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ORIGINAL ARTICLE

Strengthening sleep–autonomic interaction via acoustic enhancement of slow oscillations

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Abstract

Slow-wave sleep (SWS) is important for overall health since it affects many physiological processes including cardio-metabolic function. Sleep and autonomic nervous system (ANS) activity are closely coupled at anatomical and physiological levels. Sleep-related changes in autonomic function are likely the main pathway through which SWS affects many systems within the body. There are characteristic changes in ANS activity across sleep stages. Notably, in non-rapid eye-movement sleep, the progression into SWS is characterized by increased parasympathetic activity, an important measure of cardiovascular health.

Experimental manipulations that enhance slow-wave activity (SWA, 0.5–4 Hz) can improve sleep-mediated memory and immune function. However, effects of SWA enhancement on autonomic regulation have not been investigated. Here, we employed an adaptive algorithm to deliver 50 ms sounds phase-locked to slow-waves, with regular pauses in stimulation (~5 s ON/~5 s OFF), in healthy young adults. We sought to determine whether acoustic enhancement of SWA altered parasympathetic activity during SWS assessed with heart rate variability (HRV), and evening-to-morning changes in HRV, plasma cortisol, and blood pressure.

Stimulation, compared with a sham condition, increased SWA during ON versus OFF intervals. This ON/OFF SWA enhancement was associated with a reduction in evening-to-morning change of cortisol levels and indices of sympathetic activity. Furthermore, the enhancement of SWA in ON intervals during sleep cycles 2–3 was accompanied by an increase in parasympathetic activity (high-frequency, HRV). Together these findings suggest that acoustic enhancement of SWA has a positive effect on autonomic function in sleep. Approaches to strengthen brain–heart interaction during sleep could have important implications for cardiovascular health.

Statement of Significance

We present the first evidence that acoustic enhancement of slow-wave activity (SWA) during sleep enhances parasympathetic activity, a physiological measure of the cardio-restorative role of sleep. Results in healthy young adults showed an increase of up to 25% in parasympathetic activity assessed with heart rate variability. Results also showed that higher SWA during acoustic stimulation blocks was associated with lower sympathetic activation in the morning shortly after awakening, a time of high vulnerability for adverse cardiovascular events. Our findings indicate that the autonomic nervous system serves as an important signaling pathway that facilitates the interaction between sleep and cardiovascular function. Improving sleep quality through SWA acoustic enhancement represents an innovative, noninvasive approach for improving cardiovascular health and more broadly, physiological homeostasis.

Key words: slow wave activity; parasympathetic activity; acoustic stimulation; sleep; autonomic nervous system

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Introduction

Sleep and the autonomic nervous system (ANS) are tightly coupled and regulated through shared physiological and neurochemical pathways [1]. The major evidence for a link between the two systems is the demonstration of their synchronous fluctuations across sleep stages [2]. Sleep is a dynamic process composed of two distinct states: rapid eye movement (REM) and non-REM (NREM) sleep, occurring in cycles lasting around 90 min. Studies using heart rate variability (HRV) [3, 4] and microneurography [5] have shown that NREM-REM cycles are coupled with a synchronous oscillation of sympathetic-parasympathetic activity. Entrance into NREM sleep is characterized by a shift toward parasympathetic (vagal) dominance that is most prominent during slow-wave sleep (SWS). This parasympathetic shift is accompanied by a reduction in heart rate (HR) and blood pressure (BP), and the enhancement of protective factors at the cardiac and vascular level (e.g., decrease in vascular resistance and cardiac work load and reduction in the aggregation of platelets) [2].

The integrity of sleep, in particular SWS, has been shown to be critical for the regulation of many physiological processes, including cardio-metabolic and immune functions [6–8] and cognition [9]. Findings by Tasali *et al.* [7] further substantiate the critical role of SWS for cardiovascular regulation. In their study, the authors showed that the selective disruption of SWS led to detrimental cardio-metabolic consequences, including an increase in insulin resistance and sympatho-vagal balance, defined as an increase in the low frequency to high frequency ratio (LF:HF) of HRV, during morning waking hours. This evidence reinforces the view that SWS is a physiological state that affects multiple systems within the brain and body [6]. Moreover, it shows that the impact of sleep-related changes on ANS function is not limited to the sleep period but also influence cardiovascular function during the day [6].

Given that SWS disruption has a negative impact on ANS function, it is plausible that enhancing SWS, quantified via measurement of slow-wave activity (SWA, 0.5–4 Hz), will have beneficial effects on ANS activity. Acoustic stimulation during sleep has emerged in the last years as an innovative and effective method to enhance SWA [10–12] which in turn can improve sleep-dependent memory consolidation [13–15] and sleep-regulated endocrine and immune function [16]. However, the exact mechanisms by which SWS/SWA regulates these physiological functions remain to be elucidated. Sleep-dependent change in ANS activity has been postulated as a primary pathway through which sleep can modulate biological systems within the body [6], including in the regulation of immune, cardiovascular, metabolic, and cognitive functions [17, 18].

We have previously described [12] an automated adaptive algorithm to deliver sounds phase-locked to sleep slow-waves, capable of enhancing SWA in young and older adults [12, 15, 19]. A distinctive feature of this algorithms is that sounds are delivered at regular intervals of ~5 oscillations (ON interval) followed by a pause of ~5 oscillations (OFF interval). This approach offers the unique opportunity to investigate the parallel changes in SWA and ANS function over selected periods of acoustic stimulation (ON and OFF intervals). Therefore, in the present study, we tested the hypothesis that enhancement of SWA with acoustic stimulation during sleep would increase parasympathetic activity, assessed with HRV, during SWS across cycles of sleep. We also assessed the effect of acoustic stimulation during overnight sleep on evening-to-morning changes in HRV and other autonomic markers, including BP and cortisol.

Methods

Participants

Twenty healthy participants between the ages of 18 and 35 years (mean: 24.7 years, 75% female, BMI: 25 ± 3.5 kg/m^2) participated in this randomized crossover study (see Experimental Design section). Exclusion criteria included unstable medical or psychiatric conditions, history of seizures, history of cardiac arrhythmia or other heart conditions, hearing loss, shift work or other types of self-imposed irregular sleep/wake cycles, or history of sleep disorder. The STOP-BANG questionnaire [20] was used to screen for obstructive sleep apnea and those with a medium to high risk were excluded. The Institutional Review Board at Northwestern University approved this study. Before study participation, written informed consent was obtained from all participants.

Experimental design

The study employed a randomized crossover design with two 2-night visits separated by a 2-week washout period (Figure 1). The first night of each visit was an adaptation night, followed by an intervention night. Participants were randomized to either receive stimulation or sham stimulation on the first intervention night. Participants were given an 8-h sleep opportunity, based on their habitual bedtime, determined from actigraphy and sleep diaries obtained during the week immediately before their admission to the clinical research center.

Phase-locked auditory stimulation during sleep

A phase-locked auditory stimulation procedure was employed during sleep. Details of the automated algorithm have been extensively described elsewhere [12]. Briefly, single input from the midline frontopolar (Fpz) channel was used to phase-lock a 50 ms pulse of pink noise (1/f) to the up state of slow-waves (30 degrees to the peak) primarily during NREM stage 2 and 3 of sleep. As shown in Figure 2, pulses were delivered in blocks of five oscillations (ON interval) followed by a pause for five oscillations (OFF interval). During the ON interval, the interstimulus interval was ~1 s between each pulse and varied based on the frequency of the tracked slow-waves. Stimulation continued until there was an increase in alpha or beta activity coinciding with a reduction in delta activity, reflecting a change of sleep stage to either REM or wake. SHAM stimulation consisted of an identical setup; the phase-locked loop tracked sleep as in STIM condition, with indicators of where stimulation would have occurred, but no sound was delivered. Before sleep, volume was titrated for each individual to ensure that it was at a comfortable level that was audible but would not wake the participant.

Sleep polysomnography recording

Electroencephalographic (EEG) recordings were obtained from nine channels (international 10–20 system: Fpz, F3, F4, C3, C4,

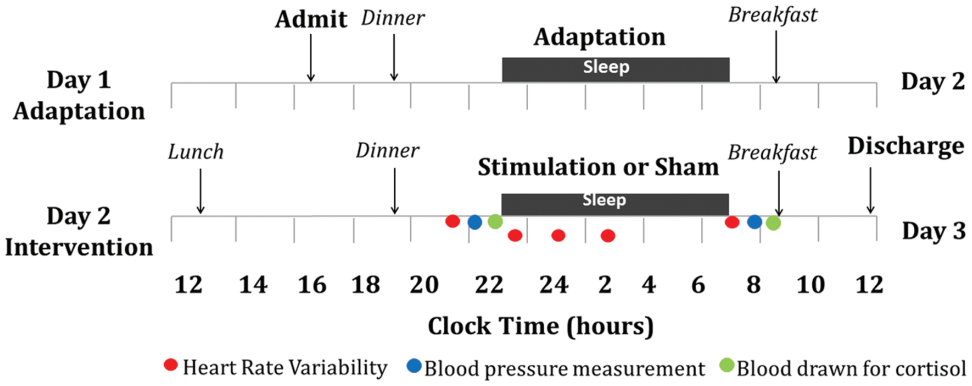

Figure 1. Inpatient study timeline. Participants completed two 2-night inpatient visits, each with one night of adaptation and one intervention night. Heart rate variability (red dots) measurements were assessed 30 min before lights out (10 min assessment), overnight during the first three cycles of sleep (5 min assessment), and immediately after lights on (10 min assessment). In the morning, a 5 five-min orthostatic test followed the supine HRV measurement. Blood pressure (blue dots) was measured 15 min before lights out and 30 min after lights on. Cortisol (green dots) samples were collected 10 min before lights out and 30 min after lights on.

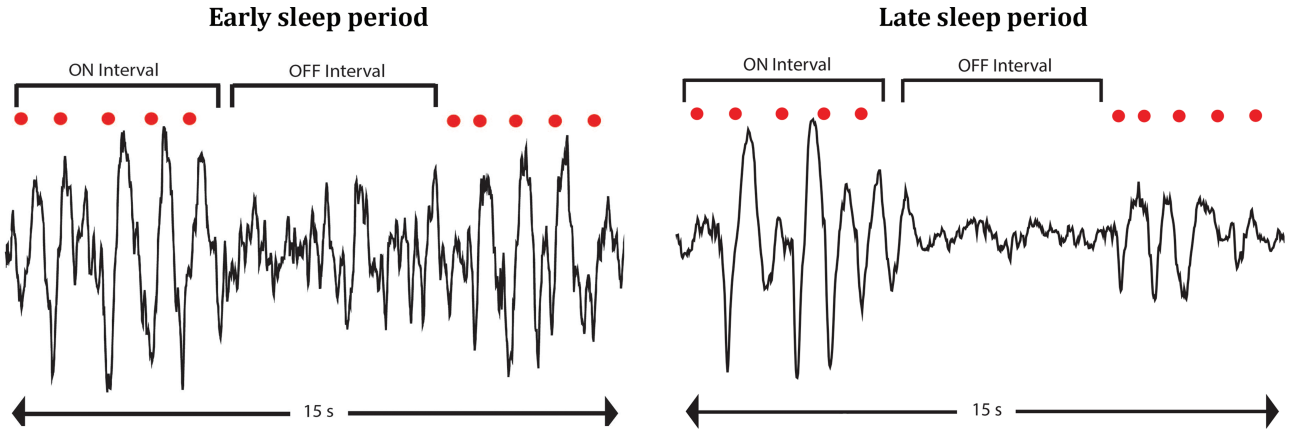

Figure 2. Examples of EEG data for phase-locked loop pulse delivery using a block design with ON and OFF intervals. Figures are representative of acoustic stimulation delivered in the first (left panel) and fifth (right panel) sleep cycle in one subject. Red dots indicate 50 ms pulses of pink noise.

P3, P4, O1, O2) referenced to left mastoid. Left and right electro-oculograms, lateral to each eye, three chin electromyogram (EMG), one thoracic belt and one electrocardiogram (ECG) lead placed one inch under the left collarbone, were also obtained. The PSG data were collected using Brain Vision software at a sampling frequency of 500 Hz, filtered offline between 0.3 and 35 Hz, and down-sampled to 250 Hz. Offline scoring was done using Polysmith reading software (v.8.0, Nihon Kohden) by two experienced raters (D.G. and S.M.A.) using the American Academy of Sleep Medicine (AASM) scoring criteria [21] with an average interscorer agreement of 90%. Scoring from a single rater, who was blinded to experimental condition, was used for all analysis. Sleep PSG features were calculated for total sleep time, time spent in each stage of sleep (N1, N2, N3, REM sleep), and arousal index (number per hour) for each stage of sleep [21].

Subjective sleep assessment

Subjective sleepiness was evaluated 15 min after wake using the Karolinska Sleepiness Scale [22], a nine-point scale, with answers ranging from 1 = extremely alert, 3 = alert, 5 = neither alert nor sleepy, 7 = sleepy, to 9 = extremely sleepy. Subjective sleep quality was evaluated 75 min after wake, using the Karolinska Sleep Diary [22], a five-part questionnaire asking the participants to rate subjective sleep quality (e.g., "Did you sleep soundly?," "How easy was it for you to fall asleep?") on a scale from 1 to 5. The sum of the scores was reported, with a minimum score of 5 indicating poor sleep quality and a maximum score of 25 indicating good sleep quality.

Power spectral analysis

EEG spectral analysis was performed in Matlab on artifact free data from channel F3. A Fast Fourier Transform (4 s window, 50% overlap) was applied and mean power spectral estimates were extracted in 30 s epochs by sleep stage. SWA was quantified as spectral power in the delta frequency band (0.5–4 Hz). Spectral power was also extracted for theta (>4–8 Hz), alpha (>8–12 Hz), sigma (>10–15 Hz), and beta (>16–20 Hz) frequency bands. Cycle analysis was used to examine the time course of SWA decline throughout the night. Sleep cycles were defined using modified Feinberg and Floyd criteria [23]. Five cycles of sleep were included in analysis. Average SWA was calculated for each cycle of sleep and normalized to the average of the entire night SWA during NREM sleep. Mean and normalized power were also extracted from channel Fpz for ON and OFF intervals of the STIM and SHAM nights. Mean power was normalized to the total power in the ON and OFF intervals for that specific frequency.

Percent power change was then calculated between ON and OFF intervals [(ON interval – OFF interval) / OFF interval × 100%].

The time course of SWA change during ON and OFF intervals was calculated for the five cycles of sleep. The amount of delta power in ON and OFF intervals during each cycle was calculated and normalized to the entire night amount of delta power in ON plus OFF intervals.

Heart rate variability before sleep and after morning awakening

Thirty minutes before lights out, participants were asked to rest supine without moving for 10 min, keeping their eyes open and breathing naturally. Light levels were kept at ~50 lux. The same test was repeated in the morning immediately after the awakening. In the morning an orthostatic test was also performed at the end of the 10 min supine period. Participants were asked to gradually transition from supine to a standing position that was maintained for 5 min during which they were instructed to remain quietly, breathing naturally, without moving or talking [24]. ECG, EEG, and breathing activity were recorded throughout these procedures. HR and HRV analysis were quantified during a 5-min period selected toward the end of the 10-min period for the supine condition and during a 3-min period selected toward the end of the 5-min orthostatic test. The changes in HR and HRV from evening-to-morning (postsleep – presleep) and from supine-to-standing (standing – supine) were calculated.

Heart rate variability during sleep

The analysis focused on the first three cycles of sleep since ~80% of acoustic stimulation occurred during that time. For each cycle, HRV was performed on 5-min periods during stable SWS and REM sleep. Segments were selected close to the midpoint of the sleep cycle during SWS when acoustic or sham stimulation was delivered.

Heart rate variability analysis

Preprocessing of the RR interval time series and computation of time and frequency domain measures of HRV were performed using Kubios HRV premium 3.0 software (Kubios Ltd., Kuopio, Finland) in accordance with standard guidelines [25]. The same investigator (D.G.) visually selected the segments before and during sleep to use for HRV analysis. To be included in the analysis, segments had to be free from microarousals, artifacts, movements, and ectopic beats. Five-minute periods were selected for analysis in which the 30 s epoch before and after were artifact free to ensure a stationary signal [25].

The square root of the mean of the squares of the successive differences between adjacent R-R intervals (RMSSD) was chosen as time domain index of HRV, since it is strongly correlated with the parasympathetic modulation of HR [25]. Spectral analysis of HRV was performed using a Fast Fourier Transform and spectral power was calculated in the high frequency band (HF: 0.15–0.40 Hz) reflecting mostly parasympathetic activity, and in the low frequency band (LF: 0.04–0.14 Hz) reflecting a combination of vagal and sympathetic activities [25]. The LF: HF ratio was also calculated as indicator of the sympatho-vagal balance [25]. Data from one female participant were excluded from the analysis due to the presence of sinus arrhythmia defined as >10% of variability in RR interval in the absence of changing P-wave morphology or axis [26]. HRV analysis was thus conducted on 19 out of 20 participants included in the study.

Blood pressure and plasma cortisol assessment

BP measurements were performed 15 min before lights off and 30 min after morning awakening from the nondominant arm. Participants were maintained for 5 min in a sitting position, after which BP was recorded using an ambulatory monitoring device. Immediately after BP measurements, blood samples for plasma cortisol were drawn. Blood samples were centrifuged immediately after collection and stored at –70°C until further processing. Plasma cortisol was measured using enzyme-linked immunosorbent assay (ELISA, Roche Diagnostics Elecsys Cortisol II) with an intra-assay variability of 1.7%.

For BP analysis, data from one subject were excluded as evening BP was not obtained, so a total of 19 participants were included. The evening-to-morning changes in BP and cortisol levels were calculated as (postsleep – presleep).

For the cortisol analysis, data were excluded for three subjects (two subjects during both STIM and SHAM conditions and one subject only during the SHAM condition) due to technical issues with blood draws. As a result, a total of 17 participants were included for the between conditions comparison of cortisol levels.

Statistical analysis

Comparison of night-to-night PSG sleep variables, evening-to-morning change of HRV, BP and plasma cortisol levels, and HRV changes during orthostatic testing were assessed using either a paired *t*-test for normally distributed variables or the paired Wilcoxon signed-rank test for non-normally distributed variables, Normality assumptions for pairwise differences were first checked using "shapiro.test" function in R.

To analyze the time courses of the spectral power and HRV outcomes as a function of sleep cycle, generalized estimating equations (GEE) [27] for repeated measures were used to model the outcome as a function of condition (STIM vs. SHAM), a third-order polynomial in time, and the interaction between them. An exchangeable within-subject covariance matrix was estimated to account for the possible correlation between outcome measurements from the same subject, and robust standard errors of the model parameters were computed using the method of Diggle *et al.* [28], which yields consistent estimates even when the within subject covariance matrix is mis-specified. Significance of the condition, time, or the interaction between the condition and time was assessed using the Wald test. Significance of main effects is reported, adjusting for other main effects, and significance of the interaction is reported adjusting for all main effects (Type II SS). Within each time point (sleep-cycle), spectral power features and HRV characteristics were compared between the two conditions, using the nonparametric Wilcoxon rank-sum test for paired data. A Bonferroni correction was applied to adjust for the number of time points considered within each group of analyses. HF and LF of HRV were analyzed as relative power (percentage of total HRV power). Nonparametric Spearman rank correlation coefficients were computed to

analyze the relationship between the overnight change in PSG, spectral power, and autonomic variables. Data are presented as mean ± standard deviation unless otherwise noted. Statistical analyses were carried out in R [29], using the geepack library [30] for the GEE models.

Results

Phase targeting and temporal distribution of acoustic stimulation by sleep stage

Acoustic stimulation successfully targeted the upstate of slow-waves. For pulses across all slow-waves, the mean instantaneous phase of pulse delivery for STIM was 331.8 (SD = 61.8) degrees.

The targeted acoustic stimulation occurred primarily during NREM sleep. The breakdown was: 46% ± 14% stage N2; 50% ± 14% stage N3; 0.32% ± 0.58% stage N1; and 2.6% ± 1.8% REM. The duration of stimulation for the combined ON and OFF intervals was similar between STIM and SHAM (163 ± 44 min and 176 ± 43 min, respectively; $t(19) = -1.40$, $p = 0.17$, paired t-test). Moreover, changes in stimulation across cycles of sleep were similar in STIM and SHAM, with both conditions characterized by a progressive reduction across the night, mimicking the physiological dissipation of sleep slow-waves (percentage of stimulation in cycle 1: STIM = 30% ± 2%, SHAM = 29% ± 2%; cycle 2: STIM = 27% ± 1%, SHAM = 26% ± 2%; cycle 3: STIM = 18% ± 3%, SHAM = 20% ± 2%; cycle 4: STIM = 15% ± 2%, SHAM = 15% ± 2%; cycle 5: STIM = 10% ± 2%, SHAM = 10% ± 2%. Condition: $p = 0.43$, cycle: $p < 0.001$, condition × cycle interaction: $p = 0.11$).

Sleep macrostructure

There were minimal differences in sleep macrostructure between STIM and SHAM nights (Table 1). An exception was that stage N1 sleep was 3 min longer during STIM ($W = 43.5$, $p = 0.04$, Wilcoxon signed-rank test), which was likely to due to a 3-point increase in the arousal index during stage N2 sleep in the STIM condition ($W = 43$, $p = 0.02$, Wilcoxon signed-rank test). The arousal index during stage N1, N3, and REM sleep comparing STIM and SHAM conditions were not statistically different ($W = 59/68/88$, $p = 0.09/0.18/0.55$ for N1/N3/REM, respectively; Wilcoxon signed-rank test).

When analyzing the changes in number of arousals across the cycles of sleep during stage N2 and N3 (Supplementary Figure S1), we did not find significant differences between STIM and SHAM (N2: group $p = 0.11$, cycle $p < 0.0001$, group × cycle $p = 0.83$; N3: group $p = 0.12$, cycle $p < 0.0001$, group × cycle $p = 0.57$).

Self-reported sleepiness and quality

In the morning following the intervention nights, comparisons between STIM and SHAM nights showed no differences in self-reported sleepiness (Karolinska Sleepiness Scale: STIM: 4.2 ± 2.1, SHAM: 4.3 ± 1.8, $W = 7.5$, $p = 0.77$, Wilcoxon signed-rank test) or in self-reported sleep quality (Karolinska Sleepiness Diary: STIM: 16.4 ± 2.8, SHAM: 16.6 ± 2.5, $W = 12.5$ $p = 0.83$, Wilcoxon signed-rank test).

Power spectral analysis and cycle analysis

Analyses focused on normalized spectral power during ON and OFF intervals for STIM and SHAM are shown in Figure 3A. There was an increase in SWA during ON intervals and a reduction in SWA during OFF intervals in STIM compared with SHAM ($W = 210$, $p = 1.9e\text{-}06$, Wilcoxon signed-rank test). Acoustic stimulation led to an average 40% increase in SWA during ON versus OFF intervals that was not present in the sham condition (STIM: +44.5 ± 8 μV2/Hz, SHAM: −1.2 ± 0.9 μV2/Hz, $W = 210$, $p = 1.9e\text{-}06$, Wilcoxon signed-rank test). The amount of SWA across the night was similar in STIM and SHAM (Figure 3B; $W = 4.0$, $p = 0.89$, Wilcoxon signed-rank test). This increase during ON intervals and decrease during OFF intervals suggests a reorganization of SWA power.

An analysis of SWA in ON intervals as a function of sleep cycle across the night (Figure 4A) showed that the STIM/SHAM effect was evident in all cycles except the first, although the interaction with cycle was nonsignificant (condition: $p < 0.0001$, cycle: $p < 0.0001$, condition × cycle interaction: $p = 0.11$). This effect was clearest during cycles 2 to 4 ($p = 0.024$, 0.069,

Table 1. Polysomnography features during STIM and SHAM

	STIM ($n = 20$)	SHAM ($n = 20$)	P
Wake (min)	38.9 (40)	58.1 (52.5)	0.09
TST (min)	454.4 (15.8)	452.7 (18.2)	0.54
Sleep latency (min)	8.8 (9.1)	9.1 (7.6)	0.89
NREM stage N1 (min)	20.8 (7.3)	17.8 (5.9)	0.04*
NREM stage N2 (min)	203.5 (46)	213.4 (29.3)	0.29
NREM stage N3 (min)	112.6 (40.5)	105.8 (26.2)	0.32
REM (min)	117.5 (28.1)	115.7 (15.6)	0.61
WASO (min)	16.4 (11.4)	18 (16.6)	0.60
Sleep efficiency (%)	94.7 (3.3)	94.3 (3.8)	0.49
Stage N1 arousal index (n/h)	48.4 (15.8)	43.2 (14.5)	0.09
Stage N2 arousal index (n/h)	12.1 (4.2)	9.2 (3.0)	0.02*
Stage N3 arousal index (n/h)	3.8 (2.2)	2.7 (2.1)	0.18
REM arousal index (n/h)	10.4 (7.3)	9.8 (6.1)	0.55

Data are mean ± (SD).
TST = total sleep time; NREM = nonrapid eye movement sleep; REM = rapid eye movement sleep; WASO = wake after sleep onset; arousal index (n/h) = number of events per hour of sleep.
*$p < 0.05$, paired Wilcoxon signed-rank test (unadjusted).

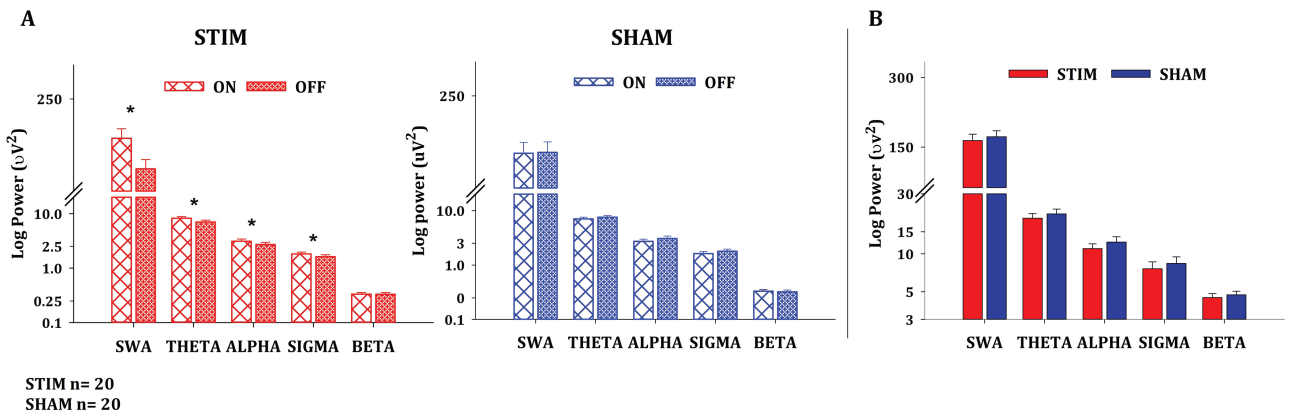

Figure 3. Spectral power during ON/OFF intervals and NREM sleep. The figure shows log-transformed average spectral power in different frequency bands. The analysis was conducted on the average spectral power in ON and OFF intervals normalized to the total power for each frequency band. (A) Spectral power in the SWA, theta, alpha, and sigma bands increased during STIM in ON vs OFF intervals, compared with SHAM. (B) Spectral power during NREM across the entire sleep period was similar between STIM and SHAM nights. Asterisks indicate $p < 0.05$ (Wilcoxon signed-rank test) following Bonferroni adjustment for multiple comparisons. Error bars represent standard error of the mean.

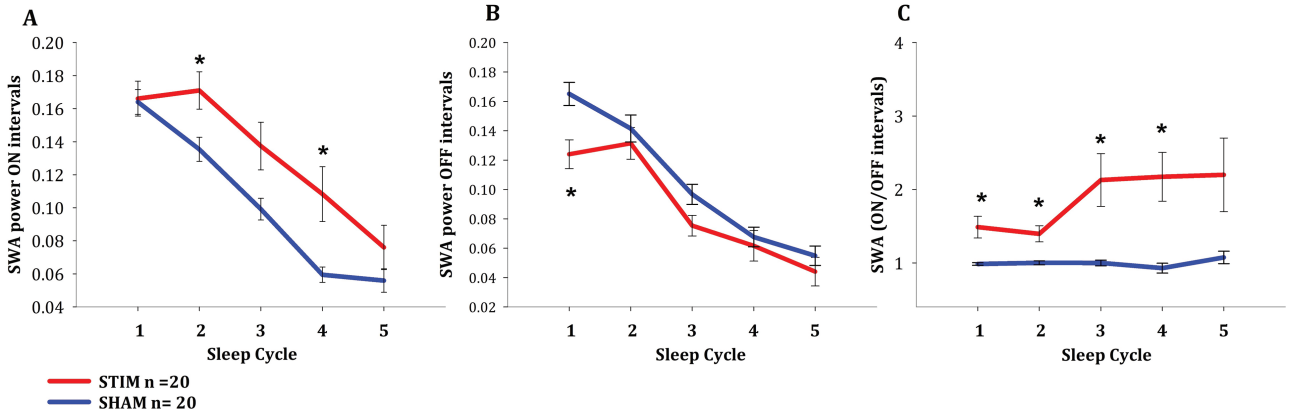

Figure 4. Changes in SWA during ON, OFF, and ON/OFF intervals through the cycles of sleep. The amount of SWA (Fpz, 0.5–4 Hz) in ON and OFF intervals during each cycle was calculated and normalized to the amount of SWA in all intervals (ON and OFF for entire night). (A) In ON intervals, the amount of SWA was higher during STIM compared with SHAM, mainly evident in cycle 2 and 4. (B) In OFF intervals, the amount of SWA was reduced in STIM compared with SHAM mainly during the first cycle of sleep. (C) Change in SWA between ON/OFF intervals was significantly higher during STIM compared to SHAM from the first until the fourth cycle of sleep. Asterisks indicate $p < 0.05$ (Wilcoxon signed-rank test) following Bonferroni adjustment for multiple comparisons. Error bars represents standard error of the mean.

0.012, respectively after Bonferroni adjustment). During OFF intervals (Figure 4B), SWA was lower in STIM compared with SHAM (condition: $p = 0.0001$, cycle: $p < 0.0001$, condition × cycle interaction: $p = 0.25$) but a significant reduction was only achieved during the first sleep cycle after adjusting for multiple comparisons ($p = 0.02$).

As a result of changes in ON and OFF intervals, the ratio of SWA during ON compared to OFF intervals (Figure 4C) was significantly higher from the first to the fourth cycle of sleep during acoustic stimulation compared with SHAM (condition: $p < 0.0001$, cycle: $p = 0.30$, condition × cycle interaction: $p = 0.02$). Since two subjects in the STIM condition and two subjects in the SHAM condition had only four cycles of sleep, the reduced number of observations in cycle 5 likely contributed to the lack of significance despite the similar magnitude in SWA change.

When looking at the entire amount of SWA across the cycles of sleep, there was no difference between STIM and SHAM, suggesting that acoustic stimulation did not alter the overall temporal pattern of SWA dissipation (condition: $p = 0.79$, cycle: $p < 0.0001$, condition × cycle interaction: $p = 0.38$). Average cycle duration was also similar between STIM and SHAM (condition × cycle interaction: $p = 0.81$).

When examining the changes in the higher frequency bands an increase in theta, alpha, and sigma activity in ON versus OFF intervals was also present during the STIM night (Figure 3A). Changes in theta, alpha, sigma, and beta power bands across the cycles of sleep are shown in Supplementary Figure S2.

Heart rate variability during sleep

As shown in Figure 5, frequency-domain analyses showed the expected increase in parasympathetic activity during SWS, indexed by HRV HF relative power (HF%), compared with baseline.

Further, HF% was significantly higher for STIM versus SHAM during cycles 2 and 3 ($p < 0.01$), but not in cycle 1 (condition: $p = 0.004$, cycle: $p < 0.0001$, condition × cycle interaction: $p = 0.40$). A concomitant reduction in LF relative power (data not shown) was observed during acoustic stimulation in cycles 2 and 3 of sleep compared with SHAM (condition: $p = 0.019$, cycle: $p = 0.010$, condition × cycle interaction: $p = 0.29$), significant only in cycle

3 (p = 0.02). There was no difference in LF:HF between STIM and SHAM (condition: p = 0.113, cycle: p = 0.0002, condition × cycle interaction: p = 0.403, data not shown). The changes in HR and RMSSD during the first three cycles of SWS, were similar in STIM and SHAM conditions (HR: condition: p = 0.69; cycle: p = 0.007, condition × cycle interaction: p = 0.83; RMSSD: condition: p = 0.90, cycle: p = 0.05, condition × cycle interaction: p = 0.91).

HR and RMSSD changes during the three cycles of REM sleep were similar in STIM and SHAM (HR: condition: p = 0.78, cycle: p = 0.55, condition × cycle interaction: p = 0.41, RMSSD: condition: p = 0.39, cycle p = 0.25, condition × cycle interaction: p = 0.63). Similarly, frequency-domain indices of HRV were also comparable during STIM and SHAM (HF%: condition: p = 0.99, cycle: p = 0.02, condition × cycle interaction: p = 0.78; LF%: group: p = 0.99, cycle: p < 0.001, group × cycle: p = 0.65; LF:HF: condition: p = 0.80, cycle: p = 0.001, condition × cycle interaction: p = 0.68).

Respiratory rate across the three cycles of sleep was comparable in STIM and SHAM during SWS (condition: p = 0.93; cycle: p = 0.18, condition × cycle interaction: p = 0.82) and REM (condition: p = 0.46; cycle: p = 0.19, condition × cycle interaction p = 0.54).

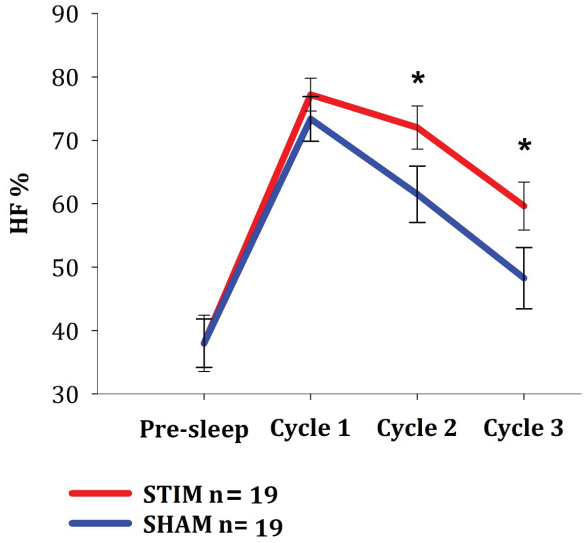

Figure 5. HRV high-frequency relative power (HF%) in the evening assessment before sleep (presleep) and during SWS in the first three cycles of sleep where ~80% of acoustic stimulation occurred. HF% in the presleep assessment was similar in STIM and SHAM. During sleep, HF% was significantly higher during cycles 2 and 3 in the STIM night. One subject was excluded from HRV analysis due to the presence of sinus arrhythmia. Asterisks indicate p <0.05 (Wilcoxon signed-rank test) following Bonferroni adjustment for multiple comparisons. Error bars represents standard error of the mean.

Heart rate variability before sleep and after morning awakening

HR, time- and frequency-domain indices of HRV, and respiratory frequency were similar in STIM and SHAM condition before sleep (Table 2). The evening-to-morning changes in HR and HRV were also similar in STIM and SHAM, as were the changes from supine to standing (Table 3). Only in the STIM condition, however, the SWA increase in ON versus OFF intervals was significantly correlated with a reduction in the evening-to-morning change of LF:HF values (Figure 6A; R = –0.60, p = 0.010, Spearman rank correlation). Likewise, the SWA increase in the STIM condition in ON versus OFF intervals, correlated with the reduction in the supine-to-standing change of LF:HF values (R = –0.52, p = 0.032, Spearman rank correlation). However, significant correlations were not present with any other spectral EEG characteristics or sleep PSG features during STIM.

We assessed whether SWA enhancement in sleep cycles 1–3 as compared with sleep cycles 4–5 was driving the observed reduction in evening-to-morning LF:HF changes (Supplementary Figure S3, A and B). The average SWA increase in ON versus OFF intervals during both periods of the night was significantly correlated with the evening-to-morning reduction in LF:HF (cycles 1–3: R=-0.57, p= 0.003, cycle 4–5: R=-0.73, p < 0.0001, Spearman rank correlations).

Blood pressure

Systolic and diastolic BP (SBP, DBP) before sleep were similar in STIM and SHAM [SBP: STIM= 113 ± 13 mmHg, SHAM = 111 ± 11 mmHg, p = 0.33, t(18) = –1.0, paired t-test p = 0.33; DBP: STIM = 66 ± 7 mmHg, SHAM = 65 ± 8, t(18) = –0.95, p = 0.35, paired t-test]. The evening-to-morning SBP change (postsleep – presleep) was comparable in the two conditions [STIM = –1.5 ± 11 mmHg, SHAM = –1.8 ± 9, p = 0.92, t(18) = –0.09, p = 0.92, paired t-test]. The evening-to-morning DBP change showed a trend toward a reduction when participants received acoustic stimulation (–1.2 ± 7 mmHg) compared with the mild increase observed after SHAM [+3.3 ± 8 mmHg, t(18) = 1.89, p = 0.07, paired t-test p = 0.07]. BP changes were not associated with any spectral EEG characteristic or sleep PSG feature.

Plasma cortisol

Cortisol levels before lights off were similar on STIM and SHAM nights (STIM = 3.2 ± 3.5 pg/mL, SHAM = 2.6 ± 2.2 pg/mL, W = 25.5, p = 0.33, Wilcoxon signed-rank test) as was the morning increase in cortisol (STIM = 19.6 ± 5.2 pg/mL, SHAM = 19.7 ± 4.4 pg/mL,

Table 2. Heart rate and HRV measures before sleep in STIM and SHAM

	STIM (n = 19)	SHAM (n = 19)	P
Mean HR (bpm)	59.26 (7.22)	58.19 (6.89)	0.65
RMSSD (ms)	70.02 (33.77)	63.88 (32.46)	0.65
HF relative power (%)	39.98 (19.32)	38.01 (16.69)	0.73
LF relative power (%)	29.44 (20.00)	32.10 (21.53)	0.74
LF:HF	1.59 (2.27)	1.75 (3.64)	0.83
Respiratory frequency (Hz)	0.18 (0.04)	0.20 (0.05)	0.28

Data are mean ± (SD). Pairwise comparisons were performed using Wilcoxon signed-rank test.
HR = heart rate; RMSSD = square root of the mean of the squares of the successive differences between adjacent R-R intervals; HF% = high frequency relative power (% of total HRV power); LF% = low frequency relative power; LF:HF = low frequency to high frequency ratio.

Table 3. Heart rate and HRV measures during evening-to-morning and supine-to-standing changes in STIM and SHAM

	Evening-to-morning change (n = 17)			Supine-to-standing change (n = 14)		
	STIM	SHAM	P	STIM	SHAM	P
HR (bpm)	6.6 (4.6)	6.2 (6.3)	0.83	34.3 (12.1)	33.6 (3.3)	0.83
RMSSD (ms)	−10.5 (20.5)	−10.3 (19.14)	0.69	−37.0 (33.1)	−42.5 (31.3)	0.57
HF%	−14.8 (14.5)	−10.9 (24.5)	0.78	−17.5 (12.5)	−18.6 (21.9)	0.78
LF%	−1.3 (15.3)	−3.4 (11.7)	0.75	22.4 (19.1)	20.03 (17.9)	0.84
LF:HF	0.6 (1.0)	0.6 (1.2)	0.43	11.9 (11.3)	13.0 (12.1)	0.91

Data are mean ± (SD). Pairwise comparisons were performed using Wilcoxon signed-rank test. Respiratory frequency during morning supine and standing test was similar in STIM and SHAM ($p > 0.05$, Wilcoxon signed-rank test).
HR = heart rate; RMSSD = square root of the mean of the squares of the successive differences between adjacent R-R intervals; HF% = high frequency relative power (% of total HRV power); LF% = low frequency relative power; LF:HF = low frequency to high frequency ratio.

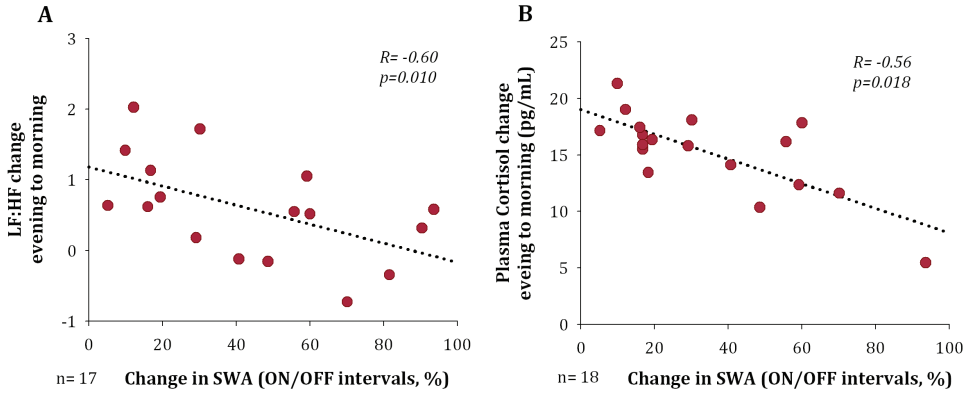

Figure 6. Nonparametric correlations between percent change in SWA in ON/OFF intervals and evening-to-morning change in LF:HF and cortisol during acoustic stimulation. Evening-to-morning changes were calculated as postsleep – presleep. (A) A larger reduction in evening-to-morning change of LF:HF was significantly associated with an increase in SWA in the STIM night during ON versus OFF intervals. (B) A significant association was present between the increase in SWA during ON versus OFF intervals and the reduction in evening-to-morning rise in cortisol plasma levels.

W = 12, p = 0.64, Wilcoxon signed-rank test p = 0.74). The evening-to-morning change of cortisol was also similar across the two conditions (STIM = +16.4 ± 5.2 pg/mL, SHAM = +15.1 ± 6.6 pg/mL, W = 23, p = 0.33, Wilcoxon signed-rank test).

Although there was no difference in overall amount of cortisol between STIM and SHAM, the increase in SWA during ON versus OFF intervals significantly correlated with a reduction in the evening-to-morning increase in cortisol levels in the STIM condition (Figure 6B, R = −0.57, p = 0.018, Spearman rank correlation). This association was not present in SHAM (R = −0.32, p = 0.21, Spearman rank correlation). There were no associations between cortisol levels and other spectral EEG characteristics or sleep PSG parameters.

Additionally, we examined the correlation between SWA increase in ON versus OFF intervals in sleep cycles 1–3 and 4–5 with evening-to-morning cortisol changes (Supplementary Figure S3, C and D). Using the Spearman rank correlation, we found a significant association with stimulation in sleep cycles 1–3 (R = −0.69, p = 0.004) and a trend in cycles 4–5 (R = −0.43, P = 0.052).

Discussion

The present study provides novel insight into the relationships between sleep, particularly sleep slow-waves, and autonomic function. Enhancement of SWA using acoustic stimulation resulted in higher parasympathetic activity during SWS, identified by an increase in the HF power of HRV. In addition, higher SWA was associated with a reduction in the evening-to-morning change in autonomic measures of HRV and plasma cortisol levels, which together indicate a decrease in sympathetic nervous system activity. The balance between the sympathetic and parasympathetic regulation of the ANS is an important biomarker of physiological and pathological responses by the cardiovascular system. Given that dysregulation between the two branches of the ANS has been implicated in the pathophysiology of cardio-metabolic disorders and may predict poorer clinical outcomes [31], strengthening the interplay between sleep and ANS regulation using acoustically induced enhancement of SWA may have implications for improving cardiovascular health.

The primary finding of this study is that acoustic stimulation of sleep slow-waves resulted in a parallel increase in SWA during the ON intervals of stimulation and parasympathetic activity (HF of HRV). The analysis of SWA changes during ON intervals further reveals that increase in SWA and HF power were seen only after the first cycle of sleep, compared with SHAM. On average, during acoustic stimulation, HF increased by 17% in the second sleep cycle and by 24% in the third sleep cycle, compared with SHAM. Given that participants were young and healthy, we hypothesize that the homeostatic drive for SWA during the first cycle of sleep may have reached a ceiling effect, such that acoustic stimulation could not produce additional enhancement.

Our results shed light on possible mechanisms whereby acoustic stimulation can influence brain–cardiac functional

coupling. Recent theoretical models developed to explain relationships between EEG and cardiac function emphasized how oscillatory patterns in both systems underlie their synchronization [32]. Lechinger *et al.* [33] used heartbeat-evoked potentials during SWS to show that the probability of ECG R peak occurrence was highest at the positive up-state of the slow-wave, suggesting that cardiac activity can modulate or be modulated by the ongoing oscillatory brain activity. Because phase-locked acoustic stimulation targeted the positive up-state of slow-waves, it is plausible that acoustic stimulation strengthened the coupling between cortical and cardiac oscillations, reflected in the concomitant changes in SWA and HRV. Although our results do not address the issue of directionality, it is likely that the communication between neural oscillations in sleep and ANS activity is bidirectional.

Some findings in the field suggest that the heartbeat may serve as a synchronizer for slow-waves [24]. Following this idea of a cardiac influence on brain function, the enhancement of ANS activity induced by acoustic stimulation in our study may have contributed to the increase in SWA. This possibility is also substantiated by evidence that sounds can modulate HR and HRV via interaction with the respiratory system [34]. The neural pathway for such an interaction is well established: the auditory and respiratory systems share a common neural relay through the medulla, specifically the rostro ventrolateral medulla (RVLM), with integrated projections to the sympathetic nervous system [35, 36]. Further investigations of the heart-brain-sleep connection using advanced real-time signal processing are needed to better understand the functional directionality.

Another important finding of our study is the novel relationship demonstrated between SWA enhancement and evening-to-morning ANS changes. ANS activity during sleep has been shown to impact cardiovascular function during wake [1]. In particular, selective SWS disruption has been shown to increase sympatho-vagal balance (LF:HF) during wake [7]. Although acoustic stimulation produced no differences in evening-to-morning HRV changes between STIM and SHAM, even after challenging the ANS (orthostatic test), there was a correlation between the amount of SWA increase during ON versus OFF intervals and the reduction in evening-to-morning and supine-to standing LF:HF changes.

Similar to LF:HF, a significant correlation was present between SWA increase in ON versus OFF intervals and the reduction of the normal evening-to-morning increase of plasma cortisol. Cortisol secretion and sleep structure are closely linked. Both the impairment of sleep quality (i.e., increased sleep fragmentation and increased amount of lighter sleep stages) and an inadequate sleep duration can enhance the stress reactivity of the hypothalamus-pituitary axis and promote cortisol increase during wake [37]. Sleep-induced changes in ANS are suggested as the main mediators of cortisol changes [38]. This hypothesis is also consistent with evidence that basal sympathetic nervous system activity strongly influences cortisol secretion [39]. Accordingly, both the LF:HF and cortisol changes associated with the transient SWA increase during acoustic stimulation likely reflect a reduction in sympathetic nervous system activity.

We did not find an association between BP changes and SWA enhancement as seen in LF:HF and cortisol. This may be due to individual variation in other mechanisms, such as peripheral vascular regulation that helps maintain a narrow blood pressure range in healthy young adults. The reduction of LF:HF and plasma cortisol indicates that enhancement of SWA influences sympathetic activity immediately following awakening. This result is of particular interest since morning hours have been shown to be the period of highest vulnerability for adverse cardiovascular events [40].

Several key questions surrounding brain physiology in response to auditory stimulation remain open for discussion. Due to the relatively few studies in this area of research, there is a lack of consensus on the optimal duration, number of pulses, and timing of stimulation [41]. In the present study, acoustic stimuli were delivered across the entire night. This is in contrast to other paradigms in which stimulation was limited to the first part of the night [10, 13, 16], presumably to maximize stimulation in SWS and avoid eliciting arousals during the time of higher arousability in the second half of the night [42]. While we observed a modest increase in the number of arousals during stage N2, primarily in the first three cycles of sleep, the number of arousals was similar between STIM and SHAM condition. Furthermore, SWA enhancement in ON versus OFF intervals in both cycles 1–3 and cycles 4–5 was significantly associated with the evening-to-morning reduction in LF:HF. The association was also significant for SWA enhancement in cycles 1–3 and the evening-to-morning reduction in cortisol and neared statistical significance in cycles 4–5. Together, these data suggest that the effect of stimulation across the entire night, rather than any specific sleep cycle, contributed to the magnitude of the overnight autonomic changes.

In our study, acoustic stimulation did not alter the total amount of NREM SWA but rather increased SWA during ON intervals of stimulation compared with OFF intervals. Importantly, this modulation of SWA drove the evening-to-morning reduction in indices of autonomic activation. This finding is consistent with previous results in older adults, demonstrating that the increase in SWA in ON versus OFF intervals with acoustic stimulation was associated with overnight memory improvement [15]. It is biologically plausible that phase-locked acoustic stimulation delivered in a block design result in a functional reorganization of SWA, which affects cognitive and autonomic functions.

Evidence also suggests that cortical excitability follows a cyclic modulation at 0.02 to 0.2Hz [43] and that infra-slow neural oscillations (0.02 Hz) are coordinated with cardiac modulation during NREM sleep in animals and humans [44]. Diencephalic and brainstem circuits, where the integration of audition and autonomic outputs co-occur [35, 36], have been proposed as possible generators of the infra-slow rhythm and modulators of cortical excitability [45–47]. Whether the stimulation algorithm presented here inadvertently locks to the presence of delta bursts, or it actually entrains the EEG oscillatory rhythm remains unknown. Future studies are needed to provide further quantification of the reorganization observed in SWA following acoustic stimulation in order to understand its potential physiological function.

There are several limitations in this study. First, our approach included only one night of acoustic stimulation in healthy young adults. Moreover, 75% of the subjects in our study were female. Therefore, the results may not be generalizable to the general population, including older adults who have impairment in both sleep and ANS function. Second, in the present study, we did not obtain PSG measures to quantify sleep-related respiratory events. However, 85% of the participants scored between 0 and 2 on the STOP-BANG screening questionnaire. The high sensitivity

of the questionnaire to exclude OSA with AHI ≥5 for scores within this range [48], combined with fact that participants were young and lean, makes it unlikely that sleep-related respiratory events significantly affected our findings.

Third, the use of HRV to assess ANS function has limitations [49]. Whereas LF:HF is commonly used to quantify the sympatho-vagal balance, it cannot provide a precise quantification of sympathetic nervous system activity [50–52]. Other approaches such as microneurography, beat-to-beat BP monitoring and catecholamine assessment can provide a more precise quantification of the ANS sympathetic function [49], but the more invasive nature of these methods often limit their application to sleep studies [49]. Nevertheless, our study shows methodological strengths in HRV analysis that support the reliability of the findings and their interpretation. In particular, participants were studied under rigorously controlled conditions and a meticulous selection of the ECG signal was preformed to ensure stationary segments for analysis [25]. Furthermore, we assessed respiratory activity and showed that HRV effects were not ascribed to respiratory changes.

Finally, the effect on ANS activity was assessed at one time point after awakening. Future research should address the effects of multiple nights of stimulation on ANS function during sleep and wake across the day.

In conclusion, enhancement of SWA in our study led to improved autonomic regulation of cardiac function, specifically by favoring the balance toward parasympathetic activity during SWS. Intriguingly, the reorganization of SWA characterized by brief intermittent boosts of slow-waves induced by acoustic stimulation, was associated with a reduction in the evening-to-morning change in HRV and cortisol, implicating SWA in the reduction of autonomic sympathetic drive. These findings suggest that enhancement of SWA with acoustic stimulation during sleep can have important preventive and therapeutic implications for cardiovascular health. Moreover, given that the ANS is an active modulator of a wide range of physiological functions, approaches to manipulate sleep quality, and specifically SWA, have the potential to improve overall physiological homeostasis.

Supplementary material

Supplementary material is available at *SLEEP* online.

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