# Supplementary Information
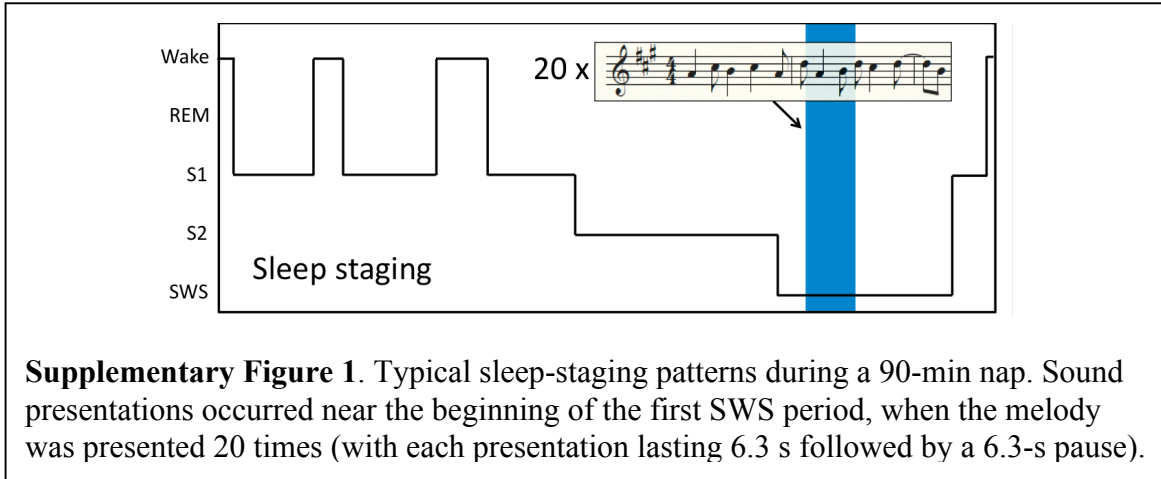## Cued Memory Reactivation During Sleep Influences Skill Learning

James W. Antony[1], Eric W. Gobel[1], Justin K. O'Hare[2], Paul J. Reber[1,2], and Ken A. Paller[1,2]
[1]Interdepartmental Neuroscience Program and [2]Department of Psychology,
Northwestern University, Evanston, IL 60208, USA

**Supplementary Figures**



**Supplementary Figure 1**. Typical sleep-staging patterns during a 90-min nap. Sound presentations occurred near the beginning of the first SWS period, when the melody was presented 20 times (with each presentation lasting 6.3 s followed by a 6.3-s pause).



**Supplementary Figure 2.** Recognition test materials for one sequence. Among the five melodies presented in this test, four were foil melodies composed using variations in tonal and rhythmic patterns from the original.
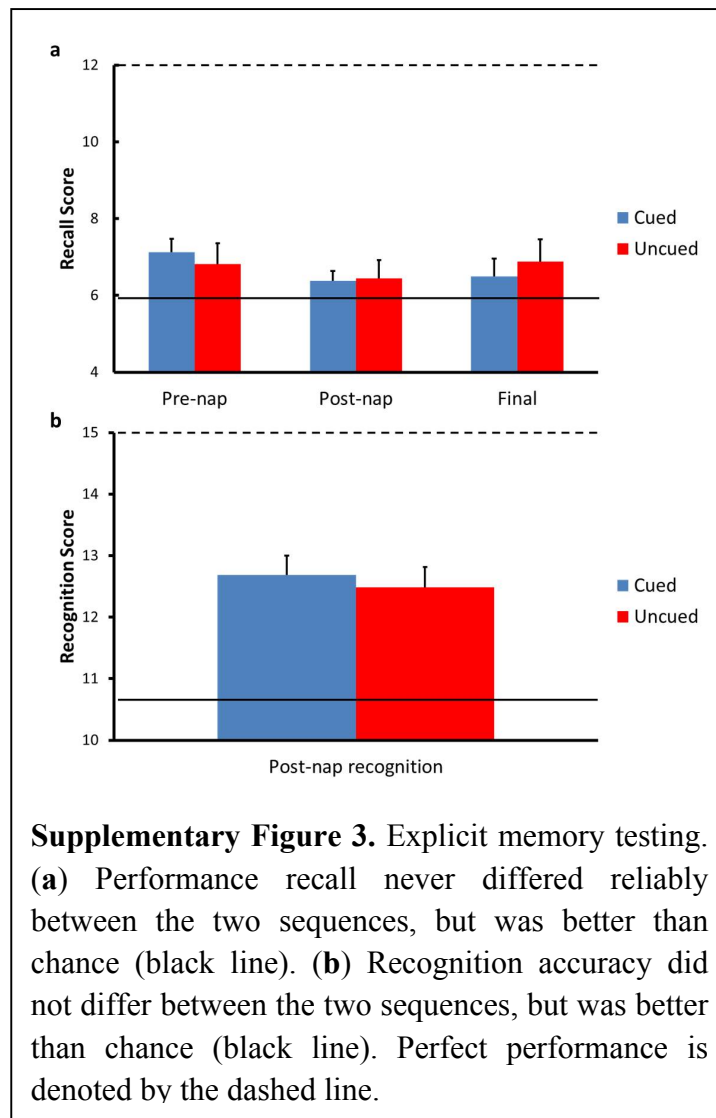
**Supplementary Figure 3.** Explicit memory testing. (**a**) Performance recall never differed reliably between the two sequences, but was better than chance (black line). (**b**) Recognition accuracy did not differ between the two sequences, but was better than chance (black line). Perfect performance is denoted by the dashed line.
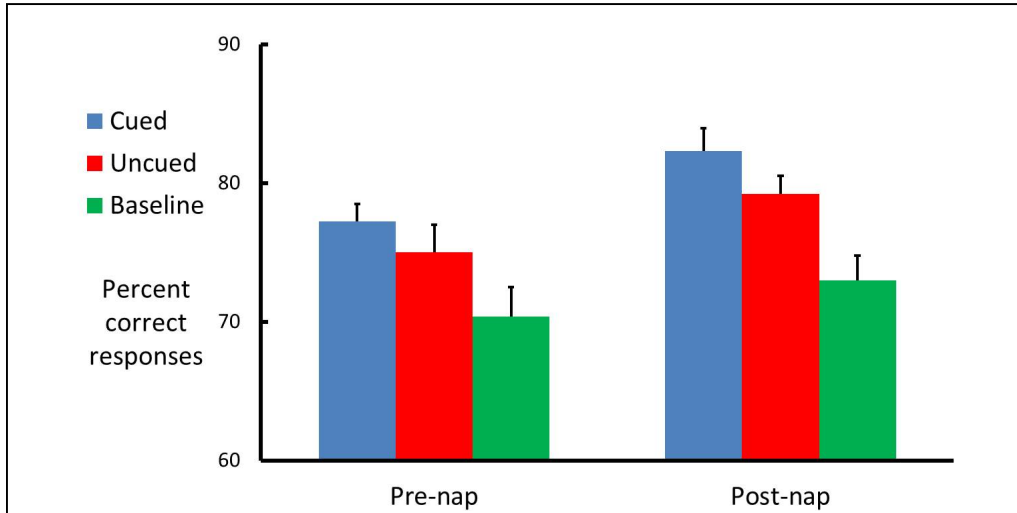
**Supplementary Figure 4.** Accuracy results from the waking protocol. No significant differences were observed between cued and uncued sequences.
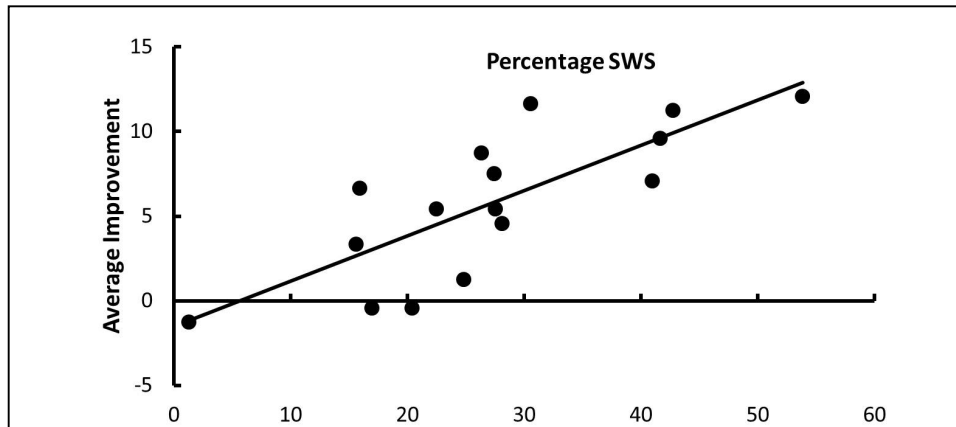


**Supplementary Figure 5.** Across-subject correlation between average improvement score in the sleep no-sounds group and the percentage of time in SWS ($N = 16$, $r = 0.78$, $P < 0.001$).

**Supplementary Table 1.** Time in each sleep stage (min ± *SE*).

| Group | S1 | S2 | SWS | REM | Total sleep |
|---|---|---|---|---|---|
| Sleep Sounds | 5.2 ± 1.1 | 28.6 ± 4.1 | 28.6 ± 3.7 | 2.4 ± 1.2 | 64.8 ± 5.6 |
| Sleep No Sounds | 5.1 ± 0.8 | 38.4 ± 3.3 | 17.8 ± 2.5 | 5.0 ± 1.6 | 66.3 ± 3.4 |

## Supplementary Results

**Explicit Memory Tests.** Performance recall scores (**Supp. Fig. 3a**) were far from perfect but tended to be above chance levels ($P = 0.03$). Recall accuracy did not differ between cued and uncued melodies at any time point (all $P > 0.5$). Likewise, mean scores after the nap in the paper recall test did not differ between cued and uncued melodies ($P = 0.44$), averaging $7.1 \pm 0.5$ for the cued melody and $6.5 \pm 0.5$ for the uncued melody. Recognition accuracy (**Supp. Fig. 3b**) was above chance ($P < 0.001$) and did not differ between the two trained melodies ($P = 0.69$). Although no influence of sleep cuing was found in any of the explicit memory tests, these findings show that subjects had some explicit knowledge of what they had learned.

**Explicit-Implicit Interactions.** Subjects intentionally learned to produce the melodic sequences, and they showed clear evidence of above-chance explicit knowledge. However, it is unclear whether accurate performance in making the correct responses in time with visual cues depends on explicit sequence knowledge. To explore possible implicit-explicit interactions, correlations were computed between task performance and the explicit memory measures. In keeping with previous findings[1], correlations were nonsignificant (Pearson correlation, all $r < 0.2$, $P > 0.3$), suggesting that successful task performance may not have relied heavily on explicit knowledge of the melody. Additionally, pre-nap explicit memory did not appear to influence performance improvement across the nap, nor did pre-nap performance correlate with explicit memory change (both $r < 0.2$, $P > 0.3$). Nonetheless, it remains possible that above-chance explicit memory is important for the impact of sleep cues on performance, especially considering the importance of explicit knowledge with respect to the sleep-dependence of procedural memory in other tasks[2].

**Debriefing.** No subjects reported awareness of sleep cues. One subject described task-related sleep cognition upon awakening in the form of a dream experience with "the red and blue dots" (from the task). Paralleling a previous account relating task-related dreaming to improved memory consolidation[3], this subject exhibited the greatest reactivation advantage of all subjects (28.3%).

**Wake Experiment.** Whether cues during waking are effective may depend on how they are interpreted. For example, if subjects regard melodies as reminders to rehearse motor patterns from training, an improvement in performance is likely. As in the sleep protocol, we conducted the experiment with no instructions with regard to sound presentations. At debriefing, all subjects claimed to have noticed the sounds.

Subjects performed the working-memory task at above-chance levels ($P = 0.02$), but only achieved an average accuracy of $63.7 \pm 4.3\%$. A comparison of second-run accuracy as a function of whether the melody was playing or not showed a negligible difference ($64.6 \pm 4.7\%$ vs. $63.3 \pm 4.4\%$, respectively, $P = 0.51$). A decline in performance during the melody might be expected, to the extent that attention was diverted away from the task by the melody. However, there was no evidence in task performance that tones were attended.

Results obtained during production of the melodies (**Supp. Fig. 4**) showed sequence-specific knowledge for the trained melodies in comparison to the baseline melody ($P = 0.01$). There were

no significant accuracy differences between cued and uncued melodies before ($P = 0.25$) or after the delay ($P = 0.18$). Explicit memory likewise did not differ between the two sequences for any test (all $P > 0.05$), although there were some trends for effects (e.g., final recall tests tended to be better for the cued than the uncued sequence, $P = 0.07$). These results cannot show whether sound cues improve musical performance during waking generally. Rather, as in previous attempts with declarative memories[4,5,6], we conclude that sound cues in a waking protocol do not necessarily produce the relative alterations in performance found with the sleep protocol.

An additional analysis for the wake group and the sleep sounds group confirmed that performance was better on the post-delay test compared to the pre-delay test. The improvement across the delay ($P < 0.001$, including cued, uncued, and baseline conditions) was similar whether the delay included sleep or not ($P = 0.32$). This improvement may be attributable to the fact that learning took place during each performance opportunity, including the pre-delay test. Also, the long break prior to the final test may have produced a reduction in fatigue. Whereas larger improvements after sleep than after wake have often been observed, this study examined a relatively short retention interval (90 min).

Other types of action-related stimuli, like visual observation of repetitive thumb movements, can aid motor learning[7], even with a concurrent task and in the absence of conscious strategies to practice the task[8]. Intentionally listening to melodies can also improve subsequent performance[9], although this has only been shown to occur with intervening sleep.

**Sleep No-Sounds Experiment.** As in the other groups, learned melody performance was significantly better than baseline performance before ($P = 0.03$) and after ($P = 0.01$) the nap interval. The average improvement for the two learned melodies was 4.4% ± 1.8, which was a significant improvement ($P < 0.001$) but not significantly different from either the cued condition in the sleep group (7.9% ± 2.4, $P = 0.22$) or from the uncued condition in the sleep group (2.6% ± 1.5, P = 0.31). These numerical trends are suggestive of an effect of sleep cues in the main experiment that involved a bias of memory processing toward the cued melody, although an influence on only one melody cannot be ruled out. Interestingly, more SWS was observed in the latter part of the nap (after the time when sleep cues began or would begin) in the sleep sounds group than in the sleep no-sounds group (12.6 ± 3.4 min vs. 4.1 ± 1.1 min, respectively, P = 0.02).

**Sleep Electrophysiology.** In the sleep sounds group, standard sleep scoring[10] (**Supp. Table 1**) confirmed that sleep cues were presented entirely during SWS for all subjects except one who received presentations during 3 min of SWS and 1 min of stage-2 sleep and another with 2.5 min of SWS and 1.5 min of stage-2 sleep. Sleep records from all subjects were sufficient for verifying the timing of sound delivery during sleep and staging the reactivation period, but data from two were excluded from further physiological analyses because computer records were partly corrupted. The average time from sleep onset to cue onset was 39.8 ± 5.1 min.

In the sleep no-sounds group, each participant reached SWS (**Supp. Table 1**). The average improvement across both sequences correlated with percentage of time in SWS (**Supp. Fig. 5**).

Given previous results suggesting differential contributions to sensorimotor learning performance from fast (13.5-15 Hz) and slow (12-13.5 Hz) spindles[11], we investigated reactivation advantage correlations for both types of spindle. When fast and slow SWS spindles at the F4 scalp location were computed separately, both revealed positive correlations with the reactivation advantage ($r = 0.71$, $P = 0.004$ and $r = 0.58$, $P = 0.03$, respectively).

We also computed separate correlations for cued and uncued melodies with percentage SWS time and SWS spindles at F4. The cued sequence improvement across the nap showed a positive correlation with the percentage of time in SWS ($r = 0.66$, $P = 0.009$); the uncued sequence improvement showed a nonsignificant negative correlation ($r = -0.25$, $P = 0.39$). The cued sequence improvement correlated positively with total SWS spindles over F4 ($r = 0.70$, $P = 0.005$); the uncued sequence improvement showed a marginal negative correlation ($r = -0.46$, $P = 0.09$). These findings suggest that sleep cues may have both improved cued sequence performance and impaired uncued sequence performance, but the most well-supported conclusion is that SWS was critical for producing a performance improvement for the cued sequence.

**Supplementary References**

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