**Phase-locked Auditory Stimulation of Theta Oscillations during** **Rapid Eye Movement Sleep**

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**Abstract**

Auditory closed-loop stimulation is a non-invasive technique that has been widely used to augment slow oscillations during non-rapid eye movement sleep. Based on the principles of closed-loop stimulation, we developed a novel protocol for manipulating theta activity (3-7 Hz) in rapid eye movement (REM) sleep. Sixteen healthy young adults were studied in two overnight conditions: Stimulation and Sham. In the Stimulation condition, 1 s of 5 Hz amplitude-modulated white noise was delivered upon detection of two supra-threshold theta cycles throughout REM sleep. In the Sham condition, corresponding time points were marked but no stimulation was delivered. Auditory stimulation entrained EEG activity to 5 Hz and evoked a brief (~0.5 s) increase in theta power. Interestingly, this initial theta surge was immediately followed by a prolonged (~3 s) period of theta suppression. Stimulation also induced a prolonged (~2 s) increase in beta power. Our results provide the first demonstration that the REM sleep theta rhythm can be manipulated in a targeted manner via auditory stimulation. Accordingly, auditory stimulation might offer a fruitful avenue for investigating REM sleep electrophysiology and its relationship to behaviour.

**Keywords:** Auditory stimulation; theta; rapid eye movement sleep

**Significance Statement**

Auditory stimuli have been used to enhance EEG slow oscillations, a hallmark feature of non-rapid eye movement sleep. Whether auditory stimuli can also be used to enhance oscillatory activity during rapid eye movement (REM) sleep, however, is unknown. We employed a novel auditory stimulation protocol to manipulate the REM sleep theta rhythm. Stimulation entrained EEG activity to the theta frequency (5 Hz), and modulated EEG power in both the theta and beta frequency bands. These findings suggest that auditory stimulation might provide an effective tool for studying the physiology and functions of REM sleep.

**Introduction**

Human sleep can be broadly classified into two distinct stages: non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep. The electroencephalogram (EEG) of deep NREM sleep (stage N3) is characterised by the occurrence of high amplitude slow oscillations (SOs; <1 Hz) and thalamocortical spindles (~11–16 Hz) [1,2]. Researchers have taken considerable interest in manipulating NREM sleep electrophysiology, and have employed a variety of techniques to stimulate SOs, spindles, and associated memory processes [3–21; for review see 22]. While the sleep engineering literature has grown rapidly in recent years, efforts have thus far been directed solely at NREM sleep. Accordingly, it is unknown whether oscillatory activity during REM sleep can also be manipulated in a targeted manner.

The REM sleep EEG is composed of irregular, high frequency and low amplitude activity; similar to that of wakefulness [23,24]. Scattered amongst these features are periods of 3–7 Hz theta activity [25,26]. Although theta oscillations are a less prominent feature of REM sleep than the SOs that characterise N3, they might occur with enough regularity to be malleable via sleep engineering.

Studies designed to boost NREM SOs have used transcranial direct current stimulation (tDCS) [15,16], transcranial magnetic stimulation [17], and sensory stimulation such as acoustic tones [18–20]. Similarly, in bids to boost NREM spindles, researchers have employed transcranial alternating current stimulation [4], oscillating sounds [14], and other sensory stimuli [27]. These studies were largely successful in manipulating NREM oscillations, but some have been criticised for disregarding the endogenous oscillatory activity generated by the sleeping brain, and imposing oscillations that are not necessarily synchronised to the brain’s natural rhythm.

More recently, a seminal technique known as auditory closed-loop stimulation has proven effective in enhancing endogenously-generated SOs [21]. By monitoring EEG activity in real time, closed-loop stimulation delivers auditory clicks in phase with the positive peaks of algorithmically-detected SOs. These clicks lengthen ongoing “trains” of SO cycles and heighten SO amplitude, thereby enhancing the SO rhythm [5–12,21].

Although human studies have used auditory stimuli in REM sleep to a) prolong REM sleep duration [28–30], and b) cue memory replay (i.e. targeted memory reactivation) [31–35], closed-loop stimulation techniques have not, to our knowledge, been employed to target specific oscillatory phenomena during this sleep stage. In the present study, we sought to manipulate theta oscillations during REM sleep using a technique modelled on the principles of auditory closed-loop stimulation [21]. Specifically, we delivered 1 s of 5 Hz amplitude-modulated white noise upon algorithmic-detection of heightened theta activity.

Interestingly, theta activity in REM sleep has been linked to the retention of memories for emotionally aversive experiences (so-called “emotional memories”) [36,37; also see 38]. It has been suggested that REM sleep theta oscillations promote the consolidation of emotional memories while concurrently downregulating their affective tone [39]. Accordingly, we also examined the impact of auditory theta stimulation on the retention and affective evaluation of emotional memories.

**Methods**

**Participants**

Sixteen healthy adults (1 male; age: M=20.06, SD=2.02 years) entered a within-subjects crossover design. Participants had no history of neurological, psychiatric, or sleep disorders, maintained a regular sleep/wake pattern, were free of psychoactive medication, were non-smokers, and agreed to abstain from alcohol and caffeine for 24 h prior to each experimental session. Wristwatch actigraphy (Actiwatch, Philips Respironics, USA) was used to ensure that participants awoke by 08:30 on the morning of each session and did not nap during the day. Written informed consent was obtained in line with the requirements of the University of York’s Department of Psychology Research Ethics Committee, who approved the study.

**Procedure**

Participants were tested in two experimental conditions: Stimulation and Sham. Conditions were separated by one week and the order was counterbalanced. Other than auditory stimulation in REM sleep, the conditions followed identical procedures. Participants arrived at 20:00 and electrodes were attached for overnight polysomnographic recording. They then completed the first part of an emotional memory task before going to bed at 23:00. In the Stimulation condition, an algorithm for theta detection and auditory stimulation was activated by a researcher whenever participants exhibited REM sleep, and deactivated when REM sleep ceased. In the Sham condition, theta detection was initiated during REM sleep, but no auditory stimuli were delivered; although points at which stimulation would have taken place were marked in the EEG recording. Participants were awoken the next morning at 07:00, and electrodes were removed. After breakfast, participants completed the second part of the emotional memory task.

 Participants completed an adaptation night at least one week before the first experimental condition, where they slept in the laboratory between 23:00 and 07:00. Polysomnographic sleep recording was carried out using the same procedures as the experimental conditions. The adaptation night was used to familiarise participants with the laboratory environment and to collect the EEG data necessary to adapt the theta detection algorithm to the individual theta characteristics of each participant.

**Polysomnographic recording**

Sleep monitoring was carried out using an Embla N7000 polysomnography system with RemLogic version 3.4 software (Embla Systems, Broomfield, CO, USA). Gold-plated electrodes were attached using EC2 electrode cream (Grass Technologies) after the scalp was cleaned with NuPrep exfoliating agent (Weave and Company). Scalp electrodes were attached at 11 locations in accordance with the International 10-20 System [40]: F3, F4, FCz, C3, C4, T3, T4, P3, P4, O1, and O2; each referenced to the linked mastoids. The electrooculogram and electromyogram were also recorded for online and offline sleep scoring. All electrodes were verified to have a connection impedance of <5k Ω. All signals were digitally sampled at a rate of 200 Hz.

**Online detection of theta oscillations and phase-locked auditory stimulation**

EEG data from the adaptation night was scored as REM or NREM (N1, N2 or N3) sleep in accordance with standardised criteria [41]. From the REM sleep EEG data, each participant’s peak theta frequency was extracted from channel FCz, and this frequency ±1.5 Hz was used to create participant-specific theta bands for online detection of theta oscillations (e.g. peak theta frequency = 6 Hz, theta band = 4.5–7.5 Hz). Channel FCz was selected because REM sleep theta power is typically maximal at the fronto-central midline [42,43]. On experimental nights, each participant’s EEG data (channel FCz) was band-pass filtered within their individual theta band using a separate EEG system (Digitimer D360 EEG amplifier and Cambridge Electronic Design Micro1401-3 data acquisition unit). Theta detection thresholds were calculated as the 75th percentile of the band-pass filtered signal (see Table S1 for individual theta detection parameters). In the Stimulation condition, auditory stimulation was initiated upon algorithmic-detection of two supra-threshold theta cycles (Spike2 version 8.17, Cambridge Electronic Design, Cambridge, UK; Figure 1A). More specifically, stimulation could only occur when the local maximum in the filtered signal was larger than the theta detection threshold, and was followed by one local minimum and a second local maximum that both also exceeded the detection threshold within 250 ms (corresponding to a lower boundary of 4 Hz). TTL triggers were sent with each stimulation to allow for event-related EEG analyses. Theta detection was paused for 5 s after each stimulation event. The Sham condition followed identical procedures with the exception that no auditory stimuli were delivered.

The detection algorithm was activated whenever participants exhibited REM sleep (at any point in the night), but paused when they transitioned into a different sleep stage or wakefulness, or showed signs of arousal. Offline analysis showed that the algorithm was mostly activated during REM sleep. Across both conditions, a mean of 480.43 [SEM=30.49] theta detections occurred [Stimulation condition: M=485.27, SEM=35.67; Sham condition: M=475.60, SEM=49.42; *t*(14)=0.20, *p*=.844], and 87.95% [SEM=1.67%] of these occurred during REM sleep [Stimulation condition: M=85.15%, SEM=2.49%; Sham condition: M=90.75%, SEM=2.06%; *t*(14)=1.59, *p*=.134].

*Auditory stimulus*

A 1 s stimulus of 5 Hz amplitude modulated white noise was presented binaurally via in-ear headphones (Sony MDR-EX110, Japan) at a volume of ~45 dB. To mitigate the potential for arousals in response to unexpected auditory stimuli, participants were advised that stimulation would occur during both experimental nights (Stimulation and Sham). Seven of the 16 participants reported some awareness of the auditory stimuli during the Stimulation night. None reported awareness of the stimuli during the Sham night.

*Data analysis*

EEG analyses were restricted to REM sleep epochs (scored using standardised criteria [41]). Because of a technical fault, TTL triggers were unavailable for one participant. Hence, EEG analyses were performed on the remaining 15 participants (1 male; age: M=20.20, SD=2.01 years).

 Artefacts were visually rejected, which led to an average of 3.60 (SEM=0.46) min of data being excluded [Stimulation: M=3.06, SEM=0.39; Sham: M=4.21, SEM=0.82; t(14)=1.28, p=.222]. EEG channels were then band-pass filtered between 0.3 and 30 Hz. ERPs time-locked to stimulation onset (-1 to 5 s time window) were high-pass filtered at 3 Hz, facilitating visualisation of the effects of stimulation in the theta range. See Figure S1 for the ERPs determined in the full 0.3–30 Hz frequency range. Statistical analysis of the ERPs was conducted with a two-tailed paired-samples t-test of the Stimulation and Sham conditions (false discovery rate [FDR]-corrected significance threshold *p*<.05).

Time-frequency representations (TFRs) were calculated using Morlet wavelets, time-locked to stimulation onset with a time-window of -1.5 to 5 s in 10 ms steps. Analysed frequencies ranged from 2 to 30 Hz in 1 Hz steps. The number of cycles was set adaptively to half of the corresponding frequency (or rounded up to the next integer value) with a minimum of 5 cycles, resulting in time windows of ~500 ms. For frequency bins corresponding to 2, 3 and 4 Hz, cycle numbers were set to 2, 3 and 4, respectively, to reduce the required window size for these low frequencies. All TFRs were transformed with respect to the relative change from baseline (-1.5 to -1 s).

TFRs were analysed with non-parametric cluster-permutation statistics [44]. Samples were selected that showed significant differences in power (two-tailed paired-samples t-tests, sample-level alpha = .05). In the resulting statistical map, adjacent samples were grouped into positive and negative clusters for which cluster-level statistics were calculated by summing up the t-values within each cluster. Clusters were tested against a reference distribution generated by randomly shuffling the association of data and condition (1000 permutations) and, for each permutation, taking the maximum statistic among all clusters (cluster threshold *p*<.05; two-tailed).

To ensure that the effect of auditory stimulation on EEG theta power was not overshadowed by evoked responses in other frequencies, frequency bands for which the TFR revealed significant effects were subjected to a more fine-grained analysis. EEG signals were separately band-pass filtered between 3 to 7 Hz (theta band) and 10 to 30 Hz (beta band) before the root mean square (RMS) was calculated based on 200 ms (theta band) and 100 ms (beta band) time-windows. Smoothing with a moving average was then applied using windows of the same length. Statistical analysis of Stimulation versus Sham conditions was based on a two-tailed paired-samples t-test with an FDR-corrected significance threshold of *p*<.05.

Generalised effects of auditory stimulation on EEG power were examined with spectral analysis. All artefact-free REM sleep epochs were applied to a Fast Fourier Transformation with a 10.24 s Hanning window and 50% overlap. Power in the theta (3–7 Hz) and beta (10–30 Hz) bands was determined by averaging across the corresponding frequency bins. Although participant-specific theta bands were used for online detection of theta oscillations, they were not used for offline analyses.

EEG data were analysed using the Fieldtrip toolbox [45] in MATLAB 2018a (Mathworks, Natick, MA) and Spike2 version 8.17 (Cambridge Electronic Design, Cambridge, UK).

**Emotional memory**

*Stimuli*

Seven hundred and twenty images were selected from standardised image batteries [46,47] for use in two valence categories: ‘negative’ and ‘neutral’. Based on the ordinal data accompanying the image batteries, negative images were selected on the basis of their low valence [M=3.10, SEM=0.03] and medium-to-high arousal scores [M=6.05, SEM=0.04], whereas neutral images were selected on the basis of their medium valence [M=5.10, SEM=0.02] and low-to-medium arousal scores [M=4.76, SEM=0.04].

The images were divided into six sets of 120 (60 negative, 60 neutral), which did not differ significantly with regard to valence [Set: *F*(5,295)=0.26, *p*=0.94; Set\*Valence: *F*(5,295)=0.30, *p*=.92] or arousal [Set: *F*(3.88,229.15)=0.42, *p*=.79; Set\*Valence: *F*(3.19,188.23)=0.02, *p*=0.99*, Greenhouse-Geisser corrected*]. As expected, the negative and neutral image categories did differ significantly with regard to overall valence [*F*(1,59)=2979.58, *p*<.001, *ƞp2*=.98] and arousal scores [*F*(1,59)=299.98, *p*<.001, *ƞp2*=.82].

Three image sets were assigned to the Stimulation condition and the other three to the Sham condition (assignment counterbalanced). Within each condition, two image sets were used as targets and the remaining set was used as foils.

*Encoding*

Participants provided affect ratings for 240 images (two sets). On each trial, a randomly selected image was presented for 1 s. An affect rating scale was then displayed, which ranged from 1 (corresponding to a sad face on the far-left of the scale) to 9 (corresponding to a smiling face on the far-right of the scale). Participants were required to select the number that best corresponded to the feelings they experienced upon encountering the image. There was no time limit, but participants were asked to provide their rating quickly and spontaneously.

*Recognition*

An immediate recognition test took place straight after encoding. This included 120 ‘target’ images (one of the two sets presented at encoding) and 60 ‘foils’ (half of the unseen set). On each trial, a randomly selected image was presented for 1 s. Participants were then asked to indicate whether the image was ‘old’ (i.e. they recognised the image from encoding) or ‘new’ (i.e. they did not recognise the image). There was no time limit, but participants were asked to respond quickly and accurately. After making their old/new response, participants provided an affect rating for the image, following the same procedures as at encoding.

A delayed recognition test took place the following morning. Procedures were identical to those at the immediate test, with the exception that the other set of encoding images were used as targets, and the other half of the unseen images were used as foils.

Behavioural tasks were executed using E-Prime version 2.0 (Psychology Software Tools) on a desktop computer with a 20” flat-screen monitor. Participant responses were collected using the keyboard.

*Alertness*

In the evening and following morning (before each recognition test), participants completed a 5 min psychomotor vigilance test (PVT) [48] and rated their sleepiness using the Stanford sleepiness scale (SSS) [49].

*Data analysis*

Recognition trials were classified as hits (targets correctly identified as old), misses (targets incorrectly identified as new), correct rejections (foils correctly identified as new) or false alarms (foils incorrectly identified as old). The sensitivity index (*d’*) was calculated as [Normalized (hits/(hits + misses)) – Normalized (false alarms/(false alarms + correct rejections))]. We adopted a log-linear approach to safeguard against errors arising from 0 and 1 values: 0.5 was added to the total hits and total false alarms, and 1 was added to the total signal (old) trials and total noise (new) trials [50]. Memory retention scores were calculated by subtracting immediate *d’* from delayed *d’* and applied to a 2 (Condition: Stimulation, Sham) x 2 (Valence: Negative, Neutral) repeated-measures ANOVA.

 Affect change scores were calculated by subtracting mean affect ratings for the target images at encoding from the mean affect ratings for the corresponding images at recognition (higher scores = greater positive change). This was done separately for immediate and delayed recognition. Affect change scores were applied to a 2 (Condition: Stimulation, Sham) x 2 (Valence: Negative, Neutral) x 2 (Test: Immediate, Delayed) repeated-measures ANOVA. Data was analysed using JASP version 0.10.2.

**Results**

**EEG**

ERPs indicated that auditory stimulation during REM sleep rapidly entrained EEG activity to the 5 Hz target frequency. Significant differences were observed between the Stimulation and Sham conditions, most notably within the first ~0.5 s of the 1 s stimulation period (Figure 1B). The amplitude of the evoked response showed a steady decline during stimulation, potentially reflecting an acute habituation to the auditory stimulus.

Time-frequency analysis (Stimulation > Sham) confirmed that stimulation evoked a rapid increase in theta power that lasted for ~0.5 s (Figure 2A; see Figure S2A for RMS theta signal). Intriguingly, this initial theta surge was followed by a strong and prolonged *suppression* of theta power, which began shortly before stimulus offset and continued for a further 3 s. Stimulation also evoked a rapid increase in beta power, which lasted for ~2 s (see Figure S2B for RMS beta signal). The suppression of theta power and increase in beta power survived cluster-based permutation correction, but the initial theta increase did not (Figure 2B).

Across all REM sleep epochs, there was no difference in theta power between the Stimulation and Sham conditions at FCz [*p*=.766] or any other channel [all *p*>.05]. There were also no between-condition differences in beta power at any channel [all *p*>.05]. Sleep macrostructure was unaffected by stimulation (Table 1): no between-condition differences emerged for time spent in REM sleep or NREM sleep stages N1, N2 or N3, or total sleep time [all *p*>.05].

**Behaviour**

Memory retention scores were unaffected by stimulation, irrespective of image valence [Condition: *F*(1,15)=0.09, *p*=.764; Condition\*Valence: *F*(1,15)=0.40, *p*=.538; Figure 3A], and were generally unaffected by image valence [Valence: *F*(1,15)=3.02, *p*=.103]. Recognition data for the immediate and delayed tests is available in Table S2.

 Affect change scores were unaffected by stimulation, irrespective of image valence and recognition test [Condition: *F*(1,15)=2.16, *p*=.163; Condition\*Valence: *F*(1,15)=0.48, *p*=.500; Condition\*Test: *F*(1,15)=1.22, *p*=.287; Condition\*Valence\*Test: *F*(1,15)=0.07, *p*=.789; Figure 3B]. Affect change scores were generally higher for negative than neutral images [Valence: *F*(1,15)=17.17, *p*<.001, *ƞp2*=.53], but this was not influenced by test time [Valence\*Test: *F*(1,15)=0.01, *p*=.934]. There was no overall effect of test time [Test: *F*(1,15)=2.69, *p*=.152]. Affect rating data for each session is available in Table S3.

**Alertness**

Neither PVT reaction times nor SSS ratings differed significantly between the Stimulation and Sham conditions in the evening [PVT: *t*(15)=1.39, *p*=.185; SSS: *t*(14)=0.52, *p*=.610] or morning [PVT: *t*(15)=1.10, *p*=.289; SSS: *t*(15)=1.00, *p*=.333]. Note that the evening SSS rating was not collected for one participant due to experimenter error.

**Discussion**

We sought to manipulate REM sleep theta activity via phase-locked auditory stimulation. Stimulation entrained EEG activity to the 5 Hz target frequency and evoked a brief increase in theta power. Interestingly, this initial theta surge was immediately followed by a marked suppression of theta activity, which lasted for several seconds. The effects of stimulation were not confined to the theta frequency: beta power was also strongly elevated in response to stimulation. Previous studies have shown that it is possible to manipulate oscillatory activity during NREM sleep using acoustic stimuli [5–11,21]. The present study is the first to extend this line of work to REM sleep.

The entrainment of EEG activity to the 5 Hz stimulation frequency began to subside shortly after stimulation onset. This ‘tapering’ of the evoked theta response is consistent with studies of repetitive auditory stimulation in NREM sleep, which show that the amplitude of evoked SOs rapidly diminishes with each individual stimulation event [5,11]. It has been suggested that this dwindling SO response reflects refractoriness of the oscillation-generating networks to acoustic stimuli, potentially protecting the system from hypersynchrony and epileptiform activity [5,51]. Although the diminished theta response observed in the current study might simply reflect habituation to the auditory stimulus, a more speculative possibility is that refractoriness of oscillation-generating networks to sustained acoustic stimulation also occurs during REM sleep.

The suppression of theta power that followed the initial increase is also reminiscent of effects that have been observed in response to phase-locked auditory stimulation during NREM sleep. Santostasi et al. (2016) delivered acoustic stimuli in blocks (Stim-ON) that were segmented by blocks of silence (Stim-OFF). Relative to a Sham control condition, delta power was decreased during Stim-OFF blocks, suggesting that SO activity was temporarily suppressed in the wake of stimulation [see *ref.* 9: Figure 18]. Similarly, Ngo et al. (2015) found that the interval between successive SOs was longer when stimulation evoked a train of multiple SOs, as compared to a single SO [5]. Such suppressive knock-on effects of stimulation might explain why acoustic stimuli delivered in NREM sleep have no significant effect on overall SO power [5,8,21]. In other words, stimulation-evoked oscillations may come at a cost to those generated endogenously. A similar phenomenon could underlie our observation that 5 Hz auditory stimulation did not boost overall theta power; theta suppression may have counteracted the initial theta surge producing a relative equilibrium.

Intriguingly, 5 Hz auditory stimulation increased beta power throughout and beyond the stimulation period. It is important to stress that sleep arousals - which are often associated with beta activity [52] - were rejected prior to all EEG analyses, and are thus unlikely to account for the observed beta power increase. It is interesting to note that the onset of the beta response seemed to coincide with the initial increase in theta power (see Figure 2A). In a recent intracranial EEG study [26] it was found that, during REM sleep, bursts of theta and beta activity often occurred in concert within fronto-cortical regions, particularly in dorsolateral prefrontal cortex and anterior cingulate cortex. This work throws into contention the possibility that the generation of theta and beta oscillations may be underpinned by a common network, and that the surge in theta power elicited by 5 Hz auditory stimulation evoked a concurrent increase in beta power.

In a bid to optimise our theta detection algorithm, we used participant-specific theta bands for online detection of theta oscillations. To facilitate the use of ERPs as a means of detecting oscillatory entrainment, however, we used a pre-defined stimulation frequency (5 Hz) across all participants. Although the average peak theta frequency was close to 5 Hz and did not vary dramatically between participants (M=5.15, SD=1.01; see Table S1), it would be interesting in future work to examine whether employing participant-specific stimulation frequencies would improve the efficacy of auditory stimulation techniques.

5 Hz auditory stimulation had no effect on the retention or subjective evaluation of aversive images. Our behavioural findings are in keeping with those of prior work that attempted to manipulate theta activity in REM sleep via electrical stimulation. Johnson and Durrant (2018) delivered 5 Hz tDCS during REM sleep in a bid to inhibit theta activity, but stimulation had no impact on the recognition of emotionally negative words [53], suggesting that REM sleep theta oscillations do not support affective memory processing.

There are nevertheless several other factors that might explain our null behavioural results. For example, stimulation did not elevate overall theta power during REM sleep, possibly due to theta suppression counteracting the initial theta surge. Moreover, although recognition paradigms like the one used in this study have been successfully deployed in previous work linking REM sleep theta activity to emotional memory consolidation [36,37], our task might not have been sensitive to the potentially more subtle effects of auditory stimulation on retention [54]. Finally, because our primary objective concerned the electrophysiological effects of REM sleep theta stimulation, our behavioural analyses might have been insufficiently powered to detect differences between the stimulation and sham conditions.

**Limitations**

An alternative interpretation of our findings is that 5 Hz auditory stimulation did not truly influence the ongoing theta oscillation, precluding any change in affective memory processing. Instead, the observed theta and beta responses to acoustic stimulation might correspond to the typical ERP components of REM sleep. It is noteworthy that higher-frequency auditory stimulation delivered in REM sleep is associated with a different profile of EEG responses to those observed in the current study [33]. Specifically, while Sterpenich et al. [33] found that 220-240 Hz stimulation evoked a prolonged increase in theta, alpha and beta power, our 5 Hz stimulation evoked only a brief increase in theta power (followed by a prolonged decrease) and had little effect on alpha power (although there was a sustained increase in beta power). These differences might suggest that our observed effects cannot be solely explained by the typical ERP components of REM sleep, though it should be noted that ERPs evoked by stimulation are known to vary with the frequency of the stimulus [55]. Direct comparisons of our current stimulation protocol and stimulation at various other frequencies are required to ascertain precisely which features of the observed EEG response depend on the 5 Hz stimulation frequency.

A second, related limitation is that the initial increase in theta power during 5 Hz auditory stimulation might reflect a type of auditory steady-state response (ASSR). To test this possibility, the effects of phase-locked stimulation could be compared to those arising from random stimulation delivered at any point during REM sleep. If the observed theta response to phase-locked stimulation was due to an ASSR, then random stimulation would presumably prompt a similar increase in theta power, relative to baseline. It is important to note, however, that our reported increase in theta power was immediately followed by a prolonged suppression of theta activity, which began during stimulation and persisted for several seconds afterwards. This pattern of activity is inconsistent with that of waking ASSRs, where power increases in the targeted frequency range remain constant throughout the stimulation period [56,57].

**Conclusion**

To conclude, we show for the first time that theta oscillations in REM sleep can be manipulated via phase-locked, non-invasive auditory stimulation. 5 Hz auditory stimulation evoked a brief enhancement and then prolonged suppression of theta power, and also a prolonged increase in beta power. These findings might provide the foundations for a novel and effective research tool for investigating the functions of REM sleep in humans.

**Acknowledgements**

The authors would like to thank Marit Petzka for valuable discussions of the data.

**Funding**

This research was supported by Medical Research Council (MRC) Career Development Award MR/P020208/1 to S.A.C. and a Radboud University Radboud Excellence Fellowship to H-V.V.N.

**Notes**

*Financial Disclosure:* None; *Non-financial Disclosure*: None.

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**Figure captions**

**Figure 1.** Stimulation procedures and event-related potentials (ERPs). **A.** Online detection of two supra-threshold theta cycles triggered a 1 s stimulus of 5 Hz amplitude-modulated white noise. **B.** ERPs for the Stimulation (Stim) and Sham conditions (channel FCz). Vertical dotted lines indicate stimulus onset (0 s) and offset (1 s). Horizontal black lines at the top of the figure indicate significant differences [*p*<.05].

**Figure 2.** Time-frequency representations. **A.** Stimulation > Sham contrast. Contours indicate significant differences (uncorrected). **B.** Stimulation > Sham contrast after statistical thresholding [*p*<.05]. Vertical dotted lines indicate stimulus onset (0 s) and offset (1 s).

**Figure 3.** Behaviour. **A.** Memory retention scores. **B.** Affect change scores. Data points represent individual participants. Data are shown as mean ± SEM.

**Tables**

**Table 1.** Sleep stage data.

|  |  |  |  |
| --- | --- | --- | --- |
|  | ***Stimulation*** | ***Sham*** | ***p value*** |
| ***TST***  | 435.22 (± 9.27) | 446.94 (± 6.64) | .096 |
| ***N1***  | 33.22 (± 3.82) | 29.22 (± 3.37) | .233 |
| ***N2***  | 216.34 (± 9.06) | 222.75 (± 5.97) | .372 |
| ***N3***  | 104.16 (± 6.02) | 107.91 (± 7.93) | .425 |
| ***REM***  | 81.50 (± 4.07) | 87.06 (± 5.36) | .289 |

Data are shown in minutes (mean ± SEM). *p* values are shown for two-tailed paired-samples t-tests comparing the Stimulation and Sham conditions. TST, total sleep time; N1, N2 and N3, stages of non-rapid eye movement sleep; REM, rapid eye movement sleep.