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Repetitive paired-pulse TMS increases motor cortex excitability and visuomotor skill acquisition in young and older adults

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Transcranial magnetic stimulation (TMS) over primary motor cortex (M1) recruits indirect (I) waves that can be modulated by repetitive paired-pulse TMS (rppTMS). The purpose of this study was to examine the effect of rppTMS on M1 excitability and visuomotor skill acquisition in young and older adults. A total of 37 healthy adults (22 young, 18–32 yr; 15 older, 60–79 yr) participated in a study that involved rppTMS at early (1.4 ms) and late (4.5 ms) interstimulus intervals (ISIs), followed by the performance of a visuomotor training task. M1 excitability was examined with motor-evoked potential (MEP) amplitudes and short-interval intracortical facilitation (SICF) using posterior–anterior (PA) and anterior–posterior (AP) TMS current directions. We found that rppTMS increased M1 excitability in young and old adults, with the greatest effects for PA TMS at the late ISI (4.5 ms). Motor skill acquisition was improved by rppTMS at an early (1.4 ms) but not late (4.5 ms) ISI in young and older adults. An additional study using a non-I-wave interval (3.5 ms) also showed increased M1 excitability and visuomotor skill acquisition. These findings show that rppTMS at both I-wave and non-I-wave intervals can alter M1 excitability and improve visuomotor skill acquisition in young and older adults.

Key words: Transcranial magnetic stimulation; Aging; Plasticity; Skill acquisition.

Introduction

The human brain and neuromuscular system allow performance of remarkable motor acts that involve highly skilled movements, which are usually honed through practice and learning. However, the ability to learn complex motor skills generally declines into older age (Voelcker-Rehage 2008), which can alter the performance of new motor tasks and influence our ability to live independently. One possible reason for this decline is an age-related change in the ability of neuronal synapses to modify their connections (Burke and Barnes 2006; Mahncke et al. 2006), a process referred to as neuroplasticity. In the short term (minutes to hours), this process involves strengthening of synapses through long-term potentiation (LTP), which is known to be important for learning and memory (Rioult-Pedotti et al. 2000). In the human motor system, noninvasive brain stimulation techniques such as transcranial magnetic stimulation (TMS) have been used to show a decline in short-term neuroplasticity in the primary motor cortex (M1) of older adults under some circumstances (Zimerman and Hummel 2010; Semmler et al. 2021), but the mechanisms that contribute to this decline, and how it impacts motor skill acquisition in older adults, are poorly understood.

Although the exact neural elements that are targeted by TMS remain unknown (see Siebner et al. 2022 for review), the response to a TMS pulse applied to M1 is characterized by a complex corticospinal descending volley that consists of a series of synchronized waves, known as indirect (I)-waves. These I-waves are separated from each other by about 1.4 ms and are numbered in order of their appearance (termed I1, I2, and I3), with the early (I1) and late (I2 and I3) waves thought to represent independent motor networks that have unique synaptic input pathways to corticospinal neurons (Di Lazzaro and Ziemann 2013; Ziemann 2020). Although TMS cannot activate separate interneuron networks, it is well known that single-pulse (SP) TMS with different directions of induced current produces a motor-evoked potential (MEP) in the target muscle that comprises different combinations of I-waves (Di Lazzaro et al. 2001; Di Lazzaro and Rothwell 2014). For example, TMS with a conventional current that flows in the posterior–anterior (PA) direction in the brain across the central sulcus preferentially activates inputs to corticospinal neurons that involve early (I1) waves (referred to as PA-sensitive circuits). In contrast, TMS with a current flowing in the anterior–posterior (AP) direction preferentially evokes less synchronized and delayed corticospinal inputs that involve later (I3) waves (referred to as AP-sensitive circuits). These early and late I-waves are known to be differentially sensitive to specific noninvasive brain stimulation protocols and have varying importance in different types of motor skill acquisition (Hamada et al. 2013; Hamada et al. 2014), suggesting that they represent an appropriate target to manipulate different motor behaviors.

Several previous studies have used different noninvasive brain stimulation interventions prior to a motor task to improve motor skill acquisition in young adults (see Müller-Dahlhaus and Ziemann 2015 for review), but the effects on older adults have been mixed. For example, motor skill acquisition can be improved in older adults when primed with transcranial direct current stimulation (Fujiyama et al. 2017), but it is impaired with paired associative stimulation (PAS; Opie et al. 2019), although there is greater motor skill retention after multisession PAS and training in older adults (Opie et al. 2020b). An alternative noninvasive brain stimulation technique that induces neuroplasticity by targeting I-waves is a repetitive paired-pulse TMS (rppTMS) paradigm at I-wave intervals. In its traditional form, rppTMS involves paired suprathreshold TMS at an early I-wave latency delivered repetitively for 15–30 min (Thickbroom et al. 2006). This I-wave intervention is known to produce a robust increase in MEP amplitude that lasts for up to 50 min (see Kidgell et al. 2016 for review) and can influence motor behavior in healthy young participants (Benwell et al. 2006; Teo et al. 2012). Furthermore, rppTMS at a late I-wave interval (4.3 ms) is effective at increasing the excitability of AP-sensitive interneuron circuits, while concurrently improving voluntary motor output, in healthy individuals and those with spinal cord injury (Long et al. 2017). This latter finding is particularly relevant for older adults, as there is an age-related change in late I-wave characteristics as assessed through short-interval intracortical facilitation (SICF; Opie et al. 2018), with this response predicting reduced manual performance in older adults (Opie et al. 2020a). However, it is unknown whether rppTMS at different interstimulus intervals (ISIs) can modulate I-wave circuits in older adults, and whether this can alter motor skill acquisition.

The purpose of this study was to examine the effect of rppTMS at different ISIs on M1 interneuron excitability and motor skill acquisition in young and older adults. In order to assess the effect of rppTMS on MEPs that preferentially involve either early or late I-waves, M1 interneuron excitability was assessed with both PA (targeting early I-wave circuits) and AP (targeting late I-wave circuits) coil orientations. We aimed to strengthen interneuron networks responsible for early and late I-waves using rppTMS at ISIs that coincide with early (1.4 ms) and late (4.5 ms) I-wave facilitation, to determine if this had any differential effect on M1 interneuron excitability and motor skill acquisition. In a separate study, we also examined the effect of rppTMS at an ISI that does not coincide with I-wave firing (3.5 ms ISI), to determine if the temporal precision involving I-wave facilitation was important for M1 plasticity and motor skill acquisition. We selected a motor task that involved visuomotor skill acquisition, as the improvement in performance of this model-based task is known to involve late I-waves (Hamada et al. 2014), and these are the interneuron circuits that are altered with advancing age (Opie et al. 2018). Our central hypothesis was that rppTMS will increase M1 interneuron excitability and improve visuomotor skill acquisition in young and old adults. Furthermore, we expected that rppTMS at a late ISI (4.5 ms) would result in a greater increase in excitability of AP-sensitive interneuron circuits, and would produce a greater effect on visuomotor skill acquisition compared with rppTMS at a short ISI (1.4 ms). Finally, we expected that the change in AP-sensitive interneuron circuits and the improvement in visuomotor skill acquisition after rppTMS would be reduced in older adults, due to altered excitability of late I-waves (Opie et al. 2018) and reduced physiological plasticity in the aging brain (Zimerman and Hummel 2010; Freitas et al. 2013).

Materials and methods

A total of 59 healthy adults were recruited from the university and wider community to participate in this study. After exclusions and withdrawals (see Results), 22 young (mean ± SD; 24.2 ± 3.8 years [range 18–32], 10 female) and 15 older adults (mean ± SD; 67.9 ± 5.4 years, [range 60–79], 9 female) contributed data to the study. These participants reported no diagnosed neurological disease, no history of concussion, and no ongoing use of psychoactive medication (antidepressants, sedatives, etc.). All experimentation was approved by the University of Adelaide Human Research Ethics Committee and conducted according to the Declaration of Helsinki. Each participant provided written, informed consent prior to inclusion in the study.

Experimental arrangement and procedures

Each participant attended 1 to 4 experimental sessions, with multiple sessions separated by at least 1 wk and performed at the same time of day. Each session involved measures of TMS and motor skill training, but applied a different TMS intervention. The interventions were performed in random order and consisted of rppTMS at 1.4 ms (rppTMS$_{1.4}$), rppTMS at 4.5 ms (rppTMS$_{4.5}$), and an SP control session (Fig. 1), with an additional session at 3.5 ms (rppTMS$_{3.5}$) performed in some participants (see Study 2 below). For all sessions, subjects were seated in a comfortable chair, with the right hand on a benchtop oriented to apply force to a transducer (MLP-25; Transducer Techniques, United States of America) in a pinch grip position. Surface electromyography (EMG) was recorded from the first dorsal interosseous muscle of the right hand using 2 Ag-AgCl electrodes in a belly-tendon montage and a strap around the wrist as a ground electrode. EMG signals were amplified (300×) and band-pass filtered (20 Hz high pass, 1 kHz low pass) using a CED1902 signal conditioner (Cambridge Electronic Design, Cambridge, United Kingdom) and digitized at 2 kHz using a CED1401 interface (Cambridge Electronic Design). To maintain consistency between sessions, all measures within the experiment were conducted by the same experimenter, with data stored on a computer for offline analysis.

Transcranial magnetic stimulation

In all sessions, TMS was applied to the left M1 using a branding iron figure-of-eight coil connected to 2 Magstim 200^2 magnetic stimulators through a Bistim module (Magstim, Dyfed, United Kingdom). TMS was applied with PA and AP orientations. PA orientation involved an anterior-medially directed current within M1, where the coil was held with an alignment of ∼5 deg to the midline and tangentially to the scalp over the optimal location for producing MEPs in the target muscle. This location was marked on the scalp for reference and checked throughout the experiment. AP orientation was performed by rotating the coil 180º, to achieve a posterior-laterally directed cortical current. In line with previous studies (Hamada et al. 2013; D'Ostilio et al. 2016; Long et al. 2017), the same hotspot was used for PA and AP TMS as it has been shown previously that the direction of current does not significantly influence the hotspot location (Sakai et al. 1997; Arai et al. 2005). For both orientations, TMS was delivered at 0.2 Hz with a 10% variation between trials to avoid anticipation of the stimulus.

At baseline, resting motor threshold (RMT) in PA and AP orientations was measured and defined as the minimum stimulus intensity required to produce an MEP with an amplitude of ≥50 μV in at least 5 out of 10 consecutive trials in the relaxed first dorsal interosseous muscle. Subsequently, a test TMS intensity
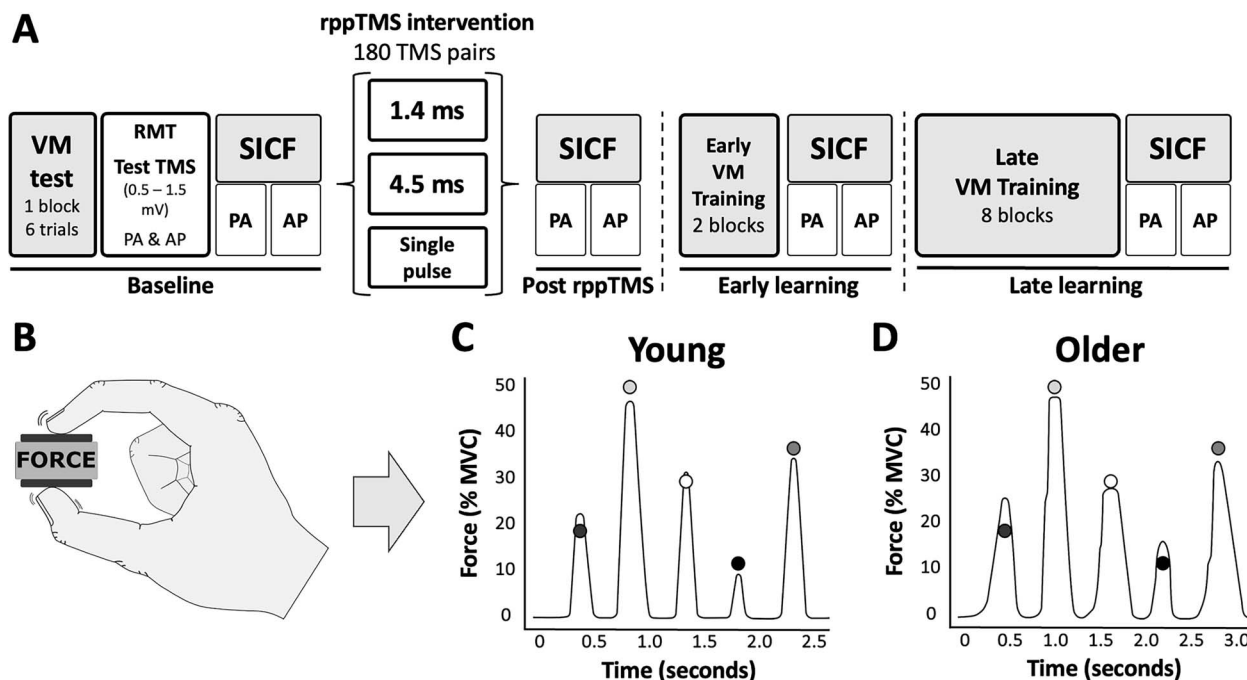
Fig. 1. Experimental protocol and visuomotor task. Timeline of events during each experimental session (A). Representative hand position during visuomotor training (B). Raw force data from a single visuomotor task trial in a young (C) and older (D) participant in a late learning trial, with the target zones depicted by circles (C). Abbreviations: VM, visuomotor; RMT, resting motor threshold; TMS, transcranial magnetic stimulation; PA, posterior–anterior coil orientation; AP, anterior–posterior coil orientation; SICF, short-interval intracortical facilitation; rppTMS, repetitive paired-pulse TMS.

was measured as the stimulus intensity required to produce a peak-to-peak MEP amplitude of ∼1 mV (range, 0.5–1.5 mV) when averaged over 15 trials. Paired-pulse TMS was used to assess SICF, which involved a subthreshold conditioning stimulus of 90% RMT, applied either 1.4 ms ($SICF_{1.4}$) or 4.5 ms ($SICF_{4.5}$) following a preceding test TMS pulse (Ortu et al. 2008). At baseline, a paired-pulse test block involving 15 trials for each condition (test TMS, $SICF_{1.4}$ and $SICF_{4.5}$ [45 trials total]) was completed for PA and AP orientations separately. To assess rppTMS- and motor training-induced changes in MEP and SICF, these testing blocks were repeated after the rppTMS intervention, and again after both early (2 training blocks) and late training (8 training blocks; Fig. 1A). Early and late training was assessed in this manner as there is evidence of a differential modulation of PA and AP circuits at these time points (Spampinato et al. 2020).

Study 1: rppTMS at I-wave intervals

Plasticity within M1 circuits was induced using rppTMS, consisting of 180 pairs of stimuli applied with a PA coil orientation every 5 s for 15 min. The intensity of TMS was equal for each stimulus in the pair (conditioning and test TMS) and was set to produce a paired-pulse MEP of 0.5–1.0 mV prior to commencing rppTMS. Two sessions involved rppTMS interventions where the ISI was designed to target either early (1.4 ms ISI; $rppTMS_{1.4}$) or late I-waves (4.5 ms ISI; $rppTMS_{4.5}$), whereas the third session involved SP TMS (SP session). These sessions were initially delivered in random order, but some participants did not complete all sessions, so additional participants were recruited for specific sessions (see Results). Ice packs were placed on the coil when necessary to mitigate excessive coil heating during rppTMS.

Visuomotor skill task

Following rppTMS, a target-based visuomotor task was used to assess motor skill acquisition. The task used was a modified

version of a sequential visuomotor isometric pinch task that is known to increase M1 excitability and alter intracortical inhibition (Coxon et al. 2014), which are considered key mechanisms of use-dependent plasticity in M1 (Liepert et al. 1998; Bütefisch et al. 2000). For this task, the participant grasped a force transducer between the index finger and thumb in a pinch grip. Prior to commencing the task, maximal voluntary contraction force was assessed through 3 separate attempts, separated by 30 s, with the highest recorded force used for the task parameters. The participants were instructed not to pinch the transducer ballistically, but to progressively apply pressure until a maximal level was reached. For the visuomotor task, a computer screen displayed a target scale with 5 separate color zones aligned vertically (Fig. 1B). Participants were required to control the movement of a cursor to reach all 5 color target zones in a particular order (consistent within each session) while returning to baseline between each target. This was achieved by modulating isometric force by pinching the transducer as quickly and accurately as possible. The baseline position was set at 0% of maximum force and the highest target was set at 45% of maximum force, with an example force trace from a single trial shown in Fig. 1C. The color targets would disappear at the end of each trial (once the participant had attempted to move the cursor to each of the 5 targets), and would reappear ∼3 s later for the start of the next trial. To increase the difficulty of the task and avoid crossover between sessions, 3 separate nonlinear force transforms were utilized for each session (exponential for $rppTMS_{1.4}$, logarithmic for $rppTMS_{4.5}$ and sigmoidal for SP). Additionally, the color order displayed on the target scale varied between sessions. At baseline, visuomotor skill was assessed with a single block of 6 trials (Fig. 1A). Subsequently, visuomotor training was split into early training (2 blocks of 12 trials) and late training (8 blocks of 12 trials). To encourage continual improvements during training, participants were provided feedback on performance after each block.

Study 2: rppTMS at non-I-wave intervals

In order to examine the effect of non-I-wave periodicity rppTMS on M1 excitability and visuomotor skill acquisition, we included an additional experiment that involved rppTMS at 3.5 ms (rppTMS$_{3.5}$), which is a paired pulse ISI that is outside of the facilitatory peaks in the SICF curve (Long et al. 2017). Using a protocol similar to Study 1 (see Fig. 1), the effect of rppTMS$_{3.5}$ on M1 excitability (PA and AP MEPs) and visuomotor skill acquisition (sigmoidal force transform) was examined in 14 young (mean ± SD; 24.6 ± 3.5 years [range 18–32], 9 female) and 7 older adults (mean ± SD; 66.9 ± 3.7 years, [range 63–73], 4 female) who also participated in the main study.

Data analysis

For all TMS data, MEPs were measured peak-to-peak for each trial and expressed in mV. Trials containing EMG activity >10 μV (peak-to-peak amplitude) in the 100 ms before TMS application were discarded from the analysis, resulting in the removal of less than 3% of all trials. To assess the influence of SICF on MEP amplitude, individual MEPs recorded in response to paired-pulse TMS were expressed as a percentage of the mean test TMS amplitude recorded at baseline. Accordingly, normalized values greater than 100% reflect facilitation, whereas normalized values less than 100% reflect inhibition. To identify rppTMS-induced changes in M1 excitability and SICF, normalized MEPs from single- and paired-pulse TMS blocks recorded after rppTMS were expressed as a percentage of the mean values from baseline.

To investigate the effect of visuomotor skill training on motor performance, skill scores from each training block (10 blocks total) were normalized to the mean skill scores from baseline. Skill scores were calculated for each visuomotor block of 12 trials using the speed and accuracy components of the movement. Trials were removed from the analysis if they were completed incorrectly, such as if the force trace did not return to zero between targets. Speed was measured as the mean movement time for each trial, while accuracy was calculated using the mean error values of each trial, which were quantified by the average Euclidean distance between the true force output and the force output required to reach the center of the target (Stavrinos and Coxon 2017). For assessment of visuomotor performance, skill scores were calculated using the following formula (Reis et al. 2009).

$$Skill = \frac{(1 - error)}{error \left(\ln \left(movement \; time \right)^b \right)}$$

The dimensionless free b parameter was set at 1.627, as this has been shown to be insensitive to changes in performance (Stavrinos and Coxon 2017). To assess motor skill acquisition, skill scores from all training blocks were normalized to the average skill score from the baseline block. Finally, to investigate training-induced changes in MEP amplitude and SICF, the amplitude of post-training single- and paired-pulse MEPs were expressed as a percentage of the mean values from the post-rppTMS time point.

Statistical analysis

Visual inspection of the data residuals were implemented for all data. Gaussian distributions were only demonstrated for baseline TMS intensities, expressed as the percentage of maximum stimulator output (% MSO), for measures of RMT and test MEP. Accordingly, comparisons of baseline TMS intensities were compared between groups and sessions using linear mixed model analyses.

All other TMS and motor training data demonstrated a significant positive skew. Therefore, generalized linear mixed model (GLMM) analyses, which account for non-Gaussian distributions of data residuals (Lo and Andrews 2015), were used for all other analyses.

Baseline test MEP amplitude and SICF were compared between AGE groups (young, older) and rppTMS sessions (rppTMS$_{1.4}$, rppTMS$_{4.5}$ and SP) using separate 2-factor GLMM analyses. To investigate the change in corticospinal excitability and SICF after rppTMS, single- and paired-pulse MEPs recorded at the post-rppTMS time point (normalized to baseline) were compared between AGE groups (young, older) and rppTMS sessions (rppTMS$_{1.4}$, rppTMS$_{4.5}$ and SP) using separate 2-factor GLMM analyses. To assess changes in visuomotor skill with training, normalized skill measures from motor training were compared between AGE groups, rppTMS sessions and training blocks (10 blocks total; Block factor) using a GLMM with repeated measures for Block (GLMM$_{RM}$). To investigate use-dependent changes in MEP amplitude and SICF, TMS measures recorded after visuomotor training (normalized to post-rppTMS) were compared between AGE groups, rppTMS sessions, and TIME (early training, late training) using a 3-factor GLMM$_{RM}$.

Spearman's correlation was used to investigate the relationship between rppTMS-induced changes in TMS measures (MEPs and SICF) and changes in visuomotor skill (final 2 blocks of motor training normalized to baseline), as well as between use-dependent changes in TMS measures and changes in skill with motor training. All Spearman's correlation tests were completed separately for each rppTMS session and AGE group, as well as with AGE groups combined. Benjamini–Hochberg adjustment for multiple comparisons was implemented for Spearman's correlation tests to control the false discovery rate.

To examine the effect of non-I-wave periodicity TMS (Study 2 involving rppTMS$_{3.5}$), the change in MEP amplitude following rppTMS$_{3.5}$ was compared with data from the SP session, where SP MEPs recorded at the post-rppTMS time point (normalized to baseline) were compared between AGE groups (young, older) and rppTMS sessions (rppTMS$_{3.5}$ and SP) using separate 2-factor GLMM analyses. To assess changes in visuomotor skill with training, normalized skill measures from motor training were compared between AGE groups, rppTMS sessions and training blocks (10 blocks total; Block factor) using a 3-factor GLMM$_{RM}$.

All tests were completed separately for data collected with PA and AP TMS orientations. For all GLMM and GLMM$_{RM}$ analyses, gamma distributions were fitted based on the pattern of data residuals. For each analysis, the model was optimized by testing 3 separate covariate structures (AR(1): Heterogeneous, Diagonal and Scaled Identity), with the best model fit (based on Bayesian Information Criterion) utilized for each test. An identity link was utilized for or all baseline (non-normalized) data, whereas a log link was implemented for post-rppTMS and training data (Lo and Andrews 2015). All statistical models were completed separately for all models, subject was included as a random effect, and significant main effects and interactions were further investigated using custom contrasts with Bonferroni correction. For all significant effects and interactions, the estimated mean difference (EMD) and associated 95% confidence interval from post hoc pairwise comparisons are reported in the text as EMD (lower 95% CI, upper 95% CI), which provides a non-standardized measure of effect size. Tables and figures display mean values of all participants included in the corresponding analysis. Data are displayed as estimated marginal mean ± 95% confidence interval unless stated otherwise.

Table 1. Baseline TMS data in young and older adults.

		Young			Older		
		$rppTMS_{1.4}$	$rppTMS_{4.5}$	SP	$rppTMS_{1.4}$	$rppTMS_{4.5}$	SP
PA TMS	Participants (n)	18	18	18	15	15	14
	RMT (%MSO)	48.5	47.3	47.5	48.8	47.7	47.7
		(45.1, 51.9)	(43.8, 50.7)	(44.2, 50.9)	(44.8, 52.7)	(43.8, 51.7)	(43.7, 51.6)
	Test TMS intensity (% MSO)	57.5	56.5	58.4	62.3	61.3	61.0
		(52.9, 62.2)	(51.9, 61.2)	(53.9, 62.8)	(57.1, 67.5)	(56.1, 66.5)	(55.8, 66.3)
	Test MEP amplitude (mV)	0.9	0.7	0.8	0.9	0.6	0.8
		(0.8, 1.0)	(0.6, 0.9)	(0.7, 1.0)	(0.7, 1.0)	(0.5, 0.8)	(0.6, 1.0)
	$SICF_{1.4}$ (% test)	238.1	262.4	187.1	217.7	297.4	242.2
		(178.8, 297.5)	(202.9, 322.1)	(129.0, 245.3)	(151.8, 283.6)	(230.2, 364.7)	(174.2, 310.2)
	$SICF_{4.5}$ (%test)	119.7	145.5	111.8	126.1	133.3	125.9
		(98.6, 140.7)	(123.4, 167.6)	(91.3, 132.4)	(102.6, 149.7)	(109.3, 157.4)	(101.6, 150.3)
AP TMS	Participants (n)	18	18	18	15	15	13
	RMT (%MSO)	60.7	59.0	63.2	63.4	62.3	63.6
		(56.1,65.3)	(54.5, 63.6)	(58.8, 67.6)	(58.2, 68.6)	(57.1, 67.6)	(58.3, 68.8)
	Test TMS intensity (% MSO)	73.6	73.5	77.6	79.3	79.6	80.0
		(68.1, 79.2)	(67.8, 79.1)	(72.1, 83.0)	(72.8, 85.7)	(73.2, 86.0)	(73.6, 86.5)
	Test MEP amplitude (mV)	0.9	0.9	*0.9	0.6	0.6	0.7
		(0.7, 1.0)	(0.7, 1.0)	(0.8, 1.1)	(0.5, 0.8)	(0.5, 0.7)	(0.6, 0.9)
	$SICF_{1.4}$ (% test)	237.3	196.6	152.1	210.8	219.8	171.0
		(193.3, 281.3)	(153.8, 239.5)	(110.6, 193.7)	(162.7, 258.8)	(171.5, 268.0)	(121.3, 220.8)
	$SICF_{4.5}$ (%test)	128.1	112.6	109.7	112.0	119.4	101.5
		(109.9, 146.3)	(95.4, 129.8)	(92.8, 126.6)	(93.3, 130.7)	(100.3, 138.6)	(82.3, 120.8)

Data represent mean (lower 95% CI, upper 95%CI). ∗ $P = 0.005$ compared with older group. Abbreviations: rppTMS, repetitive paired-pulse TMS; RMT, resting motor threshold; MEP, motor-evoked potential; MSO, maximum stimulator output; SICF, short-interval intracortical facilitation; PA, posterior–anterior; AP, anterior–posterior.

Results

From a total of 59 participants, 15 were excluded because AP MEPs were less than 0.5 mV with TMS at 100% MSO (7 older adults), 4 withdrew due to the time commitment involved (1 older adult), and 3 participants withdrew due to the discomfort with high-intensity AP TMS (3 older adults). Therefore, data were collected from 22 young and 15 older participants for the main experiment ($rppTMS_{1.4}$, $rppTMS_{4.5}$, SP sessions). For the young adults, 14 participated in all 3 sessions, 4 participated in both rppTMS sessions, and 4 participated in the SP control session. For the older adults, 14 participated in all sessions and 1 participated in both rppTMS sessions. Some participants were not able to contribute to all sessions due to disruptions related to COVID-19, with participant numbers for each condition provided in Table 1. In addition, 14 young and 7 older adults who participated in the main experiment also participated in an extra session involving $rppTMS_{3.5}$.

Study 1: I wave periodicity rppTMS ($rppTMS_{1.4}$, $rppTMS_{4.5}$, SP)
Baseline comparisons

There were no differences in baseline visuomotor skill between AGE groups (young, older) ($F_{1, 92} = 0.001$, $P = 0.9$); however skill scores varied between rppTMS sessions ($F_{2, 92} = 4.4$, $P = 0.014$). Post hoc tests revealed baseline skill scores were significantly greater for the $rppTMS_{4.5}$ session compared with both $rppTMS_{1.4}$ (EMD = 1.0 [0.1, 1.9], $P = 0.02$) and SP (EMD = 1.0 [0.1, 1.8], $P = 0.02$), but no difference between $rppTMS_{1.4}$ and SP ($P = 0.8$). Baseline skill scores for young adults were 2.7 (95% CI: 1.9, 3.4) for $rppTMS_{1.4}$, 3.4 (2.4, 4.3) for $rppTMS_{4.5}$ and 2.3 (1.6, 2.9) for the SP session. For older adults, baseline skill scores were 2.1 (1.4, 2.8) for $rppTMS_{1.4}$, 3.5 (2.5, 4.5) for $rppTMS_{4.5}$ and 2.7 (1.8, 3.5) for the SP session.

Group and session averages for baseline TMS data are displayed in Table 1, with test statistics and associated *P*-values for comparisons of these data are shown in Table 2. The only significant effect of AGE occurred for test MEP amplitude with an AP orientation, where baseline MEP amplitude was significantly greater in young participants (EMD = 0.2 mV [0.1, 0.4], $P = 0.005$).

For PA TMS, significant main effects between rppTMS sessions were observed for test MEP amplitude ($P = 0.002$) and $SICF_{1.4}$ ($P = 0.02$). Post hoc analysis showed that mean MEP amplitude was greater in the $rppTMS_{1.4}$ session compared with $rppTMS_{4.5}$ (EMD = 0.2 mV [0.1, 0.4], $P = 0.002$) and there was greater $SICF_{1.4}$ in the $rppTMS_{4.5}$ session compared with SP (EMD = 65% [6.7, 124], $P < 0.001$). For AP TMS, significant main effects between rppTMS sessions were observed for RMT ($P = 0.03$) and $SICF_{1.4}$ ($P = 0.002$). Post hoc analysis showed that RMT was greater in the SP session compared with $rppTMS_{4.5}$ (EMD = 2.7% [0.2, 5.2], $P < 0.05$), and $SICF_{1.4}$ in the SP session was reduced compared with both $rppTMS_{1.4}$ (EMD = −62% [−106, −19], $P = 0.002$) and $rppTMS_{4.5}$ (EMD = −47% [−87, −6.0], $P = 0.02$). As shown in Table 2, there were no significant interactions between AGE group and rppTMS session at baseline.

Effect of rppTMS on MEPs and SICF

Test statistics and associated *P*-values for comparisons of TMS data recorded following rppTMS (normalized to baseline) and visuomotor skill training (normalized to post-rppTMS) are shown in Table 3. MEP and SICF data (normalized to baseline) recorded following the application of rppTMS for each session and TMS coil orientation are shown in Fig. 2. For PA MEPs, there was a significant main effect of rppTMS session ($P < 0.001$). Post hoc analysis revealed that MEP amplitude following $rppTMS_{4.5}$ was increased compared with both $rppTMS_{1.4}$ (EMD = 33% [13, 54], $P < 0.001$) and

Table 2. Statistical results of baseline TMS data using GLMM analyses.

	Variable	Model term	df	F	P
PA TMS	**RMT (%MSO)**	AGE	1, 35	0.02	0.9
		rppTMS	2, 36	1.5	0.2
		AGE x rppTMS	2, 36	0.02	0.9
	Test TMS intensity (% MSO)	AGE	1, 35	1.7	0.2
		rppTMS	2, 38	0.4	0.7
		AGE x rppTMS	2, 38	0.4	0.6
	Test MEP amplitude (mV)	AGE	1, 1143	0.7	0.4
		rppTMS	2, 1143	**6.1**	**0.002**
		AGE x rppTMS	2, 1143	0.3	0.8
	SICF$_{1.4}$ (% test)	AGE	1, 1139	0.4	0.5
		rppTMS	2, 1139	**4.1**	**0.02**
		AGE x rppTMS	2, 1139	1.3	0.3
	SICF$_{4.5}$ (%test)	AGE	1, 1142	0.05	0.9
		rppTMS	2, 1142	2.7	0.07
		AGE x rppTMS	2, 1142	1.0	0.4
AP TMS	**RMT (%MSO)**	AGE	1, 35	0.4	0.5
		rppTMS	2, 39	**3.7**	**0.03**
		AGE x rppTMS	2, 39	1.2	0.3
	Test TMS intensity (% MSO)	AGE	1, 35	1.4	0.2
		rppTMS	2, 32	2.1	0.1
		AGE x rppTMS	2, 32	1.1	0.4
	Test MEP amplitude (mV)	AGE	1, 1110	**7.8**	**0.005**
		rppTMS	2, 1110	1.7	0.2
		AGE x rppTMS	2, 1110	0.05	0.9
	SICF$_{1.4}$ (% test)	AGE	1, 1117	0.04	0.8
		rppTMS	2, 1117	**6.3**	**0.002**
		AGE x rppTMS	2, 1117	1.2	0.3
	SICF$_{4.5}$ (%test)	AGE	1, 1117	0.4	0.5
		rppTMS	2, 1117	1.7	0.2
		AGE x rppTMS	2, 1117	1.1	0.3

Bold indicates statistical significance ($P < 0.05$). Abbreviations: rppTMS, repetitive paired-pulse TMS; RMT, resting motor threshold; MEP, motor-evoked potential; MSO, maximum stimulator output; SICF, short-interval intracortical facilitation; PA, posterior–anterior; AP, anterior–posterior.

SP (EMD = 56% [34, 78], $P < 0.001$; Fig. 2A). Furthermore, normalized MEPs were significantly greater for the rppTMS$_{1.4}$ session compared with SP (EMD = 23% [8.2, 37], $P = 0.002$). There were no significant main effects for AP MEPs (both $P > 0.05$); however a significant interaction between AGE group and rppTMS session was present ($P < 0.001$). Post hoc analysis revealed significantly increased MEP amplitude for the young group in the rppTMS$_{1.4}$ session compared with both rppTMS$_{4.5}$ (EMD = 26% [1.5, 50], $P = 0.04$) and SP (EMD = 36% [9.0, 64], $P = 0.004$). For the older group, normalized MEPs were greater in the rppTMS$_{4.5}$ session compared with rppTMS$_{1.4}$ (EMD = 30% [0.5, 60], $P < 0.05$); however there was no difference between SP and either rppTMS$_{1.4}$ or rppTMS$_{4.5}$ (both $P > 0.1$).

For PA SICF$_{1.4}$, analysis revealed a significant main effect of rppTMS session ($P < 0.001$). Post hoc analysis revealed that SICF following rppTMS$_{1.4}$ (EMD = 21% [10, 32], $P < 0.001$) and rppTMS$_{4.5}$ (EMD = 19% [9, 28], $P < 0.001$; Fig. 2C) was increased compared with SP, but there was no difference between rppTMS$_{1.4}$ and rppTMS$_{4.5}$ ($P = 0.6$). A significant interaction between AGE group and rppTMS session was also present ($P = 0.02$). Post hoc analysis revealed significantly increased SICF$_{1.4}$ for the young group in the rppTMS$_{4.5}$ session compared with SP (EMD = 21% [4.5, 37], $P = 0.007$), but no difference between rppTMS$_{1.4}$ and rppTMS$_{4.5}$ or SP (both $P > 0.1$). For the older AGE group, normalized SICF$_{1.4}$ was greater for rppTMS$_{1.4}$ compared with both rppTMS$_{4.5}$ (EMD = 14% [3.4, 25], $P = 0.01$) and SP (EMD = 31% [15, 47], $P < 0.001$). Furthermore, values were greater for rppTMS$_{4.5}$ compared with SP (EMD = 17% [4.4, 29], $P = 0.005$). For AP SICF$_{1.4}$, analysis revealed a significant main effect of rppTMS sessions ($P = 0.04$); however no

pairwise comparisons were statistically significant (all $P > 0.07$). A significant interaction between AGE group and rppTMS session was also present ($P = 0.01$). Post hoc comparisons indicate significantly reduced SICF$_{1.4}$ for the young group in the SP session compared with both rppTMS$_{1.4}$ (EMD = −18% [−32, −4.4], $P = 0.006$) and rppTMS$_{4.5}$ (EMD = −24% [−41, −6.8], $P = 0.002$), but no difference between rppTMS$_{1.4}$ and rppTMS$_{4.5}$, or between any rppTMS sessions for the older AGE group (all $P > 0.1$). Furthermore, normalized SICF$_{1.4}$ was greater for the older compared with the young AGE group (EMD = 35% [8, 61], $P = 0.01$).

For PA SICF$_{4.5}$, values differed between rppTMS sessions ($P < 0.001$). Post hoc analysis revealed that SICF following rppTMS$_{1.4}$ (EMD = 19% [5.8, 32], $P = 0.002$) and rppTMS$_{4.5}$ (EMD = 33% [18, 49], $P < 0.001$; Fig. 2E) was increased compared with SP. Furthermore, values were greater in the rppTMS$_{4.5}$ session compared with rppTMS$_{1.4}$ (EMD = 15% [0.8, 28], $P = 0.04$). For AP SICF$_{4.5}$, there was no difference between AGE groups or rppTMS sessions (both $P > 0.1$); however, a significant interaction between factors was present ($P < 0.001$), with post hoc comparisons revealing reduced SICF$_{4.5}$ in the young group for the SP session compared with both rppTMS$_{1.4}$ (EMD = −26% [−46, −5.9], $P = 0.008$) and rppTMS$_{4.5}$ (EMD = −31% [−55, −7.3], $P = 0.005$.), but no difference between rppTMS$_{1.4}$ and rppTMS$_{4.5}$ (EMD = −5.0% [−26, 16], $P = 0.6$) and no rppTMS session differences for the older group (all $P > 0.4$). Furthermore, AP SICF$_{4.5}$ was significantly greater for the older compared with the young group for the SP session (EMD = 49% [15, 83], $P = 0.005$), but there were no significant differences between groups for the rppTMS$_{1.4}$ or rppTMS$_{4.5}$ sessions (both $P > 0.6$).

Table 3. Statistical results of TMS measures recorded following rppTMS and visuomotor skill training in study 1 using GLMM analyses.

		Variable	Model term	df	F	P
rppTMS effect	PA TMS	Test MEP amplitude (mV)	AGE	1, 1135	0.02	0.9
			rppTMS	2, 1135	**23.6**	**< 0.001**
			AGE x rppTMS	2, 1135	0.6	0.6
		SICF$_{1.4}$ (% test)	AGE	1, 1144	0.04	0.9
			rppTMS	2, 1144	**13.3**	**< 0.001**
			AGE x rppTMS	2, 1144	**4.0**	**0.02**
		SICF$_{4.5}$ (%test)	AGE	1, 1138	0.5	0.5
			rppTMS	2, 1138	**17.0**	**< 0.001**
			AGE x rppTMS	2, 1138	1.5	0.2
	AP TMS	Test MEP amplitude (mV)	AGE	1, 1111	0.5	0.5
			rppTMS	2, 1111	2.5	0.08
			AGE x rppTMS	2, 1111	**6.8**	**< 0.001**
		SICF$_{1.4}$ (% test)	AGE	1, 1113	2.5	0.1
			rppTMS	2, 1113	**3.3**	**0.04**
			AGE x rppTMS	2, 1113	**4.4**	**0.01**
		SICF$_{4.5}$ (%test)	AGE	1, 1117	1.9	0.2
			rppTMS	2, 1117	1.3	0.3
			AGE x rppTMS	2, 1117	**7.1**	**< 0.001**
Skill training effect	PA TMS	Test MEP amplitude (mV)	AGE	1, 2294	0.7	0.8
			rppTMS	2, 2294	1.3	0.3
			AGE x rppTMS	2, 2294	**5.8**	**0.003**
		SICF$_{1.4}$ (% test)	AGE	1, 2297	0.3	0.6
			rppTMS	2, 2297	2.9	0.06
			AGE x rppTMS	2, 2297	0.9	0.4
		SICF$_{4.5}$ (%test)	AGE	1, 2285	0.2	0.6
			rppTMS	2, 2285	1.7	0.2
			AGE x rppTMS	**2, 2285**	**9.3**	**< 0.001**
	AP TMS	Test MEP amplitude (mV)	AGE	1, 2224	0.6	0.4
			rppTMS	2, 2224	**8.3**	**< 0.001**
			AGE x rppTMS	2, 2224	**19.1**	**< 0.001**
		SICF$_{1.4}$ (% test)	AGE	1, 2217	0.7	0.4
			rppTMS	2, 2217	**6.8**	**< 0.001**
			AGE x rppTMS	2, 2217	**5.0**	**0.007**
		SICF$_{4.5}$ (%test)	AGE	1, 2219	0.4	0.5
			rppTMS	2, 2219	**5.7**	**0.003**
			AGE x rppTMS	2, 2219	**13.0**	**< 0.001**

Bold indicates statistical significance ($P < 0.05$). Abbreviations: rppTMS, I-wave periodicity repetitive TMS; RMT, resting motor threshold; MEP, motor-evoked potential; MSO, maximum stimulator output; SICF, short-interval intracortical facilitation; PA, posterior–anterior; AP, anterior–posterior

Visuomotor skill training

A small number (<5%) of visuomotor skill trials were removed from the analysis as they were not completed correctly (e.g. if the force trace did not return to zero between targets), with no difference in the proportion of trials removed between age groups of sessions (all $P > 0.1$). Skill scores recorded during motor training were not different between AGE groups ($F_{1, 918} = 1.4$, $P = 0.2$), but did vary between rppTMS sessions ($F_{2, 918} = 6.8$, $P < 0.001$). Post hoc tests revealed that skill scores following rppTMS$_{1.4}$ were increased compared with both rppTMS$_{4.5}$ (EMD = 45% [0.8, 89], $P < 0.05$) and SP (EMD = 75% [24, 126], $P = 0.001$; Fig. 3C), but no difference was observed between rppTMS$_{4.5}$ and SP ($P > 0.05$). A significant effect of training Block ($F_{9, 918} = 16.7$, $P < 0.001$) was also present. Post hoc tests revealed a lower skill score in Block 1 compared with all other training Blocks (all $P < 0.05$), and training Block 10 to have greater skill scores than Blocks 1–3 (all $P < 0.005$). Spearman's analyses with Benjamini–Hochberg correction revealed no significant correlations between changes in MEP or SICF and skill acquisition (all corrected $P > 0.05$).

Effect of visuomotor skill training on MEP amplitude

Figure 4 shows MEP and SICF data recorded following training (early and late training combined), relative to the post-rppTMS time point. For PA MEPs, values were not different between AGE groups or rppTMS sessions (both $P > 0.2$); however, a significant interaction between factors was present ($P = 0.003$). Post hoc comparisons revealed that normalized MEP amplitudes were increased in the rppTMS$_{4.5}$ session compared with SP for young adults only (EMD = 24% [4.4, 43], $P = 0.01$; Fig. 4A).

For AP MEPs, a significant main effect was present for rppTMS session ($P < 0.001$), with post hoc tests revealing that MEP amplitude was reduced after SP compared with rppTMS$_{1.4}$ (EMD = −25% [−40, −9.6], $P < 0.001$) and rppTMS$_{4.5}$ (EMD = −17% [−31, −3.4], $P = 0.01$; Fig. 5B), but there was no difference between rppTMS$_{1.4}$ and rppTMS$_{4.5}$ ($P = 0.2$). Differences between rppTMS sessions also varied by AGE group ($P < 0.001$). Post hoc tests revealed that post-training MEP amplitude for the rppTMS$_{1.4}$ session was greater in the older compared with the young AGE group (EMD = 59% [26, 92], $P < 0.001$; Fig. 4B), whereas there was no difference between groups for the rppTMS$_{4.5}$ or SP sessions (both $P > 0.4$). For the older group, responses following rppTMS$_{1.4}$ were increased compared with rppTMS$_{4.5}$ (EMD = 46% [21, 70], $P < 0.001$) and SP (EMD = 63% [36, 89], $P < 0.001$; Fig. 4B), but were not different between rppTMS$_{4.5}$ and SP ($P > 0.05$). In contrast, MEPs in the young group were increased by rppTMS$_{4.5}$ compared with rppTMS$_{1.4}$ (EMD = 24% [5.2, 44], $P = 0.007$; Fig. 4B), but no
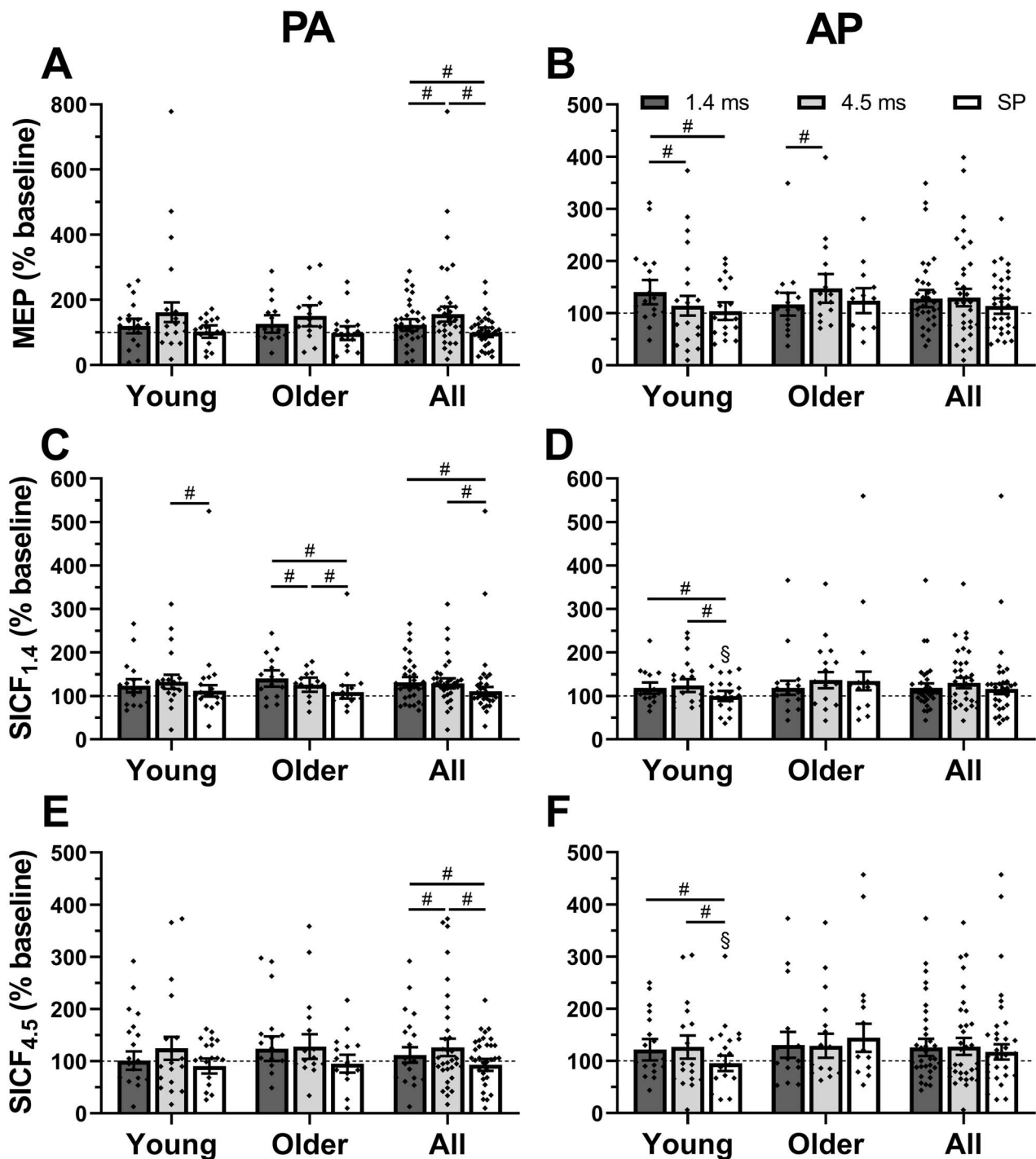
Fig. 2. Effect of rppTMS at 1.4 and 4.5 ms compared with single-pulse TMS (SP) on MEP amplitude and SICF in young and older adults. Data were recorded at the post-rppTMS time point and represent the mean ($\pm$ 95% confidence interval) test MEP amplitude (top row; A, B), $SICF_{1.4}$ (middle row; C, D), and $SICF_{4.5}$ (bottom row; E, F) for PA coil orientation (left column) and AP coil orientation (right column), expressed relative to baseline. The filled circles display individual subject means. Data points above 100 indicate a greater response compared with baseline. # $P < 0.05$; § $P < 0.001$ compared with older group.

differences were observed between SP and either $rppTMS_{1.4}$ or $rppTMS_{4.5}$ (both $P > 0.05$).

Effect of skill training on SICF

For PA $SICF_{1.4}$, analysis revealed no main effects or interactions (all $P > 0.05$). In contrast, AP $SICF_{1.4}$ differed between rppTMS sessions ($P < 0.001$), with post hoc tests revealing significantly greater upregulation of AP $SICF_{1.4}$ in the $rppTMS_{1.4}$ session

compared with $rppTMS_{4.5}$ (EMD = 15% [4.7, 24], $P < 0.001$) and SP (EMD = 10% [0.5, 20], $P = 0.04$; Fig. 4D) sessions. No difference was observed between $rppTMS_{4.5}$ and SP sessions ($P = 0.3$). The effect of session also varied by AGE group ($P = 0.007$). Post hoc comparisons revealed that upregulation of AP $SICF_{1.4}$ for the older group was significantly greater in the $rppTMS_{1.4}$ session compared with $rppTMS_{4.5}$ (EMD = 28% [12, 43], $P < 0.001$) and SP (EMD = 21% [5.4, 36], $P = 0.005$). No significant difference was
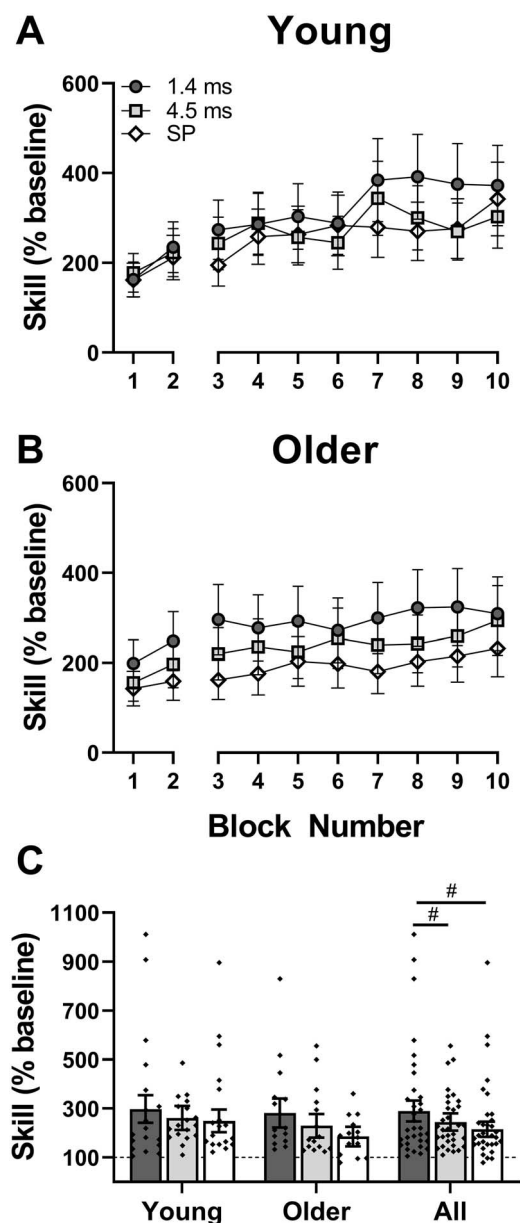
Fig. 3. Effect of rppTMS on visuomotor skill training. Data show mean (± 95% confidence interval) improvement (relative to baseline) in skill scores for 2 early training and 8 late training blocks in young (A) and older participants (B), and pooled for all training blocks (C) after rppTMS at 1.4 and 4.5 ms compared with single-pulse TMS (SP). The filled circles display individual subject means. Data points above 100 indicate a greater skill score compared with baseline. # $P < 0.05$.

observed between $rppTMS_{4.5}$ and SP sessions ($P = 0.2$), and no differences were observed between sessions for the young group (all $P > 0.9$).

For PA $SICF_{4.5}$, analysis revealed no effect of AGE group or rppTMS session (both $P > 0.1$); however a significant interaction between factors was present. Post hoc comparisons revealed significantly greater values for the older compared with the young group in the SP session (EMD = 36% [3.3, 69], $P = 0.03$). For the young group, upregulation of PA $SICF_{4.5}$ was significantly greater in the $rppTMS_{4.5}$ session compared with SP (EMD = 26% [6.2, 45], $P = 0.005$); however there was no difference between $rppTMS_{1.4}$ and either $rppTMS_{4.5}$ or SP (both $P > 0.05$). For the older group, values were significantly greater in the SP session

than both $rppTMS_{1.4}$ (EMD = 27% [5.1, 50], $P = 0.01$) and $rppTMS_{4.5}$ (EMD = 24% [3.0, 45], $P = 0.02$). Upregulation of AP $SICF_{4.5}$ varied by rppTMS sessions ($P = 0.003$), with post hoc comparisons revealing significantly greater values for $rppTMS_{4.5}$ compared with SP (EMD = 23% [6.1, 40], $P = 0.003$), but no difference between $rppTMS_{1.4}$ and either $rppTMS_{4.5}$ or SP (both $P > 0.09$; Fig. 4F). Differences between rppTMS sessions also varied by AGE group ($P = 0.001$), with post hoc analysis revealing significantly greater normalized AP $SICF_{4.5}$ in the older compared with the young AGE group following $rppTMS_{1.4}$ (EMD = 48% [8.7, 88], $P = 0.02$), but no difference between groups in the $rppTMS_{4.5}$ or SP sessions (both $P > 0.3$). Furthermore, for the older group, values were significantly greater in the $rppTMS_{1.4}$ compared with SP session (EMD = 35% [8.2, 61], $P = 0.005$), but there was no difference between $rppTMS_{4.5}$ and $rppTMS_{1.4}$ or SP (both $P > 0.05$; Fig. 4F). For the young group, upregulation of AP $SICF_{4.5}$ was greater in the $rppTMS_{4.5}$ session compared with both $rppTMS_{1.4}$ (EMD = 44% [22, 67], $P < 0.001$) and SP (EMD = 33% [11, 56], $P = 0.002$); however there was no difference between $rppTMS_{1.4}$ and SP ($P = 0.2$).

Influence of training stage on MEP and SICF

To examine the effect of training stage on MEP and SICF within each rppTMS session, the TIME factor (early, late training) was included in the AGE and rppTMS analysis (Fig. 4), and only significant TIME effects (or interactions) are reported in Fig. 5. For PA MEPs, there was a significant interaction between TIME and rppTMS session ($F_{2, 2294} = 3.1$, $P = 0.04$), with post hoc tests revealing greater upregulation of MEP amplitude for the $rppTMS_{4.5}$ compared with $rppTMS_{1.4}$ session at the late training TIME point (EMD = 21% [1.9, 40], $P = 0.03$). All other main effects and post hoc comparisons for PA MEPs were not significant (all $P > 0.05$). For AP MEPs, analysis revealed significantly greater amplitudes at the late compared with the early training TIME point ($F_{1, 2224} = 5.0$, EMD = 11.1% [1.3, 21], $P = 0.03$; Fig. 5B), however no significant interactions were present (all $P > 0.05$).

For PA $SICF_{1.4}$, analysis revealed no significant effect of TIME ($F_{1, 717} = 1.0$, $P = 0.3$); however there was a single significant interaction between AGE group, rppTMS session and TIME ($F_{2, 2297} = 4.4$, $P = 0.01$). Post hoc analysis revealed that for the young group in the $rppTMS_{1.4}$ session, upregulation of $SICF_{1.4}$ was greater at the early compared with the late training TIME point (EMD = 12% [2.0, 22], $P < 0.02$). In contrast, for young group in the SP session, normalized $SICF_{1.4}$ was greater at the late compared with the early TIME point (EMD = 15% [4.5, 25], $P < 0.005$). For AP $SICF_{1.4}$, facilitation was significantly greater at late compared with early training ($F_{1, 2217} = 4.5$, EMD = 7.0% [0.5, 13], $P = 0.03$; Fig. 4D), and an interaction between TIME and AGE group was found ($F_{1, 2217} = 5.0$, $P = 0.03$). Post hoc comparisons revealed significantly greater normalized AP $SICF_{1.4}$ for the older group, at late compared with early training (EMD = 15% [4.7, 26], $P = 0.004$), but no difference was observed between time points for the young group ($P > 0.9$). Additionally, there was a significant interaction between AGE group, rppTMS session and TIME ($F_{2, 2217} = 5.0$, $P = 0.007$), with post hoc comparisons revealing that for the young group in the SP session, normalized AP $SICF_{1.4}$ was greater at the late compared with the early training TIME point (EMD = 16% [1.6, 30], $P = 0.03$); however there were no significant differences between TIME points for the $rppTMS_{1.4}$ or $rppTMS_{4.5}$ session (both $P > 0.05$). Finally, for the older group in the $rppTMS_{1.4}$ session, upregulation of AP $SICF_{1.4}$ was greater at the late compared with the early training TIME point (EMD = 29% [10, 49], $P = 0.003$). There were no significant effects or interactions involving the TIME factor (all $P > 0.1$) for either PA or AP $SICF_{4.5}$.
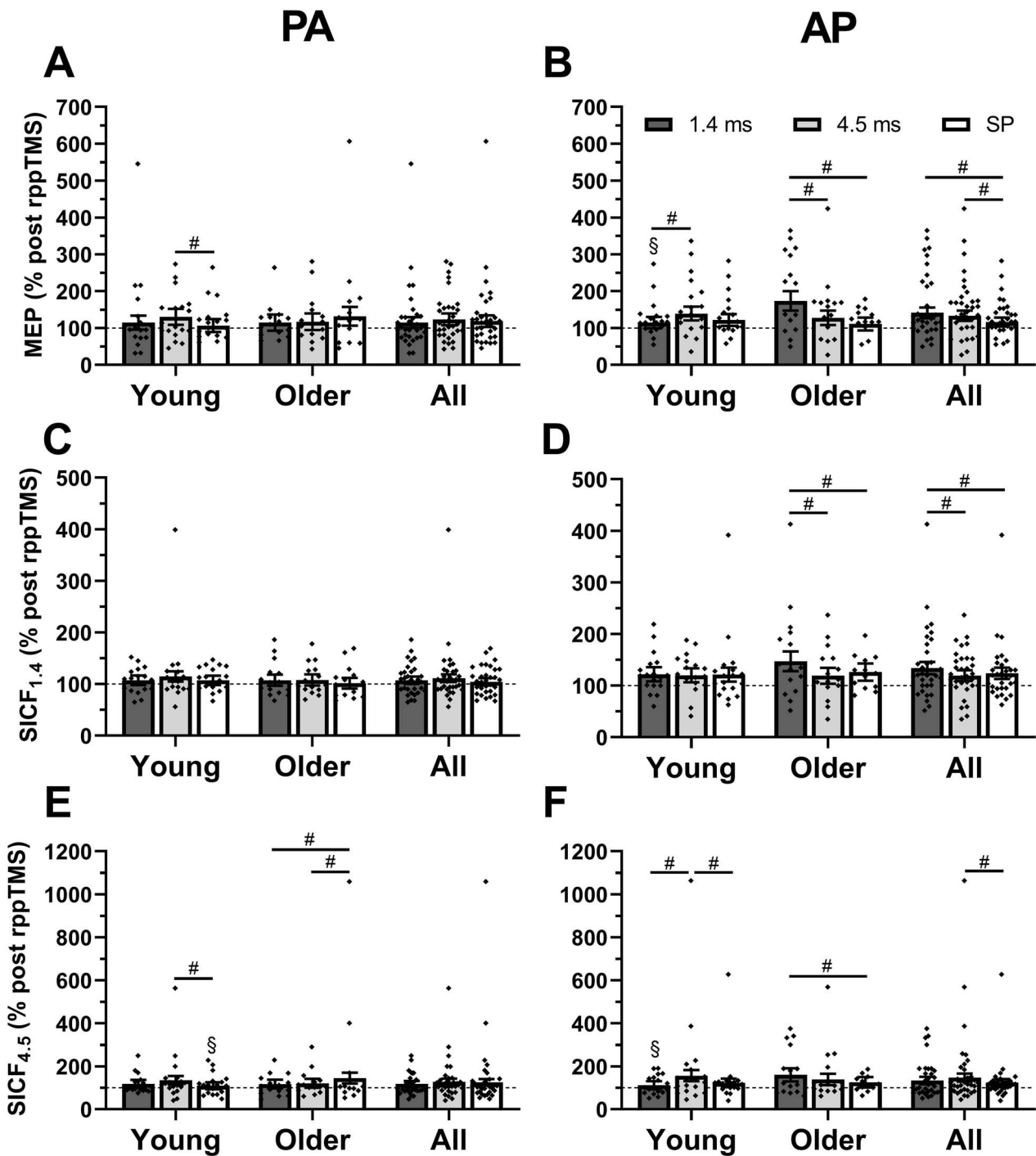
Fig. 4. Effect of visuomotor skill training on MEP amplitude and SICF after rppTMS at 1.4 and 4.5 ms compared with single-pulse TMS (SP) in young and older adults. Data show mean (± 95% confidence interval) test MEP amplitude (top row; A, B), $SICF_{1.4}$ (middle row; C, D), and $SICF_{4.5}$ (bottom row; E, F) for PA coil orientation (left column) and AP coil orientation (right column). The filled circles display individual subject means. Data were pooled for early and late training measurements, with values above 100 indicating a greater test TMS response (A, B) or SICF (C, D, E, F) compared with the post-rppTMS time point. # $P < 0.05$; § $P < 0.05$ compared with older group.

Study 2: Non-I-wave periodicity rppTMS (rppTMS_{3.5} compared with SP)

For PA MEPs recorded after $rppTMS_{3.5}$, comparisons with the SP session revealed no difference between AGE groups ($F_{1,610} = 0.3$, $P = 0.6$); however normalized MEPs were significantly greater following $rppTMS_{3.5}$ compared with SP ($F_{1,610} = 10.6$, EMD = 30% [10, 50], $P = 0.001$; Fig. 6A). There were no significant effects for AP

MEPs and no significant interaction for either coil orientation (all $P > 0.1$; Fig. 6B).

For motor skill acquisition data, there were no differences in baseline visuomotor skill between AGE groups (young, older) ($F_{1,29} = 0.94$, $P = 0.34$), rppTMS sessions ($F_{1,23} = 3.0$, $P = 0.1$), and no interaction between factors ($F_{1,23} = 0.02$, $P = 0.88$). Statistical analysis revealed significantly greater normalized skill scores in the
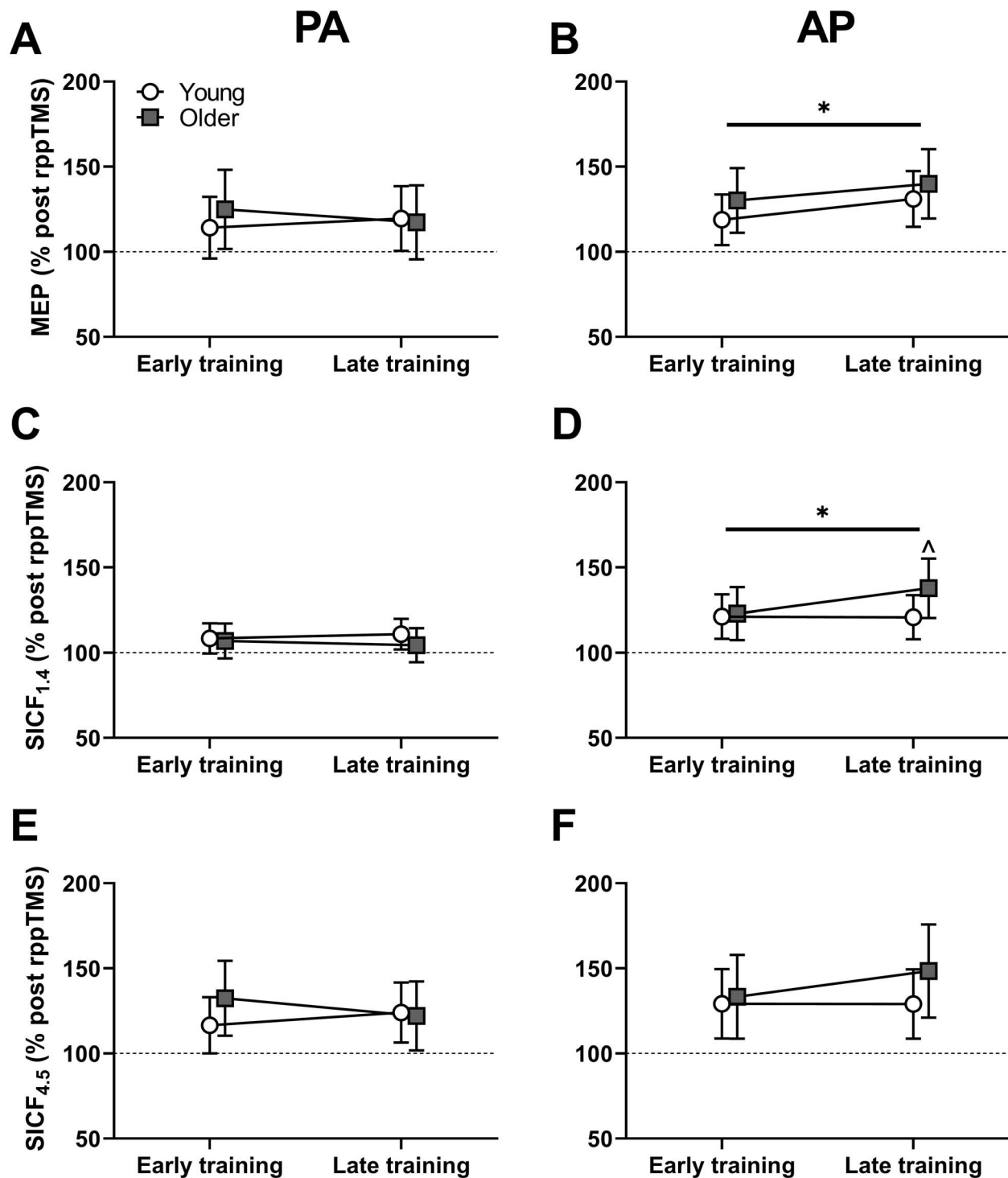
Fig. 5. Effect of visuomotor skill training on MEP amplitude and SICF during early and late training in young and older adults. Data show mean (± 95% confidence interval) for test MEP amplitude (top row; A, B), $SICF_{1.4}$ (middle row; C, D), and $SICF_{4.5}$ (bottom row; E, F) for PA coil orientation (left column) and AP coil orientation (right column), normalized to post-rppTMS. Data points above 100 indicate a greater MEP amplitude (A, B) or SICF (C, D, E, F) compared with the post-rppTMS time point. $*$ $P < 0.05$ between time points (groups pooled); $\wedge$ $P < 0.005$ compared with early training (older group).

young, compared with the older AGE group ($F_{1, 478} = 4.1$, EMD = 80% [3.1, 157], $P < 0.05$; Fig. 6C), and following $rppTMS_{3.5}$ compared with SP ($F_{1, 478} = 50.1$, EMD = 59% [39, 79], $P < 0.001$). A significant effect of training Block ($F_{9, 478} = 13.7$, $P < 0.001$) was also present. Post hoc tests revealed a lower mean skill score in Block 1 compared with all other training Blocks (all $P < 0.005$), whereas training Block 10 demonstrated greater skill scores than Blocks 1–3 (all $P < 0.001$).

Discussion

The purpose of this study was to examine the effect of rppTMS on M1 interneuron excitability and visuomotor skill acquisition in young and older adults. We examined M1 excitability with single (MEP) and paired-pulse TMS (SICF) in both PA and AP coil orientations, as these were expected to preferentially activate different combinations of early and late I-waves (Di Lazzaro et al. 2001; Di Lazzaro and Rothwell 2014). Furthermore, we compared
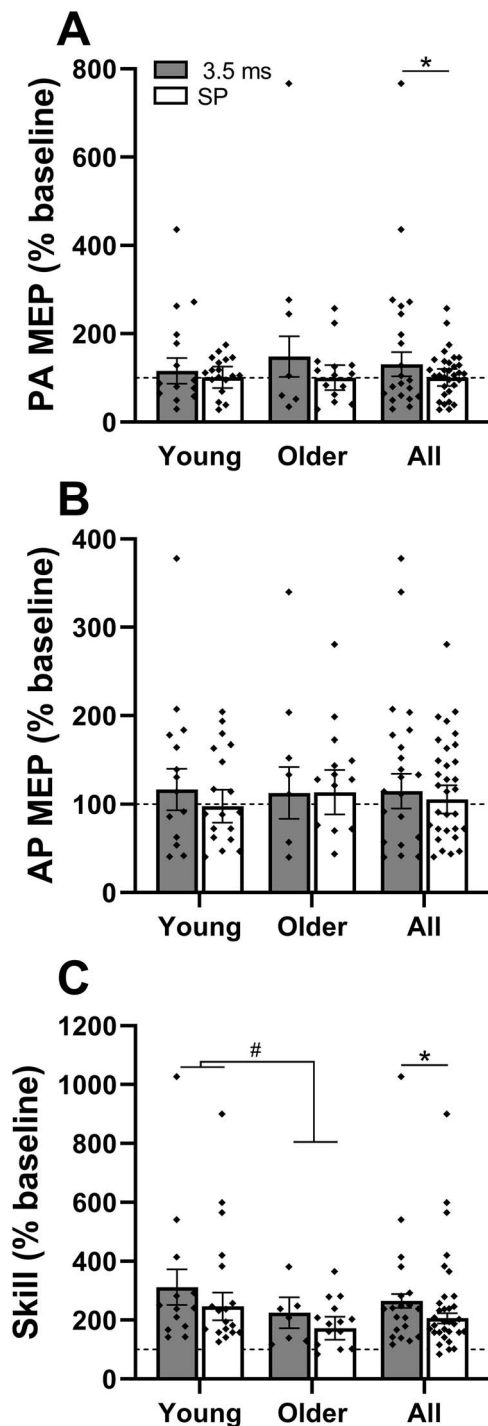
Fig. 6. Comparison of rppTMS at 3.5 ms and single-pulse TMS (SP) on MEP amplitude and skill acquisition in young and older adults. Data show mean (± 95% confidence interval) PA test MEP amplitude (top, A), AP test MEP amplitude (middle, B) recorder after rppTMS, as well as motor skill (bottom, C). All data has been normalized to baseline. The filled circles display individual subject means. Data points above 100 indicate a greater MEP amplitude (A, B) or skill (C) compared with baseline. * $P < 0.05$ between rppTMS sessions (rppTMS$_{3.5}$ and SP); # $P < 0.05$ between young and older groups.

the effects of rppTMS on M1 excitability and visuomotor skill acquisition in young and older adults using ISIs that coincide with early (1.4 ms) and late (4.5 ms) I-wave facilitation. Using this approach, we were able to identify 3 important novel findings: (1) rppTMS increased M1 excitability in young and old adults, with

the greatest effects for PA TMS at the late ISI; (2) Visuomotor skill acquisition was improved by rppTMS at the early ISI in young and older adults; and (3) rppTMS and visuomotor training increased M1 excitability for AP TMS in older adults. Performing an additional study using a non-I-wave coincident interval (3.5 ms) also showed an increase in M1 excitability and skill acquisition. These findings suggest that rppTMS at both I-wave and non-I-wave intervals can be used to alter specific features of M1 excitability and improve visuomotor skill acquisition in young and older adults.

rppTMS at I-wave intervals increases M1 excitability in young and older adults

SP TMS over M1 results in a corticospinal descending volley that consists of multiple I-waves that are separated by ~1.4 ms. The excitability of early (I1) and late (I2, I3) waves can be indirectly assessed by changing the orientation of the TMS coil, where a PA current preferentially activates early I-waves, and an AP current preferentially activates late I-waves. The mechanisms that generate I-waves are still unclear, but is likely to involve synaptic input from specific excitatory interneuron circuits onto corticospinal output cells that are modulated by GABAergic interneurons (see Ziemann 2020 for review). Irrespective of the mechanism, I-wave periodicity reflects a time window of increased excitability where a second TMS pulse is more likely to excite corticospinal neurons due to I-wave interactions at the cortical level. When this is delivered repetitively (i.e. rppTMS) at intervals matching early (see Kidgell et al. 2016 for review) or late I-wave intervals (Opie et al. 2021), there is a robust increase in MEP amplitude that outlasts the intervention, reflecting the process of neuroplasticity. Using this approach, we found that rppTMS resulted in substantial facilitation of PA MEPs (compared with SP control intervention) in young and old adults, but this effect was most pronounced for the late (4.5 ms) compared with the early (1.4 ms) ISI. However, we note that MEP facilitation from the early ISI was reduced relative to what has been observed in previous studies (30–500% increase, see Kidgell et al. 2016). Despite this, we also found that rppTMS increased PA SICF (both SICF$_{1.4}$ and SICF$_{4.5}$), with greater facilitation for SICF$_{4.5}$ with rppTMS at the late ISI. These findings suggest that rppTMS at the late ISI may be more effective at modulating M1 excitability compared with conventional rppTMS (at an early ISI). Given that rppTMS is applied at suprathreshold intensities, it is possible that some of these changes in MEPs are due to changes in spinal excitability. However, it has previously been shown that rppTMS increases MEPs without influencing spinal cord excitability (as assessed with cervicomedullary junction stimulation; Di Lazzaro et al. 2007), suggesting that the changes in MEP amplitude observed here are likely to involve a major contribution from cortical circuits.

It is commonly accepted that M1 plasticity assessed with TMS declines with advancing age (Zimerman and Hummel 2010; Freitas et al. 2011), although more recent evidence shows that this may depend on the noninvasive brain stimulation technique used to induce plasticity (Semmler et al. 2021). Despite numerous studies with rppTMS in young participants (see Kidgell et al. 2016 for review), no studies have previously examined the effect of age on M1 plasticity using conventional rppTMS (with an early ISI). Furthermore, only one study has compared age-related differences in M1 plasticity with rppTMS at late ISIs (Opie et al. 2018). In this previous study, there was a decline in M1 plasticity in older adults with rppTMS (4.1 ms ISI), but this difference was removed when the ISI was adjusted (4.9 ms ISI) to account for the delayed late I-wave facilitation in older adults (Opie et al. 2018). Using both early (1.4 ms) and late (4.5 ms) ISIs, we show no

age-related differences in MEP amplitude modulation when using a PA current, which matches the findings from the delayed ISI (4.9 ms) in the previous study (Opie et al. 2018). These findings with PA TMS indicate that rppTMS induces similar M1 plasticity in young and older adults, suggesting that plasticity in the I-wave circuits targeted by rppTMS remains intact with advancing age. One caveat to this finding is that we did not obtain detailed demographic information or perform cognitive assessments on our older adults, so we are not able to determine whether our healthy older participants were representative of the general population, or were a sample of successful agers that might not demonstrate obvious age-related changes in brain function.

In an attempt to examine the effect of rppTMS on different interneuronal circuits, we also examined the change in MEP and SICF using an AP coil orientation, which is known to preferentially activate late I-waves (see Di Lazzaro and Rothwell 2014). In contrast to a previous study (Long et al. 2017), we found only modest effects of rppTMS on AP-sensitive circuits, and these effects differed between young and older adults. For example, the change in AP MEPs were greatest after rppTMS at 1.4 ms in young participants, but were greatest after rppTMS at 4.5 ms in older adults. Furthermore, AP SICF (1.4 and 4.5) was greater after rppTMS performed at 1.4 and 4.5 ms in young adults, but it was not modulated after rppTMS (compared with SP) in older adults. These findings suggest that the effects of rppTMS (delivered with a PA current) appear to be more sensitive to modulation of AP circuits in young compared with older adults.

rppTMS improves visuomotor skill acquisition in young and older adults

rppTMS has been shown to influence certain aspects of motor behavior. For example, when rppTMS is applied before a motor task, there is an attenuation of the force decline generally associated with muscle fatigue (Benwell et al. 2006), improved rate of repetitive finger movements (Teo et al. 2012), and increased voluntary motor output and hand dexterity in patients with spinal cord injury (Long et al. 2017). However, no previous studies have examined the effect of rppTMS on motor skill acquisition in young or older adults. Given that rppTMS has been shown to influence both early and late interneuron networks (Cash et al. 2009; Long et al. 2017), which are thought to be important for different types of motor skill acquisition (Hamada et al. 2014), we expected that rppTMS would improve performance on the visuomotor skill task. Our findings support this hypothesis, showing improved visuomotor skill acquisition after rppTMS at the early ISI in young and old adults. This represents a novel and exciting finding, as previous studies using priming noninvasive brain stimulation (involving PAS) have been unable to improve visuomotor skill acquisition in older adults over single or multiple training sessions (Opie et al. 2019; Opie et al. 2020b). It is currently unclear why priming with rppTMS is effective in older adults and PAS is not, given that both rppTMS and PAS are thought to involve spike-timing-dependent plasticity that influences late I-waves (Di Lazzaro et al. 2009). However, it could be that the effects of rppTMS are more widespread and additionally involve circuits beyond those involved in I-wave generation (Di Lazzaro et al. 2007), such as those acting on the broader motor network to improve visuomotor performance. When noninvasive brain stimulation is used prior to motor training, homeostatic and non-homeostatic plasticity are potential factors that are known to modulate subsequent motor skill acquisition (Jung and Ziemann 2009). Given that rppTMS facilitates MEPs, and this was accompanied by subsequent improvements in skill acquisition, it is possible that non-homeostatic plasticity may have contributed to this improvement in skill acquisition with rppTMS (Müller–Dahlhaus and Ziemann 2015).

Although rppTMS improved motor skill acquisition in young and old adults, the effects were only observed for early (1.4 ms) but not late (4.5 ms) rppTMS ISIs. We expected greater improvement for the late ISI, given that late I-waves are important for the performance of model-based learning tasks such as visuomotor adaptation (Hamada et al. 2014), and rppTMS at a late ISI (4.3 ms) has previously been shown to influence AP-sensitive (late I-wave) circuits and improve manual performance (Long et al. 2017). However, we were unable to consistently modulate AP circuits with rppTMS at the late ISI, suggesting that the late I-waves were not preferentially modulated by rppTMS with this approach. Furthermore, the greatest change in PA MEPs occurred for rppTMS at the late rppTMS ISI, but the improvement in visuomotor skill occurred after rppTMS at the early ISI, suggesting a mismatch between the neurophysiological and behavioral outcomes following rppTMS. These divergent findings are not unexpected, as the neurophysiological factors that contribute to the MEP are complex, and the neuronal circuits that are activated by TMS only represent a subgroup of the broader neuronal network that contributes to motor behavior (Bestmann and Krakauer 2015). Nonetheless, a change in the MEP with rppTMS indicates that there are physiological changes in the corticospinal neurons that are activated by TMS, but how this contributes to the changes in motor behavior remains unknown.

One potential confound for the differences in visuomotor skill acquisition after rppTMS relates to differences in skill performance at baseline. For example, visuomotor skill at baseline (before any intervention) was greatest for the logarithmic task (rppTMS 4.5 ms session) in young and older adults, suggesting that it was easier to perform than the exponential (rppTMS 1.4 ms session) and sigmoid (SP control session) visuomotor tasks. This potentially makes it more difficult for rppTMS to improve visuomotor performance with training at the late ISI (4.5 ms), but it does not explain the improvements in skill acquisition for the exponential (rppTMS 1.4 ms session) compared with the sigmoidal tasks (SP control session), where there was no difference in visuomotor performance at baseline. Another consideration is that there might be differences in skill acquisition based on which session was performed first. For all participants in Study 1, 14 participants (7 young, 7 older) completed the rppTMS 1.4 ms session first, 16 participants (10 young, 6 older) completed the rppTMS 4.5 ms session first, and 7 participants (5 young, 2 older) completed the SP session first. Given that an equivalent number of participants completed the 1.4 and 4.5 ms sessions first, this is unlikely to account for the greater improvement in skill acquisition that we observed in the 1.4 ms rppTMS intervention.

M1 plasticity after rppTMS and visuomotor skill acquisition in young and older adults

Motor training and practice is associated with an increase in MEP amplitude that reflects use-dependent plasticity in M1 circuits (Bütefisch et al. 2000; Muellbacher et al. 2001) and is thought to occur through LTP-like mechanisms (Hess and Donoghue 1994; Rioult-Pedotti et al. 2000). Previous studies have shown that skill acquisition results in an increase in MEP amplitude for both PA and AP circuits, and this effect is similar in young and old adults (Cirillo et al. 2011; Mooney et al. 2019), suggesting that LTP of early and late I-waves accompanies the increase in skill acquisition in young and older adults. As an extension of these

studies, we found that AP MEP facilitation after visuomotor training was increased in older adults, but only when preceded by rppTMS at the early ISI (compared with SP control intervention). Furthermore, a similar finding was observed for SICF, where there was increased AP SICF (1.4 and 4.5 ms) in older adults after visuomotor training and rppTMS at the early ISI compared with the control intervention. These findings suggest that AP-sensitive circuits in older adults may be more amenable to modulation with visuomotor skill acquisition when preceded by rppTMS. The functional significance of this effect in older adults is unclear, given that the improvement in visuomotor skill acquisition with rppTMS was similar in young and older adults, and the modulation of AP-sensitive circuits after rppTMS and skill acquisition in young adults occurred at the late ISI. Nonetheless, the effects on AP-sensitive circuits in older adults were most pronounced for rppTMS at the early ISI, which is where significant improvements in performance were observed, and provides further evidence that rppTMS at late ISIs does not preferentially influence AP-sensitive circuits.

It has previously been shown that PA- and AP-sensitive circuits may be differentially modulated based on the stage of training, with AP-sensitive circuits more excitable in the later stages of skill acquisition when performance is being optimized (Spampinato et al. 2020). For this reason, we examined the change in M1 excitability for PA- and AP-sensitive circuits after 2 training blocks (early training) and after an additional 8 training blocks (late training). We found that there was no effect of training stage on PA-sensitive circuits, but there was a significant modulation of AP MEPs with late training (compared with early training). Furthermore, there was greater AP SICF (1.4 ms) in late training, with this effect due to an increase in AP SICF in older adults. These findings were not influenced by the rppTMS intervention, suggesting that they provide further support for a role of AP-sensitive circuits in the later stages of visuomotor training (Spampinato et al. 2020). Given that AP TMS is thought to activate axons that originate in premotor cortex (Aberra et al. 2020), and that premotor-M1 excitability is reduced in older adults (Ni et al. 2015), it is possible that the increased AP response in older adults reflects a compensatory mechanism (possibly via the cerebellum) to increase premotor-M1 connectivity in an effort to optimize performance in the late stages of visuomotor training (Spampinato et al. 2020).

rppTMS at non-I-wave facilitation intervals (rppTMS at 3.5 ms)

To examine whether the effect of rppTMS is temporally specific to intervals involving I-wave periodicity, we included an additional experiment where rppTMS was performed with an interval of 3.5 ms, which is outside of the facilitatory peaks in the SICF curve (Ziemann et al. 1998; Opie et al. 2018). This 3.5 ms ISI has previously been used as an rppTMS control condition where there was no modulation of AP MEPs or motor function in healthy and spinal cord injured patients (Long et al. 2017). However, we found greater modulation of PA MEPs and improved visuomotor skill acquisition after rppTMS at 3.5 ms (compared with the SP control intervention) in young and older adults. One potential confound to our findings on skill acquisition is that the same force transform (sigmoidal) was used for the 3.5 ms and SP sessions. However, there was no significant difference in baseline skill performance between sessions, and less than half of the participants completed both sessions, suggesting that a carry-over effect of performance did not influence the outcome. Nonetheless, this finding indicates that rppTMS at a late ISI (3.5 ms), which does not coincide with I-wave periodicity (SICF trough), can modulate M1 excitability and

visuomotor skill acquisition. This finding is supported by a recent study showing that paired-pulse TMS can increase M1 excitability at both I-wave (SICF peak) and non-I-wave (SICF trough) intervals, with these effects occurring through 2 different mechanisms (Kesselheim et al. 2023). Although the non-I-wave mechanism is poorly understood, the paired-pulse facilitation at a non-I-wave latency (SICF trough) may occur through slower conducting and indirect corticospinal projections that show no I-wave periodicity, or it may weaken the strength of intracortical inhibition and shift the excitation-inhibition balance toward greater facilitation (Kesselheim et al. 2023). Additional studies are therefore needed to explore these potential mechanisms of non-I-wave periodicity rppTMS and how they influence motor performance. Furthermore, to better establish the role of non-I-wave periodicity to the improvements in skill acquisition, it would be useful in future studies to adjust the ISI for individual I-wave periodicity (SICF peaks and troughs), which has been shown previously to enhance synaptic plasticity within the submillisecond range for rppTMS at an early ISI (Sewerin et al. 2011).

TMS coil orientation considerations

An important assumption of this study is that PA and AP currents preferentially activate different I-waves, and these arise from distinct sources of excitatory inputs (see Spampinato 2020; Opie and Semmler 2021 for reviews). However, TMS with a PA current at the intensities used here is likely to activate a combination of I-waves (Di Lazzaro et al. 1998), and the recruitment of early and late I-waves by TMS can vary between individuals (Hamada et al. 2013). In addition, it is necessary to use a greater TMS intensity to activate AP circuits, and this is likely to activate other neuronal populations, including those involved with PA TMS. Furthermore, our assessment included AP MEP amplitudes that were significantly smaller at baseline in older adults (Table 1). Therefore, the comparisons between PA and AP TMS must be interpreted with the appropriate caution. Additional work has indicated that AP-sensitive circuits can be more readily recruited with short (30 μs) TMS pulses (D'Ostilio et al. 2016), with even greater selectivity being achieved with short pulses during voluntary muscle activation (Hannah and Rothwell 2017), suggesting that these approaches may improve selectivity when activating different I-waves. However, muscle activation is known to influence the response to some noninvasive brain stimulation protocols (Goldsworthy et al. 2014; Goldsworthy et al. 2015), making the interpretation of noninvasive brain stimulation effects with muscle activation more challenging. It is also important to note that the rppTMS intervention used here involved a PA current, and we have recently shown that rppTMS is more effective at modulating M1 excitability when an AP current is used (Sasaki et al. 2023). However, AP rppTMS is complicated by the increased TMS intensity required to activate AP circuits, and is not feasible in participants with high AP TMS thresholds due to some hardware limitations (e.g. coil overheating). Furthermore, our studies did not use a neuronavigation system to ensure consistent placement of the TMS coil between PA and AP coil orientations. Although we are highly experienced at performing TMS, this may have contributed to some of the variability observed both within and between sessions.

In conclusion, we used a novel rppTMS intervention with early and late ISIs to alter M1 excitability and examine the effect on visuomotor skill acquisition in young and older adults. We found that rppTMS increased M1 excitability in young and old adults, with the greatest effects for PA-sensitive circuits (early I-wave facilitation) at a late (4.5 ms) ISI. Furthermore, the rppTMS

intervention at an early ISI improved visuomotor skill acquisition, and the improvement was similar in young and older adults. The improved visuomotor skill acquisition was also accompanied by plasticity of late I-wave circuits in older adults. Finally, rppTMS at a non-I-wave interval (3.5 ms) also modulated M1 excitability and visuomotor skill acquisition, suggesting that I-wave interactions may also occur outside of the main facilitatory peaks. Nonetheless, these findings suggest that rppTMS can be used to increase I-wave excitability and improve visuomotor skill acquisition in young and older adults. Interventions that increase I-wave excitability may therefore provide a promising tool to improve motor performance in age-related neurological conditions where motor function is impaired.

Author contributions

Brodie J. Hand (Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing—original draft, Writing—review & editing), Ashley Merkin (Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing—review & editing), George Opie (Funding acquisition, Investigation, Methodology, Supervision, Validation, Visualization, Writing—review & editing), Ulf Ziemann (Conceptualization, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing—review & editing), John Semmler (Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing—original draft, Writing—review & editing).

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Data availability

Data from this study will be made available to qualified investigators upon reasonable request to the corresponding author.

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