Exploring the effects of acute partial sleep restriction and subsequent caffeine ingestion on neurovascular coupling and cognitive function

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Abstract

Longitudinal, cross-sectional, and acute experimental research demonstrate that a short sleep duration is associated with an increased risk of both cardiovascular and cerebrovascular disease. Chronic habitual short sleep duration is associated with an increased dementia risk, however a quarter of the population fail to meet sleep duration guidelines. It is thought that alterations in cerebrovascular function precede symptoms of cognitive decline and dementia. Currently, no evidence exists on measures of cerebrovascular function, such as neurovascular coupling (NVC), and habitual sleep across the lifespan. The association of chronic poor sleep and dementia is likely due to the repeated exposure to a poor night's sleep. Therefore, there is value in performing controlled, interventional work to understand the acute responses to a single night of sleep restriction. Furthermore, following a night of poor sleep, individuals commonly consume caffeine to increase alertness, but caffeine is known to simultaneously reduce brain blood flow. It is unknown how caffeine affects cerebrovascular function in a sleep restricted state. The purpose of this thesis was therefore to explore 1) the impact of one night of partial sleep restriction on NVC and, 2) whether caffeine ingestion affects NVC after partial sleep restriction in young healthy adults. NVC was determined using transcranial Doppler ultrasound (TCD) to measure blood velocity through the posterior cerebral artery (PCAv) during a visual search task. TCD was also used to measure blood velocity through the middle cerebral artery (MCAv) during cognitive function tests, both of which were assessed before and after normal sleep and partial sleep restriction, and following caffeine ingestion. This study found that NVC was unaltered following partial sleep restriction, and that the magnitude of the NVC response was unchanged after caffeine ingestion in both the

rested and sleep restricted state. However, both absolute PCAv and MCAv were lower after caffeine, though this response did not differ between normal and restricted sleep. This study was the first to examine the effects of partial sleep restriction and subsequent caffeine ingestion on a measure of cerebrovascular function. However, sleep restriction was only studied in the form of a 50% sleep restriction, with sleep taking place in the second half of the night. Future research should explore whether the relationship between sleep and NVC is dependent on how sleep is accrued throughout the night, for example fragmented/broken sleep.

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Abbreviations

AChEI – acetylcholine esterase inhibition

ANOVA – analysis of variance

ASL – arterial spin labelling

AUC – area under the curve

BMI – body mass index

BOLD – blood oxygenation level dependent

CI – confidence intervals

CO_2 – carbon dioxide

$CMRO_2$ – cerebral metabolic rate of oxygen

CPAP – continuous positive airway pressure

CV – coefficient of variation

ECG - electrocardiogram

fNIRS – functional near infrared spectroscopy

HHb – deoxyhaemoglobin

iAUC – incremental area under the curve

ICC – intraclass correlation coefficient

MAP – mean arterial pressure

MCA – middle cerebral artery

MCAv – middle cerebral artery blood velocity

MD – mean difference

MRI – magnetic resonance imaging

NIRS – near infrared spectroscopy

NO – nitric oxide

NOS – nitric oxide synthase

NORM_PL – normal + placebo

NORM_CAF – normal + caffeine

NVC – neurovascular coupling

OSA – obstructive sleep apnoea

O_2Hb – oxyhaemoglobin

$PaCO_2$ – arterial partial pressure of carbon dioxide

PCA – posterior cerebral artery

PCAv – posterior cerebral artery blood velocity

ΔPCAv-peak – absolute change in PCAv from baseline to peak

%ΔPCAv-peak – percent change in PCAv from baseline to peak

PET – positron emission tomography

$P_{ET}CO_2$ – end tidal carbon dioxide

PSQI – Pittsburgh Sleep Quality Index

PSR – partial sleep restriction

SD – standard deviation

τ – time constant

TCD – transcranial doppler ultrasound

TD – time delay

tAUC – total area under the curve

Chapter 1: Introduction

1.1. Introduction

Sleep is undoubtedly a fundamental physiological process. During an 85-year life span, a person could expect to accrue ~250,000 hours, or more than 10,000 full days, of sleep. While the specific reason of why we sleep remains unknown, and people may undervalue time spent asleep as 'lost time', in the words of sleep scientist Prof Allan Rechtschaffen "*If sleep does not serve an absolutely vital function, then it is the biggest mistake the evolutionary process has ever made*" (Rechtschaffen, 1971).

Research is routinely discovering what happens during sleep, the importance of attaining sufficient sleep each night, and the consequences of when we fail to do so. Acute sleep loss has far reaching effects, from impaired glucose metabolism and appetite regulation (Knutson, Spiegel, Penev, & Van Cauter, 2007), negative mood (Watling, Pawlik, Scott, Booth, & Short, 2017) and deteriorated cognitive functioning (Lo et al., 2012), to tragic traffic related accidents (Komada, Asaoka, Abe, & Inoue, 2013). In addition to the acute effects, habitual poor sleep is associated with a plethora of diseases, including cardiovascular (Fernandez-Mendoza, He, Vgontzas, Liao, & Bixler, 2019; Krittanawong et al., 2019; Tobaldini et al., 2017) and cerebrovascular disease (Lutsey et al., 2018; Sabia et al., 2021; Wu, Sun, & Tan, 2018). Cross-sectional and prospective studies have demonstrated that individuals reporting poor sleep across the lifespan have a greater risk of developing dementia, independent of other confounding illnesses (Sterniczuk, Theou, Rusak, & Rockwood, 2013; Vaou, Lin, Branson, & Auerbach, 2018). Not only is this a concern since a quarter of adults in the United Kingdom fail to meet the National Sleep Foundation's recommended 7 – 9 hours of sleep per night (Chaput, Dutil, & Sampasa-Kanyinga, 2018; Hirshkowitz et

al., 2015; Kocevska et al., 2021), but with an ageing population, the number of people living with dementia has increased over recent decades to approximately 50 million people worldwide (Li, Qin, Zhu, & Jia, 2021), with numbers expected to nearly double every 20 years (Prince et al., 2013). In addition to this, there is still currently no 'cure' for dementia. It is therefore of great importance to understand the association between sleep and dementia from a primary prevention standpoint.

The precise temporal and spatial matching of cerebral perfusion to cerebral metabolism, termed NVC (Squair et al., 2020), is a fundamental component of cerebrovascular function, and alterations in this process are thought to be an important mechanism which precedes dementia (Beishon, Haunton, Panerai, & Robinson, 2017; Zhu, Neuhaus, Beard, Sutherland, & DeLuca, 2022). No observational evidence on NVC and habitual sleep across the lifespan exists, however, the chronic association of life-long poor sleep and dementia may be related to the repeated, acute exposure of sleep loss. Thus, a controlled experimental design might have value when trying to understand the role of NVC alterations in dementia. At present, only one study has assessed the relationship between sleep and NVC, and observed that NVC is altered after 24 hours of sleep deprivation (Csipo et al., 2021). Specifically, these authors demonstrated that young healthy adults experience a smaller haemodynamic response during a motor task after one night of total sleep deprivation as compared to pre-sleep deprivation. However, no study to date has considered the effects of one night of *partial* sleep restriction on NVC, which might reflect a more typical dose of sleep achieved. This thesis therefore strives to understand the consequences of acute partial sleep loss on NVC in young, healthy adults, providing a starting ground for future research to explore in both healthy older adults, and older adults with dementia.

One way modern society seeks to remedy acute sleep loss is through the consumption of caffeine. Importantly, there is thought to be a ‘J-shaped’ association between coffee intake and future incidence of cognitive disorders (i.e. Alzheimer’s disease, dementia, cognitive decline, and cognitive impairment), with the smallest risk at 1-2 cups of coffee per day (Wu, Sun, & He, 2017). Despite the relationship between caffeine consumption and cognitive disorder risk, and the positive effect acute caffeine consumption has on cognitive functions and alertness (Fisone, Borgkvist, & Usiello, 2004; McLellan, Caldwell, & Lieberman, 2016), caffeine acutely decreases resting cerebral blood flow (Addicott et al., 2009; Field, Laurienti, Yen, Burdette, & Moody, 2003). This reduction in blood flow in turn causes an ‘uncoupling’ between cerebral blood flow and metabolism (Chen & Parrish, 2009; Zhang, Lee, Kwan, Schwartz, & Fawzi, 2020). However, it is currently unknown how caffeine affects NVC in the partially sleep restricted state. Such context is important to understand, given that this is likely the state in which adults will most typically consume caffeine.

1.2. Thesis aims

In light of the above, there is a clear rationale to study the effects of partial sleep restriction, reflecting typical sleep loss experienced by the general population, and subsequent caffeine ingestion on NVC. This thesis therefore aims to investigate 1) the impact of one night of 50% sleep restriction on NVC, and 2) whether caffeine ingestion affects NVC after partial sleep restriction.

Chapter 2: Literature Review

2.1. Sleep and cardiometabolic health

The National Sleep Foundation recommend that adults aged between 18 – 65 years old should aim to sleep for 7 – 9 hours per night (Hirshkowitz et al., 2015). However 25% of adults fail to routinely achieve this, with more than 5% report sleeping less than six hours per night, and a further 13% reporting poor quality sleep (Chaput et al., 2018; Kocevska et al., 2021). This is a concern given that both short (< 7 hour per night) and long (> 9 hours per night) sleepers, as compared to those meeting the sleep guidelines, are at an increased risk of all-cause mortality (+ 22 to 26% for short sleep and + 17 to 24% for long sleep) (Gallicchio & Kalesan, 2009; Hublin, Partinen, Koskenvuo, & Kaprio, 2007). Such risk could be attributed to many different interactions between sleep duration and morbidity. For example, the associations between short sleep duration and an increased incidence of obesity (Cappuccio et al., 2008), cardiovascular (Fernandez-Mendoza et al., 2019; Krittanawong et al., 2019; Tobaldini et al., 2017) and cerebrovascular disease (Lutsey et al., 2018; Sabia et al., 2021; Wu et al., 2018). Combined with the impact of chronic short sleep on productively levels within the workforce, our inability to meet sleep guidelines is thought to have a considerable impact on the national economy. The United Kingdom is estimated to lose approximately 200,000 working days per year due to poor sleep duration, at an estimated expense to the economy of £40 billion per year - 1.86% of the country's gross domestic product (Hafner, Stepanek, Taylor, Troxel, & Van Stolk, 2017).

Individuals habitually sleeping 7 hours or less per night are at a greater risk of developing coronary heart disease and/or stroke (Cappuccio, D'Elia, Strazzullo, &

Miller, 2010), which may in part be explained by the increased risk of obesity among short sleepers (Cappuccio et al., 2008). Poor sleep is also associated with vascular remodelling (Wolff et al., 2008). Specifically, a J-shape relationship exists between sleep duration and carotid intima-media thickness, where thickness is lowest among participants with an average sleep duration of 7 – 8 hours per night, but increased with shorter and longer sleep duration (Wolff et al., 2008). Studies have attempted to understand the mechanisms behind the relationship between sleep and cardiovascular disease in cross-sectional investigations. A usual sleep duration above or below the median of 7- 8 hours per night is associated with an increased incidence of hypertension, which remains significant after adjustment for age, sex, race, apnoea-hypopnea index, and body mass index (Gottlieb et al., 2006). Furthermore, chronic sleep loss has been suggested as a risk factor for weight gain, insulin resistance and Type 2 diabetes (Spiegel, Knutson, Leproult, Tasali, & Cauter, 2005), an additional problem given that multiple midlife cardiovascular risk factors increase the risk of cognitive impairment later in life (Virta et al., 2013). This is supported by the findings of Jefferson et al. (2015) who reported that a high Framingham Cardiovascular Risk Profile score (a score encompassed of factors such as blood pressure, diabetes and cholesterol) is associated with worsening cognitive abilities, both in a cognitively healthy sample, as well as in patients with mild cognitive impairment (Jefferson et al., 2015). Thus, habitual sleep loss may indirectly contribute to dementia risk through unfavourable associations with cardiometabolic indices of health.

2.2. Sleep and cerebrovascular function

In addition to these associations with cardiometabolic risk factors for dementia, there is concern that habitual poor sleep might directly influence the cerebrovasculature. Research investigating the effects of persistently sleeping less than 6 hours per night

on cerebrovascular function is limited to studies of sleep disordered breathing. Importantly, in a meta-analysis by Shi et al. (2018), sleep disordered breathing was found to be a risk factor for all-cause dementia, Alzheimer's disease, and vascular dementia. However, no significant independent relationship was found between vascular dementia and insomnia after adjusting for confounders related to cardiovascular, cerebrovascular, and metabolic disease.

Obstructive sleep apnoea (OSA) involves periods of interrupted breathing during sleep due to airway obstruction with an ongoing respiratory effort, and thus results in disrupted, poor quality sleep, where total sleep time is attenuated. Such events also have considerable, acute effects on brain blood flow. Specifically, cerebral blood velocity through the MCAv has been reported to increase by up to 200% of baseline velocity during an episode of OSA (Bålfors & Franklin, 1994; Durgan & Bryan Jr, 2012). Following termination of apnoea, MCAv frequently falls below baseline values for approximately one minute. Although research in this area is limited, studies suggest that resting cerebral blood flow is chronically lower, both during sleep and wakefulness, in individuals with OSA as compared to those without (Durgan & Bryan Jr, 2012; Fischer, Chaudhary, Taormina, & Akhtar, 1992; Meyer, Ishikawa, Hata, & Karacan, 1987; Meyer, Sakai, Karacan, Derman, & Yamamoto, 1980; Urbano, Roux, Schindler, & Mohsenin, 2008). This decreased cerebral blood flow in patients with OSA is consistent with the literature exploring the acute effects of sleep restriction on brain blood flow. Poudel and colleagues (2012) restricted 20 healthy young adults, aged 20 – 37 years with no history of sleep disorders and a usual time in bed of 7 – 8.5 hours, to four hours in bed as part of a sleep restriction protocol. The following day participants were scanned during wakefulness using arterial spin labelling (ASL) perfusion imaging. The authors reported a global decrease in cerebral blood flow,

however other measures of cerebrovascular function were not considered in this study.

Cerebrovascular function can also be assessed in terms of cerebrovascular reactivity, which gives an index of reactivity of the intracranial vessels in response to a stimulus, such as ventilatory alterations in the partial pressure of carbon dioxide ($PaCO_2$) (Willie et al., 2011). However, the research in OSA patients with respect to hypercapnic (elevated $PaCO_2$) challenges is inconclusive and yields conflicting findings. Foster et al. (2009) assessed the cerebrovascular and ventilatory responses to hypercapnia in eight male patients aged 18 – 50 years with OSA, and 10 healthy age and sex matched controls. The OSA patients were assessed before after 4-6 weeks of continuous positive airway pressure (CPAP) therapy. No differences were found in the cerebrovascular and ventilatory response to hypercapnia between OSA and controls at baseline or follow-up. Similarly, Urbano et al. (2008) found no difference in the hypercapnic response between OSA and control patients. In contrast, Reichmuth et al. (2009) found that the cerebrovascular response to hypercapnia was smaller in 20 patients with OSA as compared to 20 controls, and that CPAP resulted in a trend towards improvement in the hypercapnic response. Comparable findings were reported by Diomedi et al. (1998) whereby participants with OSA had lower cerebrovascular reactivity to hypercapnia than healthy controls. Furthermore, Morgan et al. (2010) sought to explore whether cerebrovascular reactivity was altered in patients with less severe sleep-disordered breathing. A rebreathing protocol was used to induce hypercapnia (+ 10 mmHg) during wakefulness in 373 participants of the Wisconsin Sleep Cohort (a random sample of 1550 males and females of the Wisconsin state who underwent polysomnographic examinations at 4-year intervals) whilst TCD was used to assess MCAv. Linear regression analysis showed a positive

correlation between cerebrovascular carbon dioxide (CO_2) responsiveness and mean oxygen saturation during sleep, which remained significant after adjustment for known confounders (Morgan et al., 2010). However, it cannot be concluded that these findings are necessarily attributable to poor sleep, given the likelihood that respiratory factors involved in OSA and sleep disordered breathing are also affecting the cerebrovasculature, as highlighted by the findings of Morgan et al. (2010). People with OSA may routinely experience elevations in arterial carbon dioxide ($PaCO_2$), and it is known that populations who are characterised by elevated $PaCO_2$, such as those with chronic obstructive pulmonary disease, also present with altered cerebrovascular reactivity (Bernardi et al., 2008; Hlavati, Buljan, Tomić, Horvat, & Butković-Soldo, 2019).

2.3.1. Neurovascular coupling

Another fundamental regulatory process that can be assessed as measure of cerebrovascular function is NVC. In 1948, it was demonstrated that cerebral blood flow increases with global brain hyperactivity and is attenuated when brain activity is suppressed (Kety & Schmidt, 1948). Despite the brain accounting for as much as 20% of resting metabolism (Sokoloff, 1989), it has very little capacity to store energy or oxygen. Therefore, it is of critical importance that alterations in cerebral metabolism can be quickly matched by changes in cerebral perfusion. This functional hyperaemia describes NVC, where temporal and regional increases in cerebral blood flow occur in order to provide increased nutrient delivery and waste product clearance.

Multiple, interrelated mechanisms are involved in the matching of cerebral blood flow to metabolism. Increases in cerebral perfusion are controlled by feedforward and

feedback mechanisms within the neurovascular unit (Iadecola, 2017). The neurovascular unit (Figure 2.1) is a functional entity comprised of neurons, glia/astrocytes, vascular smooth muscle, and blood vessels, which emphasises the unique, interdependent, relationship between brain cells and the cerebral vasculature. It has been suggested that the metabolic feedforward mechanism triggers exaggerated cerebral blood flow to the region, and in turn neural feedback mechanisms fine tune blood delivery to the needs of the tissue (Iadecola, 2017). This aligns with the observation that cerebral blood flow typically peaks at the onset of an experimental cognitive or visual challenge, then adjusts to a lower perfusion (Smirl, Wright, Bryk, & van Donkelaar, 2016).

Astrocytes (specialised glial cells that are connected to blood vessels and neurons) are also involved in the control of cerebral metabolites (Iadecola & Nedergaard, 2007). These cells are well tuned to their microenvironment and play a key role within the neurovascular unit. Another mechanism in which the NVC response may be regulated, involves the autoregulatory actions of astrocytic mechanosensors in response to changes in tissue pressure (Kim, Diaz, Iddings, & Filosa, 2016). Whether generated by neurons, astrocytes or endothelial cells, signals act on the vasomotor apparatus to stimulate vasodilation, decrease vascular resistance and increase cerebral blood flow (Iadecola, 2017). Vasomotor responses are controlled by the constricting and relaxing of smooth muscle cells lining resistance vessels, which are in turn controlled by neural activity, vasoactive compounds (Iadecola, 2017) and intravascular pressure (Longden, Hill-Eubanks, & Nelson, 2016).

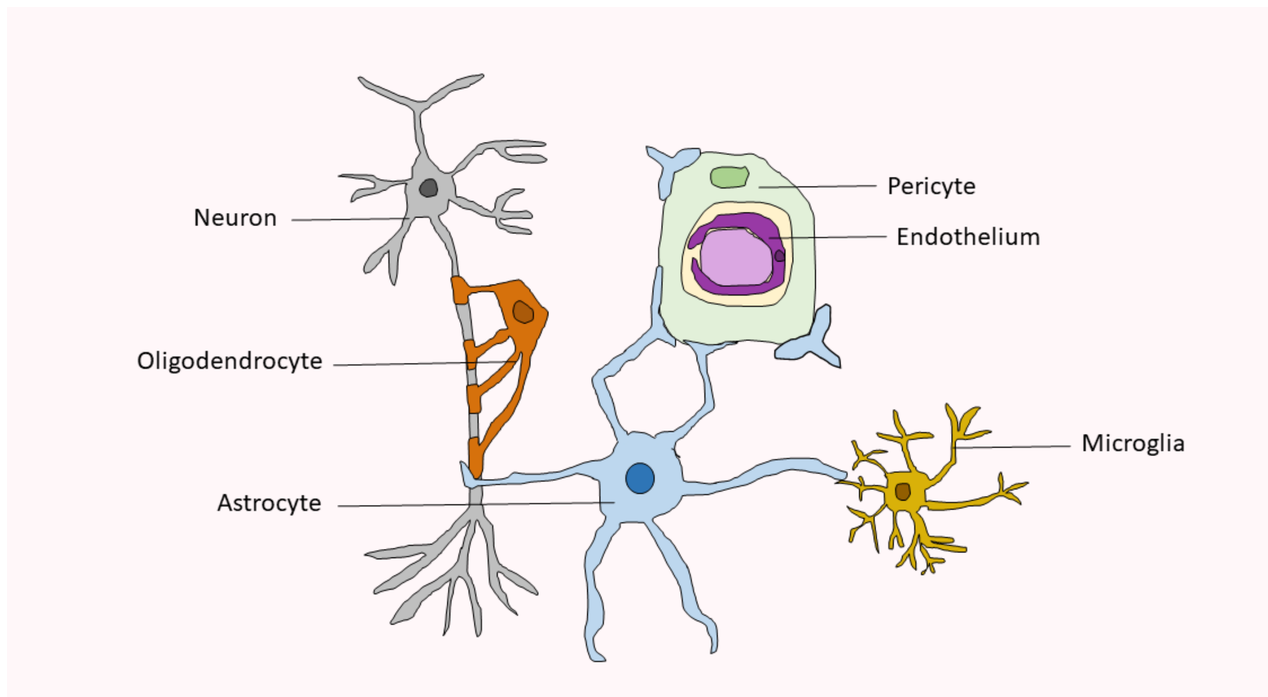

Figure 2.1. The neurovascular unit at the level of the brain is comprised of vascular cells (pericytes and endothelial cells), glia (astrocytes, oligodendrocytes, and microglia), and neurons. Adapted from Nelson, Sweeney, Sagare, & Zlokovic, (2016).

The feedback mechanism refers to the clearance of waste product metabolites, e.g., lactate, CO_2, amyloid-β peptide and tau (Figure 2.2), with the latter two implicated in the development of dementia (Janelidze et al., 2016). Some of these metabolites, including adenosine, CO_2, hydrogen, and lactate, are in themselves vasodilators (Freeman & Li, 2016; Ko, Ngai, & Winn, 1990). However, based on the evidence that the initial increase in cerebral blood flow is greater than the oxygen needs of the tissue (Raichle & Mintun, 2006), and that a rise in cerebral blood flow is also seen under conditions of excess oxygen and glucose (Attwell & Iadecola, 2002), it has been proposed that blood flow is also regulated by a neural feedforward mechanism (Figure 2). Neurons act on local blood vessels to initiate the local vascular response. Neurovascular signalling pathways, including the complex activity of glutamatergic synapses (Attwell et al., 2010), elicit the release of vasoactive compounds, including

potassium, nitric oxide (NO) and prostanoids (Attwell et al., 2010; Attwell & Iadecola, 2002; Drake & Iadecola, 2007). Similarly, endothelial cells play a key role in regulation of cerebral blood flow in response to chemical and mechanical signals, with the ability to produce various vasoactive compounds, also including NO, prostanoids, and endothelium derived hyperpolarising factor.

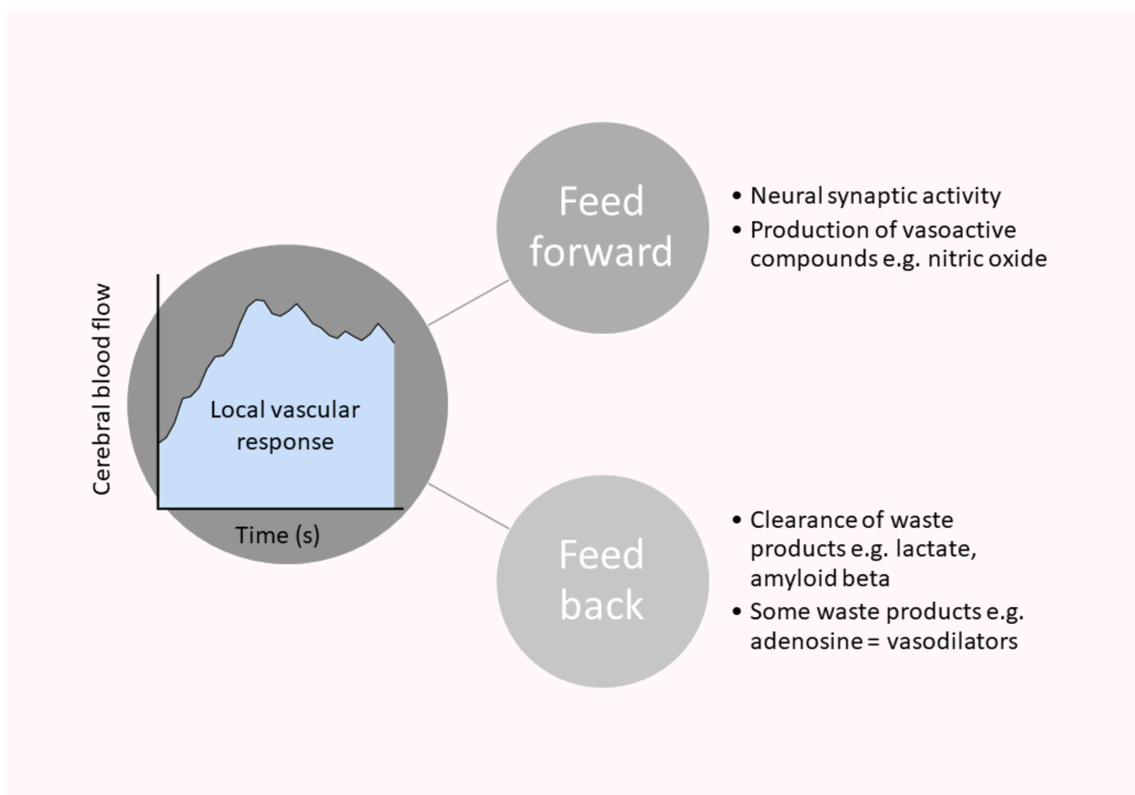

Figure 2.2. Potential feed forward and feedback mechanisms driving the local vascular response. Adapted from Iadecola (2017).

Recently, NO has been shown to play a role in NVC in humans. Hoiland et al. (2020) infused a non-selective NO blocker in young healthy males and found a 30% attenuation in peak NVC. Such findings were substantiated by Carter and colleagues (2021) who found a decrease in cerebral blood flow following infusion of non-selective nitric oxide synthase (NOS). Three NOS isoforms exist, endothelial NOS, neuronal

NOS and inducible NOS isoforms (De Silva & Faraci, 2020). Upon neuronal activation, neuronal NOS is produced and has been reported to decrease cerebral blood flow by ~4% (O'Gallagher et al., 2021). Therefore, while the exact contributions different NOS isoforms play in NVC remains to be elucidated, there is evidence to suggest that NO production is not limited to endothelial NOS.

Importantly, deficits in vascular function mediated by NO have been reported in ageing and disease (Toth, Tarantini, Csiszar, & Ungvari, 2017). The review by Toth et al. (2017) extensively discusses the association between ageing and endothelial dysfunction, highlighting various mechanisms for this association. For example, there is an increased production of reactive oxygen species in the vasculature of the brain with age, as well as an increased production of superoxide which readily reacts with NO to form peroxynitrite, thus lessening the bioavailability of NO (Toth et al., 2017). In addition to this, these age related impairments in endothelial dysfunction are exacerbated by comorbid conditions. In support of this, Lavi et al. (2016) investigated whether cerebrovascular reactivity was associated with endothelial dysfunction in 16 patients with diabetes mellitus and/or hypertension, compared to 12 age- and sex-matched healthy controls. The study found that cerebrovascular reactivity was impaired in the patient population as compared to controls, and that a NO donor offset this disparity. Furthermore, age-related endothelial dysfunction is thought to play a role in the chronically reduced cerebral blood flow seen in ageing, and subsequent cerebral dysfunction and cognitive decline (Toth et al., 2017). This is comprehensively reviewed by Di Marco et al. (2015), highlighting the circular role of vascular endothelium-mediated mechanisms in the pathogenesis of Alzheimer's disease. The transcription of endothelial NOS is dependent on sufficient cholinergic innervation of micro-vessels and loss of such cholinergic function is a key symptom in Alzheimer's disease

(Lawrence & Sahakian, 1995). Therefore, Rosengarten et al. (2006) investigated the role of acetylcholine esterase inhibition (AChEI) in NVC (assessed as the cerebral artery blood velocity in response to a visual stimulus using TCD, see *2.3.2*) in a study of patients with Alzheimer's disease who had no vascular risk factors, and a group of healthy elderly participants. The study found that while patients with Alzheimer's disease showed the typical damping in cerebral blood velocity responses (see *2.5.2*), these were restored to the same as control participants in a dose-dependent manner under AChEI. The authors therefore concluded that the changes in NVC seen in patients with Alzheimer's disease is possibly due to decreased levels of endothelial NOS, given the effects of AChEI on NVC (Rosengarten et al., 2006). Similarly, in a cell culture, cells treated with amyloid-β resulted in inhibited production of endothelial NO (Sutton, Hellermann, & Thomas, 1997).

2.3.2. Assessing neurovascular coupling with transcranial Doppler ultrasound

Given its high temporal resolution and non-invasive nature, TCD can be used to measure static and dynamic blood velocities within the major cerebral arteries. Insonation of these arteries is possible through 'acoustic' windows – thinner regions of the skull (Willie et al., 2011). Sound waves are emitted from the Doppler probe and as these reflect of dynamic red blood cells, the waves are detected by the transducer and the subsequent Doppler-shift is proportional to cerebral blood velocity (Willie et al., 2011). This tool is therefore useful across various assessments of cerebrovascular function, including cerebrovascular reactivity, autoregulation and NVC. TCD is safe for use in both healthy and diseased states and can thereby be used not only to document differences in populations (LaRovere & O'Brien, 2022; Silvestrini et al., 1993;

Silvestrini, Troisi, Matteis, Cupini, & Caltagirone, 1995), but also in the clinical setting to assess various cerebrovascular pathologies, including vasospasm, stenosis and thrombosis (Rasulo, De Peri, & Lavinio, 2008; Willie et al., 2011).

Neurovascular coupling can be indirectly assessed using TCD in humans by measuring baseline cerebral blood velocity through both the MCA and/or the PCA. Given that the PCA is the primary artery supplying blood to the occipital lobe and thus the visual processing areas of the cerebral cortex, PCAv can be recorded during a visual processing task as an indirect assessment of NVC. TCD can be used to assess changes in cerebral blood velocity to a stimulus and therefore to assess NVC due to its high temporal resolution (Aaslid, Markwalder, & Nornes, 1982). The TCD assessment of NVC can be observed when participants transition from eyes-closed to eyes-open to a visual stimulus. Previously this visual stimulus has been varied, for example reading (Azevedo, Rosengarten, Santos, Freitas, & Kaps, 2007; Rosengarten, Aldinger, Kaufmann, & Kaps, 2001) or viewing a checkerboard patterns (Zaletel, Štrucl, Rodi, & Zvan, 2004). However, recently a more complex visual search task has been recommended when assessing NVC due to its ability to evoke a more robust and reliable cerebral blood flow response (Smirl et al., 2016). This larger response is likely due to a greater visual search challenge which requires more saccadic eye movements and extended fixation durations in comparison to e.g., reading (Henderson & Luke, 2014). In support of this, Burma et al. (2021) reported that increasing the speed of a simple shape visual presentation task did not result in concurrent increases in the PCAv NVC response, however a complex visual search task invoked a greater response than all speeds of the simple shape tasks. In addition to this, participants reported an engagement score for each speed of the simple shapes task and for the complex visual search task. These measures of engagement

displayed moderate correlations with NVC as assessed as area under the PCAv curve versus time (AUC) (Burma, Wassmuth, et al., 2021).

Typically, NVC, assessed via TCD, is quantified as both absolute and percent increase in PCAv from an eyes-closed baseline to average eyes-open, as well as from baseline to peak eyes-open (Burma, Macaulay, et al., 2021; Burma, Van Roessel, Oni, Dunn, & Smirl, 2022; Burma, Wassmuth, et al., 2021; Smirl et al., 2016). To quantify total activation, the total AUC (tAUC) versus time for the eyes-open PCAv response is calculated, along with incremental AUC versus time (iAUC), subtracting the eyes-closed baseline period from the curve (Figure 2.3).

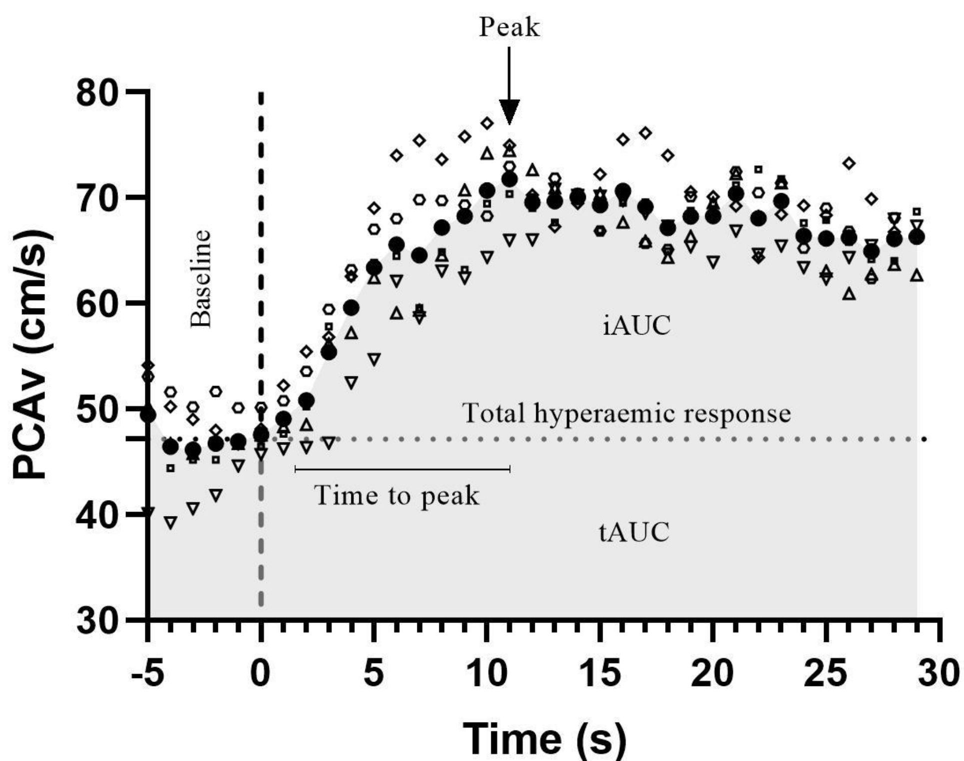

Figure 2.3. A representative trace illustrating cerebral blood velocity through the posterior cerebral artery (PCAv) with traditional neurovascular coupling (NVC) analysis methods and outcomes, including the time to peak and peak PCAv, incremental area under the curve (iAUC) and total area under the curve (tAUC). Baseline represents the final five seconds of eyes-closed, and the vertical dashed line indicates eyes-open to a visual search task. The horizontal dotted line demonstrates the average PCAv during the baseline period. Data is displayed for each of the five trials along with the ensemble average (closed circles).

An important component of NVC is the ability to *rapidly* react to an increase in metabolic demand. Previously, in attempt to calculate the speed of the response, studies have reported the time from onset of visual stimulus to peak velocity and the time from stimulus onset to an elevation in velocity >1% above the eyes closed baseline period (Smirl et al., 2016). While this does give an indication of how quickly PCAv starts to respond and peak, it provides only limited insight into the time course of the response. For example, two individuals can have the same onset and time to peak PCAv response, but have difference temporal trajectories in blood flow. A novel development in this field is applying kinetic modelling in order to characterise the reactivity of cerebral blood flow. For example, this has been applied in studying the changes in MCAv in response to hypercapnia (Koep, Bond, et al., 2022; Koep, Weston, et al., 2022) and the onset of exercise (Billinger et al., 2017; Ward et al., 2018; Weston et al., 2022). In so doing, this approach has been able to distinguish differences in cerebral blood flow, and its regulation, between groups, which otherwise might not be apparent from traditional metrics (Koep, Bond, et al., 2022; Tallon, Barker, Nowak-Flück, Ainslie, & McManus, 2020).

Novel kinetic analysis of the NVC response may be possible using a mono-exponential model, characterising both the amplitude and time constant (τ) (Figure 2.4). Where necessary, a time delay (TD) can be added to the model. The identification of a TD as part of this model may be more accurate than previous methods where the onset of the response was characterised as a >1% increase in velocity (Smirl et al., 2016). As PCAv is typically ~30-40 cm/s when resting with eyes closed, a 1% increase could be < 1 cm/s, which is hard to truly identify given that the beat-to-beat variation in baseline PCAv may also be < 1 cm/s (e.g. Table 3.2). Analysis of the kinetic onset response of

PCAv to a visual stimulus may also avoid any confounding influences of variability observed in the latter half of the response due to lapses in engagement in the visual task (Burma, Wassmuth, et al., 2021).

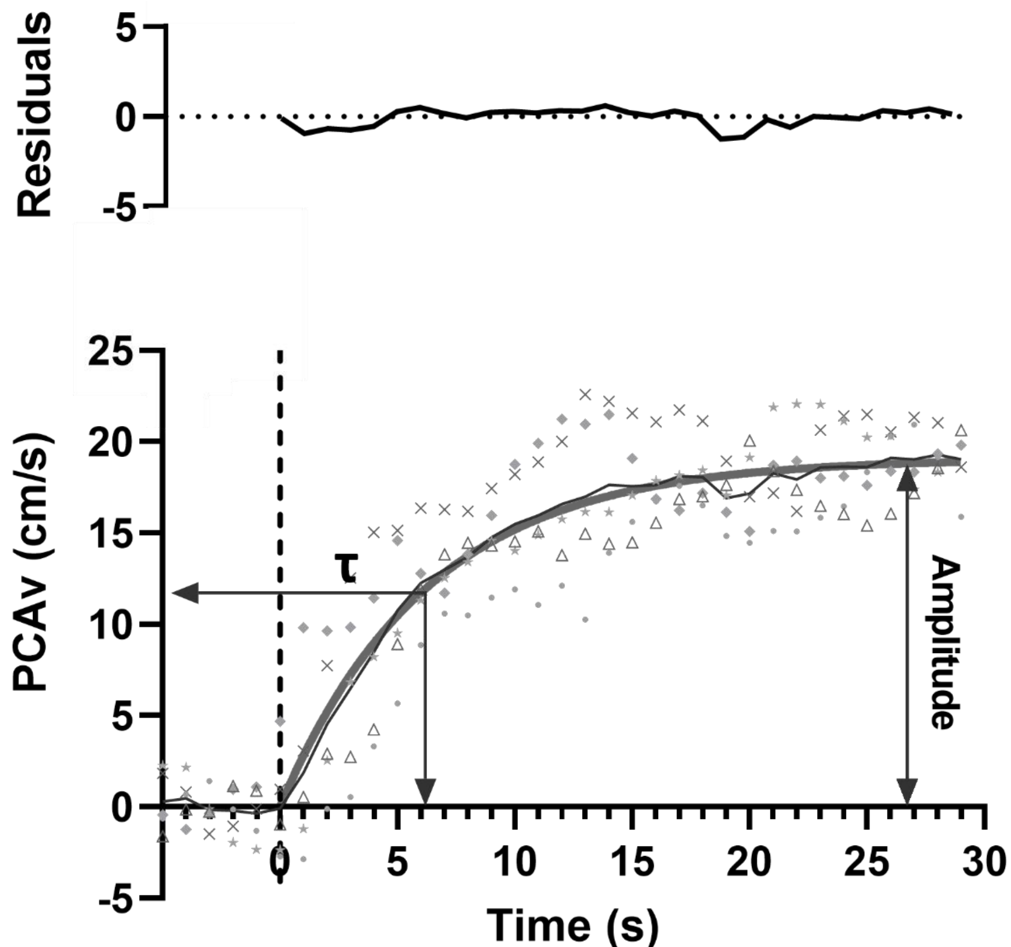

Figure 2.4. A representative trace illustrating cerebral blood velocity through the posterior cerebral artery (PCAv) using novel neurovascular coupling (NVC) kinetic analysis methods. Residuals of model fit are also presented. Baseline represents the final five seconds of the eyes-closed period, and the vertical dashed line indicates the onset of eyes-open to the visual search task. Data has been baseline corrected to show the increase in PCAv. The thin black line represents the ensemble average of the five trials, while the bold grey line shows the fitted mono-exponential model of this ensembled average. Data is also displayed for each of the trials (grey crosses, diamonds, triangles, stars and dots). Amplitude of this response = 19.1 cm/s, τ 6.3 s.

While TCD provides a useful tool for quantifying cerebral artery blood velocity, it is also important to note its limitations. In certain populations, for example elderly

participants, only one acoustic window may be viable for artery insonation, due to thickening of the skull across other windows, resulting in either no insonation or a very scattered, poor quality image (Aaslid, 2012). In addition to this, the principal limitation of TCD is its lack of spatial resolution that can be offered by other imaging modalities, such as magnetic resonance imaging (MRI), with each TCD probe limited to imaging one cerebral artery at any one time (Wintermark et al., 2005). Finally, as the diameter of the insonated vessel is unknown, TCD only measures cerebral blood velocity and not absolute, volumetric flow (Willie et al., 2011). Therefore, based on the Hagen-Poiseuille law, cerebral blood velocity can only be used as a valid surrogate of cerebral blood flow if the diameter of the insonated vessel remains constant. $PaCO_2$ is known to be a potent vasodilator, and so studies have attempted to account for changes in vessel diameter. This can be done via measurement of changes in end tidal concentrations of CO_2 ($P_{ET}CO_2$), given the strong correlation between these two outcomes (McSwain et al., 2010). The extent to which the major cerebral vessels undergo changes in diameter in response to cerebrovascular challenges remains debated (Brothers & Zhang, 2016; Hoiland & Ainslie, 2016). In 2000, using a 1.5-T clinical MRI scanner, Serrador et al. found that the MCA diameter did not change with hyper- (+ 8 mmHg $P_{ET}CO_2$) nor hypocapnia (– 13 mmHg $P_{ET}CO_2$). However, contradictory findings were later reported by Coverdale et al. (2014) using a more powerful 3T MR scanner with an 8% increase in MCA diameter during hypercapnia and a 4% decrease during hypocapnia. Smaller changes in vessel diameter were also reported by Verbree et al. (2014), with a 1.5% increase in MCA diameter at + 7.5 mmHg $P_{ET}CO_2$, and 7% increase at + 15 mmHg $P_{ET}CO_2$, although only the latter reached statistical significance. These conflicting findings are likely a result of differing MRI approaches and assumptions, the magnitude and delivery of CO_2 challenges, and

variability in the reliability of $P_{ET}CO_2$ to accurately predict $PaCO_2$ (Ainslie & Hoiland, 2014; McSwain et al., 2010).

However, these aforementioned studies only assessed changes in diameter to the MCA, rather than the PCA. Previous work has shown that cerebrovascular reactivity is not homogenous across the cerebral arteries, with the MCA exhibiting greater reactivity to $P_{ET}CO_2$ stimuli than the PCA (Bruce et al., 2016; Skow et al., 2013). This may be explained by a greater propensity for the MCA to change diameter than the PCA. A recent study demonstrated that under hypocapnic conditions (−10 mmHg $P_{ET}CO_2$), the MCA constricts by 5.7% while the PCA decreases by 4.2%, and under hypercapnic conditions (+ 7 mmHg $P_{ET}CO_2$) the dilation of the MCA is more than double the dilation of the PCA (+ 7.4% and + 2.6%, respectively) (Al-Khazraji, Shoemaker, Gati, Szekeres, & Shoemaker, 2019). While changes in $P_{ET}CO_2$ can therefore influence PCA diameter, tests of NVC do not tend to elicit significant changes in $P_{ET}CO_2$. Previous studies utilising TCD in assessment of NVC have demonstrated that $P_{ET}CO_2$ remains well within ± 5 mmHg of eucapnia during NVC tasks involving reading text, complicated visual search, viewing coloured dots and viewing a checkboard pattern (Smirl et al., 2016; Zaletel et al., 2004). Therefore, changes in diameter of the vessel are unlikely to affect PCAv during such tests.

2.4. Sleep and neurovascular coupling

Unlike other measures of cerebrovascular function, such as cerebrovascular reactivity (Diomedi et al., 1998; Foster, Hanly, Ostrowski, & Poulin, 2009), NVC has not been studied in the context of OSA, nor habitual sleep duration. Furthermore, only one interventional study to date has assessed the interaction between sleep and NVC,

specifically measuring the influence of one night of total sleep deprivation (Csipo et al., 2019). In this study, cognitive performance and NVC assessments were conducted in ten healthy, male participants prior to, and after 24 hours of complete sleep deprivation. NVC was characterised using TCD during a n-back cognitive function test (a working memory challenge), as well as dynamic retinal vessel analysis during flicker light stimulation, and functional near infrared spectroscopy (fNIRS) during a finger tapping motor task. Near-infrared spectroscopy (NIRS) is a non-invasive tool that can be used to determine changes in oxygenation patterns in different tissues in the human body. Specifically, NIRS can be used to distinguish between oxyhaemoglobin (O_2Hb) and deoxyhaemoglobin (HHb), as they are the main chromophores absorbing near-infrared light, and demonstrate distinct absorption spectrums (Csipo et al., 2019). Given the shared developmental, anatomical and physiological origins, retinal vessels may act as a surrogate vascular bed in understanding the cerebral microvasculature, both in structure and in function (Lipecz et al., 2019), and may predict cerebrovascular pathologies (de Jong et al., 2011; Heitmar, Kalitzeos, Patel, Prabhu-Das, & Cubbidge, 2015; McGrory et al., 2017). Analogous to the NVC responses in the brain, the retinal microcirculation is also dependent on close interactions between neuronal activity, activation of glial cells, and endothelium-dependent vasodilation (Metea & Newman, 2006, 2007). Flicker light stimulation can therefore be used in dynamic retinal vessel analysis to evoke NVC in retinal vessels (Csipo et al., 2021; Csipo et al., 2019; Lipecz et al., 2019). After complete sleep deprivation, Csipo et al. (2021) reported decreased cognitive performance assessed as reaction time and sustained attention. MCAv during the n-back test was also lower as compared to pre-sleep deprivation. However, baseline MCAv, as well as NVC responses measured by dynamic retinal vessel analysis did not change significantly from pre to post sleep deprivation. Csipo et al.

(2021) therefore concluded that 24 hours of sleep deprivation led to cognitive deficits and altered cerebral blood flow and cortical NVC related haemodynamic responses in the brain.

The methods used by Csipo et al. (2021) to characterise NVC using TCD were not typical of those in the field. Firstly, the researchers removed the first 10 seconds of MCAv data from the response to eliminate motion artefacts, however, the peak cerebral blood velocity response typically occurs within the first 10 – 15 seconds of stimulus presentation (Smirl et al., 2016). It is therefore possible that the study did not capture the peak response, and differences in peak amplitude between the rested and sleep deprived measures may have been attenuated. In addition to this, the authors did not measure the change from baseline to during the cognitive function test, but the raw velocity during baseline and during the n-back test independently. Furthermore, no time based parameters, such as time until peak, were reported, which again may have differentiated between the rested and sleep deprived assessments. Another consideration is the assessment of cerebral velocity through the MCA, as opposed to the PCA. While previous research has utilised measurements of MCAv during n-back or similar working memory tests as an assessment of NVC, these tends to yield a smaller magnitude of response in comparison to assessing PCAv during visual stimulation tests (Smirl et al., 2016), minimising the opportunity to see differences across conditions. For example, Smirl et al. (2016) saw a 16% greater increase in PCAv than in MCAv during visual stimulation tests, thus demonstrating a superior signal to noise ratio for PCAv during NVC assessments, and in turn advocating for this method of assessing NVC in future studies.

A final consideration of the study conducted by Csipo et al. (2021) is the use of a total sleep deprivation protocol. Instructing participants to remain awake for >24 hours is

not reflective of the sleep loss typically experienced by the general population, and therefore lacks generalisation and ecological validity. Nonetheless, as this is the first study in this field, this research is still of substantial value, providing considerable scope to further this work and better understand the relationship between sleep loss and NVC.

2.5.1. Dementia

Dementia is an umbrella term for memory loss and other cognitive impairments which are severe enough to interfere with daily life and are generally associated with abnormal brain changes (Chertkow, Feldman, Jacova, & Massoud, 2013). The most common forms of dementia are Alzheimer's disease, vascular dementia and Lewy body dementia. Whilst average life expectancy has increased over recent decades, as has the number of people living with some form of dementia. Approximately 50 million people worldwide currently live with dementia, with numbers expect to almost double every 20 years (Li et al., 2021; Prince et al., 2013). In addition to the devastating nature of this disease for individuals and their families, dementia is estimated to cost the UK economy £34.7 billion each year (Wittenberg, Hu, Barraza-Araiza, & Rehill, 2019). Despite this economic burden and substantial progress in understanding the disease, there is still no 'cure' for dementia. Accordingly, the World Health Organization has declared dementia a global health priority, with the goal of identifying disease modifying therapies by 2025. Research is therefore needed to identify effective primary prevention strategies, and approaches to slow the progression of neurodegenerative processes in those at risk.

2.5.2. Dementia and cerebrovascular dysfunction

Cerebrovascular pathologies are thought to precede amyloid-β aggregation and accumulation, and therefore these vascular changes may be an appealing early target in dementia research and diagnosis (Beishon et al., 2017). Since multiple studies have reported that both vascular dementia and Alzheimer's disease is characterised by a decreased blood supply at rest (Bateman, Levi, Schofield, Wang, & Lovett, 2006; Claassen, Diaz-Arrastia, Martin-Cook, Levine, & Zhang, 2009; Farkas & Luiten, 2001; Vicenzini et al., 2007) Beishon et al. (2017) conducted a meta-analysis investigating the use of cerebral haemodynamics and oxygenation, specifically through use of TCD and NIRS, as novel biomarkers of mild cognitive impairment. The review revealed decreased tissue oxygenation index, cerebral blood flow and velocity and cerebrovascular reactivity in mild cognitive impairment compared to controls, with mixed results with respect to neuroactivation (a technique often used to study NVC). The authors concluded that, despite heterogeneity across the studies reviewed, the analyses showed clear alterations in cerebral haemodynamic and oxygenation patterns in this population, and that future studies should continue to investigate these outcomes as biomarkers for dementia (Beishon et al., 2017).

Not only is NVC a fundamental process, but alterations in the ability to match brain metabolism with cerebral blood flow are thought to be of clinical relevance. NVC is disrupted in dementia, specifically in Alzheimer's disease (Girouard & Iadecola, 2006; Nicolakakis & Hamel, 2011; Shabir, Berwick, & Francis, 2018; Turner, 2021). Disturbances to NVC occur much earlier in dementia than in equivalently aged individuals, highlighting the progressive neuronal deterioration, vascular dysfunction and cognitive impairments associated with dementia (Nelson et al., 2016; Turner, 2021; Zimmerman, Rypma, Gratton, & Fabiani, 2021). Varying techniques and models have been used to assess the alterations in NVC with dementia. A study of the

interaction between dynamic cerebral autoregulation and NVC in healthy older adults and patients with mild cognitive impairment and Alzheimer's Disease, found that cerebral autoregulation efficiency was reduced during task-activation in healthy but not cognitively impaired participants, suggesting processing differences in NVC between the populations (Beishon, Intharakham, Haunton, Robinson, & Panerai, 2021). Using retinal vessel response to flickering lights, one of the methods utilised by Csipo et al. (2021) to assess NVC following sleep restriction, Kotliar et al. (2017) found a delayed and more emphasised reactive dilation in patients with mild-moderate Alzheimer's disease, as compared to elderly healthy controls. The authors suggest that these alterations in NVC may be explained by damaged feedback loops, or abnormally high activity of retinal neurons in Alzheimer's disease. Such alterations in NVC were also reported in patients with Alzheimer's disease by Hock et al. (1997). In that study, NVC was assessed during a verbal fluency task using NIRS to measure cerebral haemoglobin oxygenation changes in the frontal and parietal cortex, and positron emission tomography (PET) scans to measure cerebral blood flow, respectively. The researchers reported decreased parietal cerebral haemoglobin oxygenation and cerebral blood flow during the verbal fluency task in those with Alzheimer's disease, compared to healthy elderly participants. These findings were replicated by Mentis et al. (1998) using PET scans in healthy elderly participants, and patients with both mild and moderate-severe Alzheimer's disease. Whilst all groups had similar responses to low frequency flashing lights, patients with Alzheimer's disease had a smaller cerebral blood flow response than the control group at higher frequencies of flashing lights. Likewise, patients with moderate-severe stages of disease had smaller responses than those with mild disease.

Mouse models have also demonstrated that NVC is reduced both in cognitive impairment and prematurely with ageing in Alzheimer's disease (Tarantini et al., 2015; Turner, Degan, Hoffmann, Galeffi, & Colton, 2021). Tarantini et al. (2015) demonstrated that selective pharmacological disruption of NVC is associated with impaired cognitive and sensorimotor function, indicating a causal link between NVC impairment and cognitive decline. Such animal models are useful as, not only do they allow for targeted experiments, but comparable to humans, age is a critical factor in progression of dementia in mouse models, and animal age in these models can be translated to severity of degeneration (Turner et al. 2021).

2.5.3. Potential mechanisms of cerebrovascular dysfunction in dementia

Why cerebrovascular dysfunction is affected in dementia has been widely studied. In early Alzheimer's disease there is reduced uptake of glucose into brain cells (An et al., 2018). Whether this is due to a reduced demand by diseased tissue, or a cerebral vasculature that is unable to efficiently increase cerebral blood flow in activated regions, remains debated. Therefore, several causes of such reduced uptake have been suggested (Turner, 2021) including abnormal NVC resulting in deficient substrate delivery (Cai et al., 2017; Kisler, Nelson, Montagne, & Zlokovic, 2017); microstructural irregularities of the blood-brain barrier (Farkas & Luiten, 2001); reduced facilitated glucose transport into the extracellular space (Winkler et al., 2015); and/or decreased cellular metabolism (An et al. 2018). Shabir et al. (2018) also argues that atherosclerosis plays a large role in cognitive decline, affecting the integrity of major blood vessels suppling the brain, which in turn reduces cerebral blood flow and

alters NVC, resulting in cerebrovascular dysfunction. This is discussed extensively in the review by Zimmerman et al. (2021) in the context of cerebrovascular dysfunction with ageing and ill-health (Figure 2.5).

Cerebrovascular dysfunction begins from arterial walls losing elasticity, in part caused by atherosclerotic narrowing of arteries from plaque development (Zimmerman et al., 2021). This in turn can trigger arterial wall inflammation, decreased capillary density and downstream ischaemia, consequently leading to slower perfusion. Such remodelling of the cerebrovasculature causes changes in the neural-glial-vascular signalling thereby resulting in impaired cerebrovascular function (Zimmerman et al., 2021). This is compounded by a leaky blood brain barrier and reduced clearance of cellular waste. Together with sodium/potassium pump dysfunction, and a loss of myelination contributing to slower signal transduction, these structural and chemical changes decrease neural processing efficiency, and thus contribute to impaired NVC mechanisms, manifesting as cognitive impairment (Zimmerman et al., 2021).

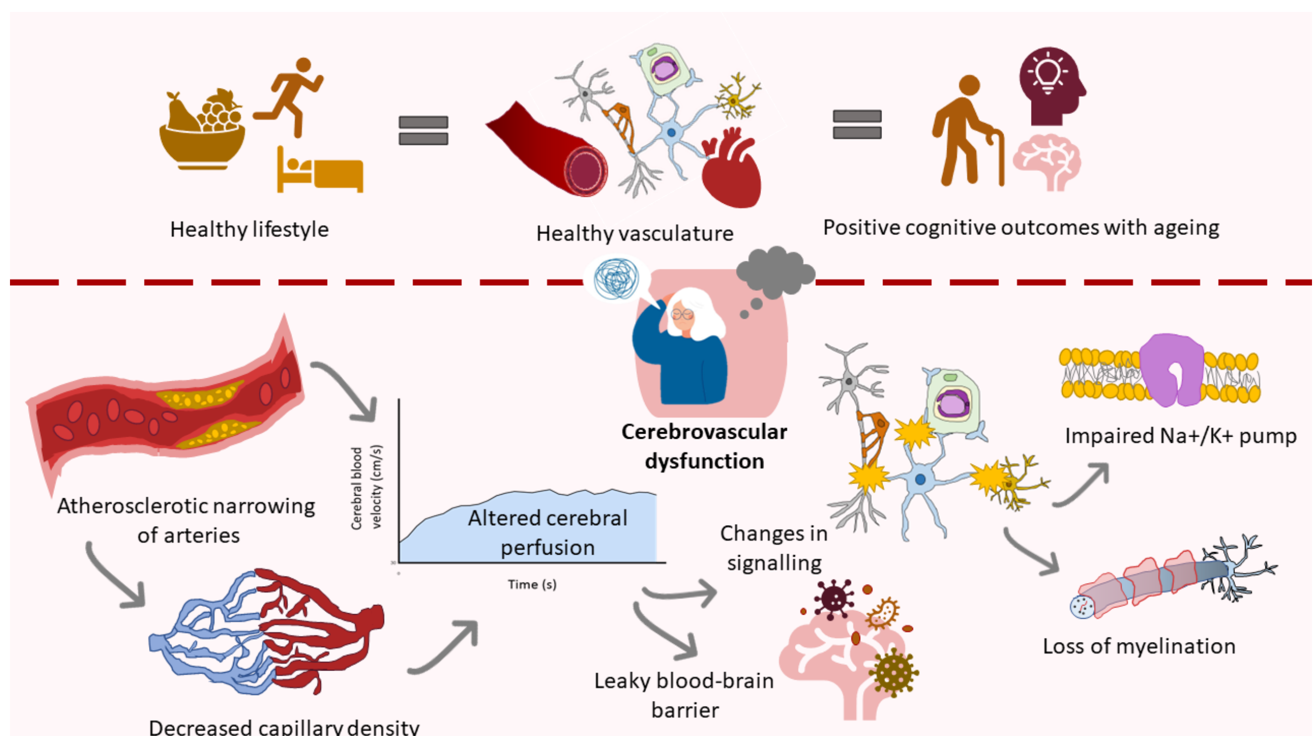

Figure 2.5. A figure depicting the process of cerebrovascular dysfunction, adapted from Zimmerman et al. (2021). Na+ sodium ions, K+ potassium ions.

2.5.4. Associations between sleep and dementia

Dementia onset and progression results from complex interactions of genetic and environmental risk factors (Migliore & Coppedè, 2022). One environmental, modifiable risk factor which is commonly associated with dementia is habitual poor sleep. Cross-sectional and prospective studies have found that those reporting poor sleep and greater sleep disturbances across the lifespan have a greater risk of developing dementia (Sterniczuk et al., 2013; Vaou et al., 2018). Using secondary analyses of sleep outcomes (sleeping problems, fatigue, taking sleeping medication, and trouble sleeping or a change in pattern) collected through the Survey of Health, Ageing and Retirement in Europe, specifically in participants who reported the absence of dementia at baseline, Sterniczuk et al. (2013) explored whether sleep-related symptoms were associated with dementia independent of their association with other illnesses. Self-reported dementia was independently predicted by each sleep measure, and a sleep disturbance index formed by a combination of the sleep outcomes, was associated with an increased risk of developing dementia, even once overall health status was added to the risk model. Furthermore, a systematic review by Wu et al. (2018) reported that the lowest incident risk of cognitive disorders (including cognitive decline, cognitive impairment, dementia and Alzheimer’s disease) was found for those who typically achieved 7-8 hours of sleep per day, and in a similar review, Vaou et al. (2018) observed that those who report severe daytime sleepiness have a greater risk of vascular dementia. Nonetheless, studies of long term occupational sleep loss have found no evidence of acute objective cognitive deficits or chronic cognitive decline (Thomas, Overeem, & Claassen, 2019; Thomas,

Overeem, Dresler, Kessels, & Claassen, 2021). However, a recent systematic review of the relationship between shift work and dementia concluded that the research is unclear, with both positive and null associations reported (Leso, Caturano, Vetrani, & Iavicoli, 2021).

Sleep loss, be that short sleep duration or poor sleep quality, can bring about immediate impairment in multiple cognitive domains, for example, alterations in working memory and executive function the next day (Scullin & Bliwise, 2015). It has also been suggested that, through repeated bouts of hypoxia, OSA may result in an accelerated amyloid-β build-up (Vaou et al., 2018), aligning with findings from a systematic review that sleep disordered breathing increases the risk of dementia (Shi et al., 2018). In this meta-analysis, the predictive roles of overall sleep disturbances, specific disturbances (e.g. insomnia, sleep disordered breathing), and other sleep difficulties (e.g. excessive daytime sleepiness) in incident all-cause dementia, Alzheimer's disease, and vascular dementia were reviewed across eighteen longitudinal studies with an average ~9.5 year follow-up period. This study also found that compared to individuals with no sleep problems, those with sleep disturbances had a higher risk of incident all-cause dementia. Furthermore, studies involving treatment of sleep disorders, including sleep disordered breathing, suggest that such treatment may delay the onset of cognitive decline (Ancoli-Israel et al., 2008).

2.5.5 Potential pathways linking sleep and dementia

The initial pathological description of Alzheimer's disease included the occurrence of amyloid plaques and neurofibrillary tangles (Lane, Hardy, & Schott, 2018), and this is still considered one of the hallmarks of the disease today. Amyloid-β is considered a metabolic waste product that is found within the interstitial fluid of the brain

(Nedergaard, 2013), however its clearance mechanisms are currently not fully understood (Mestre, Kostrikov, Mehta, & Nedergaard, 2017). The role of amyloid-β in Alzheimer's disease can be explained by the amyloid-cascade hypothesis (Querfurth & LaFerla, 2010). This cascade begins with production of amyloid precursor protein, which is also produced in the healthy brain, however in Alzheimer's disease, this can result in plaques which induce intracellular signalling and in turn the formation of hyper-phosphorylated tau proteins and neurofibrillary tangles. Amyloid-β also has the ability to incite injury via excitotoxicity and inflammatory pathways.

Research suggests that sleep performs a critical role in amyloid-β clearance (Winer et al., 2020; Xie et al., 2013). For example, a recent review highlighted that sleep deprivation has been found to both augment the production and decrease the clearance of amyloid-β production in humans (Vaou et al., 2018). Rodent and Drosophila fly models provide supporting findings of increased amyloid-β burden following chronic sleep loss (Kang et al., 2009; Tabuchi et al., 2015). Cross-sectional studies in human participants provide complimentary findings, with reports of shorter sleep duration, poorer sleep quality, and longer sleep latency often associated with greater amyloid-β burden (Brown, Rainey-Smith, Bucks, Weinborn, & Martins, 2016; Spira et al., 2013; Sprecher et al., 2015). However, this is not a conclusive finding, with a recent case control study finding that long-term sleep disruption did not result in greater concentrations of amyloid-β as compared to those with normal sleep (Thomas et al., 2020). Nonetheless, rodent models have identified that the majority of amyloid-β clearance occurs during sleep (Kang et al., 2009), and the recent discovery of the glymphatic system is thought to contribute to this clearance (Nedergaard, 2013; Xie et al., 2013). Sleep strongly activates the glymphatic system, and it has therefore been hypothesised to help in the clearance of amyloid-β (Lee et al., 2015; Xie et al.,

2013). Indeed, Ooms and colleagues (2014) found that, in healthy middle-aged men, a night of unrestricted sleep led to a decrease in amyloid-β levels as compared to the evening before, while one night of total sleep deprivation counteracted this decrease. Similarly, Shokri-Kojori et al. (2018) utilised PET imaging with a diagnostic radiotracer to measure amyloid-β burden after a night of rested sleep and a night of total sleep deprivation in twenty healthy, young participants. The researchers found that sleep deprivation resulted in greater amyloid-β burden in comparison to the control night, with such accumulations occurring specifically in the hippocampus, a key early site of change in dementia and cognitive decline, and thalamus. Similarly, baseline concentrations of amyloid-β were inversely related to the previous nights' sleep hours.

The aggregation of amyloid-β in the interstitial fluid can be considered an abnormality of clearance via the glymphatic system (Reeves et al., 2020). In 2020, Kapadia et al. proposed that the lack of clearance during sleep is due to vascular dysfunction, specifically impaired NVC. This NVC clearance of amyloid-β is thought to occur during non-rapid eye movement, slow wave sleep, with vascular activity acting to pump the cerebrospinal fluid and interstitial fluid via glymphatic routes (Kapadia et al., 2020; Winer et al., 2020). This coherent activity of electrophysiologic, haemodynamic and cerebral spinal fluid oscillations was recently demonstrated by Fultz et al. (2019). However, as discussed previously, at least in the waking state, NVC is altered in dementia (Hock et al., 1997; Kotliar et al., 2017; Mentis et al., 1998), and therefore there may be an impairment in the clearance of the interstitial fluid which may change the overall microenvironment (Kapadia et al., 2020). The sleep loss experienced by dementia patients, and subsequent time spent in slow-wave sleep, may trigger the altered NVC and impaired amyloid-β clearance.

The problems surrounding amyloid-β in the brain also appear to be reciprocal, as accumulation of this protein may impair neural pathways that regulate sleep-wake patterns (Ju, Lucey, & Holtzman, 2014). Sleep disturbances, such as circadian rhythm disorders, insomnia and decreased total night time sleep, are common early in Alzheimer's disease (de Almondes, Costa, Malloy-Diniz, & Diniz, 2016; Moran et al., 2005; Vaou et al., 2018), along with disruption of sleep architecture (which refers to the basic structural organisation of sleep stages) (Avidan, 2006). It is also important to note such sleep and circadian rhythm disorders persist in elderly people without Alzheimer's disease, though the prevalence is lower (Anderson et al., 2014). Patients with Alzheimer's disease similarly experience a degeneration of the suprachiasmatic nucleus, the area of the brain responsible for regulating the sleep-wake cycle, as well as the prefrontal cortex, which results in cognitive deterioration (Musiek & Holtzman, 2016; Van Erum, Van Dam, & De Deyn, 2018; Vaou et al., 2018). This degeneration may serve as a mechanism for the altered sleep architecture experienced by dementia patients (Musiek & Holtzman, 2016), which involves a decrease in sleep efficiency, slow wave sleep and rapid eye movement sleep, along with increased N1 (light) sleep, arousal and awakening frequency (Avidan, 2006; Tsapanou et al., 2015).

2.6. Caffeine, cerebral blood flow and dementia

Caffeine is the most popular psychoactive drug in the world, and coffee is the most commonly consumed beverage worldwide after water (Butt & Sultan, 2011; Heckman, Weil, & De Mejia, 2010). Known for its stimulating properties, it is often used as a remedy following poor sleep (Smith, 2002; Zwyghuizen-Doorenbos, Roehrs, Lipschutz, Timms, & Roth, 1990), though high consumption (more than four cups per day) can subsequently result in shorter sleep duration (Zhu et al., 2019). The ability

for caffeine to increase alertness is also at its greatest when consumed in a state of decreased alertness, such as following poor sleep (Smith, 2002).

Caffeine is able to exert its stimulating effects on the brain by acting as an antagonist at adenosine receptors (Fisone et al., 2004). There are two types of adenosine receptors: A1 receptors are accountable for the neural effects of caffeine, while A2A receptors can be characterized by their vasoconstrictive response to caffeine (Koppelstaetter et al., 2010). Previously, blood oxygenation level dependent (BOLD) imaging, as part of fMRI studies, has been used to explore the effect of caffeine on NVC (Koppelstaetter et al., 2010; Laurienti et al., 2002; Mulderink, Gitelman, Mesulam, & Parrish, 2002). However, interpretations of such studies can be difficult due to caffeine's nonspecific binding to both types of adenosine receptors. Caffeine therefore has the ability to modify the coupling between blood flow and neural activity, dependent on the proportion of each type of receptor in each brain region (Laurienti et al., 2002). The calibrated BOLD approach was introduced by Davis and colleagues in 1998, and allows the metabolic and vascular components of functional activity to be separated. Chen and Parrish (2009) utilised this calibrated BOLD approach to ascertain whether an intravenous injection of caffeine (2.5 mg/kg) changed the coupling between cerebral blood flow and global cerebral metabolic rate of oxygen consumption ($CMRO_2$) during visual and motor tasks and found that caffeine decreased the cerebral blood flow:$CMRO_2$ ratio in both the visual and motor areas. The authors suggested that since caffeine decreases baseline cerebral blood flow, the oxygen extraction fraction is expected to increase in order to meet the metabolic demands of the brain, and that future studies should aim to establish methodologies that can better understand the mechanism of caffeine (Chen & Parrish, 2009). As such, in 2015, Xu et al., conducted a study using a new MRI technique that both aimed

to examine the time-dependent changes in cerebral blood flow, $CMRO_2$, and the oxygen extraction fraction across a forty minute period following ingestion of 200 mg of caffeine. The study also aimed to determine whether any regional differences in the brain's response to caffeine existed. The authors reported that despite a significant decrease in cerebral blood flow, $CMRO_2$ was unchanged. However, to account for the decreased blood flow, the oxygen extraction fraction was increased (Xu, Liu, Pekar, & Lu, 2015). This study also found that posterior brain regions demonstrated a slower decrease in cerebral blood flow as compared to anterior regions that manifested a quicker rate of decrease across the forty minutes of assessment. Therefore, contrary to what might be expected given the relationship between caffeine and alertness, caffeine decreases cerebral blood flow (Addicott et al., 2009; Field et al., 2003; Vidyasagar, Greyling, Draijer, Corfield, & Parkes, 2013). Addicott et al. (2009) used a 1.5 T MRI scanner to assess the effects of 250 mg of caffeine in low, moderate and high caffeine users on four occasions, twice in the caffeine abstinence state and twice after normal caffeine intake. Participants consumed either caffeine or placebo in each state, and it was found that caffeine lowered cerebral blood flow by an average of 27% across both caffeine states. Despite this acute reduction, chronic caffeine consumers have increased cerebral blood flow at baseline with a positive correlation between absolute cerebral blood flow and daily caffeine use (Addicott et al., 2009; Field et al., 2003). This may contribute to meta analyses finding that chronic caffeine consumption substantially lowers the risk of developing dementia later in life (Eskelinen, Ngandu, Tuomilehto, Soininen, & Kivipelto, 2009; Kolahdouzan & Hamadeh, 2017; Liu et al., 2016; Maia & De Mendonça, 2002). For example, one analysis found that moderate coffee drinkers (3 – 5 cups per day) had a 65 – 70% decreased risk of dementia compared with low coffee consumers (0 – 2 cups per day) (Eskelinen et al., 2009),

and using a logistic regression model, another analysis reported that caffeine exposure was inversely associated with Alzheimer's disease (odds ration = 0.40), independent of other possible confounding variables (Maia & De Mendonça, 2002). However, this finding is not conclusive, with population based studies and meta-analyses of prospective studies reporting that coffee consumption is not associated with incident dementia when followed up in the long term (Larsson & Orsini, 2018; Mirza et al., 2014). Instead, the accumulated evidence suggests that there is a 'J-shaped' association between coffee intake and cognitive disorder incidence, with the lowest risk at 1-2 cups of coffee per day (Wu et al., 2017).

Despite the dependence on caffeine following a poor night of sleep by many adults, it is not known how caffeine affects NVC when consumed in the sleep deprived state. This is also of interest, as acute sleep restriction has been shown to alter the vascular response to other, non-pharmacological challenges, such as exercise (Papadakis, Forsse, & Peterson, 2020).

2.7. Sleep and circadian rhythm, caffeine, and cognitive function

The controlled relationship between circadian rhythms and sleep-wake homeostatic processes play a key role in regulating cognitive function (Borbély, 1982), with certain aspects of cognition particularly sensitive to time of day and sleep deprivation (Cajochen, Blatter, & Wallach, 2004; Graw, Kräuchi, Knoblauch, Wirz-Justice, & Cajochen, 2004; Urry & Landolt, 2014). A meta-analysis reviewing 61 studies found a significant effect of sleep restriction on cognitive processing across various cognitive domains, including executive functioning, sustained attention and long-term memory (Lowe, Safati, & Hall, 2017). The review also found that sleep latency, subjective

sleepiness and cumulative days of restricted sleep were all significant moderators of the overall effect on cognition (Lowe et al., 2017).

The neuromodulator adenosine is a by-product of metabolism and plays an important role in regulating sleep pressure (the desire to sleep), increasing in concentration during wakefulness (Urry & Landolt, 2014). As discussed, caffeine increases alertness by binding to adenosine receptors within the basal ganglia in the brain (Fisone et al., 2004). Thus, caffeine can decrease the effects of sleep deprivation on arousal and attention (Urry & Landolt, 2014). However, such effects of caffeine are limited, and may not be able to reverse the impacts of severe sleep loss on higher order cognitive functions (Urry & Landolt, 2014) such as memory encoding, consolidation and retrieval (Walker, 2008), and behavioural inhibition (Drummond, Paulus, & Tapert, 2006; Harrison, Jones, & Waterhouse, 2007), processes that typically involve the prefrontal cortex (Killgore, Kamimori, & Balkin, 2011).

2.8. Examining the cerebral blood flow response to cognitive tasks

The cerebral blood flow response to cognitive tasks can be measured using functional MRI (fMRI), specifically BOLD imaging. A strength of this imaging modality is its high spatial resolution, which is particularly beneficial during cognitive function tasks to identify the most active brain regions during different tests (Turner, 2016). However, changes in blood velocity during cognitive tasks can also be measured using TCD which may be able to provide superior temporal resolution, and can be complemented by the addition of NIRS, which has shown to be sensitive to brain oxygenation changes during cognitive activation (Fallgatter & Strik, 1997). An early bilateral TCD study by Kelley et al. (1992) measured cerebral artery blood velocity through the anterior,

middle and posterior cerebral arteries in healthy young participants, at rest and during cerebral activation in the form of a commercial video game and a mental arithmetic task. The study found a global increase in cerebral artery velocity during task performance as compared to during rest, with both the right and left MCAv, and the left PCAv experiencing a greater (+ 5, 8, 5 cm/s respectively), increase than the anterior cerebral artery (+2 cm/s). Overall, the greatest selective activation occurred in the MCAv. This is in line with the findings of Beishon et al. (2018) in another TCD study investigating the differences in NVC between healthy older and younger adults in response to cognitive tests. During bilateral insonation of the MCA, participants completed memory and visuospatial tests from the Addenbrooke's Cognitive Examination (III). The researchers found greater MCAv responses in the older adults as compared to the younger adults, though these responses varied between timepoints and hemispheres (Beishon, Williams, Panerai, Robinson, & Haunton, 2018). The use of TCD to assess cerebral blood flow during cognitive function tests has also been shown to be sensitive to a total sleep deprivation protocol as highlighted in the previously discussed study by Csipo et al. (2021). In this study MCAv was 4 cm/s lower during the n-back test after sleep deprivation, as compared to pre-sleep deprivation.

2.9. Research questions

Given the above, there is value in exploring the associations between chronic poor sleep and dementia, by using an acute intervention study to examine if sleep restriction influences various indices of cerebrovascular function. The research questions and hypotheses of this thesis are therefore as follows:

1) What are the effects of one night of partial sleep restriction, equating to half of a normal night of sleep, on NVC in healthy young adults?

   The null hypothesis was that sleep restriction would not alter NVC.

2) Does a dose of caffeine ingestion, equivalent to that of a typical cup of coffee, affect NVC and cognitive function after partial sleep restriction?

   The null hypothesis was therefore that caffeine would not change NVC outcomes following sleep restriction, as compared to after a 'normal' night of sleep; and that cognitive function would not be impaired after sleep restriction or changed following caffeine ingestion.

Chapter 3: Methodology

3.1. Participants and ethical approval

A sample size calculation was estimated using G*Power (3.1), with a power of 0.80, and an alpha error probability of 0.05. At the time of calculating the sample size, no previous work had been published exploring the effect of sleep restriction on NVC. However, work by Poudel *et al.* (2012) found that one night of partial sleep restriction decreased cerebral blood flow across specific regions of the brain, as measured using MRI scans. While the researchers did not report effect sizes, they reported standard error of the mean of cerebral perfusion for such regions which could be converted into standard deviation, and in turn a calculation of effect size. This produced a moderate dz of 0.43. However, Poudel *et al.* (2012) used MRI to measure global cerebral blood flow, while the present study used TCD to measure PCAv and MCAv in response to tasks. As such, and in the absence of knowing what a clinically relevant change in NVC is following sleep restriction, the sample size was calculated based on the smallest 'moderate' effect (a moderate $\eta_p^2 = 0.06$) and a high correlation amongst repeated measures for our primary outcome (Smirl et al., 2016). Typically, it is not always possible to gain an adequate signal for measurement of cerebral blood velocity with TCD, resulting in 10% data loss (Willie et al., 2011). To counteract any data loss due to scanning issues as well as potential participant dropout, two further participants were recruited, producing a total sample size of 20 participants.

The study was approved by the University of Exeter Sport and Health Sciences Ethics Committee (2021-M-27). Participants were recruited predominantly from the University of Exeter, but the study was also advertised outside of the university in the local area. Advertisements comprised of physical posters, social media, as well as

presentation slides during Sport and Health Sciences laboratory sessions, seminars and lectures. Participants were given a participant information sheet (Appendix 1) explaining the purpose of the research, the study protocol, the possible benefits and disadvantages of taking part, the COVID-19 secure measures in place, and the ethics surrounding withdrawal of the study and confidentiality of the research. Participants were then given time to ask the researchers any questions before providing written informed consent (Appendix 2).

Participants completed a health screening form (Appendix 3) which identified certain items on the exclusion criteria. The primary exclusion criterion was any person who could be considered a 'poor sleeper'. This was identified at three levels. Firstly, through the health screening form, identifying any individuals who suffer from diagnosed OSA, insomnia or restless leg syndrome. Secondly, during a familiarisation visit, participants completed the Pittsburgh Sleep Quality Index (PSQI) (Buysse, Reynolds III, Monk, Berman, & Kupfer, 1989) (Appendix 4) which explored different aspects of participants subjective sleep duration and quality. This was then scored by the researcher following specific criteria (Appendix 4). Participants were identified as a 'poor sleeper' and excluded from the study if they scored >5 out of a possible 21 in the PSQI, and/or stated their normal sleep duration was ≤ 6.5 hours. Finally, participants completed a diary and wore an Actiheart, a combined electrocardiogram (ECG) and accelerometer device (Actiheart, Camntech Cambridgeshire, United Kingdom) (see Section 3.3), for three days that objectively measured sleep duration. Participants were again excluded from the study if this habitual sleep assessment showed that participants slept ≤ 6.5 hours per night, however this did not lead to any exclusions due to the specific drive to recruit 'good' sleepers.

Volunteers were also excluded from participation if they suffered from any mental health illness, for example anxiety and/or depression, where symptoms are exacerbated following short or poor quality sleep (Babson, Trainor, Feldner, & Blumenthal, 2010). Other exclusion criteria included current metabolic, cardiovascular, or cerebrovascular disease, as this may have affected NVC at baseline (Girouard & Iadecola, 2006; Maeda et al., 1993). Similarly, participants were excluded if they used any supplement or medication known to influence blood vessel function or blood pressure. Finally, participants were excluded if they habitually consumed ≥600 mg/day of caffeine due to the requirement to abstain from the substance for >24 hours. It was felt that this might induce subsequent withdrawal effects, including impaired cognitive function (Rogers et al., 2005) and altered cerebral blood flow (Addicott et al., 2009). Only one person was excluded from the study, and this exclusion was based upon their diagnosis of epilepsy. There were no specific inclusion criteria, asides from being a ‘good sleeper’ (i.e. not meeting any of the criteria to be classified as a ‘poor sleeper’), and aged 18 – 40 years old. Sleep patterns are known to change across the lifespan, and baseline cerebral blood flow is also thought to change with age (Jonasdottir, Minor, & Lehmann, 2021; Li, Vitiello, & Gooneratne, 2018). Furthermore, there is some evidence to suggest that NVC during a physiological challenge might be altered by age (Nowak-Flück et al., 2018), therefore we decided to limit the age range to 18 – 40 years inclusive in order to have a homogenous group. This study aimed to understand the relationship between sleep and NVC in a young healthy population, which is a useful initial step for further work looking at change with age. A total of 17 participants completed the study, and participant characteristics are presented in Table 3.1.

Table 3.1 Participant characteristics n=17, female = 9

	Mean ± SD	Range
Age (years)	27.5 ± 5.7	20.4 – 40.3
BMI (kg/m^2)	25.9 ± 4.7	18.5 – 37.3
Habitual caffeine consumption (mg/day)	178 ± 143	0 - 462
PSQI habitual sleep duration (hours/night)	8.0 ± 0:6	7.0 – 9.0
PSQI score	3 ± 1	1 – 5
Sleep diary habitual average sleep duration (hours/night)	8.4 ± 0.5	7.2 – 9.3

Results are expressed as mean ± standard deviation. Abbreviations: BMI, body mass index; PSQI, Pittsburgh Sleep Quality Index.

3.2. Experimental overview

Participants visited the laboratory on a total of seven occasions. This consisted of one familiarisation visit and three experimental trials, each composed of two visits, one in the late afternoon/evening, and one the following morning. The sleep protocol took place at the participants' own home. The study followed a repeated measures design, in order to explore the effects of (1) normal sleep + placebo (NORM_PL), (2) normal sleep + caffeine (NORM_CAF), and (3) partial sleep restriction + caffeine (PSR), on NVC and cognitive function.

3.3. Visit 1: familiarisation

Participants were familiarised to the testing procedures ahead of completing the experimental trials. This preliminary visit also allowed for the measurement of descriptive variables, including habitual sleep duration and quality, and habitual caffeine ingestion. There were no prerequisites for this visit. Body mass (Hampel,

XWM-150K, Hampel Electronics Co., Taiwan) and stature (Seca, stadiometer, SEC-225, Seca, Germany) were measured using standard procedures, and body mass index (BMI) was calculated. Participants were then familiarised to the NVC measure (described in detail in *Section 3.5.2)*. This provided an opportunity for the researcher to ensure that the PCA could be confidently scanned and identified in the participant. No participants were excluded at this stage. The cognitive function tests were also fully explained during this preliminary visit, and participants completed a shortened version of each of the tests for familiarisation.

Subjective sleep duration and quality were assessed during this visit using the PSQI (Buysse et al., 1989). These data were not used to prescribe sleep duration/timing during the experimental visits and was used only utilised for identifying exclusion criteria and as a descriptive characteristic. Participants were then given an Actiheart for measurement of habitual sleep across three nights where they expected to sleep 'normally', i.e. they had no abnormally late nights or early mornings planned. The device was set to record for eight days following the familiarisation visit. Participants were instructed to wear the device at night only, and to complete a sleep diary (Appendix 5) across the three nights they wore the device. These data were analysed to determine the sleep duration and timing for the experimental visits, so that participants kept their normal wake time. In the instance of a single night of device failure, data was averaged across the two nights of successful data collection (this occurred for 10 participants). In the occurrence of device failure across all three nights, the participant was asked to repeat the three day assessment (this occurred for two participants).

3.4. Visits 2-7: experimental trials

The experimental trials were completed in a randomised, counterbalanced order, with six possible orders of completion. The experimental trials were separated by 13 ± 7 days (range: 3 – 41 days). For conditions where PSR was not the final trial, it was imperative that there was at least 5 days between the PSR trial and subsequent trial, to allow sufficient recovery sleep between trials. There were 15 ± 10 (range: 6 – 41) days between the PSR and subsequent trial.

Participants arrived at the laboratory for the evening visit between 16:00 and 19:00 depending on their availability, as there is not known to be a circadian effect on NVC (Burma, Macaulay, et al., 2021). However, this time of arrival was standardised for each individual participant across their subsequent evening visits (± 60 minutes). Figure 3.1 depicts a schematic of the protocol. Upon arrival, participants verbally confirmed that they had met the prerequisites of the study: no caffeine consumed on the day of the visit, no food or drink (asides from water) consumed in the two hours prior to arrival, no exercise in the six hours prior to the visit (Burma, Macaulay, et al., 2021), and a good/normal night of sleep the night before. Participants were reminded of these requirements via email the day before each of the experimental trials. If any prerequisites had not been met, the visit was rescheduled for another day. Following this, participants completed the NVC tests with the PCA insonated (see Section 3.5. for further details). The NVC protocol totalled six minutes in duration. The insonated blood vessel was then switched to the MCA, following which the participants completed four cognitive function tests, totalling ~10 minutes. Throughout the visit,

beat-by-beat fingertip blood pressure was measured continuously by finger plethysmography (NIBP, ADInstruments, Colorado Springs, CO, USA).

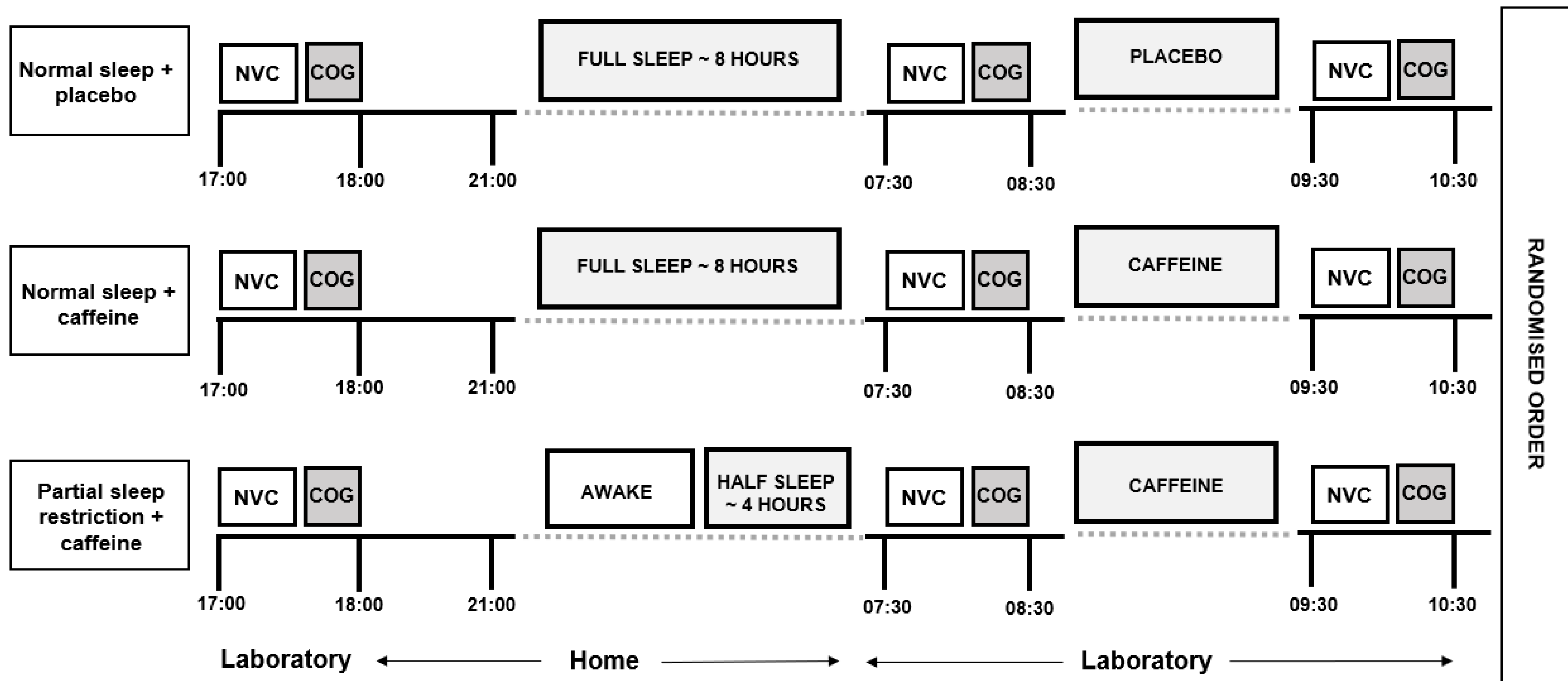

Figure 3.1. A schematic depicting the experimental trials. NVC = neurovascular coupling; COG = cognitive function tests.

Participants were reminded of which experimental sleep condition (normal sleep or PSR) to complete once they left the laboratory, and to refrain from exercising between the evening and morning visit, as well as from consuming caffeine or alcohol (see Section 2.5.6 for sleep protocol details). A food diary was retrospectively completed after the first experimental visit to allow participants to replicate their evening meal for subsequent trials.

Participants returned to the laboratory at the same time across each of the conditions, within an hour of waking (range 06:20 – 09:30) and in a fasted state, after confirmation via email that they had met the sleep requirements. The full NVC and cognitive function protocols were then repeated. Immediately after completing the final cognitive function test, participants were given a pill to consume with a glass of water. The pill was always caffeine following PSR but was either caffeine or a placebo following normal sleep. Participants were blinded to the contents of the pill, and although the study was only single blind, data were saved in a manner that meant they were analysed when blinded to pill. The caffeine and placebo pills were made on a pill-by-pill basis using respective powders. Cellulose pill capsules were filled with either 150 mg caffeine (Sigma-Aldrich, Gillingham, United Kingdom) or < 10 mg of maltodextrin (Bulk, Lancaster, United Kingdom). The caffeine dose was chosen as it reflects the typical caffeine content of a coffee, as opposed to selecting a dose that is calculated based on mg/kg body weight. This ecologically valid choice also considers that after a poor night of sleep the general population would consume a caffeinated beverage, as opposed to a specific dose of caffeine based on body weight. Furthermore, most studies assessing the relationship between habitual caffeine consumption and risk of dementia tend to correlate ‘cups of coffee’ with incident dementia risk, rather than caffeine dose *per se* (Liu et al. 2016). One hour after ingestion, participants again completed the NVC and

cognitive function tests. During the hour break, participants sat quietly and were limited to office-desk work.

3.5. Experimental measures

3.5.1. Cerebral artery blood velocity

TCD (DWL, Compumedics, Germany) was used to assess PCAv during a series of visual stimulation tasks, and MCAv throughout cognitive function tests. A 2-MHz probe was placed over the temporal acoustic windows to acquire the cerebral blood velocity in the vessel of interest. The arteries were identified and optimised based on their signal depth, waveform, angle probe and cerebral artery velocity (Willie et al., 2011). Insonation of the PCA was confirmed with visual stimulation tests (Willie et al., 2011). The position of the probe and signal depth were recorded to ensure replication of the same insonation of the arteries both within and between day. An adjustable headset was used to secure the TCD signal (DiaMon, DWL, Germany) (Figure 2.2). The within – and between – day reliability data for PCAv outcomes are presented in Table 3.2.

Figure 3.2 Transcranial Doppler ultrasound secured in place using an adjustable headset to measure cerebral artery blood velocity during the neurovascular coupling and cognitive function tests.

Table 3.2. Within – and between day reliability for PCAv outcomes.

Within – day Reliability								
	Morning Pre Placebo	Morning Post Placebo	MD	*P value*	*d*	ICC	CV (%)	*r*
Baseline PCAv (cm/s)	40.1 ± 6.2	40.8 ± 7.2	0.69	0.41	0.10	0.88	5.9	0.87
Peak PCAv (cm/s)	53.4 ± 9.9	53.9 ± 10.2	0.42	0.66	0.04	0.92	5.4	0.91
∆PCAv-peak (cm/s)	13.4 ± 5.2	13.1 ± 6.2	0.43	0.78	0.26	0.51	41.7	0.51
%∆PCAv-peak	33.1 ± 10.9	32.6 ± 13.2	0.57	0.79	0.05	0.44	45.4	0.47
PCAv iAUC	276.1 ± 116.2	268.2 ± 145.9	7.86	0.69	0.06	0.41	75.5	0.50
PCAv tAUC	1438.5 ± 254.2	1449.7 ± 271.5	11.18	0.65	0.04	0.92	5.3	0.91
PCAv Amplitude (cm/s)	13.8 ± 5.5	14.9 ± 5.0	1.17	0.18	0.22	0.86	14.5	0.83
PCAv τ (s)	5.8 ± 1.2	7.1 ± 2.1	1.36	0.10	0.78	< 0.01	31.9	< 0.01
Between – day Reliability								
	Norm_Pl Morning	Norm_Caf Morning	MD	*P value*	*d*	ICC	CV (%)	*r*
Baseline PCAv (cm/s)	40.1 ± 6.2	42.1 ± 8.1	1.99	0.19	0.28	0.74	9.0	0.72
Peak PCAv (cm/s)	53.4 ± 9.9	54.5 ± 8.3	1.10	0.45	0.12	0.80	7.0	0.80
∆PCAv peak	13.4 ± 5.2	12.5 ± 4.5	0.89	0.32	0.18	0.79	20.6	0.77
%∆ PCAv peak	33.1 ± 10.9	30.8 ± 12.2	2.37	0.26	0.21	0.73	24.9	0.72
PCAv iAUC	276.1 ± 116.2	245.0 ± 107.1	31.10	0.16	0.28	0.74	26.5	0.72
PCAv tAUC	1438.5 ± 254.2	1468.5 ± 227.6	30.0	0.45	0.12	0.81	7.6	0.80
PCAv Amplitude (cm/s)	13.8 ± 5.5	14.3 ± 5.1	0.52	0.74	0.10	0.65	24.1	0.59
PCAv τ (s)	5.8 ± 1.2	7.5 ± 2.3	1.69	0.10	0.92	0.34	36.1	0.31

Results are expressed as mean ± standard deviation. Abbreviations: MD, mean difference; ICC, intraclass correlation coefficient; PCAv, posterior cerebral artery velocity; iAUC and tAUC, incremental and total area under the curve.

3.5.2. Neurovascular coupling assessment

NVC responses were quantified using a complex visual search task, searching for characters in the "Where's Wally" books (Handford, 1987a, 1987b, 1987c, 1989), replicating the methods of Smirl et al. (2016) which have been shown to be reliable, provide a superior amplitude compared to other stimuli, and be highly engaging for the participant (Burma et al., 2022; Burma, Wassmuth, et al., 2021). Participants sat at a desk with the PCA insonated, and a one-minute eyes closed baseline was recorded. Participants completed at least five cycles 20-seconds eyes closed and 40-seconds eyes open to the visual stimulus. Five cycles were selected as this has been shown to produce reliable estimates in a young, healthy population (Burma et al., 2022) maximising the signal-to-noise ratio, given that other physiological processes, such as Mayer waves (Julien, 2006) can cause variability in each cycle. If any cycles appeared to produce poor quality data due to, for example, participants yawning or the TCD probe slipping from the centre of the acoustic window, an additional sixth cycle was completed. The Where's Wally books were positioned ~60 cm from the participants whilst they searched for the characters. The researcher chose the character that the participant had to find, and once indicated that they had found it, they moved onto the next character requested by the researcher, which ensured 40 seconds of continuous searching. A 40 second eyes-open period was selected to ensure peak PCAv occurred, which is normally achieved ~20 seconds after stimulus presentation. In order to account for potential task engagement differences between trials, for example post sleep restriction, participants rated their level of engagement on a scale of 1 – 10, where 10 was 'couldn't be more engaged in the task' and 1 was 'could not concentrate at all on the task at hand'.

3.5.3 Neurovascular coupling data handling

All cerebral blood velocity data were collected at 200 Hz using an analogue to digital converter (PowerLab, ADInstruments, Colorado Springs, USA) and stored for offline analysis using commercially available software (LabChart 8, AD Instruments, Colorado Springs, USA). Data were resampled at 1 Hz and exported to Microsoft Excel (Version 2201) for subsequent handling. All cycles were visually inspected for artefacts, time aligned and averaged to create one response per time point, per condition, per participant. In cases where PCAv data were lost across single trials during data collection, these specific trials were excluded from analyses, resulting in a total of 29 cycles removed out of a study total of 182 cycles.

The final 5 seconds of eyes-closed was averaged to create a 'baseline' value (Figure 2.3). The handled eyes-open data was capped at 30-seconds post eyes open, to minimise the influence of human in error associated with withdrawing the stimulus too early (Smirl et al., 2016). From here forwards, all data handling and analysis refers to this 30-second period, as opposed to the full 40-seconds of eyes-open data. Peak PCAv was identified as the highest PCAv value during eyes-open, and time until peak PCAv from eyes-open onset was reported. The absolute change from baseline to peak PCAv was also calculated (∆PCAv-peak), along this value as a percent change from PCAv baseline (%∆PCAv-peak). Total and incremental area under the curve (tAUC and iAUC respectively) were calculated using the trapezium rule in Prism (GraphPad Software, San Diego, CA, USA, Version 9.1.2) as an index of total activation, and iAUC was determined as the hyperaemic curve versus time above baseline PCAv. Calculating iAUC in this way minimises the effects of trial-to-trial baseline variation.

3.5.4. Kinetic analysis of the neurovascular response

Following other recent investigations which attempt to understand the time course of the cerebrovascular response (Billinger et al., 2017; Weston et al., 2022; Witte et al., 2019) kinetic analysis of the NVC response was performed using the averaged, time aligned PCAv data. Data were baseline corrected and analysed using a mono-exponential model with time delay (Equation 1) using GraphPad Prism.

$$PCAv\ (t) = \Delta PCAv_A\left(1 - e^{-(t-TD/\tau)}\right) \quad (1)$$

where PCAv(t) is the PCAv at a given time (t), $\Delta PCAv_A$ is the amplitude change of PCAv from baseline to its asymptote, TD is the time delay and τ is the time constant (Poole & Jones, 2012). However, due to the rapid onset of the response, for some time points (8) a model was more closely fitted when a time delay was not included in the equation. On such occasions the following equation (2) was used:

$$PCAv\ (t) = \Delta PCAv_A\left(1 - e^{-(t/\tau)}\right) \quad (2)$$

For both equations, the model was fitted from the start of the exponential rise in PCAv until a visual departure from steady state. The start, overall fit, and end of each exponential model was blindly verified by three researchers (AL, JK, BB), and disputes were discussed until a consensus was reached. Models were checked for approach consistency and to determine model acceptability (goodness of fit $r^2 > 0.50$, standard error of the τ, and normality of residuals).

3.5.5. Cognitive function

The international shopping list test (Lim et al., 2009) (Appendix 6) was used as an assessment of working memory with an initial test repeated three times in the evening,

and then a follow up test in the morning to measure overnight recall. The researcher read a 15-item shopping list to the participant who was then asked to recall the list in the following 60 seconds. This process was then repeated twice. Either the time taken to recite the entire list, the time when the participant indicated they could not remember anymore words, or when the 60 seconds was up, was also recorded depending on which occurred first. The data across the 3 repeats in the evening were averaged to give a single score. When completing the test the following morning, participants were first asked to recall the list without being reminded of the items, and then recall a final time after listening to the list. Four different lists were used, with lists 1, 2 and 3 to be completed on trials 1, 2 and 3 respectively. Given that participants were randomised to the order of which they completed the experimental trials, this controlled for task-related order effects. The fourth list was used if a condition had to be repeated for any reason and was thereby used on two occasions.

All other cognitive function tests were conducted on a freely available website, cognitivefun.net. A 2-back test was used as a measure of working memory (Owen, McMillan, Laird, & Bullmore, 2005). Participants watched a series of items appear in isolation on the screen and were required to click on the screen when the same item appeared that was presented two items ago. This test lasted an average of 110 seconds, and visual correct ratio and visual response time was reported. Participants then completed a 60 second modified version of the Stroop test (Bélanger, Belleville, & Gauthier, 2010; Stroop, 1992), a series of congruent and incongruent challenges, as an assessment of executive function. The percent correct, fastest, slowest, average, and standard deviation of the time to respond was reported separately for the congruent and incongruent responses. Participants also completed a 90 second go/no-go visual reaction test as a measure of inhibitory control and visual processing

speed (Garavan, Ross, & Stein, 1999; Konishi, Nakajima, Uchida, Sekihara, & Miyashita, 1998). Percent correct, fastest, slowest, average, and standard deviation of the response time were recorded.

3.5.6. Assessment of blood velocity through the MCA during cognitive function tests

MCAv was assessed during the cognitive function tests (see Section 3.5.1) (Figure 3.3). A 30-second resting baseline was recorded prior to each of the tests. Data were exported as described in Section 3.5.1. Baseline was calculated by averaging MCAv across the 30-second baseline period. On occasions where the 30-second baseline period did not reflect a 'true' baseline, for example the participant yawned, coughed or considerably altered their posture, a shorter baseline duration was used that reflected a more stable period. Each test was analysed in isolation and any obvious errant data points (caused by i.e. sudden probe movement) were removed before analysis MCAv was averaged across the test duration and subtracted from the averaged baseline to calculate an absolute and percentage change score.

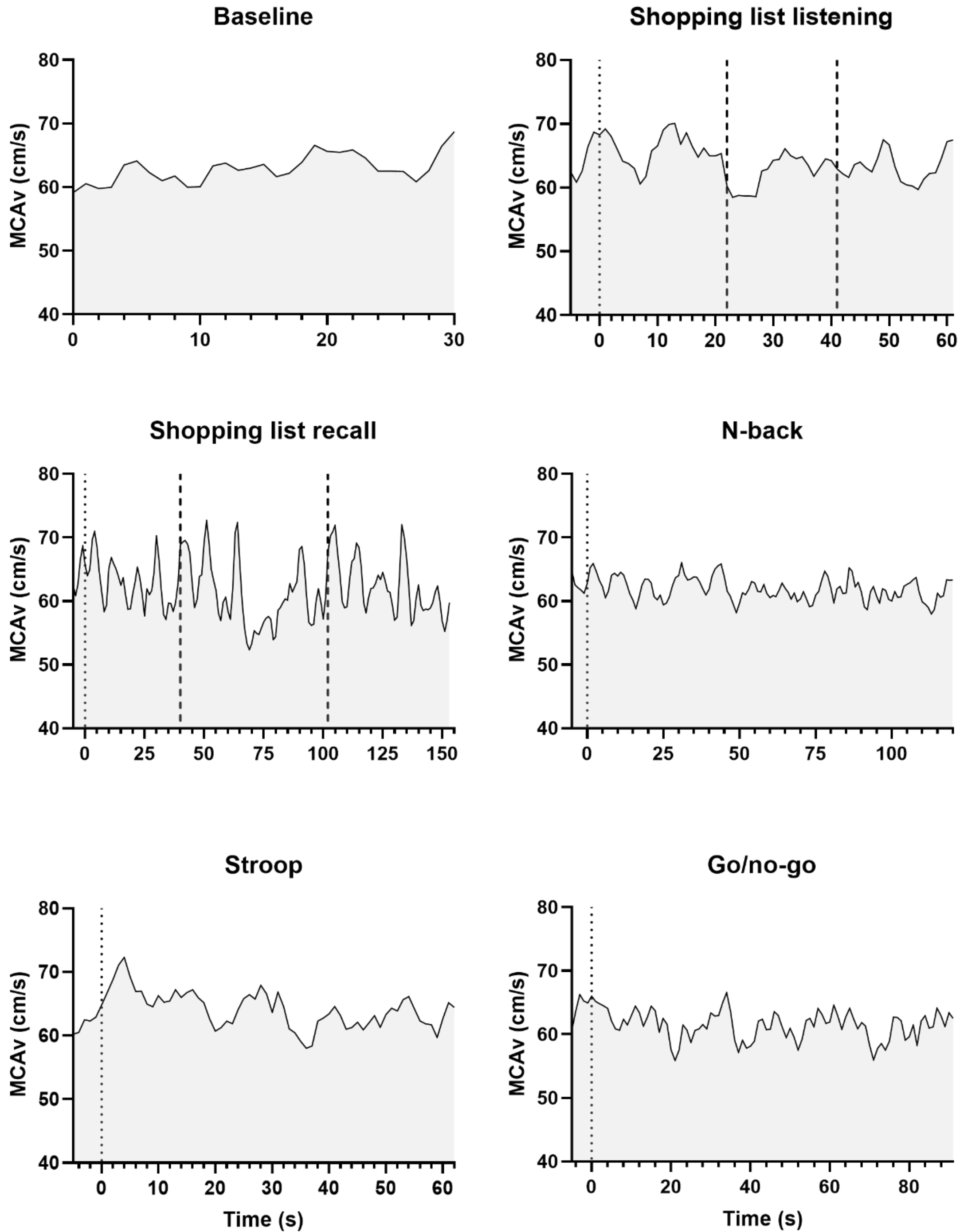

Figure 3.3. An example (n = 1) middle cerebral artery velocity (MCAv) trace at baseline and during the cognitive function tests from an evening visit. The dotted line indicates the beginning of the test, with time until this line reflecting a preceding baseline period. The dashed line during the shopping list listening and recall traces represents the start of each of listening and recall attempts respectively.

3.5.7. Blood pressure

Beat-by-beat fingertip blood pressure was continuously monitored throughout both the NVC assessment and the cognitive function tests by finger plethysmography and quantified as mean arterial pressure (MAP) (NIBP, ADInstruments, Colorado Springs, CO, USA). All data were recorded using the LabChart software, and were exported at 1 Hz.

3.5.8. Sleep assessment

For all experimental conditions, participants were told their target sleep onset time and wake up time, which were calculated from the habitual sleep assessment. Timings were the same for the two normal sleep conditions. The target sleep onset time for the sleep restriction condition was calculated as the halfway point between normal sleep onset and normal wake time, resulting in a 50% sleep restriction. For example, a participant who typically slept 00:00 – 08:00 would have a target sleep onset and wake time of 04:00 and 08:00 for their PSR trial, respectively. This later sleep onset (instead of an early wake time) was designed in attempt to specifically deprive participants of deep, slow wave sleep, as this sleep stage is thought to dominate the first half of the night on a circadian rhythm basis, as opposed to a sleep onset basis (Dijk, 2009; Léger et al., 2018). During the night of the PSR trial, participants were also instructed to remain mostly sedentary, refrain from eating during the period which they would normally be asleep and advised not to drive. Participants also emailed the researcher the word 'awake' every ~30 – 60 minutes during this period to confirm that they were still awake.

To assess sleep, participants wore an Actiheart and completed a sleep diary. The Actiheart device sampled at a rate of 250 Hz ECG, 25 Hz accelerometer, and was

attached using ECG tags. The main body of the device was attached at the sternum, and a small lead was attached on the left of the torso, below the pectoralis major muscle. Participants recorded the target sleep onset time, approximate time to fall asleep and time of waking, along with any other notes about their sleep. For the normal sleep conditions, participants also confirmed whether they felt it reflected a normal night of sleep. In circumstances where participants did not feel this was the case, the whole trial was rearranged. The Actiheart data was exported via the Actiheart software (Version 5.1.10) to produce estimations of sleep duration and sleep stages. However, data regarding sleep onset and wake time was based on participant sleep diary as opposed to Actiheart timings due to possible discrepancies between 'awake' and 'light sleep' in the Actiheart data.

3.6. Statistical analyses

Data are presented as means ± standard deviation (SD). All data were analysed in SPSS (Version 28.0.1.11, IBM, Chicago, USA). Assumptions of sphericity and normality were checked using Mauchly's and the Shapiro-Wilk tests, respectively. Differences in sleep outcomes were explored using a series of separate one-way ANOVA tests. The relationship between %ΔPCAv-peak and the mean arterial pressure (MAP) at PCAv-peak was assessed using Pearson's correlation. Differences in cerebrovascular and cognitive responses during each of the experimental trials were explored using a mixed model ANOVA, with trial (NORM_Pl, NORM_CAF, PSR) and time (evening, baseline morning, morning 1 hour post caffeine) as the main effects. Fisher's least significant difference test was used for post hoc pairwise comparisons. The confounding effect of sex was considered in the original ANOVA model. Sex never

altered the time by trial interaction ($p \geq 0.07$, $\eta_p^2 \leq 0.646$), therefore data for males and females were pooled. Statistical significance was accepted when $P < 0.05$, and effect sizes were calculated to determine the magnitude of any differences. Effect sizes (partial eta squared, η_p^2) were interpreted as small (<0.06), moderate (0.06 – 0.14) and large (>0.14) for ANOVA analyses and as small (≥0.2 – < 0.5), moderate (≥ 0.5 – < 0.8) and large (≥0.8) for pairwise comparisons (Cohen, 1988). Mean differences (MD) and 95% confidence intervals (CI) are also presented.

Chapter 4: Results

4.1. Sleep outcomes

The sleep interventions were adhered to, as confirmed verbally by participants during the morning visit, and the sleep diary and Actiheart data. Table 4.1 displays the Actiheart sleep data from each of the experimental trials. By design, time of sleep onset was significantly later in the PSR trial, as compared to the Norm_Pl and Norm_Caf trials, while time of waking remained constant.

Table 4.1. Sleep outcomes across the experimental trials

	Norm_Pl	Norm_Caf	PSR	*Trial P* (η_p^2)
Estimated sleep time (hours)	8:25 ± 0:40[a]	8:08 ± 1:00[a]	4:27 ± 1:10	**< 0.001** (0.806)
Estimated time in light sleep (minutes)	204 ± 70[a]	192 ± 61[a]	104 ± 62	**< 0.001** (0.665)
Estimated time in deep sleep (minutes)	137 ± 26[a]	135 ± 18[a]	67 ± 16	**< 0.001** (0.860)
Estimated time in REM sleep (minutes)	143 ± 44[a]	149 ± 41[a]	71 ± 36	**< 0.001** (0.669)

Data are presented as means ± SD. N = 17, female = 9 for estimated sleep time based upon data from sleep diary, n = 12, female = 6 for sleep staging data, due to loss of Actiheart data. Abbreviations: Norm_Pl, normal sleep plus placebo condition; Norm_Caf, normal sleep plus caffeine condition; PSR, partial sleep restriction plus caffeine condition; REM, rapid eye movement. [a] denotes significant difference from PSR.

4.2. Traditional neurovascular coupling outcomes

Participant engagement during the visual search task displayed a trial by time interaction ($P = 0.002$, $\eta_p^2 = 0.227$), with engagement significantly lower during the first measure in the morning after PSR as compared to NORM_PL (MD = 0.7, CI 0.1 to 1.3, $P = 0.016$, $d = 0.60$) and NORM_Caf (MD = 0.7, CI 0.2 to 1.2, $P = 0.012$, $d = 0.58$).

The %∆PCAv-peak was not related to the percent change in MAP from baseline to the time at which PCAv peak occurred ($r = 0.015$, $P = 0.936$), therefore PCAv outcomes were not normalised for MAP.

There was a trial by time interaction for PCAv baseline (Table 4.2). PCAv baseline was significantly lower 1 hour after caffeine ingestion in both the Norm_Caf (MD = 3.4 cm/s, 95% CI –6.3 to –0.5, $P = 0.024$, $d = 0.55$) and PSR (MD = 4.5 cm/s, 95% CI –7.0 to –1.9, $P = 0.002$, $d = 0.69$) conditions compared to post placebo in Norm_Pl (Figure 4.1), with no difference between Norm_Caf and PSR (MD = 1.1 cm/s, 95% CI –0.9 to 3.1, $P = 0.251$, $d = 0.21$).

There was a trial by time interaction for PCAv during the final 10 seconds of the visual search task (Table 4.2), with PCAv lower 1 hour after caffeine consumption in both the Norm_Caf and PSR trials as compared to Norm_Pl (MD = 4.6 cm/s, 95% CI –8.5 to –0.7, $P = 0.023$, $d = 0.55$; MD = 6.7 cm/s, 95% CI –10.1 to –3.4, $P < 0.001$, $d = 0.70$, respectively). Norm_Caf was not significantly different from PSR (MD = 2.1 cm/s, 95% CI –5.0 to –0.8, $P = 0.140$, $d = 0.28$).

A trial by time interaction effect (Table 4.2) also occurred for PCAv peak (Figure 4.1B). PCAv peak was lower post caffeine in both the Norm_Caf and PSR conditions compared to Norm_Pl (MD = 4.5 cm/s, 95% CI –8.4 to –0.5, $P = 0.029$, $d = 0.53$; MD = 6.6 cm/s, 95% CI –10.0 to –3.2, $P < 0.001$, $d = 0.67$, respectively). Norm_Caf was

not significantly different from PSR (MD = 2.2 cm/s, 95% CI –1.1 to 5.4, $P = 0.180$, $d = 0.27$).

There was no trial by time interaction effect for ∆PCAv-peak (Table 4.2), nor for %∆PCAv-peak ($P = 0.93$, $\eta_p^2 = 0.013$) (Figure 4.1C). There was also no trial by time interaction for the time to PCAv peak (Figure 4.1D), or iAUC (Figure 4.1E). However, there was a trial by time interaction effect for tAUC (Table 4.2), with tAUC significantly lower 1 hour post caffeine in the PSR condition compared to post placebo in Norm_Pl (MD = 162.4 cm/s/s, 95% CI –260.4 to –64.5, $P = 0.003$, $d = 0.62$) (Figure 4.1F). The tAUC was not lower after caffeine in the Norm_Caf trial compared to Norm_Pl (MD = 105.2 cm/s/s, 95% CI –212.8 to 2.4, $P = 0.055$, $d = 0.47$), but the PSR and Norm_Caf trials were also not significantly different from one another (MD = 57.3 cm/s/s, 95% CI –23.9 to 138.5, $P = 0.154$, $d = 0.27$).

Table 4.2. Traditional NVC outcomes

	Norm_Pl	Norm_Caf	PSR	ANOVA P value (η_p^2)
Baseline PCAv (cm/s)				
Evening	40.8 ± 7.3	41.7 ± 7.7	43 ± 8	**0.002** (0.227)
Morning	40.1 ± 6.2	42.1 ± 8.1	40 ± 7	
Post Pill	40.8 ± 7.2	37.4 ± 4.9*	36 ± 6#	
PCAv final 10s (cm/s)				
Evening	52.2 ± 9.7	53.0 ± 8.0	52.7 ± 10.0	**0.008** (0.190)
Morning	51.5 ± 9.8	52.0 ± 8.4	49.6 ± 8.4	
Post Pill	51.7 ± 10.3	47.1 ± 5.9*	45.0 ± 8.9#	
PCAv peak (cm/s)				
Evening	54.2 ± 10.4	55.1 ± 8.6	55.5 ± 10.1	**0.009** (0.189)
Morning	53.4 ± 9.9	54.5 ± 8.3	52.5 ± 9.8	
Post Pill	53.9 ± 10.2	49.4 ± 6.0*	47.3 ± 9.6#	
∆PCAv-peak				
Evening	13.4 ± 5.8	13.3 ± 4.6	12.8 ± 5.8	0.846 (0.021)
Morning	13.4 ± 5.2	12.5 ± 4.5	12.1 ± 6.0	
Post Pill	13.1 ± 6.2	12.0 ± 4.1	11.0 ± 5.4	
%∆PCAv-peak				
Evening	33.0 ± 12.4	32.8 ± 11.7	30.6 ± 12.4	0.93 (0.013)
Morning	33.1 ± 10.9	30.8 ± 12.2	30.3 ± 14.3	
Post Pill	32.6 ± 13.2	32.7 ± 11.0	30.0 ± 12.6	
Time to peak (s)				
Evening	17 ± 6	19 ± 9	14 ± 5	0.505 (0.05)
Morning	20 ± 7	17 ± 9	17 ± 7	
Post Pill	17 ± 7	15 ± 6	14 ± 6	
iAUC (cm/s/s)				
Evening	289.2 ± 130.4	280.2 ± 104.8	258.7 ± 131.8	0.886 (0.012)
Morning	276.1 ± 116.2	245.0 ± 107.1	233.5 ± 118.9	
Post Pill	268.3 ± 145.9	260.4 ± 96.6	235.2 ± 123.7	
tAUC (cm/s/s)				
Evening	1472.1 ± 269.4	1490.9 ± 233.3	1496.3 ± 270.1	**0.003** (0.62)
Morning	1438.5 ± 254.2	1468.5 ± 227.6	1404.6 ± 239.2	
Post Pill	1449.7 ± 271.5	1344.5 ± 162.8	1287.3 ± 250.5#	

Data are presented as means ± SD (n = 17, female = 9). Abbreviations: Norm_Pl, normal sleep plus placebo condition; Norm_Caf, normal sleep plus caffeine condition; PSR, partial sleep restriction plus caffeine condition. * Significant difference between Norm_Caf and Norm_Pl; # significant difference between PSR and Norm_Pl. ANOVA significant values are shown in **bold**.

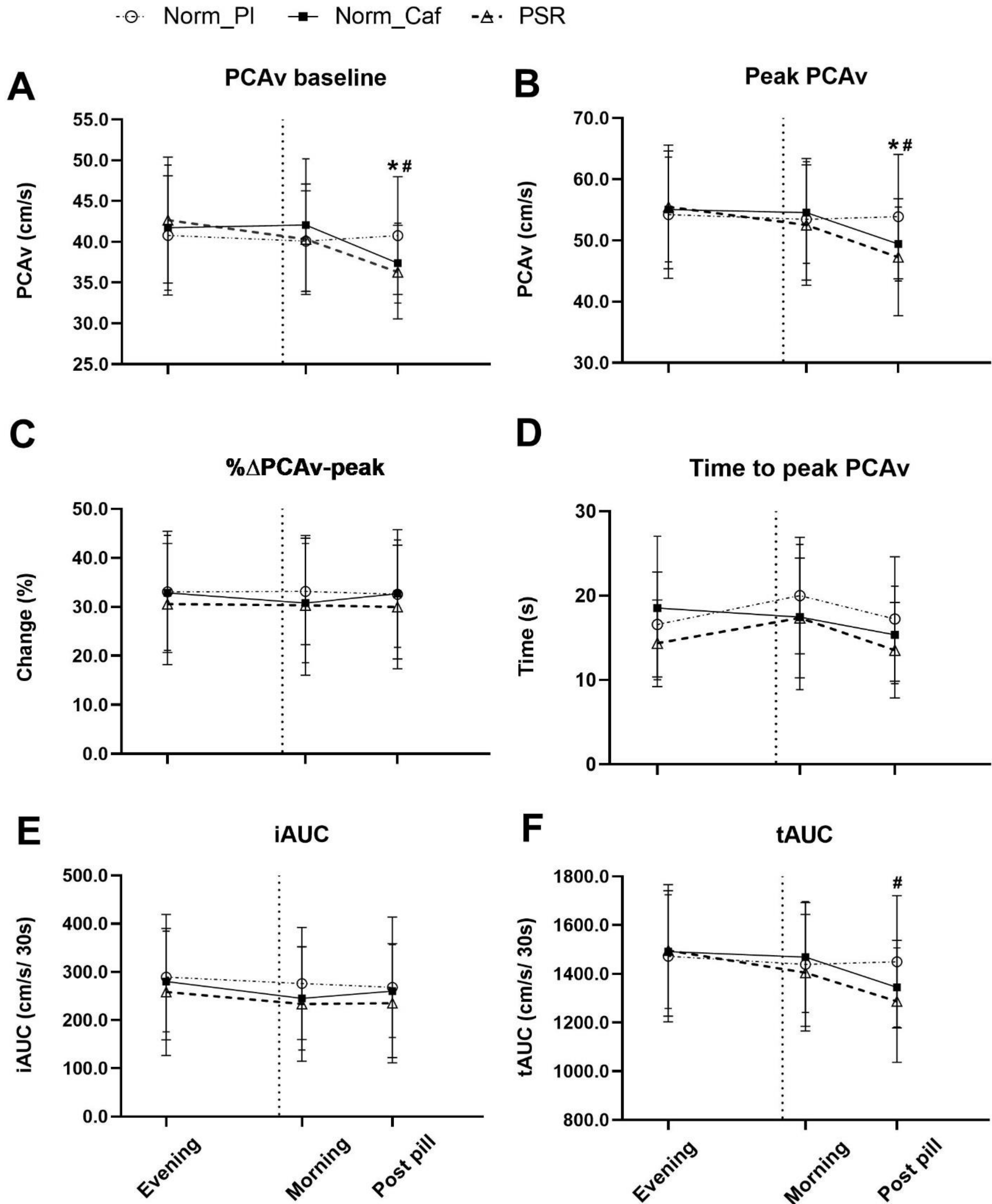

Figure 4.1. Neurovascular coupling outcomes during the evening measure, upon arrival at the laboratory after experimental sleep (morning), and 1 hour post placebo or caffeine pill ingestion (post pill) (n = 17, female = 9). The dotted line indicates the time of waking, i.e. <1 hour before the morning visit. Mean and standard deviation are presented. * Significant difference between Norm_Caf and Norm_Pl; # significantly different between PSR and Norm_Pl. PCAv, blood velocity through the posterior cerebral artery; Norm_Pl, normal sleep plus placebo condition; Norm_Caf, normal sleep plus caffeine condition; PSR, partial sleep restriction plus caffeine condition; iAUC, incremental area under the curve; tAUC, total area under the curve.

4.3. Novel kinetic neurovascular coupling outcomes

There was no trial by time interaction for PCAv amplitude as determined using a mono-exponential kinetic model, however a trial by time interaction effect existed for the τ (Table 4.3). During the evening measure, τ was quicker in the Norm_Pl as compared to Norm_Caf (MD = 1.8 s, 95% CI –3.3 to –0.3, $P = 0.028$, $d = 0.85$), whereas 1 hour after pill ingestion τ was quicker in the Norm_Caf condition than in the Norm_Pl condition (MD = 1.6 s, 95% CI –0.3 to 3.0, $P = 0.023$, $d = 0.80$).

Table 4.3. Novel kinetic NVC outcomes

	Norm_Pl	Norm_Caf	PSR	ANOVA P value (η_p^2)
τ (s)				
Evening	5.4 ± 2.0	7.2 ± 2.2*	6.0 ± 1.9	**0.008** (0.310)
Morning	5.8 ± 1.2	7.5 ± 2.3	5.6 ± 1.8	
Post Pill	7.1 ± 2.1	5.5 ± 2.0*	5.2 ± 2.6	
Amplitude (cm/s)				
Evening	14.8 ± 6.9	14.8 ± 3.2	15.0 ± 5.6	0.283 (0.127)
Morning	13.8 ± 5.5	14.3 ± 5.1	12.7 ± 4.8	
Post Pill	14.9 ± 5.0	12.7 ± 3.5	13.4 ± 6.1	

Data are presented as means ± SD (n = 17, female = 9). Abbreviations: Norm_Pl, normal sleep plus placebo condition; Norm_Caf, normal sleep plus caffeine condition; PSR, partial sleep restriction plus caffeine condition. * Significant difference between Norm_Caf and Norm_Pl. ANOVA significant values are shown in **bold.**

4.4. Blood velocity through the middle cerebral artery during cognitive function tests

There was a trial by time interaction effect for baseline MCAv (Table 4.4), with MCAv lower during both the first measurement in the morning (MD = 4.9 cm/s, 95% CI –8.5 to –1.3, $P = 0.012$, $d = 0.52$), and 1 hour post caffeine in the PSR condition compared to post placebo in the Norm_Pl condition (MD = 7.5 cm/s, 95% CI –12.2 to –2.8, $P =$

0.004, $d = 0.75$). Baseline MCAv was also lower 1 hour post caffeine in the Norm_Caf condition than in the Norm_Pl condition (MD = 6.8 cm/s, 95% CI –11.7 to –1.9, $P = 0.011$, $d = 0.63$). There was no other trial by time interaction effects for MCAv parameters during the cognitive function tests ($P \geq 0.053$, $\eta_p^2 \leq 0.174$).

Table 4.4. Baseline and percent change MCAv outcomes during cognitive function tests

		Norm_Pl	Norm_Caf	PSR	*Trial*Time* *P* (η_p^2)
Baseline MCAv (cm/s)					
	Evening	67.0 ± 9.1	69.8 ± 11.5	69.3 ± 6.2	**0.002**
	Morning	69.6 ± 8.5	67.3 ± 10.4	64.7 ± 10.0#	(0.281)
	Post pill	68.0 ± 11.7	61.2 ± 9.7*	60.5 ± 8.0#	
During cognitive tests %∆MCAv					
SL, listening	Evening	3.9 ± 5.1	– 0.50 ± 5.5	3.5 ± 5.0	0.344
	Morning	3.1 ± 5.5	3.0 ± 4.9	5.7 ± 7.6	(0.079)
SL, recall	Evening	5.6 ± 7.5	0.3 ± 6.8	4.0 ± 5.5	0.271
	Morning	2.1 ± 7.2	1.2 ± 6.0	3.7 ± 5.5	(0.096)
N-back	Evening	7.9 ± 7.8	4.8 ± 5.8	3.9 ± 4.6	0.48 (0.064)
	Morning	7.6 ± 5.3	8.6 ± 7.5	7.0 ± 7.4	
	Post pill	4.7 ± 6.1	3.1 ± 5.1	4.1 ± 5.5	
Stroop	Evening	4.6 ± 6.6	5.9 ± 9.3	7.1 ± 6.0	0.053 (0.174)
	Morning	9.4 ± 10.1	5.9 ± 8.0	5.4 ± 8.5	
	Post pill	4.7 ± 5.1	2.9 ± 6.9	6.6 ± 8.6	
Go/no-go	Evening	7.0 ± 8.3	5.0 ± 7.8	6.5 ± 7.0	0.58 (0.049)
	Morning	7.9 ± 10.7	5.7 ± 9.4	5.0 ± 8.6	
	Post pill	7.3 ± 7.7	5.7 ± 6.0	2.8 ± 4.9	

Data are presented as means ± SD (n = 14, female = 8). * Significant difference between Norm_Caf and Norm_Pl; # significantly different between PSR and Norm_Pl. Abbreviations: MCAv, blood velocity through the middle cerebral artery; Norm_Pl, normal sleep plus placebo condition; Norm_Caf, normal sleep plus caffeine condition; PSR, partial sleep restriction plus caffeine condition; SL, shopping list test. ∆ % refers to the percent change from baseline MCAv to average MCAv during the cognitive function test. ANOVA significant values are shown in **bold**.

4.5. Cognitive function

Cognitive function scores are presented in Table 4.5. There was no trial by time interaction effect for any of the cognitive function scores (*P* ≥ 0.373, $\eta_p^2 \leq 0.083$).

Table 4.5. Cognitive function test scores.

		Norm_Pl	Norm_Caf	PSR	*Trial*Time P* (η_p^2)
Shopping list Items remembered	Evening	12 ± 2	12 ± 2	12 ± 2	0.782 (0.020)
	Morning, no prompt	10 ± 3	10 ± 3	10 ± 4	
	Morning, prompt	14 ± 2	14 ± 1	14 ± 2	
N-back Visual correct ratio (%)	Evening	86 ± 17	85 ± 11	86 ± 13	0.843 (0.031)
	Morning 1	90 ± 11	87 ± 13	86 ± 15	
	Post pill	89 ± 13	85 ± 16	90 ± 7	
Stroop Stroop effect (ms)	Evening	168 ± 80	149 ± 98	179 ± 85	0.418 (0.066)
	Morning 1	179 ± 91	159 ± 89	138 ± 55	
	Post pill	153 ± 101	123 ± 99	111 ± 52	
Go/no-go Average speed (ms)	Evening	416 ± 82	425 ± 63	432 ± 60	0.373 (0.083)
	Morning 1	411 ± 63	423 ± 84	425 ± 84	
	Post pill	415 ± 60	411 ± 60	402 ± 59	
Go/no-go % correct	Evening	97.5 ± 4.3	97.0 ± 3.5	97.3 ± 3.3	0.570 (0.063)
	Morning 1	96.6 ± 4.1	93.2 ± 6.6	96.0 ± 4.3	
	Post pill	97.9 ± 3.5	97.6 ± 3.4	96.6 ± 3.2	

Data are presented as means ± SD Abbreviations: Norm_Pl, normal sleep plus placebo condition; Norm_Caf, normal sleep plus caffeine condition; PSR, partial sleep restriction plus caffeine condition. Shopping list n = 17 (female = 9), N-back n = 12 (female = 8), Stroop n = 16 (female = 9), Go/no-go n=13 (female = 9). N varies across cognitive function tests due to technical error in primary cognitive function website, with scores unavailable on secondary cognitive function website.

Chapter 5: Discussion

This study aimed to assess the acute effects of partial sleep restriction and subsequent caffeine ingestion on cerebrovascular function in healthy young adults. Specifically, the present study showed that NVC is unaltered after one night of 50% sleep restriction, and that the magnitude of the NVC response is unchanged one hour post caffeine ingestion in both the rested and sleep restricted state. However, absolute cerebral blood velocity, both in the MCA and PCA, were found to be lower after caffeine as compared to placebo, though this response did not differ between the rested and sleep restricted state.

Both ∆PCAv-peak and %∆PCAv-peak were not significantly different after partial sleep restriction as compared to after a normal night of sleep. Similarly, the total hyperaemic response as calculated as the iAUC was not significantly different between conditions. This is both contrary to our hypothesis in which we proposed that sleep restriction would alter the NVC response, and to the findings of the one existing interventional study assessing the impact of acute sleep deprivation on NVC metrics (Csipo et al., 2021). Csipo and colleagues (2021) found that one night of total sleep deprivation attenuated the haemodynamic NVC response in multiple cortical areas measured with fNIRS during a finger tapping task. A key difference between the study by Csipo et al. (2021) and the present study is that the former involved total sleep deprivation while our study involved 50% sleep restriction, with sleep occurring in the second half of the night. Our protocol is thought to reflect a degree of sleep loss that an individual may experience on any one given night. In contrast, keeping participants awake for ~ 24 hours is likely to exacerbate any alterations in NVC that may be seen as a consequence of sleep loss. Despite the lack of ecological validity of such a protocol, total sleep deprivation provides the opportunity to understand the relationship between

sleep and NVC and is still of great value given that it is the very first interventional paper in this field. Whether a dose-response relationship exists between the acute number of hours slept and the magnitude of subsequent NVC response remains to be studied.

Further key differences between the study conducted by Csipo and colleagues (2021) and our study include the method of NVC assessment, and the area of the brain assessed. Csipo et al. reported significantly smaller task-related haemodynamic responses (decreased O_2Hb and increased HHb) following sleep deprivation when utilising fNIRS in an area of the prefrontal cortex as well as an isolated area of the somatosensory cortex. fNIRS can determine changes in oxygenation patterns across the cerebral cortex, and therefore measures fluctuations in O_2Hb and HHb, as opposed to a general change in cerebral blood velocity as provided by TCD in our study. In addition to fNIRS, Csipo et al. (2021) also measured changes in cerebral blood velocity using TCD, and while they focused on MCAv as opposed to our assessed PCAv, the authors similarly did not find a significant difference between pre and post sleep deprivation at baseline. However, they did report a 4 cm/s decrease in absolute MCAv during n-back cognitive stimulation. In comparison, the visual search task used in our study provides a regional challenge, specifically activating the visual centres of the brain that are supplied by the PCA, thus during such tests, the PCAv increases to a much greater extent than the MCAv. Therefore, the MCAv cannot be used as a surrogate of PCAv. Csipo et al. (2021) also directly assessed retinal arterioles and venules in response to flicker light stimulation but did not find a significant difference between the rested and sleep deprived state. This evidence together may suggest that areas of the brain involved in visual functioning are somewhat protected following acute sleep restriction, while more frontal areas of the

brain experience greater sensitivity to sleep deprivation (Ma, Dinges, Basner, & Rao, 2015). However, this concept requires further study.

The current study also found that the magnitude of the NVC response, in terms of both ∆PCAv-peak and %∆PCAv-peak and as iAUC, was unchanged one hour after caffeine ingestion, compared to placebo, in both the rested and sleep deprived state. However, baseline MCAv and PCAv, and absolute and peak PCAv during the visual search task, were lower after caffeine as compared to placebo in both rested and sleep restricted states (ranging from ~ 8 – 13% lower). This is in line with previous research into the effects of caffeine on cerebral blood flow in the rested state (Addicott et al., 2009; Chen & Parrish, 2009; Field et al., 2003; Vidyasagar et al., 2013; Xu et al., 2015). For example, using an ASL MRI scanner, Vidyasagar et al. (2013) found both a single dose of 184 mg of caffeine, and black tea solids containing 184 mg of caffeine, decreased global cerebral blood flow by 20% and 21%, respectively, in the grey matter of healthy young men two hours after administration. Similarly, an earlier study reported a 30% decrease in whole brain cerebral blood flow using positron emission tomography following 250 mg of caffeine (Cameron, Modell, & Hariharan, 1990).

The findings of the present study are also in line with those of Addicott et al. (2009), who used MRI to assess the effects of 250 mg of caffeine in low, moderate and high caffeine users. The effects of caffeine were assessed across four occasions, twice in the caffeine abstinence state and twice after normal caffeine intake. Within each state participants consumed either caffeine or a placebo. The authors found caffeine lowered grey matter cerebral blood flow by an average of 27% across both caffeine states, and that in the abstained state, caffeine resulted in higher cerebral blood flow in the high users than the low users (Addicott et al., 2009). The authors postulated that these results could be due to the upregulation of vascular adenosine receptors during

prolonged caffeine use in order to preserve the baseline cerebral blood flow that would otherwise be expected had the individual of remained caffeine naïve. Addicott et al. (2009) suggested further support of this upregulated receptor hypothesis in that moderate and high habitual caffeine users had higher cerebral blood flow than low users during the abstained placebo condition, and a positive correlation existed between cerebral blood flow and daily caffeine use. Possibly due to an upper limit of adenosine receptors or to maximal vasodilation of blood vessels, this adenosine receptor upregulation may not be fully proportional to habitual caffeine consumption, with the authors reporting equal cerebral blood flow between moderate and high users (Addicott et al., 2009). Comparable findings were reported by Field et al. (2003) in a MRI study of dietary caffeine consumption, with post-placebo cerebral blood flow greater in high (648 mg/day) caffeine users than in low (41 mg/day) users. While our study reports participant's habitual caffeine intake with an average of 178 mg per day, this was not accounted for in the analyses as the study was not powered to do so. In addition to this, some participants consumed as little as 0 mg caffeine per day, while at the other end of the scale, one participant reported consuming ~450 mg per day. This limitation of the present study may explain some the inter-individual differences in vascular responses.

Whilst NVC can be considered as the precise matching of cerebral blood flow to cerebral metabolism, we did not measure cerebral metabolism *per se* in the present study, although measures of metabolism such as the oxygen extraction fraction can change. Instead, cerebral metabolism could only be inferred by quantifying the PCAv response to visual stimulation, which can be considered a 'metabolic challenge' for posterior brain areas (Willie et al., 2011). The assumption that changes in PCAv are proportionate to metabolism is also important in the context of caffeine ingestion, given

caffeine's effects on adenosine receptors, with research finding that to account for the decreased cerebral blood flow following caffeine ingestion, oxygen extraction fraction increases (Xu et al., 2015). It is therefore plausible that in the present study, while both absolute MCAv and PCAv following caffeine ingestion were not significantly different between the rested and the sleep restricted state, the oxygen extraction fraction may have differed. Indeed, in a review of the applications of fNIRS in fatigue, sleep deprivation and social cognition, Pan, Borragán and Peigneux (2019) highlighted that in most fNIRS studies of sleep deprivation there was a subsequent alteration in prefrontal oxygenation responses, even when participants were not fully sleep deprived. Future studies should therefore examine whether the oxygen extraction fraction is altered in response to caffeine when consumed in the sleep deprived state. Nonetheless, the PCAv increase is still of importance, given that it is valued from a disease risk perspective, reflecting the vascular responsiveness.

The data presented in the current study should be considered in the context of poor sleep, ageing, and dementia outcomes. In 2020, Kapadia et al. proposed that the underlying trigger for amyloid-β accumulation is vascular dysfunction, particularly impaired NVC, and that sleep plays an integral role in this process. Specifically, the authors discussed how individuals with Alzheimer's disease experience an accumulation and aggregation of amyloid-β due to compromised clearance of interstitial fluid. This occurs as a result of impaired microvascular autoregulation that transpires with ageing and certain environmental factors. The turnover of interstitial fluid is thought to be accomplished via tight NVC that primarily occurs during deep sleep (Kapadia et al., 2020). This sleep stage involves slow, rhythmic, neuronal oscillations and subsequent concurrent fluctuations in vascular activity (Fultz et al., 2019), which in turn pumps the cerebrospinal fluid and interstitial fluid in a coherent

manner (Kapadia et al., 2020). It is thought that this clearance, with solutes such as amyloid-β within the fluid, may occur via the glymphatic system (Kapadia et al., 2020; Reeves et al., 2020). The authors propose however, that when NVC is impaired, there is an ensuing decrease in the clearance of interstitial fluid and therefore amyloid-β (Kapadia et al., 2020). Importantly, these processes are worsened by poor sleep (total duration and/or quality) as less time is spent in this deep sleep whereby coherent activity of electrophysiologic, haemodynamic, and cerebral spinal fluid oscillations occur (Fultz et al., 2019; Kapadia et al., 2020). Therefore, even in healthy young adults, amyloid-β concentrations are elevated after sleep restriction (Shokri-Kojori et al., 2018). Kapadia et al. (2020) thereby concluded that recurrent sleep loss likely results in stepwise alterations to the microenvironment that are triggered by impaired NVC. In the present study we aimed to decrease the time spent in deep sleep, not only by halving their normal sleep duration, but also by limiting participants to sleep only during the second half of their normal sleep, as deep sleep is thought to predominate in the first half of the night (Dijk, 2009; Léger et al., 2018). This sleep staging is thought to be circadian rhythm dependent, as opposed to 'resetting' whenever sleep onset occurs (Dijk, 2009; Léger et al., 2018). While not validated for sleep staging against the gold standard of polysomnography, we attempted to measure the participants' time spent in deep sleep using Actiheart devices. Analysis of this data suggested that time spent in deep sleep was significantly lower in the partial sleep restriction condition (67 ± 16 minutes) as compared to both normal sleep conditions (placebo: 138 ± 26 caffeine: 135 ± 18 minutes). Nonetheless, we did not find that our sleep restriction protocol induced any changes in NVC.

An attenuated NVC response may have been expected following sleep restriction as such sleep loss would likely lower task engagement, and thus may result in decreased

activation of the visual cortex. Indeed, participant's self-reported engagement was lower during the initial morning visual search task after partial sleep restriction, as compared to following the normal sleep conditions. However, this decreased task engagement did not manifest as altered NVC. Thus, the reason for such null findings in the present study could be twofold. Firstly, as discussed earlier, we only captured changes in cerebral blood velocity, and did not measure any changes in $CMRO_2$ or oxygen extraction fraction, which may have differed after sleep restriction. Secondly, Kapdia et al. (2020) discussed how these changes in NVC likely accumulate with age and with repeated exposure to poor sleep, while in the present study we only assessed the effects of one night of partial sleep restriction on NVC in healthy young adults, who were typically 'good' sleepers. Supporting the former regarding changes in oxygen extraction, an animal study found that compared with mice of a similar age, a mouse model of Alzheimer's disease had reduced cortical oxygen tension during rest, while during functional activation such mice had augmented flow responses (Gutiérrez-Jiménez et al., 2018). This could suggest that metabolic feedforward regulation of flow is impaired in the resting state, while cerebral blood flow feedback regulation is maintained during neurotransmission (Gutiérrez-Jiménez et al., 2018). This study also reported a delay in the haemodynamic response during functional activation in the aged mice, suggesting capillary dysfunction may develop with age, and that in turn tissue oxygenation might be impaired (Gutiérrez-Jiménez et al., 2018). Similarly, in a study of human participants with Alzheimer's disease, impaired capillary dysfunction assessed as tissue oxygen tension was found to be associated with symptom severity and neurodegeneration (Nielsen et al., 2017). Thus, one possible reason our decreased deep sleep did not result in altered NVC, may be that we did not measure any indices of tissue oxygenation. Alternatively, poor sleep may only alter NVC as it is

accumulated across the lifespan, causing small changes to the cerebral microenvironment that amass with time.

The findings of the present thesis should not be extrapolated beyond healthy young adults as changes in NVC are apparent across the lifespan (Beishon, Clough, et al., 2021; Buckner, Snyder, Sanders, Raichle, & Morris, 2000; Gröschel et al., 2007; Nowak-Flück et al., 2018). Using functional brain imaging of young and older adults with and without dementia, Buckner et al. (2000) found that specific regions of the brain, including the visual cortex, displayed reductions in the amplitude of the haemodynamic response in older adults both with and without dementia. Similarly, Nowak-Flück et al. (2018) found that NVC was blunted in healthy older adults, compared to young adults, both at rest and during exercise, and Beishon and colleagues (2022) found age related differences in cerebral artery blood velocity in response to cognitive stimulation. On the other hand, it has also been found that the magnitude of the NVC response is independent of age, as assessed in a sample aged 21-66 years (Leacy et al., 2022). As such, in 2021, a review highlighted that studies investigating the effects of ageing on NVC have found mixed results, with reduced, equivalent and increased vascular responses to stimulation with ageing . Beishon and colleagues (2017) suggested that future studies should utilise multiple imaging techniques, for example TCD and NIRS, in an attempt to capture information on both the spatial and temporal nature of the response, thus demonstrating changes to both cerebral blood flow and metabolism. Recently, novel kinetic analyses have been applied to cerebrovascular data in order to better understand the time course of the cerebral blood velocity response (Koep, Weston, et al., 2022). In the context of cerebrovascular reactivity to hypercapnia, Koep et al. (2022) reported a positive relationship of the MCAv to cerebrovascular reactivity time constant with age, in that

the speed of the MCA response was slower with increasing age. It is therefore also possible that similar kinetic modelling of NVC may show differences with advancing age. Indeed, unpublished data from our own laboratory indicates that the NVC response is slower in older females, but not males, when analysed using a mono-exponential model. Furthermore, it has been suggested that the presence of cardiovascular risk factors may have an independent influence on the ability of cerebral vessels to react to stimulation (Gröschel et al., 2007). Whether partial sleep restriction acutely alters the NVC response in older adults, with or without cardiovascular risk factors, is unknown, and as such the findings of the present study should not be extrapolated to ‘at risk’ groups.

Acute short sleep duration is also associated with impaired cognitive function outcomes (Lowe et al., 2017). However, the present study did not find any changes in cognitive function following partial sleep restriction. This is in line with the findings of Schaedler et al. (2018) who evaluated the effects of partial morning and evening sleep restriction on executive functioning in healthy young adults. The authors utilised an independent measures study design where by one group of participants followed their normal sleep/wake cycle; one group completed morning sleep restriction, waking three hours earlier than normal; and one group completed evening sleep restriction, going to sleep three hours later normal. Participants then completed the Stroop test, the Go/No-Go test and the Iowa Gambling task. However, the study did not report any differences between the three different sleep conditions in selective attention, response inhibition and decision making abilities, as based off of performance to the aforementioned tests. While other studies utilising a longer sleep deprivation protocol have reported cognitive deficits (Cain, Silva, Chang, Ronda, & Duffy, 2011; Chuah, Venkatraman, Dinges, & Chee, 2006; Drummond et al., 2006), studies with similar

sleep restriction protocol typically report that cognitive function is unchanged (Rossa, Smith, Allan, & Sullivan, 2014; Schaedler, Santos, Vincenzi, Pereira, & Louzada, 2018). Schaedler et al. (2018) suggest that this could be due to the core sleep hypothesis, which proposes that there are two types of sleep – core sleep and optional sleep (Horne, 1988). Core sleep is the initial sleep period which is crucial for adequate daytime functioning, while optional sleep is supposedly all sleep beyond core sleep (Horne, 1988). It has been suggested that the former requires approximately four to five hours of sleep per night (Horne, 1988), and the average sleep duration for our sleep restriction protocol is line with this at 04:27 ± 1:10, which could possibly explain why we did not see any decreases in cognitive performance. Furthermore, previous research has shown that certain aspects of cognitive function are protected following acute sleep deprivation in young healthy adults in comparison to older healthy adults (Pasula et al., 2018). For example, Pasula et al. (2018) found that young adults aged 19 – 38 years outperformed older adults aged 59 – 82 years in verbal and visuospatial working memory after 36 hours of total sleep deprivation. In addition to this, Poudel et al. (2012) reported that the pattern of cerebral activity following acute sleep restriction was largely dependent on participants' self-reported drowsiness, highlighting the inter-individual effects of sleep restriction.

The present study also not find any changes in MCAv during the cognitive function tests. This is in contrast to findings of Csipo et al. (2021) who reported that MCAv was lower during the n-back test following sleep deprivation, as compared to pre-sleep deprivation. In addition to the aforementioned disparity between their use of 24 hours of sleep restriction, the reason for this contrasting finding could be twofold. Firstly, the researchers looked at absolute MCAv during the N-back test, as opposed to a change from rest. In the present study we did report that resting/baseline MCAv was lower

after sleep restriction as compared to normal sleep, but this was only significantly lower compared to one of the normal sleep conditions. This study of complete sleep restriction also reported impaired cognitive performance in terms of reaction time and sustained attention. It may therefore be that the alterations in cerebral blood velocity during cognitive tests are more likely to be seen in conjunction with impaired cognitive function, which was not seen in our sample of healthy young adults after only partial sleep restriction. Secondly, we did not consider which hemisphere of the brain the task was predominantly utilising (Beishon, Minhas, et al., 2018) and we only performed unilateral TCD measurements. We primarily scanned participants right cerebral arteries, however in two participants a more robust signal could be attained on the left, and therefore we had a final sample of fifteen participants with scans of the right MCAv and two participants with scans of the left MCAv. Given that there are key variations in activation and perfusion patterns between healthy younger and older adults, and that cognitive tasks typically lateralise to one hemisphere, (Berlingeri, Danelli, Bottini, Sberna, & Paulesu, 2013; Sorond, Schnyer, Serrador, Milberg, & Lipsitz, 2008) we may have missed the opportunity to capture any changes caused by sleep restriction in MCAv during the cognitive function tests. Specifically, the 'Compensation-Related Utilisation of Neural Circuits Hypothesis' suggests that older adults experience a loss of lateralisation that results in compensatory recruitment of additional neuronal circuits as compared to healthy younger adults (Sorond et al., 2008). Future research should therefore look to complete bilateral scans of cerebral arteries, and should also target the artery feeding the specific area of the brain that the cognitive function test activates.

Chapter 6: Considerations, future directions, and conclusion

The strengths of this study include the implementation of an ecologically valid sleep restriction protocol that reflects an amount of sleep loss an individual might experience on a given night, whether this is due to a planned short sleep in order to wake for a certain event, an unplanned worse/shorter night of sleep compared to normal, and/or the consequence of working night shifts (Chinoy, Harris, Kim, Wang, & Duffy, 2016; Garde et al., 2020; Walch, Cochran, & Forger, 2016). The only other study to have assessed the interaction between sleep and NVC utilised a total sleep deprivation protocol which is not typical of normal sleep loss (Csipo et al., 2021). Furthermore, our specific design required participants to complete the partial sleep restriction in the first half of the night, thus staying awake at the start of their typical sleep time, in an attempt to deprive them specifically of deep, slow wave sleep (Dijk, 2009; Léger et al., 2018). Using this protocol, based on recordings from Actiheart devices, we were able to more than half the total amount of time participants spent in deep sleep. This was an important part of design due to the context of changes in sleep architecture with ageing and disease. Specifically, we know that the amount of deep sleep accrued in one night decreases with age (Li et al., 2018) and is further decreased in patients with Alzheimer's disease (Avidan, 2006). Whilst the quantification of total sleep and estimation of sleep stages with the Actiheart devices is a strength of this work, future studies should either use the gold-standard method of sleep staging – polysomnography, or use devices which have been validated against polysomnography, in order to confidently measure the quantity and quality of deep sleep accrued by the participant. However, while we aimed to replicate certain aspects of the sleep of older adults (i.e., decreased deep sleep), the young age of the participants in our study can be viewed as a limitation of the work given the dementia

framework explored in the literature review. One proposed mechanism for the role of sleep in dementia progression is the clearance of amyloid-β during sleep (Winer et al., 2020; Xie et al., 2013). Though this protein has been shown to accumulate following total sleep restriction in young healthy adults without dementia (Shokri-Kojori et al., 2018), the progression of dementia is only partly explained by the amyloid-β hypothesis, and there are numerous other causes of dementia that cannot be explained by this relationship, especially in dementia asides from Alzheimer's disease, such as vascular dementia and Lewy Body dementia. It is therefore important to highlight that the relationship between sleep and dementia merely provides a framework for this study, and the results cannot be extrapolated beyond young healthy participants.

A further strength of the present study concerns the novel kinetic analysis of the NVC data. An important component of NVC is the ability to rapidly respond to an increase in metabolic demand. We fitted a mono-exponential model through our NVC data which allowed us to better quantify the time course of the response. Previously the time course has been assessed as both the time to peak PCAv, and the time from stimulus onset to the increase in PCAv by >1% above the eyes closed baseline period. The former comes with the issue that, for example, two individuals can have the same time to peak response but have different temporal trajectories in blood velocity, and the latter with the issue that a 1% increase in PCAv equates to <1 cm/s, which is hard to quantify given a beat-to-beat variation equal or greater to this. However, kinetic analysis can provide insight into these temporal trajectories in response to the increased metabolic demand. Of a possible 153 timepoints (17 participants, 3 visits, 3 conditions), 126 PCAv NVC traces were able to be fitted with the mono-exponential

model, with only 27 timepoints not feasible to fit with this model. Of these 27 timepoints, 22 were from just 3 participants who consistently did not display a typical NVC response, with small changes for all NVC metrics and thus could be considered non-responders for 2 of these 3. The remaining 5 timepoints that could not be fitted were from 4 participants, which in turn had to be excluded from statistical analysis of the kinetic outputs due to missing data points. For 3 of these participants it was evident that if more transitions had been included for each timepoint, i.e. completing 8 eyes-open to visual stimulus repetitions as opposed to 5, then the averaged response would have reflected that of a typical NVC cerebral velocity response that could be fitted with the model. Indeed, previous studies have shown that traditional NVC metrics have increased reliability with more transitions (Burma et al., 2022). Given the potential utility for kinetic analyses providing additional information which traditional cerebrovascular metrics are insensitive to (Koep, Bond, et al., 2022; Tallon et al., 2020), future work using visual search tasks might consider include more eyes-open, eyes-closed repetitions in order to ensure that a robust mono-exponential model may be fitted. Presently, the trade-off between participant burden and precision of the kinetic parameter estimates are unknown, and this remains a promising future methodological question.

One potential issue arising from kinetic analysis is the possibility that if an intervention considerably blunts the NVC response to the extent that the participant does not appear to respond, then this will render the data not fit for modelling, and thus this participant will be excluded from analysis. This may therefore result in the kinetic outcomes underestimating the findings of the intervention. This was the case for one of our participants who typically demonstrated a strong NVC response to the visual stimulus, however after completing sleep restriction, the participant’s PCAv did not

increase at all in response to the stimulus and thus this timepoint could not be fitted with the model (Figure 6.1). It is also important to acknowledge that time-based metric have greater variability, and therefore analysis requires larger sample sizes than traditional methods. Future studies should consider performing kinetic analysis of their NVC data and compute sample sizes that are primarily powered to detect changes in kinetic outcomes. It is currently unknown how many eyes-closed/eyes-open repetitions need to be averaged for kinetic analysis of one timepoint, however traditional outcomes showed that the benefit of inclusion of more than six repetitions was small (Burma et al., 2022). Studies should also consider excluding participants upon screening if they appear to be a non-responder in order to minimise data loss at the analysis stage.

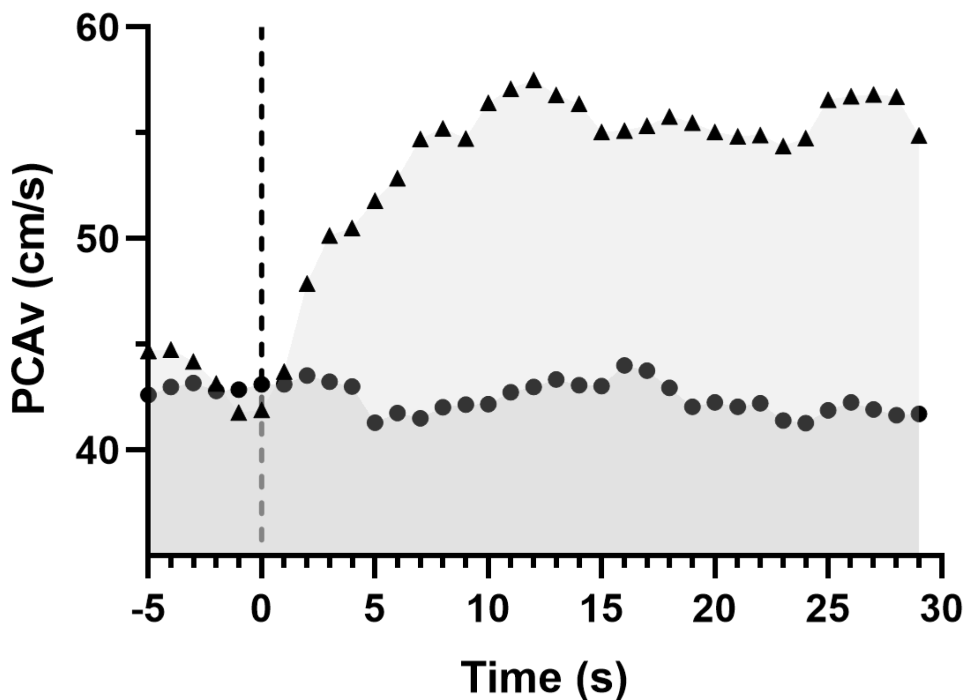

Figure 6.1. A graph depicting the NVC response of one participant who had a blunted response to the visual stimulus following the sleep restriction (circles) as compared to during the evening visit of the PSR condition (triangles). The period up to the dashed line represents the baseline eyes-closed period, with the dashed line marking the onset of the visual search task.

In addition to the previous discussion relating to kinetic data, the issue of non-responders should also be considered in the context of other NVC outcomes. A non-responder could be considered as a healthy participant who fails to show a change in cerebral artery blood velocity in response to stimulation, though the reasons for this occurring remain unclear. As previously mentioned, two participants could be considered non-responders, with inconsistent and small changes for all NVC metrics, i.e. peak PCAv, %ΔPCAv-peak and iAUC. This is common within the research field. For example, Vermeij et al. (2017) reported interindividual variability in fNIRS responses to working memory tasks, with some participants showing no clear response, irrespective of whether they were included as a healthy control participant, or a participant with mild cognitive impairment. Future work should consider employing the two-criteria method proposed by Beishon and colleagues (2020) to identify and classify non-responders to task activation measured by TCD. This study utilised files from 135 participants undergoing bilateral cerebral artery blood velocity assessment during seated rest to determine the upper limits of variability in cross-correlation function peak and variance ratio. This in turn allowed them to establish the threshold beyond which NVC responses can be considered above normal fluctuations in blood velocity. These thresholds were then applied to a subsample of participants during task-activation, and the researchers found that when applied to five different cognitive tasks, 13 – 42% of participants were classified as non-responders. This method was used in a subsequent study by Beishon et al. (2021) and was able to discriminate cognitive impairment from healthy ageing. While we did not use this method to identify our non-responders (instead considering those who had minor and inconsistent responses as non-responders), this is in line with the percentage of our sample, ~12%, who may be identified as non-responders and is a method we will utilise in future

studies. These methods also provide a useful guideline for sample size calculations with respect to considering how many extra participants should be recruited to account for those who may be considered a non-responder.

It is also important to consider the present work in light of a number of methodological considerations. Firstly, it is important to address that our main outcome of NVC was measured using TCD as an assessment of cerebral blood velocity. The limitations of TCD are discussed extensively in the literature review, but in brief, as the diameter of the insonated vessel is unknown, TCD only measures cerebral blood velocity, not absolute volumetric flow, and in turn can only be used as a surrogate of cerebral blood flow if the diameter of the insonated vessel remains constant (Willie et al., 2011). Though the cerebral circulation is regulated by $PaCO_2$, we did not measure expired gases and thus an indicator of $PaCO_2$ in the present study. The extent to which cerebral artery diameter dilates or constricts in response to changes in $PaCO_2$ remains debated (Brothers & Zhang, 2016; Hoiland & Ainslie, 2016), and these changes tend to be greater in the MCA than the PCA (Al-Khazraji et al., 2019). Furthermore, previous studies using TCD in assessment of NVC have shown that $P_{ET}CO_2$ remains well within ± 5 mmHg of eucapnia during such tasks (Smirl et al., 2016; Zaletel et al., 2004), and therefore minimal changes in PCA diameter are anticipated for in these tests. This in part influenced our decision not to record expired gases in the present study. This decision was also influenced by logistical issues of fitting a face mask and head strap to the participant who already had prefrontal cortex NIRS (though not included in the present thesis) and a TCD headset fitted and in place. A further limitation concerning the tool used to measure NVC relates to the issue of only assessing cerebral blood velocity, and not including a measure of $CMRO_2$ or oxygen extraction fraction. This is more expansively reviewed in the discussion chapter, however, future studies

assessing the effects of sleep restriction on NVC should look to also incorporate NIRS to complement the TCD data in this manner, or if available, calibrated BOLD measures in order to distinguish between changes in cerebral blood flow, $CMRO_2$ and the oxygen extraction fraction.

A further limitation of the present study is the absence of a partial sleep restriction with subsequent placebo pill experimental condition. We chose not to include this condition due to the perceived difficulties and attrition rate when requesting participants to complete two sleep restriction conditions. We also anticipated that any effects of the sleep restriction protocol without caffeine would be seen during the first morning measurement, before any pill was consumed, and that NVC would not be significantly different one hour later (post placebo) (Burma, Macaulay, et al., 2021). However, we appreciate that this limits the interpretation of the current results. Future research exploring the effects of sleep restriction and subsequent caffeine ingestion should consider including this fourth condition of sleep restriction with placebo pill ingestion.

Another limitation pertaining to the methods of the study is the possibility of attenuation of cerebral artery blood velocity responses due to the repeated measures nature of the study, both across trials and within day. During the morning visit participants are required to repeat both the visual search task and cognitive function tests within the space of a few hours. However, during the placebo condition, there was no main effect of time on any of the outcomes, suggesting that within a trial, despite the necessity to repeat tests in quick succession, there was no attenuation of responses. Similarly, engagement in the visual search task was not significantly different across the normal sleep conditions, only following partial sleep restriction.

In terms of the sleep restriction protocol, future research may also wish to explore how sleep is accrued throughout the night e.g. disrupted/fragmented sleep, and the subsequent effects on cerebrovascular function. For example, participants in our study achieved ~4.5 hours sleep. However, they could have achieved this by going to bed on time and waking up earlier or accumulated this with the use of one or more awakenings during the night. Such a design, complemented with validated sleep staging, would provide significant further insight. In addition to this, we only recruited participants with both good sleep quality and quantity, as confirmed using the PSQI, a sleep diary and Actiheart measurements of normal sleep. Measuring the effects of sleep restriction on individuals who present with irregular sleep and/or are chronically sleep deprived would have made it difficult to prescribe a 'good' night of sleep, as our experimental intervention was based on restricting the sleep of those who regularly attained 7-9 hours of sleep per day. However, as previously discussed, chronic sleep loss may result in stepwise micro-impairments to NVC that amass over time, and thus future work could consider using an observational design comparing NVC in chronic 'good' versus 'bad' sleepers, establishing whether a dose-response relationship exists with respect to sleep duration and NVC.

Similarly, it is important to consider how the ecologically valid 50% sleep restriction could be furthered by looking at multiple days of partial sleep loss. While one night of such sleep might not affect NVC in this group, whether repeated nights of partial sleep restriction alter NVC remains to be investigated. Likewise, valuable insight could be provided by an observational study of NVC in shift workers (and age and sex matched controls), who may alternate weeks of working during the day and working at night, given that such individuals are at an increased risk of developing cardiovascular disease (Torquati, Mielke, Brown, & Kolbe-Alexander, 2018; Wang, Ruan, Chen,

Peng, & Li, 2018). In addition to this, not everyone reports feeling 'drowsy' after acute sleep restriction (Poudel et al., 2012), thus future studies could also look to recruit participants who identify as being particularly sensitive to sleep loss.

One consideration of this study reviewed in depth in the discussion chapter, is the effect of habitual caffeine consumption on cerebral blood flow. Participants were instructed to refrain from consuming caffeine on the day of the evening visit and to also arrive in a fasted state the following morning. The average caffeine consumption in this study was 175 ± 140 mg/day, with a range of 0 to 462 mg/day. Despite such inter-individual variability in habitual consumption, and differences concerning the normal timing of caffeine consumption, most participants likely experienced some withdrawal effects pertaining to both to their normal cerebral blood velocity (Addicott et al., 2009; Field et al., 2003) and cognitive function (Rogers et al., 2005). It has been shown that following a period of caffeine abstinence, high caffeine users have greater cerebral blood flow than low users (Field et al., 2003). However, in a study assessing the effects of caffeine on cerebral blood velocity, it would also not make sense to allow participants to continue consuming caffeine on the day of the study. It has therefore been suggested that high caffeine use (e.g. >600 mg/day) should be an exclusionary criteria in future research (Addicott et al., 2009).

Depending on the dose, caffeine is also known to increase both systolic (3 – 8 mmHg) and diastolic (4 – 6 mmHg) blood pressure, with high inter-individual variability, typically increasing 30 minutes after consumption, and peaking at 60 – 90 minutes (EFSA Panel on Dietetic Products & Allergies, 2015). While relatively stable in normotensive individuals, blood pressure follows a circadian rhythm, rising during the morning approximately one hour before waking, and decreasing at night (Lecarpentier, Schussler, Hébert, & Vallée, 2020; Millar-Craig, Bishop, & Raftery, 1978; Weber,

Drayer, Nakamura, & Wyle, 1984). In addition to this, blood pressure is affected by sleep duration (Covassin et al., 2021). Although most studies have focused on multiple nights of partial sleep restriction or one night of total sleep restriction (as opposed to one night of partial sleep restriction) it has been found that sleep restriction also results in increased blood pressure (Carter, Durocher, Larson, DellaValla, & Yang, 2012; Dettoni et al., 2012; St-Onge et al., 2020). It is therefore likely that participants in the present study experienced fluctuations in blood pressure dependent on the time of day (evening or morning measure), sleep condition (normal or sleep restriction), and acute and chronic caffeine status (habitual caffeine consumption, and study condition of caffeine versus placebo pill). While we did not measure brachial blood pressure, a strength of the study is the measurement of fingertip blood pressure throughout both the NVC and cognitive function tests. Furthermore, the relationship between cerebral blood velocity and fingertip blood pressure was checked before performing further data analyses. Nevertheless, future studies investigating the effects of sleep/circadian rhythm and/or caffeine should record participants blood pressure throughout the study.

It is also important to note the high participant BMI range in this study. While, diagnosed OSA was an exclusion criterion, this does not account for the fact participants may have mild OSA that they are unaware of. Furthermore, we had four participants whose BMI fell within the ‘obese’ category at the time of participation, and there is a known association between obesity and OSA, with these relationships stronger in younger populations (Liu et al., 2021). Though unlikely due to screening, there is therefore the possibility of OSA affecting some of the cerebrovascular responses measured within the study (Bålfors & Franklin, 1994; Durgan & Bryan Jr, 2012).

Finally, the lack of reporting of and controlling for menstruation status (as well as menopausal status) could be considered a limitation of the study. The effects of menstrual cycle phase in premenopausal participants on cerebrovascular function remains debated (Skinner et al., 2021), with some studies reporting that cycle phase affects cerebrovascular reactivity/autoregulation (Abidi et al., 2017), and other research reporting no relationship between these measures (Favre & Serrador, 2019; Korad, Mündel, Fan, & Perry, 2022; Peltonen et al., 2016). While the effect of menstruation status on NVC is less well documented, it is recommended that testing involving cerebrovascular measures should be completed during the early follicular stage (Shechter & Boivin, 2010). Furthermore, although our sample consisted of young adults, menopausal status is known to influence NVC (Koep et al., 2023), and we cannot discount the possibility that any of our participants were experiencing an early menopause. In addition to the possible effects of menstruation status on cerebrovascular function, cycle phase can also influence sleep quality (Shechter & Boivin, 2010). Although we asked participants to confirm that they had met the sleep requirements of the experimental trials, subjective sleep assessments do not always track objective measures (Aili, Åström-Paulsson, Stoetzer, Svartengren, & Hillert, 2017), and therefore sleep quality may have been affected by menstrual phase.

Conclusions

The study of sleep quantity and quality is a rapidly growing area of research, especially regarding its influence on cerebrovascular function and dysfunction in disease. This thesis was the first study to assess the effects of sleep restriction, specifically a single night of 50% sleep restriction, on one measure of cerebrovascular function – NVC. We

found NVC outcomes were protected following such sleep restriction in healthy young adults. In addition to this, this study was one of the first to demonstrate that NVC data can be analysed using novel kinetic analysis models, which will further the field of NVC across various contexts, by quantifying the temporal cerebral blood velocity response. Furthermore, this study provides novel insight into NVC after caffeine consumption in the rested versus the sleep restricted state. We found that while the magnitude of the NVC response was unaffected by caffeine, absolute cerebral blood velocity metrics were lower following caffeine ingestion and did not differ between the rested and sleep restricted state. Future studies in this field should consider using calibrated BOLD measures to discriminate between changes in cerebral blood flow and changes in cerebral metabolism after sleep restriction. Future research could also involve observational studies of how these responses differ between those who habitually sleep well and those who report issues with sleep quantity and/or quality, as well as how the relationship between sleep and NVC changes with age and disease status. Similarly, further investigations may wish to utilise different sleep restriction protocols, for example, a disrupted sleep protocol which may reflect the sleep experienced by new parents, as well as the use of more than one night of disruption to typical sleep.

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Appendices

Appendix 1: Participant information sheet

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Participant Information Sheet

Title of Project: Exploring the effects of acute sleep restriction and subsequent caffeine ingestion on neurovascular coupling

Researcher name: *Alice Lester*

Invitation and brief summary:

We would like to invite you to take part in a research study investigating how one night of short sleep, and subsequent caffeine ingestion, affects the matching of brain blood flow to demands of the brain. Taking part in the study is entirely up to you, so before you decide, it is important for you to understand why the research is being done, and what it will involve. Please take time to consider the information carefully and to discuss it with family or friends if you wish. Please also do not hesitate to ask me any questions you have about the study, my contact details can be found at the end of the form. Thank you for taking the time to read this information.

Purpose of the research:

Poor sleep acutely reduces brain blood flow, and over a long period of time can increase the risk of dementia. Dementia is associated with an altered ability to control the delivery of blood to the brain (termed neurovascular coupling; NVC). However, we don't understand how poor sleep can influence NVC. Acute sleep loss also impairs cognitive function and arousal, and a popular remedy for this is the consumption of caffeine. However, it is not known how caffeine affects NVC following a night of short sleep. The purpose of this study is therefore to assess how one night of short sleep affects NVC, and cognitive function. A secondary aim of the study is to establish how caffeine ingestion after short sleep affects NVC and cognitive function.

Why have I been approached?

We have invited you to take part because we want to first understand the relationship between short sleep and NVC in young adults, before we upscale this work to older participants who are more vulnerable and at risk of developing dementia. In addition to this, we know that young adults have more of a certain sleep stage than older adults, which makes this sleep duration easier to manipulate in younger participants. In total, we will be inviting 20 young adults to take part.

What would taking part involve?

This study will involve one familiarisation visit to the laboratory (visit 1), and three experimental 'visits' to the laboratory (visits 2/3/4). Two of the experimental visits will involve you completing your normal night of sleep, and the other visit will involve sleeping for half of your normal sleep (this is described in more detail below). These conditions will be completed in a randomised, counterbalanced order. Please read the study protocol in detail, and see the diagrams below, which outline a timeline of what the study will involve.

Study protocol

Visit 1.

This is the shortest visit, and it will take approximately 1 hour. We will begin by making sure the consent, health screening and contact details forms have all been completed. You will then be asked to fill out a questionnaire on your habitual caffeine use and your normal sleep (the Pittsburgh Sleep Quality Index). This is to ensure you do not habitually consume more than 900 mg/day of caffeine, and to check that you qualify as a 'good' sleeper, i.e. sleeping for 7-9.5 hours for at least 5 nights a week. If any participant does not meet these criteria, we will not continue the study with them. This visit also involves some preliminary measures of height and body weight.

After completing these forms, the participants will be shown all the techniques that will be used in the study, these are all non-invasive. We will assess brain blood vessel function using ultrasound, attaching a small probe to the side of your head using an adjustable headset (pictured below). During this first visit, we will make sure we can identify both blood vessels that we will be scanning in the experimental conditions. In 10-15% of participants it is not possible to identify these blood vessels with the type of ultrasound that we will be using. If this appears to be the case after 30 minutes of trying to identify the blood vessels for a given participant, we will not continue the study with this participant. We will then practice the protocol that allows us to assess our main outcome of the study (neurovascular coupling, NVC). Whilst wearing the headset with the ultrasound attached, you will close your eyes for 20 seconds, and then open your eyes for 40 seconds. Upon opening your eyes, you will be asked to locate a person or object within a Where's Wally scene. During experimental visits, we will complete the eyes-open eyes-closed for 5 cycles. After completing the 5 cycles, we will ask you to rate, subjectively, your level of attention during the Where's Wally task on a scale of 1-10. We will also show you a probe (near-infrared spectroscopy, NIRS) that will also be attached to the forehead (under the headset). This will provide us with an indirect measure of brain activity.

The headset and ultrasound setup to measure brain blood flow.

During the experimental visits, whilst wearing the ultrasound headset, you will also be asked to complete a series of cognitive function tests. This first familiarisation visit will allow you to practice these tests so that you know what to expect during the experimental visits.

At the end of this visit you will be provided with a device (called an ActiHeart) that will help us to assess your normal sleep duration. This is a combined accelerometer and heart rate monitor that is attached to the chest via two sticky tags. You will be asked to wear this for 3 consecutive days and then return the device to the laboratory. It is important that the device is returned before you come back for your experimental visits. We ask that on these 3 days that you record your sleep, you follow a normal routine that does not involve staying out later than a normal weekday. We also ask that you avoid drinking alcohol for these 3 days. You will be given a sleep diary form, where you will be required to write down both what time you went to bed (specifically what time you started trying to go to sleep – lights off and off of all electrical devices) and what time you woke up. This will allow us to confirm the data provided by the ActiHeart on your sleep duration. We will also provide you with a food diary to record what you eat during the day of the experimental visit.

The ActiHeart device to be worn overnight

Visit 2/3/4

These visits involve two trips *each* to the laboratory: one the evening before the experimental sleep, and one the morning after. The evening visit will take approximately 2 hours, and the morning visit will take up to 4 ½ hours. You will be informed of whether you will be completing a normal sleep condition or the sleep restriction condition at least one week before the experimental visit. This is so that you can plan your day after sleep restriction appropriately (i.e. plan to avoid driving where possible).

Evening visit

You will be asked to arrive at the laboratory between 16:30 and 18:30, depending on your work schedule/what time you are able to get to the laboratory. You will be required to arrive at the laboratory at the same time for all 3 experimental visits. We will ask you to avoid food and exercise for two hours prior to the visit, and to avoid caffeine and alcohol for 12 hours prior to the visit. Upon arrival, we will attach a very small blood pressure cuff to one of your fingers to continuously measure your blood pressure at the finger. Then we will attach the NIRS and ultrasound probe to your head as described in visit 1 and complete the full NVC measurement. This will involve 5 cycles of eyes closed (20 seconds) and eyes open to the visual stimulus (40 seconds). Scanning the vessel can take between 5 and 20 minutes. The actual NVC test will total less than 10 minutes. We will then change the ultrasound to scan a different blood vessel. You will then complete a series of cognitive function tests (as practiced at the familiarisation visit). This will include memory tests (a validated shopping list recall test and an N-back test), an assessment of executive function (measured using the Stroop test), and processing speed (Go-No-Go test). This will take no longer than 15 minutes.

You will then be provided with an ActiHeart to measure your sleep back in the comfort of your own home. You will also be given another sleep diary to record approximately the time you went to sleep and the time you woke up. For the two normal sleep conditions, this will involve going to sleep and waking up at your normal time. We will discuss with you what time you normally wake up and will ask you to return to the lab within an hour of waking. The total time for this evening visit will be ~2 hours.

Sleep restriction details: For the one sleep restriction condition, you will be required to sleep for only half of your normal sleep duration. We will calculate this from the previously collected data provided by the ActiHeart and your sleep diary. For example, if your normal sleep duration is approximately 8 hours, then you will be required to sleep for only 4 hours. You will be asked to stay awake at home and remain sedentary for the first 4 hours of the night that you would normally sleep for. You will be allowed to do anything during this time that will help you stay awake, so long as the activity can be completed in a sedentary position. In order to check that you have stayed awake, you will be asked to email the lead researcher 'Awake' every 30 minutes until you start trying to sleep. You will then be required to try to sleep for the final 4 hours of the night. We appreciate that it is not possible to go to sleep on demand, so sleep will be 'capped' at 4 hours, rather than requesting you sleep for a minimum of 4 hours. Continuing with the example of sleeping for 8 hours, if you normally go to sleep at 22:30, you will be required to stay awake until 02:30, try to sleep from 02:30, and then set an alarm for 06:30.

For both the normal sleep conditions and the sleep restriction condition, you will be required to complete the food diary (given during the familiarisation visit) for the rest of the day. We also ask that you refrain from exercising and consuming caffeine and alcohol between leaving in the evening and returning to the laboratory in the morning.

Morning visit

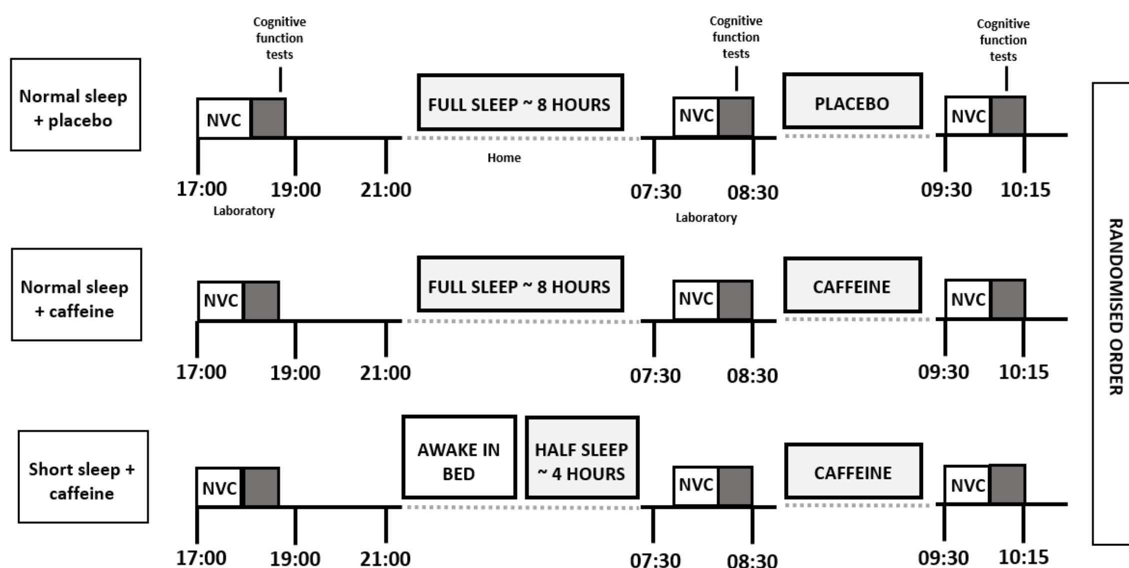
For the two normal sleep conditions, we will check that, subjectively, you felt you had a normal night of sleep before you head into the laboratory. If you know that your sleep was >1 hour less than normal for this condition, we will re-schedule the visit.

As above, you will be asked to arrive at the lab within one hour of waking. We ask that your commute does not involve exercise. Following the sleep restriction condition, you will be offered a lift to the laboratory as we recommend that you avoid driving after this condition, where possible. You will also be asked to arrive at the laboratory in a fasted state, and refrain from exercising and consuming caffeine.

For all conditions, upon arrival at the lab, we will repeat the same measures from the evening before. This includes the NVC test and cognitive function tests. This should take no longer than 2 hours. Immediately after completing this test, you will be given a pill containing either 150 mg caffeine (for the sleep restriction condition and one of the normal sleep conditions) or a placebo pill (for the other normal sleep condition). One-hour post ingestion, the NVC and cognitive function tests will be repeated. Scanning to find the vessel and repeating the tests will take less than 1 ½ hours. The end of these measures marks the end of the experimental visit. The total time for this morning visit will be ~4.5 hours.

There will be a 'washout' period of two weeks between each experimental condition. This is to allow you to fully recover from the effects of sleep restriction, should you complete this condition before the normal sleep conditions. You will then complete the alternative sleep + caffeine/placebo conditions. You will be asked to replicate your diet as closely as possible for each of the experimental visits prior to the evening visit. Excluding the sleep time/time spent at home between the evening and morning visits, the total time commitment for this study across all conditions will be a maximum of 20.5 hours

Visit 1: Familiarisation	3 days habitual sleep assessment	Visit 2: Experimental testing	~ 1 week washout	Visit 3: Experimental testing	~ 1 week washout	Visit 4: Experimental testing



What are the possible benefits of taking part?

The main benefits of this research are educational. The research will allow us to understand how short sleep affects a measure of brain function, which has never been studied before. This research will then allow us to upscale the current project to work with participants who are more vulnerable to developing dementia, a disease where we know this measure of brain function is altered. The legacy of this research therefore will deliver wider benefits to society, and some indirect benefits might be foreseeable for participants. However, it is unlikely that we can promise any specific benefits. It is hoped that the study will provide a valuable and enjoyable learning experience for you, through being exposed to novel physiology laboratory techniques. In addition to this, you will get data on your sleep, brain blood vessel function and cognitive function.

What are the possible disadvantages and risks of taking part?

There are few risks associated with this study, and all procedures are considered low risk. The assessment of brain blood flow/brain function, and the cognitive tasks are non-invasive and have been used extensively in our research laboratory. You will likely feel sleepy and have decreased alertness/general cognitive functioning following the sleep restriction condition. We highly advise that you do not drive on the day after completing this condition. We will do all we can to arrange this sleep restriction visit on a day where you do not think you will need to drive anywhere. We will also try to arrange it for when you have few commitments that require excessive levels of alertness and cognitive functioning. We will offer you a lift to the laboratory and back home during this condition if needs be.

What Covid-19 Secure measures are in place?

To minimise risk of infection to you as a participant, the research team and others, we have taken measures based on the current UK government guidance https://www.gov.uk/guidance/working-safely-during-coronavirus-covid-19

We will ask you to self-screen for new or worsening signs or symptoms of possible COVID-19 before each visit to the campus/research site.

We will ask you to consider the risk to yourself, your household, or support bubble before attending for each study visit.

We will continue to follow the working safely measures even if you have received a recent negative test result or had the vaccine (either 1 or 2 doses).

We will have an approved infection control plan for each facility involved in conducting invasive procedures with human participants, including venepuncture, biopsy or the use of any hand held or fixed apparatus (e.g. headgear or breathing devices) or samples for onward lab processing.

We will:

- **EITHER** maintain a contact record of each participant and staff member present at each visit, **OR** ask you to 'check in' using the official NHS QR code to provide a contact trace to Public Health England
- Expect anyone who is acting as a researcher and/or participant where it is considered necessary (for example in close contact activities) to provide evidence of having **2 negative lateral flow antigen (LFD) tests** within 7 days of each visit.
- Self-administered test kits or assisted tests are available to collect or book locally EITHER from the University (Staff and students only).
  https://www.exeter.ac.uk/coronavirus/staff/testing/
  OR from https://www.nhs.uk/conditions/coronavirus-covid-19/testing/regular-rapid-coronavirus-tests-if-you-do-not-have-symptoms/
  For more information about LFD testing please see
  https://www.gov.uk/guidance/understanding-lateral-flow-antigen-testing-for-people-without-symptoms
- Asymptomatic individuals who test **positive** will need to remain isolated for at least **10** days from the day of the test and anybody who lives in their household, support bubble or close contacts must also self-isolate for **10** days.

  https://www.gov.uk/government/publications/covid-19-stay-at-home-guidance/stay-at-home-guidance-for-households-with-possible-coronavirus-covid-19-infection

What will happen if I do not want to carry on with the study?

Participation in this study is entirely voluntary. Therefore, it is your decision as to whether you would like to take part or not. If you do decide to participate in the study, you will be asked to sign an informed consent form before you begin, and you will be given a copy of both the consent form and the participant information sheet for your own records. However, if you decide to take part but then change your mind, you are free to leave the study at any time without giving a reason why. Any of your data collected so far will be destroyed. This will not affect your relationship with the researcher or the university in any way.

How will my information be kept confidential?

The University of Exeter processes personal data for the purposes of carrying out research in the public interest. The University will endeavour to be transparent about its processing of your personal data and this information sheet should provide a clear explanation of this. If you do have any queries about the University's processing of your personal data that cannot be resolved by the research team, further information may be obtained from the University's Data Protection Officer by emailing dataprotection@exeter.ac.uk or at www.exeter.ac.uk/dataprotection

All confidential personal data will be anonymised and protected by linking your data using pseudonymised ID numbers e.g. 1001, 1002, etc. Personal identifying data will be stored separately from the data. If you would like to receive an email to be kept informed about your outcomes from the project, you can select to opt in on the consent form. Confidential data will be destroyed and disposed of securely once it is no longer required (and no later than 01/08/2027). The Data Protection Act and recent GDPR guidelines will be followed. When writing up the study and its results, we will not declare any participant names, meaning they will not be identifiable. Only the lead researchers and supervisors will have access to participant information.

Paper copies of participant information sheets and consent forms will be scanned and stored electronically on an encrypted device, after which paper copies will be destroyed. Any transferral of data using a memory stick or flash drive will be securely encrypted using BitLocker and a password known only to the lead researchers and supervisors. As soon as this data has been stored securely, the data will be destroyed and securely disposed of from the device. All research data will be saved and stored securely on a password protected, university owned laptop, that also has BitLocker encryption, for additional security. Data will also be backed-up online on a OneDrive cloud, registered only to my university login.

If you chose to opt in on the consent form, your (anonymised) data may be used in future research and/or may be shared with other researchers in the future. The purpose of this will be to further our understanding and will be of scientific value. Identifiable information will never be shared.

Will I receive any payment for taking part?

There is no payment for taking part in this research project.

What will happen to the results of this study?

This study will provide novel understanding of how one night of short sleep affects brain blood vessel function in young, healthy adults. We will aim to publish these results in research journals and in presentations at scientific conferences in the UK or abroad. Your data will always remain anonymous and your name will not appear on any results. However, we will explain all your results of the study to you at the end (either via email, as above, or in person) if you wish.

In addition to this, the results of the present study will allow us to upscale the current project to work with participants who are more vulnerable to developing dementia, a disease where we know the measure of brain function we are assessing in the present project, is altered.

Who is organising and funding this study?

I (Alice Lester) am the lead researcher of this project and I am primarily responsible for the organisation of this project. I am at Master's by Research student at the University of Exeter, and therefore the study has been funded by the university. My supervisors, Dr Bert Bond and Dr Gavin, Buckingham have also been highly involved in the organisation of the study. We have applied for financial support to BRACE and Nutricia Research Foundation, but we are currently awaiting their response.

Who has reviewed this study?

All research at the University of Exeter is examined and approved by an ethics committee to protect your interests. This project has been reviewed by the Sport and Health Sciences, College of Life and Environmental Sciences Research Ethics Committee at the University of Exeter (Reference Number….).

Further information and contact details

If you would like more information, or have any further questions about the study, and/or would like to take part in the study, please contact the investigators using the details below:

Alice Lester: lead researcher of present study; Sport and Health Sciences, Baring Court, St Luke's Campus, University of Exeter, EX1 2LU. Email: al801@exeter.ac.uk

Dr Bert Bond: primary supervisor of present study; Children's Health and Exercise Research Centre, Baring Court, St Luke's Campus, University of Exeter, EX1 2LU. Tel: 01392 724903. Email: b.bond@exeter.ac.uk

Gail Seymour, Research Ethics and Governance Manager
g.m.seymour@exeter.ac.uk, 01392 726621

If you are not happy with any aspect of the project, or wish to complain, please contact Dr Bert Bond (above).

Thank you for your interest in this project

Appendix 2: Consent form

CONSENT FORM

Title of Project: Exploring the effects of acute sleep restriction and subsequent caffeine ingestion on neurovascular coupling

Name of Researcher: *Alice Lester*

Please initial b

1. I confirm that I have read the information sheet dated 21/09/2021 (version no 1) for the above project. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason and without my legal rights being affected. ☐
3. I understand that relevant sections of the data collected during the study, may be looked at by members of the research team, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I understand that any information given by me may be used in future reports, articles, or presentations by the research team. ☐
5. I understand that my name will not appear in any reports, articles, or presentations. ☐
6. I understand that taking part will involve visiting the laboratory for 4 separate occasions: one familiarisation visit, and 3 experimental visits, where each experimental visit involves visiting the laboratory twice – once in the evening and once the following morning. ☐
7. I understand that for the experimental visits I must avoid caffeine for 12 hours and exercise and food for two hours prior to the start of the evening visit, and arrive fasted, avoiding caffeine and exercise before the morning visit. ☐
8. I understand that I will need to replicate my diet as close as possible for each experimental visit, prior to arrival in the evening. ☐

9.I understand that I will complete a questionnaire on my normal caffeine consumption.

10. I understand that taking part involves one night of acute sleep restriction, where I will be required to stay awake for the first half night, and then to sleep for only half of my normal sleep duration.

11. I understand that I will ingest a 150 mg caffeine pill during the morning of two experimental visits (once after sleep restriction and once after a normal sleep), and on the morning of the other visit I will ingest a placebo pill.

12. I understand that ultrasound and infrared light will be used to measure blood flow at the side of the head and the forehead respectively, during a visual stimulation task and during cognitive function tests.

13. I understand that I will complete a visual stimulation task and cognitive function tests once on each evening and twice during the morning of each experimental visit, and that after completing each visual stimulation task, I will have to rate my attention levels for that task.

14. I understand that a small cuff around my finger will measure blood pressure during all protocols.

15. I understand that I will wear a combined accelerometer and heart rate monitor for three nights prior to the experimental visits to measure my normal sleep, and then for each experimental night to record my sleep duration and stages; and that to accompany each of the nights I wear the device, I will fill in a sleep diary to record the approximate time I went to sleep and the time I woke up.

16. I understand that my data will be used for the purposes of:

Inclusion in an archive for a period of up to 5 years, securely stored on a password protected OneDrive

Shared with other researchers for use in future research projects, but identifiable information will never be shared

Teaching or training materials for use in University activities and public engagement activities

17. I agree that my contact details can be kept securely and used by researchers from the department to contact me about future research projects

18. I would like to opt in to receive an email about my outcomes from the study

19. **For Coronavirus security measures,** I understand that taking part involves me:

a. Considering the risk to me, my household, or my support bubble before attending for each study visit.
b. Self-screening for new and worsening signs or symptoms of possible COVID-19 before each visit to the campus/research site
c. Maintaining social distancing wherever possible or wearing a mask/face covering when social distancing is not possible.
d. Maintaining good personal hygiene, including proper hand washing, cough/sneeze etiquette, not touching my face, eyes nose and mouth.
e. 'Checking in' using the **NHS QR** code or providing my details for contact tracing if necessary

20. I will provide evidence of (8 in total) negative Coronavirus test samples during the study period:

a. **2 negative LFD tests** within 7 days of the lab visits. Ideally, the second test will be the day before, or the morning of your visit).
b. I am responsible for arranging my own tests.
c. I am aware that there are risks associated with this form of testing, such as a false impression of safety associated with a negative result and significant isolation requirements of any positive cases

21. I agree to take part in the above study.

Name of Participant	Date	Signature
Name of researcher taking consent	Date	Signature

When completed: 1 copy for participant; 1 copy for researcher/project file.

Appendix 3: Health screening form

HEALTH SCREENING FOR VOLUNTEERS

Participant code: …………………………………..

It is important that volunteers participating in research studies are currently in good health, and able to safely complete one night of partial sleep restriction. This is:

i) To ensure your own continuing well-being

ii) To avoid the possibility of individual health issues confounding study outcomes

Your answers to the questions in this questionnaire, are strictly confidential.

Please complete this brief questionnaire to confirm your health to participate:

1. At present, do you have any cardiovascular health problem for which you are on medication, prescribed or otherwise ………… YES □ NO □

2. Do you suffer from any of the following:
   a. A mental health illness that would become worse with acute sleep restriction (including, but not limited to, depression and/or anxiety) YES □ NO □
   b. Sleep apnoea ………………………………………… YES □ NO □
   c. Restless leg syndrome …………………………… YES □ NO □
   d. Insomnia ……………………………………………….. YES □ NO □
   e. Epilepsy………………………………………………….. YES □ NO □
   f. Lung disease ………………………………………… YES □ NO □
   g. Diabetes …………………………………………. YES □ NO □
   h. A blood disorder ………………………………. YES □ NO □
   i. Previous head injury …………………………… YES □ NO □
   j. Heart problems …………………………………… YES □ NO □

3. Do you suffer from any other medical condition that means that you should not engage in a partial sleep restriction protocol, or that could be made worse by partial sleep restriction? YES □ NO □

4. Is there any other reason that you should not engage in a partial sleep restriction protocol? YES □ NO □

If you have answered YES to any question, unfortunately you will not be able to complete this study. If this is the case, a member of the research team will be in contact to discuss the reasons for this, and how you can still benefit from engaging in the research.

Please contact a member of the research team (below) if there is anything you would like to discuss, or if you have any questions.

Thank you for your cooperation.

Primary investigator: Alice Lester; al801@exeter.ac.uk

Project coordinators: Dr. Bert Bond; B.Bond@exeter.ac.uk; 01392 724903; Dr Gavin Buckingham; G.Buckingham@exeter.ac.uk

Appendix 4: Pittsburgh sleep quality index and scoring sheets

Participant code:___________________________________ Date:_________

Pittsburgh Sleep Quality Index (PSQI)

Instructions: 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? __________________
2. During the past month, how long (in minutes) has it usually taken you to fall asleep each night? __________
3. During the past month, what time have you usually gotten up in the morning? __________________
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.) __________________

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. Have bad dreams				
i. Have pain				
j. Other reason(s), please describe:				
	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
6. During the past month, how often have you taken medicine to help you sleep (prescribed or "over the counter")?				
7. 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
8. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?				
	Very good	Fairly good	Fairly bad	Very bad
9. During the past month, how would you rate your sleep quality overall?				

	No shared bed or room	Partner/room mate in other room	Partner in same room but not same bed	Partner in same bed
10. Do you share a bed or room with another person?				
	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
If you share a room or a bed with someone, ask them how often in the past month you have had:				
a. Loud snoring				
b. Long pauses between breaths while asleep				
c. Legs twitching or jerking while you sleep				
d. Episodes of disorientation or confusion during sleep				
e. Other restlessness while you sleep, please describe:				

Scoring the PSQI

The order of the PSQI items has been modified from the original order in order to fit the first 9 items (which are the only items that contribute to the total score) on a single page. Item 10, which is the second page of the scale, does not contribute to the PSQI score.

In scoring the PSQI, seven component scores are derived, each scored 0 (no difficulty) to 3 (severe difficulty). The component scores are summed to produce a global score (range 0 to 21). Higher scores indicate worse sleep quality.

Component 1: Subjective sleep quality—question 9

Response to Q9	Component 1 score
Very good	0
Fairly good	1
Fairly bad	2
Very bad	3

Component 1 score:_____

Component 2: Sleep latency—questions 2 and 5a

Response to Q2	Component 2/Q2 subscore
≤ 15 minutes	0
16-30 minutes	1
31-60 minutes	2
> 60 minutes	3

Response to Q5a	Component 2/Q5a subscore
Not during past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3

Sum of Q2 and Q5a subscores	Component 2 score
0	0
1-2	1
3-4	2
5-6	3

Component 2 score:_____

Component 3: Sleep duration—question 4

Response to Q4	Component 3 score
> 7 hours	0
6-7 hours	1
5-6 hours	2
< 5 hours	3

Component 3 score:_____

Component 4: Sleep efficiency—questions 1, 3, and 4

Sleep efficiency = (# hours slept/# hours in bed) X 100%

hours slept—question 4

hours in bed—calculated from responses to questions 1 and 3

Sleep efficiency	Component 4 score
> 85%	0
75-84%	1
65-74%	2

< 65%	3

Component 4 score:_____

Component 5: Sleep disturbance—questions 5b-5j

Questions 5b to 5j should be scored as follows:

Not during past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3

Sum of 5b to 5j scores	Component 5 score
0	0
1-9	1
10-18	2
19-27	3

Component 5 score:_____

Component 6: Use of sleep medication—question 6

Response to Q6	Component 6 score
Not during past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3

Component 6 score:_____

Component 7: Daytime dysfunction—questions 7 and 8

Response to Q7	Component 7/Q7 subscore
Not during past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3

Response to Q8	Component 7/Q8 subscore
No problem at all	0
Only a very slight problem	1
Somewhat of a problem	2
A very big problem	3

Sum of Q7 and Q8 subscores	Component 7 score
0	0
1-2	1
3-4	2
5-6	3

Component 7 score:_____

Global PSQI Score: Sum of seven component scores:___________

Citation: Buysse, DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ: The Pittsburgh Sleep Quality Index (PSQI): A new instrument for psychiatric research and practice. Psychiatry Research 28:193-213, 1989

Appendix 5: Habitual sleep diary

THREE-DAY HABITUAL SLEEP DIARY

Participant number:

Please refer to the box at the bottom of the page to ensure you understand what each of the table columns are referring to. It is important you complete this table as honestly and as accurately as you can. Please complete in the morning as soon after waking as you can, to improve chances of remembering correctly.

On the nights that you are wearing the activity monitor and completing the sleep diary, we ask that these nights reflect your normal sleep routine. We also ask that you avoid consuming alcohol on these nights. If you have any questions, please email Alice – al801@exeter.ac.uk.

Night	Day of week	Caffeinated drinks consumed	Naps in the day	Time in bed	How long did it take you to fall asleep?	Time of waking	Do you feel this reflected a normal night of sleep?	Any other notes about your sleep?
Example	*Tuesday*	*Two coffees before midday Black tea at 1pm*	*None*	*10:30 pm*	*10 minutes*	*07:15 am*	*Yes*	*Woke once in the night to go to the toilet*
Night 1								
Night 2								
Night 3								

- If you had a nap in the day, please report the time you took that nap, and the duration of the nap
- Time in bed refers to the time you started trying to fall asleep, i.e. off phone/electronics, book down, lights out, e.g. 10 pm
- How long did it take you to fall asleep – this is an approximation in minute, from the time you started trying to fall asleep, until you feel asleep e.g. 5 minutes
- Time of waking refers to the time you woke up in the morning, and did not go back to sleep again after e.g. 06:45 am

Appendix 6: International shopping list cognitive function test example

Shopping List – V1

(Tally if word repeated)

Items	**1**	**2**	**3**	**Morning after (unprompted)**	**Morning after (prompted)**
Carrots					
Lemonade					
Bread roll					
Kitchen towel					
Salt					
Grapes					
Cheese					
Cornflakes					
Pasta					
Peas					
Salmon					
Eggs					
Mayonnaise					
Blueberries					
Cabbage					

If participant recalls all 15 words before 60 seconds are up/if they cannot remember any more

Time taken to recall					
Interruptions					