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RESEARCH ARTICLE

Closed-loop acoustic stimulation during an afternoon nap to modulate subsequent encoding

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Summary

Sleep is able to contribute not only to memory consolidation, but also to post-sleep learning. The notion exists that either synaptic downscaling or another process during sleep increase post-sleep learning capacity. A correlation between augmentation of the sleep slow oscillation and hippocampal activation at encoding support the contribution of sleep to encoding of declarative memories. In the present study, the effect of closed-loop acoustic stimulation during an afternoon nap on post-sleep encoding of two verbal (word pairs, verbal learning and memory test) and non-verbal (figural pairs) tasks and on electroencephalogram during sleep and learning were investigated in young healthy adults ($N = 16$). Closed-loop acoustic stimulation enhanced slow oscillatory and spindle activity, but did not affect encoding at the group level. Subgroup analyses and comparisons with similar studies lead us to the tentative conclusion that further parameters such as time of day and subjects' cognitive ability influenced responses to closed-loop acoustic stimulation.

1 | INTRODUCTION

The beneficial effect of sleep on subsequent learning is attributed essentially to its synaptic down-scaling function that relies on the rhythmic depolarisation Up state (neural firing) and hyperpolarisation Down state (neural quiescent) of the sleep slow oscillation (SO), the predominant rhythm of slow-wave activity (SWA; Tononi & Cirelli, 2006). Indeed, the perturbation of SWA led to poorer subsequent encoding performance (Van Der Werf et al., 2009), and interventional studies that enhanced SWA increased subsequent learning capacity (Antonenko et al., 2013). By studying the effect of sleep deprivation on post-sleep learning, the relevance of intact hippocampal function was underscored (Yoo et al., 2007). More recently post-sleep encoding performance was found to correlate with hippocampal activation, boosted during a 90-min afternoon nap (Ong et al., 2020). Furthermore, a positive correlation between sleep spindles and episodic learning after an evening nap (Mander et al., 2011) suggests a supportive function of spindle activity in post-sleep encoding.

Closed-loop acoustic stimulation (CLAS) applied during sleep is an emerging approach that targets SO depolarisation Up states to boost SO and spindle activity, and thus aims to modulate associated cognitive processes, initially sleep-associated memory consolidation (for review, see Malkani & Zee, 2020). This method employs real-time detection of ongoing neural oscillations thereby allowing acoustic stimulation to be delivered at the desired SO state (Ngo et al., 2013;

Santostasi et al., 2016). The closed-loop feature for stimulus delivery has indeed proven crucial for successfully impacting other oscillatory events and behavioural outcomes (Weigenand et al., 2016). Several studies that utilised CLAS or conceptually similar methods have uniformly demonstrated the effectiveness of CLAS in augmenting SOs and spindle activity, although behavioural findings have been inconsistent (Malkani & Zee, 2020; Ong et al., 2018). In one study in which inconsistencies in memory consolidation after non-invasive brain stimulation were assessed, cognitive ability proved to be a factor explaining variance (Koo et al., 2018). This observation underlines the possibility that cognitive ability may likewise have an impact on CLAS efficiency on learning.

The objective of this study is to investigate the effect of CLAS on SOs and spindle activity during an afternoon nap, and the potential role of induced electrophysiological changes on post-sleep encoding performance and ongoing electroencephalography (EEG) during learning tasks. Furthermore, the influence of cognitive ability on encoding performance and effects of CLAS on EEG activity during sleep are to be explored. Recently, CLAS during sleep strengthened concomitant changes in SWA and heart rate variability (Grimaldi et al., 2019), and was also linked to sleep-related cognitive performance (Naji et al., 2019). Thus, we also explored the modulation by CLAS of heart rate variability as a marker of autonomic nervous system activity, and its relationship to EEG and learning.

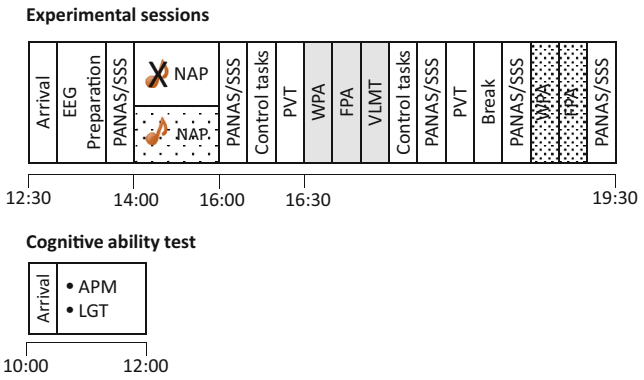

FIGURE 1 Experimental procedure. The timelines for the two experimental sessions (CLAS [closed-loop acoustic stimulation] and Sham) and subsequent assessment of cognitive ability are shown. Times given for the experimental session are exemplary. Experimental sessions were separated by at least 14 days. Filled fields correspond to learning phase (encoding + immediate recall), dotted fields correspond to delayed recall. The adaptation session is not depicted. PANAS, Positive and Negative Affect Scale; SSS, Stanford Sleepiness Scale; PVT, Psychomotor Vigilance Task served as psychometric control tasks as well as for example, word fluency. WPA, word paired-associate task; VLMT, verbal learning and memory test; FPA, figural paired-associate task. APM, Advanced Progressive Matrices to assess fluid intelligence; LGT, learning and memory test to assess memory quotient (MQ)

2 | MATERIALS AND METHODS

2.1 | Participants

Healthy subjects between 23 and 30 years were recruited largely via announcements on the university campus. Exclusion criteria included left-handedness (Nair et al., 2019), as determined by Annett's handedness questionnaire (Annett, 1970), use of medication, pregnancy, or the presence or history of any form of sleep disturbance, cognitive impairment, psychopathological disorder, brain injury or childhood seizures. A total of 37 participants were initially recruited. After screening for time spent in N3 and sleep-onset latency in the adaptation session, 23 remained. Sleep of two participants did not reveal deep enough non-rapid eye movement (NREM) sleep during the first experimental session, four were excluded due to incomparable sleep quality between sessions (one session had no sleep stage 3), and one withdrew due to a personal reason, resulting in a total of 16 participants (nine female; mean age 25.63 ± 0.58 years).

2.2 | Procedure

A within-subject crossover design was employed. After adaptation, subjects participated in two experimental nap sessions followed by a session to assess cognitive ability. Experimental sessions were pseudo-randomised and counterbalanced across participants. Participants were asked to get up 3 hr before their usual wake time or latest at 04:00 hours on the day of the nap. To monitor compliance, subjects wore an Actiwatch 2 (Resperonic Philips, Massachusetts, USA), consisting of an accelerometer and light sensor, prior to the adaptation and the experimental sessions. As shown in Figure 1, upon arrival at the laboratory (at 12:30 hours), EEG electrodes were applied, psychometric questionnaires conducted, and participants were subsequently allowed to sleep for 90–120 min. In the adaptation session, participants were given simplified versions of the learning tasks immediately after the nap and were then allowed to leave the laboratory. In the experiment sessions, subjects were given an additional 10 min after the nap to move around the laboratory to avoid sleep inertia before the learning tasks began. The order of tasks remained constant (word paired-associate [WPA], figural paired-associate [FPA], mirror tracing, verbal learning and memory test [VLMT]). We were forced to abort the procedural mirror tracing task due to the pandemic (see Supplementary Information). Electrodes were removed after learning and participants were allowed to take about a 1-hr break. During this time they were given a light dinner, and proceeded to perform the corresponding recall tasks, after which they left the laboratory. In the final session (2–7 days after the last experimental session), all participants conducted the Advanced Progressive Matrices (Raven & Raven's Progressive Matrices, 1994) and a Learning and Memory test battery (Bäumler, 1974) to measure fluid intelligence and memory quotient (MQ), respectively. All participants were informed about the study and signed a consent form before participation. The study was approved by the local ethics committee of the University of Luebeck, Germany.

3 | LEARNING TASKS

3.1 | Word paired-associate (WPA)

A total of 100 semantically non-associated word pairs were presented (encoding) on a monitor. Each pair consisted of a cue and a target word, for example, "*Question – Beginning*", shown for 3 s using a 500-ms inter-stimulus interval. At immediate recall participants recalled each target word on presentation of the cue word without time pressure or feedback. This procedure of encoding and immediate recall was repeated five times with the same 100 words resulting in five trials. After the break participants performed the recall task. Learning performance on the WPA was defined as the percentage of the number of correct words recalled on each of the five trials (mean of all trials) relative to the total number of word pairs. Retention performance was defined as the percentage of words recalled correctly at the delayed recall minus the number of correct responses at learning (mean of all trials) relative to learning.

3.2 | Figural paired-associate (FPA)

The FPA task included a total of 16 figural pairs. Each pair consisted of a cue and a target figure that were made up of geometric or non-geometric lines. During encoding, each pair appeared 3 s on the screen with a 500-ms inter-stimulus interval. Following encoding, the cue figure was presented, and participants selected the target figure from a set of seven other figures (in a recognition fashion) without time pressure or feedback. The procedure was repeated with the same pairs in a different order resulting in two learning trials. Learning on the FPA is defined as the number of correctly recalled figures at immediate recall (mean of both trials). Retention performance was defined as the percentage of correctly recalled figures at delayed-recall minus the number of correct responses at learning (mean of trials) relative to FPA learning performance.

3.3 | Verbal learning and memory test (VLMT)

The VLMT is adapted from the German version of Rey Auditory Verbal Learning Test (Helmstaedter et al., 2001). The test consists of five trials. The first three trials consist of lists of the same 15 words orally spoken each followed by an immediate verbal free recall. The fourth trial is an interference list containing 15 new spoken words, and followed by their free recall. The fifth trial requires participants to recall the 15 words from the first three trials. Words of all lists were spoken within 1 s by a pre-recorded neutral male voice. Oral free recall responses were recorded. Correctly recalled words of trials 1–3, 5 and the interference trial 4 were analysed separately. Visual and/or verbal stimuli of all three tasks were presented via E-prime 2.0 (Psychology Software Tools, Pittsburgh, PA, USA).

3.4 | EEG acquisition and preprocessing

The EEG data during the nap and the subsequent learning tasks were amplified by a compact mobile EEG amplifier (LiveAmp, Brain Products GmbH, Gilching, Germany) between 0.03 Hz and 80 Hz, applying a 50-Hz notch filter, and a sampling rate of 500 Hz. Between nap and learning subjects remained connected to the EEG device, but data were not collected. For recording, an elastic cap with 32 Ag/AgCl sintered electrodes positioned according to the 10–20 system was used. FCz served as reference, and an additional AFz electrode as ground. Two additional bipolar channels recorded an electromyography signal from the chin and a horizontal electrooculography. Impedance was kept below 5 kOhm for EEG and 10 kOhm for all other electrodes.

The EEG signals during sleep and learning were re-referenced offline to linked mastoids (TP9 and TP10) using Brain Vision Analyser software (Brain Products GmbH, Gilching, Germany). For analyses of EEG, during learning data were further band-pass filtered (0.6–45 Hz) and corrected for ocular artefacts by independent component analysis (Makeig et al., 1996). Residual artefacts were removed manually in epochs of 1-s. Lastly, data were re-referenced to the average reference, also via Brain Vision Analyser software. For EEG analyses during sleep data, originally acquired data were imported into Spike2 (Cambridge Electronic Design, Cambridge, England), after re-referencing to linked mastoids.

3.5 | EEG analyses during sleep and polysomnography

Data analyses during sleep were performed using Spike2 (Cambridge Electronic Design, Cambridge, England). To assess SO, slow and fast spindle activity, time-locked to the first stimulus of CLAS, EEG signals were first low pass-filtered at 35 Hz (FIR filter, −3 dB at 32.0 Hz) and downsampled to 100 Hz. Windows of −1 to 3 s time-locked to the first stimulus were extracted from artefact-free epochs in N2 and N3 and averaged. For spindle activity, before the 4-s windows time locked to the first stimulus were extracted, signals were bandpass filtered (slow spindle band: 9–12 Hz; fast spindle band: 12–16 Hz), and for each sample point the root mean square (RMS; time window: 0.2 s) was calculated.

Sleep EEG power spectra were obtained from artefact-free NREM sleep (N2 + N3) periods for all recording sites using Hanning tapered FFT windows of 10.24 s and 50% overlap (0.098 Hz frequency resolution), and the following frequency bands were calculated: SWA (0.5–4 Hz); Delta (1–4 Hz); Theta (4–8 Hz); slow spindles (9–12 Hz); and fast spindles (12–16 Hz).

For the detection of discrete spindles, low-pass filtered (at 35 Hz) and downsampled (to 100 Hz) EEG data were used. The spindle detection algorithm and criteria were adopted from Mölle et al. (2011). Specifically, individual fast spindle peak frequencies (range 12–16 Hz) were identified from power spectra at all channels throughout all artefact-free NREM sleep periods (N2 and N3). The mean peak frequency was 13.65 ± 0.11 Hz. A FIR band-pass filter

with a width of 3 Hz centred on the detected individual peak frequency was then applied to the EEG signals. Subsequently, a RMS representation of the filtered signal was calculated using a sliding window of 0.2 s with a step size of one sample. RMS data were smoothed with a 0.2-s-size sliding window. Time frames were considered as spindle intervals if the RMS signal exceeded a threshold of 1.5 SD of the bandpass filtered signal for 0.5–3 s. Spindle count, density (i.e. number of spindle per 30 s), mean peak to peak amplitude, mean length and mean spindle RMS throughout NREM sleep were analysed.

For polysomnography, sleep stages wake, stages N1, N2, N3 and REM sleep were determined according to guidelines of the American Academy of Sleep Medicine (AASM; Berry et al., 2012) by two independent scorers using SleepPilot (https://github.com/xuser/SleepPilot, v0.9.4-beta).

3.6 | EEG power spectra and event-related potentials (ERPs) during learning

Across the entire learning periods of each WPA, FPA and the VLMT tasks, power spectra (4 s Hanning tapered FFT windows with 50% overlap, 0.244 Hz frequency resolution), as well as theta (4–8 Hz) and alpha (8–12 Hz) frequency bands were calculated. Theta/alpha ratios were calculated to investigate frontocentral theta (FC1, FC5, FC2, FC6) synchronisation and parietal alpha (P3, P7, Pz, P4, P8) desynchronisation. Power spectrum analyses during learning were conducted with Brain Analyzer software.

During learning, only the WPA task had a sufficient number of stimuli events to perform ERP analyses. After the abovementioned pre-processing steps, time windows −500 to 2500 ms time- locked to the initial appearance of the cue-target pair (time 0) were segmented and then averaged across all stimuli for all EEG channels. Data were then baseline-normalised (−100 to 0 ms). Running *t*-tests from time −100 to 1200 ms were performed (2 ms resolution).

3.7 | Threshold determination for the CLAS during NREM sleep

In brief, data obtained from the adaptation EEG recording session were used for online NREM sleep detection in the experimental sessions. Firstly, an individual threshold value for the prefrontal delta/theta ratio was determined offline. This ratio assisted in selecting online the time periods of NREM sleep. Secondly, an individual delay time for stimulation was determined. The threshold of the delta/theta ratio was obtained by visual comparison of two histograms each showing the distribution of all delta/theta ratio values of all sleep stages, one histogram for NREM (N2 and N3) sleep and another histogram for all other sleep stages (Wake, REM, N1). Analyses to determine the delay from the negative SO half-wave peak to the succeeding depolarising Up state (SO peak) for stimulation were limited to NREM sleep epochs. Offline SO detection was performed with the same algorithm for all offline SO analyses. This algorithm is described in detail further below.

3.8 | Online SO detection and CLAS

The prefrontal (Fpz) signal was processed in real time using a custom-made script running under Spike2 software version 9 (Cambridge Electronic Design, UK) together with a sequencer in the Power1401 mk 2. For online detection of NREM sleep, the delta/theta ratio was calculated from the EEG at Fpz referenced to linked mastoids, filtered between 0.25 Hz and 45 Hz (Digitimer, Hertfordshire, UK) at a sampling rate of 200 Hz. For this calculation, every 0.5 s an FFT was applied to the EEG of the last 15 s and the ratio between the power in the delta band (0.7–3.7 Hz) and the theta band (5–8 Hz) was calculated.

Online SO detection also employed the EEG signal at Fpz referenced to linked mastoids, yet filtered between 0.25 Hz and 4 Hz (Digitimer, Hertfordshire, UK) at a sampling rate of 200 Hz. Similar to Ngo et al. (2013), once the signal surpassed an adaptive amplitude threshold, initially set at −80 μV during NREM sleep trigger initiation began. Actual release occurred however only when an additional second criterion was met: the delta/theta ratio was above the threshold for NREM sleep; then a trigger was sent to play the acoustic stimulus, unless the algorithm was paused for some reason by the experimenter. The adaptive amplitude threshold was updated every 0.5 s based on the EEG amplitude of the preceding 5 s. The stimulation was composed of two pink noise bursts (1/*f*) of 50 ms duration including 5 ms rising and falling times each. The first burst was delivered after the individually determined delay time between the detected negative SO half-wave peak and the succeeding depolarising Up state obtained from the adaption recording session (mean ± SEM: 439.25 ± 21.32 ms), and represents an estimated delay time from SO Down to Up state. The second burst followed after a fixed interval of 1075 ms. SO detection did not resume until 2.5 s after the second click. SO detection continued throughout the nap session, but was halted when the subject left N3 or N2 sleep. Acoustic stimuli were delivered via in-ear head phones. During the Sham session, SOs were detected as described above, and a TTL trigger pulse sent to the first EEG device (LiveAmp, Brain Products GmbH, Gilching, Germany) at the time determined for the acoustic stimuli.

3.9 | Psychometric tests and cognitive ability

Fluid intelligence ability was assessed using the Advanced Progressive Matrices (APM), a language-free matrix task. It assesses the ability to develop, derive or extract new insights from a given clue (Raven & Raven's Progressive Matrices, 1994). Participants were first given 12 test items (Set I) before the actual test started (i.e. Set II, consists of 36 items). Each task entails a 3 × 3 matrix of figures, of which one is blank. The subject is to fill in the matrix blank by choosing one out of eight patterns. Participants were given 30 min to complete the task. The number of correct answers was then transformed into a weighted score for further analysis. MQ was assessed by the German Learning and Memory Test battery (Bäumler, 1974). This test consists of six components that involve memorising a spatial map, word-pairs (Turkish–German), numbers, items, a short story and figures.

Participants first learned all six components and subsequently gave written responses (recall was performed as multiple choice for word-pairs, numbers and figures). Raw scores of these subtests were transformed into weighted *t*-values and again transformed into the general MQ score.

Further psychometric tests were the Positive and Negative Affect Scale (PANAS; Watson et al., 1988), the Psychomotor Vigilance Task (PVT; Roach et al., 2006) and the Stanford Sleepiness Scale (SSS; Hoddes et al., 1972), used to assess subjective mood, motivation, vigilance and feelings of tiredness. The capability to retrieve information from long-term memory expressed in the sense of word fluency, and working memory function using the digit span test (forward and backward version) were measured by the Regensburger task (Aschenbrenner et al., 2000) and the Wechsler Adult Intelligence Scale (Wechsler, 1997), respectively. The German Morningness–Eveningness Questionnaire (D-MEQ) was used to obtain chronotype (Greifahn et al., 2001). Subjects completed the Pittsburgh Sleep Quality Index (PSQI) questionnaire (Buysse et al., 1989) to assess sleep quality prior to the experiment proper.

3.10 | Electrocardiography processing

Electrocardiography (ECG) was recorded from the right arm and anterior axillary line at the level of the fifth intercostal space (V5) from the 12-lead ECG configuration, and amplified between 1 Hz and 25 Hz (LiveAmp, Brain Products GmbH, Gilching, Germany). *R* peaks were detected offline using a modified Pan–Tompkins algorithm (Sathyapriya et al., 2014). The RR signal was calculated as the temporal difference between two consecutive peaks. Intermittent values of the RR signal were determined by shape-preserving, piece-wise cubic interpolation (pchip in Matlab) with a target sampling rate of 100 Hz. Low- and high-frequency components of the RR signal were calculated using 10,001 tap Parks–McClellan band-pass FIR filters in Matlab. The low-frequency component used a pass band ranging from 0.04 Hz to 0.15 Hz with transition bandwidths of 0.03 Hz; the high-frequency component used corresponding values of 0.15 Hz and 0.4 Hz with transition bandwidths of 0.03 Hz.

3.11 | Statistical analysis

Statistical analyses on behaviour, EEG power during encoding, and NREM sleep and on spindle events were computed using SPSS (IBM SPSS Statistic, version 22.0, IBM, Armonk, NY, USA). For all learning tasks, two-factor repeated-measure (rm) ANOVAs with the ANOVA factors Condition (Cond) and Trial number (Trial) and post hoc tests were applied. Retention performance was investigated by rmANOVA with the factors Cond and Time (Learning, Delayed recall) and post hoc tests. EEG analyses employed rmANOVAS with the factors Cond and Topography (Topo). Time-locked SO and spindles RMS were compared between conditions using a running paired-sample *t*-test (uncorrected for multiple comparisons). Rm ANOVA (with factors Cond and Time) and post hoc tests were similarly applied to all psychometric measurements. Post hoc tests were conventionally conducted with *t*-tests, yet when data were not normally distributed in smaller subgroup samples Wilcoxon tests were applied. To investigate the impact of cognitive ability on CLAS, APM and MQ scores were each entered as a covariate in the rmANOVA. ECG power in low- and high-frequency bands between conditions were compared by a paired non-parametric test. All correlations were conducted using Pearson tests. All values are given in mean ± SEM.

4 | RESULTS

4.1 | Sample characteristics and psychometric measurements

Data of 16 participants were included in all analyses. MQ scores and fluid intelligence scores were 101.06 ± 4.81 (range: 70–128) and 109.44 ± 1.80 (range: 98–122), respectively. Psychometric measurements neither on sleepiness, affection nor psychomotor vigilance differed significantly between conditions (Table SI1). Participants were generally good sleepers, as confirmed by the PSQI (4.06 ± 0.54).

4.2 | Polysomnography

Participants arrived slightly sleep-deprived on both experimental days. Their total nocturnal sleep durations as assessed by actigraph were 287.53 ± 25.94 min before the Sham and 281.16 ± 14.71 min before the CLAS session ($p > 0.7$). During the nap, in CLAS, participants spent significantly more time in NREM sleep compared with Sham ($p = 0.04$), mostly at the cost of REM sleep. No other significant differences were found for any other sleep stage or latency (Table 1).

4.3 | Learning performance

The factor Trial was significant for all three behavioural tasks, indicating the expected increment in learning with repetition (each, $p < 0.05$). Yet, across all subjects, neither average nor final learning performance were affected by CLAS (WPA task, $p > 0.5$; FPA task, $p > 0.1$; VLMT, $p > 0.8$; Tables 2 and SI2). When MQ score was considered as covariate in the rmANOVA model, a significant interaction Cond × Trial × MQ for learning performance on the WPA task was revealed ($F_{4,56} = 3.61$, $p = 0.0119$). The covariate MQ exerted a significant effect on learning ($F_{1,149} = 15.23$, $p = 0.002$; as clearly seen in Figure 2), and different dynamics for the low- and high-MQ groups were observed (Cond × Trial × MQ_mediansplit, $F_{4,56} = 3.74$, $p = 0.009$). This effect was attributed to a poorer learning performance of the low-MQ group to stimulation ($F_{4,28} = 5.317$, $p = 0.003$ for Cond × Trial), which, however, at the individual trial level only reached a trend for trial 4 ($\text{Learning}_{CLAS} = 30.13 \pm 6.26$; $\text{Learning}_{Sham} = 33.73 \pm 5.15$; $Z = -1.680$, $p = 0.093$; see Figure S1 for corresponding correlations with the covariate). Values and statistical paired comparisons for learning and retention performance of all tasks across all subjects are given in

TABLE 1 Polysomnography

Measurements	Sham	CLAS	*t* (15)	*p*-Value
TST (min)	103.88 ± 3.14	105.50 ± 2.85	−0.51	0.62
TIB (min)	116.50 ± 3.10	116.66 ± 2.68	−0.05	0.96
Sleep onset (min)	4.85 ± 0.81	4.72 ± 0.85	0.16	0.88
REM onset (min)	56.00 ± 7.28	54.5 ± 8.82	0.13)	0.90
Wake (%)	5.84 ± 1.75	4.40 ± 1.28	0.76	0.46
N1 (%)	7.94 ± 1.22	8.2 ± 1.31	−0.18	0.86
N2 (%)	49.42 ± 3.30	52.16 ± 3.65	−0.82	0.43
N3 (%)	23.72 ± 3.43	26.96 ± 3.59	−1.00	0.33
REM (%)	12.62 ± 2.89	7.71 ± 1.79	1.82	0.09
NREM (%)	73.15 ± 2.61	79.12 ± 1.57	−2.23	0.04*
Arousal (%)	2.04 ± 0.57	2.25 ± 0.52	−0.26	0.80
Sleep efficiency (%)	89.37 ± 1.97	90.5 ± 1.46	−0.61	0.55

Time spent in NREM sleep differed slightly between low- and high-MQ subject groups during Sham, with high-MQ subjects (78.97% ± 3.68%) displaying more NREM sleep than low-MQ subjects (67.32% ± 2.46%; $Z = -1.995$, $p = 0.046$). Moreover, only the MQ subject group revealed a difference between CLAS and Sham ($p < 0.03$).
CLAS, closed-loop acoustic stimulation; NREM, non-rapid eye movement; REM, rapid eye movement; Sleep efficiency, amount of time spent asleep relative to the total amount of time in bed, in percent; Sleep onset, first epoch of any stage other than W, typically stage N1; TIB, time in bed; TST, total sleep time.
Percentage in W, N1–N3 and REM sleep, arousal is relative to TST, *$p < 0.05$, paired-sample *t*-test, for comparisons between conditions; uncorrected for multiple comparisons ($N = 16$). Mean ± SEM.

TABLE 2 Behavioural performance

Tasks	Sham	CLAS	*t* (1)	*p*-Value
WPA learning (%)	47.03 ± 5.50	45.15 ± 5.45	−0.92	0.37
WPA retention (%)	46.64 ± 5.07	51.51 ± 5.77	−1.03	0.32
FPA learning (%)	58.01 ± 5.11	57.42 ± 4.84	−0.16	0.88
FPA retention (%)	11.06 ± 11.04	−11.84 ± 11.16	1.60	0.13
VLMT learning (%)	78.19 ± 3.62	78.47 ± 3.19	0.10	0.92
VLMT interference	54.58 ± 4.05	56.67 ± 5.02	0.43	0.67
VLMT delayed recall	86.25 ± 4.66	87.08 5 ± 3.52	0.18	0.86

Behavioural performance on the WPA and FPA tasks as well as on the VLMT as percentage of the maximal amount of stimulus material. Learning performance represents the mean percentage of correct responses during the learning trials. Learning performance: [Mean of correct responses/Total number of trials] × 100; Retention performance: ([Delayed recall − Learning performance]/[Learning performance] × 100). Learning performance on the final trial is given in Table SI2. Mean ± SEM.
CLAS, closed-loop acoustic stimulation; FPA, figural paired-associate; VLMT, verbal learning and memory test; WPA, word paired-associate.

Tables 2 and 3. High- and low-MQ subjects did not differ in age, or the other cognitive ability measure ($p > 0.1$), but compare Table 1 for deviations in polysomnography.

4.4 | CLAS modified the EEG theta/alpha power during learning ratio, but not the ERP to stimulus onset

The theta/alpha power ratio during the WPA and VLMT learning tasks was significantly larger after Stimulation than Sham during learning (WPA: $t_{14} = -4.32$, $p = 0.001$; VLMT: $t_{14} = -3.23$, $p = 0.006$), yet there was no condition effect for the FPA task ($t_{14} = 0.52$, $p = 0.61$). The ratio was increased in the stimulation condition (Figure 3). No other frequency bands differed in power between conditions. The ERP to the presentation of cue words in the WPA to which the correct target was associated resulted in prominent N1, P2, N2, P3 and N4 components (Figure 4), but no component or time point of the ERP waveform was affected by CLAS ($p > 0.05$).

4.5 | CLAS augmented time-locked SO and spindle activity during NREM sleep

Despite a paucity of cognitive effects, across all subjects CLAS successfully augmented the EEG signal time-locked to the first acoustic stimulus during NREM sleep: two SO negative and positive half-waves

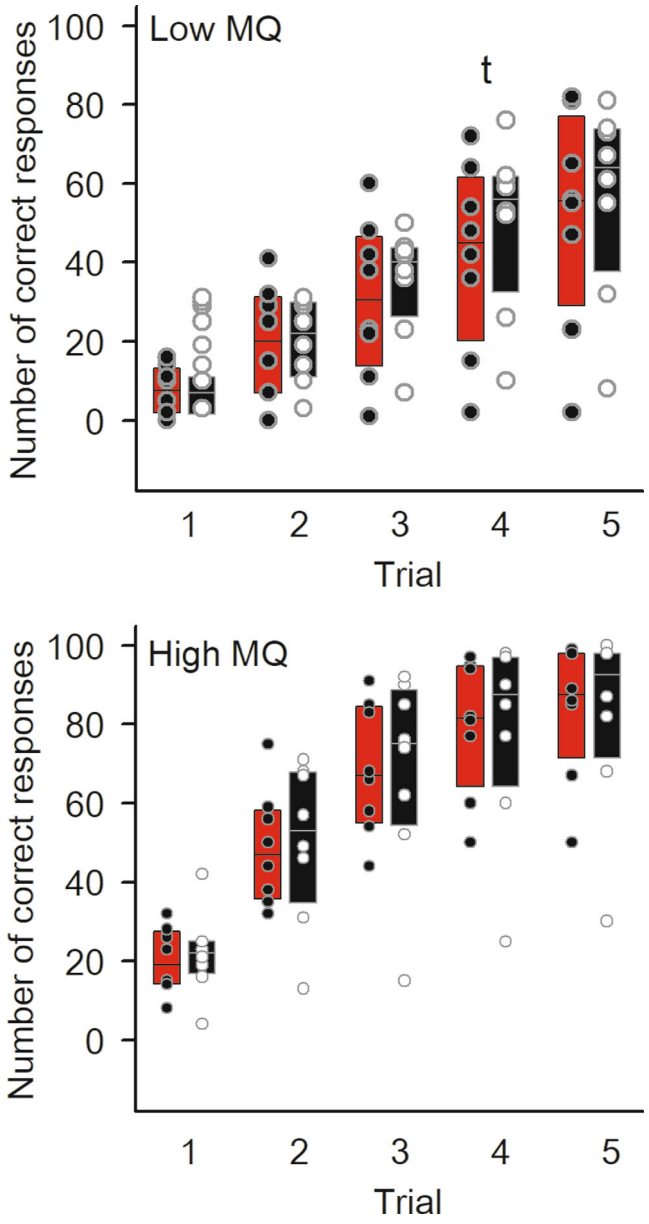

FIGURE 2 Learning performance on the word paired-associate (WPA) task for subjects with low- and high-memory quotient (MQ) scores. The low-MQ group performed slightly worse in closed-loop acoustic stimulation (CLAS) than the Sham group, which at the level of pair-wise comparisons was indicated at trial 4 (t, $p = 0.093$, $N = 8$). Results remained in the same direction after removing two outliers where their performance was very poor (< 20% of mean learning rate). Red and black bars represent CLAS and Sham, respectively

putatively reflecting SO Down and Up states, respectively, were induced (Figure 5, top). Fast spindle RMS power commencing at the SO Down-to-Up transition subsequent to the first stimulus was prominently enhanced. Slow spindle RMS commenced already on delivery of the first stimulation with the SO Up-to-Down transition. Increased values before $t = 0$ ms are likely due to the 200-ms-wide RMS window, but may also reflect a difference in slow spindle RMS already at the time of baseline normalisation (Figure 5). We next investigated for any differential responses on rhythmic brain activity to CLAS between low- and high-MQ subject groups (Figure 6). The mean time-locked SO response to CLAS was stronger in low-MQ with the differences during the hyperpolarising SO down states reaching significance. Significant differences in the response magnitude to CLAS were also observed for slow spindle RMS (Figure S2).

4.6 | Effects of CLAS on discrete spindles and relationship to MQ

The number of discrete fast spindles was significantly influenced by CLAS, as revealed by the main effect of condition (Cond: $F_{1,15} = 4.59$, $p = 0.049$, rmANOVA, over all topographies). Introducing MQ score as covariate revealed a significant effect of MQ, especially at parietal sites P3, P4, Pz ($F_{1,149} = 6.227$, $p = 0.026$ for Cond × MQ_median-split). The low-MQ group was affected by CLAS, revealing a higher number of fast spindles in CLAS than in Sham (329.3 ± 18.1 versus 275.7 ± 20.7; $Z = -2.521$, $p = 0.012$), whereas no difference was observed for the high-MQ group (275.4 ± 18.1 versus 272.9 ± 20.7; $Z = -0.28$, $p > 0.7$). The correlation of stimulation efficacy on parietal fast spindle count (count in Stimulation minus count in Sham) with MQ score reached significance ($r = -0.501$, $p = 0.048$; Figure SI3). No other discrete spindle parameters (i.e. density, length, peak to peak amplitude, RMS) were modified by CLAS. Interestingly, for frontal slow spindles throughout NREM sleep, the exploratory introduction of MQ as covariate in the rmANOVA revealed a strong dependence of density and count on MQ ($F_{1,14} \geq 8.040$, $p \leq 0.13$) independent of condition. Both parameters correlated negatively with MQ ($r \leq -|0.509|$; $p \leq 0.044$; Figure SI4).

4.7 | CLAS did not modify EEG power across the total sleep period

Although CLAS enhanced time-locked EEG activity, EEG power throughout NREM sleep was not significantly modified during any of the analysed frequency bands or at peak frequencies, as revealed by rmANOVA ($p > 0.14$; Table 4). Figure 7 indicates that NREM sleep of low MQ was affected by CLAS between slow and fast spindle frequency ranges and also around upper delta frequency.

4.8 | CLAS increased low-frequency but not high-frequency component of heart rate variability in N3 sleep

The low-frequency component was higher in CLAS (0.007 ± 0.001 ms^2 Hz^{-1}) than Sham (0.005 ± 0.001 ms^2 Hz^{-1}; $Z = -2.06$, $p = 0.039$) in N3 sleep, revealed by non-parametric Wilcoxon test ($N = 13$). The high-frequency component is, however, not significantly modified by CLAS.

TABLE 3 Number of acoustic stimuli

	Sham			CLAS		
	NREM	N2	N3	NREM	N2	N3
Sum	1966	277	1689	1615	144	1471
Mean	122.9 ± 17.7	17.3 ± 7.8	105.6 ± 19.1	100.9 ± 15.4	9.0 ± 3.6	91.9 ± 17.0
Low	139.5 ± 19.5	27.3 ± 15.1	112.3 ± 23.6	96.0 ± 18.2	15.6 ± 6.4	80.4 ± 22.1
High	106.3 ± 29.6	7.4 ± 2.8	98.9 ± 31.5	105.9 ± 25.9	2.4 ± 1.2	103.5 ± 26.7

Total number of acoustic stimuli delivered to each subject in N2, N3 or NREM sleep (top). Mean ± SEM number of stimuli delivered to subjects with low- and high-MQ score ($N = 8$ for each group), respectively.
CLAS, closed-loop acoustic stimulation; NREM, non-rapid eye movement.

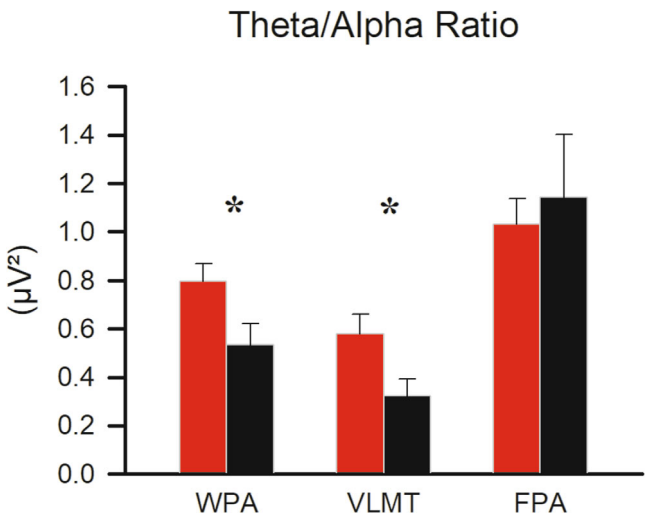

FIGURE 3 Theta/alpha ratio during learning. Closed-loop acoustic stimulation (CLAS) significantly increased the frontocentral theta/parietal alpha electroencephalogram (EEG) power ratio during learning for word paired-associate (WPA) task and verbal learning and memory task (VLMT). Red and black bars represent CLAS and Sham conditions, respectively. $N = 16$; $^*p < 0.05$

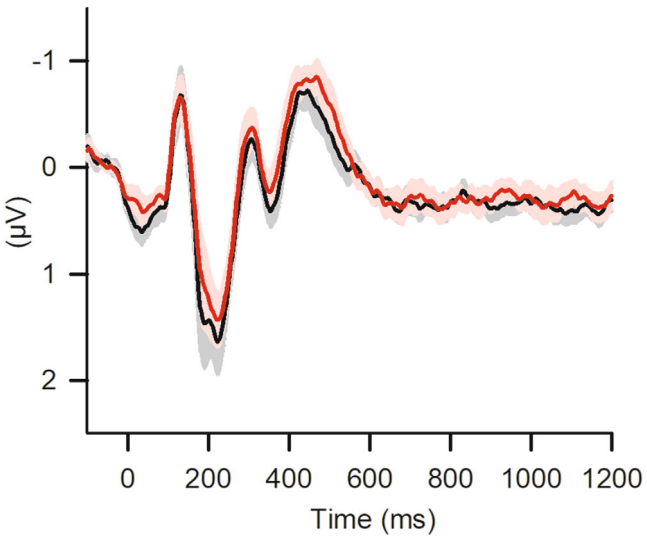

FIGURE 4 Event-related potential (ERP) of word paired-associate (WPA) learning (Cz). There was no significant difference between conditions. Red and black lines represent closed-loop acoustic stimulation (CLAS) and Sham, respectively. There was no significant difference between condition ($N = 15$). Due to a technical problem, the electroencephalogram (EEG) data of one subject during learning are missing

5 | DISCUSSION

This study applied CLAS during an afternoon nap to investigate effects on subsequent encoding capacity. In agreement with other studies, CLAS successfully boosted and induced positive and negative EEG waves corresponding to successive depolarising SO Up and hyperpolarising SO Down states, as well as time-locked spindle activity (Ngo et al., 2013; Ngo et al., 2015; Ong et al., 2016). During subsequent learning on two of the tasks ongoing EEG frontocentral theta/parietal alpha power ratio was increased, indicating differential post-sleep processing between CLAS and Sham. Frontal EEG theta is linked to working memory, episodic encoding and retrieval (Hsieh & Ranganath, 2014; Jensen & Tesche, 2002), and is often found in concert with EEG alpha suppression (Trammell et al., 2017; Woodman et al., 2022). Nonetheless, as in a previous study employing CLAS during a nap, but with a different closed-loop algorithm, overall no effect on post-sleep encoding performance was found (Ong et al., 2016).

Interestingly, a previous study that enhanced SWA using another non-invasive brain stimulation procedure, anodal slow oscillation transcranial direct current stimulation (so-tDCS), was successful in significantly enhancing post-sleep learning of hippocampus-dependent tasks (Antonenko et al., 2013), yet only an indirect comparison is warranted. Firstly, SO CLAS and so-tDCS presumably differ in their basic mechanisms. CLAS has been hypothesized to interact with the non-lemniscal pathway, a pathway that involves thalamic matrix cells projecting to cortical layer I, thereby synchronisation of large cortical populations and generating slow waves (Bellesi et al., 2014), whereby its efficiency is suggested to rely on a fine balance between activation of this non-lemniscal pathway and the arousal-related brainstem lemniscal pathway (Grimaldi et al., 2019). On the other hand, effects of so-tDCS, especially the direct current potential component, rather than activating specific pathways, affect underlying cellular activity in a field-strength-specific manner (Bikson et al., 2013). The oscillatory component of so-tDCS may act similar to transcranial alternating

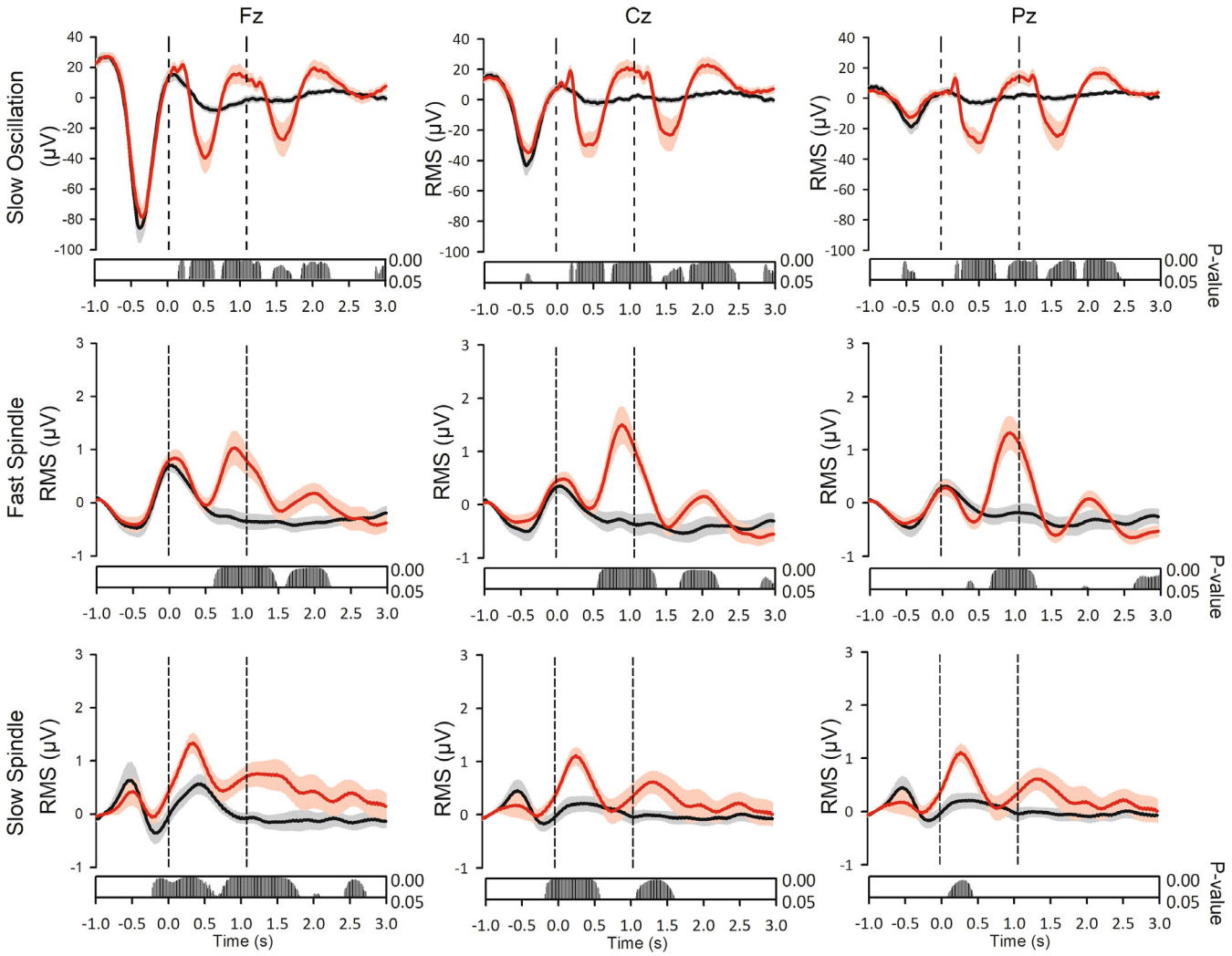

FIGURE 5 Effect of closed-loop acoustic stimulation (CLAS) on slow oscillation (SO), fast spindle and slow spindle activity; left, middle and right columns correspond to Fz, Cz and Pz, respectively. First, middle and bottom rows represent SO, fast spindle and slow spindle magnitude, respectively. Dashed lines indicate times of first and second acoustic stimulus delivery. For slow and fast spindles root mean square (RMS), baseline normalisation was from −1000 to −800 ms. Shaded areas indicate SEM. Bottom diagrams: black bars represent *p*-values for the difference between Sham and CLAS, paired *t*-test. $N = 15$, the detection of one electrode failed in the Sham condition

current stimulation and phase shift neuronal firing relative to an oscillation. Indeed, a recent study revealed that efficiency of entrainment to transcranial alternating current depended both on neuronal entrainment to the ongoing physiological oscillation and the induced electric field (Krause et al., 2022). Thus, although both non-invasive stimulation protocols can in principle affect ongoing SO, spindle activity and at least indirectly hippocampal activity (Ong 2018, cp. van der Werf 2009 for hippocampal effects of shallow sleep), disparate effects on putative synaptic downscaling function are not surprising. Although memory consolidation is not the focus of this study, differential effects of the above-mentioned stimulation approaches on reactivation processes of memory consolidation are similarly presumed.

In fact, although CLAS during NREM sleep has been shown to enhance hippocampus-dependent memory consolidation (Ngo et al., 2013; Ong et al., 2016) and/or hippocampal activation (Ong et al., 2018), when not exclusively (Henin et al., 2019), it may indeed prove suboptimal to boost putative synaptic downscaling mechanisms. A closer comparison between the data of Ong et al. (2018), ours and those of Antonenko et al. (2013) reveals that the latter boosted SWA, but failed to significantly facilitate spindle power. Also, nap duration in the present study was longer than in the study by Antoneko (116 min versus 77 min). Because a nap per se restores hippocampal function (Mander et al., 2011; Ong et al., 2016), a putative effect of stimulation may be less pronounced relative to a control of longer sleep duration. Finally, Mander et al. (2011) only found a positive correlation for spindle activity on post-sleep learning in an evening nap. Thus, it should be borne in mind that the functions of brain oscillations most likely also interact and/or are influenced by circadian and homeostatic factors. Along these lines, it remains to be investigated whether CLAS during a full nocturnal sleep period given when endogenous sleep pressure is maximum and enabling a facilitatory contribution to REM sleep (Niethard & Born, 2019) may have yielded different results.

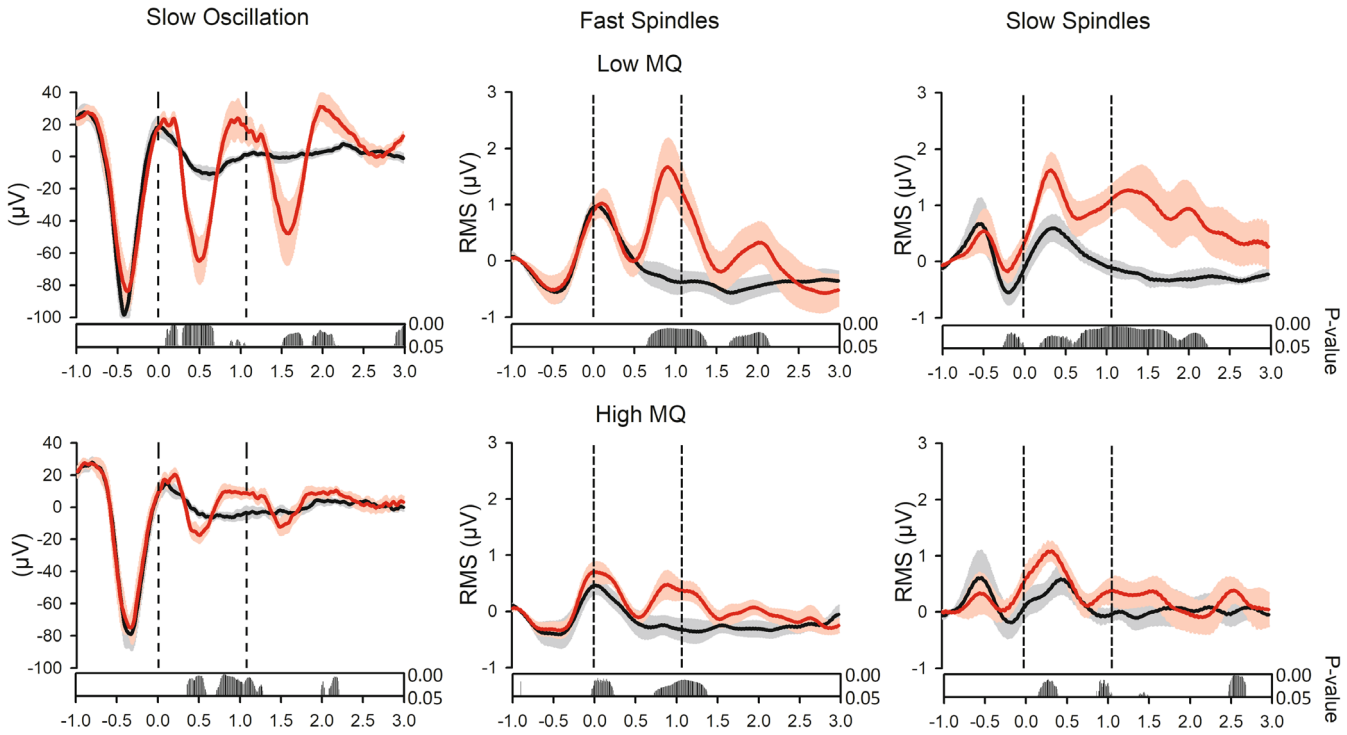

FIGURE 6 Effect of closed-loop acoustic stimulation (CLAS) on slow oscillation (SO), fast spindle and slow spindle for subjects with low- and high-memory quotient (MQ) scores. When we split the MQ into low and high groups, the low-MQ group ($N = 7$) showed a significantly stronger CLAS response in SO (0.3–0.7 s; 1.6–1.8 s) and slow spindles (Figure S2) compared with the high-MQ group ($N = 8$). Differences in fast spindles remained non-significant. Red and black lines correspond to CLAS and Sham, respectively. Shaded area indicates SEM. Bottom diagrams: black bars represent *p*-values for the difference between Sham and CLAS, paired *t*-test

TABLE 4 EEG power during NREM sleep

Frequency	Topography	Sham	CLAS	*t* (15)	*p*-Value
SO	Frontal	25.98 ± 3.53	31.80 ± 4.80	−1.05	0.31
	Central	18.94 ± 2.37	23.77 ± 4.00	−1.09	0.30
	Parietal	11.40 ± 1.74	13.18 ± 1.99	−0.70	0.49
SWA	Frontal	10.30 ± 1.32	12.35 ± 1.76	−1.08	0.30
	Central	8.22 ± 0.92	9.82 ± 1.37	−1.08	0.30
	Parietal	4.57 ± 0.61	5.15 ± 0.67	−0.73	0.48
Theta	Frontal	0.82 ± 0.09	0.88 ± 0.15	−0.80	0.44
	Central	0.90 ± 0.10	0.94 ± 0.14	−0.65	0.52
	Parietal	0.54 ± 0.06	0.59 ± 0.09	−0.95	0.36
Sspi	Frontal	0.39 ± 0.04	0.38 ± 0.04	0.23	0.82
	Central	0.37 ± 0.04	0.36 ± 0.04	0.21	0.84
	Parietal	0.23 ± 0.02	0.24 ± 0.03	−0.14	0.89
Fspi	Frontal	0.19 ± 0.02	0.18 ± 0.02	0.40	0.70
	Central	0.27 ± 0.04	0.24 ± 0.03	0.63	0.54
	Parietal	0.19 ± 0.03	0.17 ± 0.02	0.73	0.48
	Frontal	0.11 ± 0.01	0.10 ± 0.01	−1.30	0.21
Beta	Central	0.15 ± 0.02	0.13 ± 0.01	−1.52	0.15
	Parietal	0.10 ± 0.01	0.09 ± 0.01	−1.34	0.20

There was neither significant difference between conditions while using single electrodes in rmANOVA nor using pooled regions: frontal, central and parietal.
CLAS, closed-loop acoustic stimulation; Fspi, fast spindles; SO, slow oscillation; Sspi, slow spindles; SWA, slow-wave activity.

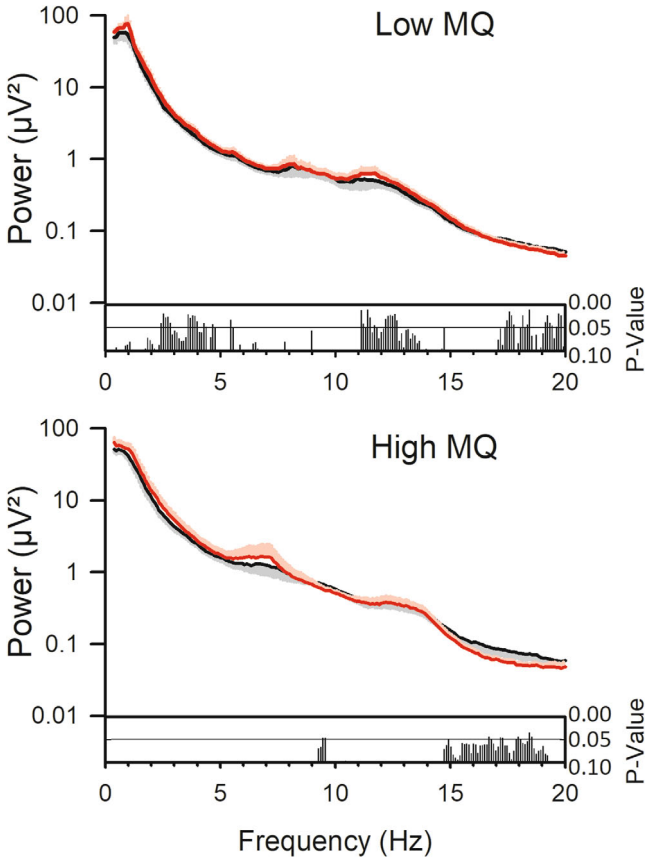

FIGURE 7 Power spectral analyses for high- and low-memory quotient (MQ) subject groups. The low-MQ group ($N = 7$) showed higher electroencephalogram (EEG) power in closed-loop acoustic stimulation (CLAS) than the Sham group within the upper delta and fast spindle frequency ranges. Red and black lines correspond to CLAS and Sham, respectively. Shaded area indicates positive or negative SEM, respectively. Spectra are from Fz. Bottom graphs represent *p*-values

A previous study of ours indicated that MQ, a measure of cognitive ability, modulated the effect of non-invasive brain stimulation on memory consolidation. Individuals with higher MQ benefitted more from stimulation on consolidation of a FPA task (Koo et al., 2018). Investigations for an influence of cognitive ability on the time-locked response to CLAS are apparent in this study. Interestingly, the subject group tending to respond to CLAS, an impairing tendency of CLAS on WPA learning, also displayed CLAS-induced hyperpolarising SO Down states of increased magnitude, and appeared to have increased parietal spindle counts with stimulation. This triad between spindles, MQ as a trait-like feature and learning as a neuroplastic phenomenon presumably possesses unknown and here uninvestigated physiological bridges. Investigations on synaptic downscaling include effects of local versus global spindles and SOs, and subsequently involve the magnitude and phase of SO–spindle coupling (Maier et al., 2019; Niethard & Born, 2019). The above triad has been more intensively investigated in the context of memory consolidation (Ackermann et al., 2015; Denis et al., 2021; Fang et al., 2020; Lustenberger et al., 2015). The correlation between discrete slow frontal spindle activity during NREM sleep and MQ scores is consistent with trait-like features previously attributed to slow spindle (Verleger et al., 2013; Yordanova et al., 2017). Future carefully planned large studies are required to validate any associations with a potential disruption in down scaling, a much larger sample size than offered by the present study. Great care for cognitive tasks is hereby not trivial as in the present data a bias may have been introduced due to a ceiling effect into the learning performance of the high-MQ subject group.

Interests in interactions between the central and autonomic nervous systems are long standing. Thus, a logical consequence is to investigate the effects of CLAS on autonomic function. In contrast to a previous finding in which CLAS led to a reduction of the so-called low-frequency and an increase in the high-frequency ECG component (Grimaldi et al., 2019), we found an increase of the low-frequency component by CLAS. The high-frequency component was not changed. This distinction may be explained by the different sleep durations and timing. Firstly, in the aforementioned study, a change in the high-frequency component was not found until the second and third sleep cycles. Secondly, they investigated nocturnal sleep so that a circadian effect on responsiveness to CLAS of the autonomic nervous system cannot be ruled out (Massin et al., 2000; Vitale et al., 2019).

From another perspective, it has been suggested that due to synaptic pruning (Li et al., 2017), REM sleep may contribute to global downscaling (Niethard et al., 2017). Interestingly, slightly increased N3 in our study went at the cost of a tendency toward reduced REM sleep in the CLAS condition. Thus, it would be interesting to investigate whether non-invasive brain stimulation targeted on enhancing theta rhythm of REM sleep may affect subsequent learning.

In conclusion, CLAS applied during an afternoon nap enhanced SO and spindle activity, but had no beneficial function on post-sleep encoding performance of associative hippocampus-dependent tasks. We speculate that the effect on spindle activity in the present study using CLAS as compared with non-invasive slowly oscillating electric stimulation, which impacts more directly endogenous SWA, may underlie the failure to find facilitated learning performance subsequent to an afternoon nap. Most importantly, the present study underscores that non-invasive stimulation can be used to probe neural network interactions.

AUTHOR CONTRIBUTIONS

This study was designed by Ping Koo-Poeggel, Matthias Mölle, Thomas Martinetz and Lisa Marshall; Soé Neuwerk and Ping Koo-Poeggel conducted the experiments. Ping Koo-Poeggel, Eike Petersen, Jan Grasshoff, Matthias Mölle and Lisa Marshall were involved in data analyses and statistics. Ping Koo-Poeggel and Lisa Marshall wrote the main portion of the manuscript, all authors contributed to the manuscript.

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DATA AVAILABILITY STATEMENT

Data available on reasonable request from the authors.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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