MONASH University

Enhancing Slow Wave Sleep, Cognition and Physiology through Acoustic Stimulation

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Abstract

Slow wave sleep (SWS) is essential for memory and learning, executive function, and neurocognitive and physiological wellbeing. While SWS deterioration is the most severe in older adults, significant reductions can be observed from midlife, with concomitant impairments in cognition and physiology. Additionally, poor sleep during midlife could lead to long-term neurobiological impairments. Slow wave activity (SWA) enhancement could provide immediate improvements to social and workplace productivity and be used as a long-term strategy to reduce cognitive decline in older age. Although previous studies have demonstrated that acoustic stimulation is a safe and accessible method of enhancing SWA, less is known about the influence on higher-order cognition and physiological health. Therefore, the overarching aim of this thesis was to assess the use of a commercially available, automated, acoustic device on SWA, executive function, attention, and cardiac outcomes.

This thesis combines experimental data from two studies, which both utilised a crossover, repeated-measures design and the same slow-wave enhancing acoustic device. The proof-of-concept study (**Chapter 3)** was the first to demonstrate that an automated, acoustic device enhances SWA over the entire night in healthy, middle-aged males. This was associated with concurrent improvement in executive function, including working memory and verbal fluency. Despite the stringent screening criteria, sample range, and protocol, there was a high degree of interindividual variability in SWA response.

The second study (**Chapter 4)** validated the device under more naturalistic settings, focusing on next-day alertness and attention outcomes in habitually sleep-restricted males and females. This was the first study to directly compare changes in SWA with consecutive nights of acoustic stimulation. As with the first study, there was an increase in SWA after one night of stimulation, while remaining stable after the second. There were improvements in subjective alertness following

both nights of stimulation, and attention outcomes improved over the course of the day after the second night. Again, there was a wide range in interindividual responses to SWA enhancement. Study 2 also revealed a diverse range in intraindividual responses from night 1 to night 2.

Using data collected in study 1, **Chapter 5** explored the potential impact of SWA enhancement on cardiac outcomes as a function of the autonomic nervous system. There was an overall improvement in heart rate variability, in both time and frequency domains. These results suggest that acoustic stimulation may have a secondary benefit of reducing cardiac risk, specifically by improving parasympathetic control during SWS.

This body of work demonstrates that acoustic stimulation benefits SWA, executive function, attention outcomes and cardiac health in middle-aged adults. Moreover, it is the first to examine consecutive nights of stimulation, which is critical to consider for everyday use given the homeostatic regulation (and potential rebound) of SWS. These findings highlight the importance of taking interindividual differences into account to optimise acoustic stimulation for improving SWS, cognition and cardiac health. Overall, this thesis provides evidence that the acoustic enhancement of SWA may be a viable intervention for alleviating the cognitive and physiological declines associated with poor sleep and advancing age.

List of Publications and Presentations related to Candidacy

Peer-Reviewed Journal Articles

Diep, C., Ftouni, S., Manousakis, J. E., Nicholas, C. L., Drummond, S. P. A., & Anderson, C. (2020). Acoustic slow wave sleep enhancement via a novel, automated device improves executive function in middle-aged men. *Sleep, 43*(1). doi:10.1093/sleep/zsz197

Diep, C., Garcia-Molina, G., Jasko, J., Manousakis, J., Ostrowski, L., White, D., & Anderson, C. (2021). Acoustic enhancement of slow wave sleep on consecutive nights improves alertness and attention in chronically short sleepers. *Sleep Med, 81*, 69-79. doi:10.1016/j.sleep.2021.01.044

Oral Presentations

Diep, C., Garcia-Molina, G., Jasko, J., Manousakis, J., Ostrowski, L., White, D., Anderson, C. (2019). Acoustic slow wave sleep stimulation improves daytime sleepiness and sustained attention in chronically sleep-deprived adults. Fast Talk. Presentation given at Australasian Cognitive Neurosciences Society Conference, Tasmania, Australia.

Diep, C., Ftouni, S., Manousakis, J., Nicholas, C., Drummond, S.P., Anderson, C. Executive function improves following acoustic slow wave sleep enhancement with a novel, automated device. (2018). Presentation given at Sleep DownUnder, Australasian Sleep Association Annual Meeting, Brisbane, Australia.

Poster Presentations

Diep, C., Garcia-Molina, G., Jasko, J., Ostrowski, L., White, D., Anderson, C. (2019). Improved alertness and sustained attention following acoustic slow wave sleep stimulation in

chronically sleep-deprived adults. Poster presented at the World Sleep conference, Vancouver, Canada.

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Other Published Work

McMahon, WR, Ftouni, S, **Diep, C**, et al. The impact of the wake maintenance zone on attentional capacity, physiological drowsiness, and subjective task demands during sleep deprivation. J Sleep Res. 2021; 00:e13312. https://doi.org/10.1111/jsr.13312

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes two original papers published in peer reviewed journals and one prepared for publication. The core theme of the thesis is to examine the impact of acoustic stimulation on slow wave sleep, executive functioning, alertness, and cardiovascular outcomes in healthy adults. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the School of Psychological Sciences at Monash University under the supervision of Associate Professor Clare Anderson, Associate Professor Joanne Fielding, and Doctor Suzanne Ftouni.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of **Chapters 3-5**, my contribution to the work involved the following:

Thesis Chapter	**Publication Title**	**Status**	**Nature and % of student contribution**	**Co-author name(s) Nature and % of Co-author's contribution***	**Co-author(s), Monash student Y/N***
Three	Acoustic slow wave sleep enhancement via a novel, automated device improves executive function in middle-aged men	Published	60% Formulated original study design, oversaw data collection and integrity, formulated research question and analysis plan, and drafted original manuscript	1) Suzanne Ftouni, formulated original study design, oversaw data collection and integrity, critical review of manuscript: 10% 2) Jessica Manousakis, provided assistance in analysis plan, interpreted results and critically reviewed manuscript: 7% 3) Christian Nicholas, provided assistance in analysis plan, and critically reviewed manuscript: 4% 4) Sean Drummond, oversaw data collection and integrity, provided assistance in developing research question, and critically reviewed manuscript: 4%	N N N N

				5) Clare Anderson, formulated original study design, oversaw data collection and integrity, provided assistance in developing research question and analysis plan, interpreted results, edited original manuscript and, critically reviewed manuscript: 15%	N
Four	Acoustic enhancement of slow wave sleep on consecutive nights improves alertness and attention in chronically short sleepers	Published	50% Data integrity, formulated research question and analysis plan, and drafted original manuscript	1) Gary Garcia-Molina, formulated original study design, oversaw data collection, provided assistance in developing research question and analysis plan, interpreted results and critically reviewed manuscript: 10% 2) Jeff Jasko, formulated original study design, oversaw data collection, provided assistance in developing research question and analysis plan, interpreted results and critically reviewed manuscript: 6% 3) Jessica Manousakis, provided assistance in developing research question and analysis plan, interpreted results and critically reviewed manuscript: 5% 4) Lynn Ostrowski, formulated original study design, oversaw data collection, provided assistance in developing research question and analysis plan, interpreted results and critically reviewed manuscript: 6% 5) David White, formulated original study design, oversaw data collection, provided assistance in developing research question and analysis plan, interpreted results and critically reviewed manuscript: 8% 6) Clare Anderson, provided assistance in developing analysis plan, interpreted results edited original manuscript, and critically reviewed manuscript: 15%	N N N N N N
Five	Heart rate variability remains stable following slow wave sleep enhancement	Under Review	62% Formulated original study design, oversaw data collection and integrity, formulated research question and analysis plan, and drafted original manuscript	1) Suzanne Ftouni, formulated original study design, oversaw data collection and integrity, critical review of manuscript: 10% 2) Gary Garcia-Molina, provided assistance in developing research question and analysis plan, interpreted results and critically reviewed manuscript: 8% 3) Sean Drummond, oversaw data collection and integrity and critically reviewed manuscript: 4% 4) Clare Anderson, formulated original study design, oversaw data collection and integrity, provided assistance in developing research question and analysis plan, interpreted results and edited original manuscript critically reviewed manuscript: 16%	N N N N

I have not renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student signature: **Date:** 01/06/2021

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author, I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor signature: **Date:** 01/06/2021

Acknowledgements

Completing a PhD is very much like the five stages of grief. You begin with blissful denial about the challenges ahead. Next is anger as the PhD consumes your life. There's lots of bargaining with your supervisors about papers and conferences and deadlines. Depression sinks in as your funding and time runs out (while people keep asking what's next, or when do you finish?). Finally, there's acceptance when your thesis is finished, and you can start to feel hope again. Unsurprisingly, a strong support network is essential for handling both.

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List of Abbreviations

General Terms

Aβ – Amyloid-beta

ADA – Adenosine deaminase

ANS – Autonomic nervous system

BDNF – Brain-derived neurotrophic factor

BMI – Body mass index

CNS – Central nervous system

CSF – Cerebrospinal fluid

GABA – Gamma-aminobutyric acid

GH – Growth hormone

HPA – Hypothalamic–pituitary–adrenal

HF – High frequency

HR – Heart rate

HRV – Heart rate variability

ICC – Intra-class correlations

LF – Low frequency

PER3 – PERIOD3

pNN50 – Proportion of NN50 divided by the total number of NN (RR) intervals

RR – Inter-beat

SDNN – Standard deviation of NN (normalised RR) intervals

TMS – Transcranial magnetic stimulation

tDCS – Transcranial direct current stimulation

tACS – Transcranial alternating current stimulation

Neuropsychiatric and Neurocognitive Terms

DASS – Depression anxiety stress scale

D-KEFS – Delis-Kaplan Executive Function System

GNG – Go/No Go

KDT – Karolinska Drowsiness Test

KSS – Karolinska Sleepiness Scale Page

MSLT – Multiple Sleep Latency Test

PHQ – Patient Health Questionnaire

PVT – Psychomotor Vigilance Task

SP-FS – Samn-Perelli Fatigue Scale

TOL – Tower of London

Sleep and Circadian Terms

AASM – American Academy of Sleep Medicine

AHI – Apnoea-Hypopnoea Index

CBT – Core Body Temperature

EEG – Electroencephalography

ECG – Electrocardiography

EMG – Electromyography

EOG – Electrooculography

ESS – Epworth Sleepiness Scale

HBT – Habitual bedtime

ISI – Insomnia Severity Index

NREM – Non-rapid eye movement (sleep)

NREM1 – Stage 1 of non-rapid eye movement sleep (also N1)

NREM2 – Stage 2 of non-rapid eye movement sleep (also N2)

NREM3 – Stage 3 of non-rapid eye movement sleep (see also N3, SWS)

OSA – Obstructive sleep apnoea

PSQI – Pittsburgh Sleep Quality Index

PSG – Polysomnography

REM – Rapid Eye Movement

RLS – Restless Legs Syndrome

SOL – Sleep Onset Latency

SWA – Slow wave activity

SWE – Slow wave energy

SWR – Sharp wave ripple

SWS – Slow wave sleep

TST – Total Sleep Time

WASO – Wake After Sleep Onset

Chapter 1

Introduction

Even a soul submerged in sleep is hard at work

and helps make something of the world.

– Heraclitus, 1755

After over a century of research, the quote and theory of the Greek Philosopher Heraclitus is now supported by empirical evidence. While the body appears quiescent, sleep is a dynamic state characterised by spontaneous and simultaneously predictable patterns of electrophysiological brainwaves, metabolic regulation and hormonal activity. Decades of research has demonstrated the critical role of sleep in physical, mental, and cognitive health, such that sleep loss and/or its disruption is associated with a myriad of adverse outcomes for health and performance. Despite this, sleep loss is endemic in modern society, with a significant proportion of the population not obtaining the recommended seven hours per night (Adams et al., 2017; Hafner, Stepanek, Taylor, Troxel, & van Stolk, 2017; Liu et al., 2016), largely due to work or social obligations. This trend shows little evidence of changing, with studies reporting decreases in sleep duration over time (Ford, Cunningham, & Croft, 2015; Matricciani et al., 2017; Roenneberg, Allebrandt, Merrow, & Vetter, 2012). This has led to the development of sleep interventions to mitigate these issues, including the use of pharmacological drugs, behavioural interventions, and acoustic stimulation. Not all sleep is the same, however. The deepest stage of sleep, slow wave sleep (SWS), is proposed to be especially critical for memory and learning, executive function, and aspects of brain health including synaptic plasticity and clearance of waste products that may lead to neurodegeneration. With this, scientists have developed techniques to enhance SWS, in a bid to enhance cognitive function and other aspects of physiological and brain health.

The first part of this review discusses sleep architecture and the role each stage plays in cognitive performance and physiological functioning, with a focus on SWS. From there, the negative impact of ageing and sleep restriction on both SWS and cognition will be explored. This review ends

with an evaluation of the SWS enhancing approaches available, with a specific focus on acoustic stimulation.

1.1 Sleep Architecture: Characteristics and Function

Hans Berger pioneered the use of electroencephalography (EEG) to capture the first recording of human brain waves (Berger, 1929). This discovery led to a series of major advances for sleep research, including continuous EEG recordings (Loomis, Harvey, & Hobart, 1935), the detection of rapid eye movement (REM) sleep (Aserinsky & Kleitman, 1953) and the formal recognition and experimentation of sleep disorders. Today, polysomnography (PSG) uses a combination of EEG, electromyography (EMG, muscle activity), and electrooculography (EOG, eye movements) to provide a holistic view of sleep macrostructure. There are four distinct sleep stages – REM, and non-rapid eye movement (NREM), with NREM further divided into three stages of varying sleep depth: N1, N2, and N3 (Berry et al., 2015). The original sleep scoring manual by Rechtschaffen and Kales (Rechtschaffen & Kales, 1968) categorized SWS as Stage 3 and 4 based on the percentage of slow wave activity (SWA), i.e. 20–50% and >50%, respectively within a single (30 second) epoch. With the publication of the American Academy of Sleep Medicine (AASM) some 40 years later, Stages 3 and 4 were combined into N3, where the epoch comprised at least 20% of SWA. In addition, SWA was evaluated from the frontal channels (as opposed to central), "movement time" was removed, scoring criteria were streamlined, and recommended derivations, sampling rates and filter settings were introduced to encourage standardised assessment (Moser et al., 2009). The sleep structure across a typical night's sleep in a young, healthy adult is shown in Figure 1. Sleep in humans tends to cycle through each progressively deeper stage of NREM and finish with REM, with each cycle lasting about 90–120 minutes (Carskadon & Dement, 2011). Initial cycles are normally dominated by N3, while later cycles in the sleep period contain more REM.

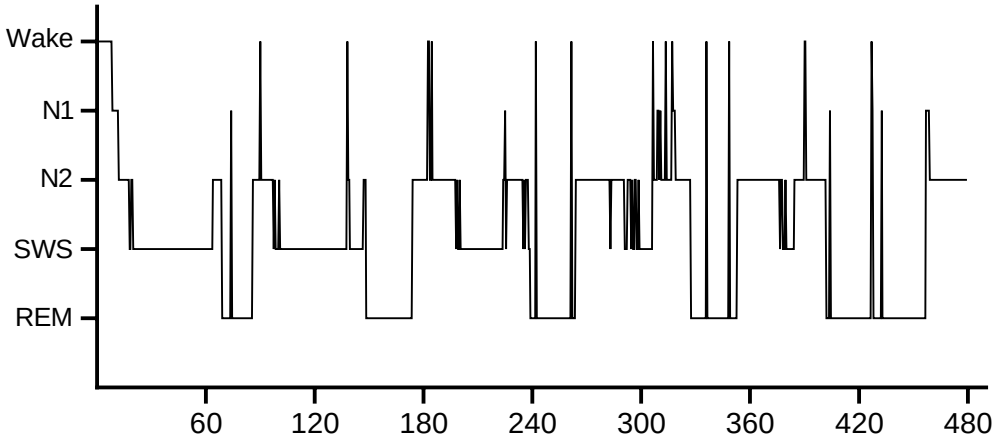

Figure 1. Hypnogram from a healthy, younger adult showing typical sleep stage duration and distributions during an overnight sleep period, based on AASM scoring criteria.

1.1.1 N1

N1 is defined by low-amplitude, mixed-frequency activity between 4–7 Hz for more than 50% of the epoch. It is characterised by slow eye movements (SEM) and vertex sharp waves, which are clearly distinct from background EEG as 'V' shaped waveforms between 100–200 μV. As the lightest stage of sleep, N1 is considered a transition stage from wake to sleep. It is the easiest stage of sleep to generate an arousal in an individual possibly because there are no "protective" mechanisms in place, such as spindles or K-complexes, see below – Section 1.6. Currently, evidence suggests that N1 does not appear to be essential to any form of physiological repair, or cognitive development, and is generally linked to cognitive decay (Song et al., 2015), in that N1 indicates an inability to reach a deeper level of sleep.

1.1.2 N2

N2 makes up approximately 50% of the sleep period. An epoch is generally labelled as N2 once a K-complex or sleep spindle appears within the epoch. Both defining features of N2 are associated with various aspects of cognition. Sleep spindles are visualised as a burst of sinusoidal waves with a frequency between 11–16 Hz and a duration of at least 0.5 seconds. They have been

associated with consolidating motor sequence learning, such that the frequency of spindles during N2 increased when re-exposed to olfactory cues presented during the initial learning task, leading to improved performance the following day (Laventure et al., 2016). K-complexes are a large negative sharp wave followed by a positive wave. Evidence suggests that they play a role in synaptic downscaling and are associated with memory consolidation and executive function (Ramakrishnan, Sartory, van Beekum, Lohrmann, & Pietrowsky, 2012). While not used as a scoring criterion, hippocampal sharp wave ripples (SWRs) (80–140 Hz), also occur during N2 (and N3) sleep, and are similarly involved with cognitive function.

1.1.3 N3

N3 is also known as SWS, due to characteristic low frequency, high amplitude (75 Hz) oscillations known as delta waves (1–4.5 Hz), or slow oscillations (0.3–1 Hz), which are collectively known as slow waves. These oscillations represent the membrane potential of cortical neurons alternating between synchronous periods of depolarisation "up states" and hyperpolarisation "down states" (Steriade, 2006). Delta waves originate from both the neocortex and the thalamus and tend to dominate the prefrontal cortex (Murphy et al., 2009). Unlike other sleep frequencies, slow oscillations are solely generated by the cortex, and generally dominate the parahippocampal gyrus, cerebellum, and brainstem (Steriade, Nunez, & Amzica, 1993). Although slow waves can be generated from almost anywhere in the brain, they preferentially originate from the frontal regions, and move in an anteroposterior direction (Massimini, Huber, Ferrarelli, Hill, & Tononi, 2004). Slow wave activity is also commonly generated from pyramidal layer V neurons (Timofeev & Chauvette, 2011). There are three leading theories on the origin of "active states". Each theory is based on the likelihood of an initial set of neurons firing to generate a wave of activity from the point of origin: neurons that receive the largest excitatory inputs, "spontaneous release"; neurons that are perpetually in a phase of depolarisation, "layer V neurons"; or that some neurons are more capable of spontaneous firing even during silent states, "selective synchronisation" (Timofeev & Chauvette, 2011). It is likely that the

theories work in harmony (Bazhenov, Timofeev, Steriade, & Sejnowski, 2002), and ultimately, SWA is most commonly generated in the prefrontal cortex and from layer V neurons as (i) there are the greatest density of neurons in the frontal regions and (ii) layer V neurons have the largest number of synapses; i.e., regions most likely to have depolarising events.

Slow oscillations entrain other EEG rhythms, including delta waves, spindles and K-complexes. They are also essential for optimal cognitive and physiological function. Given the focus of this thesis, predominantly SWS function will be referred to in the remainder of this review.

1.1.4 REM

In contrast to N3, REM sleep is known for its desynchrony, and is visualised as low-amplitude, mixed frequency EEG activity (4–7 Hz), with trains of saw tooth waves (sharply contoured and serrated waves between 2–6 Hz). REM is largely regulated by the pons and parts of the midbrain, which also send outputs to the lower brainstem and spinal cord to induce muscle atonia and the eponymous rapid eye movements, defined by the AASM as "conjugate, irregular sharply peaked eye movements". It has been proposed that REM sleep works in conjunction with SWS to process memory consolidation (Diekelmann & Born, 2010). REM sleep also modulates emotional memory and procedural memory (Ackermann & Rasch, 2014).

1.2 Slow Wave Sleep and Cognition

Sleep is essential for optimal cognitive functioning (Lo, Groeger, Cheng, Dijk, & Chee, 2016). Total sleep deprivation impairs attention, memory and executive function, to the extent that 24 hours awake is equivalent to a 0.10% blood alcohol content on reasoning tasks and response latency (Lamond & Dawson, 1999). Significant impairments are also seen following chronic sleep restriction in measures of sustained attention and executive function (Alhola & Polo-Kantola, 2007; Lim & Dinges, 2010). However, while lack of sleep impairs cognition, sleep itself is a process that actively benefits cognition, and this is particularly true of SWS.

There are two leading models explaining the neural mechanisms underpinning the relationship between SWS and cognition: active systems consolidation and the synaptic homeostasis hypothesis. The synaptic homeostasis hypothesis posits that during wake, synaptic connections are formed and strengthened, followed by a generalised pruning during SWS (Tononi & Cirelli, 2003). This leads to the down-selection of weaker synapses while stronger connections are maintained, improving the signal-to-noise ratio. This theory is supported by reduced noradrenaline and the expression of long-term potentiation genes during SWS. In contrast, active consolidation argues that memories are reactivated during SWS, and are redistributed from the hippocampus to the cortex (Diekelmann & Born, 2010). In line with this theory is the reduced cholinergic tone during SWS, which facilitates the replaying of memories in the hippocampus (Gais & Born, 2004) by synchronising SWA, sleep spindles and SWRs (Diekelmann & Born, 2010). It has been suggested that unifying the models provides a more accurate picture of the SWS impact on cognition (Wilckens, Ferrarelli, Walker, & Buysse, 2018), such that replaying memories assists to strengthen consolidation throughout synaptic downscaling (Diekelmann & Born, 2010).

1.2.1 Learning and Memory

Memory encompasses the ability to learn, preserve and recover information over time. Long term memory is split into declarative memory – the conscious and explicit recollection of facts (semantic memories) and events (episodic memory) and non-declarative memory, which are unconscious or implicit thoughts and behaviour, including procedural memory, perceptual skills and conditioning. Long term memory is formed through three main processes: encoding, consolidation and retrieval. During encoding, a memory trace is formed but is highly susceptible to being forgotten; during consolidation, the memory is converted into a long-term, stable form and during retrieval, the stable memory is recalled.

When compared to the same period awake, sleeping after learning improves retention for both declarative and non-declarative memory (Plihal & Born, 1997; Rasch, Büchel, Gais, & Born, 2007). It has been proposed that this is merely due to reduced interference, with some studies demonstrating that a period of quiet wakefulness offers similar improvements (Brokaw et al., 2016; Martini, Heinz, Hinterholzer, Martini, & Sachse, 2020). However, SWS also plays an active role in memory consolidation (Rasch & Born, 2013). Regions of the brain activated during learning are associated with increased SWA in the same areas during sleep (Huber et al., 2006; Huber, Ghilardi, Massimini, & Tononi, 2004; Mascetti et al., 2013; Peigneux et al., 2004), and lead to improved performance on the same tasks the following day. In contrast, inhibiting cortical activity, as shown in Huber et al. (2006)'s arm immobilisation study, led to a decrease in SWA in equivalent areas during sleep. Experimentally manipulating SWA also has direct effects on encoding (Wilckens et al., 2018). Enhanced SWA leads to improved declarative memory, as demonstrated with acoustic stimulation (Ngo, Martinetz, Born, & Mölle, 2013; Ong et al., 2016) or transcranial stimulation (Antonenko, Diekelmann, Olsen, Born, & Mölle, 2013; Marshall, Molle, Hallschmid, & Born, 2004). Conversely, when SWA is experimentally disrupted, hippocampal-dependent encoding (Werf et al., 2009) and consolidation are impaired (Landsness et al., 2009). Such evidence supports the notion of a unified theory of synaptic homeostasis and active consolidation, as both the capacity to encode new information, and the strengthening of existing knowledge are facilitated by SWA. When assessing micro sleep architecture, spindles and SWRs increase after learning, and are thought to indicate memory reactivation or processing of local information (Wilckens et al., 2018). Interestingly, as SWRs also occur during wake, they could be dynamically involved with decision-making, planning and active recall, all processes involved with executive functions (Joo & Frank, 2018).

1.2.2 Executive Function

Executive functions refer to a set of top-down processes that direct deliberate goal-oriented behaviour (Diamond, 2013). Executive functions are broken down into three core components:

inhibition, working memory and cognitive flexibility (Miyake et al., 2000). While each function can be isolated, they are frequently used collectively when performing higher-order cognitive tasks and skills, such as problem solving, planning, and exercising discipline (Diamond, 2013). Other models of executive function also include abilities such as sustained attention, multitasking and verbal fluency (Chan, Shum, Toulopoulou, & Chen, 2008).

Inhibition, or inhibitory control, refers to the ability to control and overcome predisposed impulses while instead directing attention, behaviour, thoughts and/or emotions towards an appropriate or necessary task at hand (Diamond, 2013). This includes the inhibitory control of attention, that is, selectively attending to one stimuli while suppressing attention allocation to irrelevant information. Most laboratory tasks measure the competition between inhibitory 'top down' control and reflexive 'bottom-up' attention allocation, such as the Go/No Go, Flanker Task or anti-saccade tasks, which generally instruct the participant to focus on one particular stimuli and perform an action, while ignoring other stimuli. However, inhibition can guide long-term behaviour, such as self-control over behavioural habits (Diamond, 2013).

Working memory involves retaining *and* manipulating information in your mind (Diamond, 2013), either through verbal or visuo-spatial means. It is essential for understanding and completing tasks that occur over a period of time and impacts informed decision making, reasoning and reading. Laboratory tasks tap into working memory with measures such as the N-back, which presents the participant with a sequence of letters to which they must press a button when a character reappears an *n* number of times back (Drummond et al., 2013), or mental arithmetic tasks (Diamond, 2013). Working memory supports inhibitory control by enabling the retention of rules and relevant information while completing a task.

Cognitive flexibility is the ability to alter thought processes and adjust to changing problems, tasks, rules or priorities (Diamond, 2013). It builds on both inhibitory control and working memory as, when changing perspectives, it is necessary to inhibit the pre-existing view and activate a different

perspective. Common measures employ task-switching and set-shifting tasks, such as the Wisconsin Card Sorting Task, which requires the participant to sort playing cards by colour, shape, or number according to a hidden rule that can be deduced by feedback provided by the examiner (Grant & Berg, 1948). The rule changes throughout the task, thus challenging the participant to constantly update their mental set. Cognitive flexibility and executive functioning can also be assessed using verbal fluency tasks (Diamond, 2013). The Delis-Kaplan Executive Function System (D-KEFS) Verbal Fluency test involves naming as many items from a particular criterion as possible within a time limit (e.g. items starting with the letter 'F' or types of animals), while the final subtask requires constant set-shifting, as it requires the participant to alternate between naming items from two different categories (Delis, Kaplan, & Kramer, 2001).

1.2.2.1 Impact of Slow Wave Sleep on Executive Functions

Neurophysiological, neuroimaging, and neuropsychological studies have shown that executive functions are primarily mediated by the dorsolateral prefrontal cortex (Alvarez & Emory, 2006; Diamond, 2013; Stuss & Alexander, 2000). Evidence suggests that SWS supports the prefrontal cortex in performing executive functions (Wilckens, Erickson, & Wheeler, 2012). For example, SWS appears to mediate performance on tasks assessing working memory, inhibition and more complex executive functions such as planning (Anderson & Horne, 2003; Ferrarelli et al., 2019; Pugin et al., 2015). Slow wave activity is associated with lower dorsolateral prefrontal metabolism during sleep but higher during wakefulness (Wilckens et al., 2016), suggesting that the neural synchrony during SWS may enhance cortical connections, or that its restorative nature improves waking function. In support of this, SWS deprivation studies show impaired performance on visuomotor and visuoperceptual tasks, and sustained attention, even when controlling for sleep timing, sleep efficiency, and age (Aeschbach, Cutler, & Ronda, 2008; Groeger, Stanley, Deacon, & Dijk, 2014; Landsness et al., 2009).

1.2.3 Attention

Attention is a broad term that, according to the Posner model, encompasses three major attention networks: the alerting network, orienting network and executive network system (Petersen & Posner, 2012). The alerting network, controlled predominately by the ascending arousal tract in the brain stem and the right hemisphere, is responsible for detecting and maintaining attention. It is commonly assessed through sustained reaction time tasks such as the Psychomotor Vigilance Task (PVT) (Dinges & Powell, 1985). The orienting network is slightly more complex and processes directional responses by coordinating information through the frontoparietal network, which controls both the top-down allocation and bottom-up reorienting of attention. Frontoparietal networks also control the executive network system to maintain focussed, controlled attention in conjunction with the frontostriatal system regulating behavioural inhibition (Corbetta, Patel, & Shulman, 2008; Ptak, 2011; Rae, Hughes, Anderson, & Rowe, 2015). For context, alerting *detects* a change in the environment, orienting *directs* attention selectively to the change, and executive control *inhibits* attention to other stimuli and manages conflict resolution. Ocular motor tasks are often used to assess the cognitive control of attentional inhibitory control in clinical populations. While not as commonly utilised in the sleep field, studies using ocular motor tasks have found that orienting and the executive control of attention are vulnerable to sleep restriction (J. Lee, Manousakis, Fielding, & Anderson, 2015), total sleep loss and circadian modulation (Collet et al., 2020). Selective SWS disruption and enhancement studies suggest that SWS moderates sustained attention (Ferrara, De Gennaro, Casagrande, & Bertini, 2000; Walsh, Randazzo, Stone, et al., 2006). While fewer manipulation studies have examined orienting and executive attention, reduced SWS has been found to be associated with poorer executive control of attention in patients with insomnia (Y. Li et al., 2016).

1.3 Slow Wave Sleep and Physiology

In addition to its benefits on cognition, SWS plays a role in regulating physiological processes throughout the brain and body (Tasali, Leproult, Ehrmann, & Van Cauter, 2008; Xie et al., 2013). The following section of this review briefly discusses the key restorative properties of SWS, with a focus on heart rate variability (HRV).

1.3.1 Clearance of Metabolites

Perhaps in its most exciting role, a landmark study revealed that SWS is associated with the clearance of metabolites in mice (Xie et al., 2013), including beta amyloid (Aβ), a key protein involved in the development of Alzheimer's disease. Xie et al. reported that during SWS, the glymphatic system actively encourages shrinkage of astroglial cells to facilitate interchanging cerebrospinal fluid (CSF) and interstitial fluid. While not directly replicable in humans, there is substantial evidence to infer that (i) increased sleep disturbances are associated with increased risk of dementia (Shi et al., 2018), Aβ deposits (Sprecher et al., 2015) and brain atrophy (Sexton, Storsve, Walhovd, Johansen-Berg, & Fjell, 2014); (ii) sleep is necessary for clearing metabolites using equivalent glymphatic systems (Abbott, Pizzo, Preston, Janigro, & Thorne, 2018; Holth et al., 2019; Lucey et al., 2018; Ooms et al., 2014; Shokri-Kojori et al., 2018); and (iii) clearance in humans also appears to be driven by SWS (Fultz et al., 2019; Ju et al., 2017), with low SWS duration associated with high CSF levels of Aβ (Varga, Wohlleber, et al., 2016). Although this work is in its infancy, future work around the role of SWS in brain health and neurodegeneration is of vital importance, given the increasing prevalence of neurodegenerative disorders such as dementia.

1.3.2 Peripheral Effects of Slow Wave Sleep

There is a sensitive and bidirectional relationship between SWS and the autonomic nervous system (ANS) (Dijk, 2008). Compared to wake and REM sleep, there is a shift from sympathetic to parasympathetic dominance during NREM sleep (Trinder et al., 2001). As a result, global changes

arise throughout the body that impact hormonal and cardiovascular control. A consolidated bout of SWS is essential for full restorative function of physiological outcomes, as fragmented SWS, for example during sleep apnoea, disrupts the balance between parasympathetic and sympathetic activation and is associated with negative health outcomes, including increased risk of cardiovascular disease, and higher body mass index (BMI) (Rao et al., 2009). Slow wave sleep is associated with low hypothalamic-pituitary-adrenal (HPA) axis activity (Buckley & Schatzberg, 2005), leading to a cascading effect on the secretion of growth hormone (GH) (Dunleavy, Oswald, Brown, & Strong, 1974; Van Cauter et al., 1997), prolactin, glucose/insulin (Tasali et al., 2008), cortisol and other immunosupportive hormones (Buckley & Schatzberg, 2005), among others (Van Cauter, Spiegel, Tasali, & Leproult, 2008). Again, studies directly manipulating SWS highlight its importance in these respects – SWS disruption led to poorer insulin regulation (Herzog et al., 2013; Tasali et al., 2008), while enhanced SWS improves endocrine and immune function (Besedovsky et al., 2017). The effect of SWS on the ANS is summarised in Figure 2.

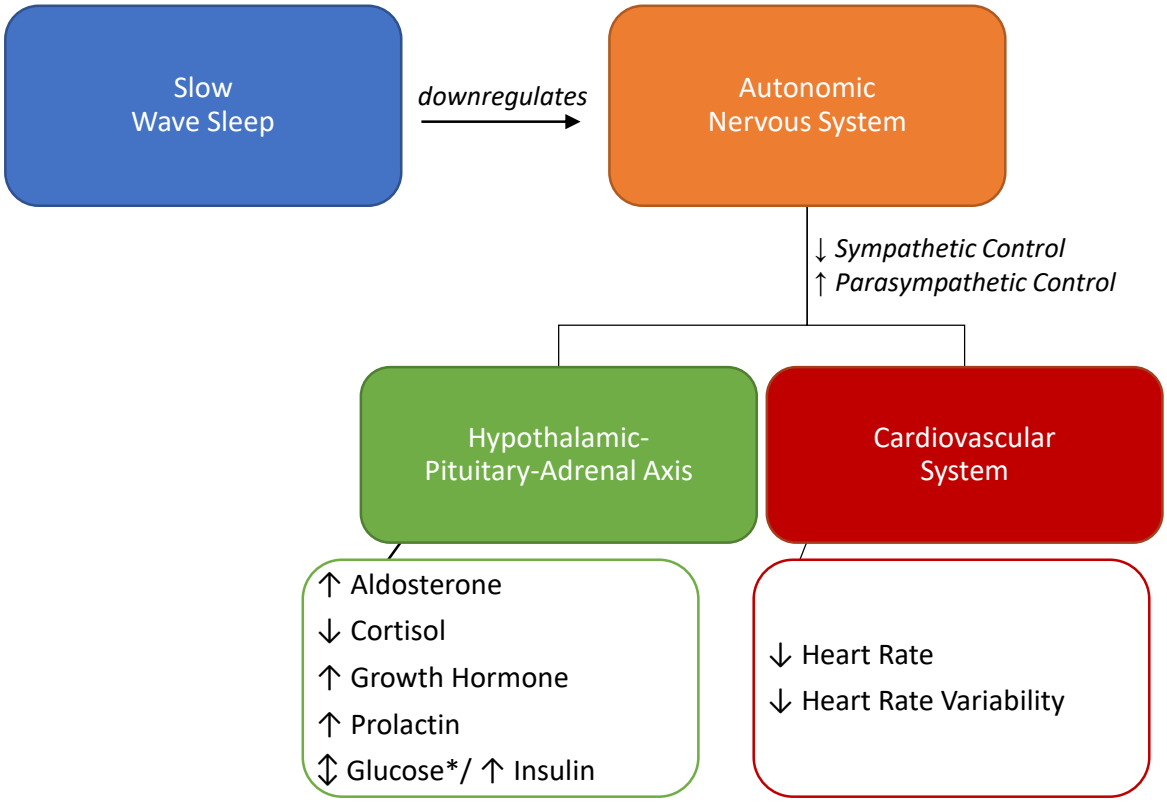

Figure 2. A simplified diagram depicting the impact of SWS on the ANS, and the subsequent effects on hormonal and vagal activity. *Changes in glucose are dependent on by location, insulin sensitivity, and whether discussing free glucose or glucose metabolism. For example, brain glucose utilisation and energy expenditure decreases, leading to increased blood glucose. Disrupting SWS reduces insulin sensitivity, which also increases blood glucose.

1.3.2.1 Cardiovascular function

Slow wave sleep and cardiovascular function are closely interrelated. Heart rate (HR) can be used to predict individual slow waves (Mensen, Zhang, Qi, & Khatami, 2016), while HRV is commonly used to observe the relationship between vagal control and SWA as they wax and wane in synchrony during sleep (Brandenberger, Ehrhart, Piquard, & Simon, 2001; Vanoli et al., 1995). Acute sleep deprivation, and long-term restricted sleep, are each associated with negative cardiovascular outcomes, including decreased heart rate (HR) (Vaara, Kyröläinen, Koivu, Tulppo, & Finni, 2009),

hypertension and coronary heart disease (Ayas et al., 2003; Wingard & Berkman, 1983). This is likely due to the reduction in SWS disrupting sympathovagal balance (Tasali et al., 2008).

Previous studies have found that disruptive acoustic tones that trigger arousals can lead to adverse heart outcomes, including decreased HR (Silvani et al., 2003), lower HRV (Chaicharn, Carrington, Trinder, & Khoo, 2008) and increased mean arterial pressure (Bangash et al., 2008). In contrast, very few studies have examined the benefits enhancing SWS on HRV. Grimaldi et al. (2019) reported that enhancing SWA led to increased parasympathetic activity, suggesting that acoustic stimulation can strengthen the relationship between SWS and autonomic function. Moreover, improving neural oscillations during wake also appears to improve cardiovascular regulation (Shaltout et al., 2018).

1.4 Regulation of Slow Wave Sleep

The disruption of SWS via experimental manipulation, lifestyle choices, sleep disorders or its natural decline as a function of ageing (See Section 1.5), is associated with impaired cognitive and physiological outcomes. As seen throughout this review, various studies have used acoustic tones to selectively disrupt SWS to observe the subsequent effect on sleep homeostasis (Akerstedt, Kecklund, Ingre, Lekander, & Axelsson, 2009; Ferrara, De Gennaro, & Bertini, 1999), endocrine and metabolic regulation (Ju et al., 2017; Tasali et al., 2008), cardiovascular function (Sayk et al., 2010), pain thresholds (Lentz, Landis, Rothermel, & Shaver, 1999), daytime sleepiness (Groeger et al., 2014), and cognitive outcomes (Ferrara et al., 2000). Chronic disorders such as sleep apnoea and fibromyalgia generally result in higher sleep fragmentation and lower SWS (Choy, 2015; Redline et al., 2004), as do acute episodes of stress – apprehension about the next day and high levels of cortisol lower SWS (Hirotsu, Tufik, & Andersen, 2015; Kecklund & Åkerstedt, 2004). When examining short-term changes, the quality and quantity of SWS changes from night to night as it is naturally modified by the intensity of the waking day.

1.4.1 Homeostatic Regulation of Sleep Pressure

The most widely accepted paradigm of sleep regulation is the two-process model. SWS represents accruing sleep pressure with each passing hour awake, known as the homeostatic "Process S". This combines with the second process, the circadian timing system "Process C" (Borbely, 1982) such that the timing and organisation of sleep is controlled by the inter-play between these two processes. The impact of Process C on SWS is not explored by this thesis (all studies were conducted on nocturnal sleep periods) and thus will not be reviewed in detail. However, as a note, while circadian rhythms have less influence on SWS relative to Process S, forced desynchrony protocols have shown that they do modulate the amplitude, frequency and slope of SWA (Lazar, Lazar, & Dijk, 2015). The impact of SWS on homeostatic regulation is widely supported by a series of observations: SWS *increases* in duration and intensity (1) in proportion to prior wakefulness (Dijk, Brunner, Beersma, & Borbely, 1990), (2) with fragmented sleep (Bonnet, 1987) and, (3) following a period of restricted sleep (Akerstedt et al., 2009), although evidence is mixed (Brunner, Dijk, & Borbély, 1993). Moreover, SWS *reduces* (4) across the course of the night (Borbély, Baumann, Brandeis, Strauch, & Lehmann, 1981) and, (5) following naps prior to the next sleep period (Werth, Dijk, Achermann, & Borbély, 1996). It has been relatively unexplored whether *enhancing* SWA during a normal night of sleep would lead to a decrease the following night, although data suggests there is no relative rebound, or is at the very least maintained, following continued enhancement (Debellemaniere et al., 2018; Garcia-Molina et al., 2018).

1.4.1.1 Impact of Acute and Partial Sleep Deprivation

The effect of acute sleep deprivation on SWS and the resulting consequences on cognition and physiology has been extensively documented (Borbély et al., 1981; Reynolds & Banks, 2010; Vaara et al., 2009). However, in reality it is more likely for individuals to suffer from chronic bouts of restricted sleep, (Adams et al., 2017; Hafner, Stepanek, Taylor, Troxel, & van Stolk, 2017; Liu et al., 2016) due to work or social obligations. Chronic sleep restriction is associated with increased risk

of obesity, hypertension, cardiovascular disease and diabetes, as well as impaired daytime sleepiness and cognitive outcomes, leading to motor vehicle and workplace accidents (Liu et al., 2016). There does appear to be a moderate SWS rebound with repeated nights of partial sleep deprivation (Akerstedt et al., 2009; Plante et al., 2016), however it is unknown whether this is sufficient for recovery.

1.5 Sleep, Ageing and Cognition

Sleep patterns change dramatically over the lifespan, including sleep duration, latency, efficiency and staging (Mander, Winer, & Walker, 2017). Both subjective and objective sleep quality declines, even within healthy ageing (Varga, Wohlleber, et al., 2016). Notably, significant changes occur from midlife (Campbell & Murphy, 2007; Landolt, Dijk, Achermann, & Borbély, 1996). This is particularly evident in the decreasing quantity and quality of SWS; while initially accounting for 20% of the sleep period during prepubescence, it decreases by 2-3% every decade beyond the age of 20, making up approximately 4% of sleep by late adulthood (Ohayon, Carskadon, Guilleminault, & Vitiello, 2004; Van Cauter, Leproult, & Plat, 2000). Decreases in SWS are accompanied by impaired cognitive performance, attention deficits and poorer mental and physiological health outcomes (Mander et al., 2013; Mander et al., 2017).

1.5.1 Age-Related Changes in Sleep

Beginning with sleep patterns, sleep latency appears to be the most stable, with significant decreases only seen when comparing young and very old adults (Floyd, Janisse, Medler, & Ager, 2000). In comparison, sleep duration decreases by approximately one hour per age bracket. When comparing spontaneous sleep duration in young (<30 years), middle-aged (31–59 years) and older adults (>60 years), young adults slept 10.5 hours, while middle-aged and older adults slept for 9.1 and 8.1 hours, respectively (Campbell & Murphy, 2007). Studies report that sleep duration decreases by approximately 10 minutes per decade (Ohayon et al., 2004). Sleep efficiency also worsens over

time, with more micro-arousals (Redline et al., 2004) and wake after sleep onset (WASO) increasing by 10 minutes per decade (Ohayon et al., 2004). The differences in sleep architecture between young, middle-aged, and older adults are depicted in Figure 3.

While REM sleep remains relatively stable until late adulthood, SWS duration declines with age to be replaced by lighter NREM stages. Slow wave amplitude and density also declines (Ujma, Simor, Steiger, Dresler, & Bódizs, 2019), and again, significantly so from middle-age (Carrier et al., 2011; Dijk, Beersma, & van den Hoofdakker, 1989; Landolt et al., 1996; Varga, Wohlleber, et al., 2016). This deterioration is pronounced over the prefrontal cortex and in the first NREM cycle. Several hypotheses have been put forward to explain this decrease. First, there is a reduction in homeostatic sleep pressure with age. For instance, the dissipation of SWA across the night is shallower in older adults relative to younger adults (Landolt & Borbély, 2001); older adults also do not accumulate subjective or objective sleepiness at the same rate as younger adults, and have dampened homeostatic increases in SWS following extended wakefulness (Münch et al., 2004); when given equal sleep opportunities, older adults sleep less than their younger counterparts (Klerman & Dijk, 2008). The second theory posits that SWS/SWA loss is due to age-related brain atrophy. Slow wave amplitude and density are associated with structural grey matter density and volume in the prefrontal cortex in young adults (Saletin, van der Helm, & Walker, 2013). Atrophy in these same areas predicts the severity of slow wave impairment (while age-related atrophy in other brain regions are not predictive) (Mander et al., 2013).

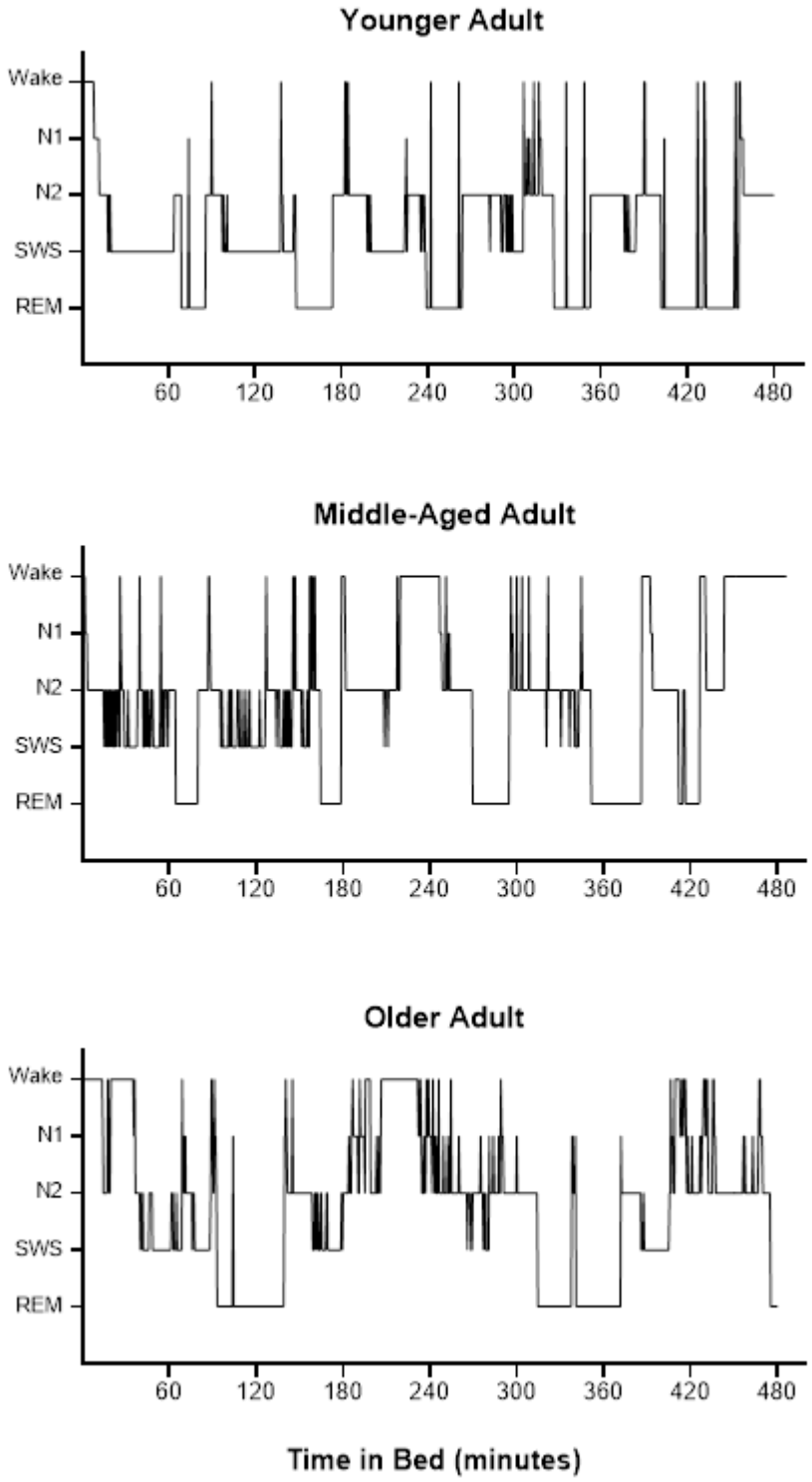

Figure 3. Hypnograms for younger (top), middle-aged (middle), and older (bottom) adults over an 8-hour sleep opportunity. Reduced N3 and increasing fragmentation (awakenings) are hallmark signs of ageing. Scoring is based on standard AASM criteria.

1.5.2 Age-Related Changes in Sleep and Cognition

Increasing age is associated with impaired cognitive performance, even in the absence of underlying pathology. While difficult to attribute causality to any one variable, several factors have been implicated, including the accumulation of neurodegenerative pathology such as Aβ and tau neurofibrillary tangles, grey matter atrophy and decreasing sleep quality. Sleep fragmentation is associated with impaired executive function (Anderson & Horne, 2003; Wilckens, Woo, Kirk, Erickson, & Wheeler, 2014). Several electrophysiological markers of sleep that decline with ageing have been associated with a parallel decline in cognition, including sleep spindles (Taillard et al., 2019), slow waves (Mander et al., 2013), and SWRs (Kanak, Rose, Zaveri, & Patrylo, 2013; Wiegand et al., 2016). Notably, SWS decline mediates age-related changes in executive function, learning, overnight memory consolidation (Mander et al., 2013; Varga, Ducca, et al., 2016) and attention outcomes. It has been theorised that poor sleep in midlife could lead to a cascading effect of neurobiological impairments (Scullin & Bliwise, 2015) – i.e., short sleep duration is linked to greater cortical Aβ burden (Spira, Chen-Edinboro, Wu, & Yaffe, 2014) leading to cognitive impairments later in life (Ferrie et al., 2011).

1.5.2.1 Sleep, Ageing and Executive Function

Given that executive functions are controlled predominately by the prefrontal cortex, SWS preferentially originates from the prefrontal cortex, and both decline with age (even within healthy ageing) (Mander et al., 2013; Verhaeghen & Cerella, 2002), it is perhaps unsurprising that they are linked (Anderson & Horne, 2003; Horne, 1993). Slow wave and spindle density predict verbal fluency performance (Lafortune et al., 2014), specifically slow oscillations, which also predict nonverbal planning ability, working memory and cognitive flexibility (Anderson & Horne, 2003; Wilckens et al., 2017). Executive functions may be particularly vulnerable to ageing as they require consolidated bouts of SWS for optimal performance, which is harder to achieve with increasing WASO with increasing age (Holanda & de Almondes, 2016; Wilckens et al., 2014).

1.5.2.2 Sleep, Ageing and Memory

While essential across all age groups, the role of SWS and SWA in memory appears to shift across the lifespan. In young adults, SWS is associated with improved retention (Backhaus et al., 2007), recall (Rasch et al., 2007), and declarative memory (Leminen et al., 2017), while this appears to be diminished in middle-aged to older adults (Backhaus et al., 2007). In older adults, overnight memory consolidation appears to be specifically mediated by SWA (Kawai et al., 2020), possibly due to epochs not reaching criterion for SWS, which highlights the importance of examining micro sleep structure. Nevertheless, in a seminal study, Mander et al. (2013) demonstrated that, despite no significant differences in pre-sleep memory between younger and older adults, the age-related prefrontal atrophy resulted in reduced SWA, leading to poorer overnight memory consolidation. Posteriorly-based regions of the brain such as the cerebellum and putamen remain relatively intact in older adults, which may explain why similar age-related reductions in procedural memory consolidation are not observed (Gui et al., 2017).

1.5.3 Sleep, Ageing and Physiology

1.5.3.1 Hormonal changes

As described earlier, SWS has an intricate relationship with the HPA axis, and mediates the secretion or suppression of countless hormones, having a bidirectional relationship with many. For example, the majority of GH is secreted during nocturnal sleep, with the amount also coinciding with SWS. Inhibiting GH also reduces SWS and both decay at a similar rate over the lifespan (Van Cauter et al., 2000). Similarly, SWS inhibits cortisol secretion, but may also be dampened by high levels of cortisol (particularly as cortisol increases with age) (J. Li, Vitiello, & Gooneratne, 2018). Sex hormones may have a protective effect on SWS, as sleep fragmentation in men increases as testosterone levels decrease (Wittert, 2014), while women have more sleep complaints following menopause (i.e., when estradiol levels decrease).

1.5.3.2 Cardiovascular changes

The severity of cardiac risk fluctuates depending on the measurement used – for example, standard deviation of all normal sinus inter-beat (RR) intervals (SDNN) indicates that HRV decreases slowly (dropping 40% by the tenth decade). In contrast, percentage of successive normal sinus RR intervals >50 ms (pNN50) suggests that HRV drops 76% by the *sixth* decade (Umetani, Singer, McCraty, & Atkinson, 1998). Nevertheless, it is generally agreed upon that HRV decreases with age, even within healthy ageing, i.e., independent of pathological disease and medication use (Jandackova, Scholes, Britton, & Steptoe, 2016). When sleep is taken into consideration, the decrease in SWS with age is associated with an increasing prevalence of hypertension, and risk of cardiovascular disease (Fung et al., 2011), likely due to the loss of parasympathetic control. Another factor could be increased WASO limiting consolidated SWS and increasing fluctuation between sleep stages (Trinder et al., 2001; Vanoli et al., 1995). It seems as though HRV is very susceptible to auditory stimuli, as music therapy has previously been used to improve HRV (Valenti et al., 2012), and some studies have specifically targeted SWS to enhance cardiac outcomes (Grimaldi et al., 2019), which is particularly interesting in light of new research suggesting that HRV is associated with memory outcomes (van Schalkwijk et al., 2020; Whitehurst, Chen, Naji, & Mednick, 2020).

1.6 Slow Wave Sleep Enhancement

With the critical role of SWS for physiological, brain and cognitive health, and its observed decline across the lifespan, a new focus for research has been developed based on the enhancement of this important 'restorative' aspect of sleep. A plethora of alternate methods for enhancing SWS have been developed, including pharmacological (Walsh et al., 2008), behavioural (Wilckens et al., 2018), brain stimulation (Marshall et al., 2004), sensory stimulation (Horne & Shackell, 1987), and acoustic stimulation (Tononi, Riedner, Hulse, Ferrarelli, & Sarasso, 2010). Each method varies in terms of effectiveness, invasiveness, and overall feasibility for use within the home, as summarised

in Table 1. The final section of this review will compare the strengths and limitations of each method, with a focus on acoustic stimulation.

Table 1. Summary of Slow Wave Enhancement Techniques and associated advantages/disadvantages for use as a targeted intervention.

Feature / Method	Effective	Safe	Affordable	Non-invasive	User-friendly	Readily available
Transcranial magnetic stimulation	✓	×	×	×	×	×
Transcranial direct current stimulation	✓	×	×	×	×	×
Sleep deprivation (rebound)	✓	×	✓	✓	✓	✓
Heating	✓	✓	✓	✓	✓	✓
Pharmaceutical	✓	×	×	×	✓	×
Sensory stimulation	✓	✓	✓	✓	✓	×
Acoustic stimulation	✓	✓	✓	✓	✓	✓

1.6.1 Pharmaceutical Agents

A number of drugs have been identified as successfully promoting SWS, including tiagabine (Walsh, Randazzo, Griffin, et al., 2006), sodium oxybate (Walsh et al., 2010), gaboxadol (Walsh et al., 2008), baclofen, olanzapine, and interleukin-6 (Walsh, 2009). Interestingly, each agent has a

unique mechanism of action, although they can be broadly categorised as gamma-aminobutyric acid (GABA) promoters (Gottesmann, 2002), 5-HT_{2C} antagonists (Sharpley, Vassallo, & Cowen, 2000), α2-δ calcium channel ligands (Foldvary-Schaefer et al., 2002) or those which target multiple receptor sites (Walsh, 2009). Pharmaceutical methods of enhancing SWS offer the benefits of being highly effective, user friendly, widely available, and non-invasive. On the other hand, they can be expensive, and are susceptible to issues associated with dependence and tolerance. As some drugs have a long half-life, they may not be conducive to shorter sleep periods resulting in daytime sleepiness (Walsh, 2009), and may not be appropriate for use in vulnerable populations, such as older adults.

1.6.2 Brain Stimulation

Transcranial stimulation techniques such as transcranial magnetic stimulation (TMS), transcranial direct current stimulation (tDCS), and transcranial alternating current stimulation (tACS), induce localised slow waves by applying low frequency electrical or magnetic pulses that stimulate neurons below and near the coil (Huber et al., 2007; Marshall, Kirov, Brade, Mölle, & Born, 2011; Marshall et al., 2004; Massimini et al., 2007). TMS is capable of triggering slow waves across the brain (Massimini et al., 2007). While this method can be used with naps, or short sleepers, and is relatively safe for healthy adults (Rossi, Hallett, Rossini, & Pascual-Leone, 2009), it has a moderate effect size with mixed effectiveness (Sahlem et al., 2015), and as it is a particularly fastidious technique, cannot be operated by the average consumer at home (although caps for home use are under development (S. Lee, Jang, Yoon, & Chae, 2019)). It is also difficult to gauge the true efficacy of these techniques as it is difficult to separate the EEG signal from the electrical artifacts produced by the stimulation. With that said, using transcranial stimulation is not completely implausible as its effects can last for up to 24 hours, and can also be used during wakefulness (Saeki et al., 2013). Perhaps as evolving technology renders TMS to be more accessible, utilising regular stimulation sessions may be useful in promoting SWA, although more work is needed to ascertain its long-term

effectiveness and safety (Grimaldi, Papalambros, Zee, & Malkani, 2020; Thut & Pascual-Leone, 2010).

1.6.3 Behavioural and Sensory Stimulation

Thermoregulation was one of the earliest identified methods of modifying SWS, and by far the most accessible, cost-effective and easiest to implement. SWS is sensitive to changes in both ambient and internal body temperature (Wilckens et al., 2018). Several studies have reported that increasing internal body temperature before bedtime increases both SWS power and duration (Horne & Shackell, 1987; Jordan, Montgomery, & Trinder, 1990). Unlike other methods of enhancing SWS, body heating has been shown to have a cumulative effect, as SWS increased during a 3-week intervention where participants were required to take a warm bath five times a week (Silva et al., 2013). Increased core body temperature (CBT) activates warm-sensitive hypothalamic neurons that subsequently increase SWA (Lan, Tsuzuki, Liu, & Lian, 2017; Wilckens et al., 2018). While exercise also increases CBT, it also promotes sleep by stimulating the release of adenosine triphosphate, adenosine and sleep regulating cytokines (Park et al., 2021). Ambient temperature also appears to impact SWS. In one study, participants exposed to 26°C had an extra 30 minutes of SWS compared to those at 23°C, and 40 minutes compared to those at 30°C (Lan, Pan, Lian, Huang, & Lin, 2014). However, there is contradictory evidence to suggest that individuals exposed to *colder* temperatures or lower CBT during sleep have increased SWS (Haghayegh, Khoshnevis, Smolensky, Diller, & Castriotta, 2019; Herberger et al., 2020; Sewitch, Kittrell, Kupfer, & Reynolds Iii, 1986). While fewer studies have explored the use of olfactory and vestibular stimulation, there is evidence to support that they can be used to increase SWS and SWA (Bayer et al., 2011; Perl et al., 2016).

1.6.4 Acoustic Stimulation

Acoustic tones were conventionally used in the past to assess wake threshold levels, or to deliberately disrupt sleep and slow waves. However, tones that are administered below the arousal

threshold and above the auditory threshold can be used therapeutically. Today, there are a multitude of studies reporting acoustic stimulation successfully improves SWS, cognition, and physiology. Acoustic stimulation enhances SWS by playing short tones (~50ms) phase-locked to individual slow waves (Tononi et al., 2010). It offers the advantage of being effective, non-invasive, easy to use and to date, has no known side effects. Emerging technology in the form of take-home devices has rendered this approach easily accessible by the average consumer. Some concerns have been raised over the relationship between acoustic stimulation and HRV, notably over whether artificially increasing SWS overly dampens autonomic activity. However, there is currently no evidence to show that acoustic stimulation is harmful, and may in fact be beneficial to cardiovascular health (Grimaldi et al., 2019). A major limitation with acoustic stimulation is that it does not appear to uniformly increase SWA, either across the night, with many studies reporting a redistribution of SWA instead (Ong et al., 2016; Papalambros et al., 2017). Nevertheless, it is important to understand the underlying mechanisms and features of acoustic stimulation to optimise it for the wider population.

1.6.4.1 Mechanisms Involved in Acoustic Stimulation

As with most other sensory modalities, the auditory system can be split into lemniscal (primary) and non-lemniscal (secondary) thalamocortical pathways. The primary pathway carries auditory information ascending from the cochlear nuclei, while secondary pathways carry information from more diffuse areas within the posterior thalamus (Hu, 2003). Additionally, the presentation of the auditory information differs, with primary pathways focusing on transmitting fast, specific features of the stimulus, and secondary pathways carrying more general information about changes in the environment. Finally, lemniscal and non-lemniscal neurons have differing membrane properties that affect their excitability and rate of discharge (Hu, 2003). These differences result in the activation of discrete neuronal populations within the thalamus that ultimately allow for a holistic approach to detecting, locating, and identifying auditory information. During sleep, when presented with neutral auditory stimuli, the brain generates K-complexes or slow oscillations in an effort to

maintain sleep continuity (Forget, Morin, & Bastien, 2011). It has been proposed that acoustic stimulation activates non-lemniscal pathways to synchronise the depolarisation of cortical neurons, leading to enhanced slow waves in terms of amplitude and slope and subsequently increased SWS (Bellesi, Riedner, Garcia-Molina, Cirelli, & Tononi, 2014). As non-lemniscal auditory pathways overlap considerably with arousal promoting systems, care must be taken when developing acoustic stimulation so as not to accidentally create an arousal. This, along with other key factors, are discussed in the following section.

1.6.4.2 Development and Optimisation of Acoustic Stimulation

Bellesi et al. (2014) originally described four key features of acoustic stimulation to be taken into consideration to maximise slow wave enhancement: intensity, frequency, timing, and entrainment. *Intensity* refers to the volume of the tone, which must be above the auditory threshold to elicit a slow wave, and below the arousal threshold to avoid disturbing sleep. They suggested that one way of identifying individuals who are resilient to perturbing stimuli could be detecting high spindle density and frequency patterns during NREM sleep. Another somewhat more straightforward albeit basic method could be playing a series of tones at differing volumes to gauge an individual's arousal threshold and noting it for future sleep periods. With more sophisticated technology, this process could be automated through machine learning. *Frequency* describes the rate at which tones are played, ideally in a randomised manner to prevent habituation of non-lemniscal pathways. This could be accomplished by changing the sound frequency (i.e., pitch, rather than timing) of the tone. With that said, the *hertz* of the tones should still fall within the bandwidth of naturally occurring slow waves (Tononi et al., 2010). Alternatively, the number of tones played during each sequence could be modified, as even a single tone is effective at producing an effect (Grimaldi et al., 2020). *Timing* refers to the ideal slow wave phase in which the tone is presented. When a tone is administered at the optimal phase of each wave, more neurons are recruited to produce a larger amplitude and further induce a train of oscillations. This was previously identified as specifically during the up-state of the

slow oscillation (Ngo, Martinetz, et al., 2013). Finally, *entrainment* refers to the rhythmicity of the presented tones to maximise the slow wave response, not unlike how slow oscillations synchronise other EEG waves (Timofeev & Chauvette, 2011). Again, this appears to be the most effective by mimicking the natural frequency of slow oscillations, between 0.5-1 Hz (Ngo, Claussen, Born, & Mölle, 2013).

Once these features have been established, the next step is to consider the model that will encapsulate them. The most common design is the closed-loop paradigms, which allows the system to constantly adjust the stimulation according to EEG patterns dynamically, rather than operate according to one set of rules (as with open feedback loops). This is particularly beneficial in preventing arousals leading to wake as it detects alpha and beta waves and ceases stimulation until the reappearance of delta waves (Ngo, Martinetz, et al., 2013). The main concern with closed-loop systems is the delay between detecting the slow wave and the presentation of the stimulus. As this method generally administers tones until a higher EEG frequency appears, it may result in tone delivery during the wrong slow wave phase (which could lead to disrupted sleep). This could be remedied with the use of phase-locked loops, which lock onto the "correct" phase prior to delivering a series of tones, followed by a short period of quiescence (Santostasi et al., 2016). Phase-locked loops offer more flexibility as they frequently readjust where it locks on the slow wave, while closed-loop systems are designed to deliver tones at fixed inter-tone intervals. The closed-loop paradigm and acoustic stimulation features are summarised in Figure 4.

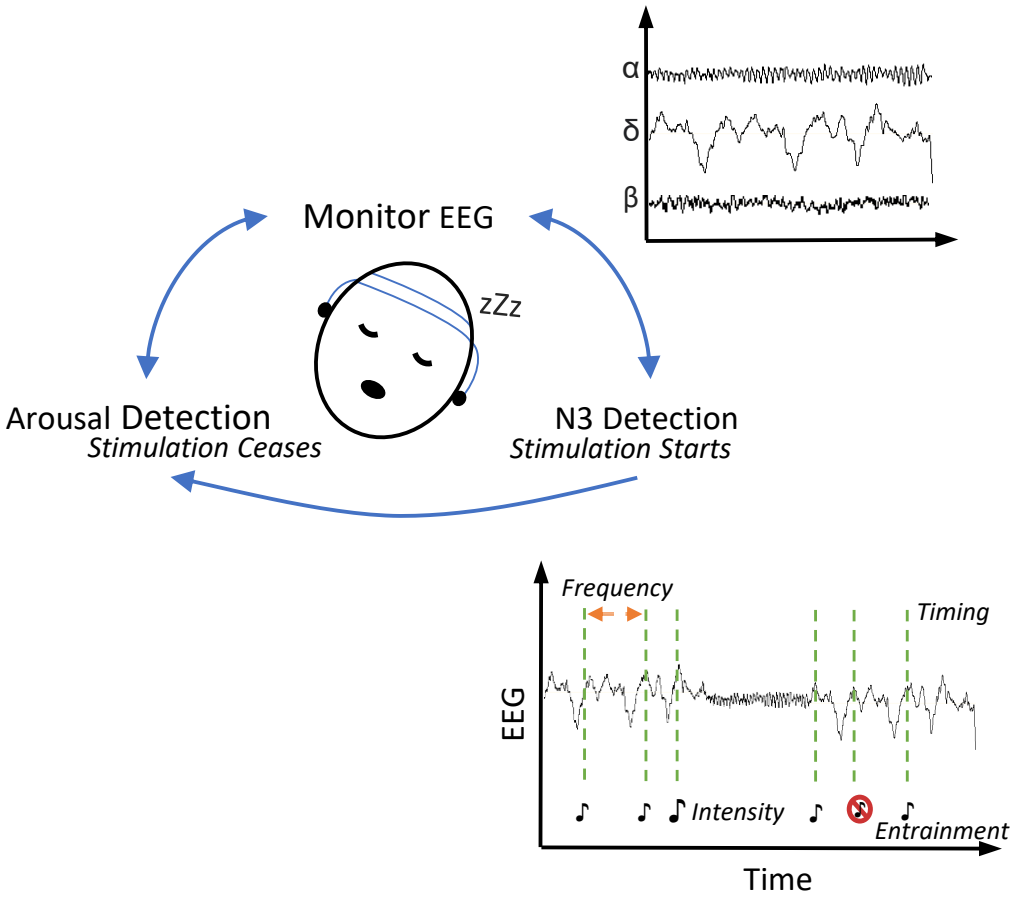

Figure 4. Example of closed-loop paradigms and acoustic stimulation factors. Algorithms constantly monitor EEG searching for the presence of delta, beta, and alpha waveforms. If N3 is detected, acoustic stimulation starts, otherwise no tones are presented. Similarly, if alpha or beta is detected, no tones are presented. When tones are presented, timing (green dashed lines), frequency (distance between green lines), intensity (loudness), and entrainment (switching number of beats) are optimised to enhance SWA.

1.6.5 Interindividual Differences and Lifestyle Factors

Despite the plethora of SWS enhancement methods available, none uniformly increase or enhance slow waves. Just as there are interindividual differences in the presentation of SWS (Erwin et al., 2020; Tucker, Dinges, & Van Dongen, 2007), there are likely interindividual responses to SWS enhancement. Therefore, the final factor for consideration is *who* would benefit the most from *what*

technique. For example, pharmaceutical drugs are more likely to clash with medications and/or cause side effects, and so may be less suitable for older adults or individuals who are on other prescriptions. With relatively long half-lives, drugs may also not be suitable for shift workers. As current acoustic stimulation techniques generally require a minimum threshold to recognise and lock onto slow waves, they also may not be as suitable in older adults with diminished SWA, although some studies have successfully implemented the use of acoustic stimulation (Papalambros et al., 2017). Consequently, heating or TMS paradigms may be the best option for older adults. Women also need to consider *when* the most appropriate time is for enhancement, as there is significant SWS disruption later in life, particularly during perimenopause. Along the same vein, the hot flashes associated with menopause indicate heating treatments may be an incompatible method of enhancement. Men have lower levels of SWS compared to women at all age points (Carrier, Land, Buysse, Kupfer, & Monk, 2001). Although a specific reason is yet to be identified, this may be due to neuroanatomic differences (Dijk, Beersma, & Bloem, 1989). Regardless of gender, more research is needed to elucidate what influences trait differences as a whole in order to develop personalised strategies for slow wave enhancement.

1.7 Conclusions and Current Research Directions

In the modern, 24/7 society, fewer people are achieving optimal sleep due to (i) an increasingly ageing population and (ii) chronic sleep restriction arising from work and lifestyle demands, among other factors. Regardless of cause, disrupted sleep, particularly the decline of SWS, is associated with a wide range of adverse effects. Although many forms of SWS enhancement exist, acoustic stimulation remains the most ecologically viable when risk, accessibility, and effectiveness are taken into consideration. While SWS deterioration is the most severe in older adults, significant reductions in SWS can be observed from midlife. Additionally, poor sleep during midlife could lead to long-term neurobiological impairments later in life. Therefore, it is imperative to target younger

age groups for SWA enhancement to improve not only short-term goals such as social and workplace productivity, but also as a long-term strategy to diminish cognitive decline in older age. Acoustic SWS enhancement is not only beneficial for cognition; it has the potential to be used in a wide number of applications, such as improving cardiovascular and metabolic health. However, little has been done beyond examining the direct impact of a single night of acoustic stimulation on SWS and overnight memory consolidation, particularly in middle-aged adults. Therefore, the overall aim of this thesis was to investigate whether acoustic stimulation enhances SWS in middle-aged adults, and examines the impact on executive function, alertness, and cardiovascular health.

1.8 Thesis Aims

The overarching aim of this thesis is to examine the impact of acoustic stimulation on SWS and the subsequent changes in cognition and physiology. More specifically, the aims of each chapter are to:

1) Describe the methodology of two studies that contributed data to this thesis **(Chapter 2)**.
2) Determine the impact of an automated acoustic stimulation device on SWA and executive function **(Chapter 3)**.
3) Examine the changes in SWA following *consecutive* nights of acoustic stimulation, and changes in alertness and attention **(Chapter 4)**.
4) Investigate the changes in HRV following acoustic SWS stimulation **(Chapter 5).**
5) Discuss and link the findings from the experimental chapters and explore future avenues of research for acoustic SWS enhancement **(Chapter 6)**.

Chapter 1 References

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Chapter 2

Methodology

In this thesis, data were collected from two studies examining the use of an automated, acoustic device to enhance SWA, and next day performance on measures of cognition (Study 1) and alertness (Study 2). Data from Study 1 contributes to two experimental chapters (**Chapters 3 and 5**) and were collected in a crossover, double-blind randomised controlled trial conducted at the Monash University Sleep and Circadian Medicine Laboratory in Melbourne, Australia (The "PowerSleep" Study). PowerSleep examined the effect of acoustic stimulation on SWA after a single night in healthy middle-aged males (35–50 years), with a secondary aim of assessing HRV. Data from Study 2 formed the third experimental paper (**Chapter 4**) and were collected in a crossover, double-blind randomised controlled trial conducted across the United States of America (The "SmartSleep" Study). The main aim of SmartSleep was to examine the impact of two consecutive nights of stimulation on SWA and alertness in habitually sleep-restricted adults (18–55 years). Both studies used an automated, acoustic device, designed to enhance SWS, as described in the following section.

2.1 Device Functionality and Programming

The various prototypes and final, commercially available device used by the PowerSleep and SmartSleep studies are shown in Figure 1. While the hardware for each device is different, the software remained the same, described below. Both studies also programmed the device using a custom-built application (the DeltaBoost program). The DeltaBoost program was used to assess hearing and alpha-delta sleep during the screening stages (see Section 2.4.3 and 2.4.4, respectively), set the device to STIM or SHAM mode (which were labelled as A or B to maintain double-blind measures), view device impedances, and download the device data following each sleep period in the laboratory.

Prototype 1, Study 1 *Prototype 2, Study 1* *Prototype 3, Study 2* *Final Design*

Figure 1. The device prototypes used in Study 1 and Study 2, and the final commercially available device (Philips SmartSleep).

Slow waves were detected in real-time from an electrode placed on the centre of the forehead (Fpz) and referenced to a right mastoid (M2). Two electrooculographic (EOG) channels (left and right outer canthi) were used to detect eye movements. All electrodes were secured with an adjustable headband, and along with the external speakers, were connected to the main device (collectively referred to as the DeltaBoost device). The device also had lights to indicate whether it was powered on or had a poor signal. As the signal quality could not be checked remotely during the sleep period, recordings did not start until several criteria were met: (i) impedances were <5Ω, (ii) the device indicated adequate signal quality and, (iii) clear eye blinks and rolls were visible during biocalibrations (which were also done to synchronise device data with PSG data).

Electroencephalography data captured by the device was recorded in real-time at 1000 Hz, filtered with a high-pass filter at 0.3 Hz, a notch filter at 50 Hz and 60 Hz (depending on whether data was collected in Australia or the USA), then down-sampled to 100 Hz. The resulting signal was filtered to identify alpha (8–12 Hz), beta (15–30 Hz) and delta (0.5–4 Hz) waves using 2nd-order Chebyshev filters. The signals were transformed to identify whether an arousal, wake, or N3 epoch had occurred in six second blocks. Slow waves were defined as peaks that passed a negative zero-

crossing and reached at least a -40-microvolt threshold with a total duration between 200–800 milliseconds. The thresholds for identifying SWA have been tested to have high specificity (≥95%) and moderately high sensitivity (≥70%) (Garcia-Molina et al., 2018).

2.1.1 Acoustic Stimulation of Slow Wave Sleep

During STIM conditions, acoustic stimulation began once N3 was continuously detected, and an appropriate sleep-depth (log-ratio between delta and beta powers) occurred. N3 detection was defined as: if the delta root mean square value crossed a pre-determined threshold a minimum of six times in a 20-second long sliding window. Tones were locked to the up-phase of the first slow wave. Tones were 50-milliseconds long, played at 1 Hz intervals until N3 ended or a µ-arousal was detected. The volume of each tone was determined by sleep-depth and ranged from 20–65 dB. During SHAM, while the recording parameters were identical, tones were played at inaudible volumes. The hardware and algorithm have been discussed at length by Garcia-Molina et al. (2018).

2.2 Overview of Target Population

PowerSleep was designed to be a proof-of-concept study, therefore only healthy males between the ages of 35–50 were recruited to ensure no underlying conditions, sex differences or individual variation would confound the impact of the study. In contrast, SmartSleep was a proof of principle study designed to assess slow wave enhancement in a population for which this therapeutic may be targeted: habitually short sleepers. Eligibility criteria ensured they were otherwise healthy to minimise confounds but did not employ the same restrictive sleep and health criteria as PowerSleep. Subsequently, both males and females were recruited, over a wider age range (18–55). The inclusion criteria for Study 1 and 2 can be seen in Table 1.

Table 1. Inclusion Criteria for Participants in Study 1 (PowerSleep) and 2 (SmartSleep).

Criteria	PowerSleep Criteria	SmartSleep Criteria

Age	35 – 50	18 – 55
Sex	Male	Male and Female
Female Health	–	Pregnant or currently breast feeding
BMI	18.0 to 29.9kg/m^2	18.0 to 40 kg/m^2
English Fluency	Adequate fluency to undergo neuropsychological assessment and provide informed consent	Adequate fluency to provide informed consent
Bedtime	Habitual bedtime prior to 1:00am	Sleep schedule is consistent for < 3 nights during work week
Wake Time	Habitual wake time prior to 9:00am	Sleep schedule is consistent for < 3 nights during work week
Sleep duration	Reported sleep duration between 7–9h per night	Reported sleep duration between 5–7h per night during the work week, with at least a 1 hour increase during weekends
Sleep quality	Sleep efficiency above 80% (determined through actigraphy)	Sleep latency < 30 minutes; WASO < 30 minutes
Naps	No more than two naps per week	No naps during the work week
Sleep Disorders	No reported sleep disorders	No reported sleep disorders
Psychiatric Disorders	No current or previous psychiatric diagnosis; No family history of mood disorders	No current or previous psychiatric diagnosis
Medical disorders	No major or uncontrolled medical conditions	No major or uncontrolled medical conditions

Eye Disorders or Diseases	No diagnosis of eye disease; No non-correctable impairment; Not colour blind (confirmed with Ishihara at consent)	–
Ear Disorders or Diseases	No diagnosis of hearing impairments	No diagnosis of hearing impairments
Medication use	No use of any prescription or non-prescription medication	No use of any prescription or non-prescription medication
Alcohol Consumption	No current or history of alcohol consumption > 14 standard drinks per week	No current or history of alcohol consumption > 21 standard drinks per week
Caffeine Consumption	No consumption of caffeine exceeding 300mg/day	No consumption of caffeine exceeding 650mg/day
Smoking	No current smoking or use of nicotine replacement therapies	No current smoking or use of nicotine replacement therapies
Circadian Misalignment	No trans meridian travel over 2 or more time zones in past 3 months; No night shift work in past 3 months	No trans meridian travel over 1 or more time zones in past 3 months; No shift work in past 3 months
Recreational Substances	No use within last 12 months or history of abuse	No use within last 12 months or history of abuse
Female Health	–	Not currently pregnant or breastfeeding, not planning on becoming pregnant
Work Schedule	–	Working full time with a regular work schedule
Participation in previous studies	None in past one month	None in past one month (unless observational)
Insomnia Severity Index (ISI)	–	Score ≤ 21

Cambridge-Hopkins Screening Questionnaire for Restless Legs	–	High risk of Restless Legs Syndrome
Snoring, Tired, Observed, Pressure, BMI, Age, Neck Size, Gender (STOP-BANG)	≤ 5 items	≤ 4 items
Depression Anxiety Stress Scales (DASS-21)	Depression ≤ 6 Anxiety ≤ 5 Stress ≤ 10 (if only elevated score, ≤ 13)	–
Pittsburgh Sleep Quality Index (PSQI)	Global score ≤ 5	–
Epworth Sleepiness Scale (ESS)	Score ≤ 10	–
Patient Health Questionnaire (PHQ-9)	Score ≤ 5	–
Apnoea-Hypopnea Index (AHI)	Score ≤ 10	Score ≤ 15
Periodic Leg Movements (PLMs)	≤ 5 with arousals per hour	–

2.3 PowerSleep Summary

The PowerSleep study was conducted from approximately April 2015 to April 2017. PowerSleep consisted of one week at-home monitoring with actigraphy and sleep diaries, followed by a 2-night, 2-day laboratory stay. Following a minimum 5-day wash-out period, the laboratory stay was repeated with the alternate experimental condition. For both conditions, Night 1 was a baseline

night to acclimatise the participants to the laboratory, and to confirm no presence of undiagnosed sleep disorders. Participants were given an 8-hour sleep opportunity starting from their habitual bedtime (HBT) for all four nights. During Day 1, participants alertness was assessed periodically throughout the day. Experimental nights were conducted on Night 2, where the acoustic device would either play tones phase-locked to the slow waves (STIM) or monitor and play tones at inaudible volumes (SHAM). The following day, Day 2, participants completed alertness and neurocognitive test batteries from 1-hour post-wake to examine overnight memory consolidation, executive function, alertness, and attention. The PowerSleep study was approved by the Monash University Human Research Ethics Committee in accordance with the National Statement on Ethical Conduct in Human Research (approval number CF15/671) and conducted in accordance with the Declaration of Helsinki; participants gave written informed consent.

2.4 PowerSleep: Participant Screening and Eligibility Criteria

A four-step screening process was used to recruit participants, including: (1) a telephone interview to screen for basic study criteria (e.g., male, recent travel history); (2) an online questionnaire to screen for all eligibility criteria and assess risk of undiagnosed sleep disorders; (3) an in-person participant information and consent meeting; and (4) a sleep disorder screening during the baseline nights. Figure 2 summarises the number of participants screened at each stage of participant recruitment and included in the final data set.

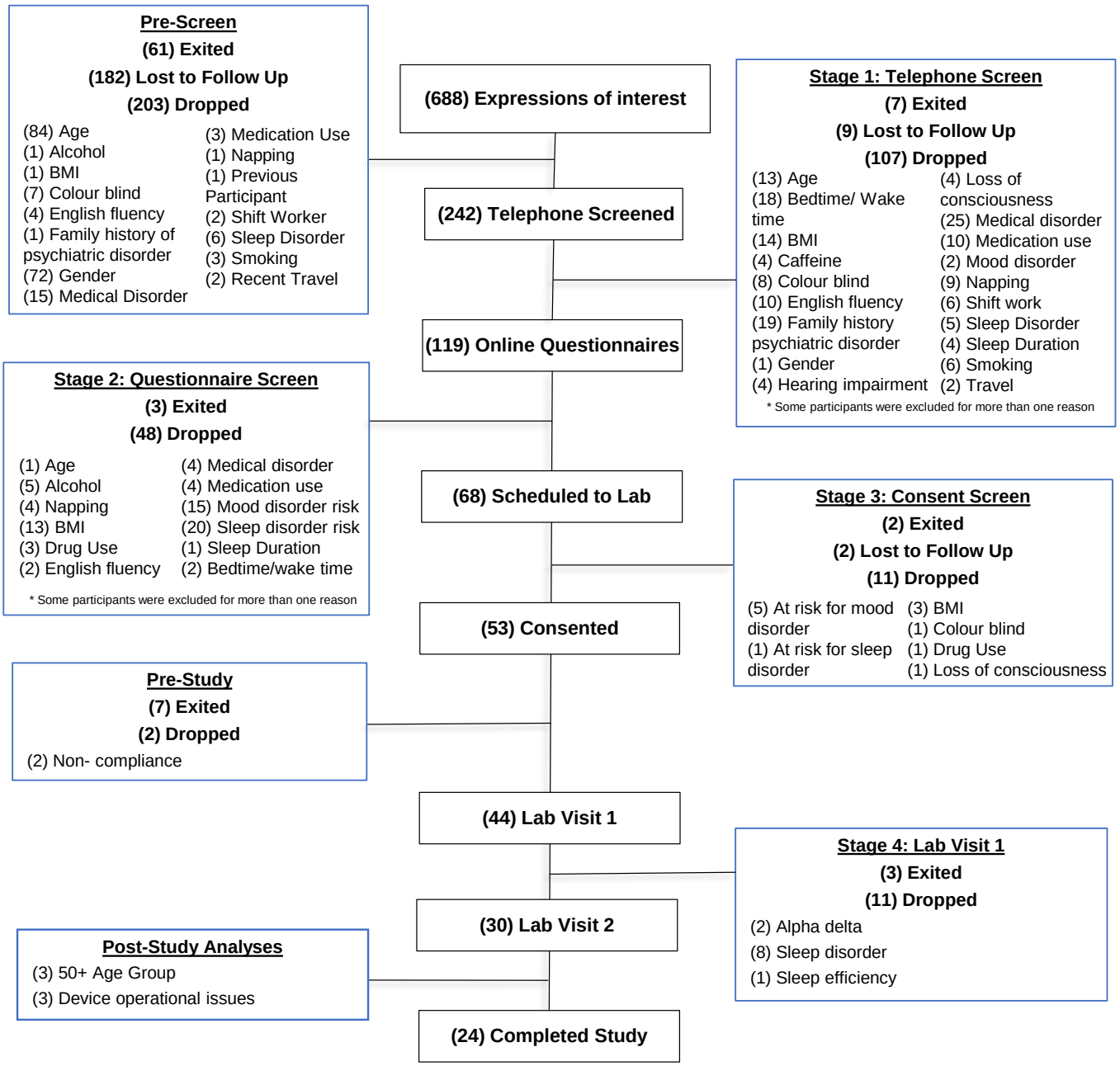

Figure 2. Flowchart of participant recruitment and screening process. The flow chart depicts participants from the initial expression of interest to the final completed dataset. Each stage summarises the reasons why participants were excluded or withdrew from the study.

N.B. Some participants were excluded for more than one reason; therefore, numbers in the brackets may not always accurately add up to the final total.

2.4.1 Stage 1: Initial Screening Criteria

During initial phone screening, basic demographic and first level eligibility information was obtained, with exclusionary criteria listed in Table 1. This included:

- Sex
- Birth date
- Primary language
- Sleep onset/offset time
- Average sleep time
- Naps per week
- Regular medications
- Cigarette/nicotine replacement therapy use
- Major health issues
- Mood disorders/psychotic disorders in immediate family
- History of loss of consciousness
- Amount of caffeine and alcohol consumed per week
- Travel outside of the state in the past three months
- Shift work in the past three years

2.4.2 Stage 2: Online Questionnaires Determining Eligibility

Eligible participants following the Stage 1 telephone screening proceeded to Stage 2 where they completed a series of online questionnaires. These included:

2.4.2.1 Mental Health Questionnaires

2.4.2.1.1 Depression Anxiety and Stress Scale (DASS-21)

The DASS-21 contains a subset of 21 items from the complete 42-item DASS-42 (Lovibond & Lovibond, 1995). The DASS-21 assesses the presence and severity of depression, anxiety, and stress experienced over the past week in three subscales. Each subscale contains 7 statements with a 4-point Likert scale ranging from 0 – "Did not apply to me at all/Never", to 3 – "Applied to me very much, or most of the time/Almost always". The Depression subscale evaluates symptoms of low mood, hopelessness, and anhedonia. The Anxiety subscale evaluates symptoms of autonomic arousal, situational anxiety, and the subjective experience of anxiousness. The Stress subscale evaluates non-specific arousal, assessing feelings such as irritability and frustration and an inability to relax. The items from each subscale are summed to create a general depression, anxiety, or stress score, with higher scores indicating greater severity. The DASS-21 has been validated in several studies, and found to have high internal consistency (Antony, Bieling, Cox, Enns, & Swinson, 1998; Henry & Crawford, 2005) and is stable across different racial groups (Norton, 2007) and sexes (Gomez, 2013). The severity ranges and cut-off values are summarised below in Table 2. Although the DASS-21 is not used for diagnostic purposes, it can be used to screen for significant indicators of depression or anxiety. Participants from PowerSleep were excluded if they scored above the "Normal" range on any of the subscales. Participants with "Severe" or "Extremely Severe" scores on the DASS-21 and who indicated having thoughts of self-harm on the PHQ-9 were followed up by phone and provided with a referral letter to their general practitioner.

Table 2. Recommended threshold criteria for the DASS-21 Subscales.

Scale Anchor	Depression	Anxiety	Stress
Normal	0 – 4	0 – 3	0 – 7
Mild	5 – 6	4 – 5	8 – 9
Moderate	7 – 10	6 – 7	10 – 12
Severe	11 – 13	8 – 9	13 – 16
Extremely Severe	14+	10+	17+

2.4.2.1.2 Patient Health Questionnaire (PHQ)-9

The PHQ-9 contains a subset of 9 items from the PRIME-MD diagnostic instrument for common mental disorders (Kroenke, Spitzer, & Williams, 2001). Each item evaluates the presence of depression symptoms over the past fortnight, such as "Little interest or pleasure in doing things?". Participants chose a statement from a 4-point Likert scale ranging from 0 – "Not at all", to 3 – "Nearly every day". Each item is summed to produce a global depression severity index, with 5 categories spanning minimal (0–4) to severe (20–27). The PHQ-9 has excellent internal validity (Kroenke et al., 2001) and has been validated across different ethnicities (Huang, Chung, Kroenke, Delucchi, & Spitzer, 2006). Participants who scored ≥5 were excluded.

2.4.2.2 Sleep Health Questionnaires

2.4.2.2.1 Epworth Sleepiness Scale (ESS)

The ESS is an 8-item questionnaire measuring trait levels of daytime sleepiness (Johns, 1991, 1992). Each item describes a situation (e.g., sitting and reading) and participants rate their likelihood of falling asleep on a 4-point Likert scale from 0 – "Would never doze", to 3 – "High chance of dozing". Each item is summed to generate the final ESS score, which falls into 5 categories ranging from <5 – "Lower Normal Daytime Sleepiness" to 16+ – "Severe Excessive Daytime Sleepiness".

The ESS has good to excellent internal validity (Johns, 1992). Participants were excluded if they showed signs of excessive daytime sleepiness (ESS ≥10).

2.4.2.2.2 Pittsburgh Sleep Quality Index (PSQI)

The PSQI is a 19-item questionnaire that assesses sleep quality and sleep disturbances over the past month (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989). There are seven components that evaluate use of medications, daytime dysfunction and sleep quality, latency, duration, efficiency, and disturbances. Each component is scored on a 3-point Likert scale ranging from 0 – "No Difficulty", to 3 – "Severe Difficulty" and summed to provide a global sleep quality index. Higher scores indicate more disturbed sleep. The PSQI has excellent internal validity across all seven components (Buysse et al., 1989) and has been validated with older adults, men and women (Beaudreau et al., 2012), and across different ethnicities (Salahuddin et al., 2017). Participants with a global sum ≥6 (indicating a "poor" sleeper) were excluded.

2.4.3 Stage 3: Pre-Study Screening

Participants attended a participant information and consent meeting at the sleep laboratory, where they were re-briefed on the study protocol and aims. Following consent, participants were examined on the following:

2.4.3.1 Body Mass Index (BMI)

Participants had their height and weight confirmed at the consent meeting to ensure their BMI was between 18 kg/m^2 and 30 kg/m^2, calculated as *weight (kg)/height ([m]^2)*. BMI was exclusionary ≥ 30 kg/m^2 as individuals with higher BMIs tend to have less SWS (Rao et al., 2009).

2.4.3.2 Ishihara Tests

As some cognitive tests used in the in-laboratory assessment relied on colour discrimination, participants were tested for colour-blindness using the Ishihara Test (Ishihara, 2008). Participants

were shown 11 different pseudoisochromatic plates. Participants were excluded if they were unable to accurately complete any of the plates (i.e., identify the anticipated number, trace the correct path).

2.4.3.3 STOP-BANG Questionnaire

The STOP-BANG is an 8-item questionnaire used as a pre-emptive screening measure for obstructive sleep apnoea (OSA) (Chung et al., 2008). Using a simple yes/no system, the questionnaire sums factors such as BMI (i.e., is BMI more than 35 kg/m^2?), snoring (i.e., do you snore loudly?) and age (i.e., are you older than 50 years old?) to generate a final risk factor, with a higher score indicating a greater risk of OSA. It has been validated across age and sex (Chung et al., 2008). The STOP-Bang has high sensitivity, but low specificity (Chung et al., 2012; Chung et al., 2008), for OSA and as participants were further screened for undiagnosed sleep disorders during the baseline night (see following Section 2.4.4), the minimum cut-off criteria was set at ≥5 (indicative of high risk of OSA).

2.4.3.4 Hearing Assessments

Hearing tests ensured that participants would be able to hear the tones during the night. This was performed using a customised program to be used in conjunction with the device, referred to as the DeltaBoost program (described in Section 2.1). Participants were seated in a sound-proof room with an air conditioner on to mimic the environment during the laboratory stay. Participants reported "when the tone reached a level that would wake them, similar to their alarm clock". To determine basic hearing thresholds, tones were administered beginning at 0.01 and the volume titrated up by 0.05 until participants indicated the sound was adequate or when the maximum level (65 dB) was reached, noted down as their maximum limit. This process was repeated in the reverse direction until participants reported not hearing a sound, noted down as their minimum limit. Both tests were repeated twice for consistency. Participants who were unable to hear any tones were excluded. Tone sensitivity would be followed up during the baseline night, described Section 2.4.

2.4.4 Stage 4 Screening: In-Laboratory Assessments

The first night of each laboratory stay was to acclimatise the participant to the suite, testing conditions, EEG equipment and to test for undiagnosed sleep disorders. From admit participants were fitted with PSG equipment based on the International 10-20 System. Electroencephalography data was recorded using a bilateral 19–channel montage (Fp1, Fp2, F3, F4, C3, C4, P3, P4, Po3, Po4, P7, P8, O1, O2, Fpz, Fz, Cz, Pz, Oz) with all electrodes being referenced to the contralateral mastoid (i.e., C3-M2; C4-M1) (Compumedics Grael, Melbourne, Victoria, Australia). Two EOG channels (left and right outer canthi) and three electromyographic (EMG) (sub-mentalis) channels were used to score for eye movements and muscle activity, respectively. Electrocardiography (ECG) data were recorded using two electrodes, placed one inch below the left collarbone, and between the lower two ribs on the right ribcage Electroencephalography data were sampled at 512 Hz, impedances were maintained at <5Ω and signals were stored unfiltered. Participants were fitted with a nasal cannula and thermistor to measure oral and nasal air flow, a pulse oximeter to measure oxygen saturation, thoracic and abdominal respiratory bands to measure respiratory effort, and two electrodes on each leg to assess periodic limb movements. Participants also wore the PowerSleep device, which was configured for the baseline night, detailed below. All PSG data was staged and scored by a certified technician according to AASM (2012) criteria. Data was filtered between 0.3–30 Hz, and a notch filter of 50 Hz was applied. Sleep parameters included: total sleep time (TST), sleep latency, minutes and percent spent in N1, N2, N3 and REM sleep, WASO and arousal index (number of arousals per hour). As updated technology and screening criteria has increased the detection and subsequent prevalence of sleep-disordered breathing, the standard exclusionary criteria of Apnea-Hypopnea Index (AHI) >5 may have been too stringent for this study and led to the exclusion of otherwise healthy individuals (Heinzer et al., 2015). Subsequently, the exclusionary criteria for OSA was raised to AHI >10. The exclusionary threshold of restless legs and period limb movements was set at >5 movements per hour

with an associated arousal as leg movements on their own are unlikely to impact sleep or daytime sleepiness (Chervin, 2001).

2.4.4.1 Exclusion of Alpha-Delta Waveforms

Alpha-delta sleep refers to alpha wave intrusions during N3 sleep. While not considered a formal sleep disorder, it is indicative of sleep disturbances (Hauri & Hawkins, 1973; Menefee et al., 2001) and may interfere with the automated identification of slow wave by the device. The baseline data collected by the device was analysed through the DeltaBoost program to calculate the presence of alpha-delta sleep. If alpha-delta sleep was identified, participants were excluded.

2.4.4.2 Tone Sensitivity

Tone sensitivity was determined at the beginning of the baseline night to ensure that the device could generate a slow wave response without arousing the participant. Once SWS was detected, the device delivered 5 short tones (< 1 second), approximately 2 seconds apart, and at differing volumes (< 65dB), while monitoring for sleep disturbances (e.g., alpha waves). Participants who were easily aroused were assigned the "sensitive" volume configuration, otherwise the "standard" configuration was used. During the STIM experimental nights, devices on the sensitive setting would have a *maximum* tone limit of 20dB, while standard tone settings were set to 65dB, however, the volume of the delivered tones were still based on sleep-depth, determined as $\log\left(\frac{\delta}{\beta}\right)$.

2.5 PowerSleep: At-Home Monitoring

Participants maintained a consistent sleep-wake schedule for at least 5 nights prior to each laboratory stay for several reasons, including: homeostatic nature of SWS (Dijk, Brunner, Beersma, & Borbély, 1990), and to maintain sleep and circadian timing between conditions, which can impact test performance (McMahon et al., 2020). Participants sleep and wake schedules for the duration of

the at-home monitoring were self-selected by the participant (as to not deviate from their usual sleep/wake times) and confirmed during the consent meeting. They remained consistent for each study visit. Self-selected bedtimes needed to be between 22:00 and 1:00 and allow for an 8-hour sleep period. Schedules were verified through (1) actigraphy, (2) sleep diaries, and (3) time-stamped voicemails made at wake time and bedtime. Actigraphy was used to objectively verify sleep/wake timing for 5 nights before each laboratory stay. Actigraphy was monitored using Actiwatch Spectrums (Phillips Respironics, BMedical, QLD, Australia). Actigraphy has been validated against the gold standard PSG and has high sensitivity and accuracy across several populations (Marino et al., 2013). Participants completed sleep diaries each morning detailing (i) what time they slept (defined as when they attempted sleep) and woke (defined as when they got out of bed), (ii) any sleep disturbances during the night and the cause of the disturbance, (iii) whether they had taken their actiwatch off during the day and why, and (iv) their sleep quality compared to normal. Participants also provided voicemails at bedtime and wake time. Actigraphy data was assessed at admit to check for compliance to the sleep/wake schedules. Where actiwatch data was unusable, a combination of sleep diaries and call-in times were used to calculate HBT and adherence to sleep/wake times. Deviations over ±15 minutes from their habitual sleep/wake times were followed up by telephone and participants with more than one deviation over ±30 minutes were either rescheduled or excluded. Participants with an average sleep efficiency under 80% were rescheduled for the following week or excluded. Total sleep time, sleep onset latency (SOL) and WASO were recorded to compare habitual sleep before each laboratory stay. The full protocol from the at-home monitoring stage for PowerSleep is shown in Figure 3.

2.6 PowerSleep: In-Laboratory Protocol

Participants were admitted the Monash Sleep and Circadian Laboratory, Victoria, Australia. Each participant room consisted of a private, sound proofed suite with a bed and ensuite bathroom. Participants were not permitted to leave the suite until the end of their laboratory stay. During their free time, participants were permitted to move around the suite and partake in non-strenuous activities, such as watching movies, reading, or light exercise. An 8-hour sleep opportunity was provided each night, beginning from HBT. To maintain consistent temperature and light conditions between visits, temperature was kept at 21°C ± 2°C and light levels at 100 or <1 lux during wake/sleep periods, respectively. Participants wore PSG equipment throughout their laboratory stay (as in Section 2.4.4). The DeltaBoost device was simultaneously set-up each sleep period.

2.6.1 Experimental Nights: Acoustic Enhancement of Slow Wave Sleep

The DeltaBoost device would be set to either SHAM or STIM configurations on the experimental nights, as described in Section 2.1.

2.6.1.1 Power Spectral Analysis of Sleep EEG Data

Power spectral analyses are a main outcome of **Chapters 3** (and reported in **Chapter 5**). To determine slow wave enhancement, using EEG as detected by the in-lab Compumedics system, power spectral analyses were performed using Curry 7® software (Compumedics Neuroscan™, Abbotsford, Australia). Data were visually cleaned to remove artefact in 5-second blocks. Artefacts included slow eye movements in the EEG channels, muscle activity and electrical noise (e.g., from impedance checks). There was minimal data loss due to artefact during the experimental nights (1.53 ± 2.21%; STIM and SHAM combined as there were no significant differences between the two conditions). EEG data was digitally re-referenced to linked mastoids and bandpass filtered with a zero phase Hanning filter between 0.3 and 30 Hz. (0.6 and 8 Hz slopes, respectively). Fast Fourier transform was

applied to decompose each EEG time series into its sine and cosine components. Power values were calculated by integrating the area under the power spectral distribution for each 0.125 Hz frequency bin (Chan, Trinder, Colrain, & Nicholas, 2015). Power spectra was calculated in non-overlapping 5-second epochs, resulting in a 0.125 Hz resolution. Total absolute power was calculated by averaging raw power across epochs within each frequency bin, and summed across frequency bands (Chan et al., 2015). Absolute power was also calculated for each cycle, defined by the first NREM epoch after sleep onset, to the last REM epoch (Chan, Trinder, Andrewes, Colrain, & Nicholas, 2013; Trinder, Stevenson, Paxton, & Montgomery, 1982). As absolute power can be impacted by artifacts, e.g., slow eye movements, relative power was used. Typically, relative power is calculated as the percentage of power as a function of total power. However, this approach results in SWA being included in both the numerator and the denominator, thus reducing the signal of any enhancement effect. To counter this, relative power was calculated by expressing SWA as a function of total power within the REM bandwidth, shown as per the formula below in lieu of traditional methods (e.g., percentage of SWA within all power spectral bands). REM was chosen as it was a period free from enhancement, while maintaining sleep-depth (as opposed to N1, which is generally punctuated with arousals).

$$\frac{(N2 + N3\ delta\ power) - mean\ REM\ delta\ power}{SD\ REM\ delta\ power}$$

Slow wave energy was calculated to capture the total SWA across each night (Plante et al., 2016), by summing corrected SWA (SWA*#minutes) across the first four cycles. Primary analyses were derived from C3 (rather than F3) as interference from the Device headband was minimal and slow waves, such as K-complexes are maximal over frontal-central sites (Cote, de Lugt, Langley, & Campbell, 1999).

2.6.1.2 Heart Rate Variability Analyses

Heart rate variability (HRV) is the main outcome of **Chapter 5**. Electrocardiography data from each experimental night were exported as .edf files, then imported into the open access application HRVTool (Vollmer, 2019) in MATLAB R2021b to analyse HRV. Data were cleaned in a three-step process. First, artifacts were removed in HRVTool using the RRfilter method. Second, the data was processed using a custom MATLAB script, with segments containing excessive noise or poor data removed. Third, the data was plotted and manually inspected for any remaining artifact in the data. A custom-built MATLAB script processed the data in clean, 5-minute segments, then resampled into 30 second intervals to oversample by a factor of ten to align with sleep staging. HRV variables included: HR, SDNN to evaluate global variability, low frequency power (LF; 0.04–0.15 Hz), high frequency power (HF; 0.15–0.40 Hz) as a measure of parasympathetic activity, and LF:HF ratio to assess sympathovagal balance. Each variable was assessed across the entire night as a global comparison between STIM and SHAM conditions, and over the first three cycles to evaluate changes in HRV over the night.

2.6.2 Cognitive Test Batteries

Next day cognitive performance was measured on Days 2 and 3 of the protocol to examine any changes in cognitive performance between STIM and SHAM. The study was designed to target cognitive tasks that were associated with SWS and/or rely on the integrity of the prefrontal areas where SWS is maximal. The test battery thus comprised an Attention and Vigilance Test Battery, a Paired Associate Learning task for overnight memory consolidation, and a split Neurocognitive Test Battery to assess executive function.

Participants completed attention and vigilance (AV) batteries on Day 1 beginning at 1.5-hours post-wake and repeated every 2-hours until bedtime. An oculomotor test battery was run at 6-hours post-wake (not discussed as it is not explored in this thesis). A Paired Associates Learning (PAL)

Task was conducted approximately 1.5 hours prior to bedtime and recalled at 1-hour post-wake. Neurocognitive test batteries were run at 2- and 4- hours post-wake, testing memory and executive function. Neurocognitive test batteries were split into two sessions to reduce participant burden. The full protocol is depicted in Figure 3 below.

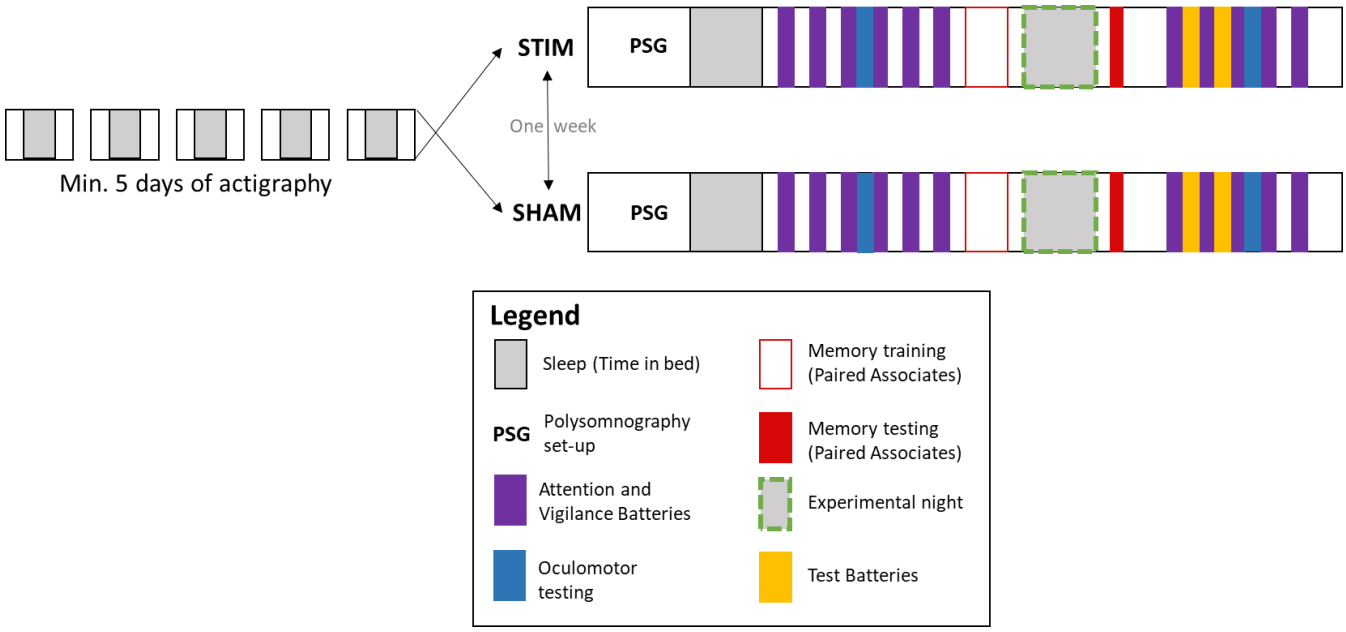

Figure 3. Study 1 in-laboratory protocol (Chapters 3 and 5). Participant sleep was monitored for a minimum 5 nights prior to their first visit to the laboratory. Participants were admitted to the laboratory approximately 5 hours prior to their HBT and provided with an 8-hour sleep opportunity at their HBT. The first in-laboratory day, participants were monitored continuously with PSG and completed a series of attention and vigilance (AV) batteries every two hours, from 1.5 hours post-wake and a practice oculomotor battery at 6-hours post-wake. The Paired Associates Learning (PAL) task was completed 2.5 hours prior to their HBT, where they were provided with a second 8-hour opportunity with the device activated to STIM or SHAM. On the second in-laboratory day, participants completed the final PAL task at 1-hour post-wake, AV batteries at 1.5 hours post-wake (and every two hours after until the end of the study), neurocognitive batteries at 2- and 4- hours post-wake, and an oculomotor test battery at 6- hours post-wake. Participants were discharged from the laboratory at 9.5 hours post-wake and returned the following week to complete the alternate condition.

2.6.2.1 Attention and Vigilance Test Battery

Attention and vigilance batteries were run periodically throughout each study day to monitor subjective and objective alertness however, they were not a main outcome within this thesis.

2.6.2.1.1 Karolinska Sleepiness Scale (KSS)

Subjective sleepiness is sensitive to SWS disruption (Dijk, Groeger, Stanley, & Deacon, 2010; Gillberg & Åkerstedt, 1994). The KSS is a one-item questionnaire that assesses subjective sleepiness (Akerstedt & Gillberg, 1990). Participants were asked "How sleepy have you been feeling in the past 5 minutes?" and rated their response based on a 9-point Likert scale ranging from 1 – "Extremely Alert", to 9 – "Extremely Sleepy". This study used the modified version, with descriptions at every point (Baulk, Reyner, & Horne, 2001). The KSS was administered as part of the full AV battery, and individually every other hour.

2.6.2.1.2 Psychomotor Vigilance Task (PVT)

The PVT is a measure of sustained attention (Dinges & Powell, 1985). PowerSleep used a 10-minute visual PVT, administered through a computer. Participants were required to respond as quickly as possible when a counter appeared at random on the screen by pressing a button on a handheld response box. The counter would display the response time briefly, before resetting to a blank rectangle. The inter-stimulus interval ranged from 2–10 seconds apart. Lapses were defined as no response within 500ms of the stimulus appearing. Responses under 100ms and anticipatory errors were removed from analyses in accordance with standard procedures (Basner & Dinges, 2011).

2.6.2.1.3 Karolinska Drowsiness Test (KDT)

The KDT is an objective measure of sleepiness (Akerstedt & Gillberg, 1990). The KDT is designed to capture a clean segment of EEG (i.e., free from artefact). Participants were instructed to

stare at a white circle on a screen while sitting as still as possible and minimising blinks for three minutes followed by sitting still with their eyes closed.

2.6.2.2 Paired Associates Learning Task (PAL)

SWS assists the conversion of hippocampal-dependent episodic memories to hippocampal-independent semantic memories. Paired associates tasks are commonly used to measure this when examining the impact of SWS on overnight memory consolidation (Marshall, Helgadóttir, Mölle, & Born, 2006; Ngo, Martinetz, Born, & Mölle, 2013; Takashima et al., 2006). A seminal paper by Mander et al. (2013) used the PAL to illustrate the association between SWS, overnight memory consolidation and ageing, with performance on paired associates tasks directly mediated SWA. The Paired Associates Learning (PAL) task is a primary measure used in **Chapter 3**.

Study 1 used a computerised 120 word-nonsense word-pairs task developed by Mander et al. (2013). An alternate form was developed for counterbalancing across STIM and SHAM. The task used nonsense words as opposed to semantically meaningful words to maximise hippocampal engagement (Mander et al., 2013). Words were 3–8 letters in length and derived from a normative set of English words, while nonsense words were between 6–14 letters in length as combinations of common phonemes. The task itself consists of 4 parts: encoding, criterion, short delay recall and long delay recall.

1) Encoding: During Encoding, all 120-word pairs were displayed sequentially on the screen for 5 seconds in 4 blocks. Participants were told to memorise each word pair as they will be tested on them later.
2) Criterion: During Criterion, participants were shown a word that was previously displayed during the Encoding stage, along with the correct nonsense word match and two new nonsense words not previously shown. After making their selection, they were given feedback for 1

second, before moving on to the next word-pair. As participants were required to correctly identify every word-pair to complete this component, trials may have been repeated.

3) Short Delay Task: The Short Delay phase started 10 minutes after Criterion was completed. Participants were shown 45 word pairs sequentially – 30 pairs were from the original Encoding phase, while 15 were new word pairs ("Foils"). Participants were given 5 seconds to choose one of the following options (i) the correct nonsense word pair ("Hit"), (ii) a nonsense word that was paired with a different word from the Encoding Phase ("Lure"), (iii) a nonsense word not shown during the Encoding phase, or (iv) "New" to indicate they had not seen the word-pair previously. No feedback was provided.
4) Long Delay Task: The Long Delay phase was conducted 1-hour post-wake the following morning. Participants were shown the remaining 90 original word-pairs, and 45 new foils.

The hit rate was calculated as *total original correct responses/total original responses*. The lure rate was calculated as *total original lure responses/total original responses*. The foil rate, which assessed false alarms was calculated as *(total foil responses - total foil correct responses)/total foil responses*. A summary memory score was generated for short and long delay tasks as *hit rate - lure rate - foil rate*. Finally, to determine the change in performance between the short and long delay tasks, the Change score was calculated as *long delay memory score - short delay memory score*.

2.6.2.3 Neurocognitive Test Battery 1

2.6.2.3.1 D-KEFS Verbal Fluency Task

Verbal fluency tasks are designed to measure cognitive flexibility and executive functioning (Delis, Kaplan, Kramer, Delis, & Kramer, 2001; Diamond, 2013), areas which appear to be facilitated by SWS (Goel, Rao, Durmer, & Dinges, 2009; Muzur, Pace-Schott, & Hobson, 2002). Slow oscillations (0.5–1 Hz) in particular are associated with verbal fluency and mental flexibility in older

adults (Anderson & Horne, 2003). Verbal fluency is a primary measure in **Chapter 3**. Study 1 administered and scored verbal fluency according to the D-KEFS Verbal Fluency Task manual (Delis et al., 2001), which contains six 60 second trials assessing phonemic fluency, semantic fluency and verbal set shifting and was counterbalanced. Trials were audio-recorded and later transcribed.

Phonemic fluency was captured over three trials (either F, A, S or B, H, R). Participants were asked to name as many words as they could that began with the target letter, so long as they were not names (i.e., Tom), places i.e., Tasmania), numbers (i.e., Twelve), or the same word with different endings (i.e., take, taking, takes). Phonemic fluency was scored according to the following rules:

1) If a generated word was either a common name or the name of a person/place (e.g., sandy, frank), the word was scored as correct, unless it was given in a list (e.g., Sandy, Sally) that clearly marked it as a name or place.
2) If a word was either a common word or number (e.g., for/four), the word was scored as correct, unless given in a list that clearly marked it as a number (e.g., four, five).
3) If the first letter of the word began with the target letter, contractions were permitted (e.g., aren't), as was the root word of the contraction (e.g., are).
4) Compound words were marked as correct but counted as a single point. Compound words with the same base word (e.g., apple, apple juice, apple pie) were marked as correct.
5) Proper nouns that were not names of people or places (e.g., months) were marked as correct.
6) Slang words that were in the Oxford dictionary (e.g., bromance) were marked as correct.

Phonemic fluency was calculated by summing all correct words from the three trials.

Semantic fluency was assessed over two trials (either animals and boys names, or items of clothing and girls names). Semantic fluency was scored according to the following rules:

1) Synonyms were marked as correct (e.g., dog, canine).

2) Words with multiple meanings were marked as correct (e.g., bat), provided they belonged to the target category.
3) Adjectives were not permitted unless it resulted in a new member of the category (e.g., big onion and small onion were not permitted, but green onion and red onion were allowed).
4) Words must be any level below the ordinate level of the target category (e.g., animal was not permitted, but canine and golden retriever were allowed).
5) Regarding animals: Different breeds e.g., Boxer, Husky), genders (e.g., bull, sow) and developmental stages (e.g., cat, kitten) were all marked as correct.
6) Regarding clothing: Clothing was defined as items typically sold in a clothing store, i.e., not jewellery or cloth.
7) Regarding names: Unisex names (e.g., Cameron), nicknames (e.g., Catherine, Kate, Kathy), and language variations (e.g., John and Juan) were all marked as correct.

   Semantic fluency was calculated by summing all correct words from both trials.

Verbal set-shifting required participants to switch between two categories (either fruit and furniture, or vegetables and musical instruments) and was scored in accordance with the semantic fluency rules in addition to:

1) A word may be a repetition error but still count towards a correct switch (e.g., in the sequence "apple, bed, orange, bed", bed is a repetition error, but was still counted as a correct switch).
2) Regarding fruits and vegetables: Items commonly regarded to belong to the other category were permitted (e.g., tomatoes).
3) Regarding furniture: Furniture was defined as any item that is commonly sold in a furniture store (i.e., lamps, rugs beds, but not televisions and refrigerators).
4) Regarding musical instruments: Instruments that could also be toys were allowed (e.g., kazoo, harmonica).

The category switching task had two main outcomes (i) total correct responses, calculated by summing all correct responses from both categories, regardless of switching accuracy and (ii) total switching accuracy, calculated by summing correct across-category switches. A correct switch was defined as naming an item from one category, followed by an item from the other category.

All tests were scored twice by the same scorer. Words that could not be clearly heard when transcribing were marked as correct. In addition to the task-specific criteria above, set-loss errors were defined as any response that violates any of the criterion rules of the condition. Repetition errors were defined as an identical word (e.g., dog and dogs would be permitted) that was repeated any time within the trial (e.g., using snakes for the "S" trial and again for the animal trial would be permitted). Repeated words with multiple meanings were marked as incorrect unless the participant specified that it was a different word. The primary measures for each sub-task were total correct responses and switching accuracy for the category switching subtask.

2.6.2.3.2 Tower of London (TOL)

The TOL task was a primary measure in **Chapter 3**. The TOL task assesses planning ability and has been associated with slow oscillation power (Anderson & Horne, 2003; Shallice, 1982). PowerSleep used a computerised version of the task with six levels of increasing difficulty and was counterbalanced. Participants were required to rearrange beads on pegs to match a target picture in a limited number of moves. If the participant chose to restart the puzzle, or went over their allotted moves, the puzzle was reset. Three attempts were allowed per puzzle. Planning time was defined as the time from when the puzzle appeared, to when the first move was played. If multiple attempts were made, planning time was summed over all attempts to generate a final score for that puzzle. Planning time was averaged across all six puzzles.

2.6.2.4 Neurocognitive Test Battery 2

2.6.2.4.1 N-back

N-back is a primary measure in **Chapter 3**. The N-back assesses working memory (Kirchner, 1958), which is mediated by SWS. Study 1 utilised a computerised version of the task with 4 levels of increasing difficulty. Participants were shown a sequence of letters (each displayed for 1 second) and indicated using a response box when they saw (i) a letter "X" on the screen (0-back), (ii) a letter repeat itself immediately (1-back), (iii) a letter that was presented two characters back (2-back), or (iv) a letter that was presented three characters back (3-back). A prompt displayed at the start of each set indicated the level of difficulty (0–3 back). Each N-back type was split into 4 sets of 11 letters, with 3 correct targets per set. Both N-back sets and letter sequences were pseudo randomly distributed. Two versions of the task were used for practice, while two were split between STIM and SHAM for counterbalancing.

Hit rate was defined as *total correct matches/12*. For instances where the hit rate was 1, hit rate was corrected to 0.999. False alarm rate was defined as *total false matches/36*. For instances where the false match rate was 0, false alarm rate was corrected to 0.001. The primary dependent variable was the discriminability index (d'), calculated by subtracting the z-score of the false alarm rate from the z-score of the hit rate. A d' score was calculated for each N-back type.

2.6.2.4.2 Go/No Go (GNG)

The GNG task is a primary measure in **Chapter 3**. The GNG is a measure of inhibitory control sensitive to changes in SWS (Mander et al., 2010). Participants were shown a sequence of items matching in either colour or shape (e.g., small pink arrow, large pink arrow, large pink cone, small pink cone; displayed for 1.5 seconds) and were required to press a button on a response box for three of the four shapes, but to withhold a response for the fourth shape (Drummond, Paulus, & Tapert, 2006). The "no go" shape was pseudo randomly distributed between the "go" shapes 57 times out of

181 trials, reflecting a 70:30 distribution of Go/No Go response. One version of the task was available for practice, with two for counterbalancing between STIM and SHAM.

Hit rate was defined as *total correct matches/124*. For instances where the hit rate was 1, hit rate was corrected to 0.999. False alarm rate was defined as *total false matches/57*. For instances where the false match rate was 0, false alarm rate was corrected to 0.001. The primary dependent variable was the discriminability index (d'), calculated by subtracting the z-score of the false alarm rate from the z-score of the hit rate.

2.7 SmartSleep Summary

The SmartSleep study was conducted from approximately March 2017 to August 2019 at the following research laboratories: Clayton Sleep Institute, St. Louis, Missouri; NeuroTrials Research, Atlanta, Georgia; Preferred Research Partners, Little Rock, Arkansas; Sleep Disorders Center of Alabama, Birmingham, Alabama; University of Pennsylvania Unit for Experimental Psychiatry, Division of Sleep and Chronobiology, Philadelphia, Pennsylvania –Site 4 and The Center for Sleep & Wake Disorders, Chevy Chase, Maryland. As SmartSleep was based on PowerSleep, the protocols are similar, in that they each assess the impact of acoustic stimulation (STIM versus SHAM design) on SWA and next day outcomes [Executive Function (**Chapter 3**), Attention and Vigilance (**Chapter 4),** and Heart Rate Variability (**Chapter 5**)]. However, the additional aim of the SmartSleep study was to examine the impact of consecutive nights of stimulation on SWA and *alertness in habitually sleep-restricted adults*. This study consisted of one week of at-home monitoring with actigraphy and sleep diaries, following by a 2-night, 1-day laboratory stay, with the alternate condition completed the following week on the same day (i.e., Tuesdays). An adaptation night occurred prior to randomisation into the study. For one week, the device would be set to play tones during SWS (STIM) during both nights; during the other week, the device would play tones at inaudible volumes (SHAM).

As all participants were working full-time, they were permitted to go to work following the first laboratory night. On the second experimental days, participants completed attention and alertness batteries at 1-, 2-, 4-, 6-, and 8-hours post-wake. The SmartSleep study was approved by the Western Institutional Review Board and conducted in accordance with the Declaration of Helsinki; participants gave written informed consent. As the SmartSleep study was designed to understand the use of acoustic stimulation in real-world settings, screening criteria were less stringent than those employed for the proof-of-concept PowerSleep study (See Table 1).

2.8 Study 2: Participant Screening and Eligibility Criteria

2.8.1 Stage 1: Initial Screening Criteria

A three-step screening process was used to recruit and screen participants: (1) a telephone interview to screen for basic study criteria, (2) an in-person participant information and consent meeting and, (3) a sleep disorder screening, with full eligibility criteria listed in Table 1. Telephone screening obtained basic demographic and study eligibility criteria including:

- English fluency
- Age
- Participation in an interventional research study in the past 30 days
- Sex
- If female, pregnant or breast feeding
- Smoking status and/or nicotine replacement use
- Work schedule (including shift work)
- Travel across time zones
- Height and weight to determine BMI
- Items to determine ISI score

- Sleep/wake habits
- Major sleep or health disorders
- Medication use impacting the CNS
- Alcohol and caffeine consumption per week
- Items to determine STOP-BANG score

2.8.1.1 Work/Sleep schedule

This study recruited individuals who reported sleeping between 5–7 hours a night during the work week, and at least an hour longer during weekends, either through long sleep periods, or daytime naps. Participants had voluntarily truncated sleep, rather than poor sleep due to sleep disorders. Participants were also required to be full time workers (e.g., 4 x 8-hour days or 5 x 10-hour days).

2.8.2 Stage 2: Pre-Study Screening

Participants attended a participant information and consent meeting at the sleep laboratory, where they were re-briefed on the study protocol and aims. Pending consent, the following were evaluated, with inclusionary criteria listed in Table 1:

- Work and non-work schedules
- Medical History Questionnaire and Review of Systems
- ESS
- Insomnia Severity Questionnaire
- Cambridge-Hopkins Questionnaire (RLS screening)
- STOP-BANG Questionnaire
- Questions about sleep schedule and sleep quality
- BMI
- Respiratory rate

- Blood pressure

2.8.2.1 Cambridge-Hopkins Screening Questionnaire

The Cambridge-Hopkins Questionnaire is a 2 to 13 item questionnaire that assesses the presence of restless legs syndrome (RLS) (Allen, Burchell, MacDonald, Hening, & Earley, 2009). The participant only continued with the questionnaire if they answered "Yes" to either of the first two questions, which asked whether they have ever had "recurrent uncomfortable feelings or sensations in their legs" or "feel the urge to move their legs while sitting or lying down". The remaining questions gauged the severity and circumstances of their symptoms, such as "Which times of day are these feelings in your legs most likely to occur?" or "When you actually experience the feelings in your legs, how distressing are they?". The Cambridge-Hopkins questionnaire has high sensitivity and specificity (Allen et al., 2009). Diagnoses are dichotomously split into "Definitely has RLS/Does not have RLS". Participants were only excluded if they were within the high-risk category, as RLS was verified during the baseline diagnostic night.

2.8.2.2 Insomnia Severity Index (ISI)

The ISI is a 7-item self-report questionnaire that assesses sleep quality and insomnia severity (Bastien, Vallieres, & Morin, 2001). Each item relates to insomnia symptoms or how the participant perceives how their symptoms impact their quality of life. All items were rated on a 5-point Likert Scale, ranging from 0–4, with higher scores indicating greater severity or distress. Items were summed to create a global insomnia score. Global scores were divided into four levels of severity. A score below 7 indicated no symptoms of clinically significant insomnia, from 8–14 indicated subthreshold insomnia, 15–21 suggested moderate insomnia and 22–28 suggested severe levels of insomnia. Scores >22 were exclusionary.

2.8.3 Stage 3: In-Laboratory Assessments

As baseline nights were used to acclimate participants to the laboratory environment and as a final screening phase for sleep disorders, they occurred immediately following their one-week of at-home monitoring. Electroencephalography data for all nights was recorded using a bilateral 7–channel montage (F3, F4, C3, C4, O1, O2, Fpz) with all electrodes being referenced to the contralateral mastoid (e.g., C3-M2; C4-M1) on the following PSG software: Sandman 10.1 SD32/Elite 9.2; Grass Aurora, Neurovirtual BWII System; Alice 6; Compumedics Siesta 802.11A; and Embla Rembrant Money. Two EOG channels (left and right outer canthi) and three EMG (sub-mentalis) channels were used to score for eye movements and muscle activity, respectively. Polysomnography equipment included a nasal cannula and thermistor to measure oral and nasal air flow, a pulse oximeter to measure oxygen saturation, thoracic and abdominal respiratory bands to measure respiratory effort, and two electrodes on each leg to assess periodic limb movements. Participants wore the DeltaBoost headband to screen for alpha-delta sleep, and to determine whether they would need the sensitive or standard tone configuration during the experimental nights.

2.9 SmartSleep: At-Home Monitoring

Eligible participants were instructed to maintain their regular sleep schedule for the duration of the study. Participants wore an actiwatch and completed a sleep diary for a minimum of 6 days prior to their baseline in-laboratory stay. This ensured they had a stable work/sleep schedules and was used to calculate their average sleep duration for the work week. The data was verified during admit on the baseline night.

2.10 SmartSleep: In-Laboratory Protocol

2.10.1 Experimental Nights

Participants returned to the laboratory for their first experimental night on Tuesday evenings to keep schedules consistent within and between participants and fitted with standard PSG and the SmartSleep device. Participants were given a sleep opportunity equivalent to their habitual sleep duration for the work week, as determined through actigraphy. During each sleep period, the device was configured to play tones phase-locked to the first slow wave on the up-phase and pulsed at 1 Hz (STIM) or inaudible tones (SHAM). For mornings after the first sleep period, participants were completed a morning alertness battery (KSS, Samn-Perelli Fatigue Scale, and Visual Analogue Scale for Sleep Quality) within 60 minutes of waking. They were permitted to go to work to emulate real-life conditions, although napping was not allowed. Participants returned to the laboratory 4 hours prior to their HBT, refitted with PSG and SmartSleep equipment, and provided with a second sleep opportunity. The full protocol for Study 2 is depicted in Figure 4.

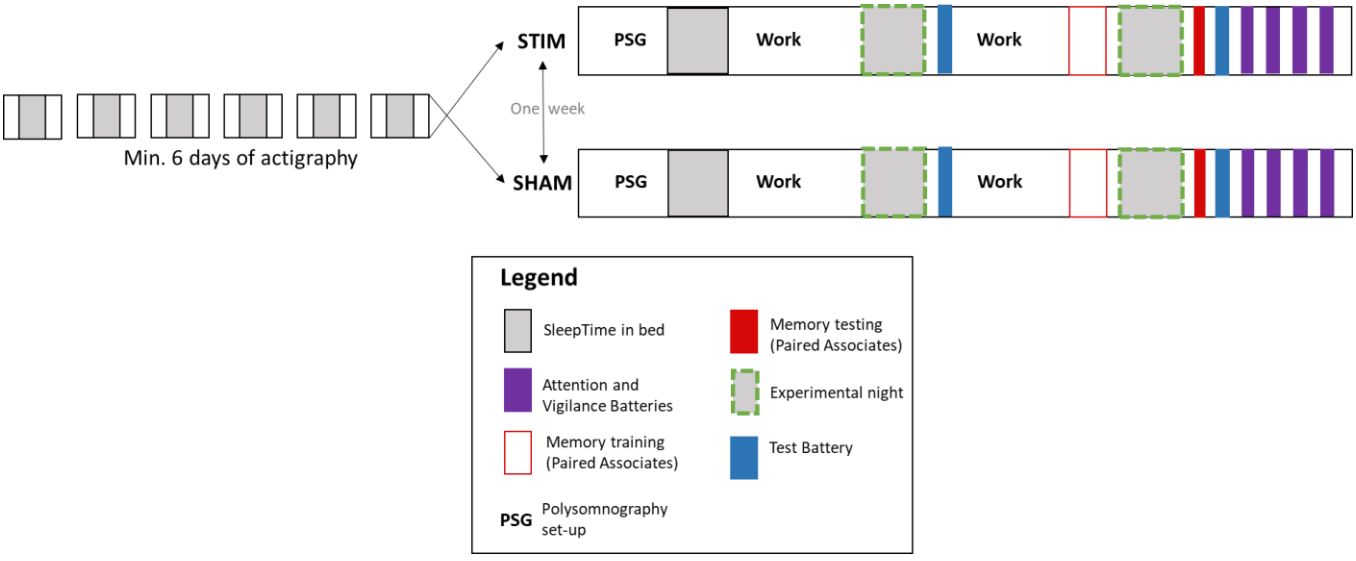

Figure 4. Study 2 in-laboratory protocol (Chapter 4). The full protocol ran over 16 days. After a minimum of 6 days of actigraphy, participants were admitted to the sleep laboratory approximately 4 hours prior to their HBT on a Tuesday. Participants were fitted with PSG and the SmartSleep device and given a sleep opportunity equal to their habitual sleep duration. Participants completed a brief alertness battery within one hour of wake-time, and were discharged for the day, returning 4 hours prior to their HBT. The same sleep conditions were repeated. On the final testing day, participants completed the full alertness battery from 2 hours post-wake, and every 2 hours until discharge at 8.5 hours post-wake. Participants returned the following Tuesday to complete the alternate condition.

2.10.1.1 Sleep and Spectral Analysis

PSG data was scored visually according to standard AASM criteria (Berry et al., 2017). Data were filtered between 0.3–35 Hz, with a 60 Hz Notch filter. Minutes of TST, N1, N2, N3, REM sleep, WASO and number of arousals were calculated from the scored data. Device data was automatically scored using an in-built scoring algorithm (Garcia-Molina et al., 2018). Device data was first validated against PSG data to compare scoring accuracy. As Study 2 aimed to replicate the user experience within the home, spectral analyses were run on device data, i.e., what was recorded when the device detected N3 and played tones, rather than the laboratory PSG (**Chapter 3 and 5**). Power spectral analyses were run using a custom MATLAB script, described in Garcia-Molina et al. (2018). Spectral analyses are a primary outcome in **Chapter 4**. Absolute and relative spectral power were reported for Night 1, Night 2 and as a sum of the two nights. SWE was calculated by summing SWA across each 6-second N3 epoch and dividing by total number of epochs. As the algorithm employed by the device is designed to only deliver tones during N3, there was no concern that there would be "enhanced" epochs included in the denominator, Subsequently, relative spectral power was calculated

as the ratio of SWE within the total spectral band from 4.5–30 Hz (as opposed to REM as in the PowerSleep study).

2.10.2 SmartSleep: Alertness and Vigilance Batteries

The day after the second experimental night, participants completed the morning alertness battery 1-hour post-wake. The remaining alertness and vigilance batteries commenced with a MSLT, followed by a PVT, KSS and Samn-Perelli Fatigue Scale at 2-, 4-, 6- and 8- hours post-wake. Participants were discharged after the completion of the final alertness battery.

2.10.2.1 PVT

The SmartSleep study used the shortened version of the PVT (PVT-B) (Basner & Dinges, 2011). The PVT-B runs for 3 minutes and has been validated against the standard PVT under well-rested, sleep deprivation, and partial sleep deprivation conditions. Tests were completed on an iPad through the Joggle Research platform (Joggle Research, Inc., Seattle, WA). PVT-B outcomes are a primary measure in **Chapter 4**. Mean reaction time (RT) and Fastest 10% RT were transformed as *1/RT*. Lapses were defined as no response over 355ms and standardised using the square root transformation *(√total lapses) + (√total lapses +1)* (Basner & Dinges, 2011).

2.10.2.2 Multiple Sleep Latency Test (MSLT)

The MSLT is a measure of objective sleepiness (Carskadon & Dement, 1982). SmartSleep used the modified MSLT, which consists of 4 20-minute nap opportunities, beginning from 2 hours post-wake and repeated every two hours. Participants completed the task in a dark room, lying on a bed, where they were instructed to "Please lie quietly, keep your eyes closed and try to fall asleep". The test ended when up to 3 consecutive epochs of sleep occurred, or after 20 minutes, whichever occurred first. MSLTs were scored using standard criteria, using a bilateral 6-channel montage (F3, F4, C3, C4, O1, O2), referenced to contralateral mastoids (e.g., C3-M2). The primary outcome was

sleep latency, defined as the time it took for the participant to reach 3 consecutive epochs of N1, or any other stage of sleep. The MSLT is reported in **Chapter 4**.

2.10.2.3 Samn-Perelli Fatigue Scale

The Samn-Perelli Fatigue Scale is a 7-point scale that assesses subjective fatigue (Samn & Pirelli, 1982). The responses range from "1 – Fully alert, wide awake", to "7 – Completely exhausted, unable to function effectively", with a mid-point of "4 – A little tired, less than fresh". The Samn-Perelli Fatigue Scale is highly associated with the ESS (Smith et al., 2018). The Samn-Perelli Fatigue Scale is a primary outcome in **Chapter 4**.

2.11 Overall Summary

In summary, the main aim of both PowerSleep and SmartSleep were to assess the impact of acoustic stimulation on SWA, and the subsequent changes in cognition. Both studies utilised a randomised, blinded, crossover design, and both studies used an automated, acoustic device. The key differences between PowerSleep and SmartSleep include (i) one versus two nights of stimulation, (ii) healthy adults versus habitually sleep restricted adults (iii) middle aged males versus males and females across a broad age range, and (iv) overnight memory consolidation and executive function outcomes versus attention and alertness outcomes.

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Chapter 3

Acoustic slow wave sleep enhancement via a novel, automated device improves executive function in middle-aged men

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Preface to Chapter 3

As reviewed in **Chapter 1**, SWS is essential for cognition. However, SWS declines steadily with ageing. Acoustic stimulation is a novel method of enhancing SWS with the benefit of being safe, accessible, and easy to use within the home. Previous studies examining the impact of acoustic stimulation have focussed on either young, or older adults. Given the significant reduction in SWA during midlife and coupled with the necessity for optimal cognitive performance in the workplace, middle-aged adults are a unique population to target for slow wave enhancement. Similarly, most outcomes centred around overnight memory consolidation, despite the role SWS plays in multiple areas of cognition. In **Chapter 3**, we present the first study to examine the impact of an automated, acoustic device on SWS and the subsequent changes in executive function in a population of healthy, middle-aged men.

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ORIGINAL ARTICLE

Acoustic slow wave sleep enhancement via a novel, automated device improves executive function in middle-aged men

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Abstract

Study Objectives: As slow-wave activity (SWA) is critical for cognition, SWA-enhancing technologies provide an exciting opportunity to improve cognitive function. We focus on improving cognitive function beyond sleep-dependent memory consolidation, using an automated device, and in middle-aged adults, who have depleted SWA yet a critical need for maximal cognitive capacity in work environments.

Methods: Twenty-four healthy adult males aged 35–48 years participated in a randomized, double-blind, cross-over study. Participants wore an automated acoustic stimulation device that monitored real-time sleep EEG. Following an adaptation night, participants were exposed to either acoustic tones delivered on the up phase of the slow-wave (STIM) or inaudible "tones" during equivalent periods of stimulation (SHAM). An executive function test battery was administered after the experimental night.

Results: STIM resulted in an increase in delta (0.5–4 Hz) activity across the full-night spectra, with enhancement being maximal at 1 Hz. SWA was higher for STIM relative to SHAM. Although no group differences were observed in any cognitive outcomes, due to large individual differences in SWA enhancement, higher SWA responders showed significantly improved verbal fluency and working memory compared with nonresponders. Significant positive associations were found between SWA enhancement and improvement in these executive function outcomes.

Conclusions: Our study suggests that (1) an automated acoustic device enhances SWA; (2) SWA enhancement improves executive function; (3) SWA enhancement in middle-aged men may be an important therapeutic target for enhancing cognitive function; and (4) there is a need to examine interindividual responses to acoustic stimulation and its effect on subsequent cognitive function.

Clinical trial registration: This study has been registered with the Australian New Zealand Clinical Trials Registry. "The efficacy of acoustic tones in slow-wave sleep enhancement and cognitive function in healthy adult males". https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=371548&isReview=true

Registration: ACTRN12617000399392.

Statement of Significance

Slow-wave sleep is critical for cognition. Middle age represents a unique target for slow-wave activity (SWA) enhancement due to depleted SWA and a need for optimal cognitive performance in the workplace. Our study describes a novel, automated acoustic stimulation device that enhances SWA and subsequently enhances executive function in middle-aged men. We provide evidence of a potential benefit of an automated device that can be readily deployed in at-home settings to enhance SWA and enhance cognitive function in the wider community.

Key words: slow-wave sleep enhancement; acoustic stimulation; cognition

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Introduction

Nonrapid eye movement (NREM) slow-wave sleep (SWS or N3) is homeostatically regulated and tightly associated with the duration and intensity of the waking day [1–4]. The electrophysiological signature of SWS is slow-wave activity (SWA), typically referred to as EEG power between 0.5 and 4.5 Hz, and its magnitude and dynamics are considered the primary marker of sleep homeostasis [1]. Beyond simply reflecting homeostatic sleep pressure, SWA serves a fundamental purpose to human function and behavior as it is critical for optimal cognitive functioning [5]. The link between SWA and cognitive function has prompted the examination of new strategies targeting the augmentation of SWA to maximize cognitive function, particularly in later life [6, 7] where SWA is severely depleted.

The link between SWA and cognitive performance has been well documented in older adults [5, 8], particularly their parallel inter-connected decline [9]. SWS however declines steadily from early adulthood [10, 11]: On average, SWS occupies 18.9% of the sleep period in young adults (16–25 years), and declines 2–3% per decade to occupy only 3.4% of the sleep period by middle age (35–50 years). Despite the marked reduction in SWA in mid-life, most studies of SWA and cognitive function have focused on either younger or older adults, where SWA is either maximal or depleted. Moreover, mid-life is a period where optimal cognitive capacity is required with respect to employment and productivity. For instance, more middle-aged adults are active in the workforce compared to younger (~30% more) and older (~60% more) adults [12], and middle-aged adults are at peak earning potential, relative to both younger and older adults [13]. Given the gradual depletion of SWA in this age group, easily employed techniques to enhance SWA and improve cognitive capability have important social and economic implications for middle-aged adults.

A number of approaches to SWA enhancement have been applied successfully, e.g., transcranial magnetic stimulation [14], transcranial direct stimulation [15], and pharmaceutical methods [16]. Although pharmacological or slow-oscillatory electrical stimulation approaches are effective at enhancing SWA, they present several limitations, including side effects such as sleep inertia, or are not feasible for application within the home. A more recent approach to SWA is acoustic stimulation. This method of enhancement provides a non-invasive tool that is cost-effective with no known side effects and with recent novel technological advancements can now be easily applied in the home setting.

Acoustic stimulation of SWA involves pulses of auditory tones specifically targeted to the upstate of the intrinsically generated 'natural' slow-wave oscillation which then entrain the endogenous slow-wave and increase its amplitude [17]. This has been shown in young [17–20] and older [7] adults. Importantly, acoustic enhancement of SWA has been associated with improved cognitive outcomes, particularly declarative memory [7, 17, 19], largely due to the important role SWA plays in the consolidation of declarative memories [5, 21, 22]. The benefit of SWA has also been linked to visuo-motor learning [23], perceptual learning [24], and executive function [8]; for which SWA-rich prefrontal areas [25] are essential for optimum function [26]. The extent to which SWA augmentation via acoustic stimulation benefits such cognitive functions beyond overnight memory consolidation is unknown.

Our study aim was to examine the effectiveness of an automated, self-regulating acoustic SWA stimulation device (SmartSleep, Philips Healthcare) in middle-aged men, and to evaluate any improvement in overnight memory consolidation and executive function. To achieve this, men between 35 and 50 years of age wore the device for two consecutive nights, repeated on two separate occasions 1 week apart: the first night acting as a baseline night and the second an experimental night where the device operated in either STIM (acoustic tones delivered) or SHAM (device operational but no audible tones delivered) mode. Day time cognitive functioning measures were evaluated following the experimental nights.

Material and Methods

Participant Screening and Recruitment

Twenty-four healthy male participants (aged 35–48 years, mean ± SD 39.92 ± 4.15 years) were recruited from the general population. Exclusion criteria included known history of any psychiatric or mood disorders including immediate family members, past or current presence of sleep disorders, current hypnotic or psychoactive drug use, hearing impairments, current smoker or use of nicotine therapies, a history of loss of consciousness greater than 15 minutes and transmeridian travel or shift work within the past 3 months of participation. Naps, alcohol, and caffeine consumption were limited to 2 naps per week, 14 standard drinks per week and 300 mg per day, respectively. Few participants napped during the study (4/24), and all naps occurred at least 3 days prior to laboratory admit and was consistent between conditions. Participants were within normal ranges for self-reported sleep quality, daytime sleepiness and mood (<5 Pittsburgh Sleep Quality Index [27]; <10 Epworth Sleepiness Scale [28], <5 Patient Health Questionnaire-9 [29], and Depression Anxiety and Stress Scale-21 (Depression: <6, Anxiety: <5, Stress: <10 [30]). As a check on hearing, all participants reported hearing the tones from the device at consent. All participants gave written informed consent and were compensated for their time. The study was approved by the Monash University Human Research Ethics Committee #CF15/671 – 2015000308.

Experimental Design

The full study protocol is summarized in Figure 1. We used a randomized, double-blind, sham-controlled, cross-over design whereby participants were exposed to a two-night protocol on two separate occasions separated by at least 5 days washout between visits. Participants not admitted on the same day of the week still had weekday admits (as opposed to weekend nights) as weekend/weekday sleep may differ [31]. As SWA is homeostatically regulated and tightly coupled to the length of the waking day [32], strict adherence to sleep/wake schedule based on their habitual sleep/wake cycle was required for 1 week prior to admittance into the laboratory. Participants were required to have a sleep efficiency of at least 80% and to comply with their sleep/wake schedule. During this period, they abstained from caffeine and alcohol for 48 hours prior to and during each laboratory visit. Upon admission to the study, participants completed a drug and alcohol screen, and sleep adherence was monitored through monitored through Actiwatch

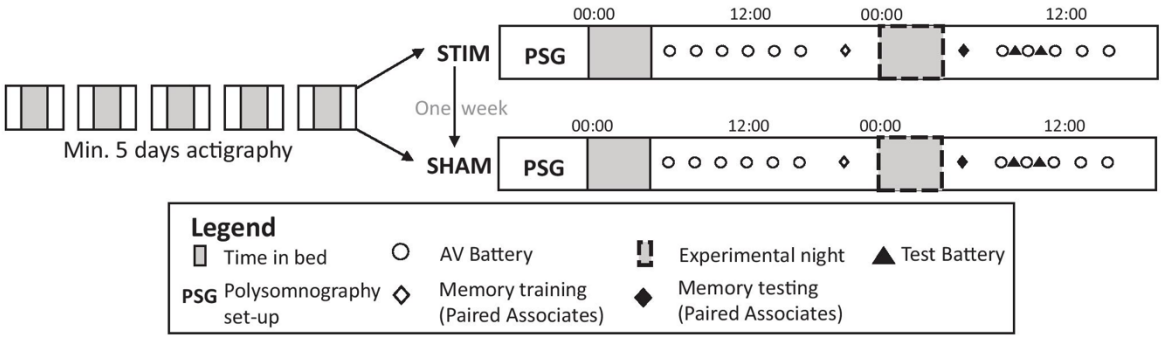

Figure 1. Depiction of laboratory protocol. On Day 1 of the protocol, participants completed Attention and Vigilance (AV) test batteries from 1.5 h post-wake and every 2 h thereafter. Karolinska Sleepiness Scale (KSS) was administered hourly. Participants undertook the encoding part of the paired associate task 2.5 h prior to habitual sleep time. On Day 2, attention and vigilance test batteries occurred at the same time. The paired associate task recall was administered at 1 h post-wake, and neurocognitive testing batteries occurred at 2 and 4 h post-wake. Participants were discharged approximately 10 h post-wake on Day 2.

Spectrums (Philips Respironics), sleep diaries and time-stamped call-ins. Full polysomnography including respiratory monitoring was conducted on Night 1 to screen for sleep apnea (Apnea–Hypopnea Index < 10) and periodic leg movements (PLMs) (PLMI with arousals < 5) with an 8-hour sleep opportunity starting at habitual sleep time.

Acoustic Stimulation

The device monitored EEG Fpz, referenced to Fp1 and M1. Acoustic volume was between 20 and 65 dB and was individualized such that the device automatically calibrated the auditory threshold on the baseline night to ensure the tone was sufficient to provide augmentation while not inducing an arousal. In the active condition (STIM), the device would administer an initial tone phase-locked to the up phase of the slow wave followed by tones at a frequency of 1 Hz, as described previously [33]. This continued until the end of the N3 period or if an arousal was detected and was repeated for each bout of SWS (N3). In the inactive condition (SHAM), the device monitored sleep without administering audible tones.

Polysomnography Recording

Electroencephalography (EEG), electrooculography, electromyography, and electrocardiography were recorded with Profusion polysomnography (PSG) 4 (Compumedics, Melbourne, Australia) and Grass gold-cup electrodes from 18 channels using the international 10–20 system (Fp1, Fp2, F3, F4, C3, C4, P3, P4, PO3, PO4, O1, O2, Fpz, Fz, Cz, Cpz, Pz, Oz). Data were sampled at 512 Hz; impedances were maintained at ≤5 Ω. On both baseline nights, thoracic and abdominal respiratory effort, airflow, finger pulse oximetry, and bilateral leg movements were monitored for diagnostic purposes.

Cognitive Tasks

Sleep-dependent memory was assessed with an episodic word-pair task with an encoding and criterion phase administered approximately 2 hours prior to bedtime, a short-delay recognition trial administered approximately 1 hour prior to bed time, and a long-delay recognition trial administered 1 hour post-wake. Other tasks were run in two test batteries to reduce any time on task fatigue [34]. The Tower of London and Verbal Fluency were administered in the first test battery (2 hours post-wake) and the Go No Go and N-Back (4 hours post-wake) were administered in the second battery. All tasks had alternate versions and order of administration was counterbalanced.

Paired Associate Learning Memory Test

The Paired Associate Learning measure of declarative memory uses 120 word-nonsense word pairs instead of semantically linked word pairs to encourage hippocampal-dependent learning [5]. The memory score for the short- and long-delay recall tasks was calculated as [number of words correctly identified – lure words – foil words]. The change score is calculated as [morning memory score – evening memory score].

Tower of London

The Tower of London measures nonverbal planning as described in Shallice [35]. The computerized version involved moving a set of blocks from a standard pattern to a target pattern in a limited number of moves. Participants completed six problems ranging in difficulty as per ref. [36] with the primary outcome measure being planning time.

Verbal Fluency

Participants had 60 seconds to verbally respond with words to a given prompt. Phonetic fluency was measured across three trials using letters as prompts (e.g. FAS and BHR), and semantic fluency was measured across two trials (animals and boys names; musical instruments and girls names), with number of correct words summed across trials. A category switching trial was administered, whereby participants had to switch between two categories (e.g. fruit and furniture), and number of accurate switches was calculated [37].

Go No Go

The Go No Go task is a measure of inhibition whereby participants must respond to three shapes (e.g. big triangle, small

triangle, big star; n = 124) and withhold response to a fourth shape (small star; n = 57), which resembles the others in form or size [38, 39]. The primary outcome measure is d', which is a ratio of correct responses to false (failure to inhibit) responses.

N-back

The *N*-back is a test of working memory, where participants are shown a randomized sequence of characters of the alphabet and must respond to characters that match those shown either immediate prior (1-back) or 2 positions back (2-back) [40].

Attention and Vigilance Battery

Each Attention and Vigilance battery consisted of a 10-minute psychomotor vigilance test, a Karolinska drowsiness test (3 minutes eyes open, 2 minutes eyes closed), and a Karolinska Sleepiness Scale at the beginning and end of each battery. This was not a primary outcome of the study but was used to check consistency between test sessions given the impact of sleep on cognitive alertness [41].

Sleep and Spectral Analysis

Sleep staging, sleep onset, and arousals were scored visually according to standard American Academy of Sleep Medicine criteria [42], channels were referenced to contralateral mastoid (e.g. C3-M1, C4-M1), and data were filtered between 0.3 and 30 Hz, with a 50 Hz Notch filter. Scorers were blinded to the experimental condition. Minutes of total sleep time, N1, N2, N3, rapid eye movement (REM) sleep, wake after sleep onset (WASO), and number of arousals were calculated from the scored data. Sleep cycles were determined according to previously used criteria [43]. Two participants had cycles 1 and 2 combined due to short (<15 minutes) first cycles (this was observed for both STIM and SHAM conditions; thus, data were treated equally for both conditions). Spectral analyses were conducted within Curry 7 software (Compumedics NeuroScan, Melbourne, Australia). Data were visually cleaned to remove artifact in 5-second windows. EEG data were digitally re-referenced to linked mastoids and then bandpass filtered with a zero-phase shift Hann filter from 0.3 to 30 Hz with 0.6 and 8 Hz slopes, respectively. Power spectra was calculated in nonoverlapping 5-second epochs, resulting in a 0.125 Hz resolution. Total absolute power was determined by averaging raw power across the night within each frequency bin and summed across frequency bands (0.5–4 Hz, etc.). As night-to-night variability in absolute power can have a large impact on EEG power [44, 45], we calculated relative power by expressing SWA as a function of total power within the REM bandwidth. Although other EEG studies may calculate relative power as a function of the total power spectra, this was problematic due to the power spectra of one condition (STIM) being systematically altered by the study condition. Expressing data relative to the full power spectra therefore would have canceled out the effect of the acoustic stimulation, i.e., if whole night data were used as the denominator for normalization, enhanced SWA would be both in the numerator and the denominator, thereby canceling itself out. Therefore, expressing EEG data relative to total power spectra in the absence of stimulation (i.e. during REM sleep) was utilized (we did not use N1 as this was associated with stage transitions, and N2 had, on occasion, acoustic tones delivered). For each frequency bin, relative EEG was calculated as absolute NREM δ power – $\bar{x}$ REM δ/SD REM δ. NREM was defined as NREM 2 + 3. As a check on the reliability of using REM for relative EEG, we replicated analyses with absolute data, checked REM power spectra across both conditions, and examined night-to-night stability in absolute delta power in NREM 2 + 3 relative to REM (i.e. to ensure one night does not involve an increase in NREM SWA which is absent in REM SWA), by assessing the ratio of NREM/REM delta power for baseline week 1 and baseline week 2.

Slow-wave energy (SWE) was calculated as SWA*#minutes N2+N3 for each cycle, which was then summed for each successive sleep cycle. As SWA reduces with each concurrent N3 cycle (due to the homeostatic reduction of SWA), examining SWA in full-night spectra can be artificially lowered for those exhibiting additional N3 sleep cycles. As this may be of benefit for those exhibiting SWA augmentation, we calculated SWE. SWE described the cumulative change in SWA (0.5–4 Hz) across the first four sleep cycles, while taking into account total number of (N2 + N3) epochs. Primary analyses are derived from C3 (except for n = 1 where C4 was substituted for both conditions due to loss of C3) as per previous studies of acoustic stimulation [20] and as k-complexes are maximal over fronto-central sites [46], and frontal sites were associated with more noise due to the placement of the acoustic stimulation headband.

Statistical Analysis

IBM SPSS Statistics V23 and GraphPad Prism V7.0 were used to run statistical analyses. Normality assumptions were checked using SPSS. Cycles were analyzed via a linear mixed model, with cycle as a fixed factor. Paired *t*-tests were run comparing SHAM and STIM if normality assumptions were met. Nonparametric data were analyzed using Mann–Whitney *U* or Wilcoxon matched-pair tests. Pearson's correlations were used to compare the relationship between SWE percent change and cognitive test percent change. Univariate outliers within cognitive results were defined as mean ± (2.5 × SD) and were corrected as next most extreme +1 [47]. Multivariate outliers were detected with Cook's distance and further examined to determine whether they were a valid case (i.e. not caused by artifact or study violations). To correct for multiple comparisons, we applied Benjamini–Hochberg's cut-offs for false discovery rate [48]. Unless stated otherwise, all results presented are mean ± SEM. Results under $p < .05$, two-tailed were considered significant.

Data Retention

One participant (4%) was not included in baseline sleep parameters due to missing data. For device operations (e.g. number of tones, Figure 2), there were missing data for two participants (total 8% data loss). One participant was excluded from analyses due to external sleep disturbances. For cognitive testing, participants were removed due to missing data or technical errors and subsequently when checked for outliers (according to Kolmogorov–Smirnov or Cooks distance). For verbal fluency, the phonetic fluency was administered incorrectly on two occasions (out of 48, 4.2% data loss) resulting in the loss of two (8.4%) (12.5%) participants. For the remaining cognitive tests, where data were statistical outliers only, results are presented with and without outliers for clarity.

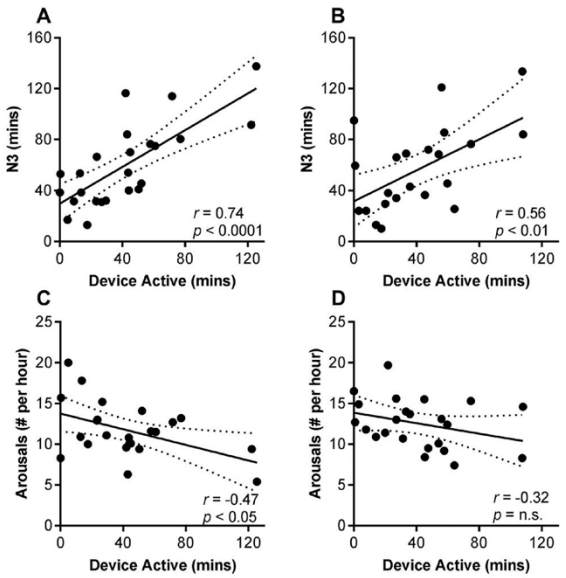

Figure 2. Relationship between device activity and sleep parameters. (A) Significant correlations between device activation and SWS were observed for STIM and (B) SHAM nights. A reduced number of arousals were associated with increased device activation for (C) STIM, but not (D) SHAM. Error is 95% confidence intervals of the regression line.

Table 1. Sleep outcomes for the one week prior to the study, and the baseline night to check consistency between conditions.

	Parameter	STIM	SHAM	P
One-week pre-study (Actigraphy)	Sleep onset	22.7 ± 0.2	22.7 ± 0.2	.3
	Sleep offset	6.3 ± 0.1	6.4 ± 0.1	.42
	Sleep efficiency	82.3 ± 1.7	83.5 ± 1.5	.92
	Sleep latency	9.8 ± 1.6	9.4 ± 1.2	.54
	WASO	56.0 ± 6.8	56.1 ± 7.9	.9
	TST	6.3 ± 0.1	6.5 ± 0.1	.8
Baseline night PSG Parameters	N1	22.4 ± 3.3	21.04 ± 2.5	.74
	N2	218.8 ± 6.8	221.89 ± 10	.76
	N3	82.2 ± 7.6	80.87 ± 7	.86
	REM	90.7 ± 4.4	91.57 ± 6.7	.9
	Sleep latency	8.72 ± 1.1	8.98 ± 1.7	.88
	Arousal Index	11.92 ± 1.2	11.56 ± 1.1	.74
	WASO	52.93 ± 7. 9	51.74 ± 6.7	.82
	TST (min)	6.9 ± 0.1	6.92 ± 0.1	.84

All results in decimal numbers unless stated otherwise. WASO, wake after sleep onset; TST, total sleep time; REM, rapid eye movement.

Results

Sleep Architecture Prior to Acoustic Stimulation

Due to the strong homeostatic regulation of SWS, we first confirmed there were no significant differences in actigraphically determined sleep during the week of at-home sleep preceding admission to the laboratory protocol ($p > .2$), nor any differences in PSG-defined sleep outcomes between STIM and SHAM for the baseline night ($p > .2$). See Table 1. In addition, there were no significant differences in relative whole night spectra when comparing the baseline nights for each condition (SHAM: 520.7 ± 59.6 μV^2 vs. STIM: 479.3 ± 65.2 μV^2, $p = .24$). See Supplementary Figure S1.

Acoustic Stimulation and Sleep Parameters—STIM Versus SHAM

We then examined the difference between STIM and SHAM device activation by examining the number of delivered tones and number of minutes of activation during each experimental night. No differences were observed in the number of tones delivered during STIM (2489.9 ± 408.5) or SHAM (2456.4 ± 373.4, $p = .38$), with a 0.56-minute difference in device activation between the two nights (STIM: 40.9 ± 6.2 minutes and SHAM: 41.5 ± 6.8 minutes), noting that the device was active, yet inaudible for SHAM. Strong correlations between STIM and SHAM for device activation ($r = .9$, $p < .0001$) were observed. As expected, given tone administration is dependent on the detection of the slow wave, device activation (number of minutes) was positively correlated with minutes of SWS during both STIM ($r = .74$, $p < .0001$) and SHAM nights ($r = .56$, $p < .01$). See Figure 2A and B, respectively. Device activation (number of minutes) was associated with a decrease in arousals during STIM ($r = -.47$, $p < .05$), but not SHAM ($r = -.32$, $p = .07$) (see Table 2). See Figure 2C and D, respectively. During STIM, increased device activation was also associated with a decrease in N1 and N2 sleep ($r = -.49$ and $-.44$, respectively, $p < .05$). No other associations were found between device activation and other sleep parameters, for STIM or SHAM mode (see Table 2).

Acoustic Enhancement of SWA

For the experimental night, we observed no difference in PSG-derived sleep parameters between STIM and SHAM conditions (see Table 3). We found no increase in absolute SWA in STIM relative to SHAM ($p = .15$) for full-night spectra, although large increases were found for cycle 4 for both delta power (0.5–4 Hz—SHAM: 185.01 μV^2 vs. STIM: 206.04 μV^2, $p < .05$, $d = 0.45$) and low-frequency delta (<1 Hz—SHAM: 143.55 μV^2 vs. STIM: 147.11 μV^2, $p < .05$, $d = 0.51$). No differences were observed in cycles 1–3 ($p > .2$), although cycle 2 had a moderate effect size ($d = 0.25$). Given that analysis of absolute data is not ideal for this study design (i.e. conditions on laboratory visits and large individual difference [44]), we focused our analyses on relative data [which was positively correlated to the absolute data for SHAM ($r = .5$, $p = .01$) and STIM ($r = .6$, $p = .003$)]. To demonstrate comparable changes in the absolute data however, we have replicated all analyses with absolute data as shown in Supplementary Figures S3–S5.

As EEG data were expressed relative to REM power spectra (as a clean, nonenhanced segment of EEG), we first ensured that changes in REM SWA were not driving any observed differences. First, we checked that there were no differences in power spectra for STIM versus SHAM for either the total REM power spectra (SHAM: 82.75 ± 6.38 μV^2 vs. STIM: 80.16 ± 6.24 μV^2, $p = .31$) or REM delta power (SHAM: 58.58 ± 6.02 μV^2 vs. 54.53 ± 4.75 μV^2, $p = .23$). Second, we demonstrated that the ratio of absolute delta power in NREM and REM sleep on the baseline nights was stable ($p > .15$), suggesting that the differences in relative SWA for STIM are not simply driven by an instability in NREM SWA and REM SWA week to week. As seen in Figure 3A, for whole night spectra we observed significantly increased power within the delta bandwidth (0.5–4 Hz) for STIM compared to SHAM ($t(22) = 2.65$, $p = .02$, $d = 0.65$). There was also decreased power in the alpha

Table 2. Pearson's correlations between device activation and PSG-sleep parameters

	STIM		SHAM	
Parameter	r	P	r	P
N1	−.49	.019	−.49	.08
N2	−.44	.035	−.44	.27
N3	.74	<.0001	.56	.003
REM	−.22	.305	−.29	.2
Arousal Index	−.47	.027	−.32	.07
WASO	−.098	.647	.16	.46
TST	−.015	.946	−.18	.23

WASO, wake after sleep onset; TST, total sleep time; REM, rapid eye movement.

Table 3. Sleep parameters for experimental nights

Parameter	STIM	SHAM	P
N1	13.33 ± 1.9	15.25 ± 1.5	.25
N2	225.21 ± 5.7	221.83 ± 7.7	.69
N3	59.67 ± 6.6	59.13 ± 7.1	.88
REM	112.63 ± 5.8	112.90 ± 5.4	.96
Sleep latency	12.94 ± 1.7	12.50 ± 1.5	.77
Arousal Index	12.52 ± 6.3	12.56 ± 2	.96
WASO	49.71 ± 4.9	53.71 ± 6	.53
TST	410.83 ±1.3	413.25 ± 32.9	.77

All results in minutes unless stated otherwise. WASO, wake after sleep onset; TST, total sleep time; REM, rapid eye movement.

(8-12 Hz) ($t(22) = 2.57$, $p = .02$, $d = 0.84$) and slow-sleep spindle (low sigma: 12–14 Hz) bandwidths ($t(23) = 2.71$, $p = .02$, $d = 0.87$). The magnitude of percentage change was high for delta, relative to other spectral bandwidths (see Figure 3B), corresponding to an 11.6% increase in delta power in STIM relative to the SHAM night. Within the delta bandwidth (Figure 3C), STIM enhanced SWA for the majority of 0.5 Hz bins (particularly <2 Hz) with small-to-moderate effect sizes ($d = 0.15$–0.52). Peak enhancement during the STIM night was found at 1 Hz ($t(23) = 2.7$, $p = .04$, $d = 0.62$), and decreased with each 0.5 Hz bin thereafter. Absolute data are shown in Supplementary Figure S3A–C. There were no significant changes observed for parietal regions (SHAM: 242.1 ± 109.5 μV^2 vs. STIM: 259.9 ± 66.9 μV^2, $t(22) = 0.69$, $p > .1$), and an increase observed in the frontal region (SHAM: 352.5 ± 28.9 μV^2 vs. STIM: 378.7 ± 29.9 μV^2, $t(22) = 2.3$, $p = .02$).

To examine changes across the night, we examined SWA in the first four sleep cycles. SWA was higher during STIM in cycle 2 (STIM: 623.05 ± 79.01 μV^2, SHAM: 512.51 ± 55.83 μV^2, Wilcoxon signed ranks test, $z = -2.68$, $p = .025$, $d = 0.71$) and cycle 4 (STIM: 270.74 ± 31.09 μV^2, SHAM: 208.62 ± 21.64 μV^2, $t(22) = 3.46$, $p = .01$, $d = 0.84$), whereas no enhancements were observed in cycle 1 ($p > .1$) or cycle 3 ($p > .1$) (see above for absolute data). Only 33% of participants in SHAM experienced a fourth N3 cycle, whereas almost twice as many of these same individuals (58%) had a fourth N3 cycle following STIM. We therefore examined SWE to show cumulative changes across the night. Here, SWE was higher in STIM relative to SHAM ($t(22) = 2.39$, $p < .5$, $d = 0.54$), with a moderate effect size (Figure 4). There were no differences in the distribution of sleep stages within each sleep cycle for STIM compared with SHAM, suggesting that the increase in SWE was not due to altered sleep staging (see Supplementary Figure S4).

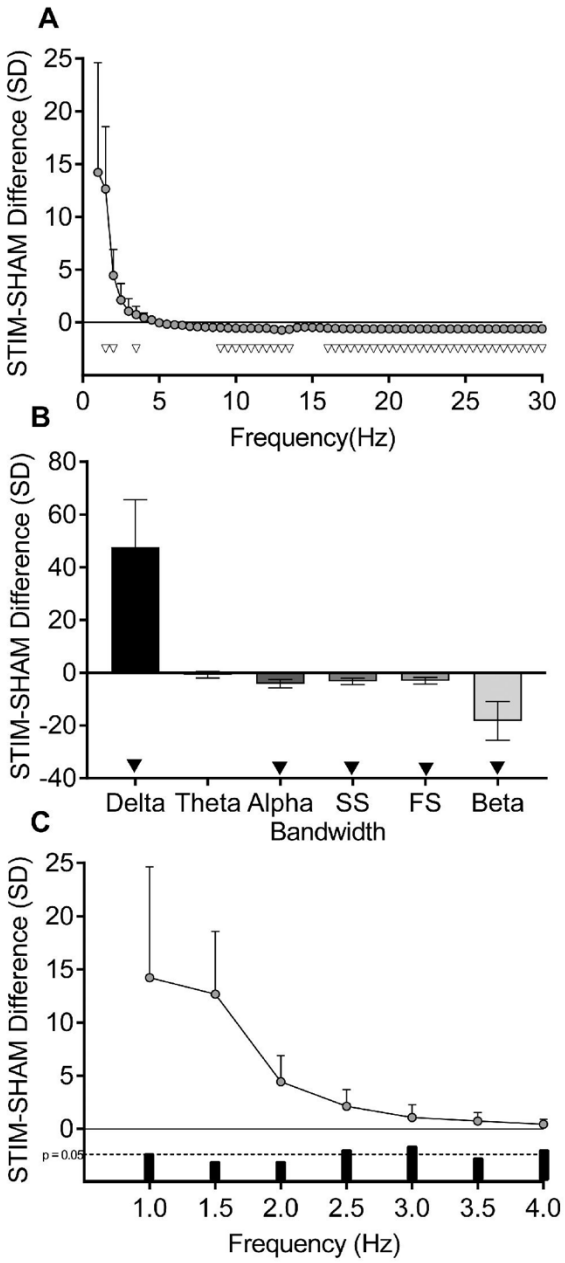

Figure 3. Acoustic enhancement of SWA during STIM relative to SHAM experimental nights. (A) Differences in relative power in full-night spectra for STIM night relative to SHAM, in 0.5 Hz bins normalized to delta power during all-night REM for all power spectra and (B) for each predetermined EEG spectral bandwidth including delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), slow spindles (SS, 12–14 Hz), fast spindles (FS, 14–16 Hz), and beta (16–30 Hz). (C) Enhancement of SWA in the delta range was greatest at 1.0 Hz, relative to other delta frequencies.

Cognitive Performance Following Acoustic Stimulation

For overnight memory consolidation, participants performed worse following a period of sleep relative to pre-sleep for both

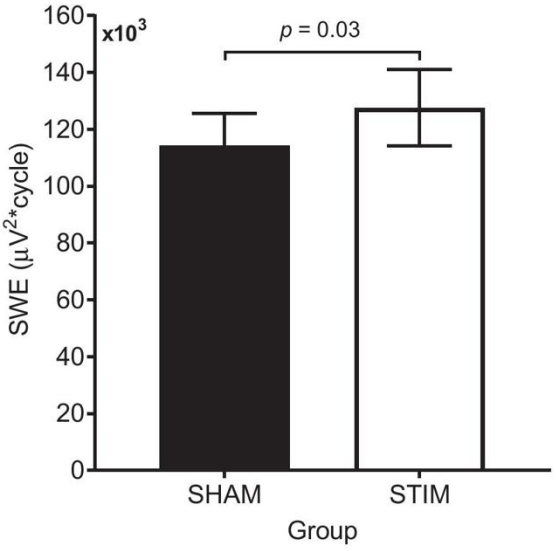

Figure 4. Change in slow-wave energy for STIM versus SHAM. (A) STIM increased slow-wave energy (SWA*#mins of N2+N3) across the full-night spectra and from the second sleep cycle compared with (B) SHAM. *$p < .05$. Error bars indicate SEM.

Table 4. Performance on Cognitive Tasks

Task	Subtask	STIM	SHAM	P
Paired associates	Change score	−0.17 ± 0.07	−0.21 ± 0.06	.71
Go No Go	d'	3.9 ± 0.18	3.75 ± 0.21	.33
N-back	0-back d'	5.84 ± 0.12	5.84 ± 0.13	.99
	1-back d'	5.34 ± 0.25	5.36 ± 0.25	.95
	2-back d'	3.75 ± 0.29	4.08 ± 0.31	.23
Tower of London	Planning	12.56 ± 1.22	13.55 ± 1.39	.5
Verbal fluency	Letter fluency	37.13 ± 2.4	37.32 ± 2.35	.37
	Category fluency	38.92 ± 1.75	38.87 ± 2.15	.8
	Switch total	13.46 ± 0.6	14.25 ± 0.62	.26

RT, reaction time; SET, set execution time.

STIM ($p = .02$) and SHAM ($p = .002$). Consistent with recognition typically being reduced following sleep, participants recognized fewer correct matches for STIM ($p = .04$) and SHAM ($p = .008$) and had higher false alarm rates (STIM: $p = .004$, SHAM: $p = .02$) on the Paired Associates task. No difference in memory consolidation was observed following STIM compared with SHAM ($p = .3$; Table 4). Similarly, no group differences were observed between STIM and SHAM on any measure of executive function ($p > .1$).

Individual Differences in Slow-Wave and Cognitive Outcomes

Over 65% of participants exhibited SWE enhancement (>3% enhancement were considered responders), whereas 30% had a decrease (>3% reduction were considered nonresponders), and 4% were stable (0% ± 3%) (Figure 5A). Due to these inter-individual differences in enhancement, examining cognitive performance at the group level may mask any cognitive improvement from SWA enhancement. For this reason, we applied individual level examination of the cognitive responses of responders against nonresponders. Participants were ranked for percentage change in SWA. Nonresponders ($n = 7$) were defined as those who had no SWE improvement on the STIM night and compared against the remaining responders ($n = 15$). Responders had, on average, a 28.36% enhancement in SWE compared with an 11.8% decrease in nonresponders (STIM median: 19.51, SHAM median: −9.12, Mann–Whitney $U = 0$, $n_1 = 15$, $n_2 = 7$, $p < .0001$; Figure 5B). Again, significant differences for these groups were observed for absolute data (STIM: 16.99 ± 3.7, SHAM: −12.19 ± 4.9, $t(20) = 5.9$, $p < .0001$) (see Supplementary Figure 4A and B). We then compared these groups for cognitive outcomes.

For cognitive outcomes (expressed as percent change following STIM relative to SHAM), responders showed greater improvement on Verbal Fluency when compared with nonresponders, for phonetic fluency ($t(18) = 2.37$, $p = .03$, $d = 1.3$) (see Figure 6A). A comparable improvement in cognition was also observed for working memory, whereby responders showed significant improvement in 2-back d', relative to nonresponders ($t(20) = 2.35$, $p < .05$, $d = 1.2$; Figure 6B). The same result was not observed for 1-back, likely due to ceiling effects for both conditions. When looking at individual performance, percentage improvement in performance was positively associated with the percentage of SWA enhancement for phonetic fluency ($r = .68$, $p < .5$, $n = 17$, with outliers [$r = .14$, $p > .5$, $n = 21$; Figure 6C], and working memory [$r = .56$, $p < .01$, $n = 222$, with outliers: $r = .4$, $p = .056$, $n = 23$]; Figure 6D). This was also observed for absolute data (see Supplementary Figure 5A–D). There were no significant differences between groups on measures of overnight memory consolidation ($p > .6$), inhibition ($p > .2$), or planning ($p > .5$).

Discussion

We describe the enhancement of SWA via a novel, automated acoustic stimulation device that can be readily deployed in at-home settings, in accordance with previous studies utilizing this specific stimulation method [33]. Moreover, we demonstrate that acoustic stimulation of SWA may lead to enhanced cognitive function, beyond sleep-dependent declarative memory, and during a stage of life marked by depleted SWA, and yet, requiring perhaps the largest cognitive capacity in the workplace. Our study therefore provides unique evidence for a wider use of acoustic stimulation within the general population to enhance SWA during sleep with an aim of improving cognitive function.

Our study in middle-aged men replicated existing literature showing SWA enhancement via acoustic stimulation in young [19] and older adults [7]. Although there was no change in the time spent in SWS (N3), SWA was enhanced due to an amplification of the slow oscillations. Taking into account previous studies of younger and older adults and notwithstanding the different methodological approaches, there does appear to be a dose–response association to the magnitude of the enhancement as a function of age. For instance, the percentage improvement in SWA was 15.6% (±5.6%) for younger adults [19], 14.3% (±5.82%) for middle-aged adults (our study), and 8% (±2%) for older adults [7]. Given the well-known depletion of SWS with age [49], and the need for the presence of SWS for the acoustic stimulation to be administered to the up phase of the slow oscillations, this would be expected. Future studies should confirm this with the use of the same experimental design and acoustic stimulation approach within a wide range of ages.

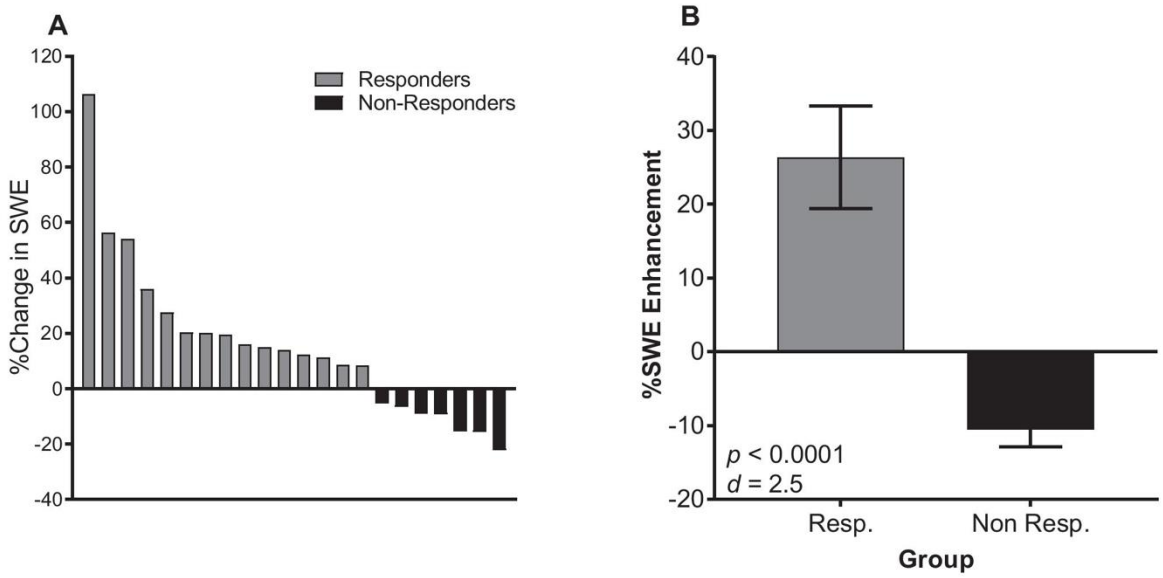

Figure 5. Individual differences in slow-wave energy (SWE) between responders and nonresponders. (A) Percent change in relative SWE (STIM-SHAM/SHAM*100) for each individual and (B) all nonresponders (< –3% change; *n* = 7) compared with responders (>3% change; *n* = 15). Error bars indicate SEM.

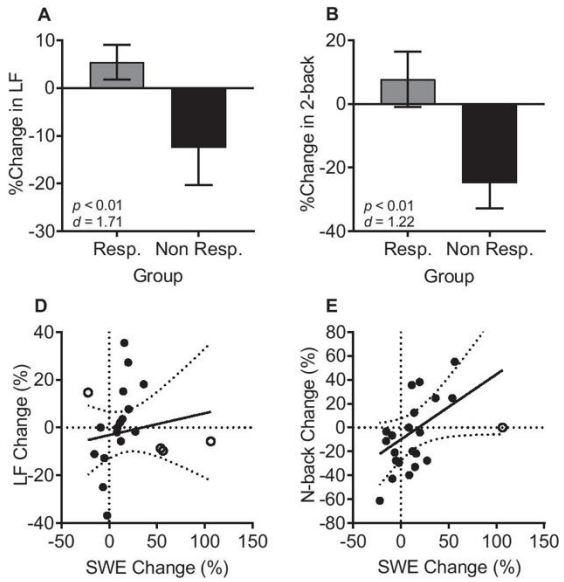

Figure 6. Changes in slow-wave energy and cognition from STIM to SHAM for responders and nonresponders. Comparison of responders (n = 15) and nonresponders (n = 7) responders on cognitive performance (top row) for (A) phonetic fluency and (B) 2-back d'. Significant positive associations were observed between magnitude of SWE change and cognitive improvement (bottom row) for (C) phonetic fluency and (D) 2-back d'. For (A) and (B), error bars indicate SEM. For (C) and (D), error is 95% confidence intervals of the regression line.

Previous studies have not shown an enhancement of SWA across the full-night spectra, but rather during STIM-ON periods versus STIM-OFF within the same sleep period (e.g. refs. [7, 19]). Contrary to this, we did report a general increase in SWA when comparing whole night spectra between STIM and SHAM. This could be driven by differing technologies [7], different analysis approaches (previous studies normalized to device-OFF periods, whereby we normalized to REM as an OFF period of device activation), or age group (although younger people appeared more responsive to SWA enhancement). Although we found relatively stable intraindividual differences in device activation (STIM vs. SHAM, albeit the latter with no sound), we did observe a wide range of interindividual responses to acoustic stimulation; this was also reported in an older age range [7], although less so in younger individuals [19].

We reported no significant improvement in cognitive outcomes at the group level (STIM vs. SHAM), which we attribute to the large individual differences in SWA enhancement (–22.04% to 106.38%). Responders however had a clear and significant improvement in multiple cognitive outcomes (phonetic fluency and working memory), compared with nonresponders. Moreover, the magnitude of SWA enhancement was positively associated with the magnitude of improvement in these executive functions: the more SWA was enhanced, the greater the improvement in next-day verbal fluency and working memory. Although we have previously demonstrated that <1 Hz SWA was linked to better performance on tasks of executive function [8], no causality could be attributed. The observation of experimental manipulation of SWA, either its depletion or enhancement, provides strong evidence of causality; thus, we provide further evidence of a causal role of SWA in these improved cognitive outcomes, which is in line with previous studies [5]. The benefit of SWS on next-day performance for cognition has been previously reported [50], which may be attributable to the reorganization of large-scale cortical networks [51] caused, in part, by the synaptic downscaling during SWS [52]. Contrary to previous studies assessing the cognitive impact of SWA enhancement, we found no improvement in sleep-dependent memory consolidation, either at the group level or at the individual level. We believe these null findings may be due to the task we used, as participants reported difficulty with the Paired Associates task (we

did report lower memory retention rates than reported previously [5]). This may have been due to the use of nonsemantically related words, which is known to reduce the capacity for mnemonic strategies [53] and which differs to previous studies of acoustic stimulation which utilize tasks with semantically meaningful words [7, 19]. This enhanced difficulty in task completion is further supported by the lack of overnight improvement in both conditions (i.e. morning recall was always worse).

Our study is the first to specifically target middle-aged men. Previous studies had targeted either healthy, young adults (which is typical for proof of concept studies) [19] or older adults [7], given the association between SWA and memory decline in later life. Middle age, however, represents a clear target for SWA enhancement. Beyond the age of 35 years old, SWA rapidly depletes [10], at a rate of 2%–3% per decade. The fourth and fifth decade of life (30–50 years) however necessitates optimal cognitive capacity with respect to employment and productivity. Our data suggest that middle-aged adults, particularly those who rely on high executive function demands, such as flexible thinking, communication and updating of information, may maximize waking outcomes with this approach. In addition, the enhancement of SWA during sleep has been argued as a potential therapeutic and preventative strategy for reducing the risk of developing dementia caused by Alzheimer's disease [54]. Although SWA does indeed improve memory function in healthy, cognitively intact older adults [7], the extent to which this approach would prevent or delay the development of cognitive pathologies is unknown. Given that the neurological indices of dementia can precede significant cognitive dysfunction (particularly memory) by over a decade [55], early intervention is essential and our data provide the first evidence of the capacity to enhance SWA in middle age. As society ages, and dementia risks continue in an upward trajectory (by 275% by 2056 in Australia [56] and 150% in the United States by 2060 [57]), acoustic stimulation may offer a viable solution to the Institute of Medicine's recommendation for targets to improve cognitive outcomes in older adults [58]. As we have shown an independent validation of a commercially available device, SWA enhancement via acoustic stimulation represents an exciting modifiable sleep target for improved cognitive function in the wider community. Future studies should examine the utility of a long-term use of an easy-to-use, at-home acoustic stimulation device on cognitive function in older adults.

Our study has a number of limitations. First, and consistent with other proof of concept studies, we employed strict screening criteria to ensure our population was as homogenous as possible. The extent to which those with poorer sleep, sleep disorders, or daytime dysfunction would benefit from this approach remains unknown. Second, due to clear sex differences in SWA [59], we chose to focus only on middle-aged men as SWA depletion is heightened for men [60]. As such, our study findings may not be applicable to women. Third, normalizing SWA power spectra to REM may not be considered a typical approach; however, this was necessary for our study design. Although relative EEG power is typically derived relative to the full power spectra, this was not appropriate in our study (see Materials and Methods). Moreover, we were unable to examine within-night, epoch-by-epoch comparisons of STIM-ON to STIM-OFF, as per previous studies (e.g. refs. [7, 17, 19]) as ours was an independent evaluation of an automated device (i.e. we had no access to that data). We do note, however, that the device we used has previously been shown to enhance SWA in ON epochs relative to OFF epochs [33]. As we report no differences in REM power spectra, demonstrate stability in NREM SWA/REM SWA ratios across nights, and that the absolute data support our relative EEG data, we do not believe this approach accounted for the observed differences in relative SWA/SWE following STIM, as described here.

Beyond addressing these limitations, our study suggests several avenues for future research. First, future studies should consider the effects of acoustic SWS augmentation on cognitive domains beyond memory consolidation. Second, and as mentioned previously [7], as this technology moves toward repeated at-home use, future studies should examine the long-term effects of acoustic enhancement on cognition and physiology more broadly in ecologically valid settings. Finally, more research needs to be conducted to identify factors that determine interindividual responses to acoustic stimulation to optimize it as a viable option for SWS enhancement in the wider community. This is particularly important as it appears that acoustic stimulation may not be effective for all individuals. As mentioned above, repeated use of acoustic stimulation would also allow us to elucidate trait characteristics between responders and nonresponders, which is a necessary next step in determining the potential positive impact of acoustic stimulation for the wider community. With further research, particularly with devices that can be readily employed outside the confines of the laboratory, the future for SWA enhancement presents as an exciting opportunity for improving memory and executive function across the lifespan, and may prove to be a critical tool for preventing or slowing down cognitive decline in later life, particularly for dementia where decaying SWA may moderate the behavioral (memory decline) and biological (beta-amyloid, tau protein) antecedents of disease progression [5, 54, 61]. Until then, we present evidence for enhancing SWA using a novel, automated device, which has a beneficial impact on next-day cognitive outcomes in middle-aged men.

Supplementary material

Supplementary data are available at *SLEEP* online.

Figure S1. Relative full-night spectra for STIM and SHAM during baseline nights. No significant differences observed in relative power between conditions.

Figure S2. Comparison between STIM and SHAM of each sleep stage in minutes for each cycle. No significant differences were observed between conditions for each sleep stage.

Figure S3. Absolute data showing (A) differences in power in full-night spectra for STIM night relative to SHAM, in 0.5 Hz bins; (B) for each pre-determined EEG spectral bandwidth including delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), slow spindles (SS, 12–14 Hz), fast spindles (FS, 14–16 Hz), and beta (16–30 Hz); and (C) SWA in the delta range;

Figure S4. Percent change in absolute SWE (STIM-SHAM/SHAM*100) for (A) each individual and (B) all nonresponders (< −3% change; n = 9) compared to responders (n = 12).

Figure S5. Changes in absolute slow-wave energy and cognition from STIM to SHAM for responders and non-responders for (A) Phonetic fluency and (B) 2-back d'. Pearson's correlations between SWE%Change and (C) Phonetic fluency and (D) 2-back d'. Error bars indicate SEM. Error is 95% confidence intervals of the regression line.

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Author Contributions

All authors have made substantial contributions to the work presented and have approved the final version of the manuscript. C.A. and S.F. designed the study with input from SPAD. Both C.D. and S.F. were responsible for the collection of data; C.D., J.E.M., S.F., C.L.N., and C.A. analyzed the data; C.D. and C.A. interpreted the data, and C.D. wrote the manuscript with edits from C.A. All authors approved the final manuscript.

Conflict of Interest statement. J.E.M., C.N., and S.P.A.D. report no competing financial interests. C.D. is a recipient of a PhD Scholarship, S.F. is a Project Leader, and C.A. is a Theme Leader in the Cooperative Research Centre for Alertness, Safety and Productivity. In the interest of full disclosure, C.A. has received a research award/prize from Sanofi-Aventis; contract research support from VicRoads, Rio Tinto Coal Australia, National Transport Commission, Tontine/Pacific Brands; and lecturing fees from Brown Medical School/Rhode Island Hospital, Ausmed, Healthmed, and TEVA Pharmaceuticals; and reimbursements for conference travel expenses from Philips Healthcare. In addition, she has served as a consultant through her institution to the Rail, Bus, and Tram Union, the Transport Accident Commission (TAC), and the National Transportation Committee (NTC). She has also served as an expert witness and/or consultant in relation to fatigue and drowsy driving.

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Chapter 3 Additional Information Supplement

The following figures were published as supplementary material to the article. They provide further details on the baseline nights, sleep staging and absolute data.

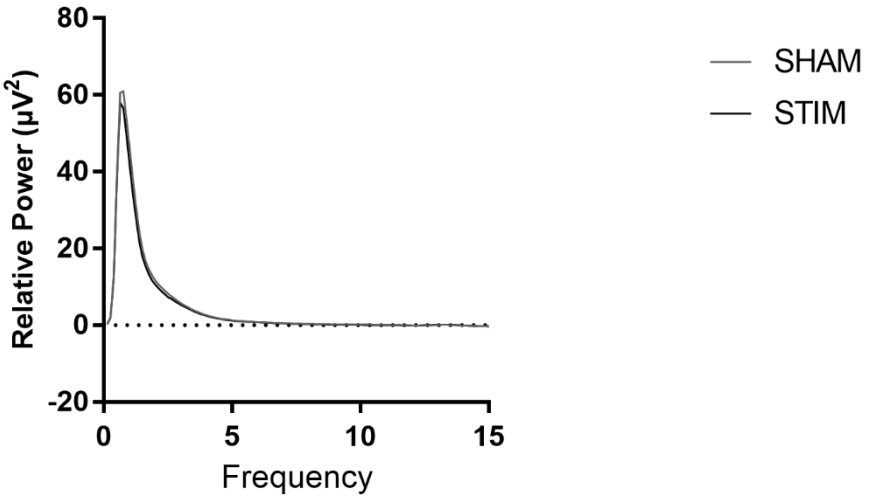

Figure S1. Relative full-night spectra for STIM and SHAM during baseline nights. No significant differences observed in relative power between conditions.

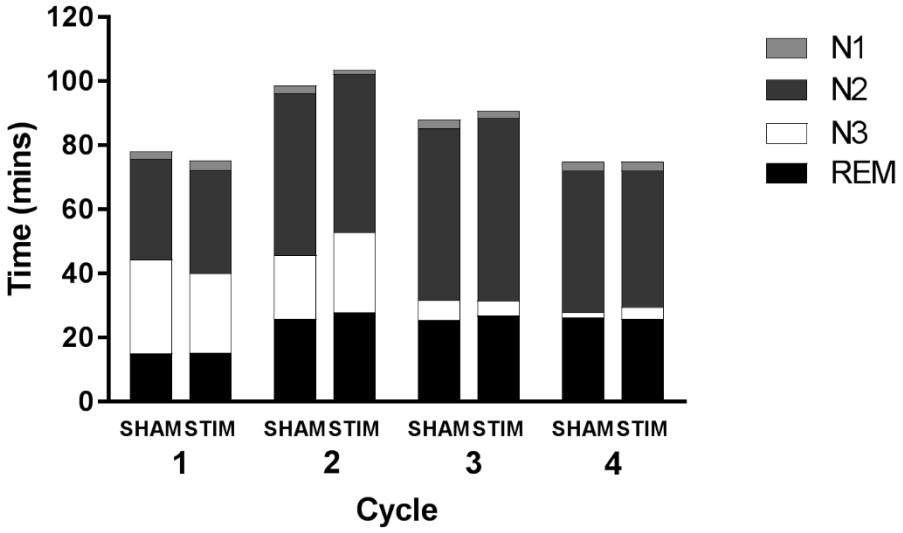

Figure S2. Comparison between STIM and SHAM of each sleep stage in minutes for each cycle. No significant differences were observed between conditions for each sleep stage.

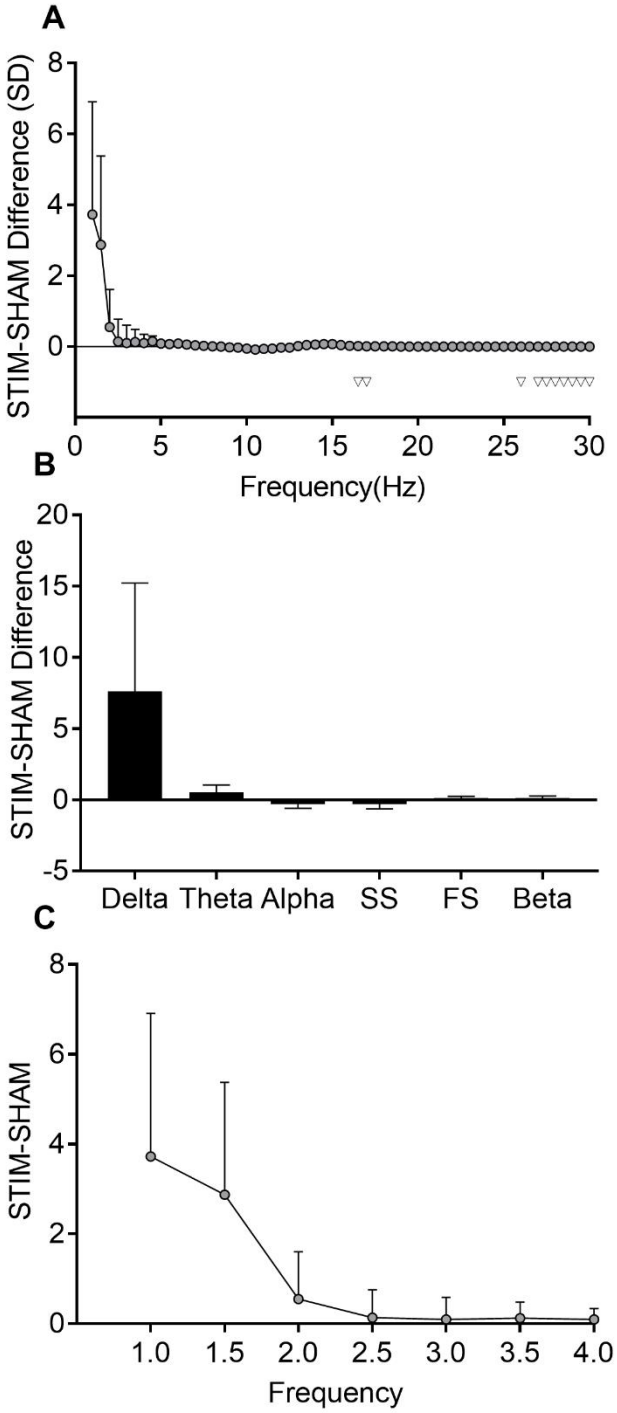

Figure S3. Absolute data showing (A) differences in power in full night spectra for STIM night relative to SHAM, in 0.5 Hz bins; (B) for each pre-determined EEG spectral bandwidth including delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), slow spindles (SS, 12–14 Hz), fast spindles (FS, 14–16 Hz), and beta (16–30 Hz); and (C) SWA in the delta range.

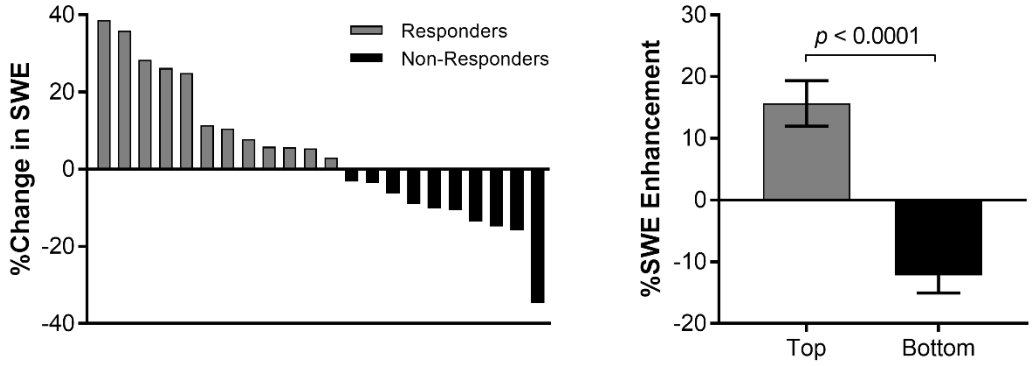

Figure S4. Percent change in absolute SWE (STIM-SHAM/SHAM*100) for (A) each individual and (B) all nonresponders (< −3% change; n = 9) compared to responders (n = 12).

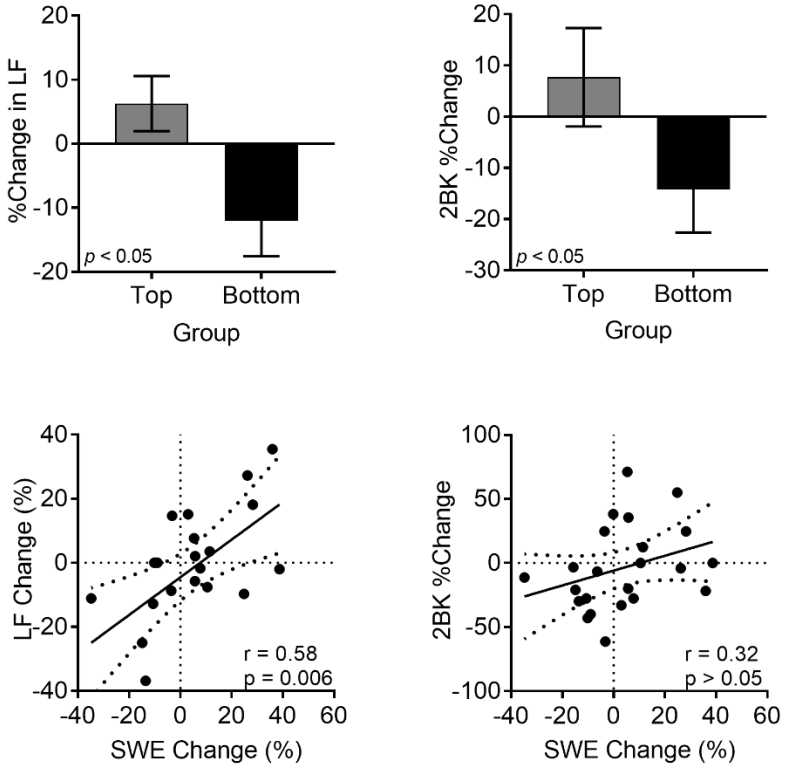

Figure S5. Changes in absolute slow-wave energy and cognition from STIM to SHAM for responders and non-responders for (A) Phonetic fluency and (B) 2-back d'. Pearson's correlations between SWE%Change and (C) Phonetic fluency and (D) 2-back d'. Error bars indicate SEM. Error is 95% confidence intervals of the regression line.

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Chapter 4

Acoustic enhancement of slow wave sleep on consecutive nights improves alertness and attention in chronically short sleepers

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Preface to Chapter 4

Chapter 3 validated the use of an automated, auditory device on SWS and executive function in healthy middle-aged men. The data in this chapter was collected across the USA in collaboration with our industry partner. As an extension of the original pilot study (described in **Chapter 3**), the protocol utilised here was similar in that it was a crossover, repeated-measures design, and also used the same acoustic stimulation device. However, the results in **Chapter 4** aimed to extend the findings presented in **Chapter 3** by looking at the impact of acoustic stimulation over consecutive nights, using a population of habitually sleep-restricted men and women, and assessed changes in attention and alertness.

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Original Article

Acoustic enhancement of slow wave sleep on consecutive nights improves alertness and attention in chronically short sleepers☆

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ABSTRACT

Introduction: Chronic sleep restriction has been linked to occupational errors and motor vehicle crashes. Enhancing slow wave sleep may alleviate some of the cognitive deficits associated with chronic sleep restriction. However, the extent to which acoustic stimulation of slow wave activity (SWA) may improve alertness and attention is not well established, particularly with respect to consecutive nights of exposure.
Methods: Twenty-five healthy adults (32.9 ± 8.2 years; 16 female) who self-restricted their sleep during workdays participated in a randomized, double-blind, cross-over study. Participants wore an automated acoustic stimulation device for two consecutive nights. Acoustic tones (50 ms long) were delivered on the up-phase of the slow wave first and then at constant 1-s inter-tone-intervals once N3 was identified (STIM), until an arousal or shift to another sleep stage occurred, or at inaudible decibels during equivalent stimulation periods (SHAM). Subjective alertness/fatigue (KSS, Samn-Perelli) was assessed across both days, and objective measures of alertness (MSLT) and attention (PVT) were assessed after two nights of stimulation.
Results: After one night of acoustic stimulation, increased slow wave energy was observed in 68% of participants, with an average significant increase of 17.7% ($p = 0.01$), while Night 2 was associated with a 22.2% increase in SWA ($p = 0.08$). SWE was highly stable across the two nights of STIM (ICC 0.93, $p < 0.001$), and around half (56%) of participants were consistently classified as responders (11/25) or non-responders (3/25). Daytime testing showed that participants felt more alert and awake following each night of acoustic stimulation ($p < 0.05$), with improved objective attention across the day following two nights of acoustic stimulation.
Discussion: Consecutive nights of acoustic stimulation enhanced SWA on both nights, and improved next day alertness and attention. Given large individual differences, we highlight the need to examine both the long-term effects of stimulation, and to identify inter-individual differences in acoustic stimulation response. Our findings suggest that the use of an acoustic device to enhance slow wave sleep may alleviate some of the deficits in alertness and attention typically associated with sleep restriction.

☆ Work was conducted in the United States of America at the Clayton Sleep Institute, St. Louis, Missouri, NeuroTrials Research, Atlanta, Georgia, Preferred Research Partners, Little Rock, Arkansas, Sleep Disorders Center of Alabama, Birmingham, Alabama, Florida Sleep & Lung Associates, Lehigh Acres, Florida, University of Pennsylvania Unit for Experimental Psychiatry, Division of Sleep and Chronobiology, Philadelphia, Pennsylvania, The Center for Sleep & Wake Disorders, Chevy Chase, Maryland and the Sleep and Circadian Medicine Laboratory, School of Psychological Sciences and Turner Institute for Brain and Mental Health, Monash University, Australia.

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1. Introduction

Insufficient sleep is associated with a wide range of adverse consequences, including poorer health outcomes [1–3], reduced cognitive and attentional capacity [4–6], and increased risk of accident and injury at work or on the road [7–9]. The Centers for Disease Control and Prevention (CDC) in the United States declared insufficient sleep a "public health problem", due to a significant proportion of the population reportedly sleeping less than the

recommended seven hours per night [10]; this is also reported across other nations, including Germany, United Kingdom, Canada, Japan, and Australia [11,12]. Lifestyle factors such as social engagements and work schedules, are major contributing factors to insufficient sleep, and appear to be worsening over time. For instance, while sleep duration on a work-free day was the same as that reported over ten years ago, over the same period of time average nightly sleep duration on work days decreased by 38 min [13]. As the opportunity for sleep decreases, and the risk for adverse consequences continue to increase, new multimodal strategies are required to ensure sleep is of maximal benefit in terms of both quantity and quality.

Numerous strategies exist to combat the effects of insufficient sleep on the waking day. The most common of these strategies include the use of caffeine [14,15], compensatory naps [14,16], or extending sleep on the weekends [17]. Recently however, studies have focused on improving daytime performance through the enhancement of sleep, specifically slow wave sleep (SWS) via boosting its electrophysiological signature, slow wave activity (SWA). SWA is visualised as high amplitude (>75 μV), low frequency waveforms (<4 Hz), indicating synchronous neocortical activity. While SWA enhancement can be successfully achieved with both pharmaceutical [18] and electrical stimulation approaches [19,20], these are not ideal for daily intervention (eg, due to the potential for side effects, or being impractical to use in an at-home setting). Enhancing SWA via acoustic stimulation provides a non-invasive, cost-effective, practical intervention for enhancing SWA, with no currently known side effects. While acoustic stimulation of SWA has been shown to enhance memory performance [21–23], and executive function [24], the impact on improvement on attention and vigilance outcomes has been relatively unexplored, despite this being the most sensitive measure of sleep-related performance impairment [6,25,26], and the underlying cause of many sleep-related accidents and errors [9,27], and motor vehicle crashes [28,29].

Demonstrating improvement in attention and vigilance outcomes would provide strong evidence for the use of acoustic stimulation as a daily intervention (alongside others) for improving daytime outcomes following insufficient sleep. To date, studies have focused on single-night applications and subsequent performance outcomes [30]. Little is known about the impact of more real-world applications, such as multiple nights of use. As SWA is homeostatically regulated [31], and tightly coupled with the duration and intensity of the waking day [32–34], the extent to which SWA can be repeatedly enhanced, and the impact on subsequent performance remains unknown. Our study therefore examined the impact of acoustic stimulation of SWA enhancement across multiple nights of stimulation, on subsequent attention and vigilance outcomes, in a sleep-restricted population.

2. Methods

2.1. Participants

Fifty-one healthy adults (29 women, 22 men) aged 22–51 years (33.4 ± 8.4 years) participated in the study. Participants were working full time with a regular work schedule (defined as four 10-h days or five 8-h days with a start time of 7am or later) with insufficient sleep based on a self-reported sleep duration between 5 and 7 h, *and* <7 h time in bed on work days *and* an increase in sleep duration by >1 h on work-free days. Further exclusionary criteria included: lack of fluency in English, presence of any major medical conditions, current shift workers, use of any medication that affect the central nervous system (eg, sedating, psychoactive), pregnant and/or breast feeding, current smokers or use of nicotine replacement therapies, BMI over 40 kg/m^2, diagnosed sleep disorders or at high risk of sleep disorders (determined through self-reported questionnaires, eg STOP-Bang), alcohol intake of over 21 drinks per week or 5 per day, excessive caffeine consumption (>650 mg/day; one 6-ounce cup of coffee or caffeine equivalent was permitted at breakfast throughout the study), presence of seizures or epilepsy, silicone/nickel/silver allergies, moderate hearing loss, any planned travel across time zones, sleep latency >30 mins, intentional naps during the work week, and presence of alpha intrusion during slow wave sleep (*alpha-delta*) [35]. All participants provided written informed consent and completed medical and sleep disorder screening questionnaires. This study was approved by the Western Institutional Review Board.

2.2. Pre-study protocol

To ensure we monitored the impact on habitual sleep, participants were asked to keep their habitual sleep schedules throughout the study (ie, the <7 h' time in bed typically experienced during the work week). This was monitored by actigraphy and sleep diaries for at least one week prior to the laboratory assessment. Participants completed a baseline night with full polysomnography to screen for undiagnosed sleep disorders (AHI ≥15 events/hour), the occurrence of alpha-delta sleep, and to determine hearing thresholds for the tones to be delivered by the device. Two volume ranges were available, sensitive or non-sensitive. For participants deemed sensitive by the algorithm or those who heard the tones during the night, the volume varied between 20 and 50 dB, while participants deemed non-sensitive had volume settings between 20 and 65 dB.

2.3. Laboratory protocol

2.3.1. Study conditions

This was a multi-site study conducted across six sites in the United States. Across all sites, participants stayed in quiet research bedrooms in accordance with standard accreditation specifications [24]. Participants completed one baseline adaptation night prior to randomization into the study to avoid any impact of a disturbed night (from the first night effect) on the main study/experimental nights. The following week, consisted of two experimental nights and two daytime outcome assessments, Fig. 1. The laboratory study commenced on a Tuesday evening (Day 0), which comprised a series of practice tests. Experimental sleep nights occurred on a Tuesday and Wednesday night (Night 1 and Night 2, respectively) and daytime outcome were assessed on a Wednesday and a Thursday (Day 1 and Day 2, respectively). This was repeated one week later with the alternate condition. On one visit, the device would play auditory tones for SWA enhancement (STIM) during the experimental nights; during the other visit, no tones were played (SHAM). These STIM and SHAM conditions were randomized, counterbalanced, and conducted under double blind conditions. As this device has previously been examined under highly controlled conditions [24,36], we wished to enhance the ecological validity of the protocol by allowing participants to leave for work between experimental nights (ie, Night 1), returning 3 h prior to habitual bed time for a controlled assessment of sleep. Participants were discharged at approximately 8-h post-wake on day 2 following alertness and vigilance testing.

2.3.2. Polysomnography and SWA enhancement

On each experimental night, participants were fitted with an early prototype of a commercial device for SWA enhancement (SmartSleep Deep Sleep Headband, Philips Healthcare). This device has been previously shown to enhance SWA across both a single night [24] and multiple nights [36], although night-to-night

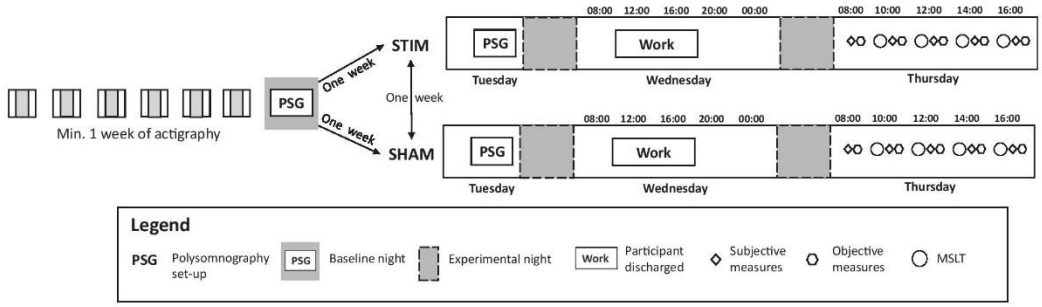

Fig. 1. Study Protocol. A randomized, counterbalanced, SHAM-controlled assessment of acoustic stimulation of SWA on subsequent daytime alertness and attention, across two consecutive nights of exposure. Actigraphy was worn throughout the study to ensure compliance. Participants completed a single adaptation and screening night at least one week prior to participation in the study. Participants were randomized to receive two nights of acoustic stimulation (STIM) or two nights of no tones (SHAM) in Week 1, followed by the alternate condition in Week 2. Subjective alertness and fatigue were evaluated in the morning of Day 1 and every two hours on Day 2. Objective alertness and attention were evaluated throughout Day 2.

comparisons were not made in the latter. The prototype consisted of two frontal electrodes (FpZ and bias electrode), two electrooculograms (E1 and E2) and right mastoid (M2). The active electrode is located on FPz and the reference on M2. An adjustable headband holds the Fpz electrode, and the speakers. Processing of the signals is performed in an integrated unit with an embedded microprocessor connected to the headband. See Fig. 2. During each experimental sleep period, the device would operate in either STIM or SHAM mode. During STIM, a 50 ms tone was phase-locked to the first slow wave on the up-phase, and pulsed at 1 Hz thereafter (STIM) [36,37] until an arousal or sleep stage change occurred. During SHAM, the device would operate in the same manner, except the volume was inaudible (this provides us with an indication of device operations, with no risk of waking the participant). As we examine data derived from the Deep Sleep Headband device, simultaneous PSG was applied to check the scoring algorithm from the device. PSG software varied according to site but included: Sandman 10.1 SD32/Elite 9.2; Grass Aurora, Neurovirtual BWII System; Alice 6; Compumedics Siesta 802.11 A; and Embla Rembrant Money. The Deep Sleep headband has been previously validated for power spectral analyses [36].

2.3.3. Alertness and vigilance outcomes

Participants were familiarized with all tests and questionnaires on the first evening of each visit (ie, on a Tuesday). Following experimental night 1 (Day 1) and night 2 (Day 2), participants completed a morning assessment for subjective alertness and fatigue, at 1 h-post habitual wake. This included the Karolinska

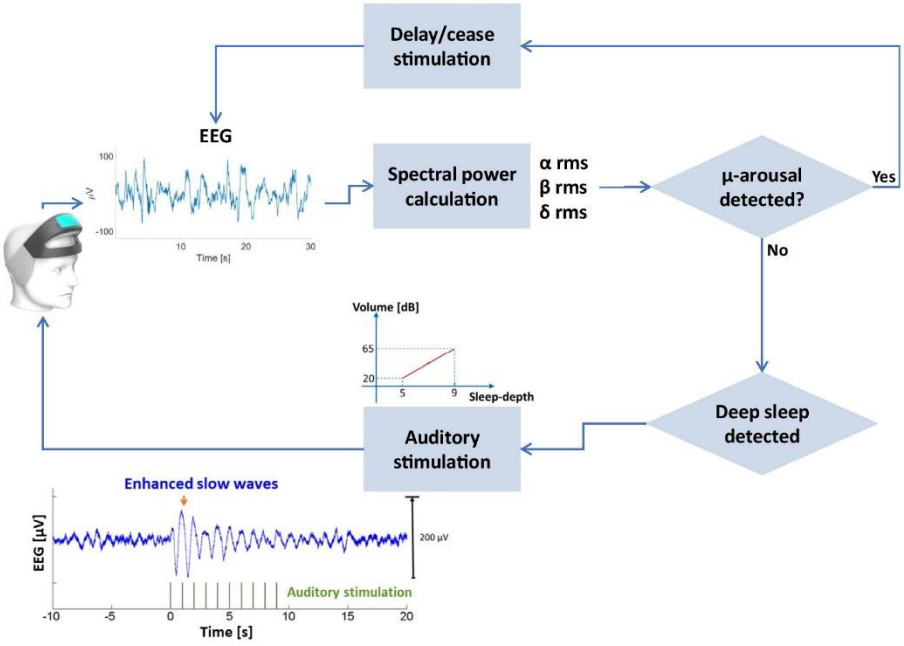

Fig. 2. SmartSleep Algorithm. Depiction of the SmartSleep stimulation process. The device detects alpha, beta and delta waves. Once 6 s of N3 is confirmed, phase-locked tones are initiated. The volume of the tones is dependent on the perceived sleep depth. If an arousal occurs, stimulation ceases until the next occurrence of N3.

Sleepiness Scale (KSS) [38] and the Samn-Perelli Fatigue Scale (SP-FS) [39]. Following experimental night 2 (Day 2), subjective alertness and fatigue (KSS and SP-FS), and objective alertness and performance were also examined at 2, 4, 6 and 8-h post wake. This included the KSS, SP-FS, a 3-min Psychomotor Vigilance Task (PVT) [40] and a standard Multiple Sleep Latency Test (MSLT, four nap sessions) [41]. Discharge was at 8-h post wake on day 2.

Karolinska Sleepiness Scale (KSS): Participants were asked to rate how sleepy they feel ranging from "1 = extremely awake" to "9 = extremely sleepy/fighting to stay awake" [38].

Samn-Perelli 7-point scale: Participants were asked to rate how alert/fatigued they feel ranging from "1 = fully alert, wide awake" to "7 = completely exhausted, unable to function effectively" [39].

Psychomotor Vigilance Task (PVT): We measured sustained attention using a 3-min PVT (Joggle Research app [40]). Participants were required to respond as quickly as possible to a counter that appeared at random intervals. Outcome measures included Mean rRT, #Lapses (responses > 355 msec) and Fastest 10% of responses.

Multiple Sleep Latency Test (MSLT): Participants were given a 20-min nap opportunity at four-intervals starting at 2-h post wake and every two hours thereafter [41]. Each time, they were instructed to lie down and attempt to fall asleep. The test was terminated following three consecutive epochs of PSG-defined sleep or after twenty-minutes, whichever occurred first. The outcome measure was average sleep latency (mins) to three consecutive epochs of N1 or one epoch of N2, N3 or REM.

2.3.4. Sleep and spectral analysis

For the PSG data, sleep staging, sleep onset and arousals were scored visually according to standard criteria [24]. Channels were referenced to contralateral mastoid (eg C3-M2, C4-M1), and data were filtered between 0.3 and 35 Hz, with a 60 Hz Notch filter. Scorers were blinded to the experimental condition. Minutes of total sleep time, N1, N2, N3, rapid eye movement (REM) sleep, wake after sleep onset (WASO) and number of arousals were calculated from the scored PSG data. The device data was automatically scored using the in-built scoring algorithm as described previously [17]. Non-REM (NREM) was defined as N2 and N3, while N1 was treated as a transitional stage and excluded from analysis. Power spectral analyses were derived from EEG from the SmartSleep device and using a custom MATLAB script as described elsewhere [36]. In brief, the power spectrum density (PSD) for each 6-s EEG epoch was estimated according to the Welch method [42] with a four-second long Hanning window (ensuring 0.25 Hz frequency resolution), a two-second-long overlap, and 1024 points to calculate the Fourier transform. Our primary output was slow wave energy (SWE) which was calculated by summing SWA across every 6-s N3 epoch and dividing by total number of epochs [36]. To control for night-to-night changes in power spectra due to recording differences (eg, impedances, noise, etc), we present both absolute (SWE_a) and relative SWE (SWE_r). SWE_r was calculated as SWE/total power spectra minus delta (4.5–30 Hz, as 0.5–4.5 Hz cannot be included in both the numerator and denominator). Data were examined as a total effect (cumulative changes across Night 1 + Night 2) and separately for Night 1 and Night 2. When examining the separate effect of Night 1 and Night 2, we compared to the SHAM average ($SHAM_{av}$) baseline to allow us to determine night-to-night changes in SWA enhancement response (rather than changes in SHAM baseline).

2.3.5. Data retention

Out of 204 experimental nights (4 nights x 51 participants), data were lost from 57 nights (27.9% loss). This includes 21 nights from SHAM (7/3.4% from Night 1 and 14/6.9% from Night 2) and 36 from STIM (21/10.3% from Night 1 and 15/7.3% from Night 2). For SHAM nights, data were lost due to excessive artefact (n = 9, 4.4%), no N3 detected or threshold too high (n = 7, 3.4%) or a protocol violation (n = 5, 2.5%). For STIM nights, data were lost due to excessive artefact (n = 11, 5.4%), no N3 detected or threshold too high (n = 13, 6.4%), a protocol violation (n = 3, 1.5%), device errors (n = 2, <1%) or the tones disturbed sleep (n = 7, 3.4%). As we examine the impact of two consecutive nights of SWA enhancement, participants were required to have a full clean dataset (ie, clean data for two SHAM and two STIM nights). Given the lost data was random, and not systematically from one participant or condition, we describe data for the ideal EEG dataset for n = 25. Beyond PSG, data was lost on one participant due to a technical problem (<2%). To check there was no bias in the dataset presented, demographic and basic sleep parameters on the available dataset versus the final dataset confirmed no differences (See Table 1).

2.3.6. Data processing and statistical analysis

PVT reaction times (RT) were normalized using a reciprocal transformation ((1/RT)*1000), and total number of PVT lapses (RT > 355 msec) were normalized using the square-root transformation [$\sqrt{(n)}+\sqrt{(n+1)}$] [43,44]. Paired samples t-tests (two-tailed) were used to compare SWE data. When data were non-normally distributed, Mann–Whitney U or Wilcoxon Matched-Pairs tests were used instead. Linear mixed models were used to examine fixed effects of condition (STIM *vs* SHAM) and time since wake (4 levels: 2, 4, 6 & 8 h post wake), plus any interaction, for subjective and objective outcomes taken on Day 2 (eg, PVT, subjective ratings). Participant was added as a random effect, and the covariance structure with the lowest BIC was utilised (Unstructured for KSS and Fastest 10% RTs, AR(1) Heterogenous for SP-FS, Toeplitz for Mean RT). To examine consistency in response across nights, we employed intra-class correlation coefficients (ICCs) with a two-way mixed effect for absolute agreement. Unless stated otherwise, all results presented are mean ± SEM. IBM SPSS Statistics V23 or V26 were used to run statistical analyses.

3. Results

3.1. Device validation and operation

To check on device accuracy in detecting N3, we compared automated detected N3 from the device against manually scored N3 from the PSG, for all available study nights (n = 34). Data from the device and PSG were aligned manually and compared on an epoch-by-epoch basis (for Wake, N1/N2, N3 and REM). Device scoring accuracy was 80.4 ± 7.7, with a sensitivity of 89.5 ± 10.1, specificity of 92.4 ± 5.7, and positive predictive value (PPV) of 70.5 ± 22.4. There was minimal low frequency artifact (eg electrodermal activity) interfering with the device, with no differences between conditions (STIM: 3.8 ± 2.03, SHAM: 5.8 ± 2.4, p = 0.1).

Table 1
Demographic data of participants in full dataset, excluded and final.

Parameter	Full	Excluded	Final	p-value
Age (years)	33.4 ± 8.4	33.9 ± 1.7	32.9 ± 1.6	0.67
Gender Ratio (F/M)	29/22	13/13	16/9	0.40
SWS; SHAM (mins)	67.0 ± 5.1	69.6 ± 8.1	64.2 ± 6.2	0.60
SWS; STIM (mins)	68.8 ± 0.7	66.5 ± 7.8	71.1 ± 6.5	0.65
Sleep Efficiency; SHAM	92.4 ± 0.7	93.1 ± 0.8	91.8 ± 1.1	0.35
Sleep Efficiency; STIM	92.7 ± 0.6	92.2 ± 1	93.1 ± 0.56	0.45
Time in bed (Actigraphy)	6:47 ± 0.12	6:51 ± 0.98	6:52 ± 0.08	0.97

t-tests were run between participants in the excluded and final datasets. Fisher's exact test run for gender ratio.

3.2. Tone delivery and sleep parameters

As a check on the stability of sleep architecture, Table 2 confirmed no significant differences in any sleep parameter for SHAM on Night 1 compared to SHAM on Night 2, ($p > 0.07$, Table 2). To enable night-to-night comparisons on the consecutive use of STIM, we compare Night 1 and Night 2 sleep parameters to the SHAM average where stated (Table 2).

3.2.1. Night 1

Number of tones delivered (with or without sound) was positively correlated with N3 minutes for both STIM ($r = 0.5$, $p = 0.005$) and SHAM ($r = 0.4$, $p = 0.04$). Compared to $SHAM_{a}v$, more tones were delivered during STIM (2536.4 ± 423.4 vs. 1867 ± 273.6, $t(24) = 1.94$, $p = 0.06$, $d = 0.42$), which may be due to more minutes of N3 being observed in the STIM condition, relative to $SHAM_{av}$ (72.7 ± 8.2 vs. 64.2 ± 6.2, $t(24) = 2.22$, $p = 0.03$, $d = 0.51$, Table 2). No other differences in sleep stages were observed between STIM and $SHAM_{av}$ ($p > 0.3$, Table 2).

3.2.2. Night 2

Similar to Night 1, the number of tones delivered was significantly correlated with N3 minutes for both STIM ($r = 0.59$, $p = 0.002$) and SHAM ($r = 0.49$, $p = 0.01$). While more tones were delivered during STIM compared to $SHAM_{av}$ (2804.16 ± 319.2 vs. 1867 ± 273.6, $t(24) = 3.83$, $p = 0.0008$, $d = 0.78$), this was not due to an increase in N3 minutes ($p = 0.13$). The number of tones was negatively correlated with arousals during STIM ($r = -0.61$, $p = 0.001$), but not SHAM ($r = -0.28$, $p = 0.18$). There was a significant decrease in WASO for STIM relative to $SHAM_{av}$ (16.7 ± 2.4 vs. 21.7 ± 2.2, $w(24) = 153$, $p = 0.03$, $d = -0.42$). No other changes were observed (Table 2).

3.3. Acoustic enhancement of SWE

3.3.1. Cumulative SWE across the two nights

SWE_a increased by 20.0% across the two nights of STIM compared to the two nights of SHAM [STIM: 2835.6 ± 436.6 μV2, SHAM: 2471.3 ± 399.3 μV2, ($t(24) = 3.1$, $p = 0.004$, $d = 0.66$); Fig. 3A]. Similar results were observed with SWE_r [STIM: 15.2 ± 2.0, SHAM: 12.9 ± 1.9, ($t(24) = 3.5$, $p = 0.002$), $d = 0.66$]. There were no significant differences between groups for other bandwidths ($p > 0.3$, Fig. 3A).

3.3.2. Night 1

Compared to $SHAM_{av}$, STIM increased SWE_a by 17.7% [STIM: 1458 ± 252 μV2, SHAM: 1236 ± 199.6 μV2, ($t(24) = 2.8$, $p = 0.01$, $d = 0.29$, Fig. 2B)] on Night 1. This increase was also consistent for SWE_r [STIM: 7.8 ± 1.2, SHAM: 6.4 ± 0.9, ($t(24) = 4.0$, $p = 0.001$, $d = 0.67$)]. Enhanced SWE was observed in 68% of participants (17/25). There were no significant differences between groups for other bandwidths ($p > 0.2$, Fig. 3B).

3.3.3. Night 2

While there was an overall 22.4% increase in SWE for STIM compared to SHAM for Night 2, this did not reach significance for SWE_a [STIM: 1378 ± 193.4, SHAM: 1236 ± 199.6, $t(24) = 1.8$, $p = 0.08$; $d = 0.20$)] or SWE_r [STIM: 7.4 ± 0.9, SHAM: 6.5 ± 0.9, $t(24) = 1.8$, $p = 0.08$, $d = 0.34$]. Enhanced SWE was observed in 64% of participants (16/25). There were no significant differences between groups for other bandwidths ($p > 0.4$; Fig. 3C).

3.3.4. Responders vs. non-responders

Intra-class correlation coefficients showed SWE_a, within individuals, was highly stable across the four nights [ICC = 0.96 (CI: 0.92–0.98), $p < 0.0001$], and for STIM nights separately [ICC = 0.93 (CI: 0.83–0.97), $p < 0.0001$] and SHAM [ICC = 0.90 (CI: 0.77–0.95), $p < 0.0001$]. On night 1, there were 17 responders (>0% increase in SWE) and 8 non-responders (>0% decrease in SWE), Fig. 4A. On average, Night 1 responders had a 33.7% enhancement effect, while non-responders decreased, on average, by 16.4%. On night 2, there were 16 responders, with an average SWE enhancement of 44.7%, while non-responders ($n = 9$) decreased on average by 17.4%, Fig. 4B. Across the two nights, 14 individuals (56%) had a consistent SWE enhancement response (44% ($n = 11$) consistent responders and 12% ($n = 3$) were consistent non-responders), while 44% ($n = 11$) showed no consistency in response, Fig. 4C. There was a significant correlation in percent change for Night 1 and Night 2 ($r = 0.59$, $p = 0.002$, Fig. 4D).

3.4. Subjective alertness following acoustic stimulation

3.4.1. Day 1

Compared to SHAM, participants felt more alert and awake in the morning after the first night of STIM as indicated by lower KSS [STIM: 3.6 ± 0.45, SHAM: 4.4 ± 0.48, $W(24) = -84$, $p = 0.01$, $d = 0.46$; Fig. 5A] and lower SP-FS scores [STIM: 2.9 ± 0.3, SHAM: 3.6 ± 0.3, $t(24) = 2.55$, $p = 0.02$, $d = 0.51$; Fig. 5B]. There were no associations between alertness outcomes and SWE_a or SWE_r ($p > 0.2$).

3.4.2. Day 2

For subjective sleepiness, there was a main effect of condition, such that participants felt more alert after a second night of STIM as indicated by the KSS [STIM: 3.1 ± 0.3, SHAM: 3.7 ± 0.3, $F(1, 48.6) = 4.33$, $p = 0.043$, Fig. 5C], however this was not seen for subjective fatigue [SP-FS scores: STIM: 2.5 ± 0.2, SHAM: 2.9 ± 0.2, $F(1, 125.5) = 2.35$, $p = 0.128$; Fig. 5D]. As expected, main effects of time since wake were found for both KSS [$F(4, 153.9) = 3.9$, $p = 0.005$] and SP-FS [$F(4, 197.5) = 2.9$, $p = 0.023$]. There was no condition*time interaction ($p > 0.28$), and an *a priori* assessment of the morning assessment (to allow comparison assimilations to be

Table 2
Sleep outcomes across all nights: Impact of stimulation.

Parameter	Baseline	SHAM 1	SHAM 2	SHAM Average	STIM 1	STIM 2
N1	39.6 ± 4.8	40.9 ± 5.3	29.4 ± 3.1	35.1 ± 4.0	32.5 ± 3.6	29.3 ± 2.7 *
N2	190.7 ± 8.1	189.4 ± 7.8	188.3 ± 6.9	188.9 ± 6.8	185.4 ± 7.2	187.1 ± 6.1
N3	57.7 ± 7.0	60.2 ± 5.9	68.1 ± 7.1	64.2 ± 6.2	72.7 ± 8.1*	69.5 ± 5.6
REM	65.4 ± 4.7	74.4 ± 5.9	80.2 ± 4.8	77.3 ± 4.6	75.3 ± 5.1	83.7 ± 4.3
Arousal Index	12.8 ± 1.4	13.0 ± 1.7	11.7 ± 1.8	12.3 ± 1.6	11.0 ± 0.8	10.4 ± 0.7
WASO	30.0 ± 5.2	23.7 ± 3.0	19.7 ± 2.0	21.7 ± 2.2	19.3 ± 2.0	16.7 ± 2.4 *[W]
TST	353.4 ± 7.1	365.0 ± 5.7	366.0 ± 6.4	365.5 ± 5.6	365.9 ± 3.7	369.6 ± 4.6

Sleep parameters in minutes±SEM. Statistical tests were run between SHAM Average and STIM 1 or 2 for each parameter. WASO, wake after sleep onset. TST, total sleep time. [W] Wilcoxon test. *$p < 0.05$.

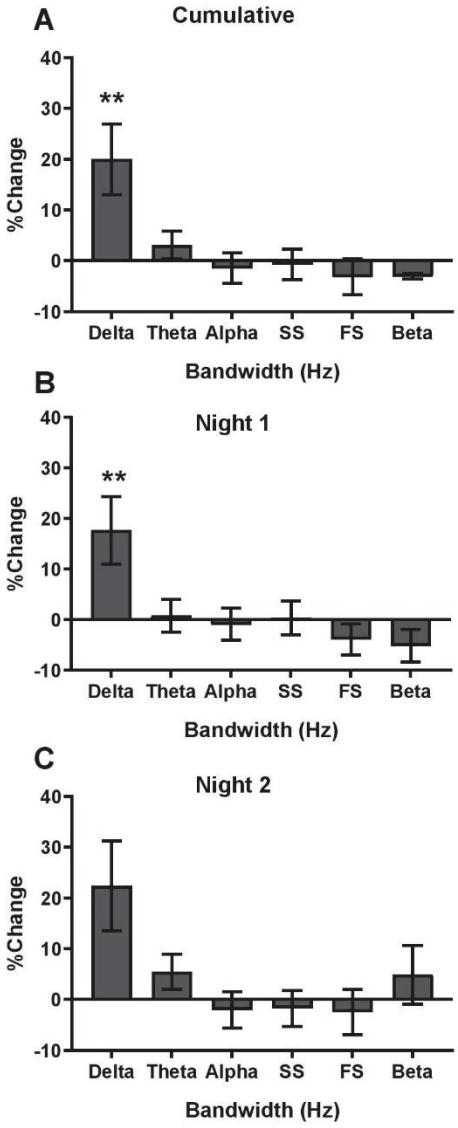

Fig. 3. Impact of Acoustic Stimulation of Power Spectra. Percent change in power spectra across all power bands including delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), slow spindles (SS, 12–14 Hz), fast spindles (FS, 14–16 Hz), and beta (16–30 Hz) for Night 1 and Night 2 combined (Upper Figure), and separately (Middle and Lower). $*p < 0.05$ $**p < 0.01$.

made to Day 1) were also not significant for both KSS and SP-FS ($p > 0.2$). Fig. 5E, F suggest improved self-reported alertness in the afternoon, although we caution against interpretation due to the insignificant interactive term.

3.5. Objective alertness and sustained attention following acoustic stimulation

Objective measures were only examined on Day 2 (following two nights of STIM). There was a main effect of condition for Mean RT [STIM: 252.5 ± 37.8, SHAM: 258.2 ± 31.6, $F(1, 23.2) = 6.4$, $p = 0.019$; Fig. 6A], and a trend toward significance for the fastest 10% of responses [STIM: 183.9 ± 17.2 SHAM: 189.9 ± 13.2, $F(1, 23.6) = 3.8$, $p = 0.064$; Fig. 6B]. As expected, significant time-since-wake effects were found for Mean RT [$F(4, 150.0) = 5.3$, $p = 0.001$] and 10% fastest RT [$F(4, 60.3) = 8.9$, $p < 0.001$]. There was no effect of condition [$F(1, 28.4) = 2.1$ $p = 0.16$] or time [$F(4, 160.0) = 1.7$ $p = 0.16$] on PVT lapses. While there were no significant condition*time interactions ($p > 0.39$), Fig. 6C, D again suggest a change in mean RT and 10% Fastest RTs across the course of the day. As an *a priori* decision to compare the Morning Assessment only (to compare to Day 1 subjective results), STIM improved performance for mean RT [STIM: 253.5 ± 7.0, SHAM: 263.8 ± 7.6, $t(1, 21) = 2.4$, $p = 0.02$, $d = 0.34$], 10% fastest RT [STIM: 187.9 ± 3.5, SHAM: 197.0 ± 3.4, $t(1, 21) = 2.3$, $p = 0.02$, $d = 0.53$] and lapses [STIM: 4.1 ± 1.0, SHAM: 6.0 ± 1.4, $t(1, 21) = 2.2$, $p = 0.03$, $d = 0.34$].

For both SHAM (92%) and STIM (88%), the majority of individuals were either moderately sleepy (MSLT 5–10 min, SHAM: 44%, STIM: 36%) or within the normal range (MSLT >10 min, SHAM: 48%, STIM: 52%). There was no difference in MSLT scores following STIM, relative to SHAM [STIM: 10.3 ± 1.0, SHAM: 10.8 ± 1.0, $t(24) = 0.58$, $p > 0.6$, $d = 0.12$], with 72% of participants remaining in the same MSLT category.

4. Discussion

We examined the impact of two consecutive nights of acoustic enhancement of SWA on next-day subjective sleepiness and fatigue, and objective alertness and attention. Acoustic stimulation enhanced SWA on Night 1 and Night 2. Acoustic stimulation significantly improved both subjective alertness and objective performance, as indicated by improved subjective sleepiness and fatigue on each day, and improved sustained attention following two nights of stimulation. To our knowledge, this is the first study to examine the impact of consecutive nights of acoustic stimulation on slow wave sleep, with a focus on alertness and attention outcomes, and in chronically sleep-deprived individuals. We therefore provide support for the potential utility of acoustic stimulation as one strategy to combat insufficient sleep.

The capacity to enhance SWA via closed-loop acoustic stimulation is consistent with previous work by us [24,36], and others [21–23,45,46]. Given acoustic stimulation devices are now commercially available, and individuals may use them night-to-night, understanding the potential impact on a subsequent night of sleep is important: firstly, it enables us to examine the potential for a rebound effect given the homeostatic regulation of slow wave sleep [31], and secondly, it provides important information on a potential trait-like response to acoustic stimulation. On average, we observed an increase in SWA of ~18% on Night 1 and 22% on Night 2, although Night 2 did not reach statistical significance. While the magnitude of SWA enhancement on Night 1 was positively correlated with the magnitude of SWA enhancement on Night 2, large individual differences were still observed. For instance, only 56% had a consistent response on each night (of which 44% were consistent responders). For the top tertile of responders on Night 1 however, only one had a negative response on Night 2. While these data suggest that there is no clear rebound following acoustically-enhanced slow wave sleep, this may be masked by a repeated night of stimulation. Future studies should therefore examine any rebound effect following acoustic stimulation on a night of sleep free from any intervention. To further elucidate any trait-like response in slow wave enhancement, future studies should examine multiple nights of acoustic stimulation, under the same conditions, but with a washout (of at least three days) between sessions. Showing a trait-like response and identifying individual factors that might predict those who benefit from acoustic

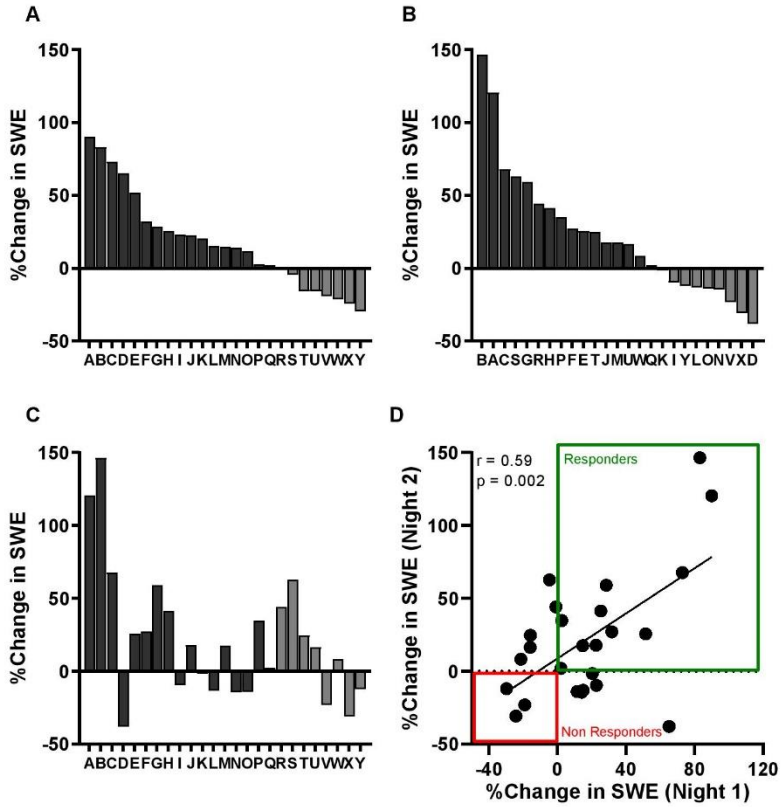

Fig. 4. Individual Differences in SWA Response. Participants' response to acoustic stimulation in rank order for Night 1 (A) and Night 2 (B). Participants' response to acoustic stimulation for Night 2 is replicated but shown in the rank order from Night 1 (C) highlighting interindividual differences across Night 1 and Night 2. The correlation between SWA enhancement response between Night 1 and Night 2 is shown in Panel D.

stimulation would provide important information regarding those who might benefit from this novel sleep therapeutic.

To date, the benefit of acoustic stimulation of SWA has largely been related to memory outcomes [21,23,45,46], and executive functions, such as semantic fluency [24]. Our data suggest that acoustic stimulation of SWA is also associated with improvements in sustained attention and subjective alertness, given participants felt more alert and less fatigued in the morning after a single night of acoustic stimulation, with improvements in sustained attention being observed after two nights of acoustic stimulation. This is consistent with pharmacologically enhanced SWA (via Sodium Oxybate and Tiagabine), which has been shown to protect against the deficits in sustained attention (PVT) typically observed during a period of chronic sleep restriction [18,47]. While the average improvement in response times were small, the range was large. For instance, for morning assessments, 60% of participants had faster RTs after STIM, with a range of −16 msec to 53 msec, while for fastest 10% of responses, 68% of participants improved, with a range of −10 msec to 58 msecs. Although these improvements remain small, and perhaps clinically not meaningful, from a performance perspective, these can be important. For example, for a car travelling at 100 kph, braking distances could be decreased by up to half a car length, while in elite sports, significant advantages can be found by as a little as 1%. For instance, as noted by Facer-Child's and colleagues, at the Beijing Olympics a 1% improvement in speed coming out of the starting blocks would have resulted in the fourth placed runner being awarded a silver medal [48]. Of note, our fastest RTs were, on average, ~5% quicker. Although our data are behavioural in nature, we suggest several possible mechanisms for how enhanced SWA may lead to improved alertness and attention outcomes. Firstly, and in line with previous pharmacological studies [18,47], acoustic stimulation of slow wave sleep may facilitate the resetting of the sleep homeostat in a shortened sleep period, thus offsetting performance deficits typically seen in chronically short sleepers. Secondly, enhanced SWA may preferentially affect the prefrontal cortex, thus leading to enhanced performance in frontoparietal networks which are critical for maintaining attention [49,50]. Of note, this interpretation may be reinforced by the device using the signal from frontal areas (Fpz) to detect and deliver the stimulation. Thirdly, enhanced SWA may decrease arousals, which are known to be associated with daytime sleepiness [51] and sustained attention [52]. For instance, we found that decreased arousal was associated with more tones for both STIM nights, as per our previous study [24], and decreased WASO during STIM relative to $SHAM_{av}$ on N2 (although we note that this does not attribute causality). Despite this, these indices of sleep consolidation were not correlated with PVT performance. Finally, the effect could be subjective in nature, such that participants may have guessed the active condition, and thereby reported improved subjective scores and even objective PVT performance [53,54]. Although we didn't ask which conditions participants thought they were in, we have previously shown participants were unable to guess when this device was active [24]. In contrast to

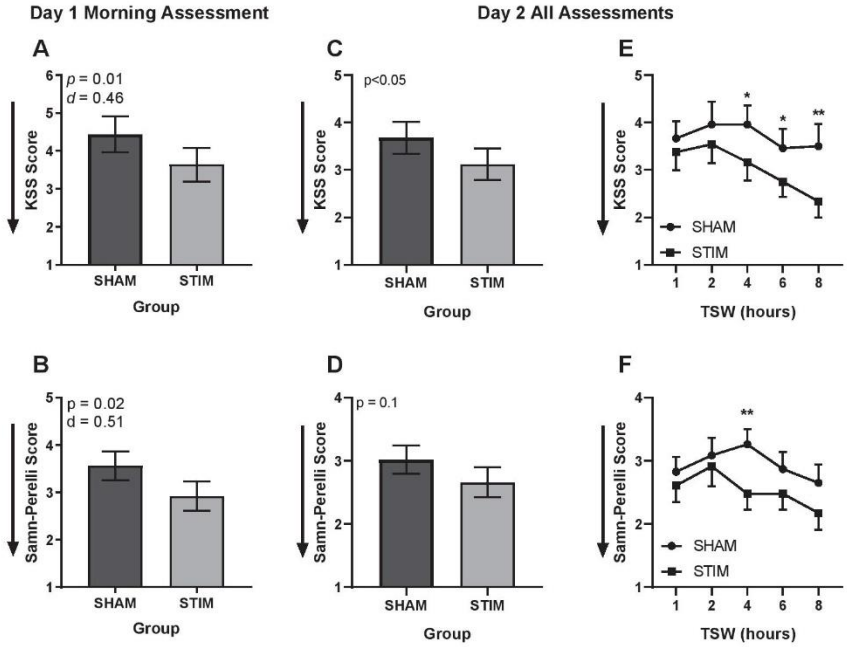

Fig. 5. Subjective Alertness and Fatigue Following Two Consecutive Nights of Acoustic Stimulation. One night of acoustic stimulation led to improved alertness (A) and fatigue (B), while two nights of acoustic stimulation led to improved alertness and fatigue across the subsequent day (C and D), which was particularly evident in the afternoon (E and F). *$p < 0.05$ **$p < 0.01$.

pharmacological studies [55], we found no change in MSLT scores following SWA enhancement; although we note that despite the substantial increase in SWS (mins) and SWA reported in these studies, only small differences were reported in MSLT scores [18,55] or no difference at all. While our finding may point to the insufficiency of the enhancement of SWA for improving MSLT-defined sleepiness, it may also be due to reduced sensitivity of the MSLT in detecting daytime sleepiness (particularly within the normal range). As the majority of our participants scored in the normal range for MSLT [41], and were thus not considered to be 'sleepy', the capacity to alleviate sleepiness is then reduced. Taken together, we propose that enhanced SWA leads to both homeostatic resetting of sleep pressure and improves sleep consolidation, in habitually short sleepers, thereby improving next day reports of sleepiness, fatigue and objective indices of sustained attention.

Sleep restriction, defined as sleep duration or quality that is insufficient to support optimal functioning [56], is endemic in modern society [10–12]. Evidence suggests that individuals obtain insufficient sleep particularly on work days [12], and the magnitude of the deficit appears to be a growing [13]. While banking sleep on weekends may be a common strategy to alleviate sleep debt, the benefit is not sufficient for the duration of the working week [17]. For individuals who experience unavoidable short sleep on a week-day, acoustic stimulation may be one strategy alongside compensatory/prophylactic napping [16,53], strategic caffeine use [12,53], and adequate recovery sleep [17], to maximise alertness and performance following insufficient sleep. Of course, these strategies remain no substitute for a sufficient sleep period long-term [57].

We note several limitations for our study. Firstly, we experienced a large drop-out rate between participants who completed the study to participants with useable data. We attribute this to a number of reasons, including the use of multiple sites with few participants (ie, several sites had lost data/data of insufficient quality for the first 1–3 participants) and excessive artefact across all nights (~10%). When examining the effect of the device itself on data loss, we were concerned with a lack of N3 being detected by the device, although this was minimal for SHAM (3.4%) and STIM (6.4%). While not detecting N3 would render the device ineffective, of greater concern is the loss of data through the device having a negative effect on sleep quality. Importantly, only 3.4% (7 nights out of a possible 204) of data was lost due to a disruptive effect of the auditory tones, which have since been further improved with updated versions of the algorithm [37]. We note here that we used the device to examine changes in alertness and attention across multiple days, rather than a direct investigation or validation of the device itself (hence we removed these 3.4% from the final analyses). Secondly, we note that participants did not complete an adaptation night prior to each laboratory stay, which may create a 'first-night effect' for the repeat visit. We believe this is not responsible for the effects we observe here however (ie, enhanced SWA, particularly on Night 1) as: the first night effect is typically associated with sleep disruption, reduced REM-sleep and prolonged sleep latency [58]; we found no difference in sleep parameters between those who experienced SHAM first, compared to SHAM second; participants had extensive screening to rule out those with any sleep or psychiatric disorders, who are most susceptible to this kind of effect [59]; one adaptation night at the beginning of the protocol should be sufficient to eradicate the first night effect [60]; and the study was counterbalanced for order of exposure. Thirdly, while our study protocol was designed to emulate real-world conditions, such that participants were permitted to leave the laboratory after the first

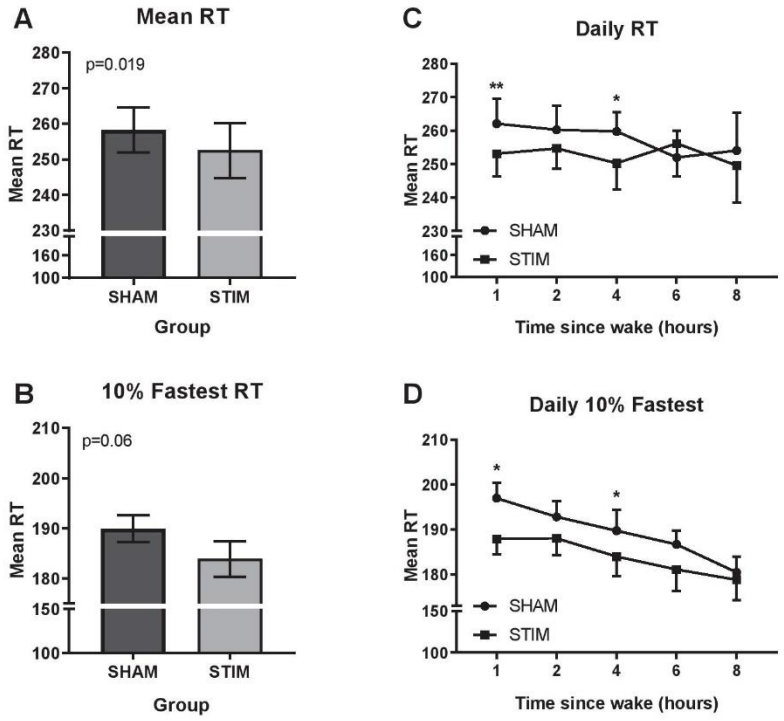

Fig. 6. Psychomotor Vigilance Performance Following Two Consecutive Nights of Acoustic Stimulation. Two nights of acoustic stimulation led to faster Mean rRT (A and C) and 10% fastest responses (B and D) on the PVT. Raw data presented in figures, analyses were run on transformed data. *$p < 0.05$ **$p < 0.01$.

night of each condition, this can be considered a limitation since it does introduce variability or noise in relation to SWA outcomes, ie, both the duration and intensity of prior wake influences SWS/SWA [32]. To limit this where possible, each visit was conducted during the work week, on the same day, such that the work environment would offer similarity between conditions.

To summarise, our data add to a growing body of knowledge with respect to the daytime benefits of acoustic stimulation of SWA. As these techniques become commerciality available, they may offer one potential solution for performance deficits in relation to memory, executive function, and attention outcomes due to insufficient sleep. To provide further evidence of acoustic stimulation of SWA as a strategy for alleviating sleep-related performance deficits, future work should examine the utility of these devices on other populations, and in relation to other cognitive domains, but in an ecological environment, ie, the environment in which these devices will be employed. As we describe large individual differences in response, future work should examine whether an acoustic SWA-phenotype exists by examining multiple, separate nights of stimulation, in a group of heterogenous individuals. Although we provide limited information on the utility of successive nights, we recommend that future work should systematically examine any rebound effect or any cumulative benefit across multiple nights, and whether devices might be most effective when used intermittently, such as when adjusting to shift work or when travelling, when used short-term such as work-schedule induced short sleep, or as a long-term strategy for alleviating SWS-related cognitive decline, such as cognitive ageing [61]. Together, these studies would reveal *who* might be benefit from acoustic stimulation of SWA and *how* they would best benefit in terms of mode of use and subsequent daytime outcomes.

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None.

Author contributions

All authors have made substantial contributions to the work. Garcia-Molina, Jasko, Ostrowski and White contributed to the study design. Diep, Garcia-Molina, Jasko, Manousakis, and Anderson analysed and interpreted the data. Diep and Anderson drafted the manuscript, with edits from Garcia-Molina, Manousakis, Jasko, Ostrowski and White. All authors approved the manuscript for publication.

CRediT authorship contribution statement

Charmaine Diep: Formal analysis, Writing - original draft. **Gary Garcia-Molina:** Formal analysis, Writing - review & editing. **Jeff Jasko:** Formal analysis, Writing - review & editing. **Jessica Manousakis:** Writing - review & editing. **Lynn Ostrowski:** Writing - review & editing. **David White:** Writing - review & editing. **Clare Anderson:** Formal analysis, Writing - original draft.

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Abbreviations List

AHI	Apnea-Hypopnea Index
AV	Attention-Vigilance
ECG	Electrocardiogram
EEG	Electroencephalogram
EMG	Electromyogram
EOG	Electrooculargram
LMM	Linear Mixed Model
MSLT	Multiple Sleep Latency Test
NREM	Non-Rapid Eye Movement
PLM	Periodic Leg Movements
REM	Rapid Eye Movement
SD	Standard Deviation
SEM	Standard Error of the Mean
SWA	Slow Wave Activity
SWE	Slow Wave Energy
SWS	Slow Wave Sleep

Conflict of interest

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: https://doi.org/10.1016/j.sleep.2021.01.044.

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Chapter 5

Heart rate variability increases following slow wave sleep enhancement

This chapter constitutes a manuscript prepared for submission to the *Journal of Sleep Research* as: Diep, C., Ftouni, S., Drummond, S.P.A., Garcia-Molina, G., & Anderson, C. (2021). Heart rate variability increases following slow wave sleep enhancement. *Journal of Sleep Research.* (Under Review).

Preface to Chapter 5

While **Chapter 3** and **4** assessed the impact of acoustic stimulation on cognitive outcomes, **Chapter 5** focussed on the physiological changes arising from acoustic stimulation, namely the changes in cardiac outcomes as a function of autonomic activity. During NREM sleep, as compared to wake, or REM sleep, there is a switch from sympathetic to parasympathetic control. This is particularly evident during SWS. With the loss of SWS due to age or disease, the clarity of the switch is diminished, and parasympathetic control is weakened, leading to decreased HRV and other adverse cardiometabolic outcomes. This includes lowered HRV during SWS itself, leading to increased cardiac risk such as atrial fibrillation. Previous studies have demonstrated that acoustic stimulation can improve these outcomes in young adults. In this chapter, we aimed to test whether acoustic stimulation could improve HRV in healthy, middle-aged men. The ECG data in this chapter was collected during the experimental nights in the *PowerSleep* study. We compared cardiac indices of autonomic activity between STIM and SHAM across time and frequency domains over the whole night, and over the first three sleep cycles.

Chapter 5 comprises of a short report prepared for *Journal of Sleep Research*. Each section is numbered for consistency and ease of navigation within the thesis.

Chapter 5 Prepared Manuscript

Title: Heart rate variability increases following automated acoustic slow wave sleep enhancement

Short title: Slow wave sleep enhancement and heart rate variability

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5.1 Abstract

Acoustic stimulation has been shown to enhance slow wave sleep and in turn, cognition, and now cardiac outcomes in young adults. With the emergence of commercial acoustic devices in the home, we sought to examine the impact of an acoustic, slow wave enhancing device on heart rate variability (HRV) in healthy, middle-aged males (n = 24, 39.92 ± 4.15 years). Under highly controlled conditions, participants were randomised to receive acoustic tones phase-locked to the up-phase of a slow wave (STIM), or no tones (SHAM) in a crossover design, separated by a 1-week washout period. STIM and SHAM were compared on measures of HRV for the whole night and over the first three sleep cycles. We found an increase in slow wave activity following STIM compared to SHAM. There was a significant increase for both time and frequency domains of HRV during the STIM condition compared to SHAM ($p < 0.05$), due to changes specifically during N3. In conclusion, HRV outcomes appear to improve following acoustic SWS enhancement.

5.2 Introduction

The autonomic nervous system is tightly coupled to sleep-wake state, with the synchronous oscillation between sympathetic and parasympathetic dominance changing with alternating Rapid Eye Movement (REM) and Non-REM (NREM) sleep states. During NREM sleep, the dominant shift toward a parasympathetic state results in reductions in blood pressure, heart rate, and systemic vascular resistance; which is particularly evident during slow wave sleep (SWS) (Mancia, 1993). This has important implications for cardiometabolic health, such that sleep insufficiency has been associated with cardiometabolic disease risk (Cappuccio & Miller, 2017). Heart rate variability (HRV) reflects the heart's ability to adapt to changing situations, with low HRV associated with increased risk of cardiac events (Tsuji et al., 1996). While suppressing SWS has been associated with reduced HRV (and other detrimental cardiometabolic events) (Tasali, Leproult, Ehrmann, & Van

Cauter, 2008), suggesting SWS may be a target for improving cardio-metabolic outcomes, there remains also a concern that enhancing SWS may further inhibit the autonomic response to physiological changes. In young healthy adults, using acoustic stimulation to enhance SWS, Grimaldi et al. (2019) reported that SWS enhancement increased measures of HRV, including high frequency (HF) power through a modulation of parasympathetic control (Grimaldi et al., 2019). With the emergence of commercial devices to detect and enhance SWS using acoustic stimulation, we sought to examine the impact of an automated, acoustic SWS enhancement device on HRV, and focussed on healthy, middle-aged men.

5.3 Methods

5.3.1 Study Design

Twenty-four healthy males (39.9 ± 4.1 years; 35-48 years), free from medical, psychiatric and sleep disorders, participated in the study. A randomised, double-blind cross over study was used to examine the impact of acoustic stimulation (STIM), compared to a SHAM control, on HRV outcomes. The full study protocol and exclusion criteria are described elsewhere (Diep et al., 2020). This consisted of a baseline night, followed by an experimental night (STIM or SHAM), repeated the following week with the alternate condition. During STIM, an automated acoustic stimulation device delivered tones (20–65 decibels depending on individualised sensitivity) phase-locked to the up-phase of the slow wave, and then pulsed at a frequency of 1Hz. During SHAM, the device operated the same but played inaudible tones (0 decibels). All participants gave written informed consent, and the study was approved by the Monash University Human Research Ethics Committee #CF15/671 – 2015000308.

5.3.2 Sleep Recording and Analysis

Electroencephalography (EEG) and electrocardiography (ECG) were recorded with Profusion PSG 4 (Compumedics, Melbourne, Australia), using the standard international 10-20 system, and two ECG channels placed one inch below the left collarbone, and between the lower two ribs on the right ribcage. ECG signals were sampled at a rate of 512 Hz and filtered between 0.05 – 100 Hz, and a 60 Hz notch filter applied. Sleep data were manually scored according to standard American Academy of Sleep Medicine criteria (Berry et al., 2017), and used to calculate total sleep time, N1, N2, N3, REM, wake after sleep onset (WASO), and number of arousals per hour.

5.3.3 Heart Rate Variability Analysis

HRV was analysed with the open access MATLAB application HRVTool (Vollmer, 2015) in clean, 5-minute segments, and resampled into 30 second periodicity to align with sleep staging using a custom-built MATLAB script to oversample by a factor of ten. Data were cleaned in a three-step process. First, artifacts were removed in HRVTool using the RRfilter method. Second, the data was then processed using a custom MATLAB script, with segments containing excessive noise or poor data removed. Third, the data was plotted and manually inspected for excessive variability in the data. HRV variables included: heart rate (HR), standard deviation of the normalised RR-intervals (SDNN) to evaluate global variability, low frequency power (LF; 0.04–0.15 Hz) and high frequency power (HF; 0.15–0.40 Hz) as a measure of parasympathetic activity, and LF:HF ratio to assess sympathovagal balance. We compared these HRV metrics between STIM and SHAM conditions over the whole night as a global measure of cardiac outcomes. For cycle-by-cycle analyses, we focussed on the first three cycles as <26% participants had N3 in cycle 4. To specifically assess changes within N3, we averaged across all available N3 epochs within each cycle. Although previous studies have examined the specific effect of stimulation by extracting 5-minute segments only (Grimaldi et al.,

2019), N3 was not consolidated in 5-minute bouts for the majority of our participants (e.g., for criteria of at least 80% of a 5-minute segment classified as N3 = ~70-74% data loss in cycle 3). Data from REM were treated in the same way for comparison/specificity. Data from one participant was excluded from analysis due to excessive artefact in the ECG recording during both experimental nights (final dataset, n = 23)

5.3.4 Statistical Analysis

GraphPad Prism V7.0 was used to run statistical analyses. Normality assumptions were checked for pairwise differences using Shapiro-Wilk's tests. Paired t-tests (or Wilcoxon matched-pair tests for non-parametric data) were used to compare whole night HRV between STIM and SHAM. Based on the cycle-by-cycle changes reported by (Grimaldi et al., 2019), we wished to follow these up in our dataset but were underpowered to run a linear mixed models with an interaction term (condition [STIM & SHAM]*cycle [cycle 1, cycle 2, cycle 3] linear mixed model using G*Power = <67% power to detect a medium-to-large effect size for all outcomes). We therefore conducted separate paired t-tests for each cycle between STIM and SHAM (i.e. Cycle 1, 2 and 3), and report both the effect size and a Benjamini–Hochberg's correction for multiple comparisons to minimise Type 1 errors. The same analyses were run for N3 and REM. Spearman's rho was used to conduct correlation analyses due to non-normality of the data. All results presented are mean ± SEM, and $p < .05$ (two-tailed) was considered significant.

5.4 Results

Whole Night Effects: There were no significant differences between STIM and SHAM for any sleep stage or metric ($p>0.25$), including N3 minutes (59.7 ± 6.6 vs. 59.1 ± 7.1, $p=0.88$). SWA (relative delta power, 0.5-4Hz) was enhanced, on average, by 11.6% across the entire night, relative to SHAM ($p<0.02$, $d = 0.65$, as described in Diep et al. (2020). Whole night changes in HRV

following STIM and SHAM are shown in Table 1. As expected, there were no differences between STIM and SHAM for any HRV outcome across the entire night ($p<0.16$).

Table 1. Heart rate variability per sleep stage

Parameter		N1	N2	N3	REM	Overall
SDNN (msec)	STIM	100.7±25.4	75.7±24.1	53.5±17.7	86.9±20.3	79.5±19.9
	SHAM	101.6±26.4	73.9±16.2	48.0±12.6	86.8±20.8	78.5±15.9
	p-value	**0.83**	**0.57**	**0.03**	**0.98**	**0.68**
LF ($msec^2$)	STIM	0.71±0.4	0.61±0.4	0.30±0.2	0.65±0.4	0.56±0.3
	SHAM	0.75±0.4	0.52±0.2	0.25±0.1	0.67±0.3	0.54±0.2
	p-value	**0.84** [W]	**0.36** [W]	**0.025**	**0.64**	**0.77** [W]
HF ($msec^2$)	STIM	0.34±0.4	0.30±0.4	0.24±0.2	0.26±0.3	0.26±0.2
	SHAM	0.33±0.5	0.23±0.2	0.18±0.2	0.20±0.2	0.22±0.2
	p-value	**0.97** [W]	**0.37** [W]	**0.034** [W]	**0.99** [W]	**0.49** [W]
LF/HF	STIM	4.1±2.1	3.5±1.7	2.3±1.5	5.1±2.3	3.8±1.7
	SHAM	4.2±2.3	3.5±1.8	2.0±1.1	4.9±2.2	3.8±1.7
	p-value	**0.58**	**0.62**	**0.77** [W]	**0.60**	**0.82**
HR (bpm)	STIM	54.9±6.4	53.9±6.7	53.1±6.4	56.9±7.0	55.0±6.7
	SHAM	54.7±5.6	53.4±5.3	52.6±5.3	56.2±6.0	54.4±5.6
	p-value	**0.36** [W]	**0.50**	**0.32** [W]	**0.03** [W]	**0.16** [W]

[W] refers to Wilcoxon matched-pairs signed rank test. P-values shown in table are uncorrected. Data presented as mean ± SD.

Sleep Stage Specific Effects: STIM versus SHAM differences were found according to sleep stage. While no changes were observed in HRV outcomes for N1, N2, or REM ($p_{adj}>0.36$), during

N3, STIM led to a significant increase in HRV outcomes, including SDNN, LF, and HF (p_{adj}<0.05), with medium to large effect sizes (d = 0.4–0.8). See Table 1 and Figure 1. At the individual level this increase in HRV outcomes was observed in most participants (70% for SDNN; 60% for HF). Furthermore, % SWA enhancement (0.5-4Hz) was positively correlated with % change in SDNN (r=0.42, p=0.058), with the top tertile of SWA responders showing significantly increased SDNN, relative to the lower tertile of SWA responders, with large effect size (32.6 ± 10.8% vs. 1.6 ± 6.0%, p=0.03, d=1.6). See Figure 2. While no correlations were reported for HF and SWA enhancement (r<0.2), the top tertile of SWA responders exhibited a greater % change in HF relative to the bottom tertile of responders, with moderate effect size (116.5 ± 178.0% vs. 14.4 ± 24.0%, p=0.007, d=0.7).

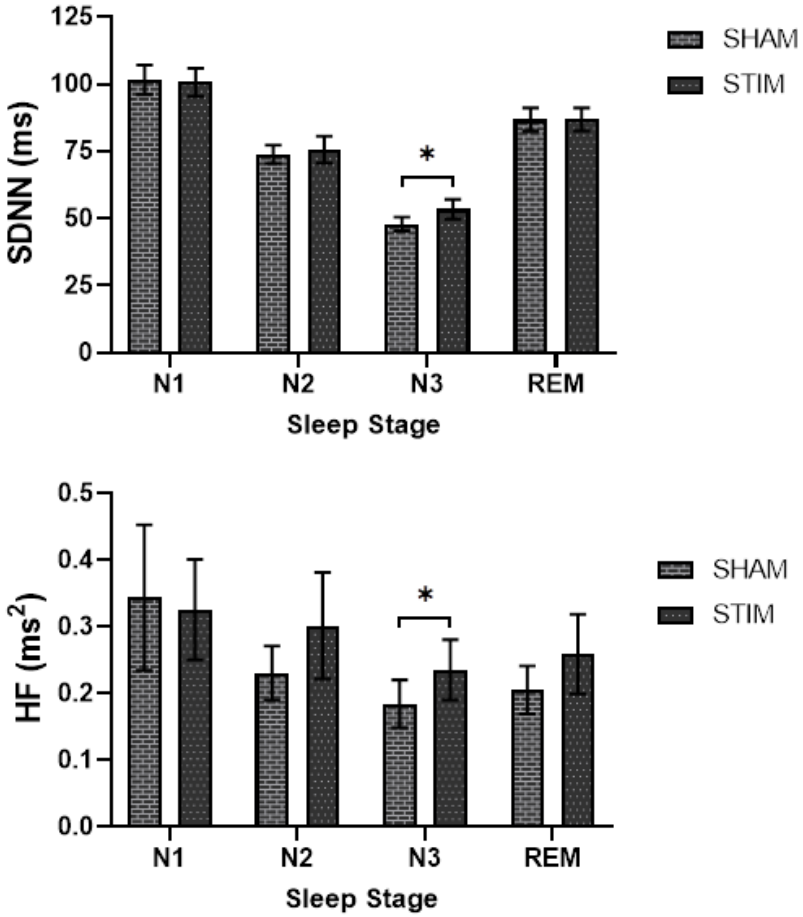

Figure 1. Changes in SDNN and HF across each sleep stage.

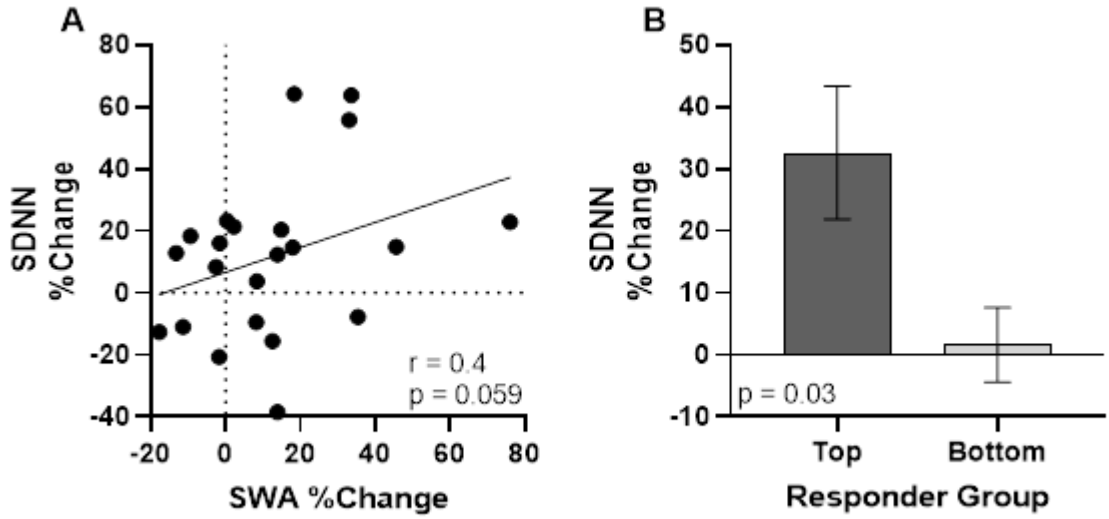

Figure 2. Changes in SDNN and SWA (4Hz) following acoustic slow wave enhancement. Left: Correlation between SDNN and SWA from SHAM to STIM. Right: Percent change in SDNN in top responders (n = 7) and non-responders (n = 7).

Sleep Cycle Effects: The largest increases from SHAM to STIM were evident during the first sleep cycle for SDNN (STIM: 55.6 ± 4.0; SHAM: 47.2 ± 2.9, p_{adj}=0.01, d = 0.65), LF (STIM: 0.34 ± 0.04; SHAM: 0.25 ± 0.02, p_{adj}^{W}=0.01, d = 0.52), and HF (STIM: 0.25 ± 0.05; SHAM: 0.18 ± 0.03, p_{adj}^{W}=0.01, d = 0.53). There were no significant changes in LF/HF (p_{adj}^{W}=0.41, d = 0.2). There were no significant outcomes observed for cycles 2 and 3 (p_{adj}>0.5, d < 0.4). There were no differences observed in REM sleep (p_{adj}>0.6, d > 0.3), as summarised in Table 2.

Table 2. Heart rate variability per cycle for N3 and REM

Parameter		Cycle 1	Cycle 2	Cycle 3
N3 SDNN (msec)	STIM	55.6±19.4	53.6±20.7	60.4±37.5
	SHAM	47.2±13.7	49.5±14.1	54.0±14.7
	p-value	**0.005**	**0.24**	**0.44**

REM SDNN (msec)	STIM	83.9±20.7	82.0±23.4	88.2±23.4
	SHAM	81.9±23.1	85.4±26.0	89.2±26.8
	p-value	**0.68**	**0.51**	**0.87**
N3 HF ($msec^2$)	STIM	0.25±0.2	0.25±0.3	0.19±0.2
	SHAM	0.18±0.1	0.17±0.2	0.15±0.2
	p-value	**0.008** [W]	**0.16** [W]	**0.06** [W]
REM HF ($msec^2$)	STIM	0.21±0.2	0.18±0.2	0.25±0.3
	SHAM	0.19±0.2	0.21±0.2	0.2±0.2
	p-value	**0.74** [W]	**0.51** [W]	**0.49** [W]

[W] refers to Wilcoxon matched-pairs signed rank test. P-values shown in table are uncorrected. Data presented as mean ± SD.

5.5 Discussion

We investigated the impact of acoustic stimulation of SWS on HRV in middle-aged men. Using an automated device, we demonstrated increased SDNN and HF during N3 sleep, signifying a potential increase in parasympathetic control during SWS enhancement. These results broadly suggest improved cardiovascular function specifically during N3 with acoustic stimulation.

Consistent with previous studies of SWS enhancement (Grimaldi et al., 2019; Shaltout et al., 2018) we found an overall increase in HRV (SDNN) and parasympathetic activity (HF) during STIM relative to SHAM. Here, both SDNN and HF power increased during STIM (14.5% and 31.8% increase, respectively), remaining within a healthy range. Importantly, those individuals with the highest SWA enhancement showed the largest improvement in SDNN and HF outcomes. This has several important implications. First, while higher HRV generally reflects better cardiac health, HRV is not always improved by an increase (e.g., stress), but should change within a healthy range [SDNN:

141±39 (ms), HF: 975±203 (ms^2), (Shaffer & Ginsberg, 2017)]. Second, HRV as indicated by SDNN is a gold standard measure of cardiac risk (albeit for 24h recordings), such that patients with pathologically low SDNN (<50ms) are 5x more likely to have a risk of mortality compared to those with high SDNN (>100ms) (Kleiger, Miller, Bigger, & Moss, 1987; Shaffer & Ginsberg, 2017). While the change in SDNN remained within the normal limits for both conditions in our study, acoustic stimulation may improve SDNN to a greater extent in clinical populations, where SDNN is low, thus increasing the capacity for improvement. Importantly, the majority of individuals (70%) showed improvements in this outcome.

Our study does differ in some of the findings reported by Grimaldi et al. (2019). First, we report an increase in LF, rather than a decrease. As LF is moderated by both the parasympathetic and sympathetic nervous system, and is not informative on cardiac health as a standalone measure, this does not change our interpretation of SDNN and HF outcomes. Secondly, while Grimaldi et al. (2019) report improvements in HRV in cycles 2 & 3, we observed changes in cycle 1 only. This may be due to a natural loss of N3 data in the second half of the night due to age; while 70% of participants contributed data to the third cycle, this comprised only 7 minutes of N3 on average, which was typically not in a consolidated bout. Moreover, our dataset was focused on men, compared to the previous study which was largely female. While this finding may also be seen as discrepant with our previous findings (e.g., we show the SWA effect to be large in subsequent cycles, whereas here we show the HRV effect to be largely focussed on cycle 1), the two studies differ in their approach (e.g., in Diep et al., we combine N2+N3 for SWA which is not recommended for HRV outcomes due to stage specific differences). Although we also urge caution to our cycle 1 findings due to reduced power (with the caveat that we observed medium effect sizes for each [$d > 0.52$]), we suggest further work to better understand the temporality of acoustic stimulation driven changes in HRV outcomes, across age, sex, and clinical samples.

Our data should be interpreted with several limitations in mind. First, participants were thoroughly screened to be as healthy as possible, which may have limited the capacity for improvement in cardiac outcomes. Second, although acoustic stimulation has been associated to both improvements in HRV during sleep and the subsequent wake period (Grimaldi et al., 2019), we were unable to examine the subsequent wake period as ECG electrodes were removed upon awakening (i.e., our study was not specifically designed to look at autonomic activity). We were therefore unable to examine whether acoustic stimulation impacts HRV during wake or exert any impact on other systems controlled by the autonomic nervous system, such as respiratory rate or cortisol. Third, our study focussed on middle-aged men to minimise age and sex differences within the dataset (which are observed for both SWS and HRV). While this is an important age group to study (e.g., SWS depletes in this group), it does restrict generalisability to the general population. Finally, we only had a single night of data of stimulation and are therefore unable to infer whether there are any long-term benefits of acoustic stimulation on HRV. As acoustic stimulation appears to improve SWS with consecutive nights of stimulation (Diep et al., 2021), and long-term sensory stimulation improves HRV (Shaltout et al., 2018), future studies might reveal important improvements in cardiac health longer-term.

To summarise, acoustic SWS enhancement does appear to benefit HRV. Of note, the increase in SDNN suggests that the use of acoustic stimulation may improve overall cardiac health and may have future application to reduce cardiovascular incidents during sleep. This is significant given that poor sleep is associated with increased cardiovascular disease (Cappuccio & Miller, 2017) and acoustic stimulation may offer an accessible, cost-effective, and free from side effects intervention for improved cardiometabolic health.

5.6 Acknowledgements

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5.7 Conflicts of Interest

G.G.M and S.P.A.D report no competing financial interests. C.D. was a recipient of a PhD Scholarship, S.F. was a Project Leader, and C.A. was a Theme Leader in the Cooperative Research Centre for Alertness, Safety and Productivity at the time of this study. No authors report any actual or potential conflicts of interests.

5.8 Author Contributions

All authors have made substantial contributions to the work presented and have approved the final version of the manuscript. C.A. and S.F. designed the study, with input from S.P.A.D. C.D. and S.F. were responsible for data collection; C.D., and G.G.M. analysed the data; C.D. interpreted the data, and C.D. wrote the manuscript with edits from C.A. All authors approved the final manuscript.

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Chapter 6

General Discussion

The aim of this thesis was to investigate the impact of acoustic stimulation via a novel, automated, auditory device on SWS, cognition and cardiovascular health. This work consists of two large studies. The first study, *PowerSleep*, examined the use of an automated, acoustic device to enhance SWA and executive function (**Chapter 3**). Using the same device, the second study, *SmartSleep*, focussed on the changes in attention and alertness outcomes following consecutive nights of use in habitually sleep-restricted adults (**Chapter 4**). Given the tightly coupled relationship between SWS and the ANS, the final aim was to explore the cardiovascular effects of SWS enhancement with data collected from the *PowerSleep* study (**Chapter 5**).

6.1 Summary of Key Experimental Findings

This thesis has made a significant contribution to the literature surrounding the utility of acoustic stimulation for enhancing SWS, cognition and aspects of physiology. The key findings include 1) acoustic stimulation using an automated device enhances SWA, which is evident in whole-night sleep EEG; 2) enhanced SWA is associated with improved executive function, and attention and alertness outcomes; 3) acoustic stimulation is beneficial for both healthy middle-aged adults, and habitually sleep-restricted adults and; 4) acoustic stimulation increases HRV in middle-aged men.

Midlife provides a unique opportunity for slow wave enhancement due to the decline in SWA but need for optimal cognitive capacity. Therefore, in **Chapter 3**, the impact of acoustic stimulation on SWS and executive function was focused on middle-aged men. While acoustic stimulation has previously been shown to enhance SWS, we provided the first evidence that an automated, acoustic device increased SWA over the *entire* night in *healthy middle-aged men*. SWA enhancement was associated with increased performance on phonetic fluency and working memory tasks, subsets of executive function. Despite the stringent screening criteria and sample range, we did not find a

uniform change in SWA across individuals, suggesting that subtle interindividual differences play a role in the response to acoustic stimulation.

To increase applicability to real-world settings, the study described in **Chapter 4** adopted a more ecologically-valid protocol with less stringent exclusionary criteria. Insufficient sleep plagues a significant proportion of the population (Hafner, Stepanek, Taylor, Troxel, & van Stolk, 2017; Liu et al., 2016), and is associated with poorer health and cognitive outcomes. **Chapter 4** examined the impact of acoustic stimulation in a population of chronically *sleep-restricted males and females*. In this paper, changes in *attention and alertness* following *two* consecutive nights of acoustic stimulation were assessed. Acoustic stimulation increased SWA on both nights, leading to improved subjective alertness and sustained attention over the course of the second day. As in **Chapter 3**, there was a broad range of interindividual responses to acoustic stimulation, such that while the majority of participants had enhanced SWA after one night of stimulation, this enhancement ranged from 2-90%. As two experimental nights were examined, we also discovered that there were significant intraindividual responses to acoustic stimulation, which appeared unrelated to SWS rebound, with 44% of participants being consistent *responders* (i.e., improved SWA during both nights of stimulation), while 12% were consistent *non-responders*. Due to the overall improvement in attention and alertness outcomes, this paper concluded that acoustic stimulation may be a practical option for alleviating some of the typical cognitive deficits that arise from insufficient sleep.

As the ANS is downregulated during SWS, **Chapter 5** examined the physiological effects of acoustic stimulation, focusing on the cardiovascular system. Using the ECG data collected in the *PowerSleep* study, we found that during N3, HRV was heightened during the STIM condition compared to SHAM. This was observed for both time and frequency domains, notably SDNN, which reflects overall cardiac health, and HF power, indicating increased vagal control. Given that SDNN

is indicative of long-term cardiac health, acoustic stimulation may be a useful therapeutic for improving cardiovascular outcomes.

In summary, **Chapters 3** and **4** illustrated that acoustic stimulation improves next-day cognition and alertness, **Chapter 5** suggests that acoustic stimulation can improve cardiac health. Additionally, **Chapter 4** demonstrated that acoustic stimulation can be operated effectively over multiple nights. Together, this thesis contributes to a body of work signifying that acoustic stimulation may have the potential use as a therapeutic to alleviate the cognitive and physiological deficits associated with slow wave decline (e.g., ageing) and/or sleep restriction.

6.2 The Impact of Acoustic Stimulation on Cognition and Physiology

6.2.1 Slow Wave Sleep and Cognition

There is a general consensus that acoustic stimulation enhances SWS, leading to improvements in cognition (Zhang & Gruber, 2019), with some exceptions (Henin et al., 2019; Weigenand, Mölle, Werner, Martinetz, & Marshall, 2016), possibly due to interindividual differences (further detailed in Section 6.3). The studies in this thesis provide further evidence that SWA plays a causal role in cognitive outcomes and that acoustic stimulation may strengthen the mechanisms underlying SWA and cognition. As described in **Chapter 1**, there are two leading theories that underpin how SWA impacts cognition, the synaptic homeostasis hypothesis (Tononi & Cirelli, 2003), and the active systems consolidation model (Diekelmann & Born, 2010).

Within the framework of the synaptic homeostasis hypothesis, acoustic SWS enhancement may improve cognitive outcomes by improving synaptic downscaling in several interrelated ways including (i) decreased acetylcholine release (Gais & Born, 2004), and (ii) strengthening long-term depression (Massey & Bashir, 2007). In support of this theory are the findings that 1 Hz activity

appear to be the focus for SWA enhancement, and executive function improved following a night of STIM in **Chapter 3**. This suggests that cortical re-organisation of waking circuits and synaptic downscaling occurring during SWA restore neural functioning for optimal next-day performance.

In line with the active consolidation model, acoustic stimulation may strengthen SWA to facilitate the reactivation of newly encoded memories and also serve to moderate and synchronise other neural drivers of cognition, including sleep spindles, hippocampal SWRs and K-complexes (Diekelmann & Born, 2010; Staresina et al., 2015). Indeed, several studies have also found concomitant increases in spindle activity following slow wave enhancing acoustic stimulation (Diep et al., 2020; Papalambros et al., 2017; Schneider, Lewis, Koester, Born, & Ngo, 2020).

Ultimately, based on the available knowledge so far, all that can be concluded is that acoustic stimulation serves to support SWS, which has a multifaceted role in maintaining cognitive health, including synaptic plasticity, overnight memory consolidation, executive function.

6.2.2 Slow Wave Sleep and Heart Rate Variability

Dynamic coupling between the central nervous system (CNS) and the ANS can be assessed by pairing EEG and cardiovascular indices (for example by observing the changes in HRV across sleep stages). The specific neural pathways underlying CNS and ANS coupling are unknown. It has been suggested however, that CNS-ANS coupling is reliant on mutual ascending and descending pathways throughout hypothalamic and brainstem circuits (de Zambotti, Trinder, Silvani, Colrain, & Baker, 2018), as both are controlled by a master conductor regulating wake, sleep and the ANS (Silvani & Dampney, 2013). As acoustic stimulation specifically enhances the slow wave (an index of the CNS), it could be used as a tool to elucidate the directionality of the relationship between the CNS and ANS.

We (**Chapter 5**) and others (Grimaldi et al., 2019) have now provided evidence demonstrating that acoustic stimulation can be utilised to regain vagal control during SWS. The loss of SWS with

age is associated with a decrease in cardiac health, even within healthy ageing (Brandenberger et al., 2003). Evidence suggests that the decrease in SWS hinders the switch between parasympathetic and sympathetic control across sleep stages (Trinder et al., 2001). Sleep restriction also significantly impairs HRV (Choudhary, Alam, Dhanvijay, & Kishanrao, 2018). Disruptions in autonomic balance is associated with the manifestation of cardiovascular diseases, and increased risk of myocardial infarctions during sleep (Marin, Carrizo, Vicente, & Agusti, 2005). Although more research is needed to elucidate the long-term changes in cardiac outcomes, acoustic stimulation may provide clinically meaningful improvements to cardiovascular health.

6.2.3 Interplay between SWS, HRV and Cognition

Throughout this thesis, the experimental chapters and broader literature review have illustrated the following: there is an association between SWS and cognition; SWS and HRV appear to have a bidirectional relationship; and acoustic stimulation impacts SWS and HRV. HRV has been linked to multiple areas of cognition independently of SWS (Elias & Torres, 2017; Forte, Favieri, & Casagrande, 2019), and interestingly, the ability to adapt to mental stressors in addition to physical ones (Hansen, Johnsen, & Thayer, 2009; Hilgarter et al., 2021). What remains to be seen is whether acoustic stimulation can moderate the relationship between SWS and HRV to further improve cognition.

6.3 Individual Differences: Responders *versus* Non-Responders to Acoustic Stimulation

A common theme that emerged from the experimental chapters was the observation of interindividual differences, and the need for future studies to understand why these occur. In **Chapter 3**, approximately 65% of participants had an increase in SWA following STIM (considered responders), while 30% had a decrease (considered non-responders). **Chapter 4** revealed that some

individuals may be *consistent* responders or non-responders. For instance, 56% of participants had a consistent SWA response, with 44% being consistent responders, and 12% were consistent non-responders. Generally, the studies in this thesis, and others have observed a broad range in SWA percent change from SHAM to STIM (Diep et al., 2020; Diep et al., 2021; Ong et al., 2018), which was related with a benefit on next-day condition. With that said, there are some conflicting reports that despite successful SWS enhancement, there were either mixed effects (Papalambros et al., 2019), or no impact on cognition (Henin et al., 2019). This may be due to differences in stimulation paradigms, or may allude to interindividual differences again, as the authors mentioned that the endogenous NREM rhythms were different between studies (Henin et al., 2019). As discussed in the introduction (**Chapter 1**), there is no "one-size fits all" method of enhancing SWS. The interindividual differences impacting the presentation and preservation of SWS have been previously described. For example, young males exposed to the same sleep deprivation protocol display interindividual variability SWA rebound during recovery sleep (Rusterholz & Achermann, 2011; Rusterholz, Dürr, & Achermann, 2010). After two nights of fragmented sleep, individuals who are morning-types have higher slow wave amplitude and steeper slopes compared to their evening-type counterparts (Mongrain, Carrier, Paquet, Bélanger-Nelson, & Dumont, 2011). The factors that control who may benefit from acoustic stimulation are yet to be determined but may be aligned with why SWA expression differs within an individual, including:

6.3.1 Genetics

Over an 11-day protocol, Tucker, Dinges, and Van Dongen (2007) systematically uncovered 17 robust sleep variables, of which SWS and delta power were the strongest. Age, ethnicity, and sex all played significant roles in the expression of SWS. While not examined in the Tucker et al. (2007) study, others have shown that there is a large genetic component to EEG (De Gennaro et al., 2008; Linkowski, 1999). Multiple genes have been implicated in SWS generation, including: PERIOD3

(PER3), such that older adults expressing the $PER3^{5/5}$ polymorphism have more delta activity compared to those with the $PER3^{4/4}$ polymorphism (Viola et al., 2012); brain-derived neurotrophic factor (BDNF), with carriers of the polymorphism *Val66Met* producing more SWS and SWA compared to those with the heterogenous *Val/Met* allele (Bachmann et al., 2012); and the c.22G>A polymorphism of adenosine deaminase (ADA), whereby individuals with the G/A genotype have more slow wave power compared to those with the G/G genotype (Bachmann et al., 2011). The extent to which acoustic stimulation can interact with these populations remains unclear. However, gene expression may indicate who may be more receptive to acoustic stimulation, particularly as it appears as though a minimum SWA threshold is required for effective stimulation, described in the following section.

6.3.2 Age

A major component impacting the effectiveness of acoustic stimulation is age. Numerous studies have demonstrated that a minimum quantity of SWA is necessary for optimal enhancement, not only when comparing young adults to older (Navarrete et al., 2020; Schneider et al., 2020), but also to middle-aged adults (Garcia-Molina et al., 2018). This can also be observed when comparing young (Ong et al., 2016), middle-aged (Diep et al., 2020) and older adults (Papalambros et al., 2017), as the magnitude of SWA enhancement decreases (Schneider et al., 2020). The significant decrease in slow wave amplitude in older adults could be countered by simply modifying the slow wave identification thresholds, but that increases the risk of overlapping with arousal thresholds (Bellesi, Riedner, Garcia-Molina, Cirelli, & Tononi, 2014).

Even when comparing within older populations, some older adults have more preserved SWA than others (Bliwise, 2011), which could be influenced by lifestyle (Naylor et al., 2000), brain structure (Mander et al., 2013), and accumulation of Aβ and tau (Liguori et al., 2014), among other factors (Li, Vitiello, & Gooneratne, 2018). Based on the knowledge that SWS is also modified by the

intensity of the waking day (Huber, Tononi, & Cirelli, 2007), acoustic stimulation may be strengthened when paired with enriched environments for older adults.

6.3.3 Sleep Continuity

Increased sleep fragmentation is associated with lower SWS. Sleep spindles show remarkable intraindividual stability (Eggert et al., 2015; Landolt, Dijk, Achermann, & Borbély, 1996) and may be a marker of sleep continuity. In contrast to alpha-delta sleep, individuals with high spindle density are more resistant to sleep disruption during auditory stimulation (Dang-Vu, McKinney, Buxton, Solet, & Ellenbogen, 2010; Dang-Vu, Schabus, et al., 2010). Similar conclusions have been made regarding K-complexes (Bastien, Ladouceur, & Campbell, 2000). Consequently, in these individuals, it is conceivable that the effects of acoustic stimulation could be extended to evoke spindles and K-complexes in lieu of an arousal to improve sleep quality and cognition, further described in Section 6.5.4 (Forget, Morin, & Bastien, 2011). Further investigation is needed, with larger sample sizes than that reported here, to identify interindividual differences between EEG signatures which may indicate whether an individual is resistant to sleep disruption.

6.4 Utility for SWA Enhancement: Daily Use of Acoustic Stimulation for Next-Day Performance

This thesis has demonstrated that acoustic stimulation provides an immediate benefit to cognitive and physiological processes. Given that the acoustic device described in this thesis is now commercially available, these findings have important implications for the everyday consumer. Understanding which consumers may benefit and under what circumstances are an important logical next step.

First: middle-aged adults. From the review presented in **Chapter 1**, many cognitive and physiological processes begin to, or are substantially deteriorated by middle age, perhaps as young

as age 40 (Singh-Manoux et al., 2012). While SWA is diminished in middle-aged adults compared to younger adults, it is not so far deteriorated that it is difficult to enhance, as with older adults. Although prior research has also indicated that both young and older adults may also benefit from slow wave enhancement (Ngo, Martinetz, Born, & Mölle, 2013; Papalambros et al., 2017), middle aged adults are more active in the workforce compared to younger (~30% more) and older (~60% more) adults (OECD, 2017). Additionally, as a large proportion of middle-aged adults will likely continue working as they move into the next age bracket, it is essential to preserve optimal cognitive capacity (Fisher, Chaffee, Tetrick, Davalos, & Potter, 2017). Outside of the workplace, implementing healthy lifestyle interventions in middle-aged adults is also essential for successful cognitive ageing (Hertzog, Kramer, Wilson, & Lindenberger, 2008). Thus, middle age provides an ideal opportunity for slow wave improvement.

Second: individuals in the workforce. SWS is essential for many cognitive functions. Given that we (Diep et al., 2020) and others (Ong et al., 2016; Papalambros et al., 2017) have shown improvements in next-day improvements in overnight memory consolidation and next-day improvements in executive function, and attention and alertness outcomes, acoustic stimulation may be beneficial for individuals who have strenuous cognitive demands in the workplace.

Third: individuals suffering from insufficient sleep. **Chapter 4** focussed on acoustic slow wave enhancement in habitually sleep-restricted adults under more ecologically valid settings. These findings are highly applicable to the 24/7 society. Given the rise in chronic sleep restriction and daytime sleepiness (Bartlett, Marshall, Williams, & Grunstein, 2008), an effective strategy to combat fatigue is essential. While shift workers may find it beneficial to use acoustic stimulation before work, or even during their break at work (as slow waves can be enhanced during a nap) to improve alertness and executive function during their shift, further work should examine whether (i) SWA can be

enhanced in sleep that is misaligned to the circadian system, and (ii) whether sleep inertia following that sleep period would be detrimental to worker performance and safety.

6.5 Limitations and Future Directions

6.5.1 Long-Term Use of Acoustic Stimulation

We (Diep et al., 2020; Diep et al., 2021), and others (Grimaldi et al., 2019; Ngo et al., 2013) have now demonstrated that acoustic stimulation improves SWA, cognition and physiology, for next-day outcomes. Needless to say, the outstanding question is: what is the long-term impact of acoustic stimulation on cognitive and physiological processes? In this thesis, stimulation was only applied for one (**Chapter 3**), or two (**Chapter 4**) nights, which restricts any inferences regarding long-term use of acoustic stimulation. Importantly, we did not observe any SWS rebound with consecutive nights of stimulation (**Chapter 4**), suggesting that acoustic stimulation may be used consistently. Further, as we (Diep et al., 2021), and others (Garcia-Molina et al., 2018) have shown that acoustic stimulation can be used for consecutive nights, acoustic stimulation may be a viable option as a regular intervention for offsetting or preventing the cognitive and physiological deteriorations associated with ageing. Nevertheless, more research is needed to elucidate the potential long-term benefits of acoustic stimulation on cognition and physiological health, or conversely whether there are any negative side-effects to using acoustic stimulation.

6.5.2 Feasibility Outside of the Laboratory

The main limitations of this work were that both studies were laboratory based, with a moderate to high level of control, which makes it difficult to infer whether acoustic stimulation applied within the home would have the same degree of success, or whether the same cognitive and physiological outcomes would be improved. Naturalistic, and mixed-environment (combining home and laboratory based) studies have now reported acoustic stimulation increases SWA (Garcia-Molina

et al., 2018), demonstrating that the everyday consumer would be able to successfully use auditory devices within the home, rather than within the confines of highly controlled laboratory environments. The next step would be to validate next-day outcomes with more ecologically valid settings and tests, such as driving fatigue or workplace performance.

6.5.3 Beyond Healthy Adults - Use in Clinical Populations

Given the relative infancy of the work in acoustic stimulation of SWS, the majority of existing literature in the field relates to reasonably healthy adults of varying ages. However acoustic stimulation may have some therapeutic benefit to non-healthy, clinical populations, given that SWS impairment is also associated with a number of neurocognitive, psychiatric and sleep disorders, including (but not limited to) depression, schizophrenia, insomnia and sleep apnoea (Anderson & Bradley, 2013).

6.5.3.1 Dementia and Neurocognitive Health

It has been predicted that by 2050, 1 in 6 people will be over the age of 65 (United Nations, 2019). The number of dementia cases in Australia are projected to increase twofold by 2058 (Brown, Hansnata, & La, 2017), and 152 million worldwide ("2020 Alzheimer's disease facts and figures," 2020), alongside parallel increases in caregiver costs from 818 billion to 2 trillion US dollars (WHO, 2017). Given that there is still no effective cure for dementia, it is pertinent to develop strategies to combat cognitive decline. Sleep has been presented as a modifiable risk factor for dementia (Minakawa, Wada, & Nagai, 2019). As described throughout this thesis, SWS mediates a multitude of cognitive and physiological processes, and all three factors deteriorate with increasing age (Brandenberger, Ehrhart, Piquard, & Simon, 2001; Mander, Winer, & Walker, 2017). Of vital importance is how SWS facilitates the clearance of metabolites from the brain (Fultz et al., 2019; Xie et al., 2013), particularly those that are implicated in neurodegenerative diseases including Aβ and

tau. SWA and Aβ have a bidirectional relationship (Wunderlin, Züst, Fehér, Klöppel, & Nissen, 2020). While Aβ deposits can disrupt SWA, the lack of SWA increases the presence of Aβ levels in CSF and Aβ deposits in the medial prefrontal cortex (Mander et al., 2015). Therefore, if this restorative role of SWS could be preserved throughout ageing, one of the main causes of AD could be ameliorated. By strengthening slow oscillations, acoustic stimulation may indirectly promote glymphatic clearance of Aβ and tau, and/or promote neuronal restoration (i.e., reduce oxidative stress and increasing cellular repair) (Mander, Winer, Jagust, & Walker, 2016).

There is some evidence suggesting that slow wave enhancing acoustic stimulation can improve memory in individuals with amnestic mild cognitive impairment (Papalambros et al., 2019), a common precursor to dementia. Ideally, given that brain pathology can precede cognitive decline by over a decade (Beason-Held et al., 2013; Risacher et al., 2009), intervention for AD-dementia should begin prior to the onset of any impairment, to delay the onset of cognitive decline, by targeting pre-symptomatic older adults, or again, middle-aged adults, as focussed here. Indeed, acoustic stimulation has had some degree of success in enhancing SWA and memory in healthy older adults (Papalambros et al., 2017), more so than in those with mild cognitive impairment (Papalambros et al., 2019). However, decline takes years to assess, and so other studies are required to first understand the long-term efficacy of slow wave intervention.

6.5.3.2 Sleep and Health Disorders

The loss of SWS with age has been implicated with the parallel decline in cardiometabolic health (Strait & Lakatta, 2012), possibly due to the loss of parasympathetic control (Brandenberger et al., 2003; Silvani & Dampney, 2013). This theory is reinforced by the fact that the experimental disruption of SWS leads to impaired metabolic outcomes (Tasali, Leproult, Ehrmann, & Van Cauter, 2008). Conversely, using acoustic stimulation to enhance SWS may improve these outcomes. Acoustic stimulation has been shown to improve cardiovascular regulation and metabolic function

by increasing indices of parasympathetic control, albeit this has only been shown in healthy young (Grimaldi et al., 2019) and middle-aged adults (Chapter 5) so far.

There is evidence that acoustic stimulation can reduce the cardiometabolic disruptions associated with some sleep disorders, such as insomnia (Tegeler et al., 2020). The specific impact of acoustic stimulation on sleep disorders such as sleep apnoea, is yet to be identified. Given that auditory stimulation increases sleep continuity, and apnoeas decrease during SWS (Ratnavadivel et al., 2009), it would not be unreasonable to posit that slow wave enhancing acoustic stimulation would also improve these outcomes. However, of note, slow wave enhancing drug studies have reported only small, or no improvements in apnoea-hypopnea symptoms (Taranto-Montemurro et al., 2017).

6.5.4 Enhancing Other Sleep Features

One of the major barriers impacting the effectiveness of acoustic stimulation is how the presence of SWA is needed to enhance SWA. This excludes individuals who arguably need acoustic stimulation the most – those who have severely depleted SWS. The logical next step would be to target other electrophysiological signatures of sleep that are (i) involved in cognition; (ii) can be enhanced via acoustic stimulation and, (iii) may benefit from enhancement (i.e., they decrease due to ageing or disease), such as sleep spindles, K-complexes, and SWRs. Sleep spindles and SWRs are particularly attractive as future candidates for enhancement as it is the functional coupling between them and slow oscillations that drive overnight memory consolidation (Diekelmann & Born, 2010; Niknazar, Krishnan, Bazhenov, & Mednick, 2015), thus providing multiple avenues for combating cognitive decline.

One clear target for dual enhancement are thalamocortical sleep spindles as they fulfil all the aforementioned criteria: sleep spindles are thought to promote memory consolidation (Fogel & Smith, 2011; Gais, Mölle, Helms, & Born, 2002) and are associated with executive functions such as reasoning and working memory (Fang, Ray, Owen, & Fogel, 2019); they increase with

pharmacological drugs (Kaestner, Wixted, & Mednick, 2013) and acoustic stimulation (Antony & Paller, 2016; Ngo et al., 2015) and finally, they decrease with increasing age (Crowley, Trinder, Kim, Carrington, & Colrain, 2002). To date, studies aiming specifically to enhance spindles and memory outcomes have yielded mixed results, with some reporting an increase (Lustenberger et al., 2016), while others report none (Ngo, Seibold, Boche, Mölle, & Born, 2019).

K-complexes also meet all of these criteria. The number and density of K-complexes decrease with age (Crowley et al., 2002). Both spontaneous and evoked K-complexes appear to be a sign of sleep maintenance (Nicholas, Trinder, & Colrain, 2002), and offer a distinct benefit to cognition (Ramakrishnan, Sartory, van Beekum, Lohrmann, & Pietrowsky, 2012) and cardiovascular health (de Zambotti et al., 2016). Additionally, as K-complexes can be evoked by sensory stimuli (Bastien et al., 2000) and are marked as slow waves if they meet the appropriate threshold (Berry et al., 2015). An interesting concept to explore would be whether K-complexes can be evoked consecutively and then further modified to essentially create SWS.

Finally, hippocampal SWRs are thought to represent the reactivation of memories during sleep to facilitate learning and memory consolidation (Eschenko, Ramadan, Mölle, Born, & Sara, 2008; Jiang et al., 2018; Joo & Frank, 2018). They are also associated with improved executive function (Buzsáki, 2015). Similar to slow waves, SWRs are highest over the prefrontal cortex (Tang & Jadhav, 2019) and decrease throughout the lifespan (Wiegand et al., 2016). To date, research regarding SWR enhancement and cognition in humans is currently inconclusive (Henin et al., 2019). Interestingly, SWRs are also seen during wake during active recall (Roumis & Frank, 2015), potentially offering the opportunity for a two-prong method of enhancing memory consolidation if they can be entrained during learning and propagated during sleep (Abadchi et al., 2020; Schlingloff, Káli, Freund, Hájos, & Gulyás, 2014).

6.6 Concluding Remarks

Ultimately, this body of work has extended on existing findings by highlighting that in addition to increasing SWS, acoustic stimulation can improve executive function, alertness, and cardiovascular health. We also emphasize the importance of identifying interindividual differences to optimise personalised healthcare plans and targeting midlife as a means of delaying the cognitive impairments associated with advancing age. Given the ubiquitousness of SWS to general brain and cognitive health, it is essential to mitigate their concurrent decline throughout life. Not only can acoustic stimulation provide an immediate benefit to health and workplace productivity, but it also offers a solid foundation for creating sustainable long-term strategies for combating cognitive and physiological decline.

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