Primary motor cortex disinhibition during motor skill learning

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Coxon JP, Peat NM, Byblow WD. Primary motor cortex disinhibition during motor skill learning. J Neurophysiol 112: 156–164, 2014. First published April 9, 2014; doi:10.1152/jn.00893.2013.—Motor learning requires practice over a period of time and depends on brain plasticity, yet even for relatively simple movements, there are multiple practice strategies that can be used for skill acquisition. We investigated the role of intracortical inhibition in the primary motor cortex (M1) during motor skill learning. Event-related transcranial magnetic stimulation (TMS) was used to assess corticomotor excitability and inhibition thought to involve synaptic and extrasyntaptic γ-aminobutyric acid (GABA). Short intracortical inhibition (SICI) was assessed using 1- and 2.5-ms interstimulus intervals (ISIs). Participants learned a novel, sequential pinch-grip task on a computer in either a repetitive or interleaved practice structure. Both practice structures showed equivalent levels of motor performance at the end of acquisition and at retention 1 wk later. There was a novel task-related modulation of 1-ms SICI. Repetitive practice elicited a greater modulation of 1-ms SICI, i.e., disinhibition, between rest and task acquisition, compared with interleaved practice. These novel findings support the use of a repetitive practice structure for motor learning because the associated effects within M1 have relevance for motor rehabilitation.

motor skill learning; repetitive practice; intracortical inhibition; GABA; transcranial magnetic stimulation

WE ARE CONSTANTLY FACED with opportunities to learn and perform motor skills, making motor control essential to almost everything we do. Neural plasticity and cortical reorganization influence how well motor skills can be acquired and learned. Use and repetition through motor practice increases corticomotor excitability of the cortical representations within M1 in combination with reduced γ-aminobutyric acid (GABA)-mediated inhibition (Butefisch et al. 2000; Liepert et al. 1998). In rodent M1 slice preparations, a reduction of GABA inhibition is a necessary condition for the induction of long-term potentiation (LTP) (Castro-Alamancos et al. 1995; Hess et al. 1996; Hess and Donoghue 1994). Magnetic resonance spectroscopy (MRS) shows that modulation of GABA is associated with LTP-like plasticity and learning in humans (Stagg et al. 2011a), particularly during the acquisition of novel motor tasks (Floyer-Lea et al. 2006).

After injury to the brain, motor practice and skill learning are essential for successful rehabilitation. Disinhibition through down-regulation of extrasyntaptic GABA-α-mediated receptor activation has been shown to promote functional recovery after experimental stroke in a rodent model (Clarkson et al. 2010). Similarly, M1 disinhibition in humans is a characteristic of subacute poststroke reorganization (Liepert et al. 2000). A better understanding of the relationship between disinhibition and motor skill acquisition may have clinical relevance.

There are multiple strategies to structure practice when a novel skill is being acquired, influencing subsequent learning. These structures can be conceptualized along a continuum. At one end, practice can be performed in a repetitive structure, where task variables are held constant from one attempt to the next. At the other end, practice can be performed in an interleaved structure, where all task variables are randomly integrated within a session so that each trial is varied. With repetitive practice, the required motor program is configured and then task focus is directed toward execution. This places a high load on local M1 processing, and learning is reliant on principles of use-dependent plasticity (Classen et al. 1998). Conversely, interleaved practice recruits a more widespread activation network, with greater involvement of prefrontal and parietal regions, due to high attentional demands and the need to reconfigure motor programs for each trial (Kantak et al. 2010; Li and Wright 2000). It has been suggested repetitive practice for novel skill learning allows for better initial performance but poorer retention, whereas interleaved practice results in worse performance initially but greater long-term skill retention (Kantak et al. 2010; Schmidt 1988).

In the present study we examined GABA-mediated intracortical inhibition to determine if it was modulated as a function of practice structure during motor learning. During acquisition of a sequential pinch-grip task, paired-pulse TMS was used to obtain measures of SICI (Fisher et al. 2002; Roshan et al. 2003) at two ISIs. SICI at 2.5-ms ISI reflects inhibition specifically involving synaptic GABA-α receptors (Ilic et al. 2002; Reis et al. 2008; Ziemann et al. 1996). SICI at 1-ms ISI may reflect extrasyntaptic GABA tone, but this is currently inconclusive (Stagg et al. 2011b). Previous studies investigating SICI in the context of motor learning have used a 2.5- to 3-ms ISI and taken measurements at rest before and after practice (Cirillo et al. 2011; Liepert et al. 1998; Perez et al. 2004; Rogasch et al. 2009; Rosenkranz et al. 2007). Repetitive practice of simple ballistic thumb movements has been associated with reduced SICI (Liepert et al. 1998; Rosenkranz et al. 2007), but there are exceptions (Rogasch et al. 2009). Interleaved practice involving more complex visuomotor tracking has also been associated with reduced SICI (Cirillo et al. 2011; Perez et al. 2004). We are not aware of any study that has investigated SICI on a trial-trial basis during motor learning, or that has directly compared disinhibition associated with repetitive vs. interleaved practice.

We used event-related TMS to investigate whether modulation of inhibition during motor skill learning was dependent on practice structure. We hypothesized that repetitive practice
would lead to a greater release of inhibition (disinhibition) compared with interleaved practice because of the known involvement of M1 in use-dependent plasticity associated with action repetition (Butefisch et al. 2000; Classen et al. 1998). Based on associations between motor learning and GABA concentration determined from MRS (Floyer-Lea et al. 2006; Stagg et al. 2011a), and between 1-ms SICI and GABA concentration (Stagg et al. 2011b), we examined whether practice structure would have a differential effect on 1-ms vs. 2.5-ms SICI.

METHODS

Participants

Twenty-four right-handed healthy young adults (12 women; mean age 22.9 yr, range 20–28 yr) without history of upper limb injury or neurological illness participated in this study. The study was approved by the University of Auckland Human Participants Research Ethics Committee in accordance with the Declaration of Helsinki. Participants gave written informed consent and were screened for contraindications to TMS by a neurologist. Right-handedness was assessed using the Edinburgh Handedness Inventory (Oldfield 1971) (mean 84%, range 62–100%).

Design

The between-subjects design randomized participants (n = 24) to interleaved (n = 12) or repetitive groups (n = 12) while minimizing differences between groups in sex, age, and time of experiment using custom software (www.rando.la). TMS was used to investigate corticomotor excitability and SICI during the acquisition session, which lasted 2–2.5 h. Retention was assessed 1 wk later, and this shorter session did not involve TMS.

Behavioral Task

All participants were naive to the behavioral task and given standardized instructions. Participants were seated at a table in front of a computer screen and held a force transducer between the nondominant left index finger and thumb (Fig. 1). Squeezing the transducer moved a computer cursor horizontally. The goal for each trial was to move the cursor sequentially into five colored targets following a specific sequence (red-blue-green-yellow-white), reaching each target in time with a 1.66-Hz metronome beat delivered via headphones (cf., Reis et al. 2009). Peak force was to coincide with each metronome beat. Visual feedback of force and timing performance for each target was provided graphically at the end of each block of 15 trials. The task difficulty was high because a logarithmic transformation was applied to the force to scale cursor movement. The farthest target was set at 45% of the individual’s maximum voluntary contraction (MVC) for pinch.

Acquisition

After the easiest target sequence orders (24 of 120 permutations) were discarded, five sequences were randomly selected. For the interleaved group, the five sequences were pseudorandomly alternated from trial to trial. For the repetitive group, the five sequences were randomized from block to block. Participants in each group performed

Fig. 1. Timeline for 1 trial of the task. The participant was required to squeeze the force transducer to move the cursor to within each of the 5 colored targets in order (always red-blue-green-yellow-white) by pinching the force transducer (left inset). Each force peak coincided with the 5 metronome beats (600 ms apart) after the warning cue turned off. The target order was displayed 2.6 s before the cursor was supposed to be within the first red target. Participants were required to relax their hand between beats, returning the cursor to the home position (0% force). Five target order sequences (right inset) were practiced, with the sequence changing from trial to trial for interleaved practice and from block to block for repetitive practice. Single- or paired-pulse transcranial magnetic stimulation (TMS) was delivered in a pseudorandom order for every trial in the acquisition session only. At the end of each block, feedback was displayed (bottom right) indicating the block score and, for both peak force and peak force timing, performance for each target over all 15 trials in the block. MVC, maximum voluntary contraction.
an equal number of trials over 16 blocks of 15 trials each. Trials lasted 5.6 s, with movement occurring during the last 3.6 s. The next trial commenced after a (pseudorandom) intertrial interval of either 1.6 or 2.2 s. Participants were given short breaks of ~2 min between blocks, during which feedback was presented. For the two types of practice, overall performance was matched for force levels produced, rate, and number of sequence repetitions.

Retention

Retention was examined 7–9 days after acquisition over 5 blocks of 15 trials each, using the same 5 sequences. The instructions were repeated, but the participant was not given any warm-up attempts. The five blocks were performed in either the structure practiced during acquisition or the unpracticed structure.

Electromyography and TMS

Surface electromyography (EMG) was recorded from the left first dorsal interosseus (FDI) using 10-mm-diameter Ag-AgCl electrodes (Ambu, Ballerup, Denmark) placed in a tendon-belly montage, following skin preparation. A ground electrode (3M Canada) was placed on the dorsum of the hand. EMG was amplified (Grass P511AC; Grass Instrument, West Warwick, RI), with 1,000 × gain, bandpass filtered (10–1,000 Hz), sampled at 2 kHz using a 16-bit analog-to-digital acquisition system (National Instruments, Austin, TX), and saved to disk for subsequent offline analysis.

Single- and paired-pulse TMS of right M1 was delivered using two MagStim 200 magnetic stimulators connected to a BiStim unit (Magstim, Dyfed, UK). A figure-of-eight coil (70-mm coil diameter) was held tangentially to the scalp and perpendicular to the central sulcus, with a posterior-to-anterior induced current flow. The coil was positioned at the site giving maximal motor evoked potentials (MEPs) in the resting left FDI muscle, and the location was marked on the scalp.

Participants rested their left hand on the force transducer and their right hand on the table surface. Active motor threshold (AMT) was defined as the minimum intensity for eliciting MEPs in 5 of 10 trials when the left FDI was preactivated with a 5% MVC squeeze on the right hand on the table surface. EMG was amplified (Grass P511AC; Grass Instrument, West Warwick, RI), with 1,000 × gain, bandpass filtered (10–1,000 Hz), sampled at 2 kHz using a 16-bit analog-to-digital acquisition system (National Instruments, Austin, TX), and saved to disk for subsequent offline analysis. Single- and paired-pulse TMS of right M1 was delivered using two MagStim 200 magnetic stimulators connected to a BiStim unit (Magstim, Dyfed, UK). A figure-of-eight coil (70-mm coil diameter) was held tangentially to the scalp and perpendicular to the central sulcus, with a posterior-to-anterior induced current flow. The coil was positioned at the site giving maximal motor evoked potentials (MEPs) in the resting left FDI muscle, and the location was marked on the scalp.

Participants rested their left hand on the force transducer and their right hand on the table surface. Active motor threshold (AMT) was defined as the minimum intensity for eliciting MEPs in 5 of 10 trials when the left FDI was preactivated with a 5% MVC squeeze on the force transducer. The test stimulus (TS) intensity was set to produce nonconditioned (NC) MEPs of ~1.5 mV at rest. The conditioning stimulus (CS) intensity was set to 90% AMT to produce near-maximal inhibition of the test responses at rest when preceding the TS by 2.5 ms. Baseline measures of resting corticocortical excitability and SICI were obtained before and immediately after task performance from 16 conditioned (C) and 16 NC MEPs at 1- and 2.5-ms ISIs.

Participants then performed simple force progressions (red-blue-green-yellow-white targets appeared in ascending and descending order) paced at a slow rate (1 Hz) to become familiar with the requirements of the task (visual display, cursor gain, auditory pacing) in pseudorandom order. The order of ISIs alternated between blocks, with the starting ISI (1 or 2.5 ms) counterbalanced across participants within each group.

Data Analysis

Behavioral performance. Acquisition was assessed from the accuracy of force peaks, calculated as the vertical difference between the participant’s peak force and the ideal force necessary to reach the target center. The Euclidean distance for the five targets was summed, resulting in a trial score, and the robust mean for each block was calculated (i.e., the mean was determined after outliers were discarded using least median squares), with smaller scores reflecting more accurate force production. “Online” performance was calculated as a percent difference between the starting score from block 1 and the mean score obtained in blocks 12–16. “Offline” performance was calculated as the percent difference between the mean score from blocks 12–16 during acquisition and the mean score from blocks 1–5 during retention. Contextual interference was calculated as the percent difference between the mean score from blocks 12–16 during acquisition and the mean score from blocks 1–5 in retention and was used to compare the cost of switching practice structures.

The frequency of sequence errors was also determined. Sequence errors are insensitive to force magnitude and timing but provide information as to whether the correct sequence structure was performed. If the target order was correct, performance was successful and a score of 1 was given; otherwise, the trial was scored 0. The percentage of sequence errors for each block was calculated as the number of incorrect sequences out of the total number of trials.

Neurophysiological measures. Peak-to-peak MEP amplitude was used as the primary measure of corticospinal excitability. Pretrigger root mean squared (rms) EMG activity was also determined for every TMS trial from a window 100 ms to 5 ms before the stimulus. The average rmsEMG of the rest data (pre and post) was calculated, with twice the average used as an individual’s pretrigger rmsEMG threshold. For the task blocks, all data above this threshold were discarded. If more than 50% of trials in a block were above threshold, the data from that block were discarded. Data were compressed into four temporally defined quartiles (Q1, blocks 1–4; Q2, blocks 5–8; Q3, blocks 9–12; and Q4, blocks 13–16), containing a possible 16 NC MEPs and 16 C MEPs each at 1 and 2.5 ms. SICI was calculated as %SICI = 100 – (%C/NC × 100).

Statistical Analysis

For force and sequence error measures at acquisition, 2 Group (interleaved, repetitive) × 16 Block repeated-measures analysis of variance (RM ANOVA) were performed. Post hoc analysis explored group differences at the beginning and end of the acquisition session. Force error was also analyzed at retention with 2 Group × 5 Block RM ANOVA. In the absence of block effects, mean retention was compared with the mean of the last 5 blocks of acquisition with 2 Group × 2 Session RM ANOVA. Two-tailed independent samples t-tests were used to compare online, offline, and contextual interference effects.

Rest NC MEP amplitudes were analyzed with 2 Group × 2 Time (pre, post) RM ANOVAs. Corticocortical excitability during acquisition was assessed with a 2 Group × 4 Time (Q1, Q2, Q3, Q4) RM ANOVA. Percent inhibition was analyzed at rest with 2 Group × 2 Time RM ANOVA and during task performance with 2 Group × 4 Time RM ANOVA separately for each ISI. We also tested for relationships between practice structure and disinhibition (∆SICI) using 2 Group × 2 Context (pre-rest, acquisition) RM ANOVAs. First, the maximum release of inhibition at any of the four quartiles was taken, indicating the greatest release of inhibition during task acquisition. Second, a release of inhibition was obtained in the first
quartile, indicating the release of inhibition in the early stage of task acquisition. Post hoc tests compared the magnitude of disinhibition across practice structures.

To determine if muscle quiescence was maintained throughout the TMS procedures, pretrigger rmsEMG was analyzed with the same RM ANOVAs as for rest, task performance, and both maximal and early change in inhibition.

An alpha level of 0.05 was adopted as the criterion for statistical significance. For all post hoc tests, the Holm-Bonferroni method was used for multiple comparisons correction and adjusted significance. For all post hoc tests, the Holm-Bonferroni method was used for multiple comparisons correction and adjusted significance. For all post hoc tests, the Holm-Bonferroni method was used for multiple comparisons correction and adjusted significance. For all post hoc tests, the Holm-Bonferroni method was used for multiple comparisons correction and adjusted significance. For all post hoc tests, the Holm-Bonferroni method was used for multiple comparisons correction and adjusted significance.

RESULTS

Behavioral Performance

Force accuracy during acquisition. There was a main effect of Block ($F_{1,22} = 22.2, \eta^2 = 0.50, P < 0.001$), with improved force accuracy over the acquisition session. A power function was fit to the force learning curves for both practice structures (Fig. 2A), with interleaved practice producing $R^2 = 0.93$ and repetitive practice producing $R^2 = 0.92$. There was a Group $\times$ Block interaction ($F_{1,22} = 3.0, \eta^2 = 0.12, P = 0.029$). Online effects were greater for participants performing interleaved practice (51% ± 4%) than for those performing repetitive practice (28% ± 4%) ($t = 4.01, d = 1.64, P < 0.001$). Post hoc analysis explored group differences at the beginning and end of the acquisition session. Force scores (arbitrary units) at the beginning of the acquisition session (mean of last 5 blocks), force scores for interleaved practice (0.16 ± 0.01) and repetitive practice (0.17 ± 0.01) did not differ ($t = 1.28, P = 0.45$).

Sequence errors during acquisition. Analysis of sequence errors showed a main effect of Block ($F_{1,22} = 15.6, \eta^2 = 0.43, P < 0.001$), with fewer sequence errors over the course of acquisition from block 1 (63% ± 4%) to block 16 (28% ± 3%). Reduction in sequence errors followed a similar power function as force improvements (Fig. 2B). Post hoc analysis confirmed sequence errors in block 1 of acquisition were more frequent for interleaved practice (73% ± 5%) than for repetitive practice (55% ± 5%) ($t = 2.40, P = 0.05$). At the end of the acquisition session (mean of last 5 blocks), the number of sequence errors produced by participants performing interleaved practice (23% ± 5%) did not differ from errors produced with repetitive practice (32% ± 5%) ($t = 1.42, P = 0.33$).

Retention and contextual interference. In the retention session, force performance was stable across the five blocks with no significant main effect of Group ($F_{1,22} = 2.4, \eta^2 = 0.09, P = 0.14$), Block ($F_{4,88} = 0.49, \eta^2 = 0.02, P = 0.74$), or their interaction ($F_{4,88} = 0.29, \eta^2 = 0.01, P = 0.88$). Mean retention performance (interleaved: 0.16 ± 0.01, repetitive: 0.19 ± 0.01; Fig. 2A) was compared with the mean performance in the last five blocks of acquisition. There was no main effect of Group ($F_{1,22} = 1.4, \eta^2 = 0.06, P = 0.25$), Session ($F_{1,22} = 3.6, \eta^2 = 0.14, P = 0.07$), or their interaction ($F_{1,22} = 0.98, \eta^2 = 0.04, P = 0.34$). Analysis of offline effects revealed a slight performance decrement that did not differ between groups (interleaved: 5% ± 5%, repetitive: 10% ± 5%; $t = 0.75, P = 0.46$). For participants who performed interleaved practice during task acquisition, the contextual interference effect was not different from the novel repetitive structure on retention (1% ± 5%, $n = 6$) compared with the familiar interleaved structure (10% ± 7%, $n = 6$) ($t = 0.98, P = 0.35$). Similarly, for participants who performed repetitive practice during task acquisition, the contextual interference effect was not different for the novel interleaved structure on retention (16% ± 9%, $n = 6$) compared with the familiar repetitive structure (5% ± 4%, $n = 6$) ($t = 1.12, P = 0.29$). The effect of switching practice structure from acquisition to retention (interleaved-repetitive vs. repetitive-interleaved) was not significant ($t = 1.45, P = 0.18$).

Neurophysiological Measures

There were no statistically significant differences between groups for AMT (38% ± 1% maximal stimulator output, MSO), CS (35% ± 1% MSO), and TS at rest (60% ± 2% MSO) (all $t < 1.14, P > 0.26$). The TS intensity was reduced slightly (56% ± 2% MSO) during the setup phase to counteract the expected increase in excitability relative to rest and was used during task acquisition blocks. For task MEP data, trials with pretrigger
Post hoc tests comparing the effect of time within groups revealed no main effect of Group (F1,22 = 3.24, F(1,22) = 1.45, P = 0.30; Time: F1,22 = 2.8, F(1,22) = 1.7, P = 0.17), or their interaction (F1,22 = 1.32, F(1,22) = 1.2, P = 0.17). Analysis of pretrigger rmsEMG (Fig. 3) revealed no main effect of Group (F1,22 = 0.39, F(1,22) = 1.7, P = 0.17), but there was a Group x Time interaction (F1,22 = 4.9, F(1,22) = 1.2, P = 0.04). MEP amplitude tended to increase over time for the repetitive group (Q1: 2.25 ± 0.35 mV; Q4: 2.54 ± 0.29 mV) and decrease for the interleaved group (Q1: 3.24 ± 0.37 mV; Q4 2.42 ± 0.27 mV). Post hoc tests comparing the effect of time within groups revealed that Q1 excitability was different from that at all other time points for the interleaved group (all t > 2.73, d > 0.74, P < 0.012) but not for the repetitive group (all t < 1.45, d < 0.24, P > 0.16). Analysis of pretrigger rmsEMG (Fig. 3) revealed no main effect of Group (F1,22 = 0.06, F(1,22) = 0.01, P = 0.12; Group x Time: F1,22 = 2.7, F(1,22) = 1.1, P = 0.11) or 2.5-ms ISI (Group: F1,22 = 0.11, F(1,22) = 0.11, P = 0.54; Time: F1,22 = 1.9, F(1,22) = 0.08, P = 0.18; Group x Time: F1,22 = 0.0, F(1,22) = 0.0; P = 0.95). However, the reduction of SICI from rest to task acquisition (Fig. 4) was of interest, particularly early in learning.

For maximal ΔSICI, there was a main effect of Context for 1-ms SICI (F1,22 = 104.6, F(1,22) = 83.8, P < 0.001) and 2.5-ms SICI (F1,22 = 200.8, F(1,22) = 80.0, P < 0.001). Post hoc tests revealed greater SICI at rest compared with acquisition for both 1 ms (rest: 8.34 ± 0.27%, acquisition: 48.02 ± 0.46%; t = 8.01, d = 1.86, P < 0.001) and 2.5 ms (rest: 77.45 ± 3.72%, acquisition: 41.73 ± 4.46%; t = 7.49, d = 1.77, P < 0.001), providing evidence of a release of inhibition during motor practice independent of ISI. There was an interaction between Group and Context for both 1 ms (F1,22 = 5.7, F(1,22) = 2.0, P = 0.026) and 2.5 ms (F1,22 = 12.7, F(1,22) = 0.37, P = 0.002). Repetitive practice produced a greater release of inhibition compared with interleaved practice for both 1-ms SICI (repetitive: −43.65 ± 5.18%, interleaved: −27.17 ± 4.60%; t = 2.38, d = 0.09, P = 0.026) and 2.5-ms SICI (repetitive: −48.71 ± 2.79%, interleaved: −26.72 ± 4.20%; t = 3.57, d = 1.45, P = 0.003). The greater disinhibition associated with repetitive practice is not simply due to increased excitability: NC MEPs were greater in the interleaved, not the repetitive, group.

To investigate whether SICI was reduced early in acquisition, ΔSICI was examined within the first quartile at both ISIs (Fig. 5, B and D). There was a main effect of Context at 1 ms (F1,22 = 1.56, F(1,22) = 0.37, P = 0.55) and 2.5 ms (F1,22 = 87.3, F(1,22) = 0.80, P < 0.001). Post hoc analyses revealed SICI was greater at rest compared with acquisition for both 1 ms (rest: 8.34 ± 0.27%, acquisition: 58.43 ± 4.66%; t = 5.23, d = 1.33, P < 0.001) and 2.5 ms (rest: 77.45 ± 3.72%, acquisition: 41.73 ± 4.46%; t = 7.49, d = 1.77, P < 0.001). There was an interaction between Group and Context for both inhibitory measures (1 ms: F1,22 = 8.9, F(1,22) = 0.29, P = 0.007; 2.5 ms: F1,22 = 9.9, F(1,22) = 0.31, P = 0.005). Repetitive practice showed a greater release of inhibition from rest to the early stage of acquisition compared with interleaved practice for both 1 ms (repetitive: −35.25 ± 6.20%, interleaved: −14.76 ± 2.96%; t = 2.98, d = 1.22, P = 0.007) and 2.5 ms (repetitive: −35.25 ± 6.20%, interleaved: −14.76 ± 2.96%; t = 2.98, d = 1.22, P = 0.007)}
The release of inhibition was therefore evident in the early stages of acquisition. For pretrigger rmsEMG, there was a main effect of Context relating to maximal disinhibition \((F_{1,22} = 23.4, \eta^2 = 0.51, P < 0.001)\) and early disinhibition analyses \((F_{1,22} = 24.9, \eta^2 = 0.53, P < 0.001)\). Post hoc analyses revealed a small but significant increase in rmsEMG during acquisition \((\text{maximal}: 8.62 \pm 0.23 \mu V, \text{early}: 8.81 \pm 0.29 \mu V)\) compared with rest \((\text{maximal}: 6.59 \pm 0.14 \mu V, \text{early}: 6.59 \pm 0.14 \mu V)\), as expected \((\text{both } P < 0.001)\). Importantly, there was no interaction between Group and Context for maximal \((F_{1,22} = 0.1, \eta^2 = 0.00, P = 0.79)\) or early disinhibition \((F_{1,22} = 0.4, \eta^2 = 0.02, P = 0.54)\), indicating the greater release of inhibition when performing repetitive practice was unlikely to be related to group differences in pretrigger rmsEMG levels.

DISCUSSION

The main findings of this study were that 1) repetitive practice led to similar levels of performance as interleaved practice by the end of acquisition, 2) excitability decreased with skill acquisition for interleaved practice and tended to increase for repetitive practice, and 3) repetitive practice elicited a greater release of inhibition within M1 during acquisition. Thus there was support for the hypothesis that repetitive task practice is capable of downregulating M1 GABAergic inhibition more so than variable practice structures. The findings may have relevance for rehabilitation after brain injury such as stroke, where the aim is to promote cortical reorganization and recovery of function via use-dependent plasticity.

Motor Learning and GABA-Mediated Disinhibition

Reduced GABAergic inhibition is associated with synaptic plasticity in animals (Castro-Alamancos et al. 1995; Hess et al. 1996; Hess and Donoghue 1994) and use-dependent plasticity in humans (Butefisch et al. 2000; Muellbacher et al. 2001; Rosenkranz et al. 2007). Human studies also indicate a relationship between GABA and motor learning through MRS (Floyer-Lea et al. 2006; Stagg et al. 2011a). However, MRS is unable to differentiate between synaptic and extrasynaptic GABA within human cortex, although extrasynaptic GABA is thought to dominate the signal (Stagg 2013). Stagg et al. (2011b) observed a correlation between MRS GABA concentration and SICI at 1-ms ISI from paired-pulse TMS. Of
interest, a similar correlation was not present between MRS GABA and SICI at 2.5-ms ISI. In this article we report complementary evidence. Repetitive practice may reflect a greater reduction of GABA within M1 compared with interleaved practice, but these effects could not dissociate extrasynaptic GABA presumed to relate to SICI at 1-ms ISI from presynaptic GABA receptor-mediated SICI at 2.5-ms ISI. Our results suggest that motor learning may be associated with disinhibition through a reduction in presynaptic GABA mechanism as well as extrasynaptic GABA.

**Motor Learning and Corticomotor Excitability**

Motor practice increases corticomotor excitability, promoting rapid expansion of functional movement representations in M1, and these effects are amplified in the context of learning (Classen et al. 1998; Muellbacher et al. 2001; Nudo et al. 1996; Pascual-Leone et al. 1995; Perez et al. 2004). In the present study, MEPs increased over time for the repetitive practice group and decreased over time for the interleaved practice group, with post hoc tests suggesting the latter was the main driver of this interaction. The increase over time for the repetitive group is consistent with previous research, but we are unaware of a precedent for the decrease observed in the interleaved group. Norepinephrine is known to increase the excitability of the corticomotor pathway without any effect on cortically mediated SICI (Plewia et al. 2001). We speculate that the highly uncertain environment during the initial blocks of interleaved practice increased participant arousal.

**Performance and Learning Across Practice Structures**

The present study shows that 45 min of either repetitive or interleaved practice in this task led to equivalent levels of performance, as demonstrated by similar force accuracy scores and sequence errors across groups by the end of the acquisition session. The complexity of interleaved practice initially caused more sequence errors and consequently less accurate force production in the initial blocks during acquisition. This was anticipated since an interleaved structure involves reparameterizing the target order for each individual trial, making the task more difficult to perform initially (Schmidt 1988). Interleaved practice led to greater online learning overall, but this is most likely a by-product of the relatively poorer initial performance. When tested 1 wk later, both groups showed similar retention scores, indicating that offline effects minimally affected learning.

Both error-based and use-dependent learning mechanisms contribute simultaneously to motor skill learning (Diedrichsen et al. 2010). Whereas the former is thought to depend critically on the cerebellum, the latter has been linked to changes in human M1 (Butefisch et al. 2000; Classen et al. 1998; Orban de Xivry et al. 2013). Here, repetitive practice was associated with a greater reduction of SICI within M1 than interleaved practice. Repeating a specific movement sequence may reinforce neural connections through a release of inhibition during initial skill acquisition. For example, learning by repetitive sequential force tracking results in decreased MRS GABA concentration within M1, whereas random force tracking without learning does not (Floyer-Lea et al. 2006). In our study, both interleaved and repetitive practice led to successful learning, but the latter was associated with a greater release of inhibition. It can be speculated that the repetitive nature of Floyer-Lea and colleagues’ (2006) task was critical to their demonstration of decreased GABA. Our study advocates that some degree of repetition in movement execution augments the release of intracortical inhibition within motor cortex.

**Practice Structure and Contextual Interference**

Interleaved practice leads to retention that is more resistant to changes in context than repetitive practice (Schmidt 1988; Shea and Morgan 1979). In the present study, contextual interference effects followed the expected pattern but were small in magnitude, intersubject variability was high, and statistical significance was not observed. One explanation for the lack of contextual interference effects is low power. Alternatively, it may relate to having experimentally controlled the temporal aspect of performance. Learning a serial reaction time task is reflected by improvements of both movement speed and accuracy (Agostino et al. 1996) and is subject to greater contextual interference for repetitive vs. interleaved schedules (Lin et al. 2010). The timing of movements is independently coded from the associated order of movements (Kornsheva et al. 2013). In our study the temporal aspect of performance was controlled by a metronome, removing the requirement to execute responses as fast as possible. External pacing allowed participants to focus on the order of the sequence, which may have been advantageous when they were required to perform the task with a novel interleaved schedule during retention.

**Potential Limitations**

There are potential limitations of the present study. First, it is unlikely that 1-ms SICI reflects solely extrasynaptic GABA, and it is currently debated as to what extent paired-pulse TMS can differentiate between extrasynaptic and synaptic GABA (Stagg et al. 2011b). This remains an intriguing hypothesis and a topic for further enquiry. Second, the analysis of MEPs in the context of motor task performance is challenging and subject to potential contamination from voluntary activation and hence modulation of neuronal excitability at subcortical levels. Although it is possible that the differential modulation of SICI from rest to acquisition can be explained by group differences in NC MEP amplitude, this seems unlikely for the following reasons: rmsEMG levels were maintained at a level below 10 μV during TMS; there was no interaction between Group and Context for pretrigger rmsEMG; subthreshold conditioning was used during SICI procedures; SICI has been shown to be insensitive to NC MEP amplitude in the range 1–4 mV (Ridding et al. 1995; Roshan et al. 2003; Wagle-Shukla et al. 2009); and excitability was greatest in the interleaved group. The argument that disinhibition simply reflects increases in excitability does not hold because it was the repetitive group that demonstrated the greatest release of inhibition. Any potential modulation of excitability at spinal levels was not investigated and cannot be known, although H-reflex and F-wave measures are unlikely to be definitive on this matter. Third, we recorded and optimized TMS for FDI, and conclusions about whether the results extend to other hand muscles therefore cannot be drawn. Fourth, the cross-over aspect of the design with respect to contextual interference is somewhat underpowered. However, this does not detract from the main SICI result obtained during acquisition.
Use of Repetitive Practice in the Context of Motor Rehabilitation

The present results may inform practice structures performed in the context of motor rehabilitation after brain injury such as stroke. Since both practice structures reached similar asymptotic levels of force performance at the end of the acquisition session that endured at retention, the repetitive structure appears to have two main advantages. First, participants are exposed to a simpler practice structure, showing superior performance when initially introduced to the task, thus avoiding the complexities and associated difficulties brought about by interleaved practice. Second, we have shown that disinhibition, a necessary precursor to the induction of plasticity, is greatest when learning a motor skill in a repetitive compared with an interleaved practice structure. Our results appear to support the use of repetitive practice for motor learning; however, it remains an open question as to how practice should be structured to obtain optimal results in a clinical setting.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.P.C., N.M.P., and W.D.B. conception and design of research; J.P.C. and N.M.P. performed experiments; J.P.C., N.M.P., and W.D.B. analyzed data; J.P.C., N.M.P., and W.D.B. interpreted results of experiments; J.P.C. and N.M.P. prepared figures; J.P.C., N.M.P., and W.D.B. drafted manuscript; J.P.C., N.M.P., and W.D.B. edited and revised manuscript; J.P.C., N.M.P., and W.D.B. approved final version of manuscript.

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