



Memory Reactivation during Sleep Improves Execution of a Challenging Motor Skill

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Memory reactivation during sleep reinforces various types of learning. Basic motor skills likely benefit from sleep. There is insufficient evidence, however, on whether memory reactivation during sleep contributes to learning how to execute a novel action. Here, we investigated motor learning in a myoelectric feedback task. Human male and female participants learned to control myoelectric activity in specific arm muscles to move a computer cursor to each of 16 locations. Each location was associated with a unique sound. Half of the sounds were played during slow-wave sleep to reactivate corresponding memories of muscle control. After sleep, movements cued during sleep were performed more quickly and efficiently than uncued movements. These results demonstrated that memory reactivation during sleep contributes to learning of action execution. We conclude that sleep supports learning novel actions, which also maps onto the learning required in certain neurorehabilitation procedures.

Key words: consolidation; EMG; motor learning; myoelectric interface; rehabilitation

Significance Statement

Prior literature on motor learning has produced much evidence supporting a role for sleep but scant evidence on the execution component. This aspect of learning is critical for many complex skills that people value in their lives. Our results not only implicate sleep in skill learning but also pinpoint a benefit for motor execution using a method for modifying memory storage during sleep. We used targeted memory reactivation (TMR), whereby a stimulus that has been associated with learning is presented again during sleep to bring on a recapitulation of waking brain activity. Our demonstration that memory reactivation contributed to skilled performance may be relevant for neurorehabilitation as well as fields concerned with motor learning, such as kinesiology and physiology.

Introduction

Gradual changes in memory storage in the brain are often required to learn to perform a complex task (McGaugh, 2000). A comprehensive understanding of these changes over time, also called memory consolidation, has not been achieved, but an intriguing insight is that they may depend on sleep (Klinzing et al., 2019). Evidence implicating sleep has been particularly contentious for the consolidation of motor skills required for performing

certain movements (Ficca and Salzarulo, 2004). Clearly sleep provides a pause in activity, allowing some protection from interference and forgetting, but sleep may do more (Walker et al., 2003).

Compelling evidence implicating sleep in consolidation derives from experimentally altering memory reactivation during sleep (Oudiette and Paller, 2013). With this method of targeted memory reactivation (TMR), memory performance can be improved as a function of sound or odor stimulation during sleep, particularly non-rapid eye movement (NREM) sleep (Rasch et al., 2007; Rudoy et al., 2009). Many studies have documented TMR improving declarative memory (Hu et al., 2020).

Does sleep-based reactivation also apply to motor skills? Whereas Rasch et al. (2007) found that TMR did not improve response speed in a finger-tapping sequence task, Antony et al. (2012) showed that TMR did benefit accuracy in a task requiring precisely timed button presses to moving visual cues. Most studies of sleep consolidation in motor learning involve finger tapping sequence learning (FTSL) or the serial reaction time task (SRTT). In both tasks, responses are preferentially speeded for repeating sequences, which implies that a sequence-specific

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representation is acquired that supports rapid execution. Prior TMR studies using these two tasks have shown both improvement (Cousins et al., 2014; Schönauer et al., 2014) and no improvement from sleep (Rasch et al., 2007; Pereira et al., 2017). Inconsistency is echoed in the broader literature on sleep-dependent skill enhancements compared with wake (Walker et al., 2005; Cai and Rickard, 2009). In fact, observed performance gains following sleep may disappear once confounding influences of fatigue build-up and time-of-day are controlled (Pan and Rickard, 2015). Designs that introduce rest periods before testing (Brawn et al., 2010), or that exclude analysis of test trials after performance deteriorates (Rickard et al., 2008), show that observed gains after sleep could be illusory.

One major limitation of FTSL and SRTT is that performance on these tasks heavily depends on choosing from multiple possible actions, or action selection, with minimal demands on the quality of performed movement, or action execution (Diedrichsen and Kornysheva, 2015). Because the simple button press actions in FTSL and SRTT are already well learned, performance in these tasks is not subject to much improvement in the quality of movement (Hardwick et al., 2013; Krakauer et al., 2019). In contrast, typical actions that people want to learn often recruit continuous adjustment and control of effectors. Because important motor tasks tend to emphasize action execution (e.g., using a scalpel to perform surgery), the requisite cognitive components and brain systems may differ.

Whereas motor-sequence learning emphasizes action selection, motor-skill learning primarily requiring action execution involves little hippocampal activation (Poldrack and Rodriguez, 2003; Albouy et al., 2008). More information is thus needed about the sleep-dependence of motor skills that emphasize action execution. TMR investigations have largely avoided such tasks, aside from two studies of ball-throwing. In one study of young adults, sleep TMR improved accuracy in this task immediately after sleep but this advantage was not long-lasting (Johnson et al., 2019). In older adults, accuracy did not improve when immediately measured after a nap (Johnson et al., 2020).

Here, we investigated motor learning that emphasizes action execution and has the advantage of connecting to the learning undertaken in motor rehabilitation protocols. We found that TMR improved performance speed and efficiency. Control of muscles improved as well, resulting in relatively greater goal-congruent muscle activity. This learning task thus provided multiple measures, both of performance and of motor activity, that were sensitive to reactivation of motor execution representations during sleep.

Materials and Methods

Subjects

All study procedures were approved by the Northwestern University Institutional Review Board. Healthy participants were recruited from Northwestern University and the Evanston community. They were instructed to wake up 1–2 h before their usual wake time and not to ingest caffeine on the day of the study. Consent was given at the start of each session and at the end participants were compensated for their time at a predetermined hourly rate. Study starting times varied based on participant availability (ranging from 7 A.M. to 5:30 P.M.), and the average duration was 6.74 ± 0.14 h. The sleep portion started ~ 5 h after study start time (ranging from 11:40 A.M. to 10:40 P.M.).

Data from 20 young adults (6 men and 14 women) ranging in age from 18 to 32 years old (mean, 20.49 ± 3.46 years) were retained for analysis. These 20 participants were asked whether they were right-arm or left-arm dominant, and all indicated they were right-arm dominant. Data from 20 other participants were excluded (seven because they could

not complete eyes-open and blind training criteria in the time allotted, five because they did not reach slow-wave sleep, seven because slow-wave sleep was too brief such that fewer than 16 sounds were presented, and one because the participant reported hearing the sounds during sleep). Hearing sounds was deemed inappropriate here given our focus on sleep-based reactivation. Several factors may have contributed to suboptimal sleep in some individuals, including the unfamiliar environment, physiological monitoring, time of day, and the sound presentations. Rules for deciding which data to discard were determined in advance; we required that participants succeed with the training and then sleep well enough for completion of the experimental manipulation, cueing half of the movements with the corresponding sounds twice each during slow-wave sleep. Failure to meet training or sleep criteria led to termination of data collection at that step. Given these procedures, generalization of the results may be limited (e.g., to individuals from the same sort of community we sampled, under some sleep deprivation, capable of learning precise muscle control, and able to achieve sustained slow-wave sleep during a nap).

Experimental design and statistical analysis

Skill learning was assessed using the myoelectric computer interface (MyoCI) task (Radhakrishnan et al., 2008), adapted from studies of post-stroke motor rehabilitation (Wright et al., 2014; Mugler et al., 2019). As shown in Figure 1, performing this task required precise control of arm-muscle activity to move a cursor to various target locations. Muscle activation was recorded using electromyographic (EMG) sensors on each arm placed in four locations: biceps, brachioradialis, extensor digitorum, and flexor carpi radialis. The EMG envelope of each muscle was automatically mapped to one of the four cardinal directions. In this way, the level of activation recorded for each muscle moved the cursor in the corresponding direction. For each arm, the cursor moved as a vector sum of all four EMG recordings.

On each trial of this task, the participant attempted to control arm-muscle activity to move a cursor from the center of a screen to one perimeter target location. Initially shown as a red square, the target became green as soon as the cursor entered it. Moving the cursor to a target in a cardinal position required selective activity in one corresponding EMG channel, whereas reaching a diagonal target required activity in a pair of EMG channels. During the training period, participants learned to independently control individual muscles as well as to co-activate pairs of muscles. Isolating the target-specified muscles but not activating ones mapped in other directions was integral to task success. A degree of activation in non-target muscles was tolerated, as long as relative amplitude in specified muscles was much greater than in incorrect ones. The edge of the screen limited cursor movement and eliminated overshooting targets.

To succeed on each trial, the participant needed to move the cursor to the target location within a 20-s time limit and hold it within the target area for 500 ms, a requirement determined from prior studies (Wright et al., 2014) and through piloting to be sufficiently challenging. Once the target was reached, if the cursor moved outside the target area before the requisite hold duration, the trial was ended and counted as a failure. In this case, the target box turned red and a buzzer sound played to indicate failure and the end of the trial. A unique sound was associated with each of the 16 targets, functioning both as a cue to move to a specific target and as feedback. Successful completion caused the sound to play once more, reinforcing that the appropriate motor action was performed. We did not instruct participants to move the cursor quickly but did instruct them to avoid the two types of failures: when the cursor left the target area prematurely and when the target was not acquired within the 20-s time limit. Before the next trial could start, the participant was required to relax muscles to bring the cursor back to center.

Training and testing on the task were conducted first for all targets for the dominant arm, and then for all targets for the nondominant arm. Mappings of cursor directions to muscles were the same in both arms. At the beginning of each trial, the yellow cursor and a red target appeared onscreen and a 1-s target sound was played. The 16 unique sounds were comprised of a diverse set of easily identifiable sounds,

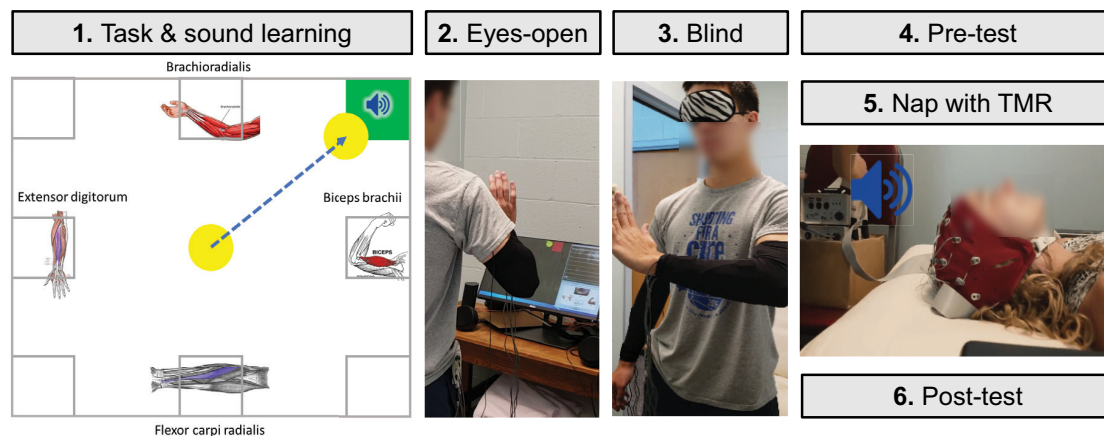
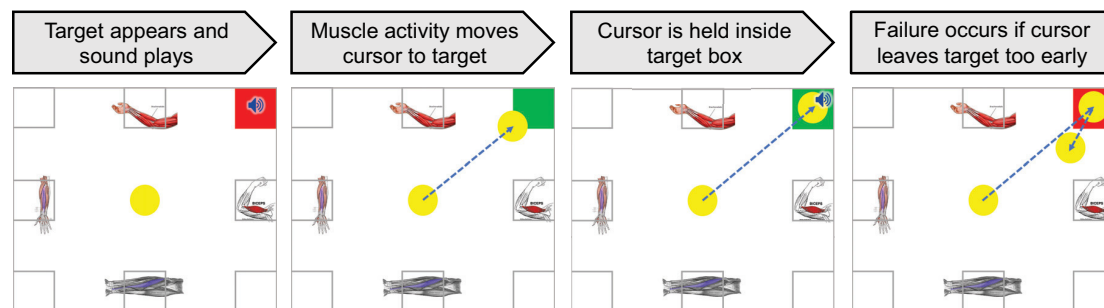
A**B**

Figure 1. Study design. **A**, Phases of the protocol were as following. (1) Muscle activity was translated to movement of an onscreen cursor. In this schematized example, the upper right target appeared with a corresponding sound signaling the participant to move the cursor from the center to the target by selectively activating two muscles. Gray square outlines show the eight target locations here, but participants saw only a single target on each trial. Likewise, participants did not see the muscle drawings shown here; they had to learn which muscle was associated with each direction. (2) Training required learning to move the cursor to eight target locations with each arm. (3) Training continued with a mask over the eyes to learn to perform the task using auditory information alone. (4) The pre-test was also conducted with only auditory instructions and feedback. Participants were tested on 16 targets (8 for each arm) with 8 trials per target. (5) A nap period included TMR for half of the targets, 4 on each arm. (6) The post-test was identical to the pre-test. **B**, Task design: at trial start, a target box appears in red with accompanying sound. Activity from muscles mapped to the up and right directions moves a cursor in the form of a yellow circle from the center diagonally to the target. Upon entry, the target turns green and the cursor is required to be held inside the box. If done so for a duration of 500 ms, the target sound plays to indicate hold success. If the cursor leaves the box before the requisite duration, the box turns red and buzzer sounds play to indicate hold failure.

including musical sounds (e.g., piano, drums), animal sounds (e.g., bark, meow), and object sounds (e.g., bell, chimes), and were randomly assigned to each target, eight for each arm, uniquely for each participant.

Participants completed three stages of training. First, they were instructed to learn the associations between the eight sounds and their respective target locations for each arm (Fig. 1A, phase 1). This was done while practicing the MyoCI task. Once participants indicated that all associations were learned, we tested memory by presenting each sound twice and requiring them to point to the appropriate target with the appropriate arm. If there were any errors, learning and testing repeated until responses were 100% correct (mean duration, 47.90 ± 4.05 min).

Participants then continued to perform the MyoCI task for all targets in training blocks consisting of four trials per target presented in pseudorandom order (Fig. 1A, phase 2). In a given block of trials, if three out of four trials for a target were successful, that target was removed from future training blocks. Training continued until this criterion was met for all targets (mean duration, 40.20 ± 2.52 min).

In the final training stage, participants performed the task guided by the auditory stimuli alone, with a mask occluding vision (Fig. 1A, phase 3). Learning in this fashion ensured that target sounds could trigger memory for the associated motor programs, as required during sleep. In the absence of visual cues, target sounds alone indicated the muscle or muscle combination to be engaged, the trial start, and successful completion, with the buzzer for trial failures. Blinded training was performed in

blocks of three trials per target for all targets. In a given block of trials, once two out of three trials for a target were successful, that target was removed from future training blocks. Blinded training concluded when all targets passed criterion (mean duration, 59.65 ± 7.04 min). Through the first three learning blocks before testing (Fig. 1A, phases 1–3, lasting ~ 2.5 h in total), participants learned to respond to sound cues with the appropriate motor act, also memorizing which sound went with which act.

In the next phase, a test consisting of 128 auditory-only trials (eight trials per target) was administered. Sleep was flanked by a pre-test and a post-test, both of which were conducted with the blindfold (Fig. 1A, phases 4–6). Based on the pre-test results, eight target sounds for TMR, four on each arm, were chosen by a computer algorithm as follows. First, the hold success rate for each target was separately quantified. Then the targets were ranked as a function of success rate, separately for each arm, with time to target used in the event of a tie. The targets in these ranked lists were then assigned to the cued and uncued conditions in an alternating fashion (first to cued, second to uncued, third to uncued, fourth to cued, and so on). With this stratification procedure, pre-test trial success was matched between the two conditions.

Following the pre-test, electrodes for polysomnography were applied and a futon was prepared to facilitate a daytime nap inside the study room. Lights in the room were turned off and the participant was given a 90-min nap opportunity. Sounds were played from a speaker with a 5-s

interval between sound onsets, beginning when the experimenter determined that the participant had reached slow-wave sleep (NREM stage 3; criteria are specified below, Polysomnography). Administration of sound cues and of concurrent time markers recorded with the EEG data were controlled using PsychoPy software (Peirce et al., 2019). Sound intensity was gradually increased to minimize sleep disruption. The final level ranged from ~40 to 50 db SPL. If sleep disruption was detected, stimulation was momentarily paused, and if stimulation was resumed, intensity was again gradually increased to the final level. At the end of the nap period, lights were turned on. However, awakening was delayed if slow-wave sleep continued, so some nap periods lasted longer than 90 min. A 15-min interval was given to remove the EEG cap and allow a bathroom break. Any lingering sleep inertia would likely have dissipated quickly during task performance, which promoted both physical and mental engagement while standing and moving. A post-test (identical to the pre-test) was administered to conclude each session.

We planned to compare performance changes for cued versus uncued items for a total of 20 subjects who met all experimental criteria (see above, Subjects). Our primary aim was to test whether cueing benefited execution of motor skill as measured by hold success rate, time to target, path length, and muscle-specificity (measures described below in Results). Statistical tests included repeated-measures ANOVAs using within-subject factors of cue condition (cued vs uncued items) and test (pre-test vs post-test) or, identically, a paired-samples *t* test comparing Δ cued items versus Δ uncued items. We were also interested in whether the number of cues or amount of slow-wave sleep, as well as arm laterality, influenced cueing benefit and used linear regression to test the significance of these predictors.

EMG processing

EMG was recorded using a Delsys Bagnoli 8 system interfacing with customized software based on the BCI2000 platform. Four active surface EMG sensors were placed on each arm above the biceps, extensor digitorum, brachioradialis, and flexor carpi radialis muscles. Bipolar signals, recorded at a sampling rate of 1000 Hz, were bandpass-filtered from 20 to 500 Hz (fourth-order Butterworth filter) and smoothed using a 100-ms moving root-mean-square (RMS) window following standard recommendations from Konrad's EMG manual (Konrad, 2005). The EMG envelope from each channel was mapped to a component of cursor movement in one of the four cardinal directions. The cursor position was mapped to the vector sum of all components. Participants stood with ample room to extend both arms, allowing for a wide range of arm movements. Onset time was defined as the delay from the trial start cue to movement onset. We defined the onset of movement as the point when EMG activity exceeded a threshold of 5% peak RMS activation using the same guidelines (Konrad, 2005).

Polysomnography

Data were recorded during the nap using a Biosemi ActiveTwo system with a sampling rate of 512 Hz. Thirty-two scalp EEG channels were referenced offline to the average of left and right mastoid recordings. A horizontal electro-oculogram (EOG) electrode was placed lateral to the right eye and a vertical EOG electrode was placed below the left eye. An EMG electrode was placed on the chin. Recordings were bandpass-filtered from 0.3 to 50 Hz.

The experimenter monitored these polysomnographic recordings during the nap to determine when to present sound cues, relying on sleep scoring rules from the American Academy of Sleep Medicine (AASM) manual (Berry et al., 2012). All 32 scalp EEG channels plus mastoid, eye, and chin channels were continuously updated onscreen. The *y*-axis scale was set to 50 μ V and gridlines were used to estimate peak-to-peak amplitude in frontal recordings, where δ activity could most easily be detected (e.g., Fz, Fp1, Fp2). Real-time classification of slow-wave sleep was performed by evaluating whether at least 20% of any continuous 30-s period included 0.5- to 2-Hz frequency oscillations with peak-to-peak amplitudes of at least 75 μ V. When this threshold was met, sounds were played until the experimenter observed a reduction of slow-wave activity below this level, such as because of momentary arousal from slow-wave sleep.

Table 1. Sleep stages averaged across participants

| Sleep stage | Mean \pm SEM (min) | Range (min) |
|-----------------|----------------------|-------------|
| Wake | 14.4 \pm 2.9 | [5.0, 47.5] |
| NREM stage 1 | 10.1 \pm 2.3 | [0.8, 37.2] |
| NREM stage 2 | 27.6 \pm 3.6 | [8.0, 55.5] |
| Slow-wave sleep | 29.2 \pm 3.5 | [7.0, 58.8] |
| REM | 0.8 \pm 0.4 | [0.0, 6.5] |

Accordingly, a quick determination of transitions in and out of slow-wave sleep was required, guided by AASM rules and expertise gained from many hours of observing sleep dynamics.

After data collection was completed, two independent coders manually classified sleep stages offline in 30-s epochs from the recorded EEG using MATLAB and sleepSMG software. Inter-coder reliability for sleep staging was 88%. Reported time spent in each stage was calculated from averaging the two reports. Conflicts were resolved by a third trained sleep scorer. Data from formal sleep scoring, which is restricted to pre-set periods of 30-s and allows checking subsequent periods before deciding on a score, was then used to determine the accuracy of online scoring for slow-wave sleep. Accordingly, 99.8% of sounds were cued within an epoch coded as slow-wave sleep offline. Some variation in sleep scoring can arise, such as because of where 30-s epochs happen to fall with respect to polysomnographic signals. Nevertheless, clear patterns of slow-wave sleep were generally observed in participants and used to systematically guide stimulus presentation.

Results

The duration of sleep across the group of 20 participants averaged over 1 h (mean \pm SEM, 67.7 \pm 4.4 min; range, 21.0–109.3 min). Average time spent in each stage of sleep is shown in Table 1. Eight of the sounds were quietly played during slow-wave sleep, as assessed via online sleep scoring. Each sound was presented on average 14.7 \pm 2.6 times. For within-subject comparisons discussed below, we calculated standard error of the mean using the Cousineau-Morey normalization method (Morey, 2008) to remove overall between-subject variability. These error values are shown in figures as error bars and correspond with error values used in statistical tests for the within-subject differences that are the focus of this experiment (e.g., cued vs uncued actions).

Cues during sleep improved task performance

MyoCI performance was tested before and after the nap. We quantified performance using time to target, path length, hold success, and speed. Time to target was defined as the time from sound cue onset to target acquisition. Successful and failed trials were included in this computation, but not trials in which the target location was not reached within the 20-s time limit.

As shown in Figure 2, responses were 142 ms faster in the post-test than in the pre-test for cued targets and 166 ms slower in the post-test than in the pre-test for uncued targets. Changes in time to target were significantly influenced by cue condition (2×2 repeated-measures ANOVA, significant cue condition \times test interaction, $F_{(1,19)} = 5.51$, $p = 0.030$). Differences between cued and uncued conditions were not apparent on the pre-test (cued, 3.77 \pm 0.10 s; uncued, 3.88 \pm 0.14 s; paired $t_{(19)} = 0.71$, $p = 0.49$), as expected given the stratification procedure by which targets were assigned to condition. Likewise, we found no evidence that cueing benefitted one arm over another (Δ cued dominant, 45 \pm 193 ms; Δ uncued dominant, 407 \pm 137 ms; Δ cued non-dominant, -346 \pm 174 ms; Δ uncued non-dominant, -70 \pm 166 ms; 2×2 repeated-measures ANOVA, nonsignificant cue condition \times arm type interaction, $F_{(1,19)} = 0.048$, $p = 0.83$). Cueing

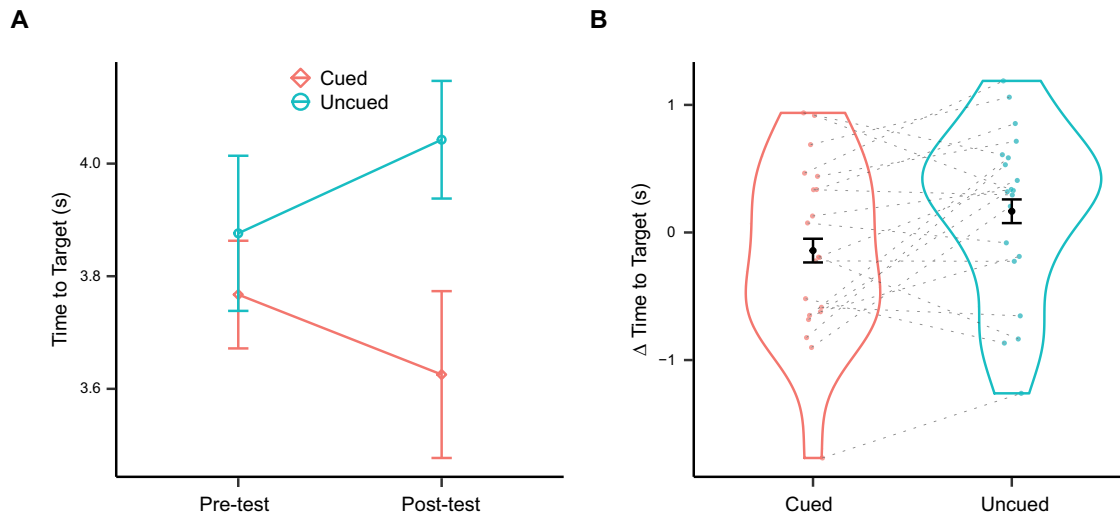


Figure 2. Cues during sleep improved performance time. **A**, A decrease in time to target was observed for actions cued during sleep, whereas performance slowed for uncued actions. **B**, Violin plots show each subject's change in time to target (post-test minus pre-test) for both cued and uncued conditions. A cueing benefit was seen in the majority of participants. All error bars represent within-subject standard error of the mean.

benefit was not dependent on number of cue presentations (linear regression, $R^2 = 0.00086$, $F_{(1,18)} = 0.015$, $p = 0.90$) nor time spent in slow-wave sleep ($R^2 = 0.0020$, $F_{(1,18)} = 0.037$, $p = 0.85$).

Given that TMR influenced the time needed to move the cursor to the correct target, it is also of interest whether or not the path traversed by the cursor changed. An analysis of the distance traversed from center start to target entry showed that the path length of the cursor changed in a manner analogous to the change in time to target (Fig. 3). Normalized path length was defined on each trial as distance traveled by the cursor (in pixels) divided by shortest distance to the target (pre-test mean, 3.61 ± 0.32). Normalized path length decreased from pre-test to post-test by 0.29 (7.88%) for cued targets and increased by 0.47 (13.63%) for uncued targets. Changes in path length were significantly influenced by cue condition ($F_{(1,19)} = 11.11$, $p = 0.004$). Differences between cued and uncued conditions were not apparent on the pre-test (cued, 3.64 ± 0.12 ; uncued, 3.48 ± 0.11 ; paired $t_{(19)} = 1.06$, $p = 0.30$).

The hold success rate was $52.8 \pm 2.0\%$ on the pre-test and $52.5 \pm 2.1\%$ on the post-test. This level of success, slightly over half of the trials, is quite impressive given the high difficulty of the task, as participants could not view the screen used in the training procedure. Instead of seeing the cursor move and knowing its position relative to the target area, they relied only on sounds to know which muscles to contract or relax. Auditory feedback signaled success or failure, but there was no signal to indicate when the cursor reached the target area and needed to be held steady there. Success rate did not differ as a function of cue condition from pre-test to post-test (cued pre-test, $52.9 \pm 1.2\%$; cued post-test, $53.1 \pm 1.6\%$; uncued pre-test, $52.8 \pm 1.0\%$; uncued post-test, $51.9 \pm 2.0\%$; $F_{(1,19)} = 0.17$, $p = 0.68$). Cued and uncued item performance did not differ at pre-test (paired $t_{(19)} = 0.19$, $p = 0.85$). Of the unsuccessful trials, the cursor did not reach the target within the 20-s time limit $3.7 \pm 1.2\%$ of the time. In the remaining trials ($43.6 \pm 1.8\%$), participants did not hold the cursor within the target area for the requisite 500 ms. Thus, participants moved the cursor to the correct target area 96% of the time.

An examination of the average cursor speed in each trial (mean over trials, 1828 ± 93 pixels/s) also showed cueing effects ($F_{(1,19)} = 7.71$, $p = 0.012$). Comparing pre-test to post-test, cursor

speed decreased for cued targets (pre-test, 1863 ± 61 pixels/s; post-test, 1824 ± 48 pixels/s; paired $t_{(19)} = 0.71$, $p = 0.49$) and increased for uncued targets (pre-test, 1764 ± 43 pixels/s; post-test, 1862 ± 63 pixels/s; paired $t_{(19)} = 1.99$, $p = 0.06$). The benefit for cued targets thus included taking a more-efficient path to the target and reaching the target more quickly, but without succeeding more often and without moving the cursor with higher speed, perhaps because of a greater emphasis on careful control of the cursor.

Cues during sleep improved selective muscle control

Acquiring targets required activating necessary muscles while suppressing activity in the other muscles. We defined muscle specificity as the RMS EMG from target-specified muscle channels divided by the total RMS value of all four muscle channels (Fig. 4A,B). Cued targets were acquired with significantly more target-specific muscle activity after the nap (cued pre-test, $63.29 \pm 0.80\%$; cued post-test, $64.03 \pm 0.82\%$; uncued pre-test, $62.79 \pm 0.77\%$; uncued post-test, $62.05 \pm 0.72\%$; $F_{(1,19)} = 4.42$, $p = 0.049$). Sleep TMR appeared to have a small but positive effect on the ability to control muscles with specificity and precision.

Cues during sleep improved execution, not premovement preparation

The total time for each trial included the time from the onset of the sound to the beginning of the movement, the time of the movement, and the time holding the cursor within the target location. Successful and failed trials were included in this computation; timed-out trials were not included in calculating movement or hold durations because the target was never reached. Total trial duration (mean, 4.62 ± 0.08 s) decreased as a function of cueing (Δ cued– Δ uncued, -334 ± 136 ms; $t_{(19)} = 2.46$, $p = 0.024$). We independently quantified the length of each period: onset time (mean, 1064 ± 66 ms), movement time (mean, 2780 ± 102 ms), and hold time (mean, 348 ± 37 ms). During the onset time, participants were likely recalling the target associated with the sound, selecting the appropriate muscles to use, and initiating cursor movement. Differences as a function of TMR were not the same for all three components, as shown in Figure 4C (onset/movement/hold by cued/uncued interaction, $F_{(2,38)} = 6.128$,

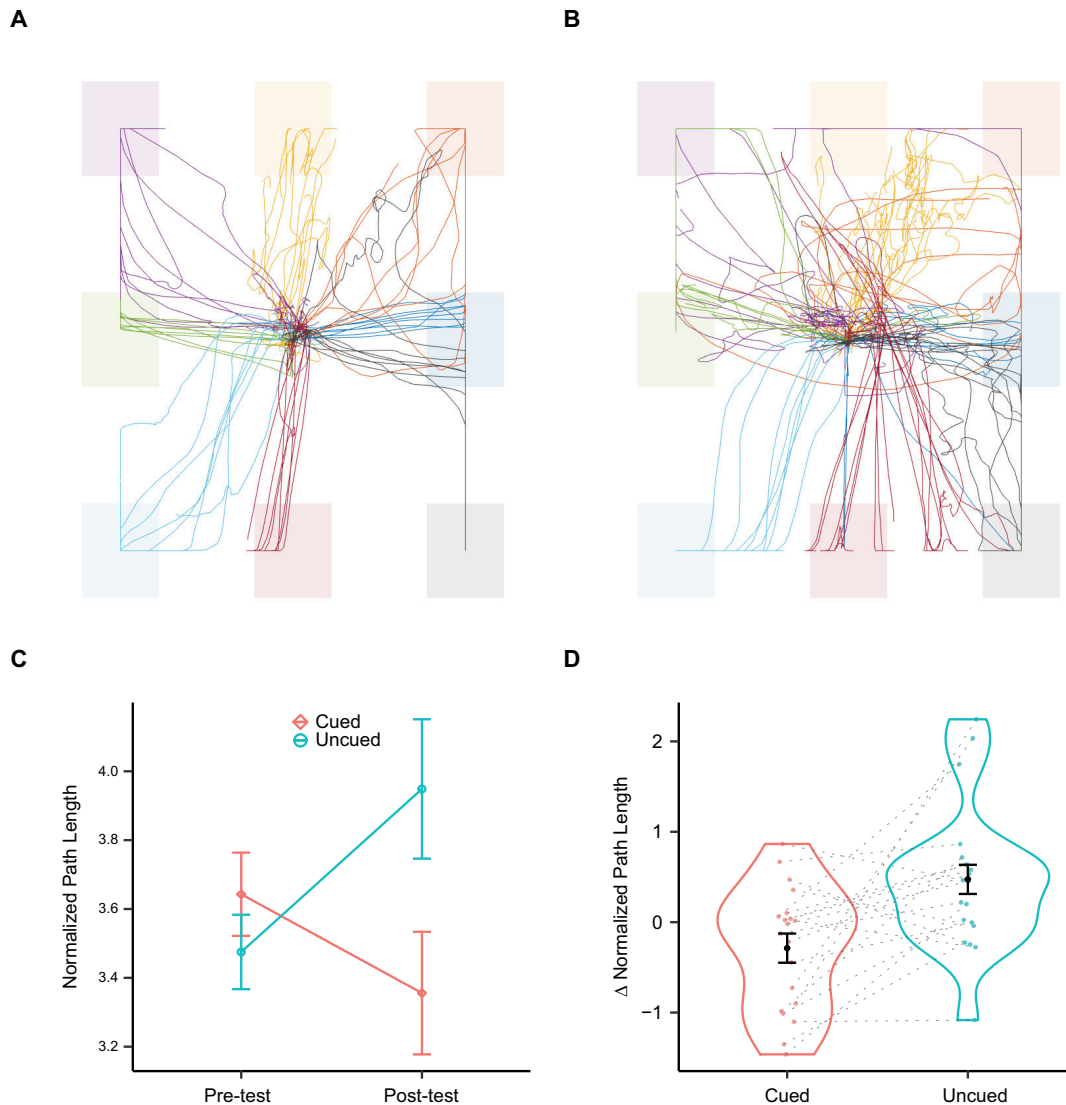


Figure 3. Cues during sleep improved movement efficiency. Representative examples of paths show that the path needed to reach the target differed between (*A*) cued versus (*B*) uncued targets. *C*, Normalized path length on the post-test decreased for cued targets and increased for uncued targets. *D*, Pre/post changes shown as Δ path length (post-test minus pre-test) were different for cued versus uncued targets for the majority of subjects.

$p = 0.005$). *Post hoc t* tests revealed that TMR reduced only the duration needed to execute cursor movement to targets (movement, Δ cued = -213 ± 129 ms; Δ uncued = 129 ± 128 ms; $F_{(1,19)} = 7.17$, $p = 0.015$), not onset time (Δ cued = 66 ± 88 ms; Δ uncued = 45 ± 77 ms; $F_{(1,19)} = 0.17$, $p = 0.69$) nor hold time (Δ cued = -7 ± 53 ms; Δ uncued = -15 ± 50 ms; $F_{(1,19)} = 0.47$, $p = 0.50$). These results suggest that sleep cues benefitted movement execution rather than the ability to recall and select the action.

Discussion

We showed that selectively reactivating a subset of motor-skill memories during sleep systematically enhanced motor execution. Specifically, a consequence of memory reactivation was an improvement in cursor movement quality for cued targets, whereas movement memories that were not reactivated showed deteriorated execution, likely because of forgetting over the interim. This improvement in cursor control was apparent in a relative reduction in both the time and the distance required to accomplish a successful movement. We also found improved

precision in muscle selectivity for cued targets compared with uncued targets. Finally, we found that the relative advantage for cued targets was because of improvement in motor execution rather than in premovement preparation. Taken together, these findings strongly indicate that execution-related aspects of motor skill can benefit from memory reactivation during sleep.

Prior studies of motor learning and sleep have predominantly emphasized learning finger-actuated sequences. Some studies considered action execution quality, focusing on motor adaptation in the form of visuomotor or force-field adaptation. The evidence for the importance of sleep in either sequence learning or motor adaptation has remained mixed, with evidence both for and against a role for sleep (Fischer et al., 2002; Walker and Stickgold, 2004; Krakauer and Shadmehr, 2006; Nemeth et al., 2010; Backhaus et al., 2016; Thüner et al., 2018). Given that most of the sleep consolidation literature on motor learning has focused on sequence learning, which prioritizes action-selection components, this literature has neglected the precision of action execution that is so important in many of the motor skills that people acquire throughout life. Here, we aimed to move beyond

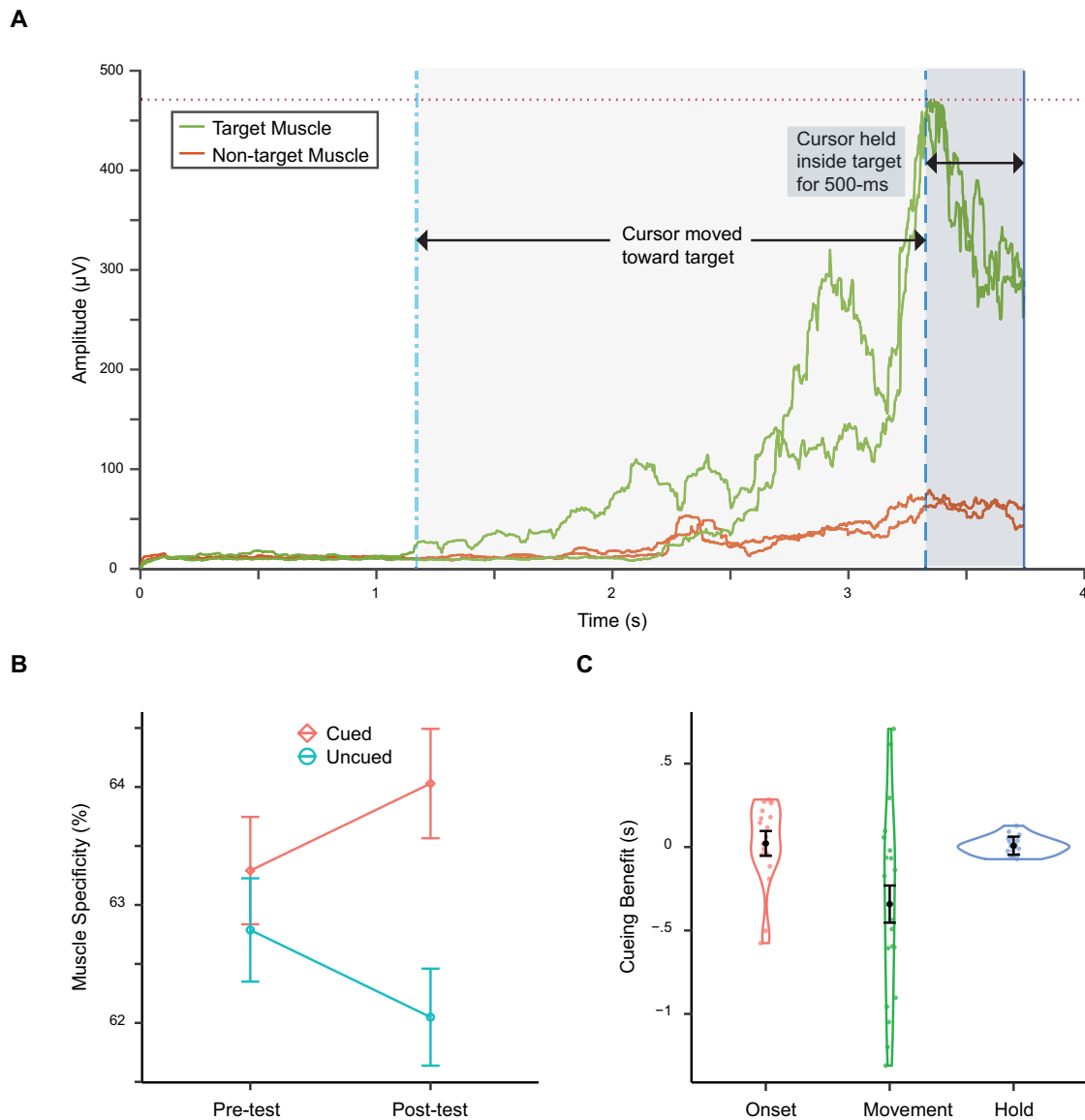


Figure 4. Cues during sleep enhanced muscle activity and motor execution. **A**, Activity in muscles specific to the target location (green lines) was necessary to acquire targets successfully. **B**, Muscle specificity improved slightly for cued items and worsened for uncued items. **C**, Sleep cueing did not impact all aspects of trial times equally. When cueing benefit (Δ cued– Δ uncued) was calculated for each trial component, only movement duration (movement) decreased, not the time needed to start movement (onset) or time spent maintaining stable activity to keep the cursor inside the target area (hold).

simple sequence performance to gain a broader understanding of the impact of sleep on motor learning. We also avoided over-learned actions, as in various skills that may already be highly familiar to participants, such as finger-tapping or ball-throwing (Johnson et al., 2019). Because the MyoCI task involved learning new visuomotor associations that combine muscle activity mapped non-intuitively to cursor direction, it was unlike any of the motor learning our participants had accomplished previously. Thus, the performance improvements observed here can be considered *de novo* learning, learning novel skills, for which there is a dearth of literature on TMR-related benefit (Hu et al., 2020). Learning to precisely control specific muscles is relevant to many real-world motor skills, including playing musical instruments or sports. We have also shown that learning to isolate specific muscles may improve motor function after stroke (Mugler et al., 2019).

By pairing sounds with skilled performance during training and then re-administering sounds during a subsequent sleep

period, we aimed to selectively reactivate memories supporting execution of specific motor skill learning. Most prior TMR studies focused on declarative memory, which is dependent on hippocampal and other cortical regions, or sequence learning, which is dominated by goal and action selection and is also at least partially dependent on hippocampal activity (Banquet et al., 2001; Poldrack and Rodriguez, 2003; Albouy et al., 2008; Lungu et al., 2014). The present findings expand the TMR literature by indicating that memory reactivation during slow-wave sleep can contribute to skill learning for a skill that emphasizes motor execution. Learning which sound goes with which action in the MyoCI task is likely hippocampal-dependent, whereas the precision learning of motor execution in the task most likely depends on networks in the motor cortex, basal ganglia, and/or cerebellum (Hikosaka et al., 2002; Hardwick et al., 2013). We thus speculate that these non-hippocampal motor networks were responsible for TMR benefits in the present study. This link with memory reactivation during sleep complements evidence of

sleep replay in the motor cortex after reaching (Ramanathan et al., 2015) and after brain-machine interface (Gulati et al., 2014) tasks.

Many questions remain about how memories are normally reactivated during sleep and about how auditory TMR engages reactivation to produce memory benefits. A reasonable scenario is that the sound cues provoke reactivation related to participants' experiences with the cues, particularly the motor-execution processing for targets associated with the sounds. Memory reactivation would thus be biased toward those motor actions, and perhaps away from other motor actions, during the sleep period.

An important consideration in studies of motor skills is whether any observed gains following sleep could be explained by recovery from fatigue rather than by consolidation. The present study avoids this challenging issue because comparisons were not based on the presence of sleep, such that differential fatigue cannot explain the performance advantage for cued targets compared with uncued targets. Furthermore, because we did not contrast the influence of a period of sleep versus a period of wake, our conclusions do not concern whether sleep is essential for consolidation. Perhaps improved motor learning would be found with relevant memory reactivation during either wake or sleep. Our results nevertheless show that memory reactivation during sleep can contribute to motor learning.

Indeed, sleep physiology may produce unique benefits. The neural mechanisms responsible for the changes we observed cannot yet be fully specified. Early studies brought forth the possibility that specific sleep stages may be linked to different memory systems, with REM linked to motor consolidation and NREM linked to verbalizable memories (Plihal and Born, 1997). However, no consensus has been reached in the intervening years regarding the dichotomy of slow-wave sleep and REM as a function of type of memory (Ackermann and Rasch, 2014). Building on prior studies (Antony et al., 2012; Johnson et al., 2019), we corroborate the notion that motor learning can benefit from memory reactivation during slow-wave sleep. Still, the magnitude of benefits we observed was unrelated to the quantity of slow-wave sleep, and REM was mostly non-existent during these daytime naps, precluding definitive statements about sleep-stage specific processing. Furthermore, sleep dynamics across participants may have been influenced by variability in study start times, altering homeostatic sleep pressure and circadian timing. The present study may thus not have been optimally designed for establishing links between specific features of sleep physiology and motor-learning benefits because of memory reactivation.

In summary, the present findings support the conclusion that execution-based components of motor skill can be reactivated during sleep, resulting in enhanced performance after awakening. By extension, activating motor control networks during sleep may be an integral part of the mechanism for consolidation of motor skills. Furthermore, these findings open the door to future applications of TMR to augment learning of a wide variety of motor skills. Nightly TMR may even be useful in a clinical context to supplement daily rehabilitation efforts for patients hoping to decrease motor impairments because of stroke or neurologic dysfunction.

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