LINKING BRAIN VASCULAR FUNCTION WITH NEUROBEHAVIORAL FUNCTION IN OSA

Protocol V1.0 April 2018

FULL TITLE:
The effects of continuous positive airway pressure (CPAP) treatment for obstructive sleep apnoea (OSA) on cerebrovascular and neurobehavioral function.

The effects of continuous positive airway pressure (CPAP) treatment for obstructive sleep apnoea (OSA) on cerebrovascular and neurobehavioral function.

Short title: **Linking Brain Vascular Function with Neurobehavioral Function in OSA**

Principal Investigator:

Associate Professor Craig Phillips, NH&MRC Dementia Research Leadership Fellow, Sleep & Circadian Research Group, Woolcock Institute of Medical Research

Co Investigators:

Professor Lindy Ray, Conjoint Senior Principal Scientist, Neuroscience Research Australia, University of NSW

Associate Professor David Wang, Clinical Associate Professor, Woolcock Institute of Medical Research and Royal Prince Alfred Hospital

Professor Lynne Bilston, Conjoint Professor, Neuroscience Research Australia, University of NSW

Dr Julia Chapman, NeuroSleep Postdoctoral Fellow, NeuroSleep CRE & CIRUS, Woolcock Institute of Medical Research

Dr Angela D'Rozario, NHMRC & ARC Dementia Early Career Fellow, Woolcock Institute of Medical Research

Dr Camilla Hoyos, NHMRC & ARC Dementia Early Career Fellow, Woolcock Institute of Medical Research

Professor Ron Grunstein, NHMRC Senior Principle Research Fellow, Woolcock Institute of Medical Research

Associate Professor Brendon Yee, Clinical Associate Professor, Woolcock Institute of Medical Research and Royal Prince Alfred Hospital

Dr Yizhong Zheng, Respiratory & Sleep Physician, Woolcock Institute of Medical Research and Royal Prince Alfred Hospital

Lead Study Coordinator:

Associate Professor Craig Phillips, NH&MRC Dementia Research Leadership Fellow, Sleep & Circadian Research Group, Woolcock Institute of Medical Research

Trial Registration: This study will be registered with the Australasian and New Zealand Clinical Trials Registry (ANZCTR) - www.anzctr.org.au

Protocol Version: 1.0 24th April 2018

Funding source: This study is funded by CIRUS, Centre for Sleep and Chronobiology

This study will be performed at the Woolcock Institute of Medical Research, University of Sydney, Glebe, NSW, The Royal Prince Alfred Hospital, Camperdown NSW, and at the Neuroscience Research Australia Imaging Centre, Randwick NSW.

Contact person: Associate Professor Craig Phillips
Email: craig.phillips@sydney.edu.au
Woolcock Institute of Medical Research
Ph: 9114 0001

PROTOCOL REVISIONS: N/A

Protocol Rev Level	Changes Made (Date)	Date ethics amendment submitted	Date ethics amendment approved	Contributors	PIS Version
0.0					

Italics denote changes to the protocol requiring ethical approval.

Table of Contents

1. Introduction

1.1. Background and rationale

OSA is a condition in which the upper airway collapses during sleep, leading to episodes of sleep fragmentation and hypoxemia (Jordan 2014). The standard treatment for OSA is continuous positive airway pressure (CPAP). OSA has been linked to a decrease in neurocognitive function in some patients and potentially to the development of dementia, however the mechanisms remain unclear. Whilst hypoxia and sleep fragmentation may be implicated, there is significant inter-individual variability in the neurocognitive effects even with a similar severity of OSA (Bucks 2012).

When healthy individuals are exposed to hypoxemia, there are auto-regulatory mechanisms in the cerebral circulation that protect the brain by causing vasodilatation and an increase in blood flow to increase oxygen supply to brain tissue. In this context, an increase in arterial stiffness and central arterial pulsatility has been implicated in cognitive decline (Webb 2016). It has been shown that OSA results in significant variations in cerebral hemodynamics (Hou et al. 2014; Pizza 2010) and it is possible that in some patients with OSA, an increase in cerebral arterial stiffness may result in the inability of the vasculature to compensate for hypoxemia during apnoeas through an increase in cerebral blood flow. This may in turn result in greater degrees of neurological deficits seen in some OSA patients (Lawley 2017) and potentially an increase in deposition of amyloid-ß – a protein linked to dementia development (Seshadri 2015). The association between sleep apnoea and dementia may also be associated with reduced clearance of amyloid-ß from the brain by the glymphatic system. The glymphatic system has been shown to be most active during sleep (Jessen et al. 2015) so it is possible that sleep fragmentation and/or cerebral vascular dysfunction leads to reduced clearance.

This clinical study will investigate the link(s) between cerebrovascular response to hypoxemia, arterial stiffness, brain tissue hypoxia, brain amyloid-ß, glymphatic clearance and neurocognitive function in OSA patients, before and after CPAP treatment.

2. Study Objectives

1. Quantify brain tissue oxygenation during hypoxia exposure, before and after treatment with CPAP.

2. Quantify arterial stiffness, before and after treatment with CPAP.

3. Quantify brain levels of amyloid-ß, before and after treatment with CPAP.

4. Quantify cerebral blood flow during sleep in untreated OSA.

5. Quantify neurobehavioral function, before and after treatment with CPAP.

6. Assess whether brain tissue hypoxia is correlated with poor neurobehavioral function in OSA.

3. Experimental Design

Pilot observational intervention study

4. Study Setting

This study will be conducted across several sites across the Sydney metropolitan area: the Woolcock Institute of Medial Research in Glebe which houses the Woolcock clinic and a purpose built 14-bed sleep and chronobiology laboratory, the Royal Prince Alfred Hospital in Camperdown and the Neuroscience Research Australia Imaging Centre in Randwick.

5. Eligibility Criteria

5.1. Inclusion criteria

- Age 20-65;
- PSG confirmed ODI $\geq$ 15 and AHI $\geq$ 30 with a diagnosis of moderate-severe hypoxemic OSA;
- Recommended for CPAP treatment by the attending sleep physician
- Ability to perform neurobehavioral tests;
- Able to give informed consent;
- Fluent in English.

5.2. Exclusion criteria

- Clinically significant co-morbidity;
- History of head injury or psychiatric/neurological disorder (including stroke);
- Use of CNS active agents;
- Heavy alcohol consumption (40g daily);
- Current shift-worker or travelled overseas within the last 2 weeks;
- Previously used CPAP;

- Excessive symptoms and/or very severe OSA which in the opinion of the treating physician requires urgent CPAP treatment

6. Study Intervention

6.1. Treatment period of continuous positive airway pressure (CPAP) therapy

Outcomes will be assessed before and after a minimum of 8 weeks of CPAP therapy.

6.2. Delivery of the CPAP intervention

The study intervention, continuous positive airway pressure (CPAP) therapy, is the routine gold-standard treatment for obstructive sleep apnoea. Its mode of delivery is through a CPAP device and a face and/or nasal mask worn by the patient nightly whilst sleeping. All participants enrolled in the study will be loaned a CPAP machine and mask for the duration of the study. Education and therapy support will be provided by CPAP therapists at the Woolcock either in person or over the phone. Therapy usage will be measured by the device and information downloaded will be used to troubleshoot and guide treatment as per usual clinical care and in study outcomes.

6.3. Discontinuing

6.3.1. *Withdrawal criteria*

Participants will be informed that they have the right to withdraw from the study at any time, without prejudice to any medical care, and are not obliged to state their reasons. Additionally the investigator may withdraw a participant at any time for the following reasons:

- Inadequate CPAP compliance assessed by CPAP therapist during follow-up care
- If any of the study exclusion criteria are diagnosed
- Protocol violations
- Serious adverse events

6.3.2. *Discontinuation of the study*

The study may be discontinued at any time on the advice of the responsible principal investigators on the basis of new information regarding safety. Additionally, the study may be terminated if progress is unsatisfactory.

In case of premature termination or suspension of the study, the investigator will inform the study participants and ensure appropriate follow up occurs. In addition, the approving ethics committee will be informed.

6.4. Procedure to withdraw

If a participant fails to return for follow-up or discontinues for personal reasons, attempts will be made to determine whether the reason for not returning is not an adverse event (bearing in mind that the participant is not obliged to state his/her reasons). Participants with clinically significant abnormalities requiring discontinuation will be followed until recovery from the abnormality, if possible. If the study is discontinued for safety reasons, the investigators will contact all affected

participants within a reasonable time frame to inform them of the termination of their involvement in the study. Participants discontinuing from the study may be replaced. A new participant number must be issued for the new participant.

7. Outcomes

7.1. Primary outcome measure:

- Change in brain tissue oxygen saturation during a hypoxemic challenge after 8+ weeks of CPAP treatment, relative to baseline.

7.2. Secondary outcome measures:

All secondary outcome measures will be collected at the 8 week time point after CPAP treatment, and expressed relative to baseline values

- Change in arterial stiffness during a hypoxemic challenge after 8+ weeks of CPAP treatment, relative to baseline.
- Multi-domain neurobehavioral performance
- Declarative memory
- Procedural memory
- Heart rate variability during overnight PSG
- Self-reported mood and sleep ratings assessed by questionnaire
- Change in amyloid-ß after 8+ weeks of CPAP treatment, relative to baseline.

8. Participant Timeline

8.1. Enrolment and screening

Patients will have undergone an overnight sleep study (PSG) and been diagnosed by a sleep physician with moderate-severe hypoxemic OSA with an Oxygen Desaturation Index (ODI) $\geq$ 15 and Apnoea Hypopnoea Index (AHI) $\geq$ 30.

If a participant is willing and eligible they will then have the study fully explained by a study investigator (a person other than the treating sleep physician) and their written informed consent to enrol in the study will be obtained at the screening visit. Potential participants will be encouraged to discuss their participation in this study with family members and/or friends who are able to support them making their decision. The screening visit will take approximately 1.5-2 hours. After consenting, a medical assessment and simple screening questionnaires will be completed.

8.2. Karolinska Sleep Diary

A 7-day sleep diary will be given to the participant to complete at the end of the screening visit to capture information about sleep-wake schedule prior to the sleep laboratory assessment.

8.3. Baseline Testing

8.2.1 Pulse wave analysis

Prior to the overnight sleep study participants will undergo pulse wave analysis to measure arterial stiffness. This will involve fitting a blood pressure cuff around the participant's arm and measuring blood pressure for approximately 1 minute.

8.2.2 Sleep Study (PSG) with NIRS and beat-to-beat finger blood pressure

Participants will undergo an overnight sleep study in the laboratory at the Woolcock Institute with NIRS to continuously monitor cerebral tissue oxygenation. NIRS sensors will be positioned on the patient's forehead superior to the temporalis muscle and lateral to the superior sagittal sinus and secured to the skin.

During the sleep study, additional physiological signals will also be recorded to discern sleep state including brain electrical activity (electroencephalogram, EEG), eye movements (electrooculogram, EOG) and chin muscle movements (electromyogram, EMG). A nasal airflow cannula and belts around the waist and chest will monitor breathing and an oximeter probe will be placed on the finger to measure oxygen levels in the blood. Finger cuffs will be fitted to two additional fingers to measure beat-to-beat blood pressure. Heart electrical activity (electrocardiogram, ECG), leg movements (EMG), sleeping position and snoring will also be recorded.

8.2.3 Neurobehavioral Performance and Memory Tasks

Participants will undertake the following tests:

- AusEd Driving Task with high-density EEG – before and after sleep
- Karolinska Sleepiness Scale (KSS) – Immediately before and after sleep
- Karolinska Drowsiness Test (KDT) with high-density EEG – Immediately before and after sleep
- Psychomotor Vigilance Task (PVT) – Prior to memory tests before and after sleep
- Declarative memory – Word-pairs Task 1-2 hours before habitual sleep and 1 hour after awakening
- Procedural memory – Finger Tapping Motor Task 1-2 hours before sleep and 1 hour after awakening

In addition to these tests, the morning after the sleep study participants will complete the following questionnaires and neurobehavioral assessments, which will take approximately 30 minutes to complete:

Questionnaires & IQ Test:

- The Epworth Sleepiness Scale (ESS)
- Insomnia Severity Index (ISI)
- Pittsburgh Sleep Quality Index (PSQI)
- Wechsler Test of Adult Reading (WTAR)
- Depression and Anxiety Severity Scale (DASS)

Neurobehavioral Assessment

- N-back (1-back and 2-back) after the PVT test
- The Stroop Task
- Letter Cancellation Task

See Neurobehavioral Performance Assessments and Memory Tasks for further details.

8.2.4 Hypoxemic challenge test

The hypoxemic challenge test will be conducted at the Royal Prince Alfred Hospital. Participants will be required to breathe through a mouthpiece attached to ventilatory response equipment which will simulate hypoxic and hypercapnic conditions (lowest PO_2= 50 mmHg) for a brief period of 5-10 mins over three sessions. Brain tissue hypoxia will be measured using a multi-probe NIRS device. Beat-to-beat finger blood pressure will also be measured.

8.2.5 MRI Sleep study

Following the sleep study and hypoxemic challenge, participants will be invited to undergo a further MRI study to assess brain blood perfusion and amyloid-ß levels. This study will occur within 2 weeks of the baseline study. Participants will be asked to come to NeuRA late in the evening, at approximately 10:00pm to undergo the MRI study. In keeping with the non-invasive principles of this study, contrast agents will not be used. Earplugs will be provided to minimise noise. Peripheral blood oxygen saturation will be recorded throughout MRI scanning using an MRI-safe finger pulse oximeter.

Several measurements will be made while the patient is awake, including:

1. Baseline phosphorylation level (^{31}P) using magnetic resonance spectroscopy (MRS) – 10 minutes
2. Scout image, high resolution T1, high angular resolution diffusion-weighted imaging (HARDI) and lactate spectrum – 25 minutes
3. Cerebral amyloid-ß levels using phase difference enhanced imaging (PADRE) – 1 minute

The patient will then be asked to try to sleep. While the patient sleeps the following measurements will be made:

1. Cerebral perfusion using 4D arterial spin labelling (4D ASL) – up to 60 minutes
2. Diffusion and lactate using MRS and HARDI – 25 minutes

The primary aim of these measurements is to measure cerebral perfusion whilst the patient is having apnoeas. The repeat lactate and diffusion scans will only be performed if time permits after successfully recording cerebral perfusion during apnoeas. Once these measurements are complete, or 90 minutes has elapsed since beginning scanning, whichever occurs first, MRI scanning will cease and the participant will be free to go home for the remainder of the night.

8.3 CPAP Intervention: Routine clinical care for commencing CPAP

8.3.1 CPAP titration study

The treatment component of this study is part of the clinical pathway for patients recommended for CPAP use. Routine clinical care involves an in-lab CPAP titration study where the pressure of the CPAP device is adjusted by a trained sleep technologist in order to determine the adequate CPAP pressure required to keep the airway open during sleep. This sleep study is not a research focused visit however information derived from the CPAP titration study (CPAP pressure and mask selection) will be used in the study protocol.

8.3.2 CPAP equipment pick up and ongoing therapy support from CPAP therapist

As part of routine clinical care and following the CPAP titration study, the CPAP therapist will review the CPAP titration study and prescribe an individual CPAP pressure for the patient. The participant will return to collect a designated CPAP device that the therapist has programmed with this pressure. After starting therapy, the participant will be asked to return at 2 weeks for a device download and any required troubleshooting, and at 6 weeks if required. Participants will continue to use their CPAP machine for an 8 week treatment period.

8.4 TREATMENT TESTS: Pulse wave analysis, neurocognitive assessments, hypoxemic challenge and MRI

Following a minimum of 8 weeks of CPAP use, participants will repeat the pulse wave analysis, neurobehavioral assessments and hypoxemic challenge. Participants will also repeat an MRI study (cerebral amyloid-ß only) if this was performed before CPAP.

8.5 Follow up with sleep physician and CPAP equipment return

The participant will see their Sleep Physician promptly following the treatment tests (same day ideally) to discuss ongoing OSA management and their treatment plan.

Participants will be asked to return the loan CPAP equipment after the study has completed. Ongoing treatment will then be managed as recommended by the treating physician. From this point, any ongoing costs associated with treatment will be the responsibility of the patient.

9 Reimbursement

Secure on-site parking is available for participants and reasonable reimbursement for transport costs will be offered. This will include assistance with attending and returning home from the NEURA centre.

Fig 1: Timeline for study protocol * Denotes overnight sleep study at Woolcock and/or NeuRA Imaging Centre.

Screening and Consent	•Medical assessment to confirm eligibility to participate in the study
Baseline Tests*	•PSG, NIRS & FBP sleep study •Karolinska Drowsiness Test and AUsEd driving task with HD-EEG •Pulse wave analysis •Hypoxaemic challenge test •Neurobehavourial Performance tasks •MRI sleep study - cerebral blood flow, amyloid-ß and glymphatic clearance
CPAP Titration*	•Education Session before overnight sleep study •Beginning of 8+ week CPAP treatment period with ongoing support at the Woolcock
Patient CPAP equipment pick up	•Equipment is on loan during patients participation in the research study (8+ weeks)
2 weeks of CPAP - w/ CPAP therapist	•CPAP download/ SEMSA questionnaire
6 weeks of CPAP - w/ CPAP therapist	•CPAP download/ SEMSA questionnaire
8+ Weeks - Treatment Tests	•Pulse wave analysis •Hypoxaemic challenge test •MRI (amyloid-ß only) •Neurobehavourial performance tasks
8+ Weeks - CPAP therapist appointment	•CPAP download •Return of CPAP equipment
8+ weeks - CPAP Follow up with Sleep Physician	•Discuss ongoing management and treatment plan

10 Sample size

In this pilot study a sample size of n=30 will be required to complete this study. Assuming a 10% attrition rate, it will be necessary to recruit 33 patients. If a higher than anticipated rate of attrition is experienced then further subjects will be recruited in order to maintain a total sample size of 30.

11 Recruitment

Number and source of participants:
Thirty-three patients who meet a clinical diagnosis of moderate-severe OSA, with ODI $\geq$ 15 and AHI $\geq$ 30 and who are recommended for CPAP treatment will be identified by sleep physicians in consultation at the Woolcock clinic. Potential participants will be initially approached by their sleep physician and will be provided with a Participant Information Sheet (PIS) for this study. Participants will have as long as they like to consider participation in this study, however the commencement of CPAP would result in their ineligibility. If patients express a desire to participate, they will be invited to attend the Woolcock Institute to provide informed consent for their participation in this study and then undergo further screening to confirm their eligibility. Suitability for participation will be assessed by sleep physicians in consensus with study investigators. Following informed consent and screening with the study coordinator, subsequent appointments for research visits will be scheduled.

12 Study Procedures and Assessments

12.2 ENROLMENT AND SCREENING

The screening visit will take approximately 2 hours. During this visit we will confirm participant eligibility through a screening checklist, medical assessment and screening questionnaires.

12.2.1 Informed consent

Each potentially eligible participant will be informed of the study's objectives and overall requirements using the Participant Information Sheet and Consent Form, and they will be provided with a copy of the forms by their treating sleep physician. If the participant is willing to participate in the study, they will be requested to give written informed consent. A researcher, other than the treating sleep physician, will be responsible for gaining patient consent. As part of the consent process potential participants will be encouraged to discuss their participation in this study with family members and/or friends who are able to support them making their decision.

Consent will be specific to this study, however participants will be asked to opt-in to provide unspecified consent for use of their MRI data and will also be asked to opt-in to being contacted by researchers for future research.

Participants will have the opportunity to opt-in to be contacted by researchers to be involved in any follow-up studies that arise from this research. Any decision to opt-in or opt-

out of being contacted about future research will not affect participant's eligibility to take part in this study.

12.2.2 Anthropometry

Anthropometric measurements such as blood pressure, height, weight, neck, hip and waist circumference as well as handedness and head measurements will be taken at the screening visit by the study coordinator.

12.2.3 Medical History and Medication

A medical history and concomitant medication information will be collected by the study coordinator and sleep physician.

12.2.4 Lifestyle, Ethnicity and Education

Questions about ethnicity, lifestyle such as exercise and activity levels, alcohol and caffeine intake, and highest level of education achieved will be asked by the study coordinator.

12.2.5 Epworth Sleepiness Scale (ESS) Questionnaire

The ESS questionnaire asks an individual how likely they are to fall asleep in 8 common every-day situations. ESS scores are calculated by taking the sum of the responses for all 8 questions, yielding a score between 0 and 24. Greater scores correlate with increased reported somnolence. Scores above 10 are usually regarded as a sign of excessive sleepiness.

12.2.6 Self-Efficacy Measure for Sleep Apnea (SEMSA) Questionnaire

This is a disease-specific measure of pre-treatment expectancies regarding OSA and CPAP treatment in adults. It is designed to assess adherence-related cognitions. It consists of 26-items with 3 subscales (Risk perception, outcome expectancies, and treatment self-efficacy).

12.2.7 Karolinska Sleep Diary

A 7-day sleep diary will be given to the participant to complete at the end of the screening visit to capture information about sleep-wake schedule prior to the sleep laboratory assessment.

12.3 SLEEP LABORATORY ASSESSMENTS

All procedures described in this section are repeated before and after treatment, with the exception of the polysomnography, cerebral blood flow and glymphatic clearance study (12.3.3, 12.3.8.1 & 12.3.8.2) which will only be done prior to treatment.

12.3.1 Sleep Symptoms and Mood Questionnaires

12.3.1.1 Epworth Sleepiness Scale

Subjective ratings of sleepiness will be measured (see 12.1.5. for ESS description)

12.3.1.2 Insomnia Severity Index (ISI)

The ISI measures the nature, severity and impact of insomnia and monitor treatment response. It is a 7-item patient reported outcome measure that probes the severity of both the night time and daytime impact of current symptoms of insomnia (the past 2 weeks) and takes around 3 minutes to complete. Each item uses a 5-point Likert scale to capture a rating (0 = no problem; 4 = very severe problem) which add up to: no insomnia (0 – 7); sub-threshold insomnia (8 – 14); moderate insomnia (15 – 21); and severe insomnia (22 – 28).

12.3.1.3 Pittsburgh Sleep Quality Index (PSQI)

The Pittsburgh Sleep Quality Index (PSQI) is a self-rated questionnaire assessing sleep quality and sleep habits during the past month. The PSQI measures sleep quality using the seven domains of subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications and daytime dysfunction.

12.3.1.4 Depression and Anxiety Severity Scale (DASS)

This shortened 21-point version of the DASS is a self-report instrument designed to measure the three related negative emotional states of depression, anxiety and tension/stress.

12.3.1.5 Karolinska Sleepiness Scale (KSS)

Participant will be asked to rate their sleepiness before and after the KDT using the KSS, a 9 point likert scale designed to assess their state of sleepiness at any given time.

12.3.2 Pulse wave analysis

Prior to the overnight sleep study participants will undergo pulse wave analysis to measure arterial stiffness. This will involve fitting a blood pressure cuff around the participant's arm and measuring blood pressure for approximately 1 minute. The participant will be required to sit comfortably in a chair for this task.

12.3.3 Polysomnography (PSG) with NIRS and beat-beat finger blood pressure

An overnight sleep study with simultaneous NIRS and beat-beat finger Blood pressure will be conducted in the ACCESS laboratory at the Woolcock Institute. Standard PSG measurements will be recorded. These include a nasal airflow piece, a belt around the waist and chest, and an oximeter probe on the finger to monitor breathing and oxygen levels in the blood. Heart rate (ECG), leg movements, sleeping position and snoring will also be recorded. NIRS sensors will be positioned on the patient's forehead superior to the temporalis muscle and lateral to the superior sagittal sinus and secured to the skin. A finger cuff will be fitted to measure beat-to-beat blood pressure.

12.3.4 Karolinska Drowsiness Test (KDT) (7.5 mins) with high-density EEG

EEG activity is recorded whilst the participant is sitting quietly and with eyes open for 2.5 mins, eyes closed for 2.5 mins, then eyes open again for 2.5 mins to assess EEG markers of

physiological sleepiness. The KDT will be administered before and after overnight sleep recordings.

12.3.5 Neurobehavioral Performance Assessments

In the morning after the PSG, participants will be asked to complete a simple IQ test (Welscher Test of Adult Reading) and perform a computerised neurobehavioral test battery, comprising of the psychomotor vigilance task (PVT), the n-back, stroop test, letter cancellation task. Participants will also undergo declarative and procedural memory (e.g. learning a list of words, and tapping fingers in sequence) testing before and after sleep. These tests will take approximately 1.5 hours to complete.

12.3.5.1 Welscher Test of Adult Reading (WTAR) (5 min)

The Wechsler Test of Adult Reading (WTAR) is a neuropsychological assessment tool used to provide a measure of premorbid intelligence, the degree of intellectual function prior to the onset of illness or disease. It is composed of 50 irregularly spelled words and begins by presenting the first word card and prompting the patient for a single pronunciation of the word. This procedure continues through all 50 word cards and is discontinued if the patient provides 12 consecutive incorrect pronunciations. Lists of acceptable pronunciations and tape recordings are provided by the publisher to account for words with multiple pronunciation variants. Each correct pronunciation is given a score of 1, with 50 as the maximum raw score which is standardized by age and demographics.

12.3.5.2 Psychomotor Vigilance Task (PVT) - (10 mins)

The PVT will be used to assess sustained attention via a simple reaction time task. The device is a hand-held box with a red light emitting diode display of a three-digit millisecond counter (PVT-192, Ambulatory Monitoring Inc, Ardsley, NY, USA). Participants are instructed to respond as fast as possible when they first see a visual stimulus appear. The time taken to respond to the stimulus is displayed in milliseconds (ms). During each 10 minute PVT session, visual stimuli appear at variable intervals between 2 to 10 seconds. The PVT response variables to be analysed are: a) mean reciprocal reaction time (RT); b) mean of the fastest 10% of RTs; c) mean reciprocal of slowest 10% of RTs; and, d) number of lapses (response time >500ms).

12.3.5.3 n-back - (8 mins)

The n-Back assesses working memory, encompassing short term memory storage and information processing, along with monitoring and updating presented information. For this visuospatial test, the 2-Back will be used as 1-Back is thought to assess vigilance only. The task concerns the order and placement of letters, displayed one at a time, on the screen. The participant is asked to compare the position of a letter displayed on the screen to the position of the letter presented 2 trials previously. For example, for 2-back, the position of the 3rd letter is compared to the position of the 1st letter and the position of the 4th letter to the 2nd letter and so on. If the position of the letters matched, the participant presses 'M' on the keyboard for 'Match' as quickly as possible. If the position of the letters did not match, the participant presses 'N' for 'No Match' as quickly as possible. The first of 50 trials

is presented after 1.5 seconds with subsequent stimulus intervals of 4.5 seconds. Each n-Back task is 4 mins in duration and the percentage of total accuracy will be calculated.

12.3.5.4 Stroop Test - (2 mins)

Stroop test assesses the ability to inhibit pre-potent responses, is typically thought to reflect executive functioning. This specific task is a two part test, which assesses reaction time to colours and words displayed on the computer screen. These tests gauge cognitive interference where processing of information is impacted by the presentation of simultaneous conflicting information. Words (red, green or blue) and three different coloured squares (red, green or blue) are displayed on the computer screen. Participants are required to click on the coloured square that matched either the MEANING (Stroop text) or the COLOUR (Stroop colour) of the word presented. Each test is approximately 45 seconds in duration and variables evaluated are: a) percentage of correct total responses; and, b) average response latency.

12.3.5.5 Letter Cancellation Task - (10 mins)

The LCT evaluates concentration, attention and visuospatial scanning ability or visuospatial neglect as well as measuring accuracy of selective attention. Participants are required to scan a large field of letters on the computer screen for a target capital letter, double space or a mixture of both of these. Participants will be asked to mark as many CAPITAL LETTERS displayed on the computer screen in 60 seconds using the mouse (this is repeated twice - trials1 & 2); then mark as many d o u b l e s p a c e s (trials 3&4), then as many CAPITAL LETTERS and d o u b l e s p a c e s (trials 5&6). On the final screen (trial 7) a combination of the two targets are displayed and participants are asked to complete the test in their own time. Variables evaluated are the mean number (average performance on trials 1-6) of: a) correctly marked targets (average hits); b) missed targets (average omissions); and, c) non-targets incorrectly marked (average commissions). Performance variables assessed during the final trial are: d) correctly marked targets (final hits); e) missed targets (final omissions); f) non-targets incorrectly marked (final commissions); and, g) final trial duration.

12.3.6 Memory Tasks

12.3.6.1 Declarative Memory Task (20-30mins)

The Word Pairs task requires the participant to learn a set of 32 pairs of words (related and unrelated), for example, maple – syrup and desk – ice. In the learning phase, each pair is presented for 5s each, continuously, in random order with blank screen between for 5s. For the first trial participants are also asked to put the words in a sentence or assess the number of syllables. During testing, the first word of a pair is presented for 12s and the participants must *recall* the corresponding word pair (all 32 presented randomly one by one). This is repeated 3 times. In the morning this is repeated and followed by a *recognition* component where they must select the correct pair out of a choice of 4 alternatives (presented for 7s).

12.3.6.2 Procedural Memory Task (6 or 12 mins)

The Finger Tapping Task (FTT) requires participants to type repeatedly a 5-element number on a standard computer keyboard with their non-dominant hand. The specific number sequence, which must be typed, is displayed in front of them on the computer screen at all times. Typing is done in 30 second trials separated by 30 second rest periods. Training (before sleep) involves 12 trials and retest (after sleep) involves 6 trials.

12.3.7 Hypoxemic challenge test

Cerebral oxygenation (ScO_2) and arterial stiffness will be assessed during hypercapnic and hypoxic ventilatory response manoeuvers using near infrared spectroscopy (NIRS) and finger blood pressure (FBP)

Participants will be connected to a rebreathing system comprised of a closed loop of pipes attached to a mouthpiece/facemask that allows control of inspired gases and measurement of end-tidal CO_2 ($EtCO_2$), respiratory rate, minute ventilation, inspired PO_2 and SpO_2. All studies will be started after 5 minutes of rest in a sitting position. Participants will be given a 15 minute break between each of the following ventilatory manoeuvers. The testing protocol has been performed in many projects over the past ten years.

1. Hypercapnic hyperoxic response manoeuver:
    a. Participants will breathe through a circuit connected to a 5 Litre reservoir bag (containing 6% CO_2, 26% O_2 and the balance N_2) via a one-way inhalation only valve. External oxygen will be fed into the circuit to keep PO2 steady at 150 mmHg during the rebreathing test.
    b. Participants will be asked to hyperventilate for 2 minutes prior to normal tidal breathing.
    c. The manoeuver will be terminated if: a) 5 minutes is reached; b) participants $EtCO_2$ reaches 10% (76mmHg); c) participant complains of discomfort; d) adequate data is obtained.
2. Hypercapnic hypoxic response manoeuver:
    a. Participants will breathe through a circuit connected to a 5 Litre reservoir bag (containing 6% CO_2, 6% O_2 and the balance N_2) via a one-way inhalation only valve. External oxygen will be fed into the circuit to keep PO2 steady at 50 mmHg during the rebreathing test.
    b. Participants will be asked to hyperventilate for 2 minutes prior to normal tidal breathing.
    c. The manoeuver will be terminated if: a) 5 minutes is reached; b) participants $EtCO_2$ reaches 10% (76mmHg) or SpO_2 falls below 80%; c) participant complains of discomfort; d) adequate data is obtained
3. Hypoxic isocapnic response manoeuver:
    a. Participants will breathe through a circuit connected to a 5 Litre reservoir bag (containing 6% CO_2, 6% O_2 and the balance N_2) via a one-way inhalation only valve.
    b. Participants will be asked to undergo normal tidal breathing.
    c. A CO_2 absorbent (soda lime) will be used to prevent CO2 accumulation

d. The manoeuver will be terminated if: a) 5 minutes is reached; b) when SpO_2 falls below 80%; c) participant complains of discomfort; d) adequate data is obtained

Changes in cerebral oxygenation (using a NIRS device - (NIRO-200NX) and finger BP (using Finapres NOVA double finger cuff system) will be recorded in real time in addition to minute ventilation, SpO_2 and $PETCO_2$. The recordings will continue for 5 minutes after termination of each ventilatory manoeuver to ensure the cerebral oxygenation (ScO2) nadir is captured.

12.3.8 MRI tests

These will be conducted at the NeuRA Imaging Centre in Randwick within 2 weeks of the baseline sleep study.

12.3.8.1 Glymphatic clearance

Glymphatic clearance will be evaluated by measuring cerebral diffusion and dynamic lactate. This will require a series of MRI scans including baseline phosphorylation (^{31}P) using MRS, scout image, high resolution T1, HARDI and lactate spectrum sequences. These sequences will be performed while the patient is awake. HARDI and lactate spectrum scans will be repeated while the patient is asleep if time permits after cerebral blood flow (see 12.3.8.2) has been measured.

12.3.8.2 Cerebral blood flow

Brain perfusion will be recorded by MRI using 4D arterial spin labelling (4D ASL) while the participant sleeps inside the MRI scanner. An MRI-safe finger pulse oximeter will be used to record peripheral blood oxygen saturation during the scanning process so that periods of hypoxemia can be identified and linked to MRI data.

12.3.8.3 Amyloid-ß

Brain amyloid-ß will be measured using MRI utilising phase difference enhanced imaging (PADRE).

13 Data Management

All data will be collected onsite at the sleep laboratory at the Woolcock Institute of Medical Research in written and computerised formats. Paper records shall be securely stored in locked cabinets for a minimum 15 years following the end of the study. Computerised data will be stored and backed-up on a secure cloud based, individual password protected database system (Research Tools^{TM}) which logs all access or changes to data back to individual users who will be given only access or change privileges to data which they require for their role. After study completion only investigators named at the front of this protocol will be allowed access to the study data under the supervision of the Principal Investigators. All data will be re-identifiable as, once randomised into the study, participants will be allocated an individual study code number. The master coding sheet will be kept in a password encrypted file and only investigators and research staff will have access to it.

However, if needed, each individual will be able to be re-identified. 15 years after completion of the study, data may be considered for disposal. Any data that is disposed of will be done in such a way that it is irreversible with no chance of recovery.

Need to add info about how data is stored at NEURA and RPA (if any).

14 Statistical Methods

Brain tissue oxygenation, arterial stiffness, performance on neurobehavioral and memory tasks, and questionnaire responses before and after CPAP therapy will be compared using paired t-tests.

The association between brain tissue oxygenation, arterial stiffness and neurobehavioral performance will be explored using multiple linear regression.

15 Adverse Events Reporting

Collection of any adverse events will occur during each visit and documented in the source notes for that visit. Each participant folder will also contain a standard adverse events form to be filled out if required.

Serious adverse events (SAE) are defined as any untoward medical occurrence that:

- Results in death
- Is an immediately life-threatening condition
- Requires hospitalisation or prolongs hospitalisation
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Results in any other important medical condition.

The Ethics Committee will be notified of any SAE within 72 hours.

16 Pathological findings on MRI scans

All MRI scans will be reviewed by a radiologist prior to release to the research group. In the event that an unexpected pathology is identified as a result of the MRI scans who is responsible for notifying the participant of the abnormal finding and assisting with the submission of the MRI report to the participant's primary health care provider.

17 Auditing

The study will not be externally audited. However, internal audits and quality control processes in accordance with the 'Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) Annotated with TGA Comments' will be adhered to.

18 Ethics and Dissemination

18.2 Study conduct/ethics approval

The study will be conducted under the ethics approval of the Sydney Local Health District HREC and will be performed in accordance with the Declaration of Helsinki, the Australian Code of Responsible Research (2007) and the 'National Statement' on Ethical Conduct in Human Research (2007). Once ethics approval has been granted, site governance approvals will be obtained at RPAH, NeuRA (UNSW) and the Woolcock Institute before research commences.

18.3 Protocol amendments

Any amendments to the protocol will be made in writing to the Sydney Local Health District Ethics Committee after discussion with all co-investigators, and then be communicated to all participants, whereby further consent will be obtained for any protocol amendments. Protocol and PIS version control will be tracked at the beginning of this document.

19 Confidentiality

Participant data will be identified by a code number that will be allocated after the participant gives consent to participate in the study. The key linking the participant's identity to the relevant code will be stored in a password encrypted file that will not be accessible from the internet. Storage of the data collected will adhere to the University regulations and the Australian Code for the Responsible Conduct of Research. De-identified data may also be made available to the public. In any publication and/or presentation, information will be provided in such a way that participants cannot be identified, except with their written, informed permission. Any information obtained for the purpose of this research that could identify participants will be treated as confidential and securely stored.

20 Declaration of Interests

None of the investigators have any pecuniary interest or academic conflict of interests in the outcomes of this study.

21 Access to Data

Only investigators and members of the study team will have access and control to any data collected from participants during this study, excluding MRI data. There are no contractual agreements that would limit access or control of the study to the investigators.

Participants will be asked to opt-in to allow their MRI data to be used for any future research purposes outside the scope of this study. Access to any stored MRI data by other researchers would be subject to approval by an appropriate HREC, and would be provided in a way such that participants are not identifiable to the researchers (but could be re-identified by NeuRA should the need arise).

22 Ancillary and Post-trial Care

Participants with be followed up with routine clinical care by the Sleep Physician. If however any harm is caused during this protocol or a medical condition becomes apparent, then

medical follow-up will be arranged with either a member of the clinical research team or the participant's normal medical practitioner.

23 Dissemination Policy

Study results will be published in peer-reviewed journals and participants will be made aware of these following publication should they desire. The Principal Investigator A/Prof Craig Phillips will be responsible for the formulation and execution of publication plans. Authorship on any manuscripts will be at the discretion of the Principal Investigator.

Appendix

Appendix A: Screening and Questionnaires

A1: Ethnicity

Ethnicity

select a partcipant... Remove

We are interested in your ancestry and ethnicity because we have observed that some sleep conditions are more common in people from certain parts of the world. Therefore, we would be grateful if you answer these questions as specifically as possible. If you do not know this information, just tick the option "Don't Know". If you do not wish to complete this section that is ok and will not influence your care.

Please select which group best describes your ancestry/ethnicity (based on a mixture of culture, religion, skin colour and language). If you come from a background of multiple ethnic ancestries please tick all the groups that best apply to you.

A. Caucasian - Australia/NZ (Anglo European), Europe (includes Russia Central and West Asia) & North Mediterranean, America, Canada, South Africa & Zimbabwe.
B. Indigenous Australian - Aboriginal, Torres Strait Islands.
C. Pacific Islander - New Zealand Maori or Pacific Islands, Hawaii, New Guinea.
D. South-East Asian - Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar/Burma, Philippines,Singapore, Thailand, Vietnam.
E. South Asian - Afghanistan, Bangladesh, Bhutan, India, Maldives, Nepal, Pakistan, Sri Lanka.
F. North East Asian - China, Hong Kong, Japan, Korea, Macau, Taiwan.
G. North Asian - Mongolia, Siberia.
H. Middle Eastern, Northern African, Somali Peninsular - Algeria, Bahrain, Djibouti, Eritrea, Ethiopia, Egypt, Israel, Iran, Iraq, Jordan, Kuwait, Lebanon, Libya, Oman, Palestinian Territories, Turkey, Turkish Cypriots, Qatar, Saudi Arabia, Somalia, Syria, Tunisia, United Arab Emirates, Yemen.
I. Sub-Saharan African - Indigenous African, African American.
J. Central/South American - Central/South America.
K. Other
L. Don't Know

Ethnicity Selection	
Select for YOURSELF	☐ A. Caucasian ☐ B. Indigenous Australian ☐ C. Pacific Islander ☐ D. South-East Asian ☐ E. South Asian ☐ F. North East Asian ☐ G. North Asian ☐ H. Middle Eastern, North Africa, Somali Peninsular ☐ I. Sub-Saharan African ☐ J. Central/South American ☐ K. Other (Please Specify Below) ☐ L. Don't Know
Other	
if **Other**, please specify	
Country of Birth	
In which country were you born?	

A2: Lifestyle

Lifestyle	
select a partcipant...	Remove
General Health	
How would you describe your general health?	☐ Excellent ☐ Very Good ☐ Good ☐ Fair ☐ Poor
Physical Activity	
How often, on average, do you do at least 30 minutes of moderate physical activity - like walking?	☐ Never ☐ Sometimes ☐ A couple of days a week ☐ Most days a week ☐ Everyday
Smoke	
Have you ever smoked?	☐ No ☐ Yes
Do you or did you smoke regularly? *("No" means less than 20 packs in a lifetime or less than 1 cigarette per day for 1 year).*	☐ No ☐ Yes
If you previously smoked but have stopped, in which year did you last smoke?	
How old were you when you first started regular cigarette smoking?	
On average, over the entire time you smoked, how many cigarettes did you smoke each day?	
Alcohol	
In a typical week during the past year, on how many days did you consume an alcoholic drink of any type? Please check the appropriate answer.	☐ 0 days / do not drink alcohol ☐ 1 day ☐ 2 days ☐ 3 days ☐ 4 days ☐ 5 days ☐ 6 days ☐ 7 days
On days when you drink alcohol, how many standard drinks of beer, wine / or other type of alcohol would you have? *(Click here for list of standard drinks)* Please check the appropriate answer.	Workday(s) ☐ None ☐ 1-2 drinks ☐ 3-5 drinks ☐ 6-9 drinks ☐ 10 or more drinks
	Non-workday(s) ☐ None ☐ 1-2 drinks ☐ 3-5 drinks ☐ 6-9 drinks ☐ 10 or more drinks
Caffeine	
In a typical day during the past week, how many caffeinated drinks did you have e.g. coffee, tea, coca cola, hot chocolate, energy drinks, soft drinks, ice teas? *(If you're unsure whether your drink contains caffeine click here to find out.)* Please check the appropriate answer.	☐ 0 drinks / I do not drink caffeine
	Workday(s) ☐ None ☐ 1-2 drinks ☐ 3-5 drinks ☐ 6-9 drinks ☐ 10 or more drinks
	Non-workday(s) ☐ None ☐ 1-2 drinks ☐ 3-5 drinks ☐ 6-9 drinks ☐ 10 or more drinks
How many hours before bedtime would you normally have your last drink containing caffeine?	Workday(s)
	Non-workday(s)

A3: Education

Education

select a partcipant... Remove

1. What is the highest grade or level of school that you have completed?

- [ ] a. Primary or some Secondary school (e.g. 8th grade or less)
- [ ] b. Some high school, but did not graduate
- [ ] c. High school graduate or HSC
- [ ] d. Tafe graduate (e.g. Certificate, Diploma)
- [ ] e. University graduate (e.g. Bachelor's degree
- [ ] f. More than 4-year University degree (e.g. Masters, PhD)

A4: Epworth Sleepiness Scale (ESS)

ESS

select a partcipant... Remove

How likely are you to doze or fall asleep in the following situations, in contrast to just feeling tired? This refers to your usual way of life in recent times. Even if you have not done some of these things recently, try to work out how they would have affected you.

	Would **never** doze	**Slight** chance of dozing	**Moderate** chance of dozing	**High** chance of dozing
1. Sitting and reading	○	○	○	○
2. Watching TV	○	○	○	○
3. Sitting, inactive in a public place (eg a theatre or a meeting)	○	○	○	○
4. As a passenger in a car for an hour without a break	○	○	○	○
5. Lying down to rest in the afternoon when circumstances permit	○	○	○	○
6. Sitting and talking to someone	○	○	○	○
7. Sitting quietly after lunch without alcohol	○	○	○	○
8. In a car, while stopped for a few minutes in traffic	○	○	○	○

A5: Karolinska Sleep Diary (7 days)

Initials: _____
Randomisation Number: _____

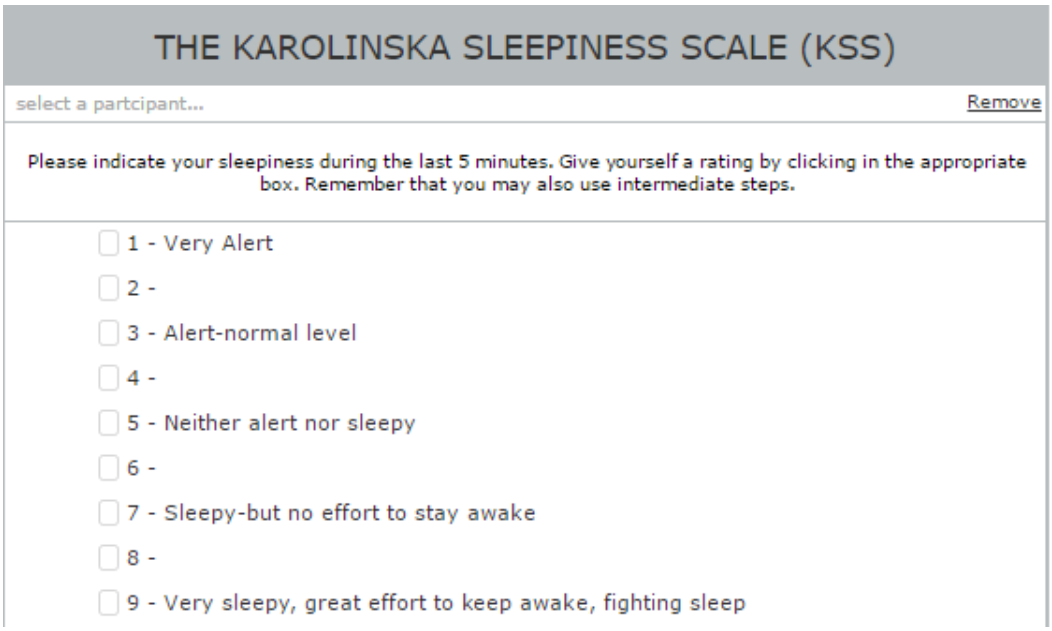
WOOLCOCK
INSTITUTE of MEDICAL RESEARCH

Karolinska Diary - ***Please fill in each morning.***

Date:_____ **Night: 1**

	Hours	**Mins**	**Comments**		
What time did you get into bed?					
What time did you attempt sleep?					
How long did it take to fall asleep?					
Time of Final Awakening					
Time of getting out of bed					
How long did you sleep?					
CIRCLE A NUMBER					
How did you sleep?	**5 = very well**	**4**	**3**	**2**	**1= very poorly**
Feeling refreshed after awakening	**5 = completely**	**4**	**3**	**2**	**1= not at all**
Calm sleep	**5 = very calm**	**4**	**3**	**2**	**1= very restless**
Slept Through	**5 = yes**	**4**	**3**	**2**	**1= woke too early**
Ease of Waking up	**5 = very easy**	**4**	**3**	**2**	**1= very difficult**
Ease of falling asleep	**5 = very easy**	**4**	**3**	**2**	**1= very difficult**
Amount of Dreaming	**5 = much**	**4**	**3**	**2**	**1= none**
Naps per day (yesterday)	**Number =**				
Caffeinated drinks yesterday (tea, coffee, cola drinks, chocolate)	**Number =**				
Alcohol yesterday (number of standard drinks)	**Number =**				
How sleepy did you feel during the day (yesterday)	**5 = very sleepy**	**4**	**3**	**2**	**1= not sleepy at all**

A6: Karolinska Sleepiness Scale (KSS)



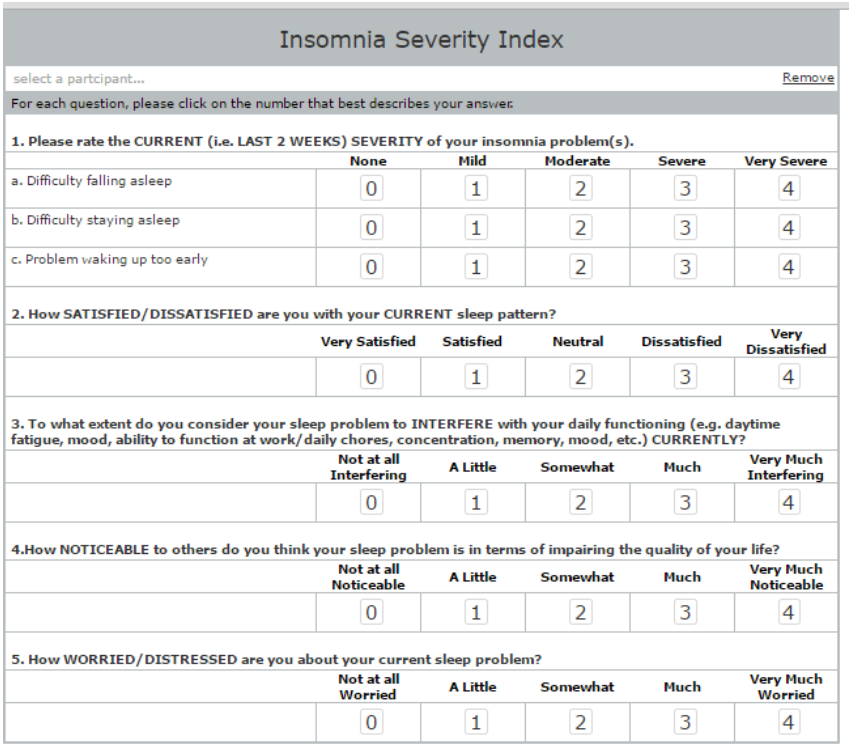
THE KAROLINSKA SLEEPINESS SCALE (KSS)

select a partcipant... Remove

Please indicate your sleepiness during the last 5 minutes. Give yourself a rating by clicking in the appropriate box. Remember that you may also use intermediate steps.

- ☐ 1 - Very Alert
- ☐ 2 -
- ☐ 3 - Alert-normal level
- ☐ 4 -
- ☐ 5 - Neither alert nor sleepy
- ☐ 6 -
- ☐ 7 - Sleepy-but no effort to stay awake
- ☐ 8 -
- ☐ 9 - Very sleepy, great effort to keep awake, fighting sleep

A7: Insomnia Severity Index



Insomnia Severity Index

select a partcipant... Remove

For each question, please click on the number that best describes your answer.

1. Please rate the CURRENT (i.e. LAST 2 WEEKS) SEVERITY of your insomnia problem(s).

	None	Mild	Moderate	Severe	Very Severe
a. Difficulty falling asleep	0	1	2	3	4
b. Difficulty staying asleep	0	1	2	3	4
c. Problem waking up too early	0	1	2	3	4

2. How SATISFIED/DISSATISFIED are you with your CURRENT sleep pattern?

	Very Satisfied	Satisfied	Neutral	Dissatisfied	Very Dissatisfied
	0	1	2	3	4

3. To what extent do you consider your sleep problem to INTERFERE with your daily functioning (e.g. daytime fatigue, mood, ability to function at work/daily chores, concentration, memory, mood, etc.) CURRENTLY?

	Not at all Interfering	A Little	Somewhat	Much	Very Much Interfering
	0	1	2	3	4

4.How NOTICEABLE to others do you think your sleep problem is in terms of impairing the quality of your life?

	Not at all Noticeable	A Little	Somewhat	Much	Very Much Noticeable
	0	1	2	3	4

5. How WORRIED/DISTRESSED are you about your current sleep problem?

	Not at all Worried	A Little	Somewhat	Much	Very Much Worried
	0	1	2	3	4

A8: Pittsburgh Sleep Quality Index (PSQI)

PITTSBURGH SLEEP QUALITY INDEX

select a partcipant... Remove

The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month.

Please answer all questions.

1. During the past month, what time have you usually gone to bed at night?
BED TIME HH:MM *24 hour clock*

2. During the past month, how long (in minutes) has it usually taken you to fall asleep each night?
NUMBER OF MINUTES

3. During the past month, what time have you usually gotten up in the morning?
GETTING UP TIME HH:MM *24 hour clock*

4. During the past month, how many hours of actual sleep did you get at night?
(This may be different than the number of hours you spent in bed.)
HOURS OF SLEEP PER NIGHT

For each of the remaining questions, tick the one best response. Please answer all questions.

5. During the past month, how often have you had trouble sleeping because you . . .	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
a. Cannot get to sleep within 30 minutes	○	○	○	○
b. Wake up in the middle of the night or early morning	○	○	○	○
c. Have to get up to use the bathroom	○	○	○	○
d. Cannot breathe comfortably	○	○	○	○
e. Cough or snore loudly	○	○	○	○
f. Feel too cold	○	○	○	○
g. Feel too hot	○	○	○	○
h. Had bad dreams	○	○	○	○
i. Have pain	○	○	○	○
j. Any other reason(s) please describe:	Please describe here			
	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
How often during the past month have you had trouble sleeping because of this?	○	○	○	○
	Very good	Fairly good	Fairly bad	Very bad
6. During the past month, how would you rate your sleep quality overall?	○	○	○	○
	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
7. During the past month, how often have you taken medicine (prescribed or 'over the counter') to help you sleep?	○	○	○	○
8. During the past month, how often have you had trouble staying awake while driving, eating meals or engaging in social activity?	○	○	○	○
	No problem at all	Only a very slight problem	Somewhat of a problem	A very big problem
9. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?	○	○	○	○
	No bed partner or room mate	Partner/room mate in other room	Partner in same room, but not same bed	Partner in same bed
10. Do you have a bed partner or room mate?	○	○	○	○
If you have a room mate or bed partner, ask him/her how often in the past month you have had . . .	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
a. Loud snoring	○	○	○	○
b. Long pauses between breaths while asleep	○	○	○	○

A9: Depression Anxiety and Stress Scale (DASS-21)

DASS **Number** **Date**

Please read each statement and circle a number 0, 1, 2, or 3, which indicates how much the statement applied to you over the past week. There are no right or wrong answers.
Do not spend too much time on any statement.

The rating scale is as follows:

0 ***Did not apply to me at all***
1 ***Applied to me to some degree, or some of the time***
2 ***Applied to me to a considerable degree, or a good part of time***
3 ***Applied to me very much, or most of the time***

1.	I tended to over-react to situations	0	1	2	3
2.	I was aware of dryness of my mouth	0	1	2	3
3.	I couldn't seem to experience any positive feeling at all	0	1	2	3
4.	I experienced breathing difficulty (eg, excessively rapid breathing, breathlessness in the absence of physical exertion)	0	1	2	3
5.	I felt that I had nothing to look forward to	0	1	2	3
6.	I found it difficult to relax	0	1	2	3
7.	I felt scared without good reason	0	1	2	3
8.	I felt that I was using a lot of nervous energy	0	1	2	3
9.	I was aware of the action of my heart in the absence of physical exertion (eg, sense of heart rate increase, heart missing a beat)	0	1	2	3
10.	I felt that I wasn't worth much as a person	0	1	2	3
11.	I found it hard to wind down	0	1	2	3
12.	I felt that I was rather touchy	0	1	2	3
13.	I felt down-hearted and blue	0	1	2	3
14.	I found myself getting agitated	0	1	2	3
15.	I experienced trembling (eg, in the hands)	0	1	2	3
16.	I felt that life was meaningless	0	1	2	3
17.	I was unable to become enthusiastic about anything	0	1	3	3
18.	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3
19.	I felt I was close to panic	0	1	2	3
20.	I was worried about the situations in which I might panic and make a fool of myself	0	1	2	3
21.	I found it difficult to work up the initiative to do things	0	1	2	3

Appendix B: IQ Test, Neurobehavioral and Memory Tasks

B1: Welscher Test of Adult Reading (WTAR)

WTAR Word List

Say, **I will show you some words that I will ask you to pronounce.** Place the WTAR Word Card in front of the examinee. As you point to the card, say, **Beginning with the first word on the list, pronounce each word aloud. Start with this word** (point to Item 1), **and go down this column, one right after the other, without skipping any. When you finish this column, go to the next column** (point to the second column). **Pronounce each word even if you are unsure. Do you understand?** When you are sure that the examinee understands the task, say, **Ready? Begin.**

	Item	Pronunciation	Score (0, 1)		Item	Pronunciation	Score (0, 1)
1.	again	uh-GEHN *or* uh-GAIN		26.	conscientious	kon-chee-EN-shus *or* kon-chee-INCH-us	
2.	address	uh-DRESS *or* AD-dress		27.	homily	HAHM-uh-lee	
3.	cough	kawf *or* kof		28.	malady	MAL-uh-dee	
4.	preview	PREE-vyue		29.	subtle	SUH-tl	
5.	although	awl-THO		30.	fecund	FE-cund *or* FEE-cund	
6.	most	mohst		31.	palatable	PAL-uh-tuh-bul	
7.	excitement	eck-SITE-munt *or* ik-SITE-munt		32.	menagerie	muh-NAJ-uh-ree	
8.	know	noh *or* no		33.	obfuscate	OB-fuh-skate *or* ob-FUH-skate	
9.	plumb	plum		34.	liaison	lee-A-zahn *or* LAY-a-zahn *or* LEE-ah-zahn	
10.	decorate	DEK-uh-rate		35.	exigency	EKS-eh-jen-see *or* ek-ZEE-jen-see	
11.	fierce	firhrss		36.	xenophobia	zen-uh-FO-bee-uh *or* zeen-uh-FO-bee-uh	
12.	knead	need		37.	ogre	OH-gur	
13.	aisle	EYE-l		38.	scurrilous	SKUR-uh-lus *or* SKUH-ruh-lus	
14.	vengeance	VEN-junts *or* VIN-junts		39.	ethereal	ih-THEER-ee-uhl *or* ih-THIR-ee-uhl	
15.	prestigious	pre-STIJ-us *or* pre-STEEJ-us		40.	paradigm	PAIR-uh-dime *or* PAIR-uh-dim	
16.	wreathe	reeTH		41.	perspicuity	pur-spuh-KYEW-uh-tee	
17.	gnat	nat		42.	plethora	PLETH-er-aah	
18.	amphitheater	AM(p)-fuh-the-uh-ter		43.	lugubrious	loo-GOO-bree-us *or* luh-GOO-bree-us *or* loo-GYEW-bree-us	
19.	lieu	loo		44.	treatise	TREET-us	
20.	grotesque	gro-TESK		45.	dilettante	DILL-uh-tahnt	
21.	iridescent	ihr-uh-DESS-unt		46.	vertiginous	vur-TI-jin-us *or* vur-TIJ-uh-nus	
22.	ballet	BA-lay *or* ba-LAY		47.	ubiquitous	you-BIC-wuh-tus *or* you-BIH-kwah-tus	
23.	equestrian	ih-KWESS-tree-un		48.	hyperbole	hi-PUR-buh-lee	
24.	porpoise	POR-pus		49.	insouciant	in-SOO-see-yunt	
25.	aesthetic	ess-THET-ik *or* ees-THET-ik		50.	hegemony	heh-JEM-o-nee *or* he-je-MO-nee	
						WTAR Raw Score	
						WTAR Standard Score	

B2: Psychomotor Vigilance Task (PVT)

PVT instructions to participants

Presentation of instructions for the Psychomotor Vigilance Task (PVT) should be standardised to the participant. Tests should be conducted in a quiet area with even lighting and a comfortable way for the subject to sit and hold the PVT-192.

The following is recommended as a minimal explanation of the task to the subject:

First indicate how you feel right now (when the Mood Word is displayed) by using the LEFT button to move the cursor closer to NO or YES. Press the RIGHT button to register your choice.

During the test, as soon as you see the red numbers in the top window, press and release the button using your RIGHT (or dominant as previously programmed) hand. You may use your thumb or finger, but use the SAME FINGER for all the tests once you have decided. The numbers in the display show how fast you responded each time (the smaller the number, the better you did). Try to do your best and get the lowest number you possibly can.

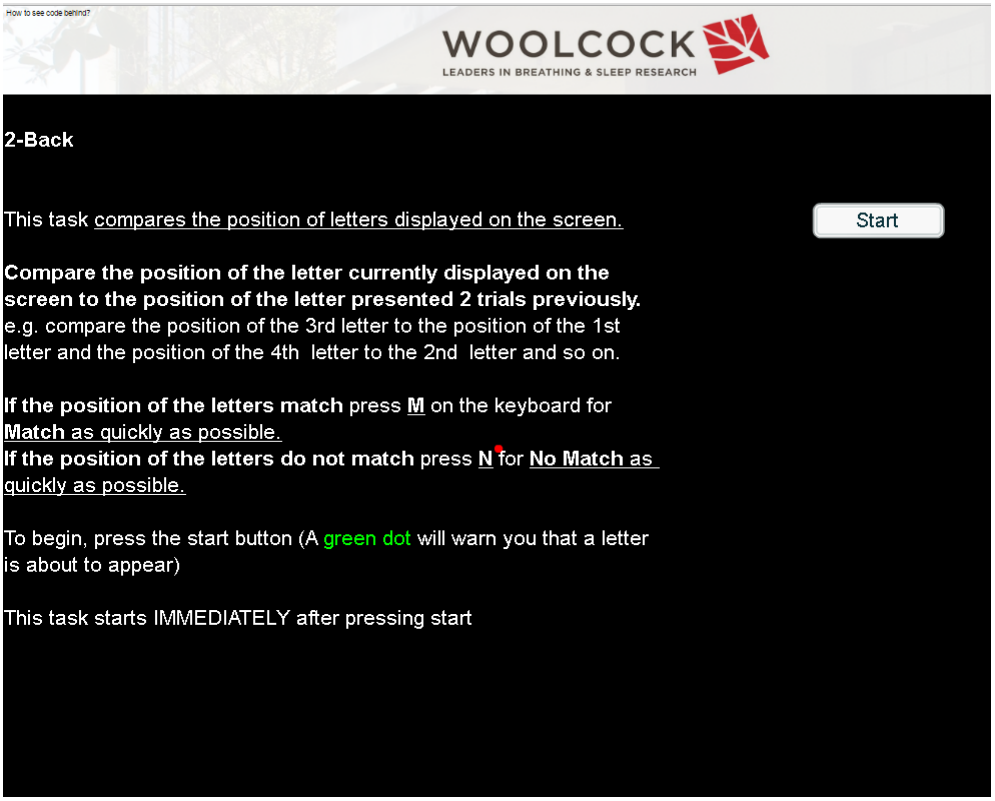
If you press too early (before the numbers appear) you will see an error message FS. If you press the other button (the one not used for this test), you will see an error message – ERR. If you forget to release the button, after a short time the test screen will remind you.

When the test is completed, the mood word will be presented again. When done, do not turn the PVT-192 off, the test administrator will do this.

NOTE: At the end of the vigilance portion of each test, the Experimenter should make sure that the PVT-192 remains turned on until the second mood scale is completed and the Select menu is redisplayed.

NOTE: The LEFT button on the PVT-192 is used to move the cursor. The RIGHT button on the PVT-192 is used to select the option.

B3: n-back


How to see code behind?
WOOLCOCK
LEADERS IN BREATHING & SLEEP RESEARCH
2-Back
This task compares the position of letters displayed on the screen.
Start
Compare the position of the letter currently displayed on the screen to the position of the letter presented 2 trials previously.
e.g. compare the position of the 3rd letter to the position of the 1st letter and the position of the 4th letter to the 2nd letter and so on.
If the position of the letters match press M on the keyboard for Match as quickly as possible.
If the position of the letters do not match press N for No Match as quickly as possible.
To begin, press the start button (A green dot will warn you that a letter is about to appear)
This task starts IMMEDIATELY after pressing start

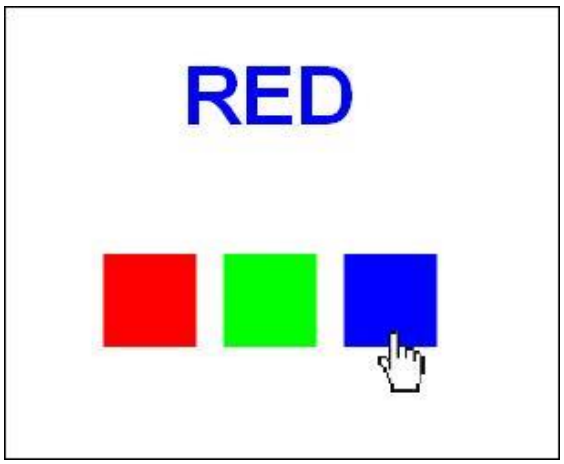
B4: Stroop Test (colour and text)

Stroop (Colour)

This task measures reaction time to colours and words.

Three different coloured squares will be displayed at the bottom of the screen.

Click on the coloured square that matches the **COLOUR** (red, blue or green) of the word above.

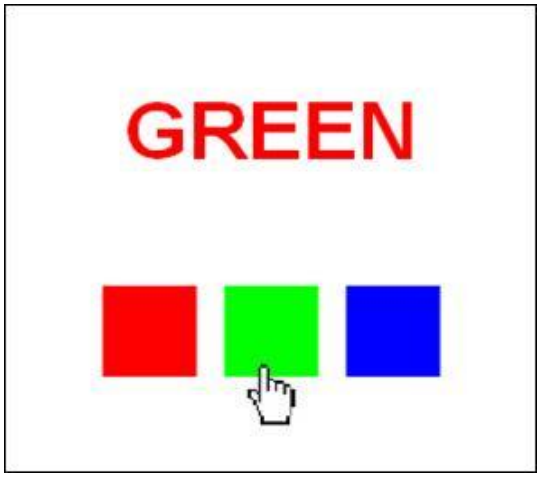


Stroop (Text)

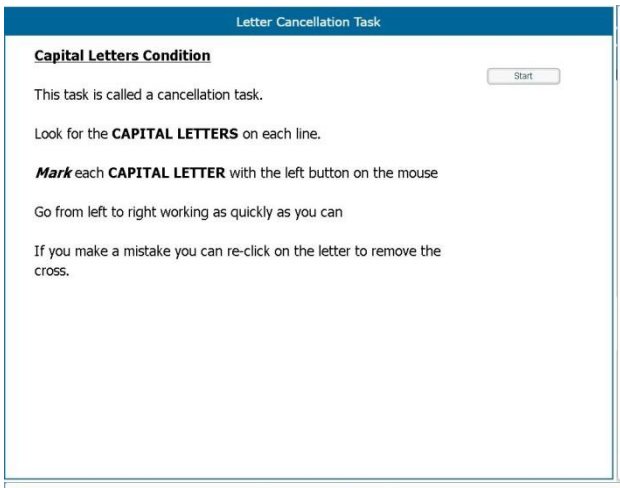
This task measures reaction time to colours and words.

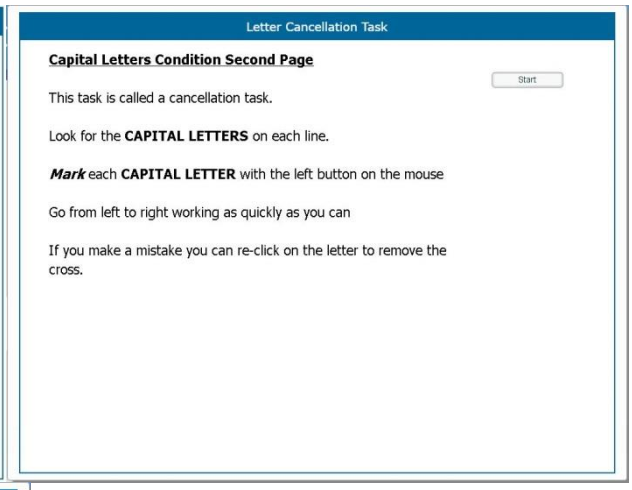
Three different coloured squares will be displayed at the bottom of the screen.

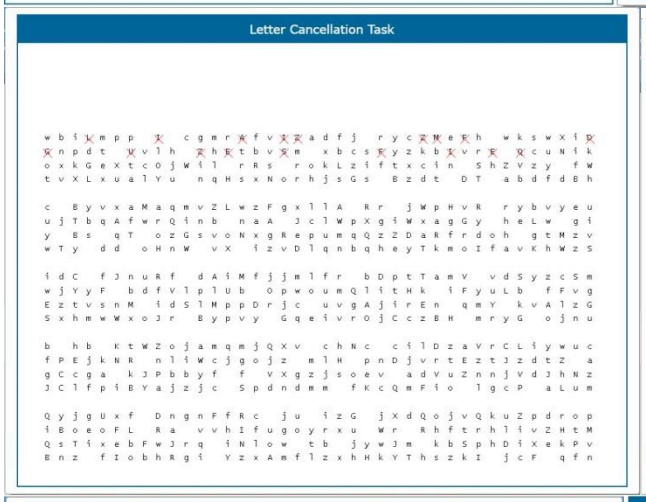
Click on the coloured square that matches the **MEANING** (red, blue or green) of the word above.

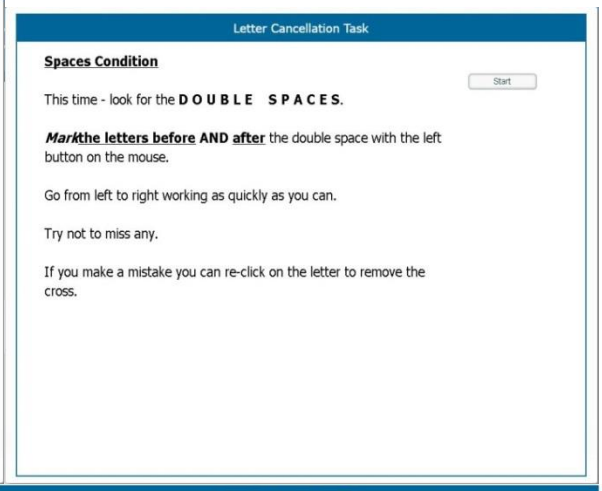


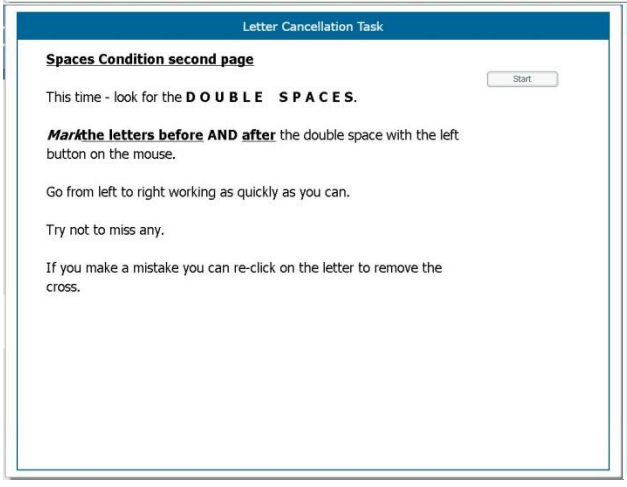
B5: Letter Cancellation Task (LCT)

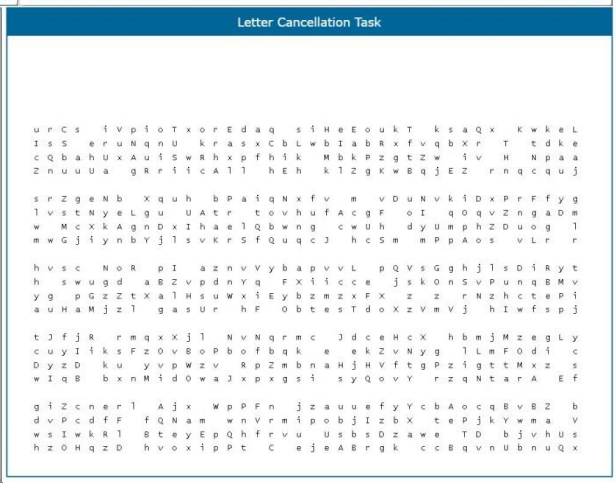


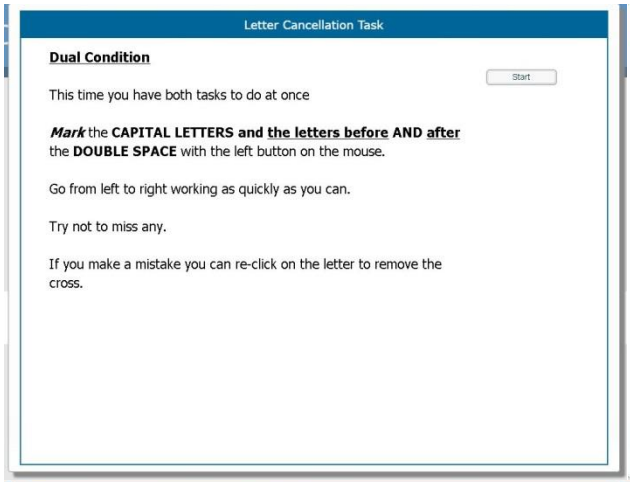


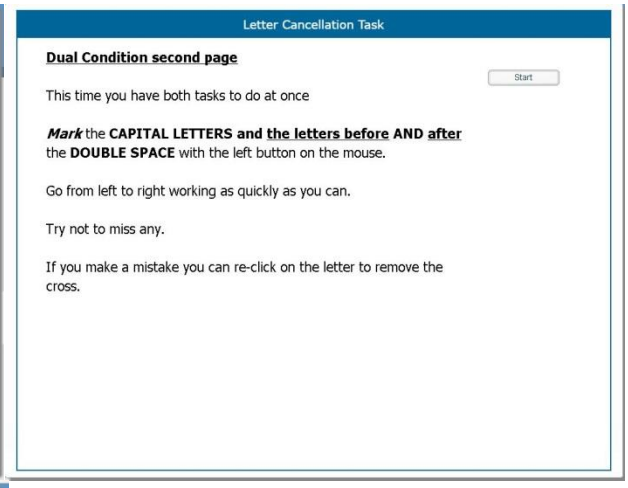


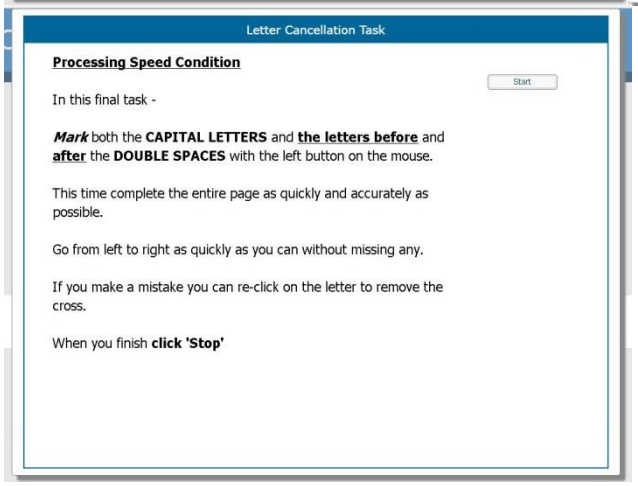







Letter Cancellation Task
Dual Condition
Start
This time you have both tasks to do at once
Mark the CAPITAL LETTERS and the letters before AND after the DOUBLE SPACE with the left button on the mouse.
Go from left to right working as quickly as you can.
Try not to miss any.
If you make a mistake you can re-click on the letter to remove the cross.

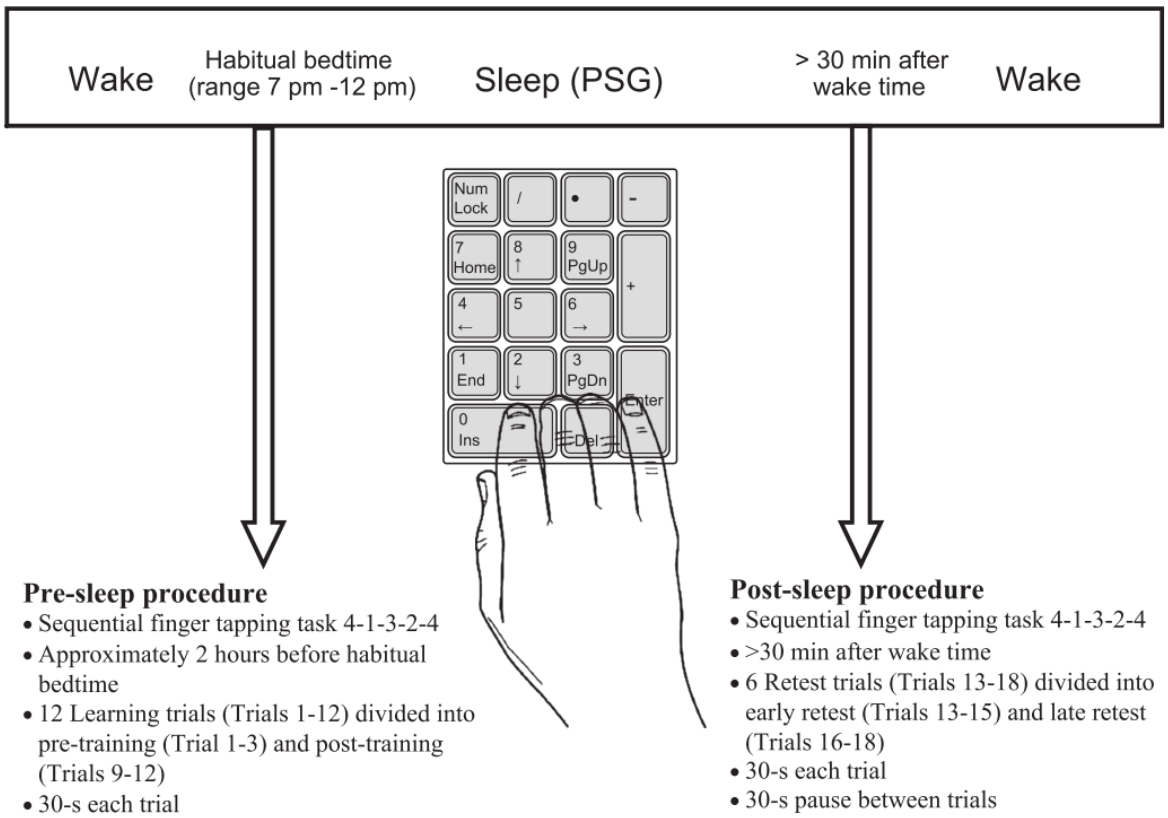

Letter Cancellation Task
Dual Condition second page
Start
This time you have both tasks to do at once
Mark the CAPITAL LETTERS and the letters before AND after the DOUBLE SPACE with the left button on the mouse.
Go from left to right working as quickly as you can.
Try not to miss any.
If you make a mistake you can re-click on the letter to remove the cross.


Letter Cancellation Task
Processing Speed Condition
Start
In this final task -
Mark both the CAPITAL LETTERS and the letters before and after the DOUBLE SPACES with the left button on the mouse.
This time complete the entire page as quickly and accurately as possible.
Go from left to right as quickly as you can without missing any.
If you make a mistake you can re-click on the letter to remove the cross.
When you finish click 'Stop'

B6: Word Pair Task (32 word pairs)

SHIRT INNOCENT	SYRUP MAPLE
Please complete the pair SHIRT - ? RUSH INNOCENT DISAPPEAR PANTS	What pair went with this word? SYRUP - ?

B7: Finger Tapping Task (FTT)



I. Karolinska Drowsiness Test (KDT)

Description and Instructions for the Karolinska Drowsiness Test (KDT, resting awake EEG recording)

Setting: Comfortable chair in a quiet room with standard ambient room lighting (~ 150 lux).

Test Summary: Participants are seated upright, on a comfortable straight-backed chair in a quiet room and asked to look at a marker (black dot) which is fixed on the wall at eye level at a distance of one meter and instructed to relax and keep as still as possible. After 2 minutes the participant is asked to close his/her eyes while remaining seated in the same position for an additional 2 minutes but to remain focused on the wall marker. Finally, another 2 minutes of resting awake EEG is recorded while the participant's eyes are open.

KDT Procedural Instructions for the Technician

N.B. Prior to beginning the KDT, ask the participant to rate their subjective sleepiness using the Karolinska Sleepiness Scale (KSS).

We are using the same recording configuration for both the sleep studies and the waking EEG.

Ensure the EEG and EOG signals are satisfactory (i.e. impedance and signal integrity). Once you are satisfied with the quality of the signal, have the participant sitting up in a chair. Ensure the lighting is adequate. Place (or find) the marker on the wall directly ahead of the participant, for them to focus on.

Instruct the Participant as Follows:

"In this test, all I would like you to do is simply look at the mark on the wall. For the next 2 and a half minutes, I want you to keep as relaxed as possible, while staying awake. Keep your shoulders relaxed, with your mouth slightly open and jaw relaxed. Keep looking at the mark until I say OK, and then close your eyes and keep them closed, but continue to imagine that you are still looking at the mark on the wall. Keep your shoulders relaxed and your mouth slightly open and jaw relaxed. This will take another 2 and a half minutes. Then lastly I will ask you to open your eyes again and look at the mark for another 2 and a half minutes. Can you tell me what you need to do? … Good, then start by looking at the mark."

Recording Procedure (with stopwatch)

- For each of the three 2.5 minute periods, we allow the last half minute for the participant to close or open his/her eyes.
- Ask the participant to start looking at the spot on the wall, with his/her eyes open.
- Start recording on the sleep recording system, and START the stopwatch.
- Record time in notes, with acquisition number.
- At 2 minutes and 10 seconds (on the stopwatch), ask the participant to close his/her eyes.
- Record time in notes.
- At 4 minutes and 40 seconds on the stopwatch, ask the participant to open his/her eyes.
- Record time in notes.
- At 7 min and 10 seconds on the stopwatch, stop the recording and then ask the participant to relax.
- Record time in notes.

24 REFERENCES

Bucks, R; Olaithe, M; Eastwood, P. 2012. 'Neurocognitive function in obstructive sleep apnoea: A meta-review', *Respirology*, 18: 61-70.

Hou, Y., Y. Shang, R. Cheng, Y. Zhao, Y. Qin, R. Kryscio, A. Rayapati, D. Hayes, and G. Yu. 2014. 'Obstructive sleep apnea-hypopnea results in significant variations in cerebral hemodynamics detected by diffuse optical spectroscopies', *Physiological Measurement*, 35: 2135-48.

Jessen, Nadia Aalling, Anne Sofie Finmann Munk, Iben Lundgaard, and Maiken Nedergaard. 2015. 'The Glymphatic System: A Beginner's Guide', *Neurochemical Research*, 40: 2583-99.

Jordan, A; McSharry, D; Malhotra, A. 2014. 'Adult obstructive sleep apnoea', *The Lancet*, 383: 736-47.

Lawley, J; Macdonald, J; Oliver, S; Mullins, P. 2017. 'Unexpected reductions in regional cerebral perfusion during prolonged hypoxia', *The Journal of Physiology*, 593: 935-57.

Pizza, F; Biallas, M; Wolf, M; Werth, E; Bassetti, C. 2010. 'Nocturnal Cerebral Hemodynamics in Snorers and in Patients with Obstructive Sleep Apnea: A Near-Infrared Spectroscopy Study', *Sleep*, 33: 205-10.

Seshadri, S; Moonis, M. 2015. 'Dipping to clear amyloid: Cerebrovascular reactivity and neurodegeneration—cause or effect?', *Neurology*, 85: 1918-19.

Webb, A; Rothwell, P. 2016. 'Magnetic Resonance Imaging Measurement of Transmission of Arterial Pulsation to the Brain on Propranolol Versus Amlodipine', *Stroke*, 47: 1669-71.