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Online feedback enhances early consolidation of motor sequence learning and reverses recall deficit from transcranial stimulation of motor cortex

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Abstract

Feedback and monetary reward can enhance motor skill learning, suggesting reward system involvement. Continuous theta burst (cTBS) transcranial magnetic stimulation of the primary motor area (M1) disrupts processing, reduces excitability and impairs motor learning. To see whether feedback and reward can overcome the learning impairment associated with M1 cTBS, we delivered real or sham stimulation to two groups of participants before they performed a motor sequence learning task with and without feedback. Participants were trained on two intermixed sequences, one occurring 85% of the time (the “probable” sequence) and the other 15% of the time (the “improbable” sequence). We measured sequence learning as the difference in reaction time (RT) and error rate between probable and improbable trials (RT and error difference scores). Participants were also tested for sequence recall with the same indices of learning 60 min after cTBS. Real stimulation impaired initial sequence learning and sequence knowledge recall as measured by error difference scores and impaired sequence knowledge recall as measured by RT difference score. Relative to non-feedback learning, the introduction of feedback during sequence learning improved subsequent sequence knowledge recall indexed by RT difference score, in both real and sham stimulation groups and feedback reversed the RT difference score based sequence

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knowledge recall impairment from real cTBS that we observed in the non-feedback learning condition. Only the real cTBS group in the non-feedback condition showed no evidence of explicit sequence knowledge when tested at the end of the study. Feedback improves recall of implicit and explicit motor sequence knowledge and can protect sequence knowledge against the effect of M1 inhibition. Adding feedback and monetary reward/punishment to motor skill learning may help overcome retention impairments or accelerate training in clinical and other settings.

Keywords

Theta burst stimulation; consolidation; motor cortex; motor skill; sequence learning; serial reaction time task

1.1 Introduction

Procedural learning, the process by which skills are acquired by practice, is a fundamental and critical function of the brain. It is a key component of higher skills, such as math, where the rules can be understood explicitly, but facility comes only with repeated problem solving (Fayol & Thevenot, 2012). The benefit of repeated practice is evident in rehabilitation strategies for brain damaged patients where outcome is improved by extensive practice of specific movements (Nadeau, 2002). It also allows acquisition of intuitive skills that help humans and animals function in uncertain environments.

One popular procedural learning paradigm is the serial reaction time task (SRTT, Nissen & Bullemer, 1987), where participants respond rapidly to a stream of cues by pressing buttons with the fingers of one hand. If a long, repeating sequence is embedded in the stream, speed improves before the participant is aware that a sequence is present. That is, the knowledge that a particular cue is likely to follow another is acquired as an unconscious motor skill through practice. This predictive knowledge, built from associations between events, could be acquired through reinforcement learning and promoted by the dopamine reward system (Schultz, 2002). An implication of this theory is that boosting the activity of the reward system could make procedural learning more efficient and aid skill acquisition. Acquisition of motor sequence learning on the SRTT is not always exclusively an implicit process, and depending on the learning environment, implicit and explicit/conscious sequence learning can occur in parallel (Willingham & Goedert-Eschmann, 1999; Willingham, Salidis, & Gabrieli, 2002). While this fact makes the task unsuitable for studying implicit knowledge in isolation, it is consistent with much learning in the real world.

The reward system is considered one of the networks involved in procedural motor sequence learning. For example, learning on the SRTT is impaired in patients with Parkinson (PD) and Huntington (HD) diseases (Doyon et al., 1997; Jackson, Jackson, Harrison, Henderson, & Kennard, 1995; Knopman & Nissen, 1991; Muslimovic, Post, Speelman, & Schmand, 2007; Wilkinson & Jahanshahi, 2007; Wilkinson, Khan, & Jahanshahi, 2009), focal lesions of the basal ganglia (Obeso et al., 2009), and traumatic brain injury (TBI) (De Beaumont, Tremblay, Poirier, Lassonde, & Theoret, 2012; Mutter, Howard, & Howard, 1994; Vakil, 2005) as well as in an animal models of dopamine depletion (Matsumoto, Hanakawa, Maki, Graybiel, & Kimura, 1999).

There is also evidence from studies in healthy humans that incentive and feedback can improve motor skill learning. For instance, adding monetary reward to a force-tracking task which incorporated a repeating pattern improved retention as demonstrated by offline gains (Abe et al., 2011). Adding similar feedback and incentive to the SRTT improved learning (Wachter, Lungu, Liu, Willingham, & Ashe, 2009). These findings imply that monetary reward might augment rehabilitation after injury or accelerate learning in healthy people.

Human learning deficits can also be produced in the laboratory. When delivered to the primary motor cortex (M1), inhibitory transcranial magnetic stimulation (TMS), in particular continuous theta burst stimulation (cTBS), reduces local cortical excitability (Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005) and temporarily impairs motor sequence learning (Rosenthal, Roche-Kelly, Husain, & Kennard, 2009; Wilkinson, Teo, Obeso, Rothwell, & Jahanshahi, 2010). The magnitude of these deficits in healthy volunteers is similar to those in patients (De Beaumont et al., 2012; Doyon et al., 1997; Jackson et al., 1995; Knopman & Nissen, 1991; Muslimovic et al., 2007; Mutter et al., 1994; Obeso et al., 2009; Vakil, 2005; Wilkinson & Jahanshahi, 2007; Wilkinson et al., 2009). However, the addition of feedback, including monetary reward, does not improve non-motor procedural learning in PD and HD (Holl, Wilkinson, Tabrizi, Painold, & Jahanshahi, 2012; Shohamy et al., 2004; Wilkinson, Lagnado, Quallo, & Jahanshahi, 2008).

Adding feedback and incentive to procedural tasks in clinical and training settings to boost learning has great appeal. Unlike interventional strategies currently under investigation, including the several forms of noninvasive brain stimulation (Reis et al., 2008; Sandrini & Cohen, 2013), there is no need for devices requiring large clinical trials and regulatory approval. There are no ethical problems posed by its use in healthy populations. However, its viability and comparative value depend on the magnitude of its effects and the ability to produce them in impaired or otherwise refractory subjects. Therefore, we decided to investigate whether adding feedback and monetary incentives to the SRTT can overcome the healthy volunteers' temporary impairment produced by inhibitory TMS and whether the size of its statistical effects is of clinical interest. We delivered sham and real cTBS to M1 just before administering a probabilistic version of the SRT, which is less likely than the conventional task to produce explicit sequence knowledge. We hypothesized that feedback would enhance sequence learning and/or recall as well as protect learning and knowledge retention from the effect of cTBS. We also tracked the development of awareness during learning closely to show whether the motor sequence learning was always unconscious or whether the procedural and declarative systems interacted during the task with and without feedback, and after inhibition of M1 via cTBS.

1.2 Materials and methods

1.2.1 Participants

We recruited 40 right-handed, healthy volunteers, all of whom met safety criteria for TMS (Keel, Smith, & Wassermann, 2001). All were free of neurological and psychiatric illness and none was on continuous medication other than oral contraceptives. We estimated intelligence with the National Adult Reading Test (NART). The study was approved by the Combined Neuroscience Institutional Review Board at the National Institutes of Health.

Written informed consent was obtained from all participants. Participants were randomly assigned to one of two treatment groups:

1. Sham cTBS ($n = 20$, 12 women) aged 22–47 years ($M = 26.60$, $SD = 7.6$)
2. Real cTBS ($n = 20$, 11 women) aged 24–53 years ($M = 27.25$, $SD = 7.1$)

1.2.2 Procedure

Participants in the sham and real groups completed the probabilistic SRTT (pSRTT; see below) with and without feedback (monetary reward/punishment) in separate sessions at least one week apart. The order of conditions (feedback or non-feedback) was randomized and counterbalanced across treatment groups and participants. At the beginning of each experimental session, after locating M1, we measured the resting and active motor evoked potential (MEP) thresholds (RMT & AMT) and baseline MEP amplitude to a stimulus delivered at 120% of RMT (see below). We then delivered real or sham cTBS before immediately retesting MEP amplitude. As soon as this was completed, participants performed 20 blocks of the pSRTT, after, MEP amplitude was retested both immediately after the learning as well 45 minutes after stimulation.

Sixty minutes after stimulation (30 min after pSRTT completion), participants performed a test of sequence knowledge retention consisting of three non-feedback pSRTT blocks. Additionally, at the end of the second pSRTT experimental session, participants performed an awareness test, the process dissociation procedure (PDP), for explicit sequence knowledge.

1.2.3 pSRTT

Stimulus presentation, response recording, and RT measurement were implemented on a notebook computer with a QWERTY keyboard layout and 19 x 26 cm color screen. Keys V, B, N & M were labeled 1–4 from left to right and corresponded to digits 2–5 of the right hand. The screen displayed four white 16 x 16 mm boxes, against a black background that corresponded to the keys. On each trial, a black X appeared in the center of one of the boxes, indicating the finger for the response. Participants were instructed to respond to this target as quickly and accurately as possible. A trial ended when the participant pressed the correct key, at which time the X disappeared from the screen. The next target appeared after a 250-msec interval. RT was measured in msec from onset of the target to button press. The pSRTT comprised 20 blocks of 100 trials.

In the pSRTT, cues appear according to two different, but matched with respect to certain properties and intermixed, sequences, which follow different rules. The rule for one, the “probable sequence” is followed more frequently (85%) than the rule for the other, “improbable” one (15%). The result is that participants learn to anticipate targets from the probable sequence. The intercalation of the two sequences creates “noise” and inhibits the development of explicit knowledge of the probable sequence without preventing the gain of implicit sequence knowledge. As sequence learning occurs, RT and errors decrease for probable relative to improbable trials, and/or increase for improbable relative to probable trials, as the probable sequence is increasingly anticipated. Therefore, comparisons of the

RT and error rate for the two sequence trial types provide measures of sequence learning, which can be observed continuously across the task. This yields a more robust and online measure of learning than in the classical or “deterministic” version of the SRTT (Wilkinson & Shanks, 2004) where RT and error rates are compared across separate random and sequence cue presentation blocks at the end of the task.

We used two pairs of different but parallel pairs of second-order conditional (SOC) sequences:

- a. Pair 1 (SOC1 = 2-4-2-1-3-4-1-2-3-1-4-3 & SOC2 = 3-4-3-1-2-4-1-3-2-1-4-2)
- b. Pair 2 (SOC3 = 3-4-2-3-1-2-1-4-3-2-4-1 & SOC4 = 3-4-1-2-4-3-1-4-2-1-3-2).

These pairs of sequences are equivalent with respect to cue frequency (each location occurs three times), first-order transition frequency (each location is preceded once by each of the other three locations), and repetitions (no repetitions in either sequence) (Reed & Johnson, 1994). Thus, the sequences in each pair differ only in their second-and-higher-order conditional structure. For instance, the SOC in each pair can each be broken into 12 identical pairs of targets (first order transitions). For SOC1 and 2, the pairs are 2-4, 4-2, 2-1, 1-3, 3-4, 4-1, 1-2, 2-3, 3-1, 1-4, 4-3, 3-2. However, the sequences *do* differ when broken into their possible triplets. In SOC1, 2-4 is always followed by 2; whereas in SOC2, it is followed by 1, and so on. Therefore, SOC 1 is differentiated from SOC 2 by (at least) second order (triplet) rather than first order information.

In our experiment, the location of the target occurred according to the probable sequence 85% of the time and according to the improbable sequence 15% of the time. Therefore, participants were exposed predominantly to the second order transition rule for the probable sequence and developed predictive behavior specific to the probable sequence. The sequences were implemented using the two most recent events to determine the next event. Thus, there was a probability of .85 that each target would be the event specified by the last two locations in the probable sequence and a probability of .15 that it would be the event specified by the last two locations in the improbable sequence. For example, a participant trained on SOC1 as the probable sequence would see the transition 4-1 followed by a target at location 2 (according to SOC1) with a probability of .85 or followed by a target at location 3 (SOC2) with a probability of .15. Sequences and sequence pairs were counterbalanced across sessions and participants. Each block began at a random point in the sequence.

1.2.3.1 Task conditions—In the feedback condition, participants received feedback on a trial-by-trial basis. They were rewarded or punished for how fast and accurate they were during learning, and monetary feedback was delivered only on probable trials. During the first learning block, we awarded \$0.10 and displayed a yellow smiley face for correct responses when the RT was < 500 msec. In subsequent blocks (2–20), \$0.10 was given and a smiley face was shown for correct responses when the RT was less than the median RT of probable trials in the previous block plus the standard deviation of the mean RT for probable trials in the previous block, divided by the ordinal number of the previous block. Thus, we made it increasingly challenging to obtain the reward on succeeding blocks. On probable

trials not meeting the above criteria, winnings were decreased by \$0.10, and a red, sad face was presented. For improbable trials, a purple, neutral face was presented, and winnings were unchanged. Participants were paid their total winnings for the feedback condition, in addition to the lump sum they were paid for participating in both feedback and non-feedback sessions.

In the feedback condition, participants were told that on the majority of trials they would see a smiley face and receive \$0.10 for fast and accurate responses; they would see a sad face and lose \$0.10 when their responses were slow or inaccurate; and that on some trials they would see a neutral face and neither win nor lose.

In the non-feedback condition, on each probable trial there was a 50% chance of seeing a smiley face and a 50% chance of seeing a sad face. Instead of winnings, running totals of sad and smiley faces were displayed. Faces were always neutral on improbable trials. In the non-feedback condition, participants were told they would see one of the three different faces after each response and that this was not related to performance.

1.2.4 Awareness testing

After the second session, participants completed the PDP. This task estimates awareness of acquired sequence knowledge and is based on the assumption that conscious knowledge is both reportable upon request and under intentional control whereas, unconscious knowledge may or may not be reportable and is not under intentional control (Destrebecqz et al., 2005; Wilkinson & Jahanshahi, 2007; Wilkinson et al., 2009). The PDP consisted of two sequence generation tests, each based on 12 six-item fragments of previously learned probable sequences. In the inclusion test designed to test reportable knowledge, participants responded to five targets and then were required to produce a single continuation response, i.e., the sixth target in the sequence or the third target in the second triplet. In the exclusion test, designed to provide a measure of whether knowledge is under intentional control, participants were told to make a response *different* from the next item of the sequence. During the inclusion test, participants were reminded that there were no repetitions in the sequence, so it would be incorrect to repeat the last item of the test sequence. In light of this instruction, there was a 1/3 probability per trial of generating the correct answer by chance, making the chance level 4/12 test items. In the exclusion test, again, participants were reminded not to repeat the last item of the test sequence in their response, and since they were required to make a response other than the correct target, there was a 2/3 probability per trial of generating the correct answer by chance, making the chance level 8/12 test items. The order of tests was counterbalanced across participants.

1.2.5 Transcranial magnetic stimulation

TMS was delivered with a Magstim Rapid® stimulator (Magstim Co., Dyfed, UK) connected to a figure-of-eight coil with an internal wing diameter of 70 mm, which was held with the handle pointing posterolaterally. The electromyogram (EMG) was recorded with a belly-tendon montage from the right first dorsal interosseous (FDI) muscle. The left M1 was located by finding the best scalp site for producing MEPs in the right FDI. We used the MRI-guided,Brainsight® TMS neuronavigation system (Rogue Research Inc.) to record

the location of M1 in each individual, to ensure the same stimulation site could be used for real and sham cTBS.

RMT was defined as the minimum stimulus intensity that produced a MEP of about 50 μ V in 5 of 10 trials with the muscle at rest. AMT was defined as the minimum stimulus intensity that produced a MEP (about 200 μ V) in 5 of 10 trials during voluntary contraction of the right FDI at approximately 10% of maximum voluntary contraction. Effort was kept constant across subjects and over time by online monitoring of the rectified EMG. cTBS was administered according to the protocol described by Huang et al. (2005). Each burst consisted of 3 pulses at 50 Hz, delivered at an intensity of 80% AMT. Bursts were given every 200 ms (i.e., 5 Hz) for a total of 600 pulses (200 theta bursts or 600 pulses). The stimulation lasted 40 sec. For sham stimulation, we used a placebo coil, which was identical to the real coil in appearance, operation, and acoustic properties but did not reproduce the physiological effect of TMS. Participants but not experimenters were blind to the stimulation condition.

The inhibitory effect of cTBS on the MEP lasts up to 60 min (Huang et al., 2005). However, as a check on its duration, we tested MEPs immediately after pSRTT completion (approximately 30 min after cTBS) and 45 min after stimulation in addition to before and immediately after cTBS. At each time, we delivered 20 single TMS pulses, 6 sec apart, at 120% RMT or the minimum intensity required to produce a consistent MEP 1 mV in that individual at the baseline measurement, and recorded mean MEP amplitude.

1.2.6 Data analysis

For each participant, mean RTs and error rates for probable and improbable trials were calculated for each pSRTT block. Trials with incorrect responses or trials with RTs either: < 200 msec or > 3 *SDs* above the individual's overall mean RT were excluded from the analysis (proportion of trials excluded, Sham: non-feedback, $M = .02$, $SD = .01$, feedback, $M = .03$, $SD = .04$. Real non-feedback, $M = .02$, $SD = .01$, feedback, $M = .02$, $SD = .01$). Significantly more trials were excluded from the feedback than the non-feedback conditions across TMS groups ($F_{(1,38)} = 6.32$, $p = .02$), but TMS group had no effect on the number of trials excluded. In all subsequent analyses, (i) data were collapsed across sequence orders; (ii) data from the first two trials of each block were excluded because their finger locations could not be predicted from sequence knowledge; and (iii) if there was a violation of the sphericity assumption, the Greenhouse-Geisser correction was used. MEP amplitudes were log-transformed for analysis (Wassermann, 2002). We used a significance criterion of $\alpha = .05$. All comparisons were two-tailed unless otherwise specified.

1.3 Results

The sham and real cTBS groups did not differ in age, estimated IQ, years of education, time between sessions, time to complete the pSRTT (30 min per group/condition), or sex. Mean winnings across learning blocks did not differ between groups: sham, $M = \$19.15$, $SD = \$9.70$, real, $M = \$22.00$, $SD = \$8.90$.

1.4.1 Learning

The main index of sequence learning in this task is the difference in RT between probable and improbable trials. We expected that RT would decrease across blocks for probable relative to improbable trials, and/or increase for improbable relative to probable trials, as participants came to anticipate the probable sequence, and that the sequence learning related changes would increase across blocks as learning develops. To examine effects of feedback and cTBS on initial sequence learning across blocks, we performed an ANOVA on mean RTs with Trial-type (probable vs. improbable), Feedback (feedback vs. non-feedback) and Block (1–20) as within-subject variables and Stimulation Group (real vs. sham cTBS) as a between groups variable. Figure 1 shows RT as function of trial-type and block separately for each feedback condition and stimulation group. During the first 1–2 blocks, there was no numerical divergence of RTs for probable and improbable trials. However, by block 3, RTs for probable trials became faster relative to improbable trials, indicating specific learning of the probable sequence. This sequence-specific learning related difference in RT between probable ($M = 363.85$, $\pm SE = 5.3$) and improbable ($M = 386.44$, $\pm SE = 5.7$) trials was significant across groups and conditions (main effect of Trial-type; $F_{(1,38)} = 188.57$, $p < .0005$). Furthermore, this RT difference changed significantly across blocks, (interaction between Trial-type x Block; $F_{(9,6,363.8)} = 14.19$, $p < .0005$), which from Figure 1, was driven by an increase in sequence learning related differences across blocks as predicted.

Changes in overall RT are indicative of nonspecific effects of task practice and therefore, *not* one of the main measures of sequence learning in this study. However, Figure 1 illustrates that, when we collapsed across probable and improbable trials, RT changed significantly over time (main effect of Block; $F_{(9,0,341.3)} = 9.68$, $p < .0005$). Overall RT was significantly faster when participants received feedback ($M = 365.41$ msec, $\pm SE = 5.0$) than when they did not ($M = 384.88$ msec, $\pm SE = 7.2$) (main effect of Feedback; $F_{(1,38)} = 10.14$, $p = .003$). Figure 1 also shows feedback caused overall RT to decline significantly faster across blocks (interaction between Feedback x Block; $F_{(1,0,378.9)} = 6.94$, $p < .0005$). Importantly, neither stimulation group nor feedback affected sequence-specific learning itself and there was no nonspecific influence of real cTBS on overall RTs that could have confounded the analysis of sequence learning: main effect of Stimulation Group ($F_{(1,38)} = .57$, $p = .46$) and the interactions between, Feedback x Trial-type x Block ($F_{(9,8,370.5)} = 1.37$, $p = .19$), Feedback x Block x Stimulation Group ($F_{(19,722)} = .67$, $p = .85$), Trial-type x Block x Stimulation Group ($F_{(19,722)} = .59$, $p = .91$), Feedback x Trial-type x Block x Stimulation Group ($F_{(19,722)} = .57$, $p = .93$), Block x Stimulation Group ($F_{(9,0,341.3)} = .42$, $p = .93$), Feedback x Trial-type ($F_{(1,38)} = .36$, $p = .55$), Feedback x Trial-type x Stimulation Group ($F_{(1,38)} = .33$, $p = .57$), Feedback x Stimulation Group ($F_{(1,38)} = .25$, $p = .62$), Trial-type x Stimulation Group ($F_{(1,38)} = .09$, $p = .76$), were non-significant.

We predicted that the error rate would decrease across blocks for probable and/or increase for improbable trials as participants came to anticipate the probable sequence, providing a second assay of sequence learning. Figure 2 shows error rate as function of trial-type and block separately for each feedback condition and stimulation group. During the first block of learning, for most groups/conditions, there was no difference in the error rates for probable and improbable trials. However, by block 2, errors became numerically faster for probable

relative to improbable trials in all groups/conditions again, indicative of learning the probable sequence specifically. An identical analysis to the above on error rates revealed significantly more errors were produced when the target appeared according to the improbable ($M = .12$, $+/- SE = .01$) than according to the probable sequence ($M = .06$, $+/- SE = .01$), indicating sequence-specific learning (main effect of Trial-type across Blocks 1–20; $F_{(1,38)} = 137.75$, $p < .0005$). As seen in Figure 2, this sequence learning related difference also became significantly greater across blocks as learning progressed (Trial-type x Block; $F_{(19,722)} = 5.32$, $p < .0005$). In addition, the sequence-specific difference in error rate decreased significantly after real cTBS regardless of feedback condition (interaction between Trial-type x Block x Stimulation Group; $F_{(19,722)} = 1.74$, $p = .03$; see Figure 3A and follow up analysis below). Overall error rate across blocks 1–20 collapsed across Trial-type (not indicative of sequence-specific learning) was significantly higher in the feedback ($M = .11$, $+/- SE = .01$) relative to non-feedback condition ($M = .07$, $+/- SE = .01$) (main effect of Feedback; $F_{(1,38)} = 16.22$, $p < .0005$) and, as Figure 2 shows, changed significantly over time (main effect of Block; $F_{(19,722)} = 17.22$, $p < .0005$). Figure 2 also, illustrates overall errors increased significantly more across blocks in the feedback condition than in the non-feedback condition (interaction between Feedback x Block; $F_{(19,722)} = 3.17$, $p < .0005$). Furthermore, overall errors increased significantly less for real cTBS than for sham stimulation conditions (interaction between Block x Stimulation Group; $F_{(19,722)} = 1.89$, $p = .01$). There was no nonspecific influence of real cTBS on overall errors (main effect of Stimulation Group; $F_{(1,38)} = .95$, $p = .34$) nor was there an effect of feedback on error-based sequence learning: Feedback x Trial type x Block x Stimulation Group ($F_{(19,722)} = 1.27$, $p = .20$), Feedback x Trial type x Block ($F_{(19,722)} = 1.05$, $p = .40$), Feedback x Trial type x Stimulation Group ($F_{(1,38)} = .85$, $p = .36$), Feedback x Trial type ($F_{(1,38)} = .38$, $p = .54$). All other interactions between Trial type x Stimulation Group ($F_{(1,38)} = 3.07$, $p = .09$), Feedback x Stimulation Group ($F_{(1,38)} = .81$, $p = .37$), Feedback x Block x Stimulation Group ($F_{(19,722)} = 1.25$, $p = .21$) were non-significant.

In light of the significant interaction between Trial-type, Block, and Stimulation Group, we calculated measures of error-based sequence learning (error difference scores) for each block by subtracting the mean number of errors for the probable trials from the mean number of errors for the improbable trials. A positive error difference score, which was also significantly greater than zero, was evidence of sequence learning. This was done for each stimulation group separately. We collapsed across feedback conditions since feedback condition did not interact with the other factors. Mean error difference scores were significantly greater for the sham than for the real group at blocks 17 ($t_{(38)} = -2.48$, $p = .02$) and 20 ($t_{(38)} = -2.78$, $p = .01$) and 16 ($t_{(38)} = -1.96$, $p = .03$, the latter comparison being one-tailed). There were no significant differences between the sham and real group for any other block. These comparisons for blocks 3 ($t_{(38)} = -1.63$), 6 ($t_{(38)} = -1.76$), 8 ($t_{(38)} = -1.06$), 12 ($t_{(38)} = -1.53$) and 19 ($t_{(38)} = 1.42$), and all other blocks (all $ts < 1$) were non-significant. In addition, in the sham group, the mean error difference scores were significantly greater than zero in all but the first block (all $ps < .02$), indicating sequence learning across the remaining blocks. However, in the real group, the mean error difference scores in the first three blocks were not significantly different from zero (all $ps > .05$), while

the remaining blocks were (all $ps < .05$). Therefore, real cTBS impaired the initial stage of sequence learning.

Notably, the pattern of overall errors and RTs during learning with feedback was identical to that seen by Wächter et al. (2009) who showed that both reward and punishment during SRTT learning made RTs drop significantly across blocks while errors increased suggesting a speed-accuracy tradeoff. In that study, this effect was present for both sequence and random blocks and was therefore related to determinants of performance other than sequence learning.

1.4.2 Recall

To look for effects of cTBS and feedback on sequence knowledge retention, we performed an ANOVA on RTs from the recall test blocks (21–23; see Figure 1). Again, mean RT was significantly faster for probable ($M = 347.40$ msec, $\pm SE = 4.3$) than improbable ($M = 386.00$ msec, $\pm SE = 4.3$) trials (main effect of Trial-type; $F_{(1,38)} = 167.46$, $p < .0005$), indicating retention of sequence-specific knowledge acquired during learning. At this time interval, however, retention was impaired by cTBS, with a significantly larger RT difference between Trial types in the sham than real group (Trial-type x Stimulation Group; $F_{(1,38)} = 4.36$, $p = .04$; see first follow up analysis below). Furthermore, there was a significant interaction between Feedback and Trial-type ($F_{(1,38)} = 18.0$, $p < .0005$) where feedback increased recall of the probable sequence (see second follow up analysis below). The size of the beneficial effect of feedback on recall in both TMS groups was also substantial (see below). The main effects of Block ($F_{(2,76)} = 2.53$, $p = .09$), Feedback, Stimulation Group ($F_s < 1$) and interactions between Trial-type x Block ($F_{(1,76)} = 2.61$, $p = .08$), Block x Stimulation Group ($F_{(2,76)} = 1.06$, $p = .35$), Feedback x Block ($F_{(2,76)} = 1.63$, $p = .20$), Feedback x Trial-type x Block ($F_{(2,76)} = 1.18$, $p = .31$), Feedback x Stimulation Group, Feedback x Trial-type x Stimulation Group, Feedback x Block x Stimulation Group, Trial-type x Block x Stimulation Group, Feedback x Trial-type x Block x Stimulation Group (all $F_s < 1$) were not significant.

To follow up the significant interactions between Trial-type x Stimulation Group and Feedback x Trial-Type, we derived an overall measure of recall performance (RT difference score) by subtracting the mean RT for probable from that for the improbable trials for each participant. A positive difference score indicated retention of sequence knowledge. Collapsed across feedback conditions, the mean RT difference for the sham group ($M = 44.83$ msec, $\pm SE = 4.4$) was significantly greater than for the real group ($M = 32.37$ msec, $\pm SE = 4.1$) ($t_{(38)} = -2.09$, $p = .04$) showing real cTBS impaired recall of sequence knowledge. Collapsed across cTBS conditions, it was evident that feedback greatly improved recall performance (feedback, $M = 47.69$ msec, $\pm SE = 4.5$; non-feedback, $M = 29.51$ msec, $\pm SE = 2.8$; $t_{(39)} = -4.27$, $p < .0001$; Cohen's $d = 0.76$).

The significant difference in error rate between probable ($M = .05$, $\pm SE = .01$) and improbable ($M = .14$, $\pm SE = .01$) trials was also present in the recall test blocks (21–23; Figure 2; $F_{(1,38)} = 82.01$, $p < .0005$). In addition, there were significantly more errors overall in the feedback condition (feedback, $M = .10$, $\pm SE = .01$; non-feedback, $M = .07$, $\pm SE = .01$; main effect of Feedback; $F_{(1,38)} = 7.09$, $p = .01$) and, as seen in Figure 2, there was a

significant decline of overall error rate across test blocks (main effect of Block; $F_{(2,76)} = 4.69, p = .01$). The error-based measure of sequence recall was unaffected by feedback (interactions between Feedback x Trial-type x Block x Stimulation Group; $F_{(2,78)} = 1.10, p = .34$, Feedback x Trial-type, Feedback x Trial-type x Stimulation Group, Feedback x Trial-type x Block (all F s < 1). Also, the interactions between Feedback x Stimulation Group; $F_{(1,38)} = 1.30, p = .26$, Block x Stimulation Group, Feedback x Block x Stimulation Group, Feedback x Block, Trial-type x Block, Stimulation Group x Trial-type x Block (all F s < 1).

However, real cTBS caused a non-significant trend for an decrease in overall errors in the test phase (real, $M = .08, \pm SE = .01$; sham, $M = .10, \pm SE = .01$; main effect of Stimulation Group; $F_{(1,38)} = 3.16, p = .08$), and a non-significant trend for a change in error-based sequence knowledge across test blocks (Trial-type x Stimulation Group; $F_{(1,38)} = 2.92, p = .09$). To follow up this interaction, we again calculated error difference scores for the two cTBS groups collapsed across feedback and test blocks. Real cTBS significantly reduced error-based sequence recall across test blocks (sham, $M = .11, \pm SE = .01$; real, $M = .09, \pm SE = .01$; $t_{(38)} = -1.78, p = .04$, one-tailed; see Figure 3B).

It is interesting that, even after feedback had been removed, overall errors were still significantly higher in the test blocks for those trained in the feedback relative to non-feedback condition. However, overall RTs were no longer reduced significantly by previous feedback. Again, our findings are identical to the lasting effects of feedback on error rate observed by Wächter et al. (2009) after feedback was removed. We assume the maintenance of a high overall error rate after learning with feedback is a priming-like effect caused by training with a high overall error rate. This effect was manifest in the error but not RT test data. Perhaps RT requires immediate incentive to be reduced by feedback. In fact, this higher overall error rate in the feedback condition across recall blocks appeared to be driven by the sham group: When their test data were analyzed separately, there was still a significant increase in overall error rate after learning with feedback (feedback, $M = .12, \pm SE = .02$; non-feedback, $M = .08, \pm SE = .02$; main effect of Feedback; $F_{(1,19)} = 5.72, p = .03$). However, this effect on the test data was not present when the real group was analyzed separately (feedback, $M = .08, \pm SE = .02$; non feedback, $M = .07, \pm SE = .02$; main effect of Feedback; $F_{(1,19)} = 1.58, p = .22$). This indicates that real cTBS abolished the feedback related priming effect, typically seen in the error test data.

A central question in this study was whether feedback could overcome the temporary learning impairment produced by real cTBS of M1. To answer this, we compared mean RT difference across recall blocks for each stimulation group and feedback condition. As Figure 4 shows, sequence knowledge recall by both stimulation groups was significantly better when acquisition was accompanied by feedback, than without feedback (real: feedback, $M = .42.93$ msec, $\pm SE = 6.4$; non-feedback, $M = 21.82$ msec, $\pm SE = 3.6$; $t_{(19)} = -3.24, p = .004$, Cohen's $d = .91$; sham: feedback, $M = .52.45$ msec, $\pm SE = 6.3$; non-feedback, $M = 37.21$ msec, $\pm SE = 3.7$; $t_{(19)} = -2.74, p = .02$). In the non-feedback condition, recall was significantly better in the sham than the real group ($t_{(38)} = -3.01, p = .01$). However, in the feedback condition, recall test scores of sequence knowledge did not differ between the sham and real groups, indicating that feedback restored the real group's sequence recall to the sham level ($t < 1$). The sham group also recalled significantly better sequence knowledge

after learning in the feedback condition than the real group did after non-feedback learning, ($t_{(38)} = -3.81, p < .0005$). Finally, the sham group's recall of sequence knowledge in the non-feedback condition was comparable to the real group's recall in the feedback condition ($t < 1$).

1.4.3 Early consolidation

We compared the effects of cTBS and feedback on RT difference scores during learning (1–20) and recall test (21–23) blocks by performing an ANOVA with Time (initial vs. recall) and Feedback as within-subject variables and Stimulation Group as a between subjects variable (see Figure 4). Across conditions and groups, sequence knowledge was significantly better on the recall test ($M = 38.60$ msec, $\pm SE = 3.1$) blocks than during learning blocks ($M = 22.60$ msec, $\pm SE = 1.6$) (main effect of Time ($F_{(1,38)} = 41.94, p < .0005$). Real cTBS significantly reduced this gain (interaction between Time x Stimulation Group ($F_{(1,38)} = 5.37, p = .03$, see follow up analysis below). Collapsed across cTBS conditions and time, overall sequence knowledge was significantly improved by feedback (feedback = $M = 34.75$ msec, $\pm SE = 2.9$; non-feedback = $M = 26.44$ msec, $\pm SE = 2.2$; main effect of Feedback; $F_{(1,38)} = 7.83, p = .01$). Feedback significantly increased the sequence knowledge gain with time (interaction between Time x Feedback; $F_{(1,38)} = 25.98, p < .0005$, see follow up analysis below). The main effect of Stimulation Group and the interactions between Feedback x Stimulation Group and Feedback x Time x Stimulation Group ($F_s < 1$) were non-significant.

In light of the significant interactions (“Time x Stimulation Group” and “Time x Feedback”), we looked separately at the effects of cTBS and feedback on the RT difference score change from learning to the recall test blocks. First collapsed across feedback conditions, both real (learning: $M = 22.09$ msec, $\pm SE = 2.3$; recall: 32.37 msec, $\pm SE = 4.1$; $t_{(19)} = 2.98, p = .01$) and sham (learning: $M = 23.10$ msec, $\pm SE = 2.4$; recall: $M = 44.83$ msec, $\pm SE = 4.4$; $t_{(19)} = 6.14, p < .0001$) groups performed significantly better on recall than learning indicating participants performed better on average on the test blocks. This performance gain was additionally supported by between groups comparisons showing the sham group did significantly better at recall than the real group did during learning ($t_{(28,4)} = -4.62, p < .0001$). Furthermore, recall in the real cTBS group was significantly better than learning in the sham group ($t_{(19)} = 2.98, p = .01$). Nevertheless (and as shown previously), the real cTBS group had significantly worse recall than the sham group ($t_{(38)} = -2.09, p = .04$), even though their performance did not differ during learning. Taken together, these findings indicate that both cTBS groups improved from learning to test, but real cTBS attenuated the gain.

Feedback had a similar but opposite effect to cTBS. Collapsed across cTBS groups, participants showed a significant performance improvement on the recall test blocks relative to learning, regardless of feedback condition (non-feedback, learning: $M = 23.38$ msec, $\pm SE = 2.3$; recall, $M = 29.51$ msec, $\pm SE = 2.8$; $t_{(39)} = -2.23, p = .03$; feedback, learning: $M = 21.82$ msec, $\pm SE = 1.8$; recall: $M = 47.69$ msec, $\pm SE = 4.5$; $t_{(39)} = -7.08, p < .001$). Moreover, recall in the feedback condition was significantly better than initial learning in the non-feedback condition ($t_{(39)} = -5.01, p < .0001$). Recall in the non-feedback condition

was significantly better than initial learning in the feedback condition ($t_{(39)} = -2.82, p = .01$). However, as mentioned previously, feedback during learning significantly enhanced recall relative to learning without feedback ($t_{(39)} = -4.27, p < .0001$). In sum, participants trained in both the feedback and non-feedback conditions showed a performance gain from learning to test, but feedback during learning enhanced this performance gain.

When the above analysis was repeated for error rate difference scores, sequence knowledge at recall ($M = .08, \pm SE = .01$) was again significantly better than during learning blocks ($M = .06, \pm SE = .01$) across conditions and groups (main effects of Time ($F_{(1,38)} = 7.78, p < .01$), indicating a consolidation effect in the error data, too (see Figure 3C). However, neither real cTBS nor feedback influenced consolidation of sequence knowledge in the error data: The main effects of Feedback ($F_{(1,38)} = 2.74, p = .11$) and Stimulation Group ($F_{(1,38)} = 1.21, p = .28$) as well as the interactions between Feedback x Stimulation Group, Time x Stimulation Group, Time x Feedback and Feedback x Time x Stimulation Group (all F s < 1) were non-significant.

1.4.4 Neurophysiological data

Four participants were excluded from this analysis due to missing data (real, $n = 1$). We also computed the mean baseline absolute MEPs across the two feedback conditions and excluded any individual who failed to produce a large enough baseline MEP (real, $n = 2$; i.e. $< 50 \mu V$ as described in the Materials and methods section) from this analysis. Therefore, we included 17 participants in the real group and 19 in the sham. We calculated mean MEP amplitude for each participant across 20 stimuli at each of the four test times: baseline (time 0), immediately after real or sham cTBS (time i), immediately after learning and 30 min after cTBS (time ii), and after learning and 45 min after cTBS (time iii; see Figure 5). This was done for each stimulation group and feedback condition. We performed an ANOVA on mean MEP amplitude (normalized to baseline) with Feedback and Time (i–iii) as within-subject variables and Stimulation Group as a between groups variable.

MEP amplitude from baseline was significantly lower in the real stimulation group ($M = -.08 \pm SE = .04$) than in the sham stimulation group ($M = .01 \pm SE = .03$) ($F_{(1,32)} = 4.34, p = .05$), an effect which persisted up to 45 min after treatment (main effect of Stimulation Group on MEP amplitude ($F_{(1,32)} = 4.34, p = .05$). The main effects of Time ($F_{(1,64)} = 2.65, p = .08$) and Feedback ($F < 1$) on MEPs were non-significant as were the interactions between Time x Stimulation Group ($F_{(2,64)} = 1.46, p = .24$), Feedback x Stimulation Group, Feedback x Time and Feedback x Time x Stimulation Group (all F s < 1).

For the real group in the feedback condition, there was a strong and unexpected association between MEP reduction 45 min after real stimulation and better RT-based sequence knowledge at recall (Figure 6; $r_{(16)} = .82, p < .0001$). As can be seen in the figure, there is one outlying point; the association remained significant when the outlying point was removed ($r = .51; p = .05$). We found no such associations for the real group at the other MEP measurement times or in the non-feedback condition. The sham group showed no associations between MEP changes and RT-based sequence knowledge.

1.4.5 Sequence awareness test

Figure 7 shows the number of correct probable sequence triplet completions generated on the inclusion and exclusion tests by stimulation group and feedback condition. To test for explicit sequence knowledge, we first compared sequence knowledge generated during the inclusion test to chance (4/12 test items correct). The sham group's sequence knowledge was significantly above chance in both the non-feedback ($M = 5.78 \pm SE = .4$; $t(9) = 4.88$, $p = .001$) and feedback ($M = 6.10 \pm SE = .7$; $t(9) = 3.12$, $p = .01$) conditions. The real group's sequence knowledge was at chance in the non-feedback ($M = 4.50 \pm SE = .6$; $t(9) = .86$, $p = .41$) but significantly above chance in the feedback ($M = 5.60 \pm SE = .6$; $t(9) = 2.76$, $p = .02$) condition.

All groups/conditions that demonstrated above-chance sequence knowledge on the inclusion test also showed significantly below-chance sequence knowledge on the exclusion test, indicating that they had "conscious control" of their sequence knowledge: Sham/non-feedback ($M = 3.44 \pm SE = .7$), Sham/feedback ($M = 3.50 \pm SE = .8$), real/feedback ($M = 3.90 \pm SE = .4$) (all $ps < .0005$). Furthermore, the real group's sequence knowledge was significantly below chance for the exclusion test in the non-feedback learning condition ($M = 3.80 \pm SE = .7$; $p < .001$). In summary, only the real cTBS group in the non-feedback condition was unable to generate sequence knowledge above the chance level on the first test of conscious recall, indicating that conscious sequence knowledge was absent in that group and condition alone.

1.4 Discussion

We set out to show that feedback could boost procedural learning and restore learning after disruptive TMS delivered to M1. We saw significant effects in the predicted directions for both feedback and real cTBS on RT-based sequence knowledge 30 min after the end of training and 1 hr. after cTBS. This time course for the effect of feedback, with the greatest effect on delayed sequence recall, is consistent with other observations on feedback and procedural learning. Abe et al. (2011) showed positive reinforcement during motor skill learning on a force tracking task did not alter performance of healthy participants during acquisition or immediate recall testing; however, it improved recall 6 hrs., 24 hrs., and 30 days later. This finding is consistent with the pattern of effects in our sham group. The timing of the effect of cTBS was different: in the non-feedback condition, there was a negative effect of real cTBS on initial error-based sequence learning and on error and RT-based sequence knowledge recall. Thus, the effect of real cTBS in the non-feedback condition on recall could be due to either an effect of real cTBS directly on recall, on initial learning, or a combination of both.

Counter to our expectations and previous studies, neither cTBS (Rosenthal et al., 2009; Wilkinson et al., 2010) nor feedback (Wächter et al., 2009) affected RT difference scores during the initial phase of sequence learning. This could stem from paradigm differences. For instance, our feedback condition, which combined monetary rewards and punishments with sad and happy faces, may have changed the pace of the task and/or distracted participants more than the simpler procedure of separating rewarded and punished feedback conditions, used by Wächter, et al. (2009). Effects on RT could thus have been suppressed

when feedback was present in the learning blocks, but unmasked in the recall blocks when the task was presented without feedback. However, Abe et al. (2011), who also employed a simpler reinforcement procedure in their study, found no effect of feedback during acquisition, nor on immediate recall without feedback. Yet, testing at later time points, also without feedback, showed significant effects. Furthermore, our participants' overall RT was lower during learning (359–416 msec) than that (400–480 msec) seen by Wilkinson et al (2010), possibly because, here, we pushed RT toward a “floor,” and reduced the chance of detecting an effect of cTBS on RT-based sequence acquisition. One reason why the overall RT during learning may have been lower than in previous studies is the presence of feedback, per se. We saw significantly lower RT and a higher error rate during learning in the feedback, than non-feedback, condition. As mentioned above, a similar pattern was reported by Wächter et al. (2009) who found reduced RT combined with increased error rate even during blocks of random stimuli and, therefore, unrelated to learning.

Even though real cTBS did not affect RT-based sequence knowledge during initial learning, as mentioned earlier it did significantly decrease the sequence learning-dependent difference in error rate between probable and improbable trials during this period, as well as during recall. Furthermore, real cTBS abolished the feedback dependent priming effect observed in the overall error data during the test blocks. Using a similar paradigm to this one, Wilkinson et al. (2010) found that cTBS over M1 also decreased sequence learning-dependent error differences during learning without feedback. Rosenthal et al. (2009), who also examined the effect of M1 cTBS on pSRTT learning did not report this kind of analysis of the error data.

Further support for the hypothesis that feedback can rescue knowledge acquired during a temporary impairment comes from the awareness analysis. When tested with probes from the non-feedback condition, our real cTBS group was the only one without some accessible sequence knowledge. This pattern is similar to the behavior of other healthy participants after cTBS over M1 (Rosenthal et al., 2009) and PD patients (Wilkinson & Jahanshahi, 2007; Wilkinson et al., 2009). In this study, the addition of feedback significantly reversed this deficit, restoring sequence knowledge to a better-than-chance level. Our findings indicate motor sequence learning is not a purely implicit task. It can engage declarative, as well as procedural, systems and the interaction between these networks during motor learning depends on the learning environment.

It is of particular interest whether the reversal of the cTBS deficit by feedback involves a specific interaction within one system or simply the addition of two opposite, but unrelated, effects. M1 is part of a motor network involving the dorsal putamen, premotor cortex (PMc), supplementary motor area (SMA), and with other regions, which has been implicated in motor sequence learning without extrinsic feedback (Doyon, Owen, Petrides, Sziklas, & Evans, 1996; Grafton, Hazeltine, & Ivry, 1995; Hazeltine, Grafton, & Ivry, 1997; Poldrack et al., 2005; Rosenthal et al., 2009; Schendan, Searl, Melrose, & Stern, 2003; Seidler et al., 2005; Wachter et al., 2009; Wilkinson et al., 2010). In addition to the classical reward projection via the ventral striatum, M1 receives a direct dopamine (DA) pathway, which appears essential for the acquisition of skilled movements in rats (Hosp, Pektanovic, Rioult-

Pedotti, & Luft, 2011; Molina-Luna et al., 2009). Therefore, real cTBS could disrupt motor loop activity, reward, or both, during learning.

Recently, we used fMRI during initial learning on the same task, without extrinsic feedback, to explore the effects of cTBS over M1. There, we found that cTBS over M1 disrupts connectivity in the motor network and shifts learning to an associative visuospatial network (Steel et al., Under review). Furthermore, the associative network does not retain knowledge as efficiently as the motor network (Hikosaka, Nakamura, Sakai, & Nakahara, 2002). This may explain the impairment of early recall observed in the current study.

PD (R. G. Brown et al., 2003; Deroost, Kerckhofs, Coene, Wijnants, & Soetens, 2006; Doyon et al., 1997; Jackson et al., 1995; Kelly, Jahanshahi, & Dirnberger, 2004; Muslimovic et al., 2007; Pascual-Leone et al., 1993; Wilkinson & Jahanshahi, 2007; Wilkinson et al., 2009) and HD (Knopman & Nissen, 1991) patients, with widespread basal ganglia and cortical pathology show impaired learning on the SRTT, although, as mentioned previously, non-motor procedural learning is not improved and, in fact, worsened, by the addition of feedback including reward (Holl et al., 2012; Shohamy et al., 2004). Presumably, the HD and PD phenotypes represent damage to both the motor and reward components. The fact that the learning deficit from real cTBS can be reversed by adding feedback suggests an effect isolated to the motor circuit, leaving the reward system intact. This indicates that M1 cTBS is not a model of PD or HD. While it remains to be seen whether feedback further impairs motor procedural learning in PD and HD patients, as it does non-motor procedural learning, it is still interesting that feedback is effective at improving learning at substantially different performance levels and does not require full function in other systems to operate.

Like PD and HD patients, individuals with TBI are impaired at SRTT learning (Mutter et al., 1994; Vakil, Kraus, Bor, & Groswasser, 2002), perhaps because cortico-striatal circuits are disrupted at the cortical level (Vakil, 2005). Therefore, it is possible that real cTBS delivered over cortical regions, is a better model of TBI than PD and HD and TBI patients may benefit more from reward and feedback than those with PD and HD.

Does added feedback improve motor sequence learning in humans via a cortical DA pathway? Wächter et al. (2009) found that the addition of reward to the SRTT improved learning and increased learning-associated activity in prefrontal, but not motor, cortex. Activity also increased in areas of the dorsal and ventral striatum, including the nucleus accumbens, suggesting that extrinsic reward was acting via subcortical pathways. Raclopride positron emission tomography in healthy individuals has also shown increased DA release in the ventral striatum when incentive feedback was added to non-motor procedural learning (Wilkinson et al., 2014). These findings and the absence of reports from human imaging studies showing effects of reward on M1 activity leave questions about the significance of human cortical DA pathways unanswered, but implicate subcortical projections in the effect of feedback on procedural learning.

A unique feature of this study was the measurement of MEPs as an independent measure of the effect of real cTBS on M1. Most importantly, it proved that the inhibitory effect of real

cTBS was present on average and persisted throughout the task. However, it also allowed us to ask if the substrates of the physiological and cognitive effects are the same. This question has particular pragmatic importance, as, lacking an online measure of cognitive effects, we dose TMS based on the MEP. A clear correlation between the strengths of the MEP depression and memory effects/learning decrement within individuals would have provided satisfying evidence in favor of overlap between neural mechanisms. Instead, as mentioned previously, there was a striking positive association between the effect of real cTBS on the MEP at 45 min post- after stimulation and sequence knowledge at recall—the exact inverse of what we expected—and only in the feedback condition. One possible explanation for this finding is that both prolonged MEP suppression and recall after feedback-aided learning represent plastic effects on neural circuits in M1, and that the association within individuals is related to the tendency to plasticity. One example of an individual factor causing individual differences in learning and the response to TMS is a variation in the brain-derived nerve growth factor gene (Kleim et al., 2006). Another possibility is that temporary impairment of a “model-based” motor learning system by cTBS proportionately unmasked the operation of a competing, reward-based system. Under normal circumstances, the systems might operate in parallel. However, if the model-based system were selectively disrupted by cTBS, there could be “release” of the competing, reward-based, system and enhanced sequence learning in the feedback condition. The degree of shift between systems might correlate with the strength of M1 inhibition. The idea that memory systems can compete in this manner has been demonstrated and discussed previously (R. M. Brown & Robertson, 2007a, 2007b; Galea, Albert, Ditye, & Miall, 2010; Keisler & Shadmehr, 2010; Robertson, 2012). While the trend was not significant, the sham group MEPs decreased after learning and increased at 45 min after real cTBS. Others (Muellbacher et al., 2002; Pascual-Leone et al., 1995; Pascual-Leone, Grafman, & Hallett, 1994) have noted changes in the MEP with sequence learning. It is possible that real cTBS suppressed this phenomenon.

The effect of exogenous reward on procedural learning raises the question of whether and how the reward system is recruited when extrinsic feedback is not added. Clearly, real world practice often provides long- and short-term reinforcement, but the pSRTT and similar tasks are specifically designed not to make success apparent to the participant. One of the salient features of reinforcement learning is that implicit associations, such as those in the SRTT, can be learned even when they are seemingly stochastic, as in the pSRTT. Therefore, it is possible that the reward system is recruited when participants “guess right” and anticipate the next cue. Such events might violate the natural assumption that the cue stream is random and unpredictable, generating a “prediction error” (Schultz, 2000) between expectations and the results of behavior, and leading to reinforcement of the association between cues. In fact, as mentioned above, recent imaging data (Steel et al., Under review) showed learning related activation in the VTA as well as in the motor loop and other regions when motor sequence learning occurs without feedback. Enhancing the salience of the behavioral outcome with feedback and money could increase the strength of the error signal.

Our RT data showed sequence knowledge improvement from the learning to the recall blocks in both cTBS groups, but only in the feedback condition. It is plausible that the presence of feedback during learning made possible early memory consolidation regardless of the effects of stimulation. M1 has been implicated in early and long-term consolidation of

procedural learning (Kantak, Sullivan, Fisher, Knowlton, & Winstein, 2010; Karni et al., 1995; Matsuzaka, Picard, & Strick, 2007; Muellbacher et al., 2002; Robertson, Pascual-Leone, & Press, 2004; Robertson, Press, & Pascual-Leone, 2005; Tecchio et al., 2010), typically in paradigms testing memory after overnight sleep (Kantak et al., 2010) or a longer daytime delay, e.g., 12 hrs. (Robertson et al., 2004; Robertson et al., 2005), see Robertson (2012) for review). However, other studies in healthy individuals (Tecchio et al., 2010) and PD patients (Bedard & Sanes, 2011) suggest that consolidation begins much earlier and on a timescale comparable to what we observed.

In conclusion, we found that incentive feedback can enhance the retention of procedural knowledge substantially and that this retention persists during temporary impairment of a contributing brain system. Procedural memory is involved in most human learning and training and has great importance in rehabilitation after illness, trauma, and the acquisition of skills by healthy individuals. Adding feedback is a simple, safe, inexpensive, and regulation-free candidate for accelerating this process and could have considerable benefits in and outside the clinic.

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Abbreviations

AMT	active motor threshold
cTBS	continuous theta burst stimulation
DA	dopamine
EMG	electromyogram
FB	feedback
FDI	first dorsal interosseous muscle
HD	Huntington's disease
M1	primary motor area
MEP	motor evoked potential
MRI	magnetic resonance imaging
NART	National Adult Reading Test
non-FB	non-feedback
PD	Parkinson's disease
PDP	process dissociation procedure

PET	positron emission tomography
PMc	premotor cortex
pSRTT	probabilistic serial reaction time task
RT	reaction time
RMT	resting motor threshold
SOC	second order conditional
SMA	supplementary motor area
SRTT	serial reaction time task
TBI	traumatic brain injury
TMS	transcranial magnetic stimulation
VTA	ventral tegmental area

References

- Abe M, Schambra H, Wassermann EM, Luckenbaugh D, Schweighofer N, Cohen LG. Reward Improves Long-Term Retention of a Motor Memory through Induction of Offline Memory Gains. *Current Biology*. 2011; 21(7):557–562.10.1016/j.cub.2011.02.030 [PubMed: 21419628]
- Bedard P, Sanes JN. Basal ganglia-dependent processes in recalling learned visual-motor adaptations. *Experimental Brain Research*. 2011; 209(3):385–393.10.1007/s00221-011-2561-y [PubMed: 21311878]
- Brown RG, Jahanshahi M, Limousin-Dowsey P, Thomas D, Quinn NP, Rothwell JC. Pallidotomy and incidental sequence learning in Parkinson's disease. *Neuroreport*. 2003; 14(1):21–24. [PubMed: 12544824]
- Brown RM, Robertson EM. Inducing motor skill improvements with a declarative task. *Nature Neuroscience*. 2007a; 10(2):148–149.10.1038/nn1836 [PubMed: 17237776]
- Brown RM, Robertson EM. Off-line processing: Reciprocal interactions between declarative and procedural memories. *Journal of Neuroscience*. 2007b; 27(39):10468–10475.10.1523/jneurosci.2799-07.2007 [PubMed: 17898218]
- De Beaumont L, Tremblay S, Poirier J, Lassonde M, Theoret H. Altered bidirectional plasticity and reduced implicit motor learning in concussed athletes. *Cereb Cortex*. 2012; 22(1):112–121.10.1093/cercor/bhr096 [PubMed: 21572090]
- Deroost N, Kerckhofs E, Coene M, Wijnants G, Soetens E. Learning sequence movements in a homogenous sample of patients with Parkinson's disease. *Neuropsychologia*. 2006; 44(10):1653–1662. [PubMed: 16674983]
- Destrebecqz A, Peigneux P, Laureys S, Degueldre C, Del Fiore G, Aerts J, Maquet P. The neural correlates of implicit and explicit sequence learning: Interacting networks revealed by the process dissociation procedure. *Learning & Memory*. 2005; 12(5):480–490. [PubMed: 16166397]
- Doyon J, Gaudreau D, Laforce R, Castonguay M, Bedard PJ, Bedard F, Bouchard JP. Role of the striatum, cerebellum, and frontal lobes in the learning of a visuomotor sequence. *Brain and Cognition*. 1997; 34(2):218–245. [PubMed: 9220087]
- Doyon J, Owen AM, Petrides M, Sziklas V, Evans AC. Functional anatomy of visuomotor skill learning in human subjects examined with positron emission tomography. *European Journal of Neuroscience*. 1996; 8(4):637–648. [PubMed: 9081615]
- Fayol M, Thevenot C. The use of procedural knowledge in simple addition and subtraction problems. *Cognition*. 2012; 123(3):392–403.10.1016/j.cognition.2012.02.008 [PubMed: 22405923]

- Galea JM, Albert NB, Ditye T, Miall RC. Disruption of the Dorsolateral Prefrontal Cortex Facilitates the Consolidation of Procedural Skills. *Journal of Cognitive Neuroscience*. 2010; 22(6):1158–1164.10.1162/jocn.2009.21259 [PubMed: 19413472]
- Grafton ST, Hazeltine E, Ivry R. Functional Mapping of Sequence Learning in Normal Humans. *Journal of Cognitive Neuroscience*. 1995; 7(4):497–510. [PubMed: 23961907]
- Hazeltine E, Grafton ST, Ivry R. Attention and stimulus characteristics determine the locus of motor-sequence encoding - A PET study. *Brain*. 1997; 120:123–140. [PubMed: 9055803]
- Hikosaka O, Nakamura K, Sakai K, Nakahara H. Central mechanisms of motor skill learning. *Current Opinion in Neurobiology*. 2002; 12(2):217–222.10.1016/s0959-4388(02)00307-0 [PubMed: 12015240]
- Holl AK, Wilkinson L, Tabrizi SJ, Painold A, Jahanshahi M. Probabilistic classification learning with corrective feedback is selectively impaired in early Huntington's disease-Evidence for the role of the striatum in learning with feedback. *Neuropsychologia*. 2012; 50(9):2176–2186.10.1016/j.neuropsychologia.2012.05.021 [PubMed: 22659110]
- Hosp JA, Pekanovic A, Rioult-Pedotti MS, Luft AR. Dopaminergic Projections from Midbrain to Primary Motor Cortex Mediate Motor Skill Learning. *Journal of Neuroscience*. 2011; 31(7):2481–2487.10.1523/jneurosci.5411-10.2011 [PubMed: 21325515]
- Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. Theta burst stimulation of the human motor cortex. *Neuron*. 2005; 45(2):201–206. [PubMed: 15664172]
- Jackson GM, Jackson SR, Harrison J, Henderson L, Kennard C. Serial Reaction-Time Learning and Parkinsons-Disease - Evidence for a Procedural Learning Deficit. *Neuropsychologia*. 1995; 33(5):577–593. [PubMed: 7637854]
- Kantak SS, Sullivan KJ, Fisher BE, Knowlton BJ, Winstein CJ. Neural substrates of motor memory consolidation depend on practice structure. *Nature Neuroscience*. 2010; 13(8):923–925.10.1038/nn.2596 [PubMed: 20622872]
- Karni A, Meyer G, Jezard P, Adams MM, Turner R, Ungerleider LG. Functional Mri Evidence for Adult Motor Cortex Plasticity During Motor Skill Learning. *Nature*. 1995; 377(6545):155–158. [PubMed: 7675082]
- Keel JC, Smith MJ, Wassermann EM. A safety screening questionnaire for transcranial magnetic stimulation. *Clinical Neurophysiology*. 2001; 112(4):720–720. [PubMed: 11332408]
- Keisler A, Shadmehr R. A Shared Resource between Declarative Memory and Motor Memory. *Journal of Neuroscience*. 2010; 30(44):14817–14823.10.1523/jneurosci.4160-10.2010 [PubMed: 21048140]
- Kelly SW, Jahanshahi M, Dirnberger G. Learning of ambiguous versus hybrid sequences by patients with Parkinson's disease. *Neuropsychologia*. 2004; 42(10):1350–1357. [PubMed: 15193942]
- Kleim JA, Chan S, Pringle E, Schallert K, Procaccio V, Jimenez R, Cramer SC. BDNF val66met polymorphism is associated with modified experience-dependent plasticity in human motor cortex. *Nature Neuroscience*. 2006; 9(6):735–737.10.1038/nn1699 [PubMed: 16680163]
- Knopman D, Nissen MJ. Procedural Learning Is Impaired in Huntingtons-Disease -Evidence from the Serial Reaction-Time-Task. *Neuropsychologia*. 1991; 29(3):245–254. [PubMed: 1829141]
- Matsumoto N, Hanakawa T, Maki S, Graybiel AM, Kimura M. Role of [corrected] nigrostriatal dopamine system in learning to perform sequential motor tasks in a predictive manner. *J Neurophysiol*. 1999; 82(2):978–998. [PubMed: 10444692]
- Matsuzaka Y, Picard N, Strick PL. Skill representation in the primary motor cortex after long-term practice. *Journal of Neurophysiology*. 2007; 97(2):1819–1832. [PubMed: 17182912]
- Molina-Luna K, Pekanovic A, Rohrich S, Hertler B, Schubring-Giese M, Rioult-Pedotti MS, Luft AR. Dopamine in Motor Cortex Is Necessary for Skill Learning and Synaptic Plasticity. *Plos One*. 2009; 4(9):e7082.10.1371/journal.pone.0007082 [PubMed: 19759902]
- Muellbacher W, Ziemann U, Wissel J, Dang N, Kofler M, Facchini S, Hallett M. Early consolidation in human primary motor cortex. *Nature*. 2002; 415(6872):640–644.10.1038/nature712 [PubMed: 11807497]
- Muslimovic D, Post B, Spelman JD, Schmand B. Motor procedural learning in Parkinson's disease. *Brain*. 2007; 130:2887–2897. [PubMed: 17855374]

- Mutter SA, Howard JH, Howard DV. Serial pattern learning after head-injury. *Journal of Clinical and Experimental Neuropsychology*. 1994; 16(2):271–288. [PubMed: 8021314]
- Nadeau SE. A paradigm shift in neurorehabilitation. *Lancet Neurology*. 2002; 1(2):126–130.10.1016/s1474-4422(02)00044-3 [PubMed: 12849517]
- Nissen MJ, Bullemer P. Attentional Requirements of Learning - Evidence from Performance-Measures. *Cognitive Psychology*. 1987; 19(1):1–32.
- Obeso JA, Jahanshahi M, Alvarez L, Macias R, Pedroso I, Wilkinson L, Rothwell JC. What can man do without basal ganglia motor output? The effect of combined unilateral subthalamotomy and pallidotomy in a patient with Parkinson's disease. *Experimental Neurology*. 2009; 220(2):283–292.10.1016/j.expneurol.2009.08.030 [PubMed: 19744484]
- Pascual-Leone A, Dang N, Cohen LG, Brasil-Neto JP, Cammarota A, Hallett M. Modulation of muscle responses evoked by transcranial magnetic stimulation during the acquisition of new fine motor skills. *J Neurophysiol*. 1995; 74(3):1037–1045. [PubMed: 7500130]
- Pascual-Leone A, Grafman J, Clark K, Stewart M, Massaquoi S, Lou JS, Hallett M. Procedural Learning in Parkinsons-Disease and Cerebellar Degeneration. *Annals of Neurology*. 1993; 34(4):594–602. [PubMed: 8215247]
- Pascual-Leone A, Grafman J, Hallett M. Modulation of cortical motor output maps during the development of implicit and explicit knowledge. *Science*. 1994; 263:1287–1289. [PubMed: 8122113]
- Poldrack RA, Sabb FW, Foerde K, Tom SM, Asarnow RF, Bookheimer SY, Knowlton BJ. The neural correlates of motor skill automaticity. *Journal of Neuroscience*. 2005; 25(22):5356–5364. [PubMed: 15930384]
- Reed J, Johnson P. Assessing Implicit Learning with Indirect Tests - Determining What Is Learned About Sequence Structure. *Journal of Experimental Psychology-Learning Memory and Cognition*. 1994; 20(3):585–594.
- Reis J, Robertson EM, Krakauer JW, Rothwell J, Marshall L, Gertoff C, Cohen LG. Consensus: Can transcranial direct current stimulation and transcranial magnetic stimulation enhance motor learning and memory formation? *Brain Stimulation*. 2008; 1(4):363–369.10.1016/j.brs.2008.08.001
- Robertson EM. New Insights in Human Memory Interference and Consolidation. *Current Biology*. 2012; 22(2):R66–R71.10.1016/j.cub.2011.11.051 [PubMed: 22280913]
- Robertson EM, Pascual-Leone A, Press DZ. Awareness modifies the skill-learning benefits of sleep. *Current Biology*. 2004; 14(3):208–212.10.1016/j.cub.2004.01.027 [PubMed: 14761652]
- Robertson EM, Press DZ, Pascual-Leone A. Off-line learning and the primary motor cortex. *Journal of Neuroscience*. 2005; 25(27):6372–6378.10.1523/jneurosci.1851-05.2005[issn0270-6474 [PubMed: 16000627]
- Rosenthal CR, Roche-Kelly EE, Husain M, Kennard C. Response-Dependent Contributions of Human Primary Motor Cortex and Angular Gyrus to Manual and Perceptual Sequence Learning. *Journal of Neuroscience*. 2009; 29(48):15115–15125.10.1523/jneurosci.2603-09.2009 [PubMed: 19955363]
- Sandrini M, Cohen LG. Noninvasive brain stimulation in neurorehabilitation. *Handb Clin Neurol*. 2013; 116:499–524.10.1016/b978-0-444-53497-2.00040-1 [PubMed: 24112919]
- Schendan HE, Searl MM, Melrose RJ, Stern CE. An fMRI study of the role of the medial temporal lobe in implicit and explicit sequence learning. *Neuron*. 2003; 37(6):1013–1025. [PubMed: 12670429]
- Schultz W. Multiple reward signals in the brain. *Nature Reviews Neuroscience*. 2000; 1(3):199–207. [PubMed: 11257908]
- Schultz W. Getting formal with dopamine and reward. *Neuron*. 2002; 36(2):241–263. [PubMed: 12383780]
- Seidler RD, Purushotham A, Kim SG, Ugurbil K, Willingham D, Ashe J. Neural correlates of encoding and expression in implicit sequence learning. *Experimental Brain Research*. 2005; 165(1):114–124. [PubMed: 15965762]

- Shohamy D, Myers CE, Grossman S, Sage J, Gluck MA, Poldrack RA. Cortico-striatal contributions to feedback-based learning: converging data from neuroimaging and neuropsychology. *Brain*. 2004; 127:851–859. [PubMed: 15013954]
- Steel A, Song SS, Bageac D, Knutson K, Gotts SJ, Saad ZS, Wilkinson L. Shifts in connectivity during procedural learning induced by non-invasive brain stimulation. (Under review).
- Tecchio F, Zappasodi F, Assenza G, Tombini M, Vollaro S, Barbati G, Rossini PM. Anodal Transcranial Direct Current Stimulation Enhances Procedural Consolidation. *Journal of Neurophysiology*. 2010; 104(2):1134–1140.10.1152/jn.00661.2009 [PubMed: 20538777]
- Vakil E. The effect of moderate to severe traumatic brain injury (TBI) on different aspects of memory: A selective review. *Journal of Clinical and Experimental Neuropsychology*. 2005; 27(8):977–1021.10.1080/13803390490919245 [PubMed: 16207622]
- Vakil E, Kraus A, Bor B, Groswasser Z. Impaired skill learning in patients with severe closed-head injury as demonstrated by the serial reaction time (SRT) task. *Brain and Cognition*. 2002; 50(2): 304–315. [PubMed: 12464197]
- Wachter T, Lungu OV, Liu T, Willingham DT, Ashe J. Differential Effect of Reward and Punishment on Procedural Learning. *Journal of Neuroscience*. 2009; 29(2):436–443.10.1523/jneurosci.4132-08.2009 [PubMed: 19144843]
- Wassermann EM. Variation in the response to transcranial magnetic brain stimulation in the general population. *Clin Neurophysiol*. 2002; 113(7):1165–1171. [PubMed: 12088713]
- Wilkinson L, Jahanshahi M. The striatum and probabilistic implicit sequence learning. *Brain Research*. 2007; 1137(1):117–130. [PubMed: 17229409]
- Wilkinson L, Khan Z, Jahanshahi M. The role of the basal ganglia and its cortical connections in sequence learning: Evidence from implicit and explicit sequence learning in Parkinson's disease. *Neuropsychologia*. 2009; 47(12):2564–2573.10.1016/j.neuropsychologia.2009.05.003 [PubMed: 19447121]
- Wilkinson L, Lagnado DA, Quallo M, Jahanshahi M. The effect of feedback on non-motor probabilistic classification learning in Parkinson's disease. *Neuropsychologia*. 2008; 46(11):2683–2695.10.1016/j.neuropsychologia.2008.05.008 [PubMed: 18585741]
- Wilkinson L, Shanks DR. Intentional control and implicit sequence learning. *Journal of Experimental Psychology-Learning Memory and Cognition*. 2004; 30(2):354–369.
- Wilkinson L, Tai YF, Lin CS, Lagnado DA, Brooks DJ, Piccini P, Jahanshahi M. Probabilistic classification learning with corrective feedback is associated with in vivo striatal dopamine release in the ventral striatum, while learning without feedback is not. *Human Brain Mapping*. 2014; 35(2):225–236.10.1002/hbm.22536
- Wilkinson L, Teo JT, Obeso I, Rothwell JC, Jahanshahi M. The Contribution of Primary Motor Cortex is Essential for Probabilistic Implicit Sequence Learning: Evidence from Theta Burst Magnetic Stimulation. *Journal of Cognitive Neuroscience*. 2010; 22(3):427–436. [PubMed: 19301999]
- Willingham DB, Goedert-Eschmann K. The relation between implicit and explicit learning: Evidence for parallel development. *Psychological Science*. 1999; 10(6):531–534.10.1111/1467-9280.00201
- Willingham DB, Salidis J, Gabrieli JDE. Direct comparison of neural systems mediating conscious and unconscious skill learning. *Journal of Neurophysiology*. 2002; 88(3):1451–1460. [PubMed: 12205165]

Figure 1.

Mean RT for probable (prob) and improbable (imp) trials across learning (1–20) and recall test (21–23) blocks, plotted by feedback condition and stimulation group. Non-feedback: non-FB; feedback: FB. Bars show standard error.

Figure 2.

Mean error rate for probable (prob) and improbable (imp) trials across learning (1–20) and recall test (21–23) blocks, plotted by feedback condition and stimulation group. Non-feedback: non-FB; feedback: FB. Bars show standard error.

Figure 3.

A. Difference in mean error difference score (higher score = better learning; see text) for learning blocks 1–20, collapsed across feedback condition, **: $p < .05$, 2-tailed, uncorrected; *: $p < .05$, 1-tailed, uncorrected. Black diamonds: not significantly > 0 ; real. Blue diamonds: not significantly > 0 ; sham. B. Difference in mean error difference score for recall test blocks, higher score = better recall, collapsed across feedback condition, *: $p < .05$, 1-tailed. C. Mean error difference scores across learning and recall test blocks. Non-feedback: non-FB; feedback: FB. Bars show standard error.

Figure 4.

Mean RT difference scores (see text) across learning and test blocks. Higher score = better learning and recall. Non-feedback: non-FB; feedback: FB. Significant comparisons between recall test performance for the two stimulation groups and for each feedback condition: *: $p < .05$, **: $p < .01$, ***: $p < .001$. Bars show standard error.

Figure 5.

Mean change in log-normalized motor evoked potential (MEP) amplitude from baseline: i) immediately after real or sham continuous theta burst (cTBS), ii) immediately after learning, and 30 min after cTBS, and iii) after learning and 45 min after cTBS, plotted by feedback condition and stimulation group: non-feedback (non-FB) and feedback (FB). Bars show standard error.

Figure 6.
Association between motor evoked potential (MEP) change from baseline at 45 min after cTBS and RT-based recall in the feedback (FB) and non-feedback (non-FB) conditions. Lines are linear regression.

Figure 7.

Mean number of correct test completions in the process dissociation procedure. Dotted line is chance performance. Non-feedback: non-FB; feedback: FB. Diamonds; different from chance. Bars show standard error.