**No benefit of auditory closed-loop stimulation on memory for semantically-incongruent associations**

Marcus O. Harringtona, Hong-Viet V. Ngob,Scott A. Cairneya,c

a Department of Psychology, University of York, Heslington, York, Y010 5DD, UK.

b Department of Psychology, University of Lübeck, Maria-Goeppert-Straße 9a, 23562 Lübeck, Germany.

c York Biomedical Research Institute, University of York, Heslington, York, YO10 5DD, UK.

Corresponding Author:Scott A. Cairney

Department of Psychology, University of York, Heslington, York, YO10 5DD

scott.cairney@york.ac.uk

+44 (0) 1904 322 863

**Abstract**

Auditory closed-loop stimulation has gained traction in recent years as a means of enhancing slow oscillatory activity and, consequently, sleep-associated memory consolidation. Previous studies on this topic have primarily focused on the consolidation of semantically-congruent associations. In this study, we investigated the effect of auditory closed-loop stimulation on the overnight retention of semantically-incongruent associations. Twelve healthy males (age: M=20.06, SD=2.02 years) participated in two experimental conditions (simulation and sham). In the stimulation condition, clicks were delivered in phase with slow oscillation up-states, whereas in the sham condition no auditory stimuli were applied. Corroborating earlier work, stimulation (vs. sham) enhanced the slow oscillation rhythm, phase-coupled spindle activity and slow oscillation power. However, there was no benefit of stimulation on overnight memory retention. These findings suggest that closed-loop stimulation does not benefit semantically-incongruent associations.

**Keywords**

Declarative memory; Semantic memory; Slow-wave sleep

**1. Introduction**

Sleep facilitates offline memory processing. Slow oscillations (SOs; <1 Hz), a hallmark EEG feature of deep, non-rapid eye movement sleep (N3), play a central role in sleep-associated consolidation (Klinzing et al., 2019). In concert with temporally-coupled spindles (~12–15 Hz) and ripples (~80–100 Hz), SOs are thought to coordinate the reactivation of newly-formed memories, supporting their migration from hippocampus to neocortex for long-term storage (Born & Wilhelm, 2012; Rasch & Born, 2013).

SO activity can be enhanced via a technique known as auditory closed-loop stimulation (CLoS). In a typical CLoS protocol, EEG data is analysed in real time and, upon algorithmic detection of a supra-threshold SO down state, 50 ms pulses of pink noise (clicks) are delivered in phase with subsequent SO up-states (Ngo et al., 2013). CLoS enhances the SO rhythm by inducing “trains” of successive SO cycles, and amplifies phase-coupled fast spindle activity (Henin et al., 2019; Leminen et al., 2017; Ngo et al., 2013, 2015; Ong et al., 2016, 2018; Papalambros et al., 2017; Schneider et al., 2020). Importantly, CLoS has been shown to improve the retention of declarative memories (Leminen et al., 2017; Ngo et al., 2013, 2015; Ong et al., 2016; Papalambros et al., 2017), supporting the view that SOs and spindles play a central role in overnight memory consolidation.

The retention of both semantically-congruent and semantically-incongruent associations has been shown to benefit from sleep (Cairney et al., 2018; Lo et al., 2014; Payne et al., 2012; Plihal & Born, 1997). Most studies indicating a memory-enhancing effect of CLoS have assessed recall of semantically-congruent associations; typically in the form of related word pairs (e.g. chair-table; Leminen et al., 2017; Ngo et al., 2013, 2015; Ong et al., 2016; Papalambros et al., 2017). The effect of CLoS on semantically-incongruent associations has received comparatively little empirical attention. In two recent studies, CLoS was found to have no significant effect on the retention of unrelated word pairs (e.g. chair-tiger; Diep et al., 2020; Henin et al., 2019, Experiment 1), suggesting that the memory benefits of CLoS might depend on the semantic relatedness of newly learned associations. It should be noted, however, that Henin and colleagues also failed to observe an effect of CLoS on the retention of related word pairs in a follow-up experiment (Henin et al., 2019, Experiment 2).

In the present study, we examined the effect of CLoS on the retention of semantically-incongruent associations. In each of two experimental conditions (stimulation and sham), participants learned unrelated word pairs before a period of overnight sleep, and their memory for the pairs was tested the following morning.

**2. Methods**

***2.1. Participants***

Twelve healthy males (age: M=20.06, SD=2.02 years) were recruited on a voluntary basis. Our original sample was N=11 (matching Ngo et al, 2013), but we recruited an additional participant after sham stimulation event markers were lost for one individual (due to a technical fault). A power analysis conducted in G\*Power 3.1.9.7 (Faul et al., 2007) indicated that a sample of N=11 (alpha = .05) provided 95% power to detect the behavioural effect of CLoS reported by Ngo and colleagues (Hedges‘ g = 1.07).

As indicated by self-report, participants had no history of sleep or psychiatric disorders, were non-smokers, and had abstained from alcohol and caffeine for 24 h prior to each session. Written informed consent was obtained in line with the Research Ethics committee of the Department of Psychology, University of York, who approved the study.

***2.2. Procedure***

Participants completed two experimental conditions (stimulation and sham) in a within-subjects, crossover design. Conditions were separated by one week and condition order was counterbalanced. Other than auditory stimulation in N3, the conditions followed identical procedures. After electrodes were attached for polysomnography, participants completed the first part of a computerised memory task, before going to sleep at ~23:00.

In the stimulation condition, CLoS was applied in the first half of the night (~23:00–03:00) when participants exhibited stable N3 sleep (Iber et al., 2007). Would-be stimulation events were marked in the sham condition, but no stimulation was applied.

Participants were awoken at 07:00 and electrodes were removed. After a ~30 min delay, they then completed the second part of the memory task.

***2.3. Memory task***

In the first part of the memory task (evening), participants were asked to memorise 80 unrelated word pairs (e.g. *chair–tiger*), which were each shown for 5 s (order randomised; fixation interval = 1.5 s). In an immediate cued recall test, they were shown the first word of each pair and asked to type the corresponding target word within 12 s. Feedback on the correct target word was shown for 3.5 s, irrespective of the participant’s response. Participants who did not achieve a cued recall score of ≥60 percent correct were required to repeat the test until they met this criterion.

In the second part of the memory task (morning), participants completed a single round of cued recall without feedback on the correct target words.

***2.4. Stimuli***

Three-hundred and twenty words were extracted from an adapted version of the University of South Florida word association norms (Maki et al., 2004) to create 160 unrelated word pairs. The word pairs were divided into two sets of 80 for use in each condition. Correspondence between word set and condition was counterbalanced.

***2.5. Polysomnography and CLoS***

Sleep was monitored with an Embla N7000 polysomnography system. Gold-plated electrodes were attached at six standardised locations (F3, F4, C3, C4, O1, O2) and referenced to the linked mastoids (impedance <5 kΩ; sample rate = 200 Hz). For real time monitoring of SOs (as required for CLoS) channel FPz, bandpass filtered at 0.25–4 Hz, was recorded with a Digitimer D360 amplifier and CED Micro 1401-3 digitizer.

The CLoS protocol was identical to that of Ngo et al. (2013). Briefly, upon algorithmic detection of a supra-threshold SO down-state, two 50 ms pulses of pink noise (clicks) were delivered binaurally (~40 dB; in-ear headphones), in phase with the ensuing SO up-states (Figure 1A).

***2.6. Data analysis***

*2.6.1. EEG*

Overnight polysomnography data was scored according to standardised criteria (Iber et al., 2007). N3 sleep epochs that occurred during the ~23:00–03:00 stimulation period were subjected to visual artefact rejection, and then taken forward for further analysis.

To visualize the effects of CLoS on the SO rhythm, EEG channels were first band-pass filtered at 0.3–30 Hz. ERPs time-locked to stimulation onset (-2 to 3 s time window) were created by averaging across all EEG channels and participants. For visualization of fast spindle activity, EEG channels were band-pass filtered at 12–15 Hz and the root-mean-square (RMS) signal (time-locked to stimulation onset, -2 to 3 s time window) was calculated based on 200 ms windows. Smoothing with a moving average was applied using windows of the same length. RMS signals were averaged across all EEG channels and participants. Statistical analyses of the ERP and RMS signals were based on paired-samples t-tests (alpha level = .05), corrected for multiple comparisons using a cluster-permutation procedure with a cluster-level alpha of .05 (Maris & Oostenveld, 2007).

The general effect of auditory stimulation on EEG power was examined with spectral analysis. Artefact-free N3 epochs were applied to a Fast Fourier Transformation with a 10.24 s Hanning window and 50% overlap. Irregular-resampling auto-spectral analysis was used to correct for 1/f noise (Wen & Liu, 2017). Power in the SO (<1 Hz), delta (1-4 Hz), theta (4-8 Hz), slow spindle (8-12 Hz), fast spindle (12-15 Hz), and beta (15-30 Hz) bands was determined by averaging across the corresponding frequency bins. EEG power was compared between the stimulation and sham conditions using two-tailed paired-samples t-tests.

*2.6.2. Behaviour*

Cued recall scores were calculated as the percentage of target words that were correctly typed, and applied to a 2 (Test: Evening/Morning) x 2 (Condition: Stimulation/Sham) repeated-measures ANOVA.

**3. Results**

***3.1. EEG***

CLoS enhanced slow oscillatory activity, evoking a train of successive SO cycles (Figure 1B), and amplified phase-coupled fast spindle activity (Figure 1C). Spectral power analysis of all N3 epochs that fell within the ~23:00–03:00 stimulation period revealed that stimulation (vs. sham) increased power in the SO [t(10)=3.33, p=.008, d=1.00, Figure 1D] and delta [t(10)=3.23, p=.009, d=0.97] bands. Power in the theta, slow spindle, fast spindle, and beta bands did not differ significantly between the stimulation and sham conditions [all p≥.280]. CLoS had no significant impact on total sleep time [t(11)=1.00, p=.339; Table 1], or time spent in any particular stage of sleep [all p≥.075].

**Table 1. Sleep parameters**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | N1 | N2 | N3 | REM | TST |
| **Stimulation** | 8.38 ± 1.09 | 247.04 ± 8.03 | 121.50 ± 7.54 | 87.08 ± 6.07 | 464.00 ± 7.35 |
| **Sham** | 10.38 ± 1.55 | 244.79 ± 9.02 | 109.38 ± 7.35 | 87.58 ± 7.24 | 452.13 ± 12.30 |

Time (minutes) spent in each stage of sleep and total sleep time. N1-N3: stages of non-rapid eye movement sleep; REM: rapid eye movement sleep; TST: total sleep time. Data are shown as mean ± SEM.

***3.2. Behaviour***

There was a general increase in recall scores between the evening and morning tests [*F*(1,11)=25.65, *p*<.001, *ƞp2*=0.70; Figure 2A], presumably due to feedback provided in the evening session. Interestingly, overnight memory gains were lower in the stimulation (vs. sham) condition (Figure 2B), but the interaction between Test and Condition was not significant [F(1,11)=3.99, p=.071, ƞp2=0.27]. There was also no significant general effect of Condition on performance [*F*(1,11)=0.01, *p*=.938].

In the stimulation condition, overnight changes in memory performance were not significantly correlated with phase-coupled spindle activity [*r*=-0.28, *p*=.411], N3 SO power [*r*=-0.22, *p*=.526], or time spent in N3 [*r*=-0.138, *p*=.670].

**4. Discussion**

We examined the effect of CLoS on the overnight retention of semantically-incongruent associations. Replicating previous findings (Henin et al., 2019; Leminen et al., 2017; Ngo et al., 2013, 2015; Ong et al., 2016, 2018; Papalambros et al., 2017; Schneider et al., 2020), CLoS enhanced the SO rhythm and amplified phase-coupled fast spindle activity. However, CLoS had no significant impact on word pair recall.

In recent work, delivering one-pulse auditory stimulation during a daytime nap was found to boost SO and phase-coupled spindle activity, but had no significant effect on the retention of unrelated word pairs (Henin et al., 2019; Experiment 1). Similarly, another study that delivered two-pulse auditory stimulation during overnight sleep also failed to find a significant memory benefit of CLoS for unrelated word pairs (Diep et al., 2020). We observed a similar pattern of results following delivery of two-pulse auditory stimulation during overnight sleep, suggesting that the memory enhancing effects of CLoS may not extend to semantically-incongruent associations.

Coherence between new information and pre-existing knowledge is thought to expedite overnight memory processing mediated by SOs and spindles, permitting rapid integration of memories into neocortex (Lewis & Durrant, 2011). From this perspective, enhancements of sleep-associated consolidation via auditory stimulation should be more discernible for semantically-congruent than incongruent associations. Consistent with this view, the retention of unrelated word pairs was not significantly influenced by CLoS in the current study. By contrast, other studies have observed sizeable improvements in memory recall for related word pairs following CLoS (Ngo et al., 2013, 2015; Ong et al., 2016; Papalambros et al., 2017).

Notably, retention of unrelated word pairs was found to profit from CLoS in one study, but only when those word pairs were linked to an anticipated reward (Prehn-Kristensen et al., 2020). Specifically, after stimulation (but not sham) healthy children exhibited superior recall performance for word pairs associated with a monetary reward, compared to unrewarded word pairs. These results indicate that an anticipated reward, which has been shown in earlier work to aid sleep-associated consolidation (Fischer & Born, 2009; Studte et al., 2017), may render incongruent associations accessible to the memory-enhancing effects of CLoS.

As reflected in our EEG results, the enhancing effect of CLoS on the SO rhythm and phase-coupled spindle activity is stable and reproducible (Harrington & Cairney, 2021). The effect of CLoS on memory retention, by contrast, is less reliable. Although the retention of related word pairs typically profits from CLoS (Ngo et al., 2013, 2015; Ong et al., 2016; Papalambros et al., 2017), negative results have also been reported (Henin et al., 2019; Papalambros et al., 2019; Schneider et al., 2020). Studies that have used other learning tasks (besides verbal paired-associates) have also produced mixed findings (Henin et al., 2019; Leminen et al., 2017; Prehn-Kristensen et al., 2020; Schneider et al., 2020). It is therefore likely that pre-existing associative knowledge is one of several factors that can influence the effect of CLoS on memory retention (Harrington & Cairney, 2021).

A limitation of the current study is that we did not directly compare the retention of related and unrelated word pairs, meaning that we cannot draw firm conclusions on the divergent effects of CLoS on newly-formed memories that do or do not conform to pre-existing knowledge. The influence of CLoS on memory for both related and unrelated information has previously been tested within the same study, but with different tasks (Leminen et al., 2017). While recall of semantically-related word pairs benefitted from CLoS (vs. sham), no such effect occurred for arbitrary face-name pairs. Because the tasks used different types of stimuli (i.e. word pairs vs. face-name pairs), it is unclear whether these divergent results were driven solely by differences in relatedness. Future studies should examine the impact of CLoS on the retention of congruent and incongruent associations within the same task.

***4.1. Conclusions***

In sum, we found that CLoS did not improve the consolidation of semantically-unrelated word pairs, despite augmenting the SO rhythm and phase-coupled fast spindle activity. These results add to a growing body of literature suggesting that memories are more likely to profit from the memory-enhancing effects of CLoS if they are consistent with pre-existing knowledge.

**Acknowledgements**

This work was supported by Medical Research Council (MRC) Career Development Award MR/P020208/1 to S.A.C.

**Author contributions**

H.V.N. and S.A.C. conceived and planned the experiment. M.O.H., H.V.N. and S.A.C. collected and analysed the data. M.O.H. wrote the manuscript. H.V.N. and S.A.C. reviewed and edited the manuscript.

**Competing interests**

None.

**References**

Born, J., & Wilhelm, I. (2012). System consolidation of memory during sleep. *Psychological Research*, *76*(2), 192–203. https://doi.org/10.1007/s00426-011-0335-6

Cairney, S. A., Lindsay, S., Paller, K. A., & Gaskell, M. G. (2018). Sleep preserves original and distorted memory traces. *Cortex*, *99*, 39–44. https://doi.org/10.1016/j.cortex.2017.10.005

Diep, C., Ftouni, S., Manousakis, J. E., Nicholas, C. L., Drummond, S. P. A., & Anderson, C. (2020). Acoustic slow wave sleep enhancement via a novel, automated device improves executive function in middle-aged men. *Sleep*, *43*(1). https://doi.org/10.1093/sleep/zsz197

Faul, F., Erdfelder, E., Lang, A. G., & Buchner, A. (2007). G\*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, *39*, 175–191. https://doi.org/10.3758/BF03193146

Fischer, S., & Born, J. (2009). Anticipated Reward Enhances Offline Learning During Sleep. *Journal of Experimental Psychology: Learning Memory and Cognition*, *35*(6), 1586–1593. https://doi.org/10.1037/a0017256

Harrington, M. O., & Cairney, S. A. (2021). Sounding it out: auditory stimulation and overnight memory processing. *Current Sleep Medicine Reports*, In Press.

Henin, S., Borges, H., Shankar, A., Sarac, C., Melloni, L., Friedman, D., Flinker, A., Parra, L. C., Buzsaki, G., Devinsky, O., & Liu, A. (2019). Closed-loop acoustic stimulation enhances sleep oscillations but not memory performance. *ENeuro*, *6*(6). https://doi.org/10.1523/ENEURO.0306-19.2019

Iber, C., Ancoli-Israel, S., Chesson, A., & Quan, S. F. (2007). *The AASM manual for the scoring of sleep and associated events rules, terminology, and technical specifications*. American Academy of Sleep Medicine.

Klinzing, J. G., Niethard, N., & Born, J. (2019). Mechanisms of systems memory consolidation during sleep. *Nature Neuroscience*, *22*(10), 1598–1610. https://doi.org/10.1038/s41593-019-0467-3

Leminen, M. M., Virkkala, J., Saure, E., Paajanen, T., Zee, P. C., Santostasi, G., Hublin, C., Müller, K., Porkka-Heiskanen, T., Huotilainen, M., & Paunio, T. (2017). Enhanced memory consolidation via automatic sound stimulation during non-REM sleep. *Sleep*, *40*(3). https://doi.org/10.1093/sleep/zsx003

Lewis, P. A., & Durrant, S. J. (2011). Overlapping memory replay during sleep builds cognitive schemata. *Trends in Cognitive Sciences*, *15*(8), 343–351. https://doi.org/10.1016/j.tics.2011.06.004

Lo, J. C., Dijk, D. J., & Groeger, J. A. (2014). Comparing the effects of nocturnal sleep and daytime napping on declarative memory consolidation. *PLoS ONE*, *9*(9). https://doi.org/10.1371/journal.pone.0108100

Maki, W. S., McKinley, L. N., & Thompson, A. G. (2004). Semantic distance norms computed from an electronic dictionary (WordNet). *Behavior Research Methods, Instruments, and Computers*, *36*(3), 421–431. https://doi.org/10.3758/BF03195590

Maris, E., & Oostenveld, R. (2007). Nonparametric statistical testing of EEG- and MEG-data. *Journal of Neuroscience Methods*, *164*(1), 177–190. https://doi.org/10.1016/j.jneumeth.2007.03.024

Ngo, H. V. V., Martinetz, T., Born, J., & Mölle, M. (2013). Auditory closed-loop stimulation of the sleep slow oscillation enhances memory. *Neuron*, *78*(3), 545–553. https://doi.org/10.1016/j.neuron.2013.03.006

Ngo, H. V. V, Miedema, A., Faude, I., Martinetz, T., Molle, M., & Born, J. (2015). Driving Sleep Slow Oscillations by Auditory Closed-Loop Stimulation--A Self-Limiting Process. *Journal of Neuroscience*, *35*(17), 6630–6638. https://doi.org/10.1523/jneurosci.3133-14.2015

Ong, J. L., Lo, J. C., Chee, N. I. Y. N., Santostasi, G., Paller, K. A., Zee, P. C., & Chee, M. W. L. (2016). Effects of phase-locked acoustic stimulation during a nap on EEG spectra and declarative memory consolidation. *Sleep Medicine*, *20*, 88–97. https://doi.org/10.1016/j.sleep.2015.10.016

Ong, J. L., Patanaik, A., Chee, N. I. Y. N., Lee, X. K., Poh, J. H., & Chee, M. W. L. (2018). Auditory stimulation of sleep slow oscillations modulates subsequent memory encoding through altered hippocampal function. *Sleep*, *41*(5). https://doi.org/10.1093/sleep/zsy031

Papalambros, N. A., Santostasi, G., Malkani, R. G., Braun, R., Weintraub, S., Paller, K. A., & Zee, P. C. (2017). Acoustic enhancement of sleep slow oscillations and concomitant memory improvement in older adults. *Frontiers in Human Neuroscience*, *11*(109). https://doi.org/10.3389/fnhum.2017.00109

Papalambros, N. A., Weintraub, S., Chen, T., Grimaldi, D., Santostasi, G., Paller, K. A., Zee, P. C., & Malkani, R. G. (2019). Acoustic enhancement of sleep slow oscillations in mild cognitive impairment. *Annals of Clinical and Translational Neurology*, *6*(7), 1191–1201. https://doi.org/10.1002/acn3.796

Payne, J. D., Tucker, M. A., Ellenbogen, J. M., Wamsley, E. J., Walker, M. P., Schacter, D. L., & Stickgold, R. (2012). Memory for semantically related and unrelated declarative information: The benefit of sleep, the cost of wake. *PLoS ONE*, *7*(3), e33079. https://doi.org/10.1371/journal.pone.0033079

Plihal, W., & Born, J. (1997). Effects of early and late nocturnal sleep on declarative and procedural memory. *Journal of Cognitive Neuroscience*, *9*(4), 534–547. https://doi.org/10.1162/jocn.1997.9.4.534

Prehn-Kristensen, A., Ngo, H. V. V., Lentfer, L., Berghäuser, J., Brandes, L., Schulze, L., Göder, R., Mölle, M., & Baving, L. (2020). Acoustic closed-loop stimulation during sleep improves consolidation of reward-related memory information in healthy children but not in children with attention-deficit hyperactivity disorder. *Sleep*, *43*(8). https://doi.org/10.1093/sleep/zsaa017

Rasch, B., & Born, J. (2013). About sleep’s role in memory. *Physiological Reviews*, *93*, 681–766. https://doi.org/10.1152/Physrev.00032.2012

Schneider, J., Lewis, P. A., Koester, D., Born, J., & Ngo, H. V. (2020). Susceptibility to auditory closed-loop stimulation of sleep slow oscillations changes with age. *Sleep*, *43*(12). https://doi.org/10.1093/sleep/zsaa111

Studte, S., Bridger, E., & Mecklinger, A. (2017). Sleep spindles during a nap correlate with post sleep memory performance for highly rewarded word-pairs. *Brain and Language*, *167*, 28–35. https://doi.org/10.1016/j.bandl.2016.03.003

Wen, H., & Liu, Z. (2017). Separating Fractal and Oscillatory Components in the Power Spectrum of Neurophysiological Signal. *Brain Topography*, *29*(1), 13–26. https://doi.org/10.1007/s10548-015-0448-0

**Figure captions**

**Figure 1. A.** Stimulation procedure. In the stimulation condition, detection of a supra-threshold slow oscillation (SO) down-state triggered two clicks (red dashed lines). The first click occurred during the predicted SO up-state based on the temporal characteristics of each participant’s SOs (obtained during an adaptation night), and the second after a 1.075 s interval corresponding to the SO period duration. In the sham condition, corresponding time points of stimulation events were marked but no stimulation was applied. The detection routine was paused for 2.5 s after the second click. **B.** Mean (± SEM) EEG signal averaged across participants and channels, time locked to the first click. Vertical red dashed lines indicate timing of the clicks. Horizontal black lines indicate significant differences. **C.** Fast spindle activity (12-15 Hz; root-mean-square ± SEM based on a 200-ms window) averaged across participants and channels, time locked to the first click. Vertical red dashed lines indicate timing of the clicks. Horizontal black line indicates significant differences. **D.** Mean SO power (<1 Hz) across all N3 epochs within the ~23:00–03:00 stimulation period, averaged across all participants and channels. Data points represent individual participants.

**Figure 2.** Cued recall scores. **A.** Percent word recall and **B.** overnight improvement. Data points represent individual participants in A and B. Error bars represent SEM in A.