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Age related differences in the neural substrates of motor sequence learning after interleaved and repetitive practices

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ABSTRACT

Practice of tasks in an interleaved order generally induces superior retention compared to practicing in a repetitive order. Younger and older adults practiced serial reaction time tasks that were arranged in a repeated or an interleaved order on 2 successive days. Retention was tested on Day 5. For both groups, reaction times in the interleaved condition were slower than the repetitive condition during practice, but the reverse was true during retention on Day 5. After interleaved practice, changes in M1 excitability measured by paired-pulse TMS were greater than after repetitive practice, and this effect was more pronounced in older adults. Moreover, the increased M1 excitability correlated with the benefit of interleaved practice. BOLD signal was also increased for interleaved compared to repetitive practice in both groups. However, the pattern of correlations between increased BOLD during practice and subsequent benefit of the interleaved condition differed by group. In younger adults, dorsolateral-prefrontal activity during practice was related to this benefit, while in older adults, activation in sensorimotor regions and rostral prefrontal cortex during practice correlated with the benefit of interleaving on retention. Older adults may engage compensatory mechanisms during interleaved practice such as increasing sensorimotor recruitment which in turn benefits learning.

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Introduction

Previous studies have shown that introducing manipulations that make performance more difficult during practice may nonetheless improve long-term retention and transfer (Schmidt and Bjork, 1992). The notion of “desirable difficulties” suggests that challenges for learners such as context shifts and retrieval during study result in enhanced learning and should be introduced into skill practice (Christina and Bjork, 1991). An example of a *desirable difficulty* 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 induces inferior practice performance but leads to 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.

The CI effect was initially demonstrated in the verbal learning literature (Battig and Berry, 1966) and was subsequently studied in learning of motor tasks (Shea and Morgan, 1979). Shea and Morgan's results demonstrated that during practice, a more difficult condition (interleaved practice) resulted in worse performance but leads to better retention and transfer compared to a less difficult condition (repetitive practice). The poorer performance during interleaved practice implies that greater effort expended in a more difficult condition may facilitate long-term retention.

Despite the age-related decline in accuracy or speed at making fine movements (Spirduso et al., 2005), healthy older adults remain readily able to acquire new skills and procedures (Kausler, 1994). Studies of motor sequence learning have demonstrated similar levels of sequence learning in older and younger adults (Fraser et al., 2009; Howard and Howard, 1989), although sequence-specific learning in older adults may be slower (Daselaar et al., 2003; Lin et al., 2010), may show less transfer (Seidler, 2006), and may not consolidate as effectively (Brown et al., 2009; Nemeth and Janacek, 2011). Another similarity in sequence learning for older and younger adults is the finding that both groups benefit equally from interleaved practice on a delayed retention test (Lin et al., 2010). These findings underscore the generality of the CI effect,

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and demonstrate that variable practice has a beneficial effect on neural plasticity in healthy aging.

Recent neuroimaging studies have demonstrated that even when behavioral performance is matched, younger and older adults show different brain activation patterns (Morcom et al., 2003). Some activation patterns in older adults are related to optimal performance, suggesting compensatory potential in the aging brain (Cabeza et al., 1997; Lin et al., 2011). There is also evidence suggesting that encoding-related activation patterns that predict subsequent successful retrieval in older adults are different from those that are associated with subsequent retrieval in young adults (Morcom et al., 2003). These data suggest that older adults may engage different neural circuitry from young adults to reach the same behavioral endpoint.

The present study was designed to address two main questions. First, does interleaved practice of sequences result in increased neural activity compared to repetitive practice in older adults, a similar pattern we have previously identified in younger adults (Lin et al., 2011)? Second, is increased neural activity during interleaved practice associated with enhanced learning and whether aging may modulate this correlation? In this study, our measures of neural activity are blood-oxygen-level-dependent (BOLD) signal and cortical motor 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.

Participants were scanned using fMRI 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 participants were at rest) before and after each fMRI practice session and before the fMRI retention test. Based on our previous work and the prediction of *desirable difficulties* in CI, we anticipated that for both age groups, 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). We anticipated that within each age group, the desirable difficulty effect of CI will manifest as greater sensorimotor activity (measured by BOLD signal) and M1 excitability (measured by ppTMS) during interleaved compared to repetitive practice given that increased task complexity typically results in increased BOLD signal during practice (Kuhnt-Buschbeck et al., 2003; Verstynen et al., 2005). However between age groups, BOLD signal during practice will be greater in older than younger adults based on previous findings showing aging-related hyperactivation on cognitive tasks (Gutchess et al., 2005). For the same reason, increased M1 excitability associated with interleaved practice was expected and that may be maintained at the retention test given that enhanced excitability associated with motor practice may constitute a necessary precursor for inducing plastic changes within the motor system (Koeneke et al., 2006; Pascual-Leone et al., 1995). Furthermore, we anticipated that for both younger and older adults, increased sensorimotor BOLD activity and M1 excitability during interleaved practice would correlate with the level of skill learning (Tamas Kincses et al., 2008). However, the brain regions that show functional correlations with enhanced learning may differ between the two age groups.

To our knowledge, this is the first study that combines fMRI and paired-pulse TMS measures as biomarkers to examine age-related changes in neuroplasticity. Our use of the CI paradigm, which has been shown to benefit learning of motor skills, allows us to identify how aging may modulate the neural mechanisms of optimized learning.

Materials and methods

Participants

Sixteen younger (9 men and 7 women, mean age 26.4 ± 3.1) and sixteen older (7 men and 9 women, mean age 66.2 ± 4.7) right-handed adults were enrolled in the study. 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 neuromuscular condition which prevents them from performing the task. Participants were also excluded for any contraindications to TMS or MRI, 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).

Study design

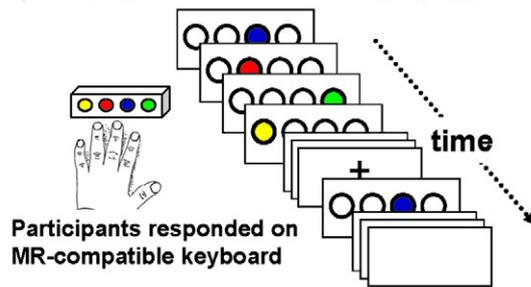
For both younger and older adults, we applied a *within-subject cross-over design* with three measurements: behavior (serial reaction time sequence learning task, Fig. 1A) (Nissen and Bullemer, 1987), cerebral hemodynamic responses by functional magnetic resonance imaging (blood oxygen level dependent signals (BOLD), fMRI), and intracortical excitability within the primary motor cortex (M1) by paired-pulse transcranial magnetic stimulation (ppTMS). Therefore, age-group is a *between-subjects factor* and practice condition is a *within-subject factor*. The participants practiced the serial reaction time (SRT) task on two consecutive training days (Days 1 and 2, Fig. 1B). To measure the effects of practice on learning, we tested the delayed retention performance on Day 5 (Cahill et al., 2001; Lin et al., 2011; Perez et al., 2005; Shea and Morgan, 1979; Wright et al., 2005) (Fig. 1B). Behavioral and fMRI data were acquired concurrently on each testing day within the MR scanner, while the paired-pulse TMS (ppTMS) was performed immediately before and after each training day and before the retention session on Day 5 in the adjacent TMS laboratory (Figs. 1B and C). ppTMS was applied to evaluate the excitability of intracortical circuits in M1 (Kujirai et al., 1993). 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. All participants were informed of the presence of sequences prior to practice. 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 allowing us to assess BOLD signal differences between practice conditions and participant groups.

Participants practiced and learned a variation of the SRT task 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 “Behavioral 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 participated in the other practice condition (i.e., Repetitive → Interleaved, or vice versa). The order of the practice conditions and the SRT sequences were counterbalanced across participants.

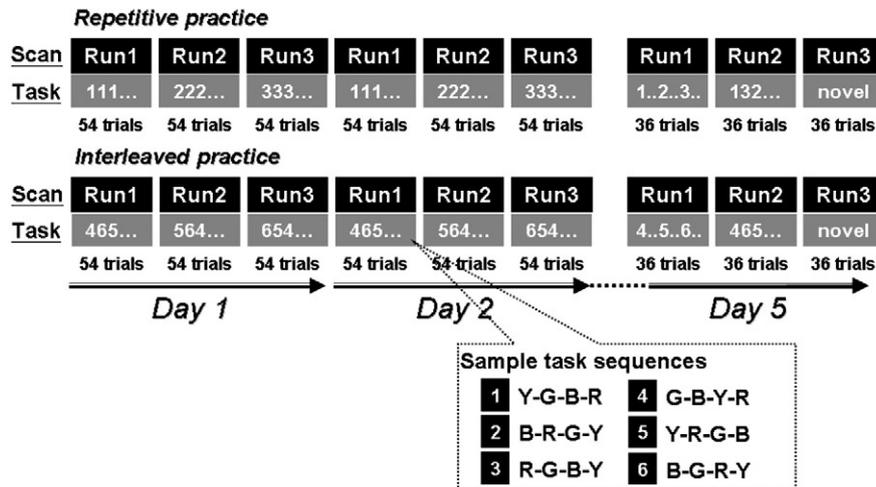
Functional images were acquired concurrently while the SRT was performed inside the MR scanner. For Days 1 and 2, there were three functional imaging runs on each day (Fig. 1B). Each run consisted of 54 movement trials, where the participants practiced one test sequence in each trial. For Repetitive practice (RP), each of the three test sequences was practiced for 54 consecutive trials (i.e., one fMRI run) before the next sequence appeared, resulting in 162 trials (54 trials \times 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 test sequences were arranged in a non-repetitive manner within each 54-trial fMRI run (Fig. 1B bottom), and 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

A) Display visual stimuli through goggle



B) 5-day regime of Repetitive or Interleaved practice



C) Timeline of behavioral task, fMRI, and TMS

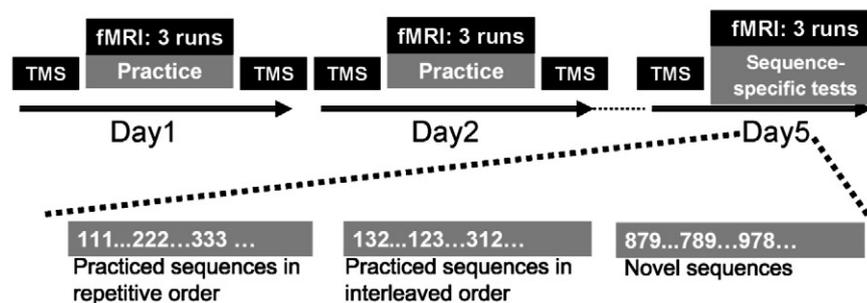


Fig. 1. The participants practiced serial reaction time sequence learning tasks in both a Repetitive or an Interleaved order (B) in the MR scanner, by pressing corresponding keys in response to visual stimuli displayed through MR-compatible goggles (A). They practiced tasks for two consecutive days and performed practiced and novel sequences in the retention tests on Day 5 (C). Image data were acquired concurrently while the participants were performing the tasks. During Repetitive practice, the participants practiced the same sequence (e.g., sequence 1, 2, or 3) repeatedly in the same scan run, while in the Interleaved practice, the participants practiced a combination of three sequences that were arranged in a pseudorandom order (e.g., sequences 4 to 6). Paired-pulse TMS was conducted before and after practice on Days 1 and 2, and before the retention tests on Day 5 (C). Two weeks after practice and retention for one practice order (e.g., the Repetitive or Interleaved order), participants returned to practice a different set of three sequences in the other practice order, i.e., Repetitive practice → Interleaved practice or vice versa. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

207 previous 2 days. In one run the practiced sequences were presented
 208 in a repetitive order (denoted by Repetitive test condition, or RC),
 209 and in the other run they were presented in an Interleaved order
 210 (denoted by Interleaved test condition, or IC). This was to ensure
 211 that practice-test compatibility was equal for either practice condition
 212 (Lee, 1988; Lee and Magill, 1983; Shea and Morgan, 1979). The
 213 order of these runs was counterbalanced across participants. Each sequence
 214 was presented for 24 trials across these two runs on Day 5 (in
 215 contrast, during practice on Days 1 and 2, each sequence was presented
 216 for 54 trials on each day). The reduced number of trials was used
 217 in order to limit further learning of the sequences (Cross et al.,

2007). To assess whether learning was specific to the trained sequences,
 218 on the third fMRI run the participants were tested with three novel,
 219 or unpracticed, test sequences (Fig. 1B). 220

221 Measures of M1 intracortical excitability by paired-pulse transcranial
 222 magnetic stimulation (ppTMS) were acquired before and after (pre and post)
 223 each fMRI session on Days 1 and 2. This allowed immediate changes in
 224 M1 excitability to be monitored as an effect of practice. Because our focus
 225 was not on the immediate changes in M1 excitability before and after
 226 retention tests, intracortical excitability was acquired only before the
 227 retention session in fMRI on Day 5. 228

229 Behavioral task

230 The SRT task during the fMRI scanning was executed as follows
 231 (Fig. 1A). Participants positioned the four fingers of the left hand
 232 (all except the thumb) on the four light-sensitive response keys of a
 233 magnet-compatible button box (Current Designs, Inc.). The left hand
 234 was used to increase the overall difficulty of the task, analogous to
 235 the approach in Cross et al. (2007) and Lin et al. (2011). Participants
 236 watched colored circles (yellow, red, blue, and green) through
 237 magnet-compatible goggles. Only one colored circle appeared at a
 238 time while the other circles were transparent (Fig. 1A). Each color
 239 was always displayed at the same circle location, thus providing a
 240 consistent spatial and color cue for the motor response on the spatial-
 241 ly corresponding response key. Participants were instructed to “re-
 242 spond as quickly as possible.” Within each sequence, one circle
 243 would be colored at a time with the next colored circle appearing as
 244 soon as the previous response was made.

245 Participants pressed four consecutive keys (four elements, equiva-
 246 lent to one sequence) to complete one task trial. To ensure that each
 247 participant practiced an equal number of trials, each sequence (four
 248 key presses) was presented for a fixed duration of 3 s. If the participant
 249 completed the four key presses before 3 s, 4 transparent circles would
 250 appear on the screen, thus controlling visual stimulation (Fig. 1A).
 251 Since we applied a blocked design for functional imaging acquisition
 252 with 18-second task blocks interleaved with 18-second rest blocks, be-
 253 havioral trials were presented as 6 sequences per task block. During the
 254 rest block, the circles would be replaced by a fixation cross in the center
 255 of the screen. Participants were instructed to remain relaxed but gaze at
 256 the fixation cross. A custom-designed computer software program writ-
 257 ten with Presentation (Neurobehavioral Systems) controlled the ap-
 258 pearance of the colored circles and recorded the participants’
 259 responses. Response time, defined as the interval between stimulus
 260 onset and key pressing, was recorded for each key press.

261 Transcranial magnetic stimulation

262 The ppTMS was applied using two Magstim Rapid stimulators
 263 connected to a Bistim module. An optimal location for magnetic
 264 stimulation (hotspot) was defined as the location where magnetic
 265 stimuli consistently elicited a maximal motor evoked potentials
 266 (MEPs) from the contralateral first dorsal interosseous (FDI) muscle.
 267 A figure-8 stimulating coil was applied (diameter: 7 cm/wing). The
 268 point of intersection of the figure-8 coil was placed against the
 269 skull and the coil was held at a 45-degree angle to the mid-sagittal
 270 plane, with the handle oriented anteromedially (Brasil-Neto et al.,
 271 1992; Kammer et al., 2001; Suppa et al., 2008). This orientation has
 272 been shown to be the most efficient one to induce MEPs comparable
 273 to those obtained using a monophasic stimulator (Lin et al., 2011;
 274 Suppa et al., 2008).

275 Electromyography (EMG) was monitored throughout the TMS ex-
 276 periment to ensure the spatial specificity of magnetic pulses. EMG
 277 signals were acquired using surface electrodes in a belly-tendon mon-
 278 tage from the first dorsal interosseous muscle of the left hand. The
 279 signals were filtered with a bandpass of 1–1000 Hz, amplified, and
 280 then digitized at 5000 Hz. The digitized EMG data were visually dis-
 281 played and stored for later analysis in 600-ms samples beginning
 282 100 ms before TMS onset (Labview, National Instruments). MRI-
 283 guided frameless stereotaxy (Brainsight Frameless; Rogue Research,
 284 Montreal, Quebec, Canada) was used to verify the position of the
 285 coil with respect to the underlying brain anatomy.

286 The ppTMS trials were conducted by delivering a conditioning and a
 287 testing pulse (CS and TS, respectively) separated by an interstimulus in-
 288 terval (ISI) through the same coil over the M1 hotspot. The intensity of
 289 the conditioning stimulus (CS) was adjusted to be 90% active motor
 290 threshold (Ilic et al., 2002; Ziemann et al., 1996). Active motor threshold
 291 (aMT) was defined as the lowest stimulus intensity, which elicited a

mean peak-to-peak MEP > 100 μ V during slight isometric contraction 292
 (5–10% of maximum voluntary contraction) from five single-trial 293
 sweeps (Rossini et al., 1994). The intensity of testing stimulus (TS) 294
 was adjusted to evoke a MEP of 1 mV amplitude from peak to peak in 295
 a relaxed left first dorsal interosseous muscle (Kujirai et al., 1993). For 296
 both age groups, the mean intensity of TS did not change over time 297
 and was not different between the two practice conditions. 298

We collected 12 TMS trials for each of the 7 settings: 6 paired-pulsed 299
 settings with the ISI at 2, 3, 4, 5, 10, and 15 ms and 1 single pulse setting 300
 with TS alone. The short-ISI pp-TMS (2, 3, 4, 5 ms) elicited short- 301
 interval intracortical inhibition (SICI) while the longer-ISI ppTMS (10, 302
 15 ms) intracortical facilitation (ICF) (Chen et al., 1998). The order of 303
 7 settings was randomized across participants. Each TMS trial was sep- 304
 arated by a minimum of 8 s. Each single TS pulse trial results in an un- 305
 conditioned MEP. Each pair of CS-TS pulses (presented during ISI 306
 trials) results in a conditioned MEP (cMEP). For each condition, peak- 307
 to-peak MEP amplitudes were averaged. Intracortical excitability for 308
 each ISI was computed as the mean cMEP amplitude for that ISI 309
 expressed as a percentage of the mean unconditioned MEP amplitude. 310

Functional magnetic resonance imaging 311

Brain images were acquired using a Siemens Trio 3.0 T MRI scanner 312
 housed in the Ahmanson-Lovelace Brain Mapping Center at UCLA. Two 313
 sets of high-resolution anatomical images were acquired for image regis- 314
 tration: (1) an MP-RAGE structural volume (TR = 1900, TE = 2.26, flip 315
 angle = 8°) with 176 sagittal slices, 1 mm thickness/0.5 mm gap, a 316
 256 × 256 matrix and 1.33 mm × 1.33 mm in-plane resolution, and (2) a 317
 T2-weighted co-planar volume (TR = 5000, TE = 34, flip angle = 90°) 318
 with 34 transverse slices covering the whole brain, 4 mm thickness/ 319
 1 mm gap, a 128 × 128 matrix and an in-plane resolution of 1.5 mm × 320
 1.5 mm. 321

Functional images were acquired while the participants performed 322
 the sequence learning task. There were three functional runs which cor- 323
 responded to the three practice sessions on each day (Fig. 1B). On Days 324
 1 and 2, each functional run lasted for 5 min and 48 s, and consisted of 325
 153 EPI volumes (gradient-echo, TR = 2000, TE = 30, flip angle = 90°), 326
 each with 34 transverse slices, 4 mm thickness/1 mm gap, and a 327
 64 × 64 matrix yielding an in-plane resolution of 3 mm × 3 mm. The 328
 first four volumes of each functional run were removed from analysis 329
 to allow for magnetization to reach equilibrium. On Day 5, the partici- 330
 pants underwent three functional runs (Fig. 1B, two runs for the practi- 331
 ced sequences and one run for the novel sequences). Each run lasted 332
 for 3 min and 48 s, and consisted of 99 EPI volumes. 333

Statistical analysis 334

Behavioral data 335

We assessed the behavioral performance based on the response 336
 time (RT) for key pressing. For each single 4-element sequence trial, 337
 RT was defined as the sum of every interval between stimulus onset 338
 and key pressing. We calculated the median of RT across every six 339
 consecutive sequence trials for subsequent analyses. 340

In our previous brain mapping study (Lin et al., 2011), we found 341
 contextual interference effects in motor sequence learning, where 342
 the Interleaved practice led to better retention than the Repetitive 343
 practice. Here we aimed to further determine (*Behavioral Aim 1*) 344
 whether such contextual interference benefits in learning differs be- 345
 tween age groups, i.e., whether the older adults may still improve 346
 their learning through the Interleaved practice condition, as we previ- 347
 ously found in the younger adults (Lin et al., 2011). In addition, we 348
 aimed to investigate (*Behavioral Aim 2*) whether the benefit of the In- 349
 terleaved practice on retention was *sequence-specific*, or simply 350
 reflected general improvement in key-pressing speed, which we de- 351
 note by *non-specific learning*. Sequence-specific learning was repre- 352
 sented by the difference in the RT between the practiced and novel 353

sequences on Day 5. Here we assumed that the participants' general performance (e.g., key-pressing speed, or familiarity with the testing environment) was the same when tested with the practiced and novel sequences, so the difference in the RT specifically reflected the learning of the practiced sequences. By contrast, non-specific learning was represented by the difference in the RT between the first block on Day 1 and the novel sequences of Day 5. Given that the participants had not practiced the sequences at these two time points, the difference in the RT then reflected the improvement of the general (non-specific) performance. Differences in the RT for sequence-specific learning and non-specific learning were further normalized to the participants' baseline performance (the mean RT of the first 6 trials) (Lin et al., 2010).

We used a repeated measures ANOVA model, with Age as the between-subjects factor, Practice condition as the within-subject factor, and Age \times Practice as the interaction term. For Behavioral Aim 1, we tested Age \times Practice condition interactions in the differences between the mean RT across 2 days of practice (i.e., interaction during the practice phase) and the mean RT of the practiced sequences during the retention test on Day 5 (i.e., interaction during the retention phase). For Behavioral Aim 2, we tested Age \times Practice condition interactions in sequence-specific and non-specific learning.

TMS data

The primary outcome measure for ppTMS was the mean conditioned MEP (cMEP). The cMEP was expressed as a percentage of the unconditioned MEP, measured in each ISI (2, 3, 4, 5, 10, 15 ms), each of the five TMS sessions (Day1-pre, Day1-post, Day2-pre, Day2-post, and Day5-pre), each practice condition (Repetitive and Interleaved), and in each age group (Older and Younger). These cMEPs were initially analyzed with a full ANOVA model with Practice condition, Sessions, and ISI as within-subject factors; and with Group (Older and Younger) as the between-subjects factor. *Post hoc* analyses of the main effects and the interaction effects were carried out with pairwise comparisons. Since our initial analysis found no significant differences between cMEPs across all ISIs (Fig. 2C), we collapsed cMEPs across all ISIs for subsequent analyses. We noted that cMEPs at different ISIs represent the excitability of different neural circuits (Chen et al., 1998), so the mean of the cMEP data we used here was arguably a reasonable measure for the overall neural excitability of the motor cortex.

To associate practice-dependent changes in intracortical excitability with the retention performance, for each practice condition we computed the difference in cMEPs, denoted by TMS (post-pre), before and after practice on Day 1 (Day1-post minus Day1-pre) and Day 2 (Day2-post minus Day2-pre).

Imaging data

Functional images were processed using the Statistical Parametric Mapping software (SPM8, 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 using 4th-degree B-Spline interpolation (Friston et al., 1995). None of the subjects had scans with head motions 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 further resampled to $3 \times 3 \times 3$ mm³ per voxel, and then spatially smoothed with an isotropic Gaussian filter with full width at half maximum (FWHM) = 8 mm. A first-level statistical analysis was 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). Moreover, the mean response time for each task block was added as an additional parametric regressor, to ensure that any

differences in brain activities during practice and retention were due to the influences of the practice conditions, but not due to differences in the response time.

Second-level group analyses in imaging data. To investigate the effects of aging on cerebral responses measured by BOLD contrasts, group comparisons (Younger vs. Older) were carried out using a second-level random-effects analysis on the contrast images (task versus rest) derived from the first-level GLM fitting (Handwerker et al., 2004; Worsley et al., 2002). This second-level analysis yielded four contrast images: (1) Younger minus Older, Repetitive practice, (2) Older minus Younger, Repetitive practice, (3) Younger minus Older, Interleaved practice, and (4) Older minus Younger, Interleaved practice.

All statistical maps were corrected for multiple comparisons using the topological false discovery rate (FDR) method (Chumbley et al., 2010). Overall significance was achieved when $FDR < 0.05$, which indicated that on average less than 5% of the significant voxels were false positive.

Age influences on the associations between condition-dependent neural changes and learning

We used a multiple regression model to determine whether age influences the associations between the learning performance and fMRI BOLD responses or M1 excitability. We set BOLD contrasts (move minus rest) of fMRI, or cMEPs measured by TMS, as the dependent variable. The independent variables included the Age group (0: older group; 1: younger group) and the Practice condition (0: Repetitive; 1: Interleaved). Here we added an additional interaction term, Age \times Practice condition, into the regression model, to test the age effects on the functional significance of neural activity changes (BOLD contrasts or M1 excitability) between the two practice conditions. For BOLD signal, the regression analysis was performed at every voxel of the brain. Maps of regions with significant Age \times Practice condition interactions were corrected for multiple comparisons using the topological FDR method (Chumbley et al., 2010). Overall significance of the maps was achieved when topological $FDR < 0.05$. Statistical comparisons involving the behavior and the TMS data were performed using SPSS 13.0 (SPSS Inc., Chicago, IL), with the significance level set at $p < 0.05$.

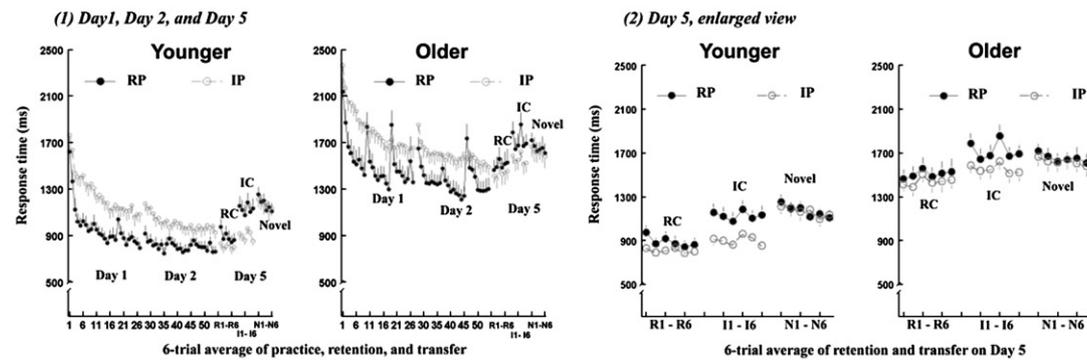
Results

Behavioral results

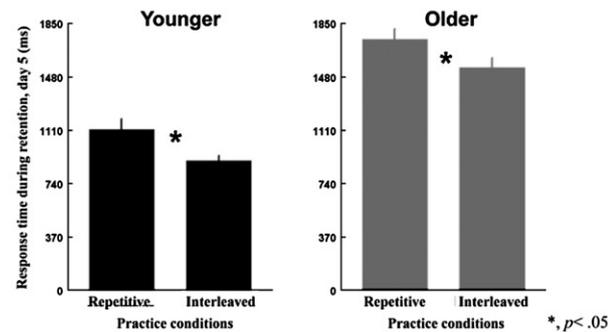
Overview

At the beginning of practice, the mean response time (RT) of the first 6 trials on Day 1 showed no significant difference between the Repetitive and Interleaved conditions within each age group ($p = .21$, Younger; $p = .25$, Older). Both younger and older participants improved performance after 2 days of practice and the mean RT across the two practice days was faster in the Repetitive than in the Interleaved condition (mean RT, Younger-Repetitive (YR) = 880.8 ± 69.4 , Younger-Interleaved (YI) = 1121.6 ± 64.7 , $p = .006$; Older-Repetitive (OR) = 1424.6 ± 69.4 , Older-Interleaved (DOYON et al., 1998) = 1700.9 ± 64.5 , $p = .038$; Fig. 2A, Day 1 and Day 2, RP was lower than IP in both younger and older participants). 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, especially when the participants were tested using interleaved sequences (mean RT, YR = 1114.9 ± 74.4 , YI = 897.4 ± 37.7 , $p < .001$; OR = 1738.6 ± 74.2 , OI = 1542.6 ± 68.8 , $p = .013$, Fig. 2B). RT was also faster at a trend level after Interleaved practice than after Repetitive practice when the participants were tested using repeated blocks of the sequences (mean response time, YR = 880.9 ± 56.1 , YI = 810.4 ± 28.2 ; OR = 1501.8 ± 79.4 , OI = 1438.3 ± 66.3 , Fig. 2B). These results replicate findings of previous work on CI effects based on various paradigms (Lee and Magill, 1983; Lin et al., 2009, 2010, 2011; Shea and Morgan, 1979), and

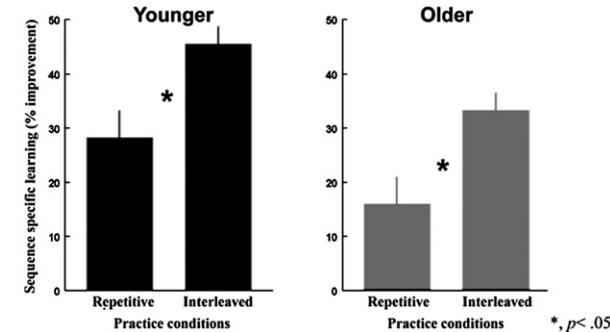
A) Practice, retention, and transfer



B) Performance during retention in the interleaved testing condition



C) Effect of practice condition on sequence specific learning



D) Effect of practice condition on non-specific learning

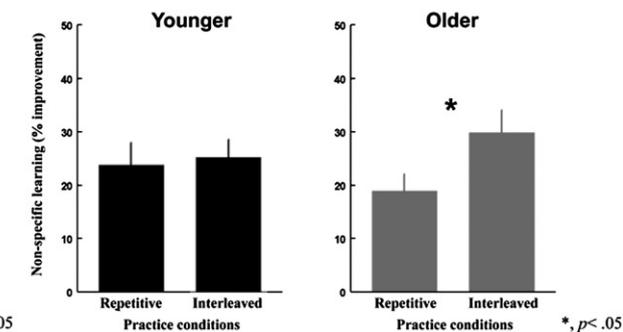


Fig. 2. Effects of practice condition on the evolution of SRT task performance across 5 days, retention of practiced sequences on Day 5, sequence specific learning, and non-specific learning for both younger and older adults. Panel A(1) shows task performance, measured by response time (mean \pm SE) on practice on Days 1 and 2 (blocks 1–54), and on retention of the practiced and novel sequences on Day 5 (A(2)). The practiced sequences in the retention tests were further arranged in either a Repetitive testing condition (RC) or an Interleaved testing condition (IC). Sessions for novel sequences are labeled as “Novel”. R1 to R6 on the x-axis label denote the 6 response time blocks for repetitive testing condition, I1–I6 for interleaved testing condition, and N1–N6 for the Novel sequences. At the baseline, there was no significant difference in the response time between the Repetitive practice (RP, filled circle) and the Interleaved practice (IP, empty circle) conditions for both age groups. Performance in the two age groups and in the two practice conditions all improved after 2 days of practice, shown by the decreased response time. During practice, the response time was shorter in the Repetitive than the Interleaved condition, while during retention on Day 5 (also refer to the illustration of A(2)), the 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 (IC). This contextual interference effect was observed in both younger and older adults, and is more clearly demonstrated in panel B. To further identify the underlying processes that improved the sequence learning, we divided the motor sequence learning into *sequence-specific learning* and *non-specific learning*. Sequence-specific learning was defined as the percentage difference in the response time between practiced and novel sequences on Day 5. Here we assumed that the participants’ level of motor skills remained unchanged on the same day, so that the shorter response time with respect to the practiced sequences than the novel sequences was due to learning of the content of the practiced sequences. Non-specific learning was defined as the percentage decrease in the response time from the first block of training (Day1 baseline) and the novel sequences on the retention tests on Day 5. This means that the participants improved in their performance on novel sequences (note that all sequences were “novel” to the participants on Day 1 before practice) due to some factors unrelated to the practiced sequences per se, e.g., they became familiar with key pressing. Panel C shows that the Interleaved practice led to better sequence-specific learning than the Repetitive practice in both younger and older adults (* indicates that the between condition difference is significant, $p < 0.05$). Interestingly, the Interleaved practice also facilitated non-specific learning in older adults (D), indicating that increase in task variability during practice improves the general motor performance in the elderly, e.g., increase in dexterity in key pressing or better adaptation to the test environment.

479 also demonstrate that motor learning in older adults can also benefit from
480 CI, particularly when the practiced skill was retained using interleaved
481 structure.

482 *Sequence-specific and non-specific learning*

483 We next investigated whether the benefit of the Interleaved prac-
484 tice on retention was *sequence-specific*, or simply reflected general
485 improvement in key-pressing speed, which we denoted by *non-*
486 *specific learning*. Fig. 2C compares sequence-specific learning between
487 the two age groups. Interleaved practice significantly facilitated
488 sequence-specific learning compared to the Repetitive practice for
489 both younger ($p = .003$, Fig. 2C, left) and older adults ($p = .015$,
490 Fig. 2C, right). By contrast, the difference in non-specific learning be-
491 tween the Interleaved and Repetitive practices was either not signif-
492 icant or at a trend level for both age groups (younger adults:
493 $p = .741$, Fig. 2D, left; older adults: $p = .04$, Fig. 2D, right).

494 *TMS results*

495 *Interleaved practice of sequences increased M1 excitability*

496 One participant in the younger age group was excluded from TMS
497 analysis due to a high motor threshold that prevented collection of inter-
498 pretable MEPs. Prior to practice on Day 1, the mean amplitude of the con-
499 ditioned MEPs (cMEPs) was not different between the two practice
500 conditions at each ISI. Both younger and older adults showed expected
501 responses to paired-pulse TMS – shorter ISIs (ISI 2–5 ms) elicited
502 short-interval intracortical inhibition (SICI), with a suppressed baseline
503 with respect to the amplitude of unconditioned MEPs, while longer ISIs
504 (ISI 10–15 ms) elicited intracortical facilitation (ICF) (Fig. 3A).

505 To provide an overview of how aging may affect changes in intra-
506 cortical M1 excitability in different practice conditions, we highlight-
507 ed TMS data on Day 1-pre in blue and on Day 5-pre in red (Fig. 3A).
508 For younger adults, the Interleaved practice, but not the Repetitive
509 practice, led to a net increase in M1 excitability (decrease in SICI
510 and increase in ICF) from Day 1 to Day 5 as demonstrated by a net
511 up-shift of the sigmoid curves (Fig. 3A). A repeated measures
512 ANOVA that included Condition (Repetitive and Interleaved), Session
513 (Day1-pre, Day1-post, Day2-pre, Day2-post, and Day 5-pre), and ISIs
514 (2, 3, 4, 5, 10, and 15 ms) as within-subject factors indicated a signif-
515 icant Condition effect ($p = .014$) and Condition by Session interaction
516 ($F(1, 11) = 6.4$, $p = .005$), supporting that practice conditions modu-
517 lated how M1 excitability evolves during practice. A similar finding
518 was present in the older adults (Fig. 3A, right), with greater increase
519 in M1 excitability during the Interleaved practice than the Repetitive
520 practice. A repeated measures ANOVA conducted in the Older group
521 also showed a significant Condition effect ($p = .006$) and significant
522 Condition by Session interaction ($F(1,12) = 8.627$, $p = .002$). More-
523 over, the CI effect on M1 excitability was more significant in the
524 older adults – a repeated measures ANOVA showed a significant in-
525 teraction between Age group and Condition, where the older adults
526 had a greater difference in cMEP between the Interleaved and the Re-
527 petitive practice than the younger adults (Interleaved minus Repeti-
528 tive cMEPs: 0.92 ± 0.20 in the older adults, and 0.31 ± 0.09 in the
529 younger adults; $F(1,30) = 7.994$, $p = .008$).

530 *Increased M1 excitability during interleaved practice is associated with 531 better learning*

532 For both younger and older adults, the increase in M1 excitability
533 after Interleaved practice over Repetitive practice was significantly
534 associated with better sequence specific learning (Fig. 3B, Younger,
535 $r = .50$, $p = .04$; Older, $r = .53$, $p = .03$). For non-specific learning,
536 such association, however, was only significant in the older adults
537 (Fig. 3C, Younger, $r = .341$, $p = .197$; Older, $r = .554$, $p = .026$).

Functional MRI

538

Age interacts with CI effects in sequence learning

539

540 We identified brain regions where the younger and the older adults
541 showed a different magnitude of the effect of practice schedule on BOLD
542 activation. For the younger adults, there was greater BOLD activity in
543 the left dorsal lateral prefrontal cortex (DLPFC) for the Interleaved over
544 the Repetitive practice condition, while this difference was not present
545 for older adults. For younger adults, the degree of increased BOLD activity
546 during interleaved practice was correlated with the subsequent benefit of
547 interleaved practice at retention (Figs. 4A1, A2). This association, howev-
548 er, was not detected in the older adults. This correlation suggests that the
549 DLPFC contributes to the CI benefit in younger, but not older adults.

550 On the other hand, in the rostral prefrontal (BA10), the primary
551 motor (M1), and the supplementary motor (SMA) cortices, the
552 older adults showed a greater increase in BOLD signal than did the
553 younger adults, and in the older adults only, these increases in
554 BOLD during interleaved practice correlated with the subsequent
555 benefits in this practice schedule on retention (Figs. 4B1–B4). The re-
556 sults from M1 and SMA suggest that older adults rely on increased
557 sensorimotor engagement during interleaved practice for the CI ben-
558 efit to learning. Our findings show that brain regions that mediate the
559 CI benefits in motor sequence learning differ with age, implicating the
560 modulatory effects of aging on neuroplasticity.

Age differences in functional activation maps during sequence practice

561

562 In order to explore the Age group \times Practice condition interaction,
563 we next examined age differences in functional activation maps for
564 the two practice conditions. Fig. 5A shows regions where BOLD acti-
565 vation was significantly different between the Older and Younger
566 groups in the Repetitive condition. MNI coordinates of the peak acti-
567 vation voxel in these regions are listed in Table 1. During the Repeti-
568 tive practice, older adults exhibited greater bilateral activity than the
569 younger adults in the medial frontal, the inferior parietal, and the
570 supplementary motor areas. These differences are further demon-
571 strated in the bar graphs in Fig. 5B, where BOLD signal during Repeti-
572 tive practice was higher in the older than younger adults in regions
573 that are part of the motor learning network: the right medial prefrontal
574 (Fig. 5B left, $p = .002$), right supplementary motor (Fig. 5B middle,
575 $p < .001$), and the right inferior parietal areas (Fig. 5B right, $p = .001$).
576 No region showed greater brain activity in the younger adults than
577 the older adults during Repetitive practice.

578 During Interleaved practice, the older adults exhibited greater activity
579 than the younger adults in the medial prefrontal, premotor, primary
580 motor, and the inferior parietal areas bilaterally, and the right superior
581 prefrontal areas (Fig. 5C, Table 2). Bar graphs in Fig. 5D further show
582 higher BOLD signal in the older than the younger adults during Inter-
583 leaved practice, in regions belonging to the frontoparietal network: the
584 right medial prefrontal (Fig. 5D left, $p = .001$), right premotor (Fig. 5D
585 middle, $p < .001$), and the right primary motor cortices (Fig. 5D right,
586 $p = .003$). No region, however, showed greater activity in the younger
587 adults than the older adults during Interleaved practice. Taken together,
588 these results show that older adults exhibit greater neural recruitment
589 than the younger adults during sequence learning. This trend was more
590 apparent during the Interleaved practice condition, consistent with the
591 higher need for task switching and executive control.

Discussion

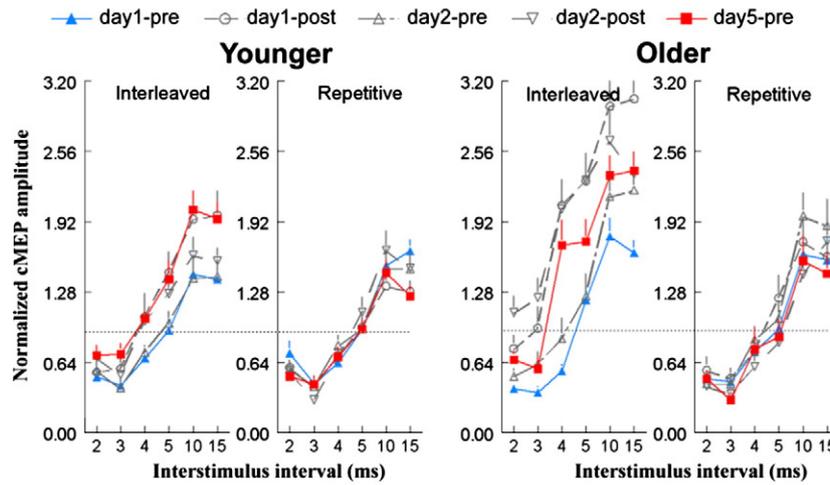
592

593 Our purpose was to combine fMRI and paired-pulse TMS to deter-
594 mine how aging affects the neural correlates of the contextual interfe-
595 rence (CI) benefit in motor sequence learning. There are four main
596 findings. First, in a within-subject design, we confirmed that the para-
597 doxical opposing effects of CI during practice and retention of motor se-
598 quences are present for both younger and older adults. A novel finding
599 is that, in older adults, part of the CI benefit at retention was mediated

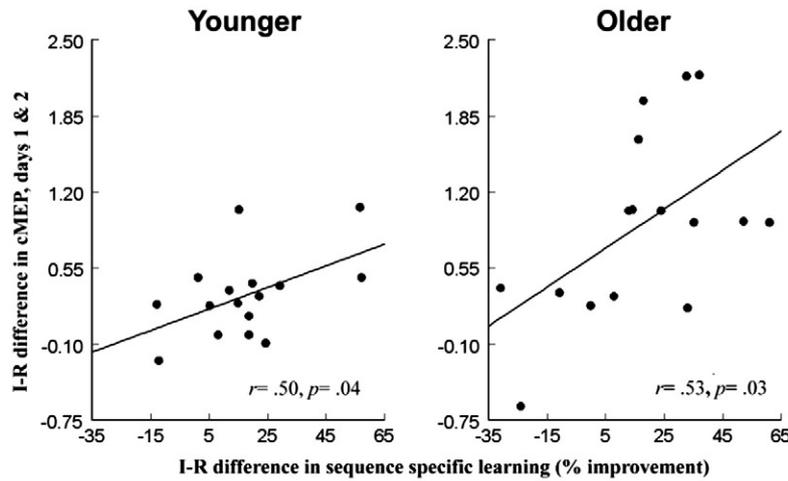
600 though motor learning that was not sequence specific. Second, older
 601 adults showed a greater short-term increase in M1 excitability after In-
 602 terleaved practice compared to the young group. Third, increased M1
 603 excitability during Interleaved practice correlated with sequence-
 604 specific learning for both age groups. However, only in older adults,
 605 this practice-related increase in M1 excitability also correlated with
 606 non-specific learning. Fourth, the CI benefit to sequence learning was

mediated through different brain regions in the two age groups as
 607 assessed by fMRI BOLD signal. The retention benefits of Interleaved
 608 practice correlated with increased recruitment of the left DLPFC in
 609 younger adults, but in older adults, these benefits correlated with great-
 610 er recruitment of the right rostral prefrontal and sensorimotor regions.
 611 Overall, we demonstrated the first evidence of age-related similarity
 612 and differences in neuroplastic changes underlying the CI benefits and
 613

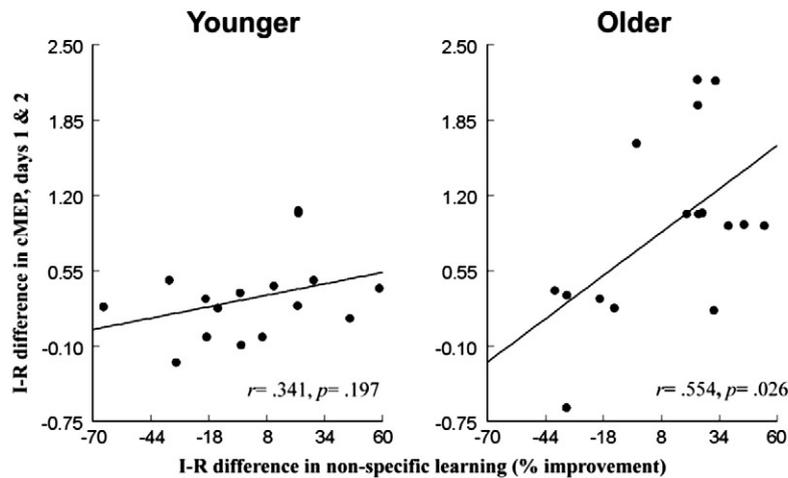
A) Intracortical excitability



B) Correlation between intracortical excitability and sequence specific learning



C) Correlation between intracortical excitability and non-specific learning



614 directly relate them to enhanced learning. Introducing the *desirable dif-*
 615 *iculty* of CI during practice induces greater neuroplastic changes in
 616 older adults, leading to more efficient long-term retrieval.

617 The CI effect may benefit older and younger adults through different
 618 cognitive routes. We identified this difference by separating behavioral
 619 gain (changes in performance between baseline and on Day 5) into se-
 620 quence specific and non-specific learning. These further analyses allow
 621 us to evaluate whether participants' behavioral improvements were
 622 based on the learning of motor sequences, or if the benefit was due to
 623 a general improvement such as becoming more familiar with moving
 624 their fingers on the keys or learning visuo-motor mappings. For both
 625 younger and older adults who were trained in the Interleaved practice
 626 condition, the sequence-specific learning was greater than that seen
 627 after Repetitive practice (Fig. 2C). But only in older adults, Interleaved
 628 practice also resulted in greater non-specific learning (Fig. 2D). Taken
 629 together, the results suggest that Interleaved practice of sequences ben-
 630 efits older adults in both a sequence-specific and non-specific manner.

631 *Age-related increase in brain activation and excitability.* Older adults
 632 exhibit greater neural recruitment for motor sequence practice.
 633 Greater brain activation in brain regions associated with executive
 634 function (the medial frontal area) and sequence organization (the
 635 supplementary motor area, SMA) was identified even when they
 636 practiced sequences in the relatively easier, Repetitive condition.
 637 When older adults had to tackle the more difficult Interleaved condi-
 638 tion, additional neural recruitment in the right medial prefrontal and
 639 bilateral sensorimotor areas was required. The functional imaging re-
 640 sults overall are consistent with the previous findings showing age-
 641 related hyperactivation (Gazzaley et al., 2005; Yassa et al., 2010).
 642 The older participants also showed greater recruitment near motor
 643 and bilateral parietal cortex (Fig. 4C), regions that have been func-
 644 tionally associated with spatial processing and sensorimotor map-
 645 ping. The additional recruitment of sensorimotor cortices in older
 646 adults may suggest increased sensorimotor recruitment as a compen-
 647 satory strategy to meet the demands of the Interleaved condition. Our
 648 previous work in younger adults has linked increased medial prefrontal
 649 activity with the CI benefits in learning (Lin et al., 2011). Since
 650 older adults already show more medial frontal activity in the Repetitive
 651 condition than the younger subjects (Fig. 4A), they may fail to
 652 further recruit medial prefrontal activity to accomplish the cognitive
 653 challenges of the Interleaved condition. Therefore, additional sensori-
 654 motor recruitment may serve as an alternative neural resource that
 655 can support the benefits of CI.

656 Older adults showed increased supplementary motor area (SMA)
 657 BOLD activity during sequence practice compared to younger adults.
 658 The SMA can be differentiated into two distinct areas according to
 659 both anatomical and physiological evidences: the rostral area called
 660 pre-SMA and the caudal area called SMA-proper, with the pre-SMA
 661 playing a greater role in sequencing and the SMA-proper being more in-
 662 volved in motor control (Coxon et al., 2010). The greater SMA activity in
 663 older adults that we report here was not clearly centered in either sub-
 664 region (Table 1). It is likely that older adults show greater engagement
 665 of both SMA subregions during sequence learning.

666 Aging effects in the hemodynamic response as a functional of prac-
 667 tice condition were also identified by the neurophysiologic TMS mea-
 668 sure. For the Interleaved, but not the Repetitive practice condition, the
 669 ppTMS curve shifted toward a more excitable state after each training
 670 day that persisted at the Day 5 retention test (Fig. 3A), suggesting
 671 both short-term (changes within the same day) and long-term
 672 (manifested 3 days after practice) modifications in intracortical circuitry
 673 within M1 (Kujirai et al., 1993). Such changes seem to involve M1 cir-
 674 cuitry of both short-interval intracortical inhibition (ISI 2–5 ms) and
 675 intracortical facilitation (ISI 10 and 15 ms). The condition-dependent
 676 change in M1 excitability was consistent across both age groups, but
 677 was more robust in the older adults. This greater increase in M1 exci-
 678 tability during Interleaved practice in older adults is consistent with
 679 their greater sensorimotor activation shown by functional imaging
 680 data (Fig. 4C), and supports the interpretation that older adults rely
 681 more on sensorimotor activation during Interleaved practice.

682 Furthermore, increased M1 excitability during Interleaved practice
 683 contributes to the CI benefit in sequence-specific learning for both age
 684 groups (Fig. 3B). This may suggest that the greater the intracortical ex-
 685 citability within the learner's motor cortex during Interleaved practice,
 686 the greater the eventual learning benefits will be. Aging does not appear
 687 to significantly impair these corticomotor processes. Nevertheless, only
 688 in older adults, M1 circuitry may also mediate the non-specific behav-
 689 ioral improvements after Interleaved practice, where higher cMEPs in
 690 the Interleaved condition over the Repetitive condition were associated
 691 with greater non-specific learning (Fig. 3C). Overall, the results of TMS
 692 measures suggest that increased M1 excitability during practice en-
 693 hances long-term retention of motor skills and that older adults may
 694 rely on this enhancement to a greater extent than younger subjects. In-
 695 creased brain excitability in older adults may also support the non-
 696 sequence specific improvements in perceptual-motor dexterity.

697 One provocative explanation for the disparate pattern of neuro-
 698 plastic changes between older and younger adults is that regions
 699 uniquely or increasingly recruited by older adults are serving a compen-
 700 satory function. Additional brain regions might be brought on-line in
 701 older adults to mediate task-relevant cognitive operations and enable
 702 optimal learning during Interleaved practice of sequences (Cabeza
 703 et al., 1997; Grady et al., 1994). The compensatory-recruitment hypothe-
 704 sis underscores the potential for brain plasticity over the life span.

705 *BOLD–ppTMS correlation.* Greater cortical recruitment during practice
 706 in young and older groups was accompanied by increased short-term
 707 M1 excitability immediately after practice and both were associated
 708 with enhanced learning on the delayed retention test following Inter-
 709 leaved practice. Previous studies have shown that on-line TMS stimula-
 710 tion targeted to a given brain region modulates the activity of remote
 711 regions that are anatomically and functionally connected to the
 712 targeted region (Platz and Rothwell, 2010). Thus, the enhanced exci-
 713 tability in M1 may reflect enhanced functional connectivity with struc-
 714 tures that project to M1, not only enhanced excitability in intrinsic M1
 715 circuitry. While functional connectivity measurements are beyond the
 716 scope of the current report, this interpretation is suggested by the sig-
 717 nificant correlation between relative BOLD contrast in primary

Fig. 3. Correlations between intracortical excitability and learning performance. Panel A shows the 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 and Repetitive practice conditions at five time points: baseline before practice (day1-pre, filled gray up-triangle in blue), Day 1 after practice (day1-post, open circle), Day 2 before practice (day2-pre, open up-triangle), Day 2 after practice (day2-post, open down-triangle), and Day 5 before retention tests (day5-pre, filled red rectangle) in the younger (A, left) and older adults (A, right). In both age groups, Interleaved practice enhanced motor cortex excitability – the intracortical facilitation (the TMS effects with respect to the ISI = 10–15 ms) increased and intracortical inhibition (the TMS effects with respect to the ISI = 2–3 ms) decreased. Even so, the effects of the Interleaved practice were stronger in the older adults, leading to greater increase in normalized cMEPs that was significant on Group (younger, older) by Practice condition (Repetitive, Interleaved) by Sessions (Day1-pre, Day1-post, Day2-pre, Day2-post, and Day5-pre) interactions. By contrast, motor cortex excitability remained unchanged following Repetitive practice in either age group. This contextual interference (CI) effect in motor cortex excitability coincided with the CI effect in sequence-specific learning. In both age groups, higher cMEPs in the Interleaved practice than the Repetitive practice were associated with better sequence-specific learning (greater percentage difference in the response time between practiced and novel sequences on Day 5; see Fig. 2 for the definition of sequence-specific learning and non-specific learning) following Interleaved practice (B). On the other hand, associations between the CI effects in motor cortex excitability and non-specific learning were only detected in the older adults (C), where higher cMEPs in the Interleaved practice than the Repetitive practice were associated with greater percentage difference in the response time between the baseline block on Day1 and the novel sequences on the retention tests on Day 5. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

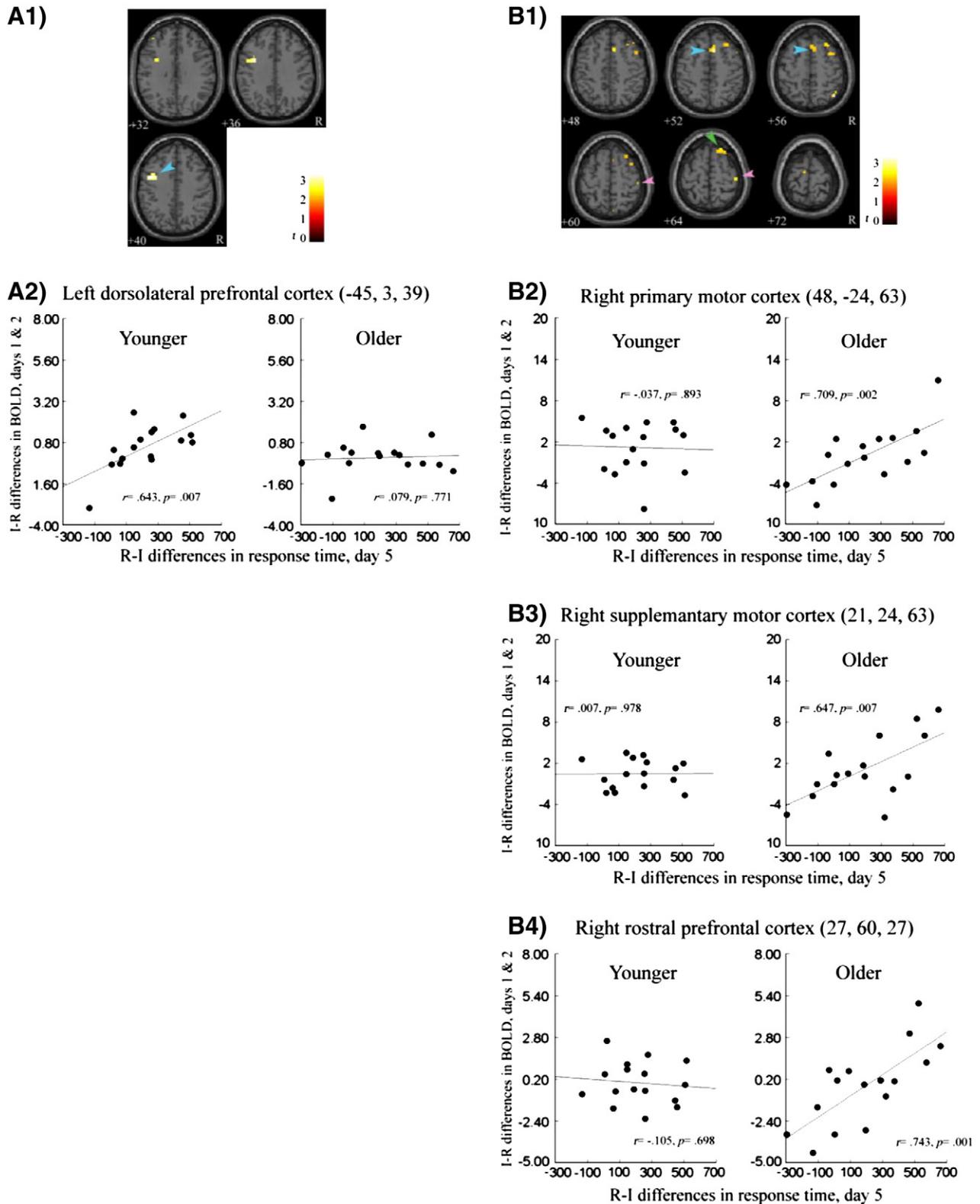


Fig. 4. Maps of brain regions where age interacts with contextual interference effects in motor sequence learning. For the younger adults, contextual interference (CI) effects in motor sequence learning were mediated by the left dorsolateral prefrontal cortex (arrowhead in A1), where greater BOLD activity during the Interleaved than the Repetitive practice was significantly associated with better retention performance (shorter response time on day 5) following the Interleaved practice. While for the older adults, the CI effects were detected in the right primary motor cortex (pink arrowheads in B1), the right supplementary motor cortex (blue arrowheads in B1), and the right rostral prefrontal cortex (green arrowhead in B1). The scatter plots compare associations for Interleaved-minus-Repetitive BOLD contrast on practice (y-axis) and the Repetitive-minus-Interleaved response time on retention (x-axis), between the younger and older adults in the above brain regions. In summary, our findings show aging may alter the processes of motor sequence learning (e.g., older adults depend more on non-specific learning than younger ones; see Fig. 2), the patterns of motor cortex excitability (Fig. 3), and the brain regions that are functionally important for better sequence learning. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

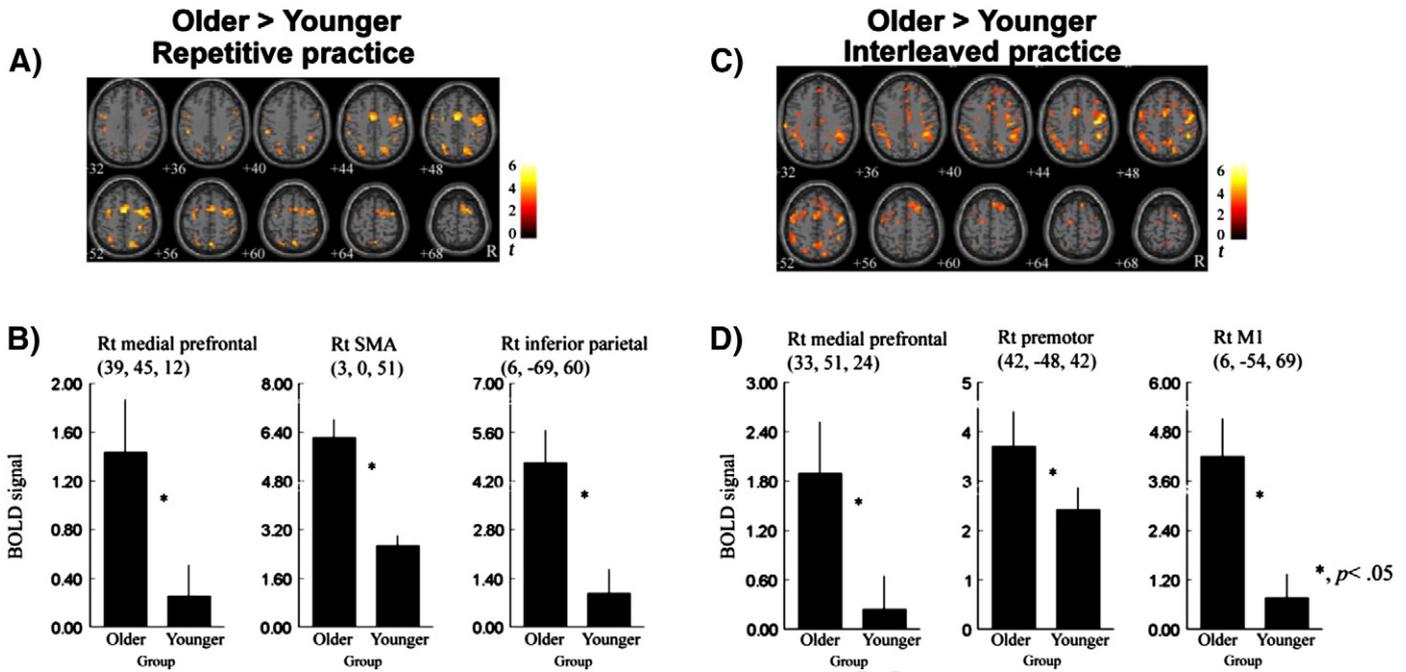


Fig. 5. Older adults require greater neural recruitment during motor sequence practice. The figure shows that fMRI BOLD activity was significantly higher in the older adults, for both Repetitive practice (A and B) and Interleaved practice (C and D) of motor sequences. Brain regions with a positive t-value were color-coded, indicating an excess in BOLD response, or greater demand in neural recruitment, in the older adults than the younger adults for motor tasks at the same level of difficulty. To correct for multiple comparisons, only voxel clusters that passed the topological FDR at $p < .05$ threshold are displayed. For the Repetitive condition, a relatively easier practice condition, the older adults still exhibited greater BOLD activity than the younger adults in the right medial frontal, bilateral inferior parietal, and bilateral supplementary motor areas (A). The group difference was further demonstrated in bar graphs of panel B, where BOLD signal (mean \pm SE) during Repetitive practice was higher in the older adults than younger adults in the right medial prefrontal, the right supplementary motor, and the right inferior parietal areas. “***” indicates that the between group difference is statistically significant ($p < .05$). During Interleaved practice (C), greater BOLD activity was detected in the older adults in the right anterior and medial frontal cortex, and bilateral sensorimotor cortex. Bar graphs in D show higher BOLD signals in the older than the younger adults during Interleaved practice, in regions belonging to the frontoparietal network: the right medial prefrontal, right premotor, and the right primary motor cortices. Our findings demonstrate that during sequence learning, the older adults required greater neural recruitment in brain regions associated with executive function (the medial frontal area) and sequence organization (the supplementary motor area). Furthermore, extra neural recruitment in the bilateral sensorimotor areas was required for older adults to tackle the more difficult Interleaved condition. The functional imaging data overall is consistent with previous neuroimaging findings in the aging literature.

Table 1
Activation locations for group comparison of sequential learning in the Repetitive condition, older group minus younger group (thresholded at $p < .05$, topological FDR corrected).

Regions	MNI coordinates			p (tFDR corrected)	t
	x	y	z		
R medial temporal gyrus	48	-63	-3	<.0001	6.22
R medial occipital gyrus	24	-90	6	<.0001	4.35
R superior temporal gyrus	55	-27	15	<.0001	5.18
L precentral gyrus	-30	-15	54	.001	3.38
R supplementary motor	3	0	51	<.0001	5.53
	5	5	57	<.0001	4.84
	2	-15	75	<.0001	4.69
R premotor cortex	21	0	69	<.0001	4.77
L premotor cortex	-30	-6	51	<.0001	4.12
	-27	3	57	.001	3.51
R medial frontal gyrus	39	45	12	<.0001	4.47
	33	51	3	<.0001	3.99
	24	54	21	<.0001	3.86
R inferior frontal gyrus	36	24	-18	<.0001	4.29
L inferior parietal lobule	-48	-33	39	<.0001	4.8
R precuneus	6	-69	60	<.0001	4.6
L precuneus	-3	-63	66	.001	3.37
R cerebellum	3	-48	-6	<.0001	3.65
L cerebellum	-9	-54	-24	<.0001	4.39
	-5	-55	-24	<.0001	4.35
L caudate	-15	24	-3	<.0001	3.69

For each cluster, all local maxima exceeding a voxel-level corrected $p < .05$ threshold are presented.

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

Table 2
Activation locations for group comparison of sequential learning in the Interleaved condition, older group minus younger group (thresholded at $p < .05$, topological FDR corrected).

Regions	MNI coordinates			p (tFDR corrected)	t
	x	y	z		
R medial temporal gyrus	48	-60	-3	<.0001	6.98
L inferior occipital gyrus	-39	-69	-9	<.0001	6.21
L medial occipital gyrus	-21	-96	6	<.0001	5.31
	-48	-72	3	<.0001	4.32
R superior temporal gyrus	63	-39	15	<.0001	5.76
R precentral gyrus	42	-18	42	<.0001	6.49
	54	-3	45	<.0001	5.72
	48	-9	48	<.0001	5.72
L precentral gyrus	-48	-6	57	<.0001	4.30
	-48	0	51	<.0001	4.21
	-39	-12	54	<.0001	3.75
R supplementary motor	9	24	50	<.0001	4.40
	5	5	51	<.0001	4.33
L supplementary motor	-3	3	45	<.0001	5.11
L postcentral gyrus	-6	-54	69	<.0001	4.32
R medial frontal gyrus	33	51	24	<.0001	5.77
	42	48	12	<.0001	5.29
L medial frontal gyrus	-39	54	12	<.0001	5.14
	-48	39	18	.001	3.53
R inferior frontal gyrus	51	24	-3	<.0001	5.38
R superior frontal gyrus	3	48	36	.001	3.47
	21	42	39	.001	3.35
R anterior cingulate gyrus	3	45	27	<.0001	4.31

For each cluster, all local maxima exceeding a voxel-level corrected $p < .05$ threshold are presented.

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

sensorimotor regions and relative M1 excitability during Interleaved compared to Repetitive practice. As such, although no practice condition differences in BOLD signal were seen in the M1 hand region (where M1 excitability is assessed), the differences in BOLD activity in related brain regions may be serving to strengthen inputs to M1 for which excitability is assessed by paired-pulse TMS. Further, these brain regions which show increased BOLD activity suggest that the network inputs to M1 appear to differ depending on age. In younger adults, increased M1 excitability following Interleaved practice was associated with hemodynamic changes in the primary motor, premotor, and parietal sensorimotor regions (Suppl. Fig. 1) while in the older adults, increased M1 excitability as a result of Interleaved training is associated with increased hemodynamic response in prefrontal and subcortical regions such as caudate (Suppl. Fig. 1). The “desirable difficulty” effect of CI may strengthen connectivity of different motor network circuits over the life span. Enhanced learning associated with Interleaved training in the younger adults may be supported by stronger M1-sensorimotor connectivity while in the older adults, it may be related to stronger M1-prefrontal or subcortical connectivity.

The dynamic and demanding nature of Interleaved practice may strengthen the synaptic efficacy within M1 and with other cerebral regions (Kujirai et al., 1993), that may consequently promote the function of frontal-parietal networks in sequence learning for the older population (Ziemann et al., 1995). Across younger and older adults, the results suggest that CI effects may be mediated by two levels of neural processes, one is actively engaging the prefrontal network during practice so task switching and executive function can be accomplished; the other is by raising the overall excitability of the brain so better consolidation has the basis to evolve.

Another possibility is that interleaved practice does differentially engage M1, compared to Repetitive practice, resulting in greater “neural efficiency” that would not result in a BOLD signal change, but would enhance specialization of M1 circuits, resulting in an increase in the MEP with ppTMS. These gains in neural efficiency may be accomplished by functional reorganization in the striatum (Olson et al., 2006; Reithler et al., 2010). Previous findings have shown that some post-learning processes may be mediated by subcortical structures such as the basal ganglia (Toni, et al. 2002; Wu, et al. 2008). The striatum may participate in developing a motor repertoire that can be initiated in response to appropriate environmental cues (Laforce and Doyon 2001). We speculate that the Interleaved condition facilitates M1-striatum interaction during sequence learning. The striatum and M1 may function jointly to optimize retrieval processing during retention by transforming a series of motor elements (e.g., the motor elements that compose a motor sequence) as one motor representation, making the retrieval performance more automatic.

Age-related differences in neural regions that support enhanced learning. By correlating the relative increase in BOLD signals during practice at every voxel of the brain with the behavioral benefits of the Interleaved practice condition, we identified encoding-related activation patterns that predict subsequent ‘successful’ memory in younger and older adults (Fig. 5). For the younger adults, learning benefits of Interleaved practice were more associated with increased BOLD signal in the left DLPFC during practice whereas for the older adults, enhanced learning depended on greater recruitment of the right rostral prefrontal, right SMA and M1 (Fig. 5). The greater dependence on the M1 and SMA in older adults supports the idea that older adults may adapt alternative sensorimotor strategies to manage increasing cognitive demand in the Interleaved condition. This trend is similar to previous studies showing that in working memory, visual attention, and episodic retrieval tasks, older adults showed stronger parietal activity than younger adults (Cabeza et al., 2004). These results support the compensatory recruitment hypothesis and challenge the idea that additional recruitment adversely affects performance. It also implies that in the aged population, those who can recruit more brain activity during practice are those who can gain the behavioral benefit of CI (Table 3).

Though increased prefrontal and parietal recruitment was identified during Interleaved practice in the older adults, we did not observe a significant increase of BOLD signal in the lateral premotor cortex, an area that is typically associated with visuo-motor transformation for the key pressing tasks. Instead, compared to younger adults, the supplementary motor (SMA) cortices in the older adults showed a greater increase in BOLD signal during interleaved practice and the increased BOLD correlated with the subsequent learning benefits on retention. Previous studies have suggested SMA as an important neural substrate for the formation and storage of motor programs. Overall, the results may suggest that instead of facilitating the visuo-motor transformation of the key pressing tasks, the Interleaved condition benefits older adults through either developing stronger internally-driven motor programs or the retrieving of motor engrams. Though lateral premotor cortex is an essential substrate for serial reaction time key pressing tasks, it may not be differentially engaged in different practice conditions.

In younger adults, the left DLPFC appears to be an important neural substrate for the CI benefits in sequence learning (see Figs. 5A1, A2 for BOLD-learning correlation). The results support previous studies, which observed increased DLPFC activity when young subjects perform working memory tasks involving contextual coding and scheduling demands (Lie et al., 2006). DLPFC activity also increases with increasing memory load or retention interval, and left prefrontal activity has been shown to predict subsequent memory in younger adults (Morcom et al., 2003). Likewise, older adults’ poorer performance in elaborative encoding is associated with under-recruitment of left prefrontal areas that have been linked to successful memory in younger adults (Logan et al., 2002).

On the other hand, recruitment of the right rostral prefrontal cortex (rPFC) supports the CI benefits in sequence learning for the older adults (Figs. 5B1, B3). Neuroimaging experiments have related episodic memory retrieval with rPFC activation (Burgess et al., 2007), and this relationship seems particularly strong for tasks that engage complex control processing (Simons et al., 2006). Given that the importance of right rPFC in the CI effect was only identified in older adults, and the known function of rPFC in memory retrieval, we speculate that instead of maintaining multiple sequences in working memory, older adults “retrieve” those sequences as an alternative strategy during Interleaved practice. Thus, while younger adults engage DLPFC during interleaved practice because of increased working memory manipulation, older adults may retrieve sequences from episodic memory so as to not overly tax working memory. Both of these potential mechanisms (refreshing items in working memory and retrieving items from episodic memory) appear to have facilitated motor sequence learning.

Table 3

Activation locations for group comparison of functional correlation between BOLD contrast and the learning benefit of Interleaved practice condition, older group minus younger group (thresholded at $p < .05$, topological FDR corrected).

Regions	MNI coordinates			p (tFDR corrected)	t
	x	y	z		
L superior occipital gyrus	-15	-99	15	.003	3.02
	-27	-93	30	.007	2.64
L precentral gyrus (M1)	48	-24	63	.002	3.16
R supplementary motor	6	15	51	<.0001	3.43
L supplementary motor	-6	-12	75	.003	2.94
R medial frontal gyrus	27	60	27	.001	3.29
R superior frontal gyrus	21	24	63	.003	2.93
R inferior opercular frontal gyrus	57	21	30	.003	2.93
L inferior parietal lobule	42	-63	57	.001	3.59
L cuneus	-3	-90	33	.008	2.6
R fusiform gyrus	24	-84	-12	.002	3.10
L lingual gyrus	-12	-54	-3	.004	2.94
R thalamus	3	-9	9	.007	2.63
L caudate	-15	0	24	.001	3.69

For each cluster, all local maxima exceeding a voxel-level corrected $p < .05$ threshold are presented.

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

Consistent with the trend observed in previous research, older adults appear to have engaged the right hemisphere to a greater extent than younger subjects. Right frontal activation has been suggested to be a characteristic finding in older adults and manifests regardless of whether participants engage in semantic processing (Logan et al., 2002). Instead of strategy variations, this right frontal activation seems to reflect episodic encoding in older adults. In addition, increased recruitment of the right M1 and SMA may suggest that older adults encounter more difficulty and expend more effort during Interleaved practice compared to younger adults. The latent potential of the non-dominant hemisphere may be a resource of neuroplasticity across the life span (Reuter-Lorenz, 2002).

Learning processes are related to complex changes as a function of practice content and duration. The sequence learning task has been well studied using different imaging techniques. For example, Karni et al. first showed an enlarged representation of the practiced sequence in M1 after 3 weeks of practice (Karni et al., 1995). Pascual-Leone et al. showed that once a motor sequence was made explicit, the area from which a response could be evoked by TMS expanded across several consecutive daily sessions (Pascual-Leone et al., 1993, 1994). Enlarged motor representation after practice may be a result of motor map reorganization and synapse formation during the consolidation of motor skill at late stages of training (Kleim and Jones, 2008; Kleim et al., 2002). This is corroborated by primate studies (e.g., Nudo et al., 1996) showing that extensive motor skill training induced reorganization of movement representations and synaptogenesis within the motor cortex. As such, increase in BOLD activity and TMS excitability in M1 after Interleaved practice may reflect strengthening of inputs to this region, and may contribute to the “slow learning” component of neural plasticity (Kleim and Jones, 2008).

Desirable difficulties in aging. Desirable difficulties such as CI during Interleaved practice result in greater cost, but this cost eventually leads to better learning (Guadagnoli and Lee, 2004; Lin et al., 2010). The greater cost is seen in behavioral performance (longer RT), hemodynamic response (increased BOLD signal), and greater intracortical excitability in M1. The CI benefit to skill learning has been explained in terms of greater information processing, such as repeated task-switching and retrieval of action plans, during the Interleaved practice condition (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 prefrontal and parietal areas (Rossi et al., 2009)), and to program a different action plan for each upcoming trial (engaging premotor areas (Gail et al., 2009)). This additional practice in motor programming may be especially beneficial for older adults given the current findings of a correlation between motor cortical activation and subsequent retention (Figs. 5B1, B2).

Summary and future directions. Using the contextual interference paradigm, we have demonstrated how aging influences neuroplasticity when skills are developed under different practice conditions. By correlating behaviorally relevant BOLD signal changes and cortical excitability, we were able to examine the effect of aging on concurrent learning-related changes at a hemodynamic network level and within the M1 circuit level. A behavioral paradigm with finer temporal resolution may be able to pinpoint the aging effect on skill learning by separating the phase of task processing, including encoding, consolidation, and retrieval. Another possible future direction may be to relate physiological effects of aging (e.g. reduced estradiol levels) with changes in patterns of neuroplasticity (Joseph et al., 2012). It may also be possible to directly test the causal significance of DLPFC and M1/SMA in younger and older adults using rTMS to disrupt these regions at different points during learning. Such data would provide a dynamic picture of the changes occurring during skill learning and how aging interacts with the CI effect on different components of motor skill learning.

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