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SHORT REPORT

Heart rate variability increases following automated acoustic slow wave sleep enhancement

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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 in healthy, middle-aged males ($n = 24$, 39.92 ± 4.15 years). Under highly controlled conditions, the participants were randomised to receive closed-loop brain state-dependent stimulation in the form of auditory tones (STIM), or no tones (SHAM), in a crossover design, separated by a 1 week washout period. STIM and SHAM were compared on measures of heart rate variability for the whole night and over the first three sleep cycles. We found an increase in slow wave activity following STIM compared with SHAM. There was a significant increase in high frequency power and standard deviation of the normalised RR-intervals (SDNN) during the STIM condition compared with SHAM ($p < 0.05$), due to changes observed specifically during N3. In conclusion, heart rate variability appears to improve following acoustic slow wave sleep enhancement.

KEYWORDS
acoustic stimulation, heart rate variability, middle-aged men, slow wave sleep enhancement

1 | INTRODUCTION

The autonomic nervous system is tightly coupled to the 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 heart rate variability associated with an increased risk of cardiac events (Tsuji et al., 1996). Suppressing slow wave sleep has been associated with reduced heart rate variability (and other detrimental cardiometabolic events) (Tasali et al., 2008), suggesting that slow wave sleep may be a target for improving cardiometabolic outcomes. Conversely, there is a possibility that enhancing slow wave sleep may further inhibit the autonomic response to physiological changes (de Zambotti et al., 2018). In young healthy adults, using acoustic stimulation to enhance slow wave sleep, Grimaldi et al. (2019) reported that slow wave sleep enhancement increased measures of heart rate variability, 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 slow wave sleep using acoustic stimulation, we sought to examine the impact of an automated, acoustic slow wave sleep enhancement device on heart rate variability, and focussed on healthy, middle-aged men.

2 | METHODS

2.1 | 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 crossover study was used to examine the impact of acoustic stimulation (STIM), compared with a SHAM control, on heart rate variability outcomes. The full study protocol and exclusion criteria are described elsewhere (Diep et al., 2020). The study consisted of a baseline night, followed by an experimental night (STIM or SHAM), repeated the following week with the alternate condition. Our study utilised a closed-loop brain state-dependent stimulation approach. 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 1 Hz. 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.

2.2 | Sleep recording and analysis

Electroencephalography (EEG) and electrocardiography (ECG) were recorded with Profusion PSG 4 (Compumedics, Abbotsford, 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. The 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 the total sleep time, N1, N2, N3, REM, wake after sleep onset (WASO), and the number of arousals per hour.

2.3 | Heart rate variability analysis

Heart rate variability was analysed with the open access Matlab application HRVTool (Vollmer, 2015) in clean, 5 min segments, and resampled into 30 s periodicity to align with sleep staging using a custom built MATLAB script to oversample by a factor of 10. Data were cleaned in a three-step process. First, artefacts were removed in HRVTool using the RRfilter method. Second, the data were then processed using a custom Matlab script, with segments containing excessive noise or poor data removed. Third, the data were plotted and manually inspected for excessive variability in the data. The eart rate variability 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 the LF:HF ratio to assess sympathovagal balance. We compared these heart rate variability 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 min segments only (Grimaldi et al., 2019), N3 was not consolidated in 5 min bouts for the majority of our participants (e.g., for criteria of at least 80% of a 5 min 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 were excluded from the analysis due to an excessive artefact in the ECG recording during both experimental nights (final dataset, $n = 23$).

2.4 | 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 they 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 the results presented are mean ± SEM, and $p < 0.05$ (two-tailed) was considered significant.

3 | RESULTS

3.1 | 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, 0.5–4 Hz) 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 heart rate variability following STIM and SHAM are shown in Table 1. As expected, there were no differences between STIM and SHAM for any heart rate variability outcome across the entire night ($p < 0.16$).

TABLE 1 Heart rate variability per sleep stage

Parameter		N1	N2	N3	REM	Whole Night
SDNN (ms)	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 (ms^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[a]	0.36[a]	0.025	0.64	0.77[a]
HF (ms^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[a]	0.37[a]	0.034[a]	0.99[a]	0.49[a]
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[a]	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[a]	0.50	0.32[a]	0.03[a]	0.16[a]

[a]Wilcoxon matched-pairs signed rank test. *p*-Values shown in table are uncorrected. Data presented as mean ± SD.

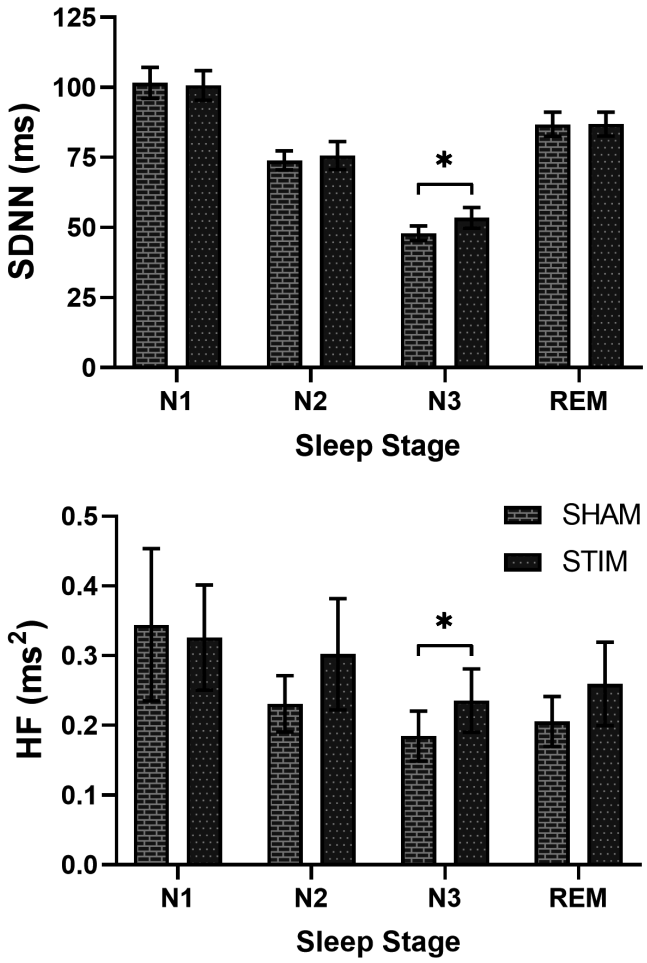

FIGURE 1 Changes in SDNN and HF across each sleep stage

3.2 | Sleep stage specific effects

STIM vs. SHAM differences were found according to sleep stage. While no changes were observed in the heart rate variability outcomes for N1, N2, or REM ($p_{adj} > 0.36$), during N3, STIM led to a significant increase in the heart rate variability 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 heart rate variability outcomes was observed in most participants (70% for SDNN; 60% for HF). Furthermore, the % SWA enhancement (0.5–4Hz) was positively correlated with the % change in SDNN ($r = 0.42$, $p = 0.059$, Figure 2a), with the top tertile of SWA responders showing significantly increased SDNN, relative to the lower tertile of SWA responders, with a large effect size (32.6 ± 10.8% vs. 1.6 ± 6.0%, $p = 0.03$, $d = 1.6$) (Figure 2b). No significant correlations were reported for HF and SWA enhancement ($r < 0.2$). However, 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%, $d = 0.7$), although this did not reach significance ($p = 0.1$).

3.3 | Sleep cycle effects

The largest increases from SHAM to STIM were evident during the first sleep NREM 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

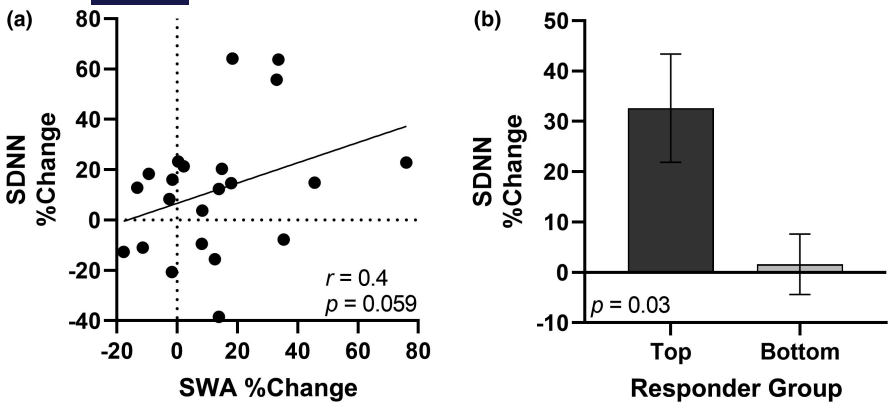

FIGURE 2 Changes in SDNN and SWA (4 Hz) following acoustic slow wave enhancement. (a): Correlation between SDNN and SWA from SHAM to STIM. (b): Percent change in SDNN in top responders ($n = 7$) and non-responders ($n = 7$)

TABLE 2 Heart rate variability per sleep cycle for NREM3 and REM

Parameter		Cycle 1	Cycle 2	Cycle 3
N3 SDNN (ms)	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 (ms)	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 (ms^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[a]	0.16[a]	0.06[a]
REM HF (ms^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[a]	0.51[a]	0.49[a]

[a]Wilcoxon matched-pairs signed rank test. *p*-Values shown in table are uncorrected. Data presented as mean ± SD.

outcomes observed for NREM 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.

4 | DISCUSSION

We investigated the impact of acoustic stimulation of slow wave sleep on heart rate variability 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 slow wave sleep enhancement. These results broadly suggest improved cardiovascular function specifically during N3 with acoustic stimulation.

Consistent with previous studies of slow wave sleep enhancement (Grimaldi et al., 2019; Shaltout et al., 2018) we found an overall increase in heart rate variability (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), and 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 heart rate variability generally reflects better cardiac health, heart rate variability 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, heart rate variability as indicated by SDNN is a gold standard measure of cardiac risk (albeit for 24 h recordings), such that patients with pathologically low SDNN (<50 ms) are 5× more likely to have a risk of mortality compared with those with high SDNN (>100 ms) (Kleiger et al., 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. This does require further investigation, however importantly, the majority of individuals in our study (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 systems, 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) reported improvements in heart rate variability in cycles 2 and 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 min of N3 on average, which was typically not in a consolidated bout. Moreover, our dataset was focussed on men, compared with the previous study which was largely female. Furthermore, while our finding may also be seen as discrepant with our previous findings (e.g., we show the slow wave sleep effect to be large in subsequent cycles, whereas here we show the heart rate variability effect to be largely focussed on cycle 1), the two papers differ in their approach (e.g., in Diep et al., we combined N2 + N3 for SWA which is not recommended for heart rate variability 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 heart rate variability outcomes, across age, sex, and clinical populations.

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 heart rate variability during sleep and the subsequent wake period (Grimaldi et al., 2019), we were unable to examine the subsequent wake period as the 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 heart rate variability during wake or exerts any impact on other systems controlled by the autonomic nervous system, such as the respiratory rate or cortisol release. Third, our study focussed on middle-aged men to minimise age and sex differences within the dataset (which are observed for both slow wave sleep and heart rate variability). While this is an important age group to study (e.g., slow wave sleep depletes in this group), it does restrict generalisability to the wider 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 heart rate variability. As acoustic stimulation appears to improve slow wave sleep with consecutive nights of stimulation (Diep et al., 2021), and long-term sensory stimulation improves heart rate variability (Shaltout et al., 2018), future studies might reveal important improvements in cardiac health longer-term.

To summarise, acoustic slow wave sleep enhancement does appear to benefit heart rate variability. 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.

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CONFLICT 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 interest.

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. and C.A. interpreted the data, and C.D. drafted the manuscript with edits from C.A. All authors approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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