Naming Test-Short Form (BNT) (18). Attentional performance is studied using the alertness test of the Test of Attentional Performance (TAP 2.3) (16). This test is conducted twice, once in the evening around 19.00 h and once in the morning around 9.00 h. In order to test overnight memory consolidation, a novel paradigm was developed based on the Doors Test, a visual recognition task developed by Baddely and colleagues (19) and extended using a validated database of 2000 pictures of doors (20). During the encoding trial, we present 120 pictures of doors that participants are instructed to remember. All targets (doors) are presented twice in a different, pseudo-randomized order. Targets are shown for 5 seconds each, separated by a fixation cross presented for 1 second (figure 3). Approximately 10 minutes after the encoding trial, participants are presented with 30 of the original doors and 15 distracters (new pictures of doors, not presented before) that are randomly mixed. During this short-term recognition test, the task is to indicate whether the door had been presented before (oral response with ‘yes’) or not (oral response ‘no’). For the long-term delayed recall, the same procedure is applied in the morning, using the other 90 original pictures, plus 45 new distracters (figure 3). Calculating the hits and false alarms, we compute the sensitivity (A′) for short-term and for long-term delayed recall

(A′= 0.5 + ((hit-rate – false alarm rate) × (1 + hit rate – false alarm rate))/ (4 × hit rate × (1 – false alarm rate)).

Figure 3: Memory Consolidation Task Design

A) Example of Encoding trials – 120 doors separated by fixation cross, B) Example of short-term recognition trials (after approx. 10 min) – oral response (pressing spacebar to move to next stimulus), 30 targets and 15 distracters, C) Example of long-term recognition trials (after one night) - oral response (pressing spacebar to move to next stimulus), 90 targets and 45 distracters

2.6. Amyloid PET-CT scan with co-registered magnetic resonance imaging (MRI)

Brain amyloid PET-CT scan will be performed to measure amyloid load. Participants will be scanned at the Radboudumc department of Radiology and Nuclear medicine on a PET scanner (Biograph mCT, Siemens, Erlangen, Germany). Subjects will receive an intravenous bolus of the well-validated PET tracer ^{18}F-flutemetamol, and static brain images will be acquired from 90-110 min post-injection (frames of 5 mins), as recommended (see SPC; http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002557/WC500172950.pdf). The individual reconstructed PET images will be co-registered with individual structural T_1 MRI scans.

The PET scans will be rated visually as positive or negative by an experienced nuclear medicine physician for the presence of amyloid depositions typical of AD. Scores will be expressed as a global SUV, which will be compared against population normative values, as earlier described (21). For quantitative purposes, grey matter volumes of interest will be defined on the individual MRIs (e.g. frontal brain areas, the precuneus, and hippocampus) as well as for cerebella grey matter (to assess non-specific uptake). The amyloid burden will be quantified using the standardized uptake value ratio, since it has been validated that this analysis method has comparable agreement with full kinetic modeling (22). Brain MRI will be performed on a 3 Tesla system (Magnetom Trio, Siemens, Erlangen, Germany). The structural T_1-weighted images will be used for co-registration purposes, and to define grey matter in the volumes of interest. In addition, these scans will be used to perform volumetric measurements (e.g. of the hippocampus). Also, arterial spin labeling (ASL) will be performed to measure global and regional cerebral blood flow (CBF), since reduced regional CBF is

an early marker of AD. Individual anatomic MRI scans will be co-registered with the individual PET scans using the image processing platform FLS (fsl.fmrib.ox.ac.uk) to calculate standard uptake value ratios. The PET-CT data will be expressed in Centiloid units to evaluate our data with historical control data as was recently validated by Battle and co-workers (23).

2.7. Future follow-up visits

Because of the insufficient knowledge about the correlation between amyloid accumulation in the brain and the actual development of Alzheimer's disease, we decided to follow the maritime pilots in a 5 year cycle in order to monitor any cognitive changes. We will contact them 3 times in total, which leaves us with a maximum follow-up period of 15 years. At each time point, they will be asked to answer 3 questions online:

1) Did you develop cognitive complaints over the last 5 years? This question will be further elaborated on with the Cognitive Failure Questionnaire (CFQ). The CFQ is a validated questionnaire that aims at detecting daily disruptions of cognitive functions. Participants are confronted with 25 statements and have to indicate how often they experience the situation that is described in the statements with a score between 0 (never) and 4 (very often).

2) Were you diagnosed with mild cognitive impairment during the last 5 years? If yes, when and what was the precise diagnosis?

3) Were you diagnosed with dementia in the past 5 years? If yes, when and what was the precise diagnosis?

If the answer is 'Yes' to one of these questions, we will reach out to the participants for clarification.

2.8. Statistical analysis

All analyses will be performed using IBM SPSS version 20.0 (SPSS Inc, Chicago, USA). Statistical significance is set at $p < 0.05$, with Bonferroni correction for multiple comparisons when appropriate, combined with reports of effect size and 95% confidence intervals. All continuous variables will be assessed for normal distribution by inspection of histograms and the Kolmogorov-Smirnov test. Levene's test will be used to assess equality of variances. We will perform an independent samples t-

test to compare all outcome parameters. The primary outcome for cognitive function will be the score on each test respectively adjusted for age and education. Regarding imaging, the primary outcome measure will be the visual read of the amyloid PET scans (positive or negative PET). Secondary outcome measurements will be quantitative PET (SUV ratios), brain volume (MRI), and CBF measurements (MRI-ASL).

3. ETHICS AND DISSEMINATION

The study protocol was approved by our institutional review board, CMO (Commissie Mensgebonden Onderzoek) (NL55712.091.16, file number 2016-2337) and performed according to good clinical practice (GCP) rules. Written informed consent will be obtained from all participants. We are planning to publish the data and results of the SCHIP study in two or three articles in 2019 and 2020.

4. DISCUSSION

In this article we presented the design and methodology of the SCHIP study. The main aim of the SCHIP study is to investigate the effect of long-term work related sleep loss on cognition and amyloid accumulation. Since previous studies assessed sleep deprivation for only one or a few days, the SCHIP study extends these studies by investigating the effect of long term sleep loss on cognitive function and amyloid accumulation. We expect that the maritime pilots have had long-term (>15 years) exposure to work related sleep loss, because of their work schedules and that this exposure has led to reduction in SWS. Every slow wave observed on an electro-encephalography (EEG) is a pause in synaptic activity (24). Synaptic activity and Aβ levels appear strongly related, as Aβ levels increase due to synaptic activity (25). More synaptic activity is observed during wakefulness, especially in the default mode network (DMN) or other highly interconnected brain areas (25). During sleep, synaptic activity is reduced, which could result in a decrease in Aβ levels in the brain (25). Therefore, sleep loss (especially poor SWS) over the course of many years could increase Aβ concentrations, which in turn could trigger AD-associated neurodegeneration and loss of cognitive function.

To test this hypothesis, we will perform extensive cognitive testing, using tests that are sensitive to subtle decline in episodic memory, which is affected early in AD. Additionally, amyloid positivity will be measured using amyloid PET-CT scans in order to explore the effect of work related sleep loss on amyloid concentration in the brain. Cerebral blood flow and cerebrovascular resistance will be investigated, as reductions in blood flow and increases in resistance occur early in the Alzheimer disease process (26). Finally, global grey matter volume and hippocampal volume will be measured. The unique cohort of maritime pilots will allow the prospective assessment of prolonged work-related sleep loss and its consequences for cognitive functioning and amyloid accumulation. The design of the SCHIP study presents a number of strengths. No other previous investigation has looked into the effect of chronic partial sleep deprivation on cognition in healthy men to this extent. Since sleep disorders might also be early symptoms of preclinical AD, it is especially important in this age group to investigate the effect of sleep loss due to an external factor and not due to intrinsic sleep disorders. All enrolled maritime pilots did not have any sleeping disorders and did not use sleep medication as confirmed by a general health questionnaire which was filled in upon screening for participation. Furthermore, we measure different domains of cognitive function (reaction time, visual memory, executive function, semantic memory and episodic memory) using sensitive and well-validated neuropsychological tasks. In addition to testing cognitive functions, we will perform brain imaging to detect amyloid accumulation as potential consequence of sleep loss. We will make use of four different instruments to get a comprehensive measure of sleeping patterns, two subjective and two objective ones. The subjective measurements consist of the PSQI and the maintenance of the sleep-wake diary. The objective assessments include the data from the Actiwatch 2 (Philips Respironics) in addition to a night of PSG. The data from the Actiwatch can be compared to the sleep-wake diary entries. These data can then be used to verify the sleeping behavior of participants, making sure they maintain regularity and consistency in their sleeping habits.

Results of the SCHIP study will give us more insights into the consequences of long term work related sleep loss on cognitive function and amyloid accumulation in an AD related context. Our results could give rise to new treatment opportunities, that aim at sleep improvement and management in order to prevent or reduce amyloid accumulation and in turn delay or even prevent the development of AD.

5. REFERENCES

1. Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP. The global prevalence of dementia: a systematic review and metaanalysis. Alzheimers Dement. 2013;9(1):63-75. e2.
2. Jagust WJ, Mormino EC. Lifespan brain activity, β-amyloid, and Alzheimer's disease. Trends Cogn Sci. 2011;15(11):520-6.
3. Korczyn AD. The amyloid cascade hypothesis. Alzheimers Dement. 2008;4(3):176-8.
4. Osorio RS, Pirraglia E, Agüera-Ortiz LF, During EH, Sacks H, Ayappa I, et al. Greater risk of Alzheimer's disease in older adults with insomnia. J Am Geriatr Soc. 2011;59(3):559-62.
5. Tranah GJ, Blackwell T, Stone KL, Ancoli-Israel S, Paudel ML, Ensrud KE, et al. Circadian activity rhythms and risk of incident dementia and mild cognitive impairment in older women. Ann Neurol. 2011;70(5):722-32.
6. Yaffe K, Laffan AM, Harrison SL, Redline S, Spira AP, Ensrud KE, et al. Sleep-disordered breathing, hypoxia, and risk of mild cognitive impairment and dementia in older women. Jama. 2011;306(6):613-9.
7. Benedict C, Byberg L, Cedernaes J, Hogenkamp PS, Giedratis V, Kilander L, et al. Self-reported sleep disturbance is associated with Alzheimer's disease risk in men. Alzheimers Dement. 2015;11(9):1090-7.
8. Sterniczuk R, Theou O, Rusak B, Rockwood K. Sleep disturbance is associated with incident dementia and mortality. Curr Alzheimer Res. 2013;10(7):767-75.
9. Hahn EA, Wang H-X, Andel R, Fratiglioni L. A change in sleep pattern may predict Alzheimer disease. The American Journal of Geriatric Psychiatry. 2014;22(11):1262-71.
10. Potvin O, Lorrain D, Forget H, Dube M, Grenier S, Preville M, et al. Sleep quality and 1-year incident cognitive impairment in community-dwelling older adults. Sleep. 2012;35(4):491-9.
11. Ju Y-ES, McLeland JS, Toedebusch CD, Xiong C, Fagan AM, Duntley SP, et al. Sleep quality and preclinical Alzheimer disease. JAMA neurology. 2013;70(5):587-93.
12. Bubu OM, Brannick M, Mortimer J, Umasabor-Bubu O, Sebastião YV, Wen Y, et al. Sleep, cognitive impairment, and Alzheimer's disease: a systematic review and meta-analysis. Sleep. 2016;40(1):zsw032.
13. Kang J-E, Lim MM, Bateman RJ, Lee JJ, Smyth LP, Cirrito JR, et al. Amyloid-β dynamics are regulated by orexin and the sleep-wake cycle. Science (80-). 2009;326(5955):1005-7.
14. Ooms S, Overeem S, Besse K, Rikkert MO, Verbeek M, Claassen JA. Effect of 1 night of total sleep deprivation on cerebrospinal fluid β-amyloid 42 in healthy middle-aged men: a randomized clinical trial. JAMA neurology. 2014;71(8):971-7.
15. Faul F, Erdfelder E, Lang A-G, Buchner A. G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007;39(2):175-91.
16. Zimmermann P, Fimm B. A test battery for attentional performance. Applied neuropsychology of attention Theory, diagnosis and rehabilitation. 2002:110-51.
17. Shokri-Kojori E, Wang G-J, Wiers CE, Demiral SB, Guo M, Kim SW, et al. β-Amyloid accumulation in the human brain after one night of sleep deprivation. Proceedings of the National Academy of Sciences. 2018;115(17):4483-8.
18. Bigler ED. Symptom validity testing, effort, and neuropsychological assessment. J Int Neuropsychol Soc. 2012;18(4):632-40.
19. Baddeley A, Emslie H, Nimmo-Smith I. Doors and People: A Test of Visual and Verbal Recall and Recognition, Thames Valley Test Company, Bury St. Edmunds, UK. 1994.
20. Baddeley AD, Hitch GJ, Quinlan PT, Bowes L, Stone R. Doors for memory: A searchable database. The Quarterly Journal of Experimental Psychology. 2016;69(11):2111-8.
21. Frings L, Hellwig S, Bormann T, Spehl TS, Buchert R, Meyer PT. Amyloid load but not regional glucose metabolism predicts conversion to Alzheimer's dementia in a memory clinic population. Eur J Nucl Med Mol Imaging. 2018;45(8):1442-8.

22. Cecchin D, Barthel H, Poggiali D, Cagnin A, Tiepolt S, Zucchetta P, et al. A new integrated dual time-point amyloid PET/MRI data analysis method. Eur J Nucl Med Mol Imaging. 2017;44(12):2060-72.
23. Battle MR, Pillay LC, Lowe VJ, Knopman D, Kemp B, Rowe CC, et al. Centiloid scaling for quantification of brain amyloid with [18 F] flutemetamol using multiple processing methods. EJNMMI Res. 2018;8(1):107.
24. Nir Y, Staba RJ, Andrillon T, Vyazovskiy VV, Cirelli C, Fried I, et al. Regional slow waves and spindles in human sleep. Neuron. 2011;70(1):153-69.
25. Kang D, Lim HK, Jung WS, Jeong J-H, Kim TW, Han J-H, et al. Sleep and Alzheimer's Disease. Sleep Medicine Research. 2015;6(1):1-9.
26. de Heus RA, de Jong DL, Sanders ML, van Spijker GJ, Oudegeest-Sander MH, Hopman MT, et al. Dynamic regulation of cerebral blood flow in patients with Alzheimer disease. Hypertension. 2018;72(1):139-50.

6. AUTHORS' CONTRIBUTION

Jana Thomas was involved in setting up the study, recruiting participants, gathering baseline characteristics, analyzing of first data and writing this manuscript.

Sharon Ooms helped with setting up the study, recruitment and design of the project and writing the first draft of the manuscript.

Marcel Verbeek contributed to the design of the study.

Jan Booij and Mark Rijpkema were major contributors in choosing and designing the right PET-CT/MRI procedure and wrote part of the manuscript.

Roy Kessels contributed to selecting the right neuropsychological tests, helped with the statistical analyzes of the first data and contributed to the revision of this manuscript.

Sebastiaan Overeem was a major contributor in setting up a collaboration with the sleeping center Kempenhaeghe (Heeze, The Netherlands) and helped revising the manuscript.

Jurgen Claassen was a major contributor in obtaining funding, setting up the study, designing the project and was extensively involved in writing and revising the manuscript.

All authors read and approved the final manuscript.

7. FUNDING STATEMENT

The SCHIP study is funded by ISAO (Internationale Stichting Alzheimer Onderzoek) (grant number 15040). Additional financial resources were provided by the Radboud University Medical Center (Nijmegen, The Netherlands).

8. COMPETING INTEREST STATEMENT

The authors declare that they have no competing interest.

Figure 1: Example maritime Pilot working schedule. Working hours indicated in red.

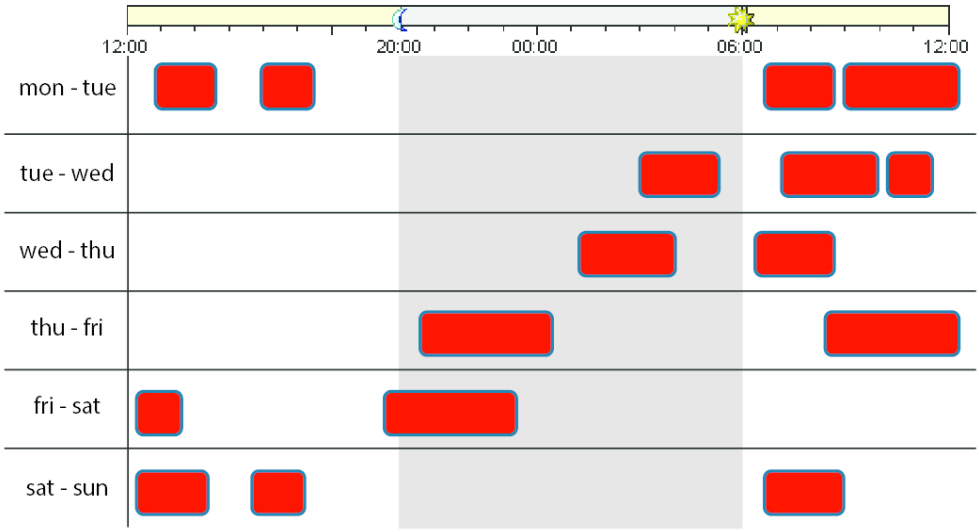


Figure 1: Example maritime pilot working schedule. Working hours indicated in red.

90x90mm (300 x 300 DPI)

Figure 2: Overview experimental design

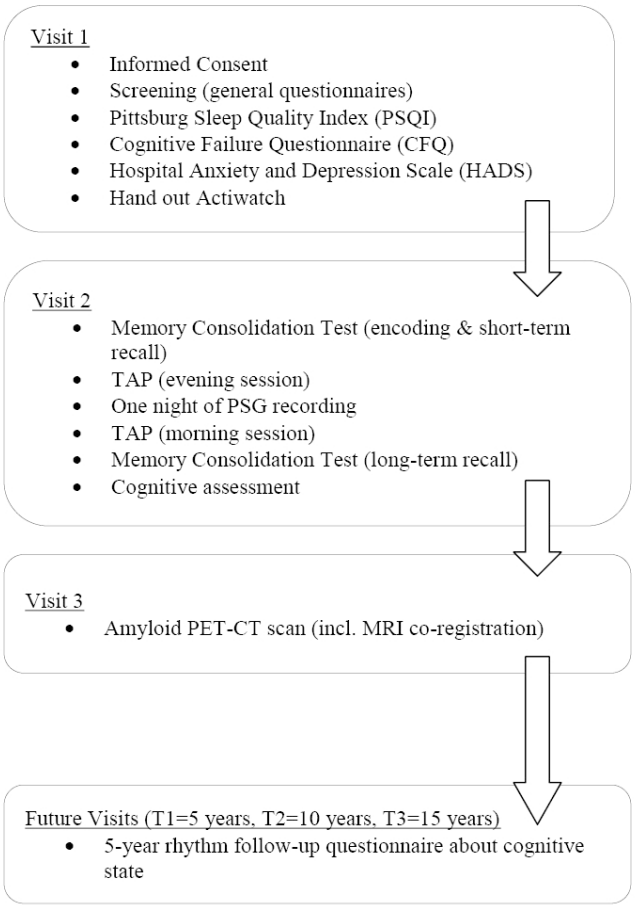


Figure 2: Overview experimental design

90x90mm (300 x 300 DPI)

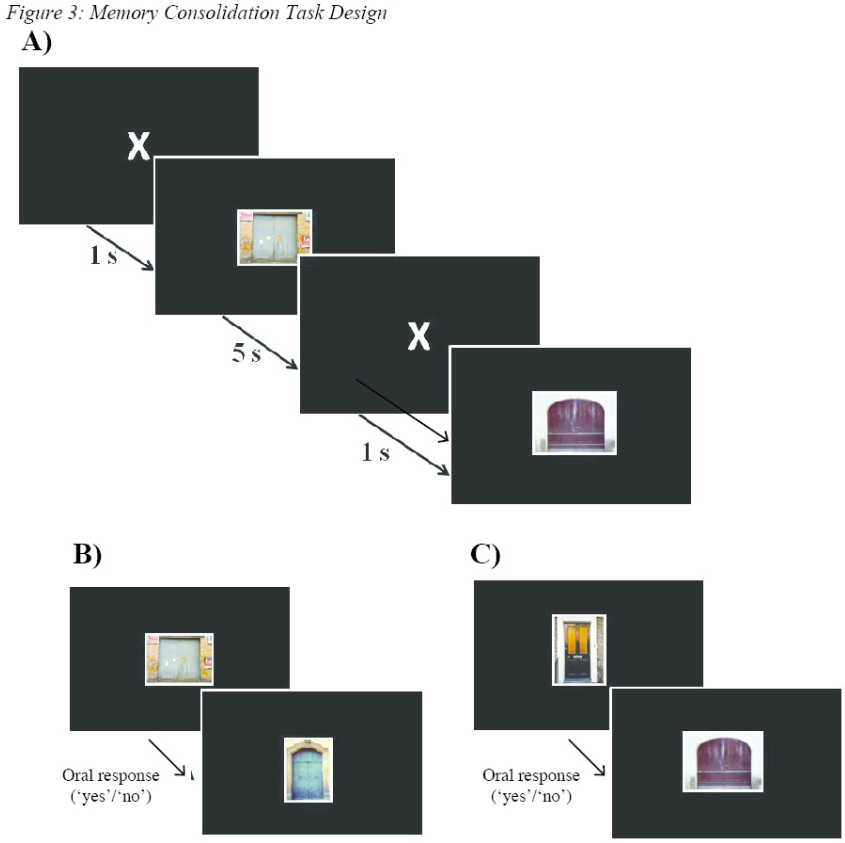


Figure 3: Memory Consolidation Task Design
A) Example of Encoding trials – 120 doors separated by fixation cross, B) Example of short-term recognition trials (after approx. 10 min) – oral response (pressing spacebar to move to next stimulus), 30 targets and 15 distracters, C) Example of long-term recognition trials (after one night) - oral response (pressing spacebar to move to next stimulus), 90 targets and 45 distracters

90x90mm (300 x 300 DPI)