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Automated selective disruption of slow wave sleep

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Abstract

Background—Slow wave sleep (SWS) plays an important role in neurophysiologic restoration. Experimentally testing the effect of SWS disruption previously required highly time-intensive and subjective methods. Our goal was to develop an automated and objective protocol to reduce SWS without affecting sleep architecture.

New Method—We developed a custom MatlabTM protocol to calculate electroencephalogram spectral power every 10 seconds live during a polysomnogram, exclude artifact, and, if measurements met criteria for SWS, deliver increasingly louder tones through earphones. Middle-aged healthy volunteers (n=10) each underwent 2 polysomnograms, one with the SWS disruption protocol and one with sham condition.

Results—The SWS disruption protocol reduced SWS compared to sham condition, as measured by spectral power in the delta (0.5–4 Hz) band, particularly in the 0.5–2 Hz range (mean 20% decrease). A compensatory increase in the proportion of total spectral power in the theta (4–8 Hz) and alpha (8–12 Hz) bands was seen, but otherwise normal sleep features were preserved. N3

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CONFLICTS: SO, YSJ, DMH, and JMZ have no relevant conflicts of interest.

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sleep decreased from 20 ± 34 to 3 ± 6 minutes, otherwise there were no significant changes in total sleep time, sleep efficiency, or other macrostructural sleep characteristics.

Comparison with existing method—This novel SWS disruption protocol produces specific reductions in delta band power similar to existing methods, but has the advantage of being automated, such that SWS disruption can be performed easily in a highly standardized and operator-independent manner.

Conclusion—This automated SWS disruption protocol effectively reduces SWS without impacting overall sleep architecture.

Keywords

sleep; spectral power; slow wave sleep; electroencephalogram; polysomnogram

1. INTRODUCTION

Slow wave sleep (SWS) is characterized by high-amplitude delta frequency range (0.5–4 Hz) electroencephalogram (EEG) waves, signifying synchronous relative quiescence of cortical neurons (Nir et al., 2011). SWS represents the deepest part of non rapid eye movement (NREM) sleep, and day-to-day variations in cortical activity affect the distribution and amplitude of SWS at night (Huber et al., 2006, Pugin et al., 2015). SWS plays a key role in synaptic downscaling and other processes that underlie plasticity and memory consolidation (Tononi and Cirelli, 2006, Tononi and Cirelli, 2014) and has been hypothesized to be important in neurophysiologic restoration. SWS is linked with lower amyloid- β ($A\beta$) levels likely due to decreased synaptically driven $A\beta$ release (Kang et al. 2009) as well as to an increased rate of glymphatic metabolite clearance in the brain, such as clearance of amyloid proteins (Xie et al., 2013). In turn, neurodegenerative amyloid plaques inhibit both the amount of NREM sleep (Roh et al., 2012) and the normal propagation of slow waves (Mander et al., 2015). Given the tight interaction between SWS and neurological physiology from the biochemical to the behavioral levels, many groups have sought to experimentally disrupt or enhance SWS in humans (Landsness et al., 2009, Ngo et al., 2013, Santostasi et al., 2016). Prior methods of SWS disruption have typically relied on highly-trained individuals subjectively interpreting EEG in real time, and delivering auditory or other stimuli to cause arousals out of deep NREM sleep. Therefore, these existing methods are highly subject to inter-operator variability in EEG scoring, selection of stimuli, and delivery of stimuli. Our aim was to develop an automated and operator-independent SWS disruption protocol, using on-line spectral power analysis and auditory tones, to reduce specifically SWS without affecting sleep architecture in healthy middle-aged participants.

2. METHODS

2.1 Participants

We recruited 11 healthy participants aged 45–65 years from a community-based research participant registry in Saint Louis, Missouri, USA. One participant was lost to follow-up prior to completing the study; therefore 10 participants were included in the final analysis. Participants had no comorbidities, took no neuro-active medications, and were cognitively

normal (Mini Mental Status Examination score 27/30). Participants had regular sleep schedules with bedtime between 8PM–12AM and wake time between 4AM–8AM, confirmed by 5 days of actigraphy immediately prior to polysomnograms. Participants did not have obstructive sleep apnea or periodic limb movement disorder, as defined as an apnea-hypopnea index ≤ 5 or periodic limb movement index ≤ 15 , during a screening unattended polysomnogram. This study was approved by the Washington University Human Research Protection Office. Written, informed consent was obtained from all participants.

2.2 Sleep studies

The first four participants each underwent two trial polysomnograms, at least one week apart. The trial polysomnograms were used to test and refine the SWS disruption protocol parameters. All ten participants then underwent two protocol polysomnograms with the final parameters. Four participants therefore underwent a total of four polysomnograms, with only the last two included in this analysis. The SWS disruption protocol was used for one polysomnogram, and a sham protocol used for the other; the order was random and participants were blinded to the condition. Sham protocol consisted of identical set up including earphones, but no noises were delivered through the earphones during the night. Standard polysomnogram channels were used, including frontal (F3, F4), central (C3, C4), and occipital (O1, O2) parasagittal electrodes referenced to the opposite mastoid (M1, M2) electrodes (Iber C et al., 2007). EEG data were acquired at 200 Hz and recorded to a MK3 TrackIt™ (Lifelines Neurodiagnostic Systems, Illinois, USA) device in European Data Format (EDF). A registered sleep technologist, blinded to the condition, performed sleep staging on 30-second epochs using standard criteria (Iber C et al, 2007). Additionally, the technologist scored movement or arousal artifact for each 10-second mini-epoch of sleep. Following polysomnograms, participants completed a questionnaire asking “Do you recall being woken during the night because of the noises through the earphones?”, and “If yes, how many times do you think you woke up because of the noises?”

2.3 Development of the SWS disruption protocol

A live, automated protocol to disrupt SWS was developed in Matlab™ (Figure 1). Every 10 seconds, or mini-epoch, the protocol accessed the EDF file, extracted the most recent EEG data, and calculated spectral power for the delta (0.5–4 Hz) and the alpha (8–12 Hz) bands from the F4-M1 electrode using a fast Fourier transform function over the most recent 10.24 seconds (2048 samples). While SWS often is calculated by the amount of N3 (NREM stage 3) sleep, due to decreasing EEG amplitude with age, many adults have little to no sleep that meets scoring thresholds for N3 (Van Cauter et al., 2000). Therefore, spectral power in the delta band—or “delta power”—serves as a more appropriate and continuous measure of SWS. The F4 electrode was chosen rather than an average of multiple electrodes because SWS is most prominent in the frontal electrodes (Finelli et al., 2001), to reduce computing requirements, and to enable adding this protocol to a standard polysomnogram montage which shows F4, C4, and O2 EEG electrodes only (Iber et al., 2007). Furthermore, recent SWS disruption protocols using visual scoring have also used single electrodes (Landsness et al 2009, Aeschbach 2008).

Two methods were used to identify artifact. First, if alpha power or delta power was above a very high fixed threshold, which is usually due to movement or sweat artifact, then the mini-epoch was categorized as artifact. Second, if the delta power was relatively higher than the immediately preceding epochs, it was also categorized as artifact. Specifically, if the delta power was greater than the running average of the delta power for the last five non-artifactual mini-epochs, multiplied by a specified “running average factor,” then the current mini-epoch was considered to be artifact. The running average factor therefore identifies as artifact those mini-epochs that demonstrate sudden bursts of spectral power, which usually correspond to arousal artifact. Since delta power progressively increases over a NREM cycle, outliers can be identified and excluded using this method that cannot be identified with the fixed thresholds in the first method. A third candidate artifact-detection method using the [delta power : alpha power] ratio, to identify EEG data with disproportionately high delta power due to eye movements, was tested but ultimately found to be ineffective and not used. The cutoff values used for artifact detection are all variables that can be adjusted through the user interface; determination of appropriate cut-off values is discussed in the next section.

If the mini-epoch was not categorized as artifact, and delta band power was above a specified threshold, then the protocol considered the EEG data to be SWS, and delivered an auditory tone through earphones. The tones were pure tones of random pitch and duration, to prevent habituation; prior studies using auditory disruption of sleep have found that there is rapid adaptation if the same tone is used throughout the night (Roehrs et al., 1994). The pitch range was determined by a hearing test at the beginning of the night, and was usually in the 25 Hz to 2000 Hz range (approximately the pitch of the lowest note on a standard piano to 2 octaves above middle C). The duration of each tone varied randomly between 1 and 5 seconds. The first tone in a series of tones would start at the lowest detectable amplitude (volume) for the participant, determined by a hearing test prior to the polysomnogram, and with each consecutive mini-epoch that met criteria for SWS, the amplitude of the tone would increase, to a maximum of ~100 dB. The maximum amplitude was set to be reached over 12 amplitude levels, or 2 minutes. Every mini-epoch that was scored as not-SWS would decrease the amplitude level by one, but no tone would be delivered. For instance, if there were 5 consecutive mini-epochs of SWS (with tones delivered at amplitude levels 1–5), and then 2 mini-epochs of not-SWS (with no tones), then the following mini-epoch of SWS would result in a tone at amplitude level of 3. This feature of the amplitude level “fading” during non-SWS mini-epochs, rather than starting over at 0, was specifically added during protocol development. We found that once a participant gets into deep SWS, it is very difficult to disrupt SWS without a strong stimulus that would actually awaken them. If the amplitude level started over at 1 after just one epoch of non-SWS, it could take up to 2 minutes for the amplitude levels to get back to where they left off, by which point the person could be in very deep SWS and not have any response to the tones. By having the amplitude level increase or decrease every 10 seconds, the SWS disruption protocol essentially “tunes” the tone amplitude to the participant’s changing arousal threshold throughout the night. The amplitude level tracker would adjust regardless of whether tones were delivered or whether the amplitude level was at maximum (12). If the amplitude level tracker reached 30 (essentially, the participant has SWS despite 5 minutes of

tones every 10 seconds), the participant was roused by the sleep technician speaking into an overhead intercom, “[Participant name], wake up.” All participants were roused at least once overnight in this manner. No participant required physical stimulation or other measures to awaken. The clock time, epoch, mini-epoch, spectral power, auditory tone information, and auditory level tracker were all written into a log file live during the polysomnogram.

The SWS disruption protocol was designed to function automatically. A technician was required for several roles. One was to assess for sweat artifact or other problems with EEG electrodes, and replace them if necessary. Technicians also were instructed to push a button on the user interface to suppress tones if it appeared that the SWS disruption protocol was delivering tones when a participant was awake. This button did not need to be pushed during any of the polysomnograms in this study. Lastly, the technician spoke into an overhead intercom if a participant remained in SWS despite 5 minutes of tones, as described above. Other than these straightforward tasks, the SWS disruption protocol did not require additional operator knowledge or training. All Matlab scripts required for the protocol are provided in the supplementary material. The main file (SWSdeprive2.m) contains a small optional modification that allows using an average of F3, F4, C3, and C4 data for the live protocol. If this modification is used, then the technician would need to add the left-sided EEG electrodes to the viewing montage and assess for problems with these electrodes during the study also.

2.4 Determination of scoring variables

PSG data from 18 healthy participants (age 45–65) from a separate study were used to determine the initial settings for the SWS disruption protocol. The settings were further refined in the first four participants during their first two polysomnograms. We elected to determine protocol parameters for the age group rather than for each individual participant, because our purpose was to develop an easy-to-use protocol that could be applied without multiple nights’ data collection. Delta power was plotted against alpha power and divided into NREM and not-NREM (REM, wake, or artifact). Values for alpha and delta power that encompass NREM were determined visually and set at 200–3450 $\mu\text{V}^2\cdot\text{s}$ for delta power and 0–275 $\mu\text{V}^2\cdot\text{s}$ for alpha power (Figure 2A). The threshold for minimum delta power to deliver auditory tones was lowered to 100 $\mu\text{V}^2\cdot\text{s}$ in the final protocol, because this improved scoring sensitivity and the degree of SWS disruption. While an epoch with delta power of 100 $\mu\text{V}^2\cdot\text{s}$ would generally be visually scored as N2 rather than N3, we found that auditory stimuli had to start at least several minutes before N3 sleep in order to effectively disrupt SWS. Various running average factor values were tested, and a value of 8 provided the best balance of sensitivity and specificity. In our participants, these settings resulted in a sensitivity of $97\pm3\%$ and a specificity of $83\pm11\%$ compared to the gold standard of visual scoring, indicating that, on an epoch-by-epoch basis, the ability of the protocol for discriminating SWS from other sleep-wake stages is excellent (Figure 2B), and superior to prior automated SWS scoring algorithms (Su et al., 2015).

2.5 Data analysis

During the live SWS disruption protocol, a rapid fast Fourier transform function (*bandpower* function in the Matlab Signal Processing toolbox) was performed on data from the F4

electrode only, to reduce computing requirements. Following data collection, a more detailed spectral analysis was performed on all EEG electrodes, including F3, F4, C3, C4, O1, O2, each referenced to the opposite mastoid (M1 or M2). Power was normalized to the total power, and taken as an average over all electrodes (proportional power). Data for each 10.24 seconds—with 0.24 seconds overlap between each pair of mini-epochs—were analyzed using Welch's method. The 2048 data points in each 10.24 seconds were divided into 4 sections of 512 data points, with 50% overlap. A Hamming window was applied prior to the discrete Fourier Transform to calculate the spectral power for every 0.5 Hz bin from 0.5 to 32 Hz. Frequency ranges were 0.5–4 Hz for delta, 0.5–2 for low delta, 2–4 for high delta, 4–8 Hz for theta, 8–12 for alpha, and 12–18 for beta.

Statistical analyses were performed with IBM SPSS version 20.0 (SPSS Inc, Chicago, USA). Statistical significance was set at $p < 0.05$. The Kolmogorov-Smirnov test was used to determine whether data were normally distributed. Differences in between SWS disruption and sham protocols were calculated using paired Student's t-tests for normally-distributed variables, and related-samples Wilcoxon signed rank test for variables that were not normally distributed, with each participant acting as his or her control.

3. RESULTS

Our research population ($n=10$) consisted of five female and five male participants, with a mean age of 53 ± 4 years, and equal number of Caucasians and African-Americans. Delta power over all non-artifactual NREM sleep calculated from the mean of the F3 and F4 electrode was significantly decreased by an average of 18% in the SWS disruption condition ($123 \pm 57 \mu V^2 \cdot s$) compared to the sham condition ($149 \pm 62 \mu V^2 \cdot s$; $p = 0.001$) (Figure 3A). This decrease was completely driven by reductions in low delta power (0.5–2 Hz), in which there was an average 20% decrease in spectral power, from $134 \pm 56 \mu V^2 \cdot s$ in the sham night to $108 \pm 53 \mu V^2 \cdot s$ in the SWS disruption protocol night ($p < 0.001$) (Figure 3B). All 10 participants had decreased delta power and low delta power during the SWS disruption protocol compared to sham. There were no significant differences in the high delta band (2–4 Hz) between conditions (Figure 3C). The four participants who had previously had polysomnograms during the protocol development phase had a 15% decrease in total delta power during the SWS disruption protocol condition compared to the sham condition, while the remaining six participants had a 19% decrease in total delta power, indicating that the protocol was effective for all participants we studied, and the protocol and parameters are not specific for the 4 participants.

Since spectral power may vary by age, sex, and other inter-individual characteristics, we normalized the data by analyzing band power as a proportion of total power in the entire (0.5–32 Hz) analyzed range. The proportion of spectral power in the low delta band during NREM sleep was again significantly decreased with the SWS disruption protocol compared to the sham condition ($64 \pm 5\%$ versus $69 \pm 5\%$, $p = 0.007$), while the proportion of high delta power was no different (Figure 4A). There was a compensatory shift of proportional power into the theta and alpha bands (Figure 4B). While data from only the F4 electrode were used during the SWS disruption protocol, off-line analysis after the polysomnogram showed there was a decrease in delta power in all EEG electrodes (Figure 5). Time in N3 sleep was

decreased during the SWS disruption protocol (20 ± 34 versus 3 ± 6 minutes). Otherwise, there were no significant differences in total sleep time (384 ± 54 versus 381 ± 67 minutes), sleep efficiency (0.79 ± 0.09 versus 0.79 ± 0.13), or time in different sleep stages between the sham and the SWS disruption condition (Table 1).

There were 1172 ± 389 tones delivered during the SWS disruption protocol nights. Of these, 350 ± 64 tones were followed by a mini-epoch categorized as “not-SWS,” and we termed these “effective” tones. On visual scoring, the most common EEG response to an “effective” tone was a microarousal, meaning a shift from a lower to a higher frequency. The majority of tones were delivered during NREM sleep, and, the majority of the time, the following mini-epoch was also NREM sleep, supporting that the SWS disruption protocol does not function by causing awakenings (Figure 6). Post-polysomnogram questionnaires assessed how aware the participants were of the tones delivered during the protocol. Of the ten participants, one participant did not fill out the questionnaire, and, among the remaining nine, two did not recall any awakenings due to the tones, while seven were aware of the tones during the SWS disruption protocol night. Of the seven who were aware of the tones, two did not specify the number of tones they recalled. Combining the five who did provide an estimated number of tones, and the two who did not recall hearing any tones, on average the participants estimated hearing 7 ± 8 tones (range 0–20), or 0.5% of the tones they actually received. As expected, no one recalled awakenings due to tones during the Sham condition.

4. DISCUSSION

Our live, automated SWS disruption protocol accurately identified and specifically suppressed SWS, while preserving normal sleep architecture. These results are similar to previous studies using non-automated methods, in which total sleep time remained the same, while delta spectral power decreased 20% (Landsness et al., 2009), and 20–30% (Aeschbach et al., 2008). With our novel SWS disruption protocol, relative spectral power shifted from the low delta range into the high delta, theta, and alpha ranges, essentially “lightening” NREM sleep, without affecting macrostructural sleep characteristics. This protocol does not exert effect by causing awakenings. Rather, participants remained in NREM sleep (Figure 6), albeit lighter NREM sleep. We suspect that only low delta power is affected by the protocol because our protocol essentially shifts the power spectrum to higher frequencies, and the majority of total delta power is within the lower delta band (as illustrated in Figure 5). Therefore the protocol “pushes” what would have been in the low delta range into the high delta and theta ranges. It would be difficult to reduce power further in the high delta band without awakening a person; some high delta activity is inevitable and necessary to score an epoch as NREM sleep. Additionally, SWS in the low delta range has also specifically been linked to memory function and the presence of A β pathology (Mander et al., 2015). Therefore we anticipate that our SWS disruption protocol that reduces low delta power would be of benefit to investigators interested in assessing the physiological effects of experimental SWS disruption.

Our automated protocol removes the subjectivity and variability inherent in prior SWS disruption methods. Our participants received 1172 ± 389 tones during the SWS disruption protocol nights, or about 150 tones per hour over an 8-hour polysomnogram. An extremely

highly skilled technician with exceptional vigilance would be required to replicate the protocol manually. All equipment and software used in our SWS disruption protocol are commercially available, the computer processing demand does not require specialized equipment, and the Matlab scripts are being shared for scientific use. No special training is required, allowing a sleep technician to simply apply the protocol during a standard polysomnogram. Subsequently, other laboratories can easily adopt the SWS disruption protocol into their investigations.

One of the major challenges of this study was identifying parameters that were suitable for a mixed population, in this case, healthy middle-aged participants. While it is possible to use an individual's EEG data from one night to set the protocol parameters for another night with SWS disruption, a requirement for multiple nights' recording limits practical application. To overcome this limitation, we have identified parameters that are effective at reducing SWS in a middle-aged group, rendering redundant a baseline recording night for each participant and thereby decreasing participant burden. We also considered using a patient's EEG data from the beginning of the study to automatically set or "tune" protocol parameters for the rest of the night. However, since sleep pressure as measured by SWS dissipates over the course of the night, particularly in the first 1–2 NREM periods, the EEG features of the first part of the night do not reflect EEG features over the entire night for an individual. Moreover, auto-tuning protocol parameters based on the beginning of the night would require the operator to live-score every 10-second mini-epoch, and then that data would need to be entered into the software and analyzed. This would add substantially to operator burden and computational load, conflicting with our goal of developing an *automated* SWS disruption protocol that could be easily added to a standard polysomnogram. Lastly, since some REM sleep would need to be collected to auto-tune the protocol parameters, and the first REM sleep period typically occurs after ~1–1.5 hours of NREM sleep (the first NREM period), that means that the SWS disruption protocol could not be applied for ~1–1.5 hours during the portion of sleep that contains the highest amount of SWS. For all these reasons, we decided against using a participant's EEG data from the first part of night to determine SWS disruption protocol parameters for the rest of the night. As investigators collect EEG data on other target populations (*e.g.* elderly, people with dementia, or children), different sets of parameters will need to be tested to optimally disrupt SWS in each population. Of course, investigators can individualize protocol parameters for a specific person by using data from a prior polysomnogram to set parameters for the SWS disruption protocol, if multiple nights' studies are practical. Furthermore, linear discriminant analysis, support vector machines, or other machine learning methods may be applied to group- or individual-level EEG data to determine protocol parameters that are more effective at automated scoring and SWS disruption than the visually- and empirically-derived parameters presented here.

Our study is limited by the small sample size, which can be justified by the larger group ($n=18$) of healthy participants that was used to derive the initial protocol parameters, which were then refined during testing. Also, the protocol parameters were targeted for an age group of 45–65 years, but the parameters can be easily altered for other populations through the user interface of the SWS disruption protocol (Figure 1). In addition, the SWS disruption protocol is not 100% automated, and some human intervention is required as described in

the methods section. However, minimal effort is required such that one individual can deliver a standardized SWS disruption protocol to several participants simultaneously.

In conclusion, our SWS disruption protocol is an automated and easy-to-use tool that effectively reduces SWS without affecting sleep architecture. This protocol will be used in future studies to investigate the role of SWS on physiological processes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

EDF	European Data Format
EEG	electroencephalogram
NREM	non rapid eye movement
REM	rapid eye movement sleep
SWS	slow wave sleep

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HIGHLIGHTS

- Easy-to-use tool that effectively and specifically reduces slow wave sleep.
- Normal sleep architecture is preserved.

Figure 1. Schematic overview of the SWS disruption program

The left box represents the operator graphical user interface, which contains two buttons (one to suppress a noise and one to end the study), noise parameters, and spectral parameters. All the white boxes can be altered. Noise parameters marked with * are determined individually at the beginning of the night to determine the participants hearing range, including minimum and maximum pitch, and minimum amplitude (loudness). The maximum amplitude used was 100 decibels (dB). The duration of noises used was 1 to 5 seconds. The same spectral parameters were used for every participant, since we determined parameters that were effective in this age group (45–65 years) as a whole. “α power max” and “δ power max” indicate values of alpha power ($275 \mu V \cdot s^2$) and delta power ($3450 \mu V \cdot s^2$), respectively, above which EEG data are scored as artifact. “Running average factor” identifies as artifact EEG data with a burst of delta power, defined as greater than the running average of the delta power for the last five non-artifactual mini-epochs, multiplied by the specified “running average factor” (8). “δ power SWS threshold” is the delta power above which noises will be delivered to disrupt SWS; a value of $100 \mu V \cdot s^2$ was used in our protocol. Every 10 seconds (mini-epoch) the slow wave sleep (SWS) disruption protocol 1) extracts electroencephalogram data; 2) performs a fast Fourier transformation to calculate delta and alpha spectral power; 3) determines whether the data represent artifact (three different methods); 4) assesses if delta spectral power meets a predetermined threshold, and, if so, 5) delivers a noise to the participant through earphones. When a mini-epoch is considered SWS, the noise level increases one level. When a mini-epoch is considered not-SWS, the noise level decreases one level (unless it is at zero), but no noise is delivered.

Figure 2. Development and accuracy of scoring parameters

Electroencephalogram delta and alpha spectral power were used for automated scoring. **A)** Data from 18 participants from another study were used to set the trial parameters. Each dot represents a 30 second epoch. The horizontal line represents the delta power threshold set at $200 \mu\text{V}^2\cdot\text{s}$. **B)** Data from the 10 study participants for both the sham and SWS disruption protocol nights. Each dot represents a 10 second mini-epoch. The majority of the REM, artifact, and wake mini-epochs in the “SWS” range (above the horizontal line, to the right of diagonal regression line) were rejected as artifact via the other artifact identification method described in Methods section 2.3. The final scoring specificity was 83% and sensitivity was 97% for detecting SWS.

Figure 3. Specific reduction in low delta band power

Change in spectral power (% compared to the sham night) over all non-artifactual NREM sleep for each individual measured from the mean of the F3 and F4 electrodes for **A)** all delta (0.5–4 Hz) **B)** low delta (0.5–2 Hz) and **C)** high delta (2–4 Hz) bands are displayed. Every participant demonstrated a decrease in delta power with the automated SWS disruption protocol (–18% change, $p = 0.001$); a reduction in the low delta band power (–20% change, $p = 0.001$) entirely accounted for the decrease in delta power. (* $p = 0.05$)

Figure 4. Shift in spectral power to higher frequencies

The proportion of power (% of total power in the 0.5–32 Hz range) is displayed for each band, for all NREM sleep. With the SWS disruption protocol, there is a significant reduction in proportional power in the low delta (0.5–2 Hz) range, and a shift of proportional power to the theta (4–8 Hz) and alpha (8–12 Hz) bands. Note the different y-axis ranges in the two graphs. Middle band indicates median, box indicates interquartile range, and whiskers indicate maximum and minimum values. (* $p < 0.05$).

Figure 5. Power spectral distributions

Power spectral distributions demonstrate the decrease in power in the low delta band, and a slight compensatory shift in power to the higher frequencies, indicating selective SWS disruption. Data are averaged for all (F3, F4, C3, C4, O1, O2) electrodes over all NREM sleep for all 10 participants. The main graph presents data with the y-axis on a log scale, while the inset graph shows the data in the lower frequency range (in dotted box) on a linear scale to demonstrate the dramatic reduction in low delta power. Shaded regions indicate standard error.

Figure 6. Stage transition due to auditory stimulation in the SWS disruption protocol

An average of a total of 1172 (± 389) tones were delivered during the SWS disruption nights. The upper bar graph demonstrates during what sleep stages tones were delivered, in percentages. The majority of tones were delivered during NREM sleep, demonstrated in shades of blue. “A” represents artifact during NREM sleep, due arousal with movement. The lower pie charts depict the sleep stage following tones, delivered during N3 (left), N2 (middle), and N1 (sleep). The vast majority of tones delivered during NREM sleep were followed by more NREM sleep, rather than wake or arousal (with movement).

Table 1

Macrostructural sleep characteristics

	Sham Mean (±standard deviation)	Range	SWS disruption protocol Mean (±standard deviation)	Range	p-value
Total sleep time (minutes)	384(±54)	302–477	381 (±67)	302–489	0.89
Sleep efficiency (%)	79 (±9)	67–94	79 (±13)	61–95	0.93
NREM (minutes)	311 (±41)	253–379	287 (±33)	245–333	0.20
N1 (minutes)	37 (±23)	16–95	50 (±25)	21–101	0.22
N2 (minutes)	250 (±47)	162–351	230 (±39)	151–279	0.28
N3 (minutes)	20 (±34)	0–99	3(±6)	0–16	0.03 *
REM (minutes)	57 (±26)	17–98	63 (±39)	7–144	0.68

* Related-samples Wilcoxon signed rank test due to non-normally-distributed data, as determined by Kolmogorov-Smirnov test. All other comparisons were done with paired t-tests, since data were normally-distributed.