Dual enhancement mechanisms for overnight motor memory consolidation

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Our brains are constantly processing past events. These offline processes consolidate memories, leading in the case of motor skill memories to an enhancement in performance between training sessions. A similar magnitude of enhancement develops over a night of sleep following an implicit task, in which a sequence of movements is acquired unintentionally, or following an explicit task, in which the same sequence is acquired intentionally. What remains poorly understood, however, is whether these similar offline improvements are supported by similar circuits, or through distinct circuits. We set out to distinguish between these possibilities by applying transcranial magnetic stimulation over the primary motor cortex (M1) or the inferior parietal lobule (IPL) immediately after learning in either the explicit or implicit task. These brain areas have both been implicated in encoding aspects of a motor sequence and subsequently supporting offline improvements over sleep. Here we show that offline improvements following the explicit task are dependent on a circuit that includes M1 but not IPL. In contrast, offline improvements following the implicit task are dependent on a circuit that includes IPL but not M1. Our work establishes the critical contribution made by M1 and IPL circuits to offline memory processing, and reveals that distinct circuits support similar offline improvements.

A memory continues to be processed after its formation. These ‘offline’ processes consolidate a memory, which can lead in the case of motor skill memories to their enhancement during sleep. Offline improvements of a similar magnitude develop following an implicit task, in which a sequence of movements is acquired unintentionally, or following an explicit task, in which the same sequence is acquired intentionally. Similar circuits may support these similar improvements. Alternatively, distinct circuits may support these improvements because they are triggered by different learning tasks. Distinguishing between these possibilities will provide insight into the organization of offline processing.

During learning, a memory for a skill is encoded across a network of brain areas. Included within this network are the primary motor cortex (M1) and the inferior parietal lobule (IPL). Subsequent offline brain activity is perhaps, at least in part, due to the processing of the representations encoded during learning. The pattern of neuronal activity during the formation of a motor memory is replayed during sleep in the rat motor cortex, and this neuronal replay is correlated with the subsequent offline improvement in performance during sleep (see review). In humans, spindle activity in M1 during a nap is related to the subsequent improvements in motor skills. Similarly, slow-wave activity in the IPL has been related to overnight motor skill improvements. Thus, M1 and IPL circuits have both been implicated in the encoding and subsequent offline processing of memories.

Potentially, M1 and IPL may operate together as a unified circuit critical for all improvements over a night of sleep. In this scenario, there is no redundancy with only a single route to the offline enhancement of a memory. Alternatively, M1 and IPL may be part of functionally distinct circuits, providing alternative routes to offline enhancements of a memory. Consolidation would then not be linked to a particular circuit, but would be a property that emerges from any circuit. This is a more complex organization than a single circuit supporting overnight improvements of all motor skill memories; yet it might have the benefit of allowing multiple skills to be consolidated simultaneously during sleep. Despite showing the same magnitude of improvement over sleep, different memory tasks may trigger distinct offline processes. In sum, the circuits supporting consolidation may be determined solely by the behavioural expression of consolidation, for example an improvement; alternatively, they may be determined by the learnt task and its properties.

To distinguish between these possibilities, we had participants learn and be tested on either an implicit or explicit task. The implicit task was introduced to participants as a test of reaction time, whereas in the explicit version of the task, participants were made aware of the underlying sequence (Fig. 1). Immediately after learning, when the encoded representation is sensitive to disruption, transcranial magnetic stimulation (TMS) was applied over M1 or IPL. Subsequent offline processing in M1 or IPL circuits may be critically dependent on this encoded information. As a consequence, disrupting this information will impair offline improvements and reveal the importance of these circuits to offline processing. We applied stimulation at 1 Hz for 10 minutes (600 pulses), which commonly leads to a decrease in cortical excitability and has been widely used to disrupt a diverse array of cognitive processes in humans. Following stimulation, participants remained awake for several hours, and then slept. After the night of sleep, we retested participants’ performance. The difference between performance at testing and retesting provided a measure of offline improvements (Fig. 1).

We found no significant difference in participants’ initial skill across the groups (analysis of variance (ANOVA), F(3,47) = 0.226, P = 0.878). The number of errors made by participants during testing also did not differ significantly across the groups (ANOVA, F(3,47) = 0.557, P = 0.646). There was also no significant difference in time asleep, time awake, sleep efficiency or the proportion of time spent in the various sleep stages (N1, N2, slow-wave sleep (SWS) and rapid eye movement (REM)) across the groups (Table 1; multivariate analysis of variance (MANOVA), F(24,105) = 0.890, P = 0.614). Nonetheless, we found that offline improvements differed significantly depending on the site of stimulation and the type of learning task (that is, site x task interaction; repeated measures ANOVA, F(1,47) = 9.945, P = 0.003; Fig. 2). This interaction was
not significant for the change in error between testing and retesting (ANOVA F(1,47) = 1.473, P = 0.231).

Offline improvements following learning in the implicit task were significantly less following IPL than following M1 stimulation (repeated measures ANOVA, F(1,24) = 4.5, P = 0.044). We found no significant offline improvements following IPL stimulation (mean ± s.e.m., 75 ± 9 ms versus 70 ± 6 ms; paired t-test, t(13) = 0.521, P = 0.611), whereas there were significant offline improvements following M1 stimulation (70 ± 6 ms versus 92 ± 9 ms; paired t-test, t(11) = 3.188, P = 0.009). These improvements were not correlated with the percentage of total sleep spent in either REM or SWS, which is consistent with their developing independently of sleep (for further analysis, see Fig. 2; REM, R = 0.289, F(1,8) = 0.728, P = 0.418; SWS, R = 0.034, F(1,8) = 0.009, P = 0.925)12,18,21. We also found significant offline improvements following sham stimulation (69 ± 14 ms versus 98 ± 11 ms; paired t-test, t(7) = 2.73, P = 0.03), and these were significantly greater than following IPL stimulation (unpaired t-test, t(20) = 2.16, P = 0.042). Thus, applying TMS to IPL but not M1 represented the development of offline improvements in the implicit task.

By contrast, offline improvements following learning in the explicit task were significantly less following M1 than IPL stimulation (repeated measures ANOVA; F(1,23) = 6.09, P = 0.021). We found no significant offline improvements following M1 stimulation (82 ± 16 ms versus 75 ± 15 ms; paired t-test, t(11) = 0.832, P = 0.423), whereas there were significant offline improvements following IPL stimulation (82 ± 17 ms versus 99 ± 18 ms; paired t-test, t(12) = 2.979, P = 0.011). These improvements were correlated with the percentage of total sleep spent in SWS, which is consistent with their being dependent on sleep (for further analysis; see Fig. 2; R = 0.578, F(1,10) = 5.51, P = 0.04)12,18,21. Offline improvements also developed following sham stimulation (77 ± 11 ms versus 99 ± 13 ms; paired t-test, t(7) = 2.78, P = 0.027), and these were significantly greater than following M1 stimulation (unpaired t-test, t(18) = 2.42, P = 0.026). Thus, applying TMS to M1 but not IPL prevented the development of offline improvements in the explicit task. Overall, different mechanistic routes, one IPL-dependent and at the other M1-dependent, lead to the development of offline improvements during sleep.

We applied TMS after learning for three important reasons. First, by applying TMS immediately after learning, we prevented stimulation from affecting the initial learning and formation of a memory. Second, a memory is frequently most sensitive to disruptive interventions such as TMS or protein synthesis inhibitors immediately after learning22. Finally, applying TMS after learning minimalized the potential for stimulation to affect subsequent sleep. Brain stimulation can affect sleep when it is applied immediately before or during sleep13–15. By contrast, in the current study several hours passed between applying TMS and the onset of sleep. We found no difference in the length of sleep or the proportion of time in each sleep stage across the groups. Thus, there was no site- or task-specific effect of stimulation on sleep, and so the current pattern of results cannot be explained by TMS affecting sleep. Overall, we applied stimulation at a time when it would affect memory consolidation and not formation, when the memory was highly sensitive to disruption, and when there was a minimal chance of stimulation affecting subsequent sleep.

Our results reveal a double dissociation. Following learning in the explicit task, a circuit containing M1, but not IPL, is critical for the development of improvements over sleep. By contrast, following learning in an implicit task, a circuit containing IPL, but not M1, is critical for the development of improvements over sleep. In both these tasks, improvements over sleep are of a similar magnitude. Thus, these results establish a degenerate organization for human memory processing, with distinct networks responsible for the same magnitude of enhancement.

The different tasks may trigger different behavioural patterns of consolidation. Improvements typically only develop over sleep, but following learning in the implicit task, improvements have also been reported to develop during wakefulness19. We examined the development of offline improvements following learning in the implicit and explicit tasks. In an additional group, participants’ skill was tested, and then retested at 8 p.m., after a 12-hour interval of wakefulness. We found no significant difference in initial skill at testing following learning in the implicit and explicit tasks (unpaired t-test, t(20) = 0.181, P = 0.858). Yet offline improvements differed significantly between the two tasks (repeated measures ANOVA, F(1,20) = 6.08, P = 0.023). Following learning in the implicit task, there were significant improvements between testing and subsequent retesting during wakefulness (72 ± 9 ms versus 100 ± 9 ms paired t-test, t(11) = 2.97, P = 0.013), whereas, following learning in the explicit task, there were no significant improvements (74 ± 11 ms versus 62 ± 13 ms paired t-test, t(9) = 0.887, P = 0.398). Overall, improvements following the implicit task develop during wakefulness, and as shown in the earlier experiments they also develop during sleep, which is a time-dependent pattern. By contrast, improvements in the explicit task do not develop during wakefulness, and instead only develop over a night of sleep, which is a sleep-dependent pattern.
but also during wakefulness. At least 4–5 hours between testing and retesting is required in the implicit task for the development of offline improvements\(^2\)\(^\text{19}\). By contrast, the explicit task shows a sleep-dependent pattern with improvements developing exclusively during sleep. Other similar tasks in which participants also intentionally learn a sequence of movements, such as a finger tapping task, show improvements over sleep\(^16\)\(^\text{17}\) (see also ref. \(^1\)). Improvements can develop rapidly within 5–30 minutes of rest after learning a finger tapping task, but these improvements are transient, whereas sustained offline improvements develop only during sleep\(^16\)\(^\text{18}\). In this explanation, time- and sleep-dependent improvements are not merely descriptions of when improvements develop, but are mechanistically distinct, describing different processing routes. One route is dependent on an IPL circuit and the other dependent on an M1 circuit.

Sleep- and time-dependent consolidation are triggered by different tasks. As a consequence, any explanation for the different circuits supporting offline improvements may lie with the tasks. Information is encoded during learning of these tasks. Applying TMS immediately after learning disrupts that information, which reveals its importance and that of the targeted circuits (M1 and IPL) to subsequent offline processing. Thus, by understanding the information encoded during learning it may be possible to explain the differential contribution of M1 and IPL circuits to subsequent consolidation of the implicit and explicit tasks.

The implicit task has multiple components\(^18\). One of these components, the goal of the movement, is enhanced over sleep, and this aspect of the memory is encoded during learning in a circuit that includes IPL\(^1\)\(^\text{3}\)\(^\text{14}\). As a consequence, applying TMS to IPL immediately after learning prevents improvements from developing over a night of sleep. Consistent with the importance of IPL for improvements over sleep, an increase in slow-wave power occurs over the parietal cortex after learning a visuomotor task\(^1\). The perturbation that participants adapt to in this task is introduced slowly over a

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**Figure 2 | A double dissociation between site of stimulation and subsequent offline improvements in different learning tasks.** We found that offline improvements differed significantly depending on the learning task (implicit versus explicit) and the site or type of stimulation (TMS to IPL versus M1 or sham; ANOVA \(F(2,61) = 5.259, P = 0.008\)). But there was no significant difference across these groups at testing (skill; ANOVA, \(F(5,61) = 0.204, P = 0.959\); bar plots display mean ± s.e.m.). a, Improvements developed over a night of sleep in the implicit task after sham stimulation (paired \(t\)-test, \(t(7) = 2.73, P = 0.03\)). b, Improvements no longer developed after applying TMS to IPL (paired \(t\)-test, \(t(3) = 3.188, P = 0.009\)). c, In contrast, improvements continued to develop after applying TMS to M1 (paired \(t\)-test, \(t(7) = 3.188, P = 0.009\)). d, The improvements that did develop were not correlated with the percentage of sleep spent in SWS in both a standard (\(R = 0.034, F(1,8) = 0.009, P = 0.925\) and robust regression \((P = 0.443)\), which is consistent with the improvements in the implicit task being time dependent, rather than related to a brain state such as sleep. There continued to be no significant correlation even when the participant showing the greatest offline improvement was removed from the analysis (\(R = 0.430, F(1,7) = 1.587, P = 0.248\)), and even without this participant there continued to be significant offline improvements (paired \(t\)-test, \(t(10) = 3.31, P = 0.009\)). e, Improvements developed over a night of sleep in the explicit task after sham stimulation (paired \(t\)-test, \(t(7) = 2.76, P = 0.027\)). f, Improvements also developed after applying TMS to IPL (paired \(t\)-test, \(t(13) = 3.222, P = 0.007\)). g, Improvements no longer developed after applying TMS to M1 (paired \(t\)-test, \(t(11) = 0.832, P = 0.423\)). There was no significant difference in sequence recall across the explicit task groups (sham versus IPL versus M1; ANOVA \(F(2,31) = 0.702, P = 0.503\)). h, The improvements following IPL stimulation were correlated with the percentage of total sleep that participants spent in SWS in both a standard \((R = 0.596, F(1,10) = 5.51, P = 0.04)\) and robust regression \((P = 0.0038)\), which is consistent with the improvements in the explicit task being sleep dependent. Only those participants with complete high-quality sleep recordings are shown in each of the correlations d and h. ∆, difference; s*, significant difference; ns, no significant difference.
number of trials, and so, as for the sequence in the implicit task, participants are unlikely to be completely aware of what they are learning. Subsequent improvements in this type of task, like the implicit task in the current study, develop during both wakefulness and sleep\textsuperscript{46}. Thus, converging evidence shows that an IPL circuit is critical for improvements during sleep. The component encoded within the M1 circuit, is enhanced during sleep, and so the IPL circuit, and not the M1 circuit, is critical for improvements during sleep.

The explicit task, unlike the implicit task, only shows improvements during sleep\textsuperscript{2,39}. An inhibition of M1 prevents offline improvements from developing during wakefulness\textsuperscript{48}. Preventing inhibition of the M1 circuits is sufficient to induce improvements during wakefulness, and likewise, all that may be necessary for improvements during sleep is the information within M1 circuits\textsuperscript{48,39}. Consistent with this idea, we show that applying TMS over M1, but not over IPL, prevents improvements from developing during sleep.

**Figure 3 | Response time changes during learning in the different tasks.** The task was introduced to participants as either a reaction time task (implicit task) or a sequence learning task (explicit task). Despite these different instructions, both tasks required participants to acquire skill at performing a sequence of movements. Following learning, TMS was applied to either M1 or IPL. a. During the initial training block, we found a significant change in response time during the sequential trials ($F(1,46.89) = 25.53, P < 0.001$), which did not differ significantly across the site of stimulation and learning tasks ($F(1,46.89) = 2.9, P = 0.093$). b. We found a similar pattern during the subsequent training block: a significant change in response time during the sequential trials ($F(2,92.139.2) = 14.1, P < 0.001$), which did not differ significantly across the stimulation sites and learning tasks ($F(2,92.139.2) = 0.229, P = 0.79$). c. Similarly, during the test block, there was a significant change in response time ($F(2.94) = 3.52, P = 0.033$), and again this was not significantly different across the sites and tasks ($F(2.94) = 0.252, P = 0.778$). The difference in response time between the final epoch of sequential trials and the subsequent random trials also did not differ significantly across the different stimulation sites and tasks at the initial block ($F(1,47) = 2.02, P = 0.162$), the training block ($F(1,47) = 0.117, P = 0.734$) or the test block ($F(1,47) = 0.039, P = 0.844$)\textsuperscript{46,54}. Together, these different measures of learning suggest that similar learning took place across the groups (that is, the task x site interaction was not significant). d. We found no significant change in response time during the sequential trials of the retest block ($F(2.94) = 2.5, P = 0.09$), and this did not differ significantly across the sites of stimulation and tasks ($F(2.94) = 1.337, P = 0.249$). Yet when the random trials were introduced at the end of the block, there was a significant change in response time differed significantly across the different sites and tasks ($F(1,47) = 4.595, P = 0.037$). The change in response time provides a measure of skill specifically for the sequence\textsuperscript{46}. Overall, there was no difference in skill across the groups at testing, and little evidence of differences in learning during retesting, so the difference in skill across the groups at retesting is most likely to be due to the offline interval. The double vertical arrow shows the skill at testing (skill1; see c) and retesting (skill2; see d) in those groups that show offline improvements (see Fig. 2). At each block, there was a significant difference in sequential response time between the two tasks (for all four blocks: $F(1,49) > 7, P < 0.01$). The shade of each symbol shows the task (implicit versus explicit), its shape shows the site of stimulation (IPL versus M1) and its value is an epoch of trials (mean ± s.e.m.). For the random trials (highlighted in grey), each epoch is 50. To divide each set of sequential trials equally required a different epoch size for the implicit (60 trials) and explicit tasks (36 trials). These epoch sizes are used in the analysis provided in this legend, whereas in the main text, the change in response time between sequential and random trials was based on uniform epoch sizes of 50 trials. Regardless of the epoch size (50 versus 60 or 36 sequential trials), our results followed a similar pattern.
Other studies have also linked M1 to the development of offline improvements during sleep. For example, patterns of M1 functional activation change following overnight improvements in a task that, like the explicit task in the current study, involved participants intentionally learning a sequence of finger movements, which only showed improvements over sleep²,³,⁴,⁶. Thus, an M1 circuit seems to be critical for supporting improvements over sleep, which is consistent with the physiological changes associated with other sleep-dependent tasks. Information encoded within, and only within, the M1 circuit is critical for subsequent offline improvements, and so when this is disrupted (for example by applying TMS), improvements no longer develop.

Different circuits are critical for the offline processing of different tasks, despite those tasks showing similar offline improvements. Offline processing is related to the tasks learned, and in turn the consolidation processes triggered (that is, sleep- versus time-dependent), as opposed to being linked simply to the end result, in this case the performance enhancement of consolidation. Different circuits are supporting the same expression of consolidation, suggesting that, at least in principle, any circuit can support consolidation. With this degenerate organization, there are alternative routes to consolidation, which may explain the diminished interference between memories over a night of sleep⁵,⁶,16.19 (see reviews³,⁴). Participants practised and were tested on one of these motor skill tasks at 8 p.m. (skill). After learning, TMS was immediately applied to either M1 or IPL. Participants subsequently slept from 11 p.m. to 7 a.m., recorded using polysomnography (PSG), and their motor skill was retested the next day at 8 a.m. (skill). Their previous night’s sleep had also been recorded in the same sleep laboratory to allow them to adapt to sleeping in a new environment. The change in motor skill between testing and subsequent retesting provided a measure of offline change in motor skill. In sum, we used a 2(task; implicit versus explicit) × 2(site: M1 versus IPL) between-subject design to understand how applying stimulation to different sites (IPL versus M1) affected subsequent changes in skill between testing and retesting across different tasks (implicit versus explicit) over sleep.

In another set of experiments, we applied sham rather than real stimulation. As in the earlier experiments, participants learnt either the implicit or the explicit task, and had their skill tested (at 8 p.m.; skill) but, immediately after learning, sham rather than real stimulation was applied. Subsequently, participants slept overnight and had their skill retested the next day at 8 a.m. (skill). In the final set of experiments, we examined the development of offline improvements during wakefulness, as opposed to over a night of sleep. Following learning of either the implicit or explicit task, participants had their skill tested (at 8 a.m.; skill), and subsequently retested, 12 hours later (at 8 p.m.; skill). Examining improvements during wakefulness complemented the other experiments examining improvements over sleep. It allowed us to determine whether tasks showed improvements over any interval, or, in contrast, one or both of the tasks showed improvements restricted to a particular interval.

Participants. We recruited only right-handed participants (defined by the Edinburgh handedness questionnaire⁴¹), 18–35 years of age, with no medical, neurological or psychiatric history, and either normal or corrected to normal vision. All participants provided informed consent for the study, which was approved by the local institutional review board.

We randomly allocated 35 participants across the four groups of the 2(M1 versus IPL) × 2(implicit versus explicit) between-subject design. Four participants allocated to the implicit task groups were able to recall four or more of the twelve items of the sequence. This higher recall modifies the task so that it comes to resemble the explicit task. The higher recall may alter the properties of offline improvements, preventing them from developing while awake, and instead they develop exclusively during sleep⁴. Disrupting training on approximately four items of a twelve-item sequence allows improvements to develop over wakefulness and, in other earlier studies, excluding participants with a higher recall has allowed the implicit task to retain its capacity to develop improvements over both wakefulness and sleep⁴,²⁴. A recall of more than four items can modify the implicit task, the properties of offline improvements and potentially the circuits supporting those improvements. Thus, those four participants with a recall of four or more items were removed from further analysis. Of the remaining 51 participants (24 male, 20.9 ± 2.4 years; mean ± 1 s.d.), 12 were allocated to each of the two M1 groups, 14 allocated to learn the implicit task in an IPL group, and 13 to learn the explicit task in the final IPL group. Of the further 21 (6 ± 0.8 years; mean ± 1 s.d.) were recruited, equally distributed and randomly allocated between the two groups that received sham stimulation.

For the final set of experiments, 25 participants were recruited; of those, three allocated to the implicit group were able to recall four or more of the twelve items of the sequence, and so were removed from subsequent analysis. Of the remaining 22 participants (9 male, 20.4 ± 2.1 years; mean ± 1 s.d.), 10 were allocated to explicit task group, and the remaining 12 were allocated to the implicit task group. In both groups, participants were trained, tested and, following a 12-hour interval of wakefulness, retested on the respective task.

For each group, the numbers of participants that we recruited were similar to or higher than those used in earlier studies to detect offline improvements (see, for example, refs ²,²⁰). Participants did not know to which group they had been assigned. Nonetheless, it was necessary for the individual running the study to know the assigned group for the appropriate task and site of stimulation to be used. Final analysis was conducted with knowledge of group allocation.

**Motor sequence learning tasks.** We used a modified version of the serial reaction time task (SRT T)²⁴. A solid circular visual cue (diameter 20 mm, viewed from approximately 800 mm) could appear at any one of four possible positions, designated 1 to 4, and arranged horizontally on a computer screen. Each of the four possible positions corresponded to one of the four buttons on a response pad (Cedrus, RB-410), upon which the fingers of the participant’s right hand rested. If a target appeared on the pad, when a target appeared, participants were instructed to respond as quickly and as accurately as possible by pressing the appropriate button on the pad. If the participant made an incorrect response, the stimulus remained until the correct button was selected. Once the correct response was made, the cue on the screen disappeared and was replaced by the next cue after a delay of 400 ms. Response time was defined as the interval between presentation of a stimulus and selection of the correct response.

We used two versions of the SRT T. In one version, the explicit task, participants were introduced to the task as a sequence learning task. Participants were instructed that a change in the colour of the stimuli from black to blue heralded the beginning of a repeating sequence (2–3–1–4–2–3–4–2–1). The colour change was exclusive used to mark the introduction of the sequence, not its removal. This colour change marked the introduction of the sequence during all the training, test and retest blocks. Participants were told neither the sequence itself nor its length. By contrast, in the implicit task, participants were introduced to the task as a test of reaction time. So, in this task, participants were not told about the sequence, and there were no cues marking the introduction of the sequence. The stimuli did not change colour during this task, instead participants were instructed to respond as quickly and as accurately as possible by pressing the appropriate button on the pad. If the participant made an incorrect response, the stimulus remained until the correct button was selected. Once the correct response was made, the cue on the screen disappeared and was replaced by the next cue after a delay of 400 ms. Response time was defined as the interval between presentation of a stimulus and selection of the correct response.

To compare the offline improvements that develop following learning in the implicit and explicit tasks, we used a design that has been successfully applied in earlier work²,²⁴. Sequence learning can occur more quickly in the explicit than in the implicit task²⁴.²⁵. Acquiring only a small amount of skill during learning may be insufficient to trigger subsequent offline improvements⁶. Conversely, substantial learning, at least in principle, may prevent or impair further improvements, as the maximum, or close to the maximum, skill has already been achieved. Thus, it was important to ensure that similar amounts of skill were acquired in both tasks. As in earlier work, we achieved this by using a different number of trials in each task²⁴.

There was an initial short training block of either 15 (implicit task) or 9 (explicit task) repetitions of the sequence; the main training block had either 25 (implicit task) or 15 (explicit task) repetitions, and then the test block had the same number of repetitions as the initial short training block. Participants were tested and subsequently retested the next morning (approximately 12 hours later, at 8 a.m.) on the same task (implicit or explicit task). The retest block had the same number of repetitions as the earlier test block. The difference between skill at testing (skill), and retesting (skill) provided a measure of offline motor skill (skill–skill).

Fifty random trials preceded and followed the sequential trials in the training and test blocks of both tasks. Within the training trials, there were no item repeats (for example, 1−1−1 was illegal), and each item had approximately the same frequency of appearance. Each set of random trials in the training and test blocks
was unique. This minimized the chance that participants might become familiar with the random trials. Nonetheless, the same random trials were used within both tasks. As a consequence, performance of the motor sequence—which was common to both tasks—was compared against a common set of random trials. We administered a free recall test when participants had completed the SRTT, following retesting. For the explicit task, participants had already been told about the sequence and so were simply asked to recall as many items of the sequence as possible. For the implicit task, participants were asked whether they had noticed anything about the visual cues, and to describe that property. If participants had realised that there was a sequence, they were asked to recall as many items of the sequence as possible. Participants accurately recalling four or more items from the twelve-item sequence were removed from further analysis. Declarative knowledge for the sequence modified the task so that any improvements are no longer able to develop during wakefulness but only develop over a night of sleep (that is, offline improvements become sleep-dependent).2,3

Transcranial magnetic stimulation. Using a Magstim 2 Super Rapid (Magstim Inc), we applied TMS at 1 Hz for 10 minutes (600 pulses) at an intensity of 90% of motor threshold. These same parameters have been widely adopted to disrupt a diverse array of cognitive processes in humans.27,28 Using a stimulation intensity beneath motor threshold minimized the movements elicited at each site of stimulation, which makes it easier to compare the effects of stimulation between these sites. Stimulation was applied immediately after one of the motor sequence learning tasks, to either the left M1 or left IPL. The left M1 was identified as the optimal location for inducing contractions in the right abductor pollicis brevis muscle, and the lowest intensity of stimulation that was capable of inducing visible muscle contractions in at least six out of ten trials was defined as the motor threshold.29 For all the participants, we determined the motor threshold before they practised the motor sequence learning task. To identify the position of the IPL, we used a magnetom Beatri 

Polysomnography recording. We recorded PSG using digital electroencephalogram (EEG), electromyogram (EMG) and electro-oculogram (EOG) signals acquired with the Embla system (sampling rate 256 Hz, high- and low-pass filter 0.3 and 35 Hz, respectively; notch filter 60 Hz). A referenced PSG electrode montage was used, including sites C3, C4, O1 and O2 of the International 10–20 system, referenced to the mastoids. We also used two EOG and two EMG channels, which were also placed in a standard manner with the EOG electrodes offset (one above and one below the eye) and the EMG electrodes placed under the chin. We recorded the PSG from before lights out (11 p.m.) until just after the lights came on in the morning (7 a.m.).

Data analysis. Polyomognography data. We scored the PSG record using standard American Academy of Sleep Medicine (AASM) criteria, with manual scoring of 30+ epochs, while blinded to participants’ behavioural performance.2,3,4 The signals were displayed on a computer monitor and rated visually, epoch by epoch, as awake, movement time, N1, N2, SWS or REM sleep. The AASM manual for scoring of sleep and associated events was followed. We used a MANOVA to compare the total sleep time, time awake and the proportion of time spent within SWS sleep stages. Converging evidence links REM and particularly SWS to motor memory consolidation, and so the difference between these response times provides a sensitive measure of sequence learning. Overall, the difference between sequential and subsequent random response time provides both a specific and a sensitive measure of sequence learning. During learning, sequential response time would be expected to decrease. Subsequently introducing random trials would increase response time. An important component of this increase is because participants inappropriately expect the visual cues to continue to follow the sequence. As a consequence, the response times during random trials can be higher than those before the sequence, because they are inflamed by participants expecting to see a cue at one location but finding it another. Thus, both the decrease in response time during sequential trials and the increase during the subsequent random trials contain a component that is due to sequence learning, and so the difference between these response times provides a sensitive measure of sequence learning. For the implicit task, participants were asked whether they had noticed anything about the visual cues, and to describe that property. If participants had realised that there was a sequence, they were asked to recall as many items of the sequence as possible. Participants accurately recalling four or more items from the twelve-item sequence were removed from further analysis. Declarative knowledge for the sequence modified the task so that any improvements are no longer able to develop during wakefulness but only develop over a night of sleep (that is, offline improvements become sleep-dependent).2,3

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to the consolidation of motor sequence and visuomotor adaptation learning.

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