



Brain–behavior correlates of optimizing learning through interleaved practice

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ABSTRACT

Understanding how to make learning more efficient and effective is an important goal in behavioral neuroscience. The notion of “desirable difficulties” asserts that challenges for learners during study result in superior learning. One “desirable difficulty” that has a robust benefit on learning is contextual interference (CI), in which different tasks are practiced in an interleaved order rather than in a repetitive order. This study is the first to combine functional imaging and paired-pulse transcranial magnetic stimulation to analyze the neural basis of the CI effect in skill learning. Difficulty during practice of a serial reaction time task was manipulated by presenting sequences of response locations in a repetitive or an interleaved order. Participants practiced 3 sequences for 2 days and were tested on day 5 to examine sequence-specific learning. During practice, slower response times (RT), greater frontal–parietal blood-oxygen-level-dependent (BOLD) signal, and higher motor cortex (M1) excitability were found in the interleaved condition compared to the repetitive condition. Consistent with the CI effect, we found faster RT, decreased BOLD signal in frontal–parietal regions, and greater M1 excitability during the day 5 retention task when subjects had practiced interleaved sequences. Correlation analyses indicated that greater BOLD signal in contralateral sensorimotor region and M1 excitability during interleaved practice were interrelated. Furthermore, greater BOLD signal in prefrontal, premotor and parietal areas and greater M1 excitability during interleaved practice correlated with the benefit of interleaved practice on retention. This demonstrates that interleaved practice induces interrelated changes in both cortical hemodynamic responses and M1 excitability, which likely index the formation of enhanced memory traces and efficient long-term retrieval.

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Introduction

Previous studies have shown that introducing manipulations that make performance more difficult during practice may nevertheless improve long-term retention (Schmidt and Bjork, 1992). Bjork proposed the notion of “desirable difficulties”; that challenges for learners such as context shifts and retrieval during study result in more robust learning and should be introduced into skill practice (Christina and Bjork, 1991). An example of *desirable difficulties* is the contextual interference (CI) effect where practice context is manipulated by presenting multiple tasks in either a repetitive (blocked)

order or an interleaved (random) order (Shea and Morgan, 1979). Practicing tasks in an interleaved order generally results in inferior practice performance but induces superior retention compared to practicing in a repetitive order (Brady, 2008). This differential effect of practice condition during practice and retention phases is an example of the distinction between performance and learning. While CI is detrimental to performance during the practice phase, it benefits the learning of skills.

The CI effect was originally demonstrated in the verbal learning literature (Battig, 1966) and was subsequently studied in motor learning tasks (Shea and Morgan, 1979). In a classic study of the CI effect, Shea and Morgan had subjects learn three arm-movement tasks presented in a repetitive (less difficult) or an interleaved (more difficult) order. Subjects were retested after a 10-min or 10-day delay with both repetitive and interleaved presentation of the practiced tasks. Subsequent transfer to a task of either the same or greater complexity than the originally learned tasks was also investigated.

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Supporting Bjork's conceptualization of desirable difficulties in cognitive learning, Shea and Morgan's results demonstrated that during practice, a more difficult condition (interleaved practice) resulted in worse performance compared to a less difficult condition (repetitive practice). In contrast, retention and transfer were greater after interleaved practice than after repetitive practice. The poorer performance during interleaved practice implies that there is greater effort expended in a more difficult condition, and this increased effort during practice leads to better long-term retention.

Despite the robust benefits of CI on learning, little is currently known about the neural basis of how CI leads to better retention. This knowledge has important implications for understanding brain mechanisms in skill learning and how these neural processes can be optimized. The present study was designed to address two main questions. First, does neural activity reflect the paradoxical effect of CI on practice and retention? That is, will increased neural activity during interleaved compared to repetitive practice be accompanied by a relative decrease in activity during retention? Second, does increased neural activity during interleaved practice predict enhanced learning as assessed on a later retention test? In this study, our measures of neural activity are bold-oxygen-level-dependent (BOLD) signal on fMRI and cortical excitability as assessed by paired-pulse transcranial magnetic stimulation (ppTMS) (Kujirai et al., 1993). As such, we were also able to relate changes between these two measures of neural activity with each other.

We applied fMRI online during 2 days of practice of a sequence learning task and during a retention test on day 5. Neurophysiologic changes in primary motor cortex (M1) excitability were evaluated by ppTMS offline (while subjects were at rest) before and after each fMRI practice session and before the fMRI retention test on day 5. Based on the prediction of *desirable difficulties* in CI, we anticipated that practicing sequences in an interleaved order would result in inferior performance during practice but would induce superior sequence-specific learning compared to practice in a repetitive order (Shea and Morgan, 1979). Since previous imaging studies have shown that increased task complexity results in increased BOLD signal during practice, we anticipated the desirable difficulty effect of CI as greater sensorimotor activity measured by BOLD signal and M1 excitability during interleaved compared to repetitive practice (Kuhtz-Buschbeck et al., 2003; Verstynen et al., 2005). We predicted that this pattern would reverse during retention for BOLD signal, indicating more efficient processing after interleaved practice (Karni et al., 1995). We hypothesized that increased excitability after interleaved practice would be maintained at the retention test as enhanced excitability associated with practice of movement tasks may constitute a necessary precursor for inducing plastic changes within the motor system (Koeneke et al., 2006; Pascual-Leone et al., 1995). Furthermore, we predicted that increased sensorimotor BOLD activity and M1 excitability during interleaved practice would correlate with the subsequent level of skill retention (Tamas Kincses et al., 2008).

Methods

Study design

We applied a within-subject cross-over design with three measurements: behavior (serial reaction time sequence learning task, Fig. 1A) (Nissen and Bullemer, 1987), cerebral hemodynamic responses by functional magnetic resonance image (blood-oxygen-level-dependent signals (BOLD), fMRI), and intracortical excitability within the primary motor cortex (M1) by paired-pulse transcranial magnetic stimulation (ppTMS). The participants practiced the serial reaction time (SRT) task on 2 consecutive training days (days 1 and 2, Fig. 1B); to measure the effects of practice on learning, we tested the delayed retention performance (Cahill et al., 2001; Perez et al., 2005; Shea and Morgan, 1979; Wright et al., 2005) on day 5 (Fig. 1B). Behavioral and fMRI data were acquired concurrently on each testing day within a MRI scanner,

while the paired-pulse TMS was performed prior to and after each training day and before the follow-up session on day 5 in the adjacent TMS lab (Fig. 1B and C). Paired-pulse TMS was applied to evaluate how intracortical circuits excitability within M1 was modulated by practice in different conditions (Kujirai et al., 1993). An extensive psychophysical and neuroimaging literature has contrasted implicit and explicit learning of sensorimotor sequences using the SRT task (Abrahamse et al., 2010). The present study did not aim to investigate the difference between implicit and explicit sequence learning, in that the sequences were short and practiced extensively so all participants became aware of them. The SRT task was chosen here to study contextual interference because one can readily create multiple sequences that can be learned in either an interleaved or repetitive order. In addition, the SRT finger tapping task is a motor task that is readily adapted to fMRI because it involves minimal motion of the upper body, thus enabling us to assess BOLD signal differences during practice and retention.

Each participant practiced and learned a variation of the serial response task (SRT) over the course of 5 days, which consisted of three different four-element sequences, presented in either a repetitive or interleaved order (Fig. 1B, also see "Behavior Task" below for details). In this within-subject cross-over design, each participant started in the first week with either the repetitive practice (RP) or the interleaved practice (IP) condition; 2 weeks later, each participant performed in the other practice condition (i.e., repetitive → interleaved, or vice versa). The order of the practice conditions and the SRT sequences was counter-balanced across participants.

Functional images were acquired concurrently while the SRT was performed inside the MR scanner. For days 1 and 2, there were three functional imaging runs on each day (Fig. 1B). Each run consisted of 54 movement trials, where the participants practiced one test sequence in each trial. For repetitive practice (RP), each of the three test sequences was practiced for 54 consecutive trials (i.e., one fMRI run) before the next sequence appeared, leading to 162 trials (54 trials × 3 test sequences) for each day (Fig. 1B top). The order of the three sequences was counter-balanced across the participants. For interleaved practice (IP), the three tested sequences were arranged in a non-repetitive manner within each 54-trial fMRI run (Fig. 1B bottom). The same arrangement of the test sequences was applied to all the participants, so that every IP participant performed the same order of test sequences.

On the retention day (day 5), the participants underwent 3 fMRI runs, with 36 trials per run (Fig. 1B, day 5). In the first two runs they were tested with the three sequences they had practiced in the previous 2 days. In one run the practiced sequences were presented in a repetitive order (denoted by repetitive test, or RT), and in the other run they were presented in an interleaved order (denoted by interleaved test, or IT). This was to ensure that practice-test compatibility was equal for either practice condition (Lee, 1988; Lee and Magill, 1983; Shea and Morgan, 1979). The order of these runs was counter-balanced across subjects. Each sequence was presented for 24 trials across these two runs on day 5 (in contrast, during practice on days 1 and 2, each sequence was presented for 54 trials on each day). The reduced number of trials was used in order to limit further learning processing for the sequences (Cross et al., 2007). To assess whether learning was specific to the training sequences, on the third fMRI run the participants were tested with three novel, or unpracticed, test sequences (Fig. 1B).

Measures of M1 intracortical excitability by paired-pulse transcranial magnetic stimulation (ppTMS) were acquired before and after (pre and post) each fMRI session on days 1 and 2. This allowed immediate changes in M1 excitability to be monitored as an effect of practice. On day 5, intracortical excitability was acquired before the retention session in fMRI. Acquiring intracortical excitability measures before task performance on each day allowed a direct comparison of persistent changes in excitability independent of transient changes that would be apparent shortly after practice.

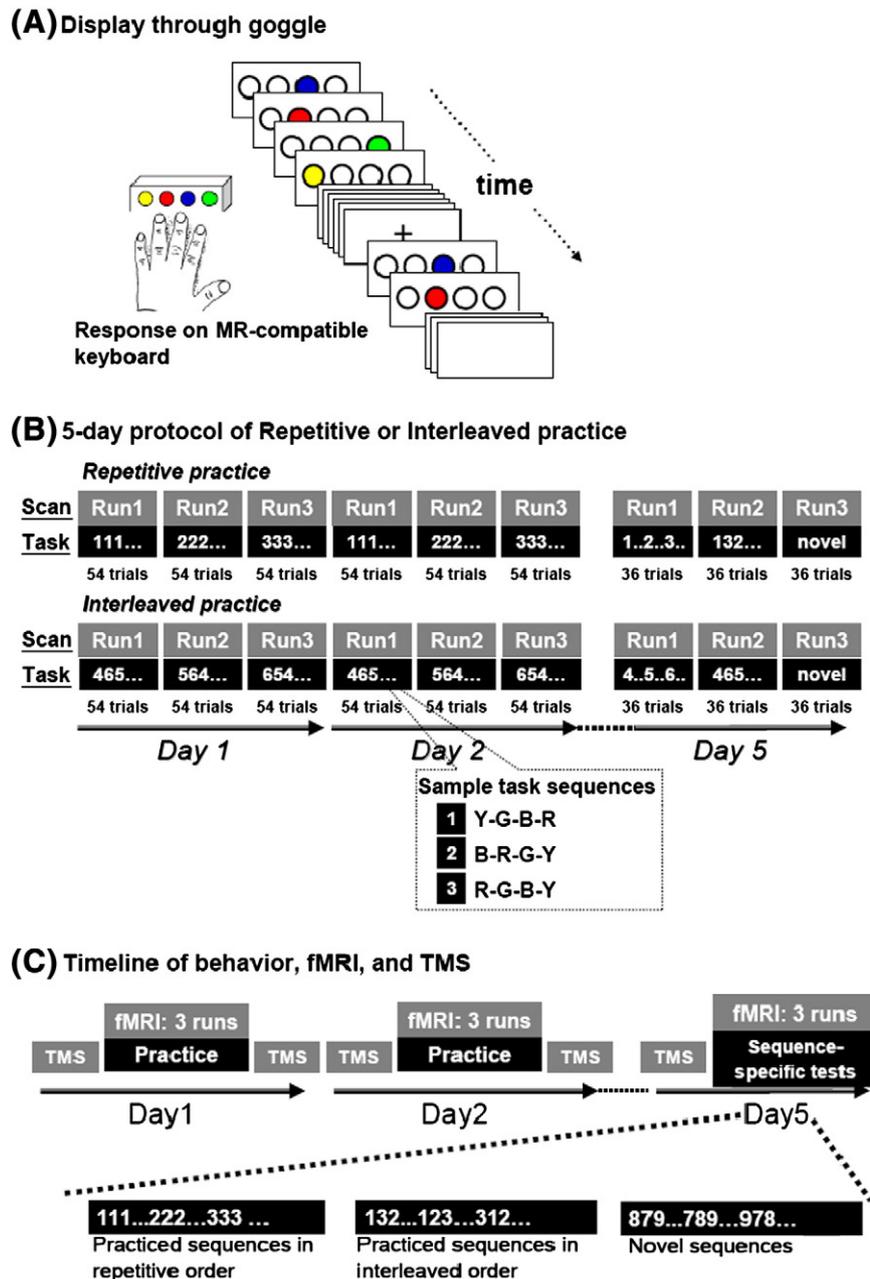


Fig. 1. illustrates that participants practiced serial reaction time sequence learning tasks (A) in either a repetitive or an interleaved order (B). Participants practiced tasks for 2 consecutive days with the retention tests of trained and untrained sequences taking place on day 5 (C). Imaging data were acquired concurrently while the participants practiced the tasks. During repetitive practice, participants performed the same sequence repeatedly for the same scan run. Paired-pulse TMS was conducted before and after each training day and before the tests on day 5 (C). Two weeks after practice and retention for one training order, participants returned to practice a different set of three sequences in the other condition (e.g., repetitive practice → interleaved practice).

Participants

Sixteen right-handed young adults were enrolled in the study (9 men and 7 women, ages 19–29). Participants were recruited from the University and adjacent community. All participants gave informed consent using an institutionally approved consent form. Participants were excluded if they had any contraindications to MRI or TMS, significant medical, neurological, or psychiatric history, a history of seizure, prescription medications, a family history of uncontrolled epilepsy, uncorrected vision loss, or scored less than 28 on the Mini-Mental State Exam (MMSE; Folstein et al., 1975).

Behavioral task

The SRT task during the fMRI scanning was executed as follows (Fig. 1A). Participants positioned the four fingers of the left hand (all except the thumb) on the four light-sensitive response keys of a magnet-compatible button box (Current Designs, Inc.). The left hand was used to increase the overall difficulty of the task, analogous to the approach in Cross et al. (2007). Subjects watched colored circles (yellow, red, blue, and green) through magnet-compatible goggles. Only one colored circle appeared at a time while the other circles were transparent (Fig. 1A). Each color was always displayed at the same

circle location, thus providing a consistent spatial and color cue for the motor response on the spatially corresponding response key. Participants were instructed to “respond as quickly as possible.” Within each sequence, one circle would be colored at a time with the next colored circle appearing as soon as the previous response was made.

Participants pressed four consecutive keys (four elements, equivalent to one sequence) to complete one task trial. To ensure that each subject practiced an equal number of trials, each sequence (four key presses) was presented for a fixed duration of 3 seconds. If the participant completed the four key presses before 3 seconds, 4 transparent circles would appear on the screen, thus controlling visual stimulation (Fig. 1A). Since we applied a blocked design for functional imaging acquisition with 18-second task blocks interleaved with 18-second rest blocks, behavioral trials were presented as 6 sequences per task block. During the rest block, the circles would be replaced by a fixation cross in the center of the screen. Participants were instructed to remain relaxed but gaze at the fixation cross. A custom-designed computer software program written with Presentation (Neurobehavioral Systems) controlled the appearance of the colored circles and recorded the participants' responses. Response times (RT) were recorded for each key press.

Functional magnetic resonance imaging

Images were acquired using a Siemens Trio 3.0-T MRI scanner. Two sets of high-resolution anatomical images were acquired for image registration. We acquired an MP-RAGE structural volume (TR = 1,900, TE = 2.26, flip angle = 8°) with 176 sagittal slices, each 1 mm thick with 0.5 mm gap and 1.33 mm × 1.33 mm in-plane resolution. We also acquired a T2-weighted co-planar volume (TR = 5,000, TE = 34, flip angle = 90°) with 34 transverse slices covering the whole brain, each 4 mm thick with 1 mm gap, a 128 × 128 matrix and an in-plane resolution of 1.5 mm × 1.5 mm.

Functional images were acquired while the participants performed the sequence learning task. There were three functional runs which corresponded to the three practice sessions on each day (Fig. 1B). On days 1 and 2, each functional run lasted for 5 min and 48 seconds, and consisted of 153 EPI volumes (gradient-echo, TR = 2,000, TE = 30, flip angle = 90°), each with 34 transverse slices, 4 mm thick, 1 mm gap, and a 64 × 64 matrix yielding an in-plane resolution of 3 mm × 3 mm. The first four volumes of each functional run were removed from analysis to allow for magnetization to approach equilibrium. On day 5, the participants underwent three functional runs (Fig. 1B, two runs for sequence specific tests and one run for the novel sequence test). Each run lasted for 3 min and 48 seconds and involved the acquisition of 99 EPI volumes.

Transcranial magnetic stimulation

Paired-pulse transcranial magnetic stimulation (ppTMS) was applied using two Magstim Rapid stimulators connected to a Bistim module. An optimal location for magnetic stimulation (hotspot) was defined as the location where magnetic stimuli consistently elicited a maximal motor MEP from the contralateral first dorsal interosseous (FDI) muscle. The stimulating coil was a figure-8 coil (diameter: 7 cm/wing). The point of intersection of the figure-8 coil was placed against the skull and the coil was held at a 45° angle to the mid-sagittal plane, with the handle oriented anteromedially (Brasil-Neto et al., 1992; Kammer et al., 2001; Suppa et al., 2008). This orientation has been shown to be the most efficient orientation to induce MEPs using a biphasic TMS unit. When the Magstim coil handle is oriented anteromedially (handle points forward), the initial phase of current flows *within the coil* toward the handle, or posterior-anteriorly. Thus, the first phase of induced current flow *within the brain* will flow anterior-posteriorly with the second phase of the biphasic pulse flowing posterior-anteriorly. Because the most

efficient stimulation direction for a biphasic pulse is the second phase, this orientation matches the most efficient stimulating current direction with the induced current from a usual monophasic TMS unit (Kammer et al., 2001). The use of biphasic stimulators has also shown to provide results comparable to that obtained with monophasic stimulators (Suppa et al., 2008).

Electromyography (EMG) was monitored throughout data collection to ensure the spatial specificity of magnetic pulses. EMG signals were acquired using surface electrodes in a belly-tendon montage from the first dorsal interosseous muscle of the left hand. The signal was filtered with a bandpass of 1–1000 Hz, amplified, and digitized at 5000 Hz. The data were visually displayed and stored for later analysis in 600-ms samples beginning 100 ms before TMS onset (Labview, National Instruments). In four subjects, MRI-guided frameless stereotaxy (Brainsight Frameless; Rogue Research, Montreal, Quebec, Canada) was used to verify the position of the coil with respect to the underlying brain anatomy.

Paired-pulse TMS trials were conducted by delivering a conditioning and a testing pulse (CS and TS, respectively) separated by an interstimulus interval (ISI) through the same coil over the M1 hotspot. The intensity of the conditioning stimulus (CS) was adjusted to be 90% active motor threshold (Ilic et al., 2002; Ziemann et al., 1996). Active motor threshold (aMT) was defined as the lowest stimulus intensity, which elicited a mean peak-to-peak MEP > 100 μV during slight isometric contraction (5–10% of maximum voluntary contraction) from five single-trial sweeps (Rossini et al., 1994). The intensity of testing stimulus (TS) was adjusted to evoke a motor-evoked potential (MEP) of 1 mV amplitude from peak to peak in a relaxed left first dorsal interosseous muscle (Kujirai et al., 1993). We collected 12 TMS trials for each of the 7 settings: 6 ISI (2, 3, 4, 5, 10, and 15 ms) and 1 TS alone. The short ISI (2, 3, 4, 5 ms) were chosen to represent short-interval intracortical inhibition (SICI) while the longer ISI (10, 15 ms) represent intracortical facilitation (ICF) (Chen et al., 1998). The order of 7 settings was randomized across participants. TMS trials were each separated by a minimum of 8 seconds. Each single TS pulse trial results in an unconditioned MEP. Each pair of CS-TS pulses (presented during ISI trials) results in a conditioned MEP. For each condition, peak-to-peak MEP amplitudes were averaged. Intracortical excitability for each ISI was computed as the mean conditioned MEP amplitude (cMEP) for that ISI expressed as a percentage of the mean unconditioned MEP amplitude.

Statistical analysis

Behavior data

Response time (RT) was defined as the time between stimulus onset to key press and was measured and stored. The total RT for each 4-element sequence trial was calculated by adding up the RT of each of the four key presses. For both practice and retention, we calculated the median total RT for every six sequence trials for use in all analyses.

To compare the effects of training conditions (repetitive versus interleaved) during practice, we averaged RT across 2 days of practice before determining the main condition effect. We also assessed how well participants performed during the retention test on day 5. The condition effect on day 5 was evaluated by comparing the averaged RT of the practiced sequences. We then investigated whether the superior performance on retention for interleaved practice was sequence-specific, or simply reflected general improvement in key-pressing speed (non-specific learning). Sequence-specific learning was defined as the averaged performance on the practiced sequences while non-specific learning was the averaged performance on the novel sequences on day 5. Full model analysis including the two practice conditions was conducted for practice and retention phases, separately. Paired sample *t*-tests were applied to contrast averaged RT between the repetitive and interleaved practice conditions on the

baseline performance, the end of practice, and performance on the trained and novel sequences on day 5.

Imaging data

Functional images were processed using the Statistical Parametric Mapping software (SPM5, Wellcome Department of Cognitive Neurology, London, UK). To correct for motion artifacts, functional image data were realigned to the first volume in each functional run and then resliced with 4th-degree B-Spline interpolation (Friston et al., 1995). None of the subjects had scans with head motion greater than 2 mm. After realignment, the resulting mean images of each subject were normalized to the standard Montreal Neurological Institute (MNI) EPI template (Evans et al., 1993). The normalization parameters were then applied to all the functional images of that subject. The normalized images were then resampled to $3 \times 3 \times 3$ mm³ per voxel, and subsequently spatially smoothed with an isotropic Gaussian filter with full width at half maximum (FWHM) = 8 mm. Statistical analysis was first carried out separately for each participant using the general linear model (GLM) (Friston et al., 1995). The fMRI data were modeled using a boxcar function that included an explicit baseline model convolved with the hemodynamic response function (HRF). An additional parametric regressor with the mean response time for each task block was applied to ensure that any differences in brain activities during practice and retention were due to the influences of the practice conditions but not to differences in the response time.

To address the CI effects on cerebral responses measured by BOLD signal, group comparisons under the four conditions below were performed using a second-level random effect analysis on the contrast images (task versus rest) derived from the above model fitting (Handwerker et al., 2004; Worsley et al., 2002): (1) interleaved practice minus repetitive practice, (2) repetitive practice minus interleaved practice (Goode et al., 2008), (3) retention BOLD response following interleaved practice minus those following repetitive practice, and (4) retention BOLD response following repetitive practice minus those following interleaved practice. All statistical maps were corrected for multiple comparisons using the false discovery rate (FDR) method (Benjamini and Hochberg, 1995). Overall significance was achieved when $FDR < 0.05$, which indicated that on average less than 5% of the significant voxels were false positive.

TMS data

For each participant, the primary ppTMS outcome measure, mean conditioned MEP (cMEP) expressed as a percentage of the unconditioned MEP, was computed for each ISI (2, 3, 4, 5, 10, 15 ms), for each of the five TMS sessions (day1-pre, day1-post, day2-pre, day2-post, and day5-pre) and for each practice condition (repetitive and interleaved). These cMEPs were initially analyzed with a full model ANOVA with Practice condition, Sessions, and ISI as within-subject factors. Post hoc analyses of main effects and interactions were carried out with pairwise comparisons. Since initial analysis found no significant interactions associated with ISI (Fig. 2C), we collapsed cMEPs across all ISIs for subsequent analyses. Because results at different ISIs represent different neural circuits (Chen et al., 1998), this consolidation of cMEP data effectively represents a measure of net neural excitability within the motor cortex in our study.

To examine the long-term effects of practice on later retention, we restricted our data analysis to measures of intracortical excitability before task performance on each practice and retention day (TMS(pre)) and for each practice condition. These three time points (day1-pre, day2-pre, and day5-pre) were not directly influenced by transient task performance effects. To demonstrate the long-term effects of interleaved practice on intracortical excitability, we compared TMS(pre) data during practice (average of days 1 and 2), and retention (day 5) between practice conditions.

To relate short-term practice-dependent changes in intracortical excitability to fMRI measures during practice and to later retention performance, we computed TMS(post-pre) for each practice condition as the cMEP difference before and after practice on day 1 (day1-post minus day1-pre), day 2 (day2-post minus day2-pre), and averaged across days 1 and 2.

Correlations between BOLD signal, excitability, and learning

We sought to determine if any differences in BOLD signal between the two conditions were associated with relative differences in M1 excitability and/or the learning benefits of interleaved practice. All correlations were performed using a contrast between practice conditions (either repetitive minus interleaved, denoted by R-I, or interleaved minus repetitive, denoted by I-R, depending on hypothesized direction of effect). Specifically, for correlations involving behavioral results, mean response time (RT) during practice was computed across day 1 and day 2 with a positive I-R contrast indicating slower RT for interleaved practice. As a measure of relative behavioral benefits of learning between practice conditions, mean response time (RT) during retention was computed on practice sequences on day 5 with a positive R-I contrast indicating faster RT (behavioral improvement) for interleaved practice.

Similar to the behavioral analysis, for practice-related BOLD contrasts, a positive I-R contrast indicates brain regions with greater BOLD signal for interleaved practice. However, for retention-related contrasts, a positive R-I contrast indicates brain regions with reduced BOLD signal (hypothesized to represent regions with increased efficiency in memory retrieval) for interleaved practice. For correlations involving M1 excitability, conditioned motor evoked potentials (cMEPs) were collapsed across all ISIs for each subject. For practice-related M1 excitability correlations, TMS(post-pre), was used to index the short-term increases in excitability induced by each practice session. For retention-related M1 excitability correlations, TMS(pre) was used to index long-term changes in excitability across days. For both practice and retention correlations in M1 excitability, positive I-R contrasts indicate that M1 excitability is greater for interleaved practice condition rather than for repetitive practice condition.

Five sets of correlation analyses, based on different between-condition contrasts on response time, BOLD signal, and TMS were performed to answer five specific questions:

- (c1) Were changes in BOLD signal and cortical excitability during the practice phase related? Relative BOLD contrast (BOLD, I-R) was correlated with the relative M1 excitability changes during practice (TMS(post-pre), I-R);
- (c2) Was the reduction in BOLD during the day 5 retention test after interleaved practice associated with enhanced retention performance? Relative BOLD contrast during retention (BOLD, R-I, positive contrasts indicate regions of greater BOLD reduction during retention tests following the interleaved practice condition) was correlated with the amount of benefit of interleaved practice on subsequent retention (RT, R-I, positive differences indicate improvement in performance following the interleaved practice condition).
- (c3) Was greater M1 excitability on the day 5 retention test associated with enhanced learning? Relative M1 excitability on day 5 (TMS(pre), I-R) was correlated with the amount of benefit of interleaved practice on subsequent retention (RT, R-I).
- (c4) Was increased BOLD signal during practice predictive of enhanced learning? Relative BOLD contrast (BOLD, I-R) during practice was correlated with the amount of benefit of interleaved practice on subsequent retention performance (RT, I-R).
- (c5) Did increased M1 excitability during practice correlate with enhanced learning? Relative M1 excitability changes between

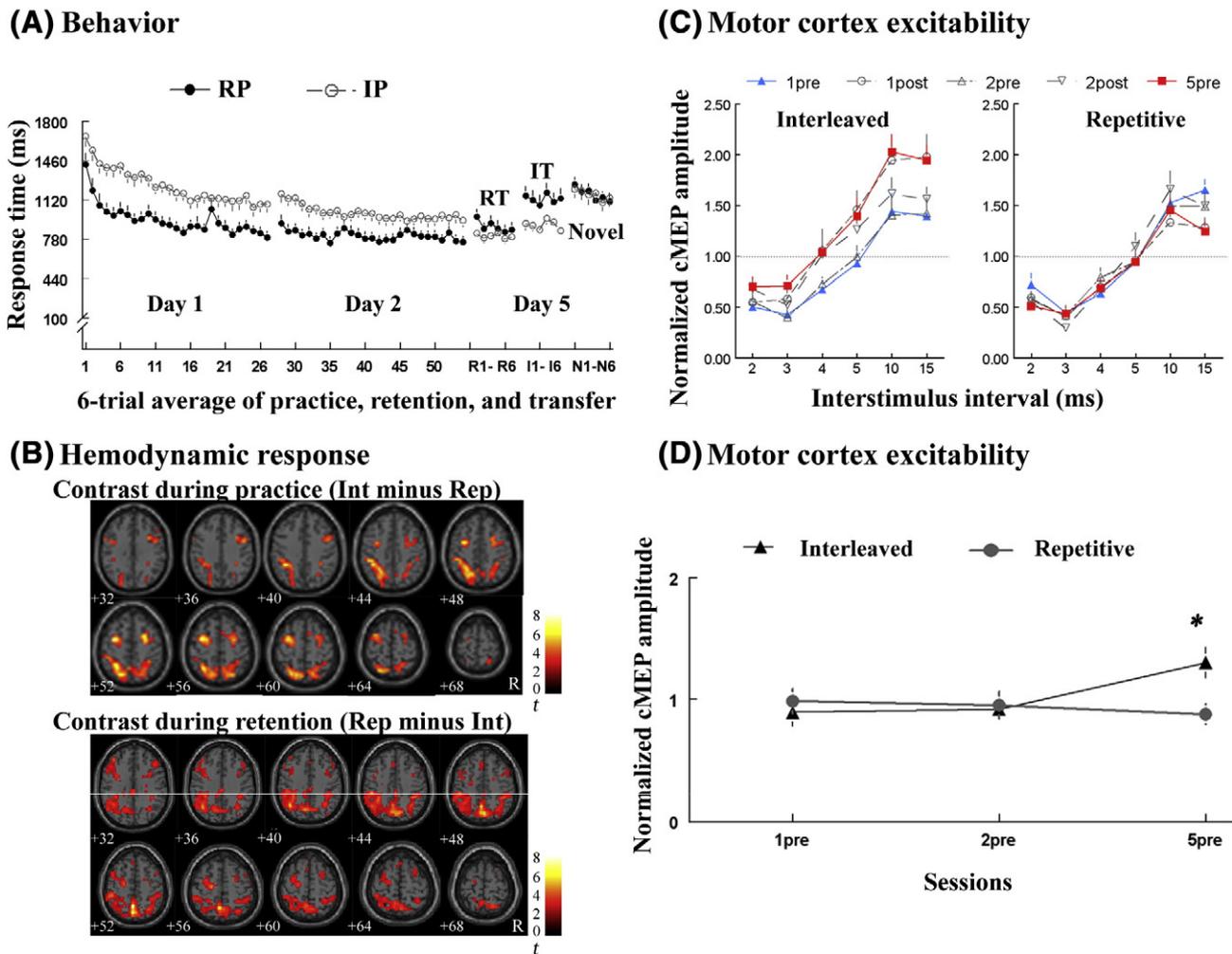


Fig. 2. Shows the effect of practice condition on behavior (A), hemodynamic response (B), and motor cortex excitability (C and D). Panel A shows task performance, measured by response time (mean \pm SE) on practice on days 1 and 2 (blocks 1–54), and on retention of practiced and novel sequences (repetitive testing condition (RT), interleaved testing condition (IT), and novel sequences, respectively) on day 5. At the baseline, there was no significant difference between repetitive (RP, filled circle) and interleaved (IP, empty circle) practice conditions. Performance in both conditions improved after 2 days of practice—the response time decreased and in general was faster in the repetitive than the interleaved condition. On day 5, performance of the trained sequences was significantly better for the interleaved than the repetitive condition, especially when the testing sequences were presented in an interleaved order (X axis label: R1–R6, averaged response time 1 to 6 of the repetitive condition; I1–I6, averaged response time 1 to 6 of the interleaved condition; and N1–N6, averaged response time 1 to 6 of the novel sequence). Performance of the novel sequences on day 5 was, however, not different between the two conditions, suggesting that the benefit of interleaved practice is sequence specific. Panel B shows the relative BOLD contrasts during practice and retention. A positive t -value means excess in BOLD responses, which may indicate greater demand in neural recruitment, and may be associated with less dexterity in task execution. The interleaved (Int) condition invoked greater sensorimotor BOLD responses than the repetitive (Rep) practice during the practice phase (upper row), but this pattern was reversed during retention (lower row). Panel C shows conditioned motor-evoked potential amplitudes (cMEP, mean \pm SE), normalized to the amplitude of a single testing pulse (TS) alone, as a function of inter-stimulus intervals (ISIs) for the interleaved (C, left) and repetitive (C, right) practice conditions at five time points: baseline before practice (1pre, filled gray up-triangle in blue), day 1 after practice (1post, open circle), day 2 before practice (2pre, open up-triangle), day 2 after practice (2post, open down-triangle), and day 5 before testings (5pre, filled red rectangle). Interleaved practice leads to increased motor cortex excitability: intracortical facilitation (the TMS effects with ISI = 10–15 ms) increased and intracortical inhibition (the TMS effects with ISI = 2–3 ms) decreased on day 5 vs. the baseline on day 1, demonstrated by the upper shift of the sigmoid curves. However, this was not observed in repetitive practice of sequences (C right). The increase in motor cortex excitability in the interleaved rather than the repetitive practice condition was found even in the pre-performance condition on every visit, as shown in D, where normalized cMEP amplitudes TMS(pre) averaged across all inter-stimulus intervals (mean \pm SE) of the interleaved (filled triangle) and repetitive (gray circle) practice conditions were presented at the baseline (1pre), day 2 (2pre), and day 5 (5pre) before performing any sequences. This pre-performance state of M1 excitability was not different between the two conditions until day 5, which may indicate a form of memory consolidation which differs between the interleaved and the repetitive practice.

the two conditions during practice (TMS(post-pre), I–R) were correlated with the benefit of interleaved practice on subsequent retention performance (RT, R–I).

For correlations involving BOLD signal, a whole-brain Pearson's correlation test was applied and corrected for multiple comparisons to identify brain regions related to the other correlational factor. Overall significance was achieved when $FDR < 0.05$. For all statistical tests in behavior and TMS data, significance level was set at $p < 0.05$. SPSS 13.0 (SPSS Inc., Chicago, IL) statistical software was used for the statistical analyses.

Results

Behavior results

At the start of practice, the average of the first 6 trials on day 1 showed no significant difference in response time between the repetitive and interleaved conditions ($p = 0.19$). Performance in both conditions improved after 2 days of practice and the overall response time was faster in the repetitive than in the interleaved condition ($p < 0.001$, Fig. 2A, day 1 and day 2, RP was lower than IP). At the end of practice on day 2, the average of the last 6 trials on day 2 continued to

show a significantly slower response time in the interleaved practice condition compared with the repetitive condition ($p=0.004$). This pattern was however reversed on the retention test on day 5, where performance of the trained sequences was faster for the sequences practiced in the interleaved than in the repetitive condition ($p=0.006$, Fig. 2A, collapsed across all sequence-specific trials). These results replicate the findings of many other studies in the CI literature (Lee and Magill, 1983; Lee et al., 1992; Lin et al., 2008; Maslovat et al., 2004; Perez et al., 2005; Wright et al., 2005).

We next investigated whether the benefit of interleaved practice on retention was sequence-specific, or if it reflected general improvement in key-pressing speed. We hypothesized that if the benefit of interleaved practice simply reflects general improvement in key-pressing speed, interleaved practice should yield faster response time for novel, untrained sequences, compared to repetitive practice. However, performance of the untrained sequences was not different between interleaved and repetitive practice ($p=0.79$, Fig. 2A, novel), suggesting that the benefit of interleaved practice is sequence-specific.

Imaging results

Whole-brain analyses

We started by identifying common cerebral substrates across participants that were involved in the sequence learning task. Table 1 presents a list of regions that were significantly different between task and rest, averaged across interleaved and repetitive practice conditions for the 2 practice days. Unless otherwise noted, all results were significant at $p<0.05$ (map-wise corrected for false discovery rate (FDR) using thresholding at $p<0.05$). Sequential key pressing engaged areas that are classically associated with executive function, movement planning and execution, including the dorsal prefrontal, dorsal and ventral premotor, supplementary motor area, and the inferior and superior parietal areas (Cross et al., 2007; Karni et al., 1998). The activation pattern is consistent with previous studies involving sequential finger pressing tasks, and the bilateral activation of these areas during left-handed key presses in right-handed subjects (Cross et al., 2007).

Effect of practice condition during practice

We next investigated the BOLD signal differences when participants practiced sequences in an interleaved compared to a repetitive practice condition. Fig. 2B shows a map of regions that were significantly different between interleaved and repetitive practice, averaged across 2 days of practice (Fig. 2B, top). Significant activations are listed in Table 2. Compared to repetitive practice, interleaved practice was associated with greater bilateral activity in occipital/

Table 1

Activation locations for comparison of sequential learning tasks versus rest (thresholded at $p<0.05$, FDR corrected).

Regions	MNI coordinates			p (FDR corrected)	t
	x	y	z		
L fusiform gyrus	-24	-63	-24	0	12.7282
R inferior parietal lobe	42	-40	35	0	12.3519
R medial frontal gyrus	3	3	57	0	12.3322
	42	51	-9	0.0094	2.6336
	42	30	27	0.0001	4.839
	40	9	46	0.0079	2.7181
L inferior occipital lobe	-30	-96	-9	0.0029	3.8094
R superior parietal lobe	-18	-69	51	0.0036	7.1896
R inferior temporal gyrus	54	-36	-15	0.0083	2.6934
	51	-30	-18	0.0151	2.3914

For each cluster, all local maxima exceeding a voxel-level corrected $p<0.05$ threshold are presented.

FDR: false discovery rate; R, right; L, left.

Table 2

Activation locations for comparison of interleaved practice versus repetitive practice (thresholded at $p<0.05$, FDR corrected).

Regions	MNI coordinates			p (FDR corrected)	t
	X	y	z		
R middle frontal gyrus	24	3	51	0.0024	8.2001
R superior frontal gyrus	39	24	12	0.0317	3.7315
R inferior frontal gyrus	42	27	18	0.023	4.0307
R postcentral gyrus	45	-33	45	0.0225	4.0619
L fusiform gyrus	-36	-57	-24	0.0279	3.8464
L parahippocampal gyrus	-18	-42	-21	0.0306	3.7632
R parahippocampal gyrus	18	-36	-21	0.0384	3.5642
L medial temporal gyrus	-48	-72	12	0.0318	3.7289
R inferior temporal gyrus	45	-63	-9	0.031	3.75

For each cluster, all local maxima exceeding a voxel-level corrected $p<0.05$ threshold are presented.

FDR: false discovery rate; R, right; L, left.

temporal cortex, sensorimotor and premotor areas, inferior and medial prefrontal areas, and the medial temporal area. Fig. 3 shows that BOLD signal (mean \pm SEM) was higher in interleaved than repetitive conditions for three regions of the motor learning network: right prefrontal (Fig. 3A left, $p=0.017$), right premotor (Fig. 3B left, $p<0.001$), and right inferior frontal areas (Fig. 3C left, $p=0.004$). There were no regions in which repetitive practice resulted in greater activity than interleaved practice.

Effect of practice condition during later retention

Previous literature and our behavioral data (Fig. 2A) demonstrate the benefit of interleaved practice in long-term retention (Brady, 2008; Lin et al., 2008; Shea and Morgan, 1979). Here we hypothesized that enhanced learning following interleaved practice might involve a more efficient cerebral network for retrieval, therefore less activity during retention testing on day 5 was expected following interleaved practice than following repetitive practice. Fig. 2B, bottom, shows a map of regions that were significantly different between interleaved and repetitive conditions when participants performed the trained sequences on day 5. Stereotactic locations for significant activations are listed in Table 3. BOLD signal during the retention test was increased after repetitive practice compared to interleaved practice. This increase was present in medial prefrontal, premotor, and inferior and posterior parietal areas. Fig. 3 shows that BOLD signal during retention (mean \pm SEM) was lower after interleaved than after repetitive practice in three cortical regions: right prefrontal (Fig. 3A right, $p=0.032$), right premotor (Fig. 3B right, $p=0.026$), and right inferior frontal areas (Fig. 3C right, $p=0.05$). There were no areas showing greater activation for sequences trained under interleaved order compared to the repetitive order during retention.

TMS results

One participant was excluded from TMS analysis because a high motor threshold prevented collection of interpretable MEPs. The averaged intensity of test stimulus (TS) for 1 mV MEPs did not change over time and was not different between the two practice conditions ($p=0.47$). Prior to practice on day 1, averaged amplitude of the conditioned motor evoked potentials (cMEPs) was not different between the two practice conditions at each inter-stimulus interval (ISI) (Fig. 2C, $p=0.158$, ISI = 2 ms; $p=0.994$, ISI = 3 ms; $p=0.676$, ISI = 4 ms; $p=0.893$, ISI = 5 ms; $p=0.745$, ISI = 10 ms; and $p=0.242$, ISI = 15 ms). Short-interval intracortical inhibition (SICI, ISI 2–5 ms) showed baseline suppression while intracortical facilitation (ICF, ISI 10–15 ms) showed facilitation compared to unconditioned MEP amplitudes.

To provide an overview of changes in intracortical M1 excitability between practice conditions, we highlighted TMS(pre) data on day 1

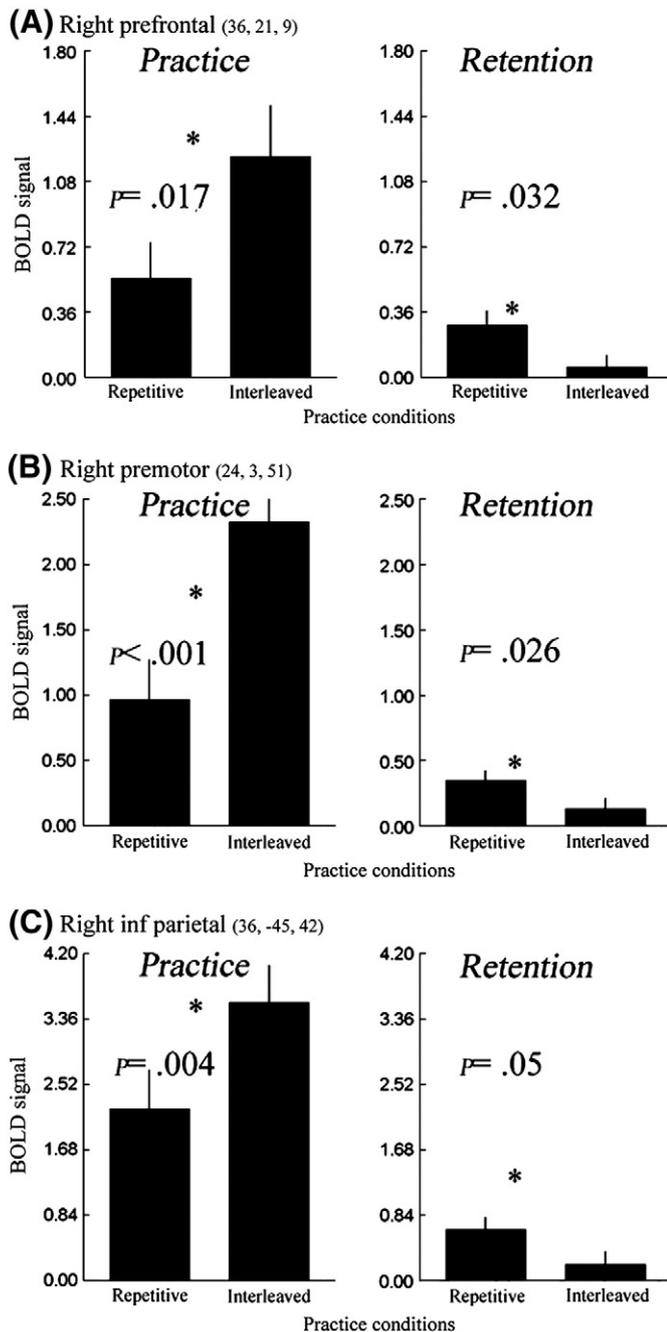


Fig. 3. Shows bar graphs of signal extracted from three regions of the motor learning network, with SE: right prefrontal gyrus (top), right premotor are (middle), and right inferior parietal area (bottom). BOLD signal during interleaved practice was higher than during repetitive practice in these three regions (left column); however, the pattern was reversed during retention (right column).

in blue and on day 5 in red (Fig. 2C). Interleaved practice led to net increased motor cortex (M1) excitability (decrease in SICI, increase in ICF) from day 1 to day 5 as demonstrated by a net up-shift of the sigmoid curves (Fig. 2C left). However, this was not observed after repetitive practice of sequences (Fig. 2C right). In fact, a full model ANOVA with repeated measures including condition (repetitive and interleaved), session (day1-pre, day1-post, day2-pre, day2-post, and day 5-pre), and ISIs (2, 3, 4, 5, 10, and 15 ms) as within-subject factors indicated a significant Condition effect ($p=0.023$) and condition by session interaction ($p=0.012$), supporting that practice conditions modulate how M1 excitability evolves during practice. To investigate if this differential modulation persisted 3 days after practice, post hoc

Table 3

Activation locations for comparison on day 5 following repetitive practice versus following interleaved practice (thresholded at $p<0.05$, FDR corrected).

Regions	MNI coordinates			p (FDR corrected)	t
	X	y	z		
R inferior parietal	-3	-57	54	0.002	9.49
	-18	-9	15	0.002	8.52
R superior frontal gyrus	-21	51	10	0.003	7.60
	27	6	60	0.027	3.56
L fusiform gyrus	27	0	54	0.034	3.52
	18	60	15	0.028	3.51
L cerebellum	-45	-54	-24	0.014	4.79
	-33	-12	-24	0.027	3.56
L supplementary motor area	-39	-75	-21	0.017	4.51
	-33	-84	-21	0.033	3.25
R middle frontal gyrus	24	39	-9	0.018	4.37
L inferior occipital gyrus	-30	-96	-9	0.023	3.89
R medial frontal gyrus	18	-6	51	0.037	3.11
R supplementary motor area	3	24	45	0.027	3.55
L supplementary motor area	-3	15	48	0.040	2.98
R inferior temporal gyrus	51	-51	-9	0.028	3.52
	63	-45	-9	0.033	3.26
L putamen	57	-60	-12	0.033	3.25
	-27	-9	-6	0.028	3.50

For each cluster, all local maxima exceeding a voxel-level corrected $p<0.05$ threshold are presented.

FDR: False discovery rate; R, right; L, left.

paired t tests showed that normalized TMS(pre) cMEP amplitudes on day 5 were greater than on day 1 (baseline) for 5 out of the 6 ISIs following interleaved practice (Fig. 2C left, $p=0.118$, ISI=2 ms; $p=0.024$, ISI=3 ms; $p=0.004$, ISI=4 ms; $p=0.04$, ISI=5 ms; $p=0.004$, ISI=10 ms; and $p=0.001$, ISI=15 ms). This pattern was however not observed for the repetitive practice condition (TMS(pre), day 5-day 1 difference: $p=0.152$, ISI=2 ms; $p=0.932$, ISI=3 ms; $p=0.554$, ISI=4 ms; $p=0.99$, ISI=5 ms; $p=0.702$, ISI=10 ms; and $p=0.002$, ISI=15 ms). Since our full model ANOVA showed no ISI interactions with practice condition ($p=0.56$) or session ($p=0.47$), we then conducted subsequent analyses using normalized cMEP averaged across ISIs.

To confirm how practice conditions modulate task-independent M1 excitability, Fig. 2D plots normalized TMS(pre) cMEP amplitude, averaged across all ISI, for the pre-practice sessions on days 1, 2, and 5. We compared the M1 excitability on TMS(pre) so that any effects observed do not reflect transient changes in excitability due to performance of the task. The interleaved-repetitive differences in TMS(pre) M1 excitability were not significant before either practice day, but significantly diverged just before the retention test on day 5 ($p=0.01$) (Fig. 2D). This delayed condition effect may represent a form of motor memory consolidation.

Changes in BOLD and M1 excitability represented interrelated forms of neural processing in sequence learning (correlation 1, c1)

Our results for both BOLD signal and M1 excitability demonstrated increased neural activity during interleaved compared to repetitive practice. We then performed a series of correlational analyses to investigate whether the increased neural activity during practice, as indexed by fMRI BOLD and pp-TMS excitability measures, represent interrelated forms of neural processing in sequence learning.

Fig. 4 shows the correlation (c1) between relative BOLD contrast (BOLD, I-R) and relative changes of M1 excitability (TMS(post-pre), I-R) during 2 days of practice. We found a significant positive correlation between BOLD, I-R and TMS(post-pre), I-R in right primary motor (Fig. 4A and B, left, $r=0.76$, $p=0.001$) and right primary sensory (Fig. 4A and B, right, $r=0.80$, $p<0.001$) regions. These findings suggest that the relative increases in BOLD signal in the right sensorimotor cortex, contralateral to the hand performing the

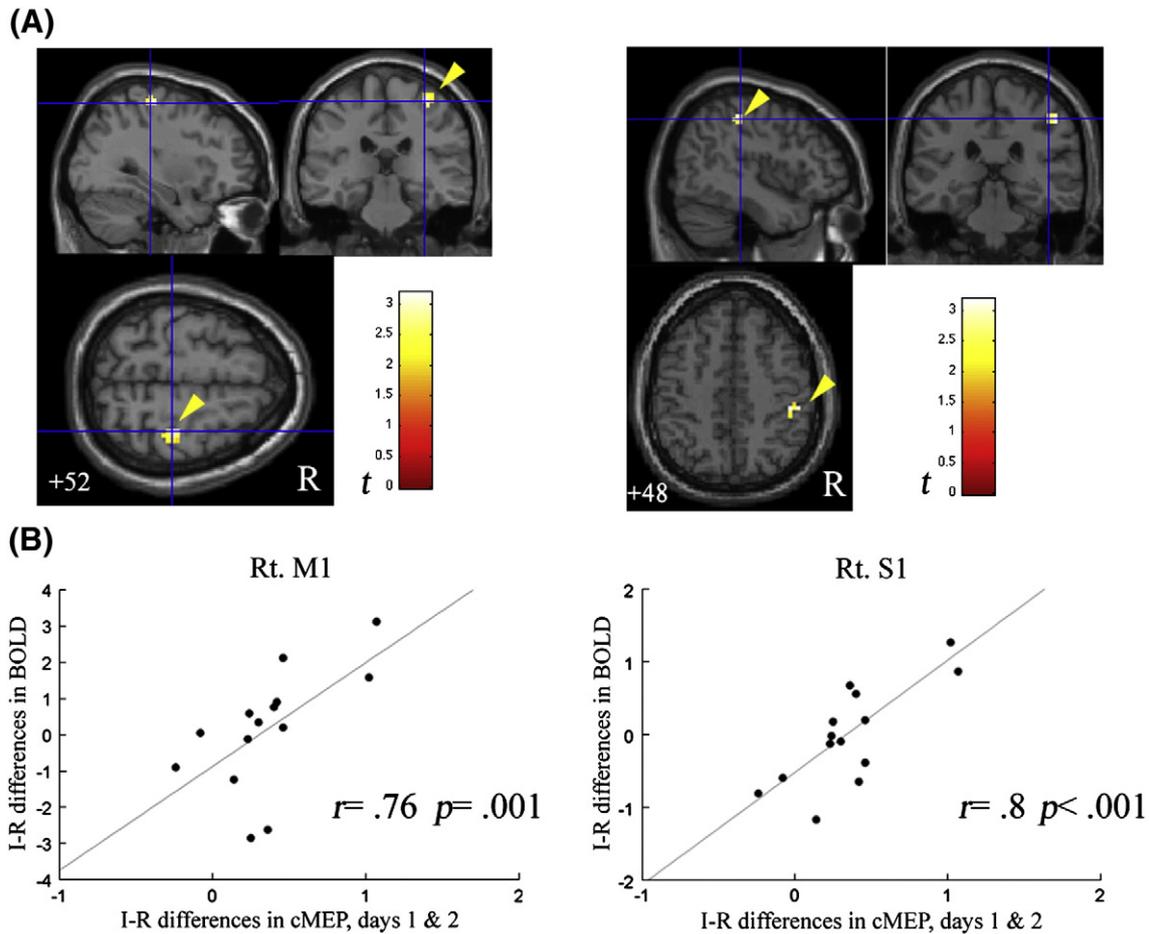


Fig. 4. Shows correlation between relative hemodynamic responses and relative M1 excitability during the practice phase. Panel A indicates that associations between relative BOLD contrast (interleaved minus repetitive contrast, days 1 and 2) and relative M1 excitability changes (interleaved minus repetitive, change scores on days 1 and 2; a positive value indicates greater changes in M1 excitability in the interleaved than the repetitive practice condition) were significant in primary motor (A, left, a positive t -value indicates greater association between relative BOLD and relative M1 excitability in the interleaved than in the repetitive practice condition) and primary sensory regions (A, right). Multiple comparisons were corrected with FDR at $p < 0.05$. Associations between relative BOLD contrast and relative M1 excitability changes were further demonstrated in the scatter plot in B, where the relative BOLD contrast during practice (interleaved minus repetitive contrast extracted from a sample voxel marked by the cross-hair in panel A (Y axis: $z = 52$, primary motor, M1, A left; $z = 48$, primary sensory, S1, A right)) were regressed against the relative M1 excitability changes between the two conditions (X axis; I-R differences during practice—a positive value represents greater M1 excitability in interleaved condition). These findings suggest that increase in BOLD and increase in M1 excitability may indicate inter-related forms of neural processing that supports motor skill encoding during the interleaved compared with the repetitive practice.

task, reflect similar relative increases in M1 excitability when practice is interleaved compared to repetitive. Increases in BOLD signal in these regions and M1 excitability may be associated with the enhancement of motor skill encoding that occurs with interleaved practice.

Reduced BOLD during retention may indicate efficient retrieval of motor memory (c2)

We previously hypothesized that enhanced learning following interleaved practice might involve a more efficient cerebral network for retrieval, therefore less BOLD activity during testing on day 5 was expected following interleaved practice than following repetitive practice. We found a significant positive correlation (**c2**) between relative BOLD contrast during the retention test (day 5, BOLD, R-I) and the learning benefits from interleaved practice (day 5, RT, R-I) in regions that are typically associated with executive function and movement planning: right medial frontal, supplementary motor, dorsolateral prefrontal and somatosensory areas (Fig. 5A). This correlation was further demonstrated in a scatter plot with relative BOLD contrast extracted from the peak voxel (Fig. 5B, $r = 0.85$, $p < 0.001$). These correlations show that, in these regions, relative

BOLD reduction for retention performance following interleaved practice conditions correlates with improvement (*shorter RT*) in retention task performance following interleaved practice. As such, the correlation suggests that these brain regions may index more efficient retrieval of motor memory following interleaved compared to repetitive practice.

Increased M1 excitability during retention may indicate more efficient memory retrieval (c3)

Changes in M1 excitability represent another measure of neural activity that may support learning. Unlike the pattern of BOLD, we found increased M1 excitability (assessed by TMS(pre)) on day 5 following interleaved practice than repetitive practice (Fig. 2D). The correlation (**c3**) between relative M1 excitability on day 5 (TMS(pre), I-R) and relative learning benefit of the interleaved condition (RT, R-I) was significant (Fig. 5C, $r = 0.52$, $p < 0.04$). This correlation shows that greater M1 excitability just prior to the retention test is correlated with relative improvement (*shorter RT*) in retention task performance following interleaved practice. As such, this correlation suggests that increased M1 excitability may be associated with improved motor memory retrieval.

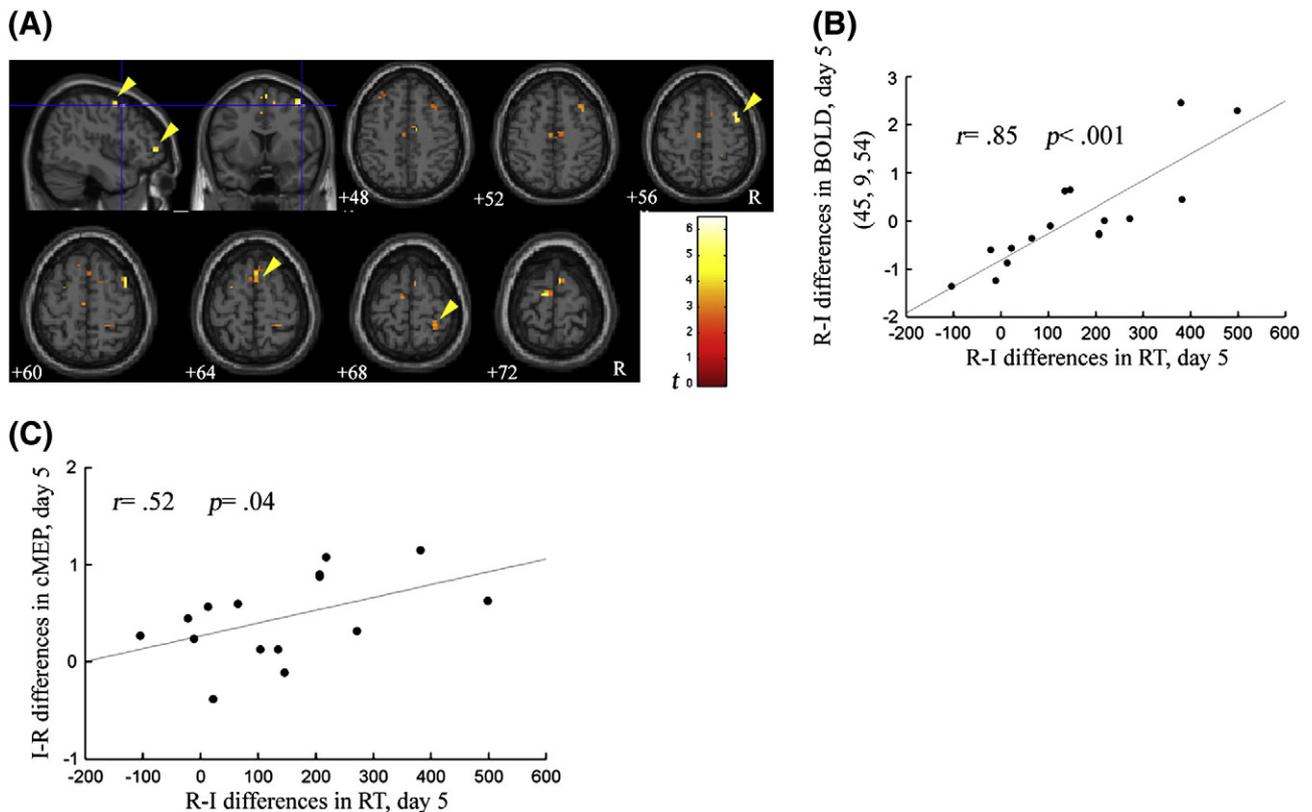


Fig. 5. Shows the neural correlates of enhanced learning on day 5. Panel A indicates that associations between relative BOLD contrast (repetitive minus interleaved contrast on day 5, a positive value represents reduction in BOLD in interleaved condition) and learning benefits following interleaved practice (repetitive minus interleaved, day 5) were significant in medial prefrontal ($z = 56$), pre supplementary motor ($z = 64, 72$), and primary sensory areas ($z = 68$). Multiple comparisons were corrected with FDR at $p < 0.05$. Associations between relative BOLD contrast (repetitive minus interleaved, R-I differences) and learning benefits from the interleaved practice were further demonstrated in the scatter plot in B, where the BOLD contrast during retention on the Y-axis (R-I differences on day 5, extracted from a sample voxel marked by the cross-hair in Fig. 4A; MNI coordinates = 45, 9, 54) was regressed against the differences in learning performance on the X-axis (R-I differences on day 5—a positive value represents longer response time in the repetitive condition and thus indicates that the interleaved condition is favorable). Panel C shows that relative M1 excitability on day 5 was also significantly associated with the learning benefits following the interleaved practice condition. The Y-axis indicates increase in M1 excitability on day 5 (interleaved minus repetitive, I-R differences), and the X-axis indicates learning benefits due to the interleaved practice (R-I differences). These findings identify cortical regions that may be responsible for consolidation of motor learning. Reduction in BOLD responses and increase in cortical excitability in these regions may indicate more efficient retrieval of motor memory following the interleaved than the repetitive practice.

Increased neural activity during practice is associated with enhanced learning (c4)

We then investigated whether increased BOLD activity during interleaved practice was correlated with enhanced learning (c4). Fig. 6A demonstrates significant correlations between relative BOLD signal during practice (days 1 and 2, BOLD, I-R) and the relative learning benefits following interleaved practice (day 5, RT, R-I) in left superior frontal gyrus, an area known for sensorimotor processing and integration (Hanakawa et al., 2008). Relative BOLD signal changes at the peak voxel in left superior frontal gyrus are shown in a scatter plot with relative RT improvement (Fig. 6B, $r = 0.73$, $p = 0.001$). These correlations show that the relative BOLD signal increases in superior frontal gyrus during interleaved practice over repetitive practice correlates with behavioral improvement (shorter RT) during retention task performance.

Increased M1 excitability during practice is associated with enhanced learning (c5)

Relative M1 excitability changes (as assessed by TMS(post-pre), I-R) during practice showed a significant correlation with the subsequent behavioral benefit of interleaved practice (RT, R-I) 3 days later during the retention tests (Fig. 6C, $r = 0.71$, $p = 0.003$) (c5). These correlations show that greater relative increases in M1 excitability during practice are associated with behavioral improvement (shorter RT) during retention task performance.

Discussion

Contextual interference (CI) is a *desirable difficulty* resulting in effective skill learning (Christina and Bjork, 1991; Shea and Morgan, 1979). Our purpose was to combine fMRI and paired-pulse TMS to identify neural correlates of the CI benefit in motor sequence learning. There are four main findings. First, we demonstrated the paradoxical opposing effects of CI during practice and retention of motor sequences. Second, these behavioral results were paralleled by cerebral hemodynamic responses. We observed BOLD signal increases in sensorimotor and prefrontal regions during interleaved practice, while in these same areas BOLD signal was reduced during retention after interleaved compared to repetitive practice. Third, interleaved practice resulted in greater short-term increases in M1 excitability after practice which persisted on day 5 retention. Fourth, greater left superior frontal gyrus BOLD signal and larger short-term excitability increases seen during interleaved practice were correlated with the behavioral benefits of interleaved practice on subsequent retention. This study demonstrates neural changes underlying the beneficial effects of CI and directly relates them to enhanced learning. Introducing the *desirable difficulty* of CI during practice induced neural changes that appear to support more efficient long-term retrieval.

The present results suggest that CI (i.e., practicing in interleaved vs repetitive order) during practice of motor sequences increases recruitment of inferior and medial frontal, premotor, and inferior parietal regions of the brain. Greater recruitment of those regions

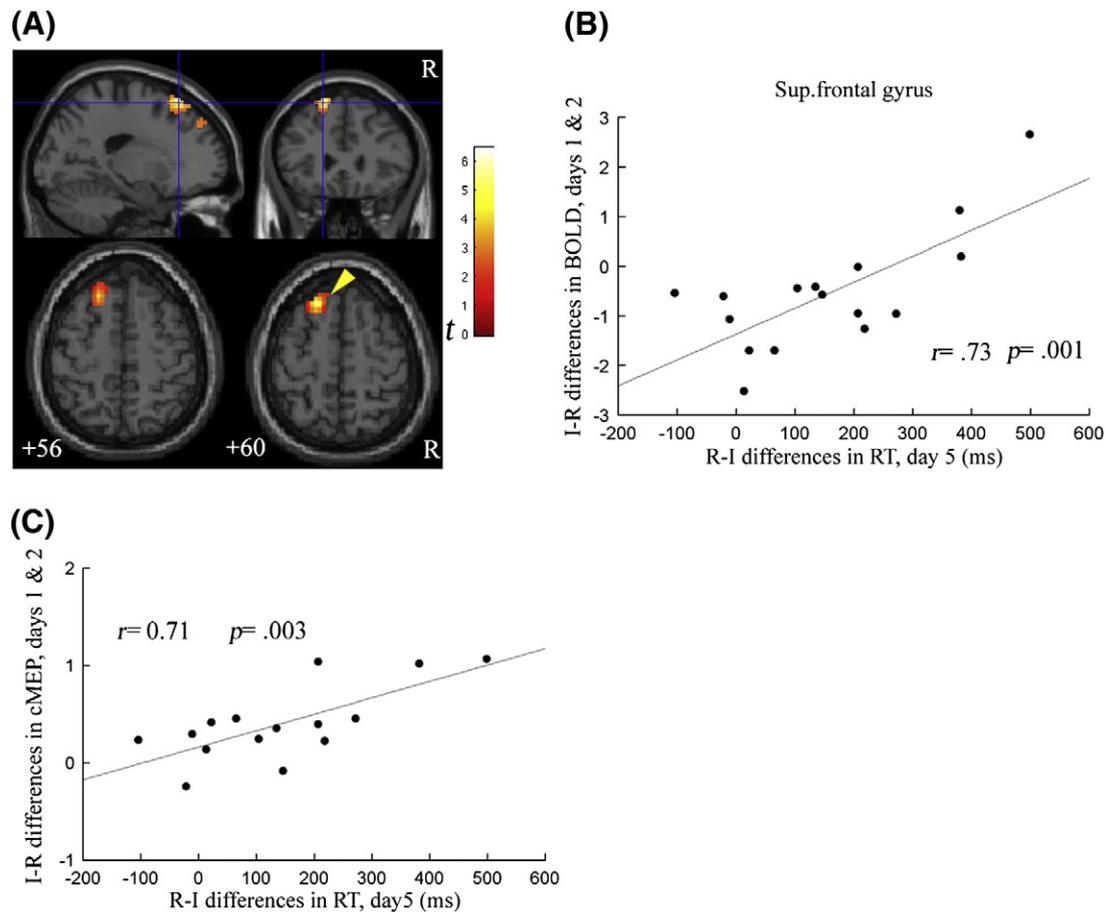


Fig. 6. Shows the functional significance of increased neural activity in sequence learning. Panel A indicates that associations between relative BOLD contrast (interleaved minus repetitive contrast, days 1 and 2) and learning benefits following interleaved practice (repetitive minus interleaved, day 5) were significant in superior frontal gyrus (A, a positive t -value indicates greater association between relative BOLD contrast and learning benefits in the interleaved than the repetitive practice condition). Multiple comparisons were corrected with FDR at $p < 0.05$. Associations between relative BOLD and learning benefits from the interleaved practice were further demonstrated in the scatter plot in B, where the BOLD contrast during practice (interleaved minus repetitive contrast, I–R differences, extracted from a sample voxel marked by the cross-hair in Fig. 5A (Y axis: $z = 60$)) were plotted against the learning benefits of interleaved condition (X axis; R–I differences during practice—a positive value represents longer response time in repetitive condition and thus indicates that the interleaved condition is favorable). Panel C shows that relative M1 excitability changes during practice were also significantly associated with the learning benefits following the interleaved practice condition. The Y-axis indicates relative M1 excitability changes on days 1 and 2 (I–R differences), and the X-axis indicates learning benefits of interleaved condition (R–I differences). These findings identify cortical regions that might be responsible for enhanced motor learning. During practice, increase in BOLD in these regions and increased M1 excitability may predict better learning following the interleaved than the repetitive practice.

during practice was accompanied by increased short-term M1 excitability immediately after practice and both were associated with enhanced learning on the delayed retention test following interleaved practice. Hemodynamic responses may reflect plasticity that results in persistent changes in neural excitability. This interpretation is supported by the significant correlation between relative BOLD contrast in primary sensory motor regions and relative M1 excitability during interleaved compared to repetitive practice.

The CI benefit to skill learning has been explained in terms of greater information processing during the interleaved practice condition. For example, the interleaved condition requires repeated task-switching and retrieval of action plans (Lee and Magill, 1983; Shea and Zimny, 1983). To accomplish multiple tasks in a non-repetitive order, learners must switch their attention and task sets (engaging medial frontal, DLPFC, and superior parietal areas (Rossi et al., 2009)), and to program an action plan for a different upcoming trial (engaging premotor areas (Gail et al., 2009)). Greater neural changes during interleaved compared to repetitive practice may support such elaborative information processing and retrieval during practice, leading to enhanced learning. The parallel dynamic process between BOLD and M1 excitability supports these psychological explanations. The exact contribution of M1 intracortical circuitry to the CI benefits remains to be determined. The dynamic and

demanding nature of interleaved practice may strengthen the synaptic efficacy within M1 and with other cerebral regions (Kujirai et al., 1993), which may consequently promote the function of frontal–parietal networks in sequence learning (Ziemann et al., 1995).

The regions we found to be involved in the CI effect are consistent with literature linking the prefrontal cortex and executive control (Abe et al., 2007) and also with the role of premotor cortex in motor planning (Schubotz and von Cramon, 2002; Seidler et al., 2004). Previous studies with event-related potentials and fMRI in humans implicate the superior frontal areas as a key node for executive control and action set selection during motor tasks (Ye and Zhou, 2009). The right inferior prefrontal cortex and pre-supplementary motor area may play a particular role in the inhibition of competing task sets related to its more general role in response inhibition (Aron et al., 2004) and may be necessary for resolving interference during interleaved practice. Inferior parietal lobe performs highly specialized spatial and sensorimotor functions and has been conceptualized in building multiple spatial representations for the guidance of both eye and limb movements (Hamilton and Grafton, 2009; Hattori et al., 2009). The fusiform gyrus has been linked to color processing and within-category identification (Olsson et al., 2008). Greater activation of these regions during interleaved practice suggests that interleaved practice of sequences recruit prefrontal–parietal and visual regions to

engage attention and to process task-related sensorimotor transformation. Functional roles of these regions support existing psychological explanations about the beneficial effects of CI on learning.

For the interleaved, but not the repetitive practice condition, the paired-pulse TMS (ppTMS) curve shifted toward a more excitable state after each training day and plateaued on day 5 (Fig. 2C). Such changes involved changes in intracortical circuits within M1 including those assumed to be related to short-interval intracortical inhibition (ISI 2–5 ms) and intracortical facilitation (ISI 10 and 15 ms). These changes suggest both short-term (changes within the same day) and long-term (manifested 3 days after practice) modifications in intracortical circuitry within M1 (Kujirai et al., 1993). Based on the correlation between learning benefits on day 5 and relative M1 excitability changes during practice, we suggest that the more the intracortical excitability within the learner's motor cortex during interleaved practice, the greater the eventual learning benefits will be.

Desirable difficulties such as CI during interleaved practice are thought to result in easier retrieval during retention, which would be reflected in both shorter response times and reduced brain activation when participants performed practiced sequences during the retention test. After regressing out the response time in the hemodynamic response function, we found a reduction in BOLD signal in sensorimotor regions on day 5 for interleaved practice compared to repetitive practice, suggesting more efficient cortical processing for retrieval. The regions showing a decrease in activation overlapped to a great extent with those that were more active during practice in the interleaved condition (Fig. 2B and Fig. 3). This reduction in BOLD signal during retention was accompanied by increased M1 excitability and both were significantly correlated with enhanced learning for the interleaved condition. By “efficient information processing”, we refer to the reduced time and/or effort it takes for learners to perform sequences during the retention test after interleaved practice. In terms of neural mechanisms, this decreased effort would be manifest as reduced BOLD signal during performance of the sequences following interleaved practice. This increased efficiency may reflect greater ease of retrieval. In conjunction with enhanced learning, reduced BOLD activity may result from improved intersynaptic communication which is induced by increased M1 intracortical excitability (Hamzei et al., 2006; Ziemann et al., 1995). This effect might be a consequence of a more effective synaptic interaction or an increase in effective connectivity within the motor learning network as a result of interleaved practice (Buchel et al., 1999).

There are distinct mechanisms attributed to different intracortical circuits at different interstimulus intervals (ISIs). As such, we have further analyzed the paired pulse data separately for long and short ISIs. Related to Waldvogel et al.'s broader finding that BOLD signal relates to excitatory synapses (Waldvogel et al., 2000), at short ISIs (ISI = 2 and 3 ms, Supplementary Fig. 1A) which index short-interval intracortical inhibition circuits, we found no significant correlation between interleaved-repetitive contrast in BOLD during practice and the averaged interleaved-repetitive difference in conditioned motor evoked potentials (cMEP) during practice. In contrast, for the long ISIs (ISI = 10 and 15 ms, Supplementary Fig. 1B) which index intracortical facilitation circuits, we found that cMEP differences between the interleaved and repetitive conditions during practice were associated with the interleaved-repetitive BOLD contrasts in the medial frontal and superior frontal regions (SFG). Interestingly, we also found increased SFG activity during interleaved practice to be predictive of the learning benefits of interleaved practice (Fig. 6). The increased relative BOLD signal during interleaved practice is therefore associated with increased intracortical facilitation at rest, assessed immediately after practice. Together, we consider both measures of neural function to contribute to greater eventual behavior gain. The subsequent analysis suggests that it is the long ISI (intracortical facilitation circuits) that is contributing most to this effect.

This does not exclude an absence of an effect of short-interval intracortical inhibition on the neural changes with practice. We found a tendency for short ISI disinhibition right after interleaved practice (Fig. 2C); however, such increase in disinhibition after interleaved compared to repetitive practice did not correlate with the contrast of BOLD (interleaved minus repetitive). There is evidence suggesting that intracortical inhibition provides powerful control over activity-dependent synaptic plasticity (Hensch et al., 1998). Also both intracortical inhibition and facilitation circuits interact (e.g., Chen, 2004) with nonlinear modulation of processes relevant for learning. For example, recent research has also emphasized the importance of homeostatic plasticity as a means of stabilizing the properties of neuronal circuits. Facilitatory preconditioning with anodal direct current stimulation (tDCS) has been shown to improve neuroplasticity (e.g., Turrigiano and Nelson, 2004). It is therefore conceivable that practicing sequences in an interleaved condition may modulate the inhibitory intracortical circuits in an indirect, nonlinear manner, by modulating its homeostatic state. The disinhibition of short ISI circuits may suggest the need to prime the motor system specifically for the interleaved practice. The prefrontal region, together with the intracortical facilitation circuits, may provide control over the state of inhibitory circuits. Such modulation may not present in the form of increased hemodynamic responses, but it may nevertheless play a role in the neural changes that support the benefit of interleaved learning.

Our fMRI data did not show a relative BOLD contrast of M1 activity during interleaved compared to repetitive practice. However, offline TMS data demonstrated increased short-term M1 excitability during interleaved compared to repetitive practice. This pattern of results suggests that interleaved practice may not differentially modulate the hemodynamic activity in M1 per se but may enhance M1's excitability response to practice due to short-term development of stronger synaptic connections to neighboring regions of the frontal-parietal network. Previous studies suggest a role of M1 in the implementation of higher order information from the frontal-parietal motor network (Deiber et al., 1997; Hikosaka et al., 2002). Our fMRI results support this functional interpretation by showing increased BOLD signal in regions neighboring M1 that are associated with executive function (DLPFC), action planning (premotor), and sensorimotor transformations (inferior parietal). M1 excitability increases may result from changes in inputs from these higher order regions.

Learning processes are related to not only BOLD reductions but complex changes as a function of practice and time. In the past 10 years, the sequence learning task has been well studied using different imaging techniques. In an investigation for the effects of long-term practice on M1 hemodynamic responses, Karni et al. have first shown an enlarged representation of the trained sequence emerged in M1 after 3 weeks of training (Karni et al., 1995). This increased motor representation is consistent with findings in primate studies by Nudo et al. (e.g., Nudo et al., 1996) and may contribute to the “slow learning” component of neural plasticity. Karni et al. (1998) further proposed the conceptual framework of temporal specificity in acquiring skilled motor performance. The temporal specificity, or time course, of skill acquisition may indicate mechanisms of neuroplasticity in human brain that support the acquisition and retention of skills. Karni et al. suggest that the “fast” learning is a within-session, habituation-like improvement, followed by a period of consolidation of several hours duration, and then “slow” learning, consisting of delayed, incremental gains in performance emerging after continued practice. Our functional imaging data during practice were averaged across both practice days prior to contrast the difference between the interleaved and repetitive conditions. This approach increased statistical power to detect the CI effect in hemodynamic responses during practice, but was inadequately informative about the time course in which cortical neuroplasticity took place.

Our study provides insight into the neuroplastic features of experience-dependent fast learning and the subsequent consolidation.

During 2 days of practice, an immediate condition effect was observed in BOLD contrast between interleaved and repetitive conditions. On day 5, after 3 days of consolidation, we hypothesized and found a significant reduced BOLD and increased M1 excitability after interleaved than repetitive practice. The results support some transcranial magnetic stimulation studies which showed that once a sequence of movements was made explicit, the area from which a response could be evoked expanded across several consecutive daily sessions (Pascual-Leone et al., 1993; Pascual-Leone et al., 1994). However, our design is limited in detecting a much slower between-session phase of learning, which cannot be accomplished without long-term practice over several weeks like Karni et al.'s work.

We also observed patterns of motor memory consolidation at both behavioral and neural levels. In the hemodynamic responses, the relative contrast between repetitive and interleaved conditions was opposite during practice and retention phases. In the TMS data, the pre-performance M1 excitability state, an index that is independent of task performance on each day, was not significantly different between practice conditions during practice, but diverged with greater excitability 3 days later following interleaved practice. As such, the TMS excitability measure reflected the CI effect during retention, but not during the practice phase. These two complimentary brain mapping methods both demonstrated continuous changes 3 days after practice. It appears that memory traces that develop during interleaved practice continue to evolve with time, resulting in stronger memory traces, better synaptic efficacy (greater M1 excitability), and less demand for sensorimotor recruitment during retrieval. Our results support a recent study by Kantak et al. (2010), which showed that the neural substrates of motor memory consolidation are modulated by practice structure.

The serial reaction time task (Nissen and Bullemer, 1987) is a paradigm frequently used to examine motor sequence learning in neurologically healthy adults (Dahlin et al., 2008; Fraser et al., 2009; Rowe et al., 2009) and patients (Vandenbosche et al., 2009). In the SRT, participants usually make sequential key press responses to cues presented in four spatial locations. A patterned sequence of locations is presented and the response time to the associated stimuli decreases after practice compared with that seen for untrained random stimuli. In the sequence learning literature, this learning was for the most part implicit, as subjects were not aware of the sequence. In our investigation, instead of having participants practice one long sequence (usually 10–14 elements in the SRT literature), participants practiced three 4-element sequences and the sequences were arranged in either a repetitive or interleaved order. Participants were aware of these sequences prior to practice, but it is not clear if this explicit knowledge contributed to the decrease in reaction times with practice. We demonstrated that subjects were able to learn multiple spatial sequences and learning was enhanced by interleaved practice. Interleaved practice of sequences benefits learners 3 days after practice, although this condition incurs a greater cost, shown by increased hemodynamic responses and corticomotor excitability in this condition. The results suggest that the benefits of CI are at least in part due to increased retrieval practice, as participants must retrieve each sequence when tasks are presented in an interleaved order as compared to a blocked repetitive order when tasks can remain in working memory. The present results cannot rule out the possibility that the CI benefit was due to greater elaborative encoding through comparing and contrasting sequences in the interleaved condition. If this mechanism contributed to the CI benefit, the present results suggest that these elaborative encoding processes were engaging prefrontal and sensorimotor regions of the brain.

The SRT is not simply a motor learning task; it has both motor and perceptual learning components. The perceptual learning component of the SRT provides an explanation for the shift in brain areas supporting SRT performance when the perceptual properties of the SRT are altered (Robertson, 2007). Our results demonstrated

increased brain activation in regions of sensorimotor perceptual regions when multiple sequences were practiced in an interleaved order (Fig. 2B and Table 2). This result supports previous work showing that the perceptual component is an important one in SRT. On the other hand, successful implementation of SRT also involves different levels of information processing, such as implicit efficiency in sensorimotor transformation, explicit awareness of practiced sequences, and strategy to optimize sequences such as chunking. For the implicit component of SRT learning, an important issue to consider is the time point when an individual becomes aware of the presence of sequences. Since our study was designed to understand the neural distinction between different practice conditions rather than the transition of implicit to explicit learning, we controlled for the individual difference in awareness of sequences by explicitly informing the participants prior to task practice. This was done to ensure any changes in brain activation we measured are less likely to be driven by a sudden awareness of sequences.

It is also likely that the results would change if we apply a task relying more in implicit memory and this can be done by increasing the length of sequences without notifying the subjects. For example, a prolonged sequence or a more implicit type of learning would evoke engagement of the medial temporal lobe (MTL) during SRT performance. The MTL has been shown to engage during the acquisition of high- but not low-order sequences even when participants are not declaratively learning any of the sequence (Schendan et al., 2003). Dorsal premotor and supplementary motor areas, the neural substrates known to play a role in sequence processing, may also show greater activation.

Our fMRI findings support a previous study (Cross et al., 2007) which examined the neural correlates of interleaved practice. Using an event-related, between-subjects design and a Go/No-Go paradigm, Cross et al. demonstrated brain activation differences in sensorimotor and premotor regions in interleaved vs. repetitive practice. However, Cross et al. did not observe differences between groups in BOLD signal during retention, and they did not report correlations between neural changes during practice and behavioral performance. To extend beyond these previous findings, we used a within-subject design and a blocked fMRI design to reduce heterogeneity and increase statistical power. We also extended practice to 2 days and evaluated sequence retention 3 days after practice in order to increase the difficulty of retrieval. We were thus able to relate BOLD signal differences to the benefit on learning, and our use of ppTMS allowed us to relate these changes to cortical excitability.

Our approach of combining fMRI and paired pulse TMS in a within-subjects design allowed us to explore the neurophysiological consequences of the learning-related changes due to differences in practice order. By correlating behaviorally relevant BOLD signal changes using fMRI and cortical excitability using TMS, we were able to gain insight into concurrent learning-related changes at a hemodynamic network level and within the M1 circuit level. Future studies could examine these changes with finer grained temporal resolution than that used here. The functional imaging in the current study was based on a blocked-design, and thus we were unable to differentiate neural processing during the different phases of each trial, including planning, execution, and consolidation. A study separating the phase of task processing may be able to pinpoint the beneficial effect of CI on skill learning. Such data would provide a dynamic picture of the changes occurring during skill learning and how an interleaved practice condition enhances these processes.

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