

## PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (<http://bmjopen.bmj.com/site/about/resources/checklist.pdf>) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

### ARTICLE DETAILS

<b>TITLE (PROVISIONAL)</b>	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
<b>AUTHORS</b>	Thomas, Jana; Ooms, Sharon; Verbeek, Marcel; Booij, Jan; Rijpkema, Mark; Kessels, Roy; Overeem, Sebastiaan; Claassen, Jurgen

### VERSION 1 - REVIEW

<b>REVIEWER</b>	Nicola Magnavita Università Cattolica del Sacro Cuore, Roma
<b>REVIEW RETURNED</b>	18-Nov-2018

<b>GENERAL COMMENTS</b>	the described method appears well designed, it should provide the expected results
-------------------------	--

<b>REVIEWER</b>	Anna Donna O'Hagan Dublin City University; Dublin, Ireland
<b>REVIEW RETURNED</b>	18-Nov-2018

<b>GENERAL COMMENTS</b>	<p>Well done on a very interesting and worthwhile study. The study is well thought-out and planned. However, some minor amendments and suggestions are proposed.</p> <ul style="list-style-type: none"><li>- The title is a little confusing. Are you referring to maritime pilots or healthy middle-aged men? Or is it health middle-aged maritime pilots? More clarity is needed here.</li><li>- Amendments are needed to the way in which you state your objective. What is meant by "prolonged abnormal sleep behaviour". This needs to be more specific as to what exactly you are referring (see comment below).</li><li>- Overall the abstract is good. However, you need to be very clear on what you mean by disturbed sleep. Throughout the piece you make reference to sleep disturbance and sleep deprivation however, these have different meanings. If you are referring to sleep loss it could have occurred due to a number of reasons - e.g., sleep disturbance, sleep restriction, sleep deprivation - you need to be very clear on which you are referring .</li><li>- In one of your limitations you suggest that your unique cohort of maritime pilots may not be comparable to the control group to</li></ul>
-------------------------	---

some extent - can you elaborate on this further as to why you think this might be the case.

- Whilst it is important to mention the effects AD has on sleeping patterns, more emphasis is needed on the proposed impact of sleep on the development of AD as this is the focus of your research.

- You say that poor sleep " could represent an early symptom that precedes the clinical manifestation of AD" - do you not mean this could be a factor that potentially contributes to the development of AD?

- I would like to see a little more clarity in your writing - this research is looking at the effects of sleep on the development of AD. Overall, the writing could be a little more concise throughout.

- You cannot state that this research "is caused by an external factor (work) and not by an intrinsic sleeping disorder" - unless you test and control for these.

- You should not make the assumption that the week off for pilots consists of sleep loss due to social activities. I would like to see a little more information on typical schedules worked by the pilots to give the reader a better indication of pilots working and sleeping schedules.

- In your methodology it is suggested that pilots work 4 days X 24 hour schedules - is this correct? This would mean a 96 hour shift with 2 hours rest in between every 2 hours? You need to make this clearer for the reader (a diagram might help here?).

- Will you be employing an upper and lower age limit (in addition to the +15 years experience)? You may need to consider the impact of age on sleep in older individuals.

- Have you considered controlling for smoking and caffeine intake during your study (or at least monitoring it)? These will have an influencing impact on alertness and performance.

- Will participants be fed during the study? It might be beneficial to provide more information on this.

- What will the findings from the Amyloid PET-CT be compared to? This needs to be made more clear for the reader.

- What is the purpose of the accelerometer? It is mentioned once but I would like to see a little more clarity for its purpose.

- The follow-up questions will provide a clearer picture on the relationship between these variables. However, I would like to see slightly more detail - e.g., what is meant by a "cognitive complaint"? Why have you decided to do this every 5 years there after?

Overall I think this is a very good and useful study. I believe it will provide beneficial information regarding the sleep and AD relationship. Some minor amendments are needed for clarity and to further enhance this study.

<b>REVIEWER</b>	Martin Olsson Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Sweden.
<b>REVIEW RETURNED</b>	20-Mar-2019

<b>GENERAL COMMENTS</b>	<p>Dear editor.</p> <p>I have read the article carefully. While I believe changes to be necessary, the concept is appealing and the research question is of great importance. I would recommend further reviewing with the goal to publish.</p> <p>There are some considerations before going further. Participants, power and longitudinal problems. The title of the paper suggests that this is a prospective study, yet the prospective part is only briefly described. No power is calculated for this part and it is not reproducible as it stands right now. Matched controls are not followed hence aging effect cannot be assessed. Without changes to the protocol, this should be considered a case-control study.</p> <p>There is also concerns regarding the power in general as this protocol aims to follow pathophysiology and not normal physiology, which is the case in previous studies made on healthy volunteers with short experimental sleep deprivation.</p> <p>Minor concerns include typos, unnecessary self-citing, lack of N in abstract and an overstated claim of importance. There is also presentation of statistics in the introduction that is more precise than there is ground for.</p>
-------------------------	--

### VERSION 1 – AUTHOR RESPONSE

Reviewer 1:

The described method appears well designed, it should provide the expected results.

-We thank the reviewer for the kind words and for taking the time to read through our manuscript.

Reviewer 2:

Well done on a very interesting and worthwhile study. The study is well thought-out and planned. However, some minor amendments and suggestions are proposed.

-We thank the reviewer for the compliments and comments that have helped us to further improve our manuscript. Below we will address your comments point-by-point.

1) The title is a little confusing. Are you referring to maritime pilots or healthy middle-aged men? Or is it health middle-aged maritime pilots? More clarity is needed here.

-We agree with the reviewer that the title is a little bit confusing, therefore we changed the title for more clarity.

2) Amendments are needed to the way in which you state your objective. What is meant by "prolonged abnormal sleep behaviour". This needs to be more specific as to what exactly you are referring (see comment below).

Overall the abstract is good. However, you need to be very clear on what you mean by disturbed sleep. Throughout the piece you make reference to sleep disturbance and sleep deprivation however, these have different meanings. If you are referring to sleep loss it could have occurred due to a number of reasons - e.g., sleep disturbance, sleep restriction, sleep deprivation - you need to be very clear on which you are referring .

-We agree with the reviewer that the usage of the wording for poor sleep varies within the text and that this can be confusing. With 'prolonged abnormal sleep behavior' we mean chronic partial sleep deprivation caused by external factors (work schedules). We took the comment into account and changed the wording to 'partial sleep deprivation' throughout the whole piece to make this more clear and consistent.

3) In one of your limitations you suggest that your unique cohort of maritime pilots may not be comparable to the control group to some extent - can you elaborate on this further as to why you think this might be the case.

-Thank you for this comment, we agree that it would be favorable to elaborate on this part, therefore we added some examples for more clarity (page 3).

4) Whilst it is important to mention the effects AD has on sleeping patterns, more emphasis is needed on the proposed impact of sleep on the development of AD as this is the focus of your research.

You say that poor sleep " could represent an early symptom that precedes the clinical manifestation of AD" - do you not mean this could be a factor that potentially contributes to the development of AD?

-Thank you for this important point, we fully agree that the emphasis within the introduction needs to be on the impact of poor sleep on the development of AD. We changed the approach of the introduction to put more emphasis on the role of poor sleep as a risk factor for the development of AD (instead of focusing on how AD can lead to poor sleep in too much detail).

5) I would like to see a little more clarity in your writing - this research is looking at the effects of sleep on the development of AD. Overall, the writing could be a little more concise throughout.

-Thank you for the recommendation on the writing. We tried to make the text a little bit more concise throughout.

6) You cannot state that this research "is caused by an external factor (work) and not by an intrinsic sleeping disorder" - unless you test and control for these.

-Thank you for your comment. We excluded sleeping disorders through a health questionnaire, and by verifying that none of them took any sleep medication. We added information on this on page 12 within the discussion in the main document.

7) You should not make the assumption that the week off for pilots consists of sleep loss due to social activities. I would like to see a little more information on typical schedules worked by the pilots to give the reader a better indication of pilots working and sleeping schedules.

-Thank you for this suggestion. This assumption was made based on subjective impressions of the participants. We agree however that this is an assumption without any measurable evidence, we deleted the sentence (see page 6).

8) In your methodology it is suggested that pilots work 4 days X 24 hour schedules - is this correct? This would mean a 96 hour shift with 2 hours rest in between every 2 hours? You need to make this clearer for the reader (a diagram might help here?).

-We agree with the reviewer that the description of the work schedules is not clear enough and we understand that we might have caused some confusion about the actual work hours. Therefore, we formulated a new description (on page 6), which gives a better understanding of their working schedules. However, a precise way of describing it is not possible due to the high irregularity of their working hours.

9) Will you be employing an upper and lower age limit (in addition to the +15 years experience)? You may need to consider the impact of age on sleep in older individuals.

-Thank you for this recommendation. A lower age limit has been given on page 6, however we also added a maximum age for participation in the revised manuscript. Most of the maritime pilots have to retire when they are 60 years old (in some cases they are allowed to work 2 or 3 more years), this is why we chose to set the age limit at 60.

10) Have you considered controlling for smoking and caffeine intake during your study (or at least monitoring it)? These will have an influencing impact on alertness and performance.

-Thank you for this suggestion. We did monitor smoking habits within the general questionnaire, however only 2 of our participants were smokers, versus 38 non-smokers. Cognitive testing will be performed in the morning, after an overnight PSG, in a controlled setting after breakfast. Maritime pilots and controls will be allowed to use their normal amount of coffee or tea with breakfast. We do not expect important differences in caffeine intake between the maritime pilots and the controls.

11) Will participants be fed during the study? It might be beneficial to provide more information on this.

-We agree with the reviewer that it is important to monitor food and beverage intake especially before the PET-CT scan. Therefore information about food intake before the PET-CT scan is added on page 7 (section 2.2 Experimental design). For the PSG, see comment above.

12) What will the findings from the Amyloid PET-CT be compared to? This needs to be made more clear for the reader.

-We agree that the rating of the PET-CT scans is not clearly described. We added some more information within the 'Amyloid PET-CT scan with co-registered magnetic resonance imaging (MRI)' section in the main document on page 10.

13) What is the purpose of the accelerometer? It is mentioned once but I would like to see a little more clarity for its purpose.

-The purpose of the accelerometer is described on page 8 in section 2.4 'Sleep measurements'. It is an additional tool to gather objective sleep measurements.

14) The follow-up questions will provide a clearer picture on the relationship between these variables. However, I would like to see slightly more detail - e.g., what is meant by a "cognitive complaint"? Why have you decided to do this every 5 years there after?

-Thank you for your feedback on this. We agree that the description of the future visits is a little vague. Therefore we added a section about how we want to identify cognitive impairment and what we actually mean with the terminology within the section 'Future visits' on page 10 and 11 in the main document.

We chose to examine the participants every 5 years to limit the burden for participants. Furthermore, we do not expect any rapid changes or fast decline in cognitive function, because the progression of Alzheimer's disease is a slow process. 5 years is a good interval to monitor any significant changes.

Reviewer 3:

Dear editor.

I have read the article carefully. While I believe changes to be necessary, the concept is appealing and the research question is of great importance. I would recommend further reviewing with the goal to publish.

There are some considerations before going further. Participants, power and longitudinal problems.

-We thank the reviewer for recognizing the importance of our research question. Below we will address your comments and our responses to them point-by-point.

1) The title of the paper suggests that this is a prospective study, yet the prospective part is only briefly described. No power is calculated for this part and it is not reproducible as it stands right now. Without changes to the protocol, this should be considered a case-control study.

-Thank you for this recommendation, we agree and changed the title to 'Methodology of a case-control study' (instead of prospective cohort study).

2) Matched controls are not followed hence aging effect cannot be assessed.

-Thank you for your comment, this is an interesting point for discussion. In the section 'Future visits' we explain that we want to follow the participants who underwent the amyloid PET-CT scan in order

to investigate the association between a positive amyloid PET-CT scan and the actual development of AD. Thus, we will not look at the aging effect in general but at the correlation between having a positive or negative amyloid PET-CT scan and what that means for future development of AD. We expect that some of the participants will have positive scan and some will have a negative scan. Therefore we can divide the group again into healthy participants (with a negative amyloid PET-CT scan) and participants at risk for developing AD (with a positive amyloid PET-CT scan).

3) There is also concerns regarding the power in general as this protocol aims to follow pathophysiology and not normal physiology, which is the case in previous studies made on healthy volunteers with short experimental sleep deprivation.

-We agree with the reviewer that the power of the PET-CT (n=20) is limited. We will take this into account while interpreting and rating the PET-CT images. A bigger sample (and therefore more power) would have been desirable, but unfortunately that was not possible due to logistic and financial issues.

4) Minor concerns include typos, unnecessary self-citing, lack of N in abstract and an overstated claim of importance. There is also presentation of statistics in the introduction that is more precise than there is ground for.

-Thank you for this comment. We added the number of participants (N) in the abstract and deleted some unnecessary quotation of statistics in the introduction (see for example page 1).

#### VERSION 2 – REVIEW

<b>REVIEWER</b>	Anna Donnla O'Hagan Dublin City University, Ireland
<b>REVIEW RETURNED</b>	22-Apr-2019

<b>GENERAL COMMENTS</b>	<p>Well done - this is much improved on the last version. Again I think this is a very interesting and worthwhile study. There are just a few minor amendments I feel are needed.</p> <p>You state that this study is looking at "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". But as you have not recorded their sleep over the past 15 years you cannot say this. What you are really looking at in this study is the effects of long-term exposure to maritime shift work schedules (i.e. 24 hour shifts over a 7-day period) and its effects on cognitive function and amyloid accumulation as a result of its impact on sleep. So in basic terms you are looking at sleep in shift work and the development of AD. I believe referring to it in this way will make you study stronger and easier to follow.</p> <p>The second thing that needs a little more clarity is your reference to sleep deprivation vs sleep loss vs sleep fragmentation. So we know these pilots have long-term exposure to these shift working schedules and as a result have experienced long periods of wakefulness and short sleep periods. Sleep loss refers to a</p>
-------------------------	--

	<p>complete lack of sleep across a certain period of time or a shorter than optimal sleep period. Sleep loss may occur as a result of total sleep deprivation (i.e., no sleep for a period of time (at least one night) resulting in prolonged wakefulness), chronic sleep restriction (i.e., shorter sleeping period which is less than an individuals typical baseline sleep or sleep required on a regular basis for optimal performance) or sleep fragmentation or disruption (i.e., the interruption or fragmentation of sleep in which regular arousals interfere with the normal dynamics of sleep). You need to be very clear as to what your participants have experienced and then use that term throughout your study as opposed to switching between different terms which mean different things. Your participants may have been exposed to more than one of these things and therefore you would refer to it as the umbrella term of sleep loss (but indicate in your work that, for example, sleep loss occurred among these participants as a result sleep deprivation and sleep restriction due to their working schedules).</p> <p>Do you have an example of a typical working schedule for a maritime pilot? This might help in the explanation for their shift working patterns.</p> <p>Having the age range up to 60 years old means you may need to take in to consideration additional aging factors and associated effects on sleep.</p> <p>Overall well done - I think this will make a great study and I look forward to reading it when it is completed!</p>
--	---

<b>REVIEWER</b>	Martin Olsson Sahlgrenska Academy, University of Gothenburg
<b>REVIEW RETURNED</b>	29-Apr-2019

<b>GENERAL COMMENTS</b>	No further editing is needed. Good luck.
-------------------------	--

### VERSION 2 – AUTHOR RESPONSE

Reviewer 2

Well done - this is much improved on the last version. Again I think this is a very interesting and worthwhile study. There are just a few minor amendments I feel are needed.

-Thank you for the kind words.

1) You state that this study is looking at "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". But as you have not recorded their sleep over the past 15 years you cannot say this. What you are really looking at in this study is the effects of long-term exposure to maritime shift work schedules (i.e. 24 hour shifts over a 7-day period) and its effects on cognitive function and amyloid accumulation as a result of its impact on sleep. So in basic terms you are looking at sleep in shift work and the development of AD. I believe referring to it in this way will make you study stronger and easier to follow.



2) The second thing that needs a little more clarity is your reference to sleep deprivation vs sleep loss vs sleep fragmentation. So we know these pilots have long-term exposure to these shift working schedules and as a result have experienced long periods of wakefulness and short sleep periods. Sleep loss refers to a complete lack of sleep across a certain period of time or a shorter than optimal sleep period. Sleep loss may occur as a result of total sleep deprivation (i.e., no sleep for a period of time (at least one night) resulting in prolonged wakefulness), chronic sleep restriction (i.e., shorter sleeping period which is less than an individual's typical baseline sleep or sleep required on a regular basis for optimal performance) or sleep fragmentation or disruption (i.e., the interruption or fragmentation of sleep in which regular arousals interfere with the normal dynamics of sleep). You need to be very clear as to what your participants have experienced and then use that term throughout your study as opposed to switching between different terms which mean different things. Your participants may have been exposed to more than one of these things and therefore you would refer to it as the umbrella term of sleep loss (but indicate in your work that, for example, sleep loss occurred among these participants as a result sleep deprivation and sleep restriction due to their working schedules).

-Thank you for your comments and helpful suggestions. These are two very important and relevant points. You suggest to use the term 'maritime shift work'. However, shift work implies at least some regularity (e.g. night shifts, early shifts or late shifts), where people for example shift their working (and waking) hours from day to night for a fixed period, but in that period will have a more or less regular sleeping period (in this case during the day). Therefore, we chose to avoid describing the maritime pilot occupation as shift work, because it would suggest more regularity than there really is. Their work schedules are completely unpredictable, as explained on page 6 in the manuscript. For that reason, we will avoid the term 'shift' when we refer to their work. However, we agree that the description of the maritime pilots as being 'chronically partially sleep deprived' is rather an assumption, because indeed, we did not record their sleep during the last 15 years. We therefore took your advice from the second comment and will stick to the umbrella term 'sleep loss' when describing the exposure of the maritime pilots to irregular work. We use this term throughout the whole manuscript instead of switching between terms. In the introduction (page 5) of the manuscript, we define what we mean by 'sleep loss', indicating that sleep loss in these maritime pilots can refer to sleep restriction, sleep deprivation or sleep fragmentation.

3) Do you have an example of a typical working schedule for a maritime pilot? This might help in the explanation for their shift working patterns.

- Good idea, thank you. We added a figure (figure 1) on page 6, describing a workweek of one of the maritime pilots to give an example of the irregular working schedule.

4) Having the age range up to 60 years old means you may need to take in to consideration additional aging factors and associated effects on sleep.

-Thank you for your comment, we agree. We added a statement about aging effects as possible limitation in the strengths and limitations section on page 3, and by indicating that we will take this into account when analyzing the results of our study.

Overall well done - I think this will make a great study and I look forward to reading it when it is completed!

-Thank you for your time and effort to review our manuscript.

Reviewer 3

No further editing is needed. Good luck.

- Thank you.

**VERSION 3 - REVIEW**

<b>REVIEWER</b>	Anna Donnla O'Hagan Dublin City University, Ireland
<b>REVIEW RETURNED</b>	21-May-2019

<b>GENERAL COMMENTS</b>	Well done - great job! Best of luck in your future research endeavours!
-------------------------	--