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Visuomotor task acquisition is reduced by priming paired associative stimulation in older adults

George M. Opie [a,c,1], Brodie J. Hand [a,1], James P. Coxon [b], Michael C. Ridding [c], Ulf Ziemann [d], John G. Semmler [a,*]

[a] Discipline of Physiology, Adelaide Medical School, The University of Adelaide, Adelaide, Australia
[b] School of Psychological Sciences, Monash Institute of Cognitive and Clinical Neurosciences, Monash University, Victoria, Australia
[c] Discipline of Obstetrics and Gynaecology, Adelaide Medical School and Robinson Research Institute, The University of Adelaide, Adelaide, Australia
[d] Department of Neurology and Stroke, Hertie-Institute for Clinical Brain Research, Eberhard Karls University of Tübingen, Tübingen, Germany

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ABSTRACT

Transcranial magnetic stimulation may represent an effective means for improving motor function in the elderly. The aim of this study was therefore to investigate the effects of paired associative stimulation (PAS; a plasticity-inducing transcranial magnetic stimulation paradigm) on acquisition of a novel visuomotor task in young and older adults. Fourteen young (20.4 ± 0.6 years) and 13 older (69.0 ± 1.6 years) adults participated in 3 experimental sessions during which training was preceded (primed) by PAS. Within each session, the interstimulus interval used for PAS was set at either the N20 latency plus 5 ms (PAS_{LTP}), the N20 latency minus 10 ms (PAS_{LTD}), or a constant 100 ms ($PAS_{Control}$). After training, the level of motor skill was not different between PAS conditions in young subjects (all p-values > 0.2), but was reduced by both PAS_{LTP} ($p = 0.02$) and PAS_{LTD} ($p = 0.0001$) in older subjects. Consequently, priming PAS was detrimental to skill acquisition in older adults, possibly suggesting a need for interventions that are optimized for use in elderly populations.

1. Introduction

A decline in motor function is perhaps one of the most characteristic aspects of the aging process. For example, older adults move more slowly and with greater variability (Darling et al., 1989; Krampe, 2002), they demonstrate increased difficulty with fine motor manipulation (Marneweck et al., 2011; Ranganathan et al., 2001) and altered motor adaptation (Buch et al., 2003; Hardwick and Celnik, 2014). Such deficits can influence the ability of older individuals to complete even the most basic tasks of daily living, such as tying a shoe lace or buttoning a shirt. These functional deficits can necessitate greater support from external sources, degrading psychological status (Forsell et al., 1994; Kennedy et al., 1990) and quality of life (Borowiak and Kostka, 2004). The development of interventions able to ameliorate age-related deficits in motor function is therefore a high priority.

Although the factors contributing to motor decline in older adults are multifactorial, growing evidence suggests that age-related deficits in neuroplasticity are likely important. Neuroplasticity refers to the brains ability to change the strength of its connections, and this process is crucial for modifying neuronal activity to achieve effective motor control (Sanes and Donoghue, 2000). This has been best demonstrated in humans by studies using noninvasive brain stimulation (NIBS) techniques such as transcranial magnetic stimulation (TMS). These techniques are able to induce long-term potentiation (LTP) and depression (LTD) like modifications to synaptic efficacy (Ziemann et al., 2008), which are key mediators of neuroplastic change (Sanes and Donoghue, 2000). Although not a universal outcome (for review, see; Berghuis et al., 2017), a number of studies using this approach have shown reduced neuroplastic capacity in the elderly (Fathi et al., 2010; Freitas et al., 2011; Müller-Dahlhaus et al., 2008; Rogasch et al., 2009; Tecchio et al., 2008; Todd et al., 2010). Consequently, bolstering the neuroplasticity response in older adults may constitute an effective means of improving motor function in older adults.

A number of factors have been shown to influence the plasticity response (Ridding and Ziemann, 2010). While some of these are not modifiable (e.g., genetics, age, sex), others can be easily manipulated, making it possible to experimentally influence plasticity induction. In particular, an extensive literature has shown that the history of synaptic activity has a powerful influence on the

* Corresponding author at: Discipline of Physiology, Adelaide Medical School, The University of Adelaide, Adelaide, South Australia 5005, Australia. Tel.: + 61 8 8313 7192; fax: + 61 8 8313 4398.
E-mail address: john.semmler@adelaide.edu.au (J.G. Semmler).
[1] These authors contributed equally to this manuscript.

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subsequent induction of synaptic plasticity (Abraham, 2008; Hulme et al., 2013). This process, known as metaplasticity, has been extensively investigated in humans using NIBS (Müller-Dahlhaus and Ziemann, 2015). Specifically, when used to modify cortical excitability before plasticity induction (via experimental or training based interventions), brain stimulation can result in a potentiated neurophysiological response and a greater response to training (for review: Karabanov et al., 2015; Müller-Dahlhaus and Ziemann, 2015). Consequently, this "priming" approach may represent a method by which motor function could be improved in older adults. In support of this, a recent study has reported comparable levels of motor learning performance between young and older adults when transcranial direct current stimulation (tDCS; an alternative NIBS technique able to induce neuroplastic change) was applied before and during the training period (Fujiyama et al., 2017). By contrast, when sham tDCS was used before training, performance was reduced in the elderly (Fujiyama et al., 2017).

While Fujiyama et al provide strong preliminary evidence for the functional utility of priming in older adults, the stimulation provided by tDCS is relatively nonspecific in nature (Datta et al., 2009; Thielscher et al., 2011). Consequently, it remains possible that the efficacy of priming in older adults could be enhanced by using an alternative priming technique that provides more specific modulation of the motor cortical areas activated during training. For example, paired associative stimulation (PAS) is a TMS paradigm that utilizes a spike-timing–dependent approach for inducing neuroplastic changes in motor cortical excitability; afferent signals generated by percutaneous electrical stimulation of peripheral nerves are coupled at high temporal resolution with corticospinal activation generated by TMS (Suppa et al., 2017). The resulting motor cortical activation generated by this approach is therefore more specific than can be provided by tDCS.

The aim of the present study was therefore to investigate the utility of priming PAS stimulation for improving the response to motor training in older adults. In addition, given that only homeostatic priming was assessed by Fujiyama et al (i.e., depressive priming protocol before an excitatory training intervention), whereas nonhomeostatic interactions may also be important (Müller-Dahlhaus and Ziemann, 2015), our secondary aim was to investigate both homeostatic and nonhomeostatic priming mechanisms. As priming PAS has previously been shown to improve the response to motor training in young subjects (Jung and Ziemann, 2009), and tDCS can improve motor skill in older adults (Fujiyama et al., 2017), we expected that priming PAS would improve the response to training in older adults.

2. Materials and methods

Fourteen young (mean ± SD; 20.4 ± 2.1 years, 7 females) and 13 older (mean ± SD; 69.0 ± 5.6 years, 6 females) adults were recruited from the university and wider community to participate in the present study. Exclusion criteria included a history of neurological or psychiatric disease, or current use of psychoactive medication (sedatives, antipsychotics, antidepressants etc.) All subjects were right-handed according to self-report. All experimentation was approved by the University of Adelaide Human Research Ethics Committee and conducted in accordance with the Declaration of Helsinki. Each subject provided written informed consent before participation.

2.1. Experimental arrangement

Each participant attended a total of 6 experimental sessions, including 3 main sessions that each had a shorter retention session 24 hours after motor training (Fig. 1A). All 3 main sessions were separated by at least 7 days, and were conducted at approximately the same time of day to control for diurnal variations in cortisol (Sale et al., 2007, 2008). During each session, subjects were seated in a comfortable chair, with their right arm and hand relaxed on a table in front of them. Surface electromyography (EMG) was recorded from the first dorsal interosseous (FDI) 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, UK), digitized at 2 kHz using a CED1401 interface (Cambridge Electronic Design) and stored in a computer for offline analysis.

2.2. Experimental procedures

2.2.1. Transcranial magnetic stimulation

TMS was applied to the hand area of the left primary motor cortex using a figure-of-eight coil connected to a Magstim 200^2 magnetic stimulator (Magstim, Dyfed, UK). The coil was held tangentially to the scalp at an angle of 45° to the sagittal plane, with the handle pointed backwards and laterally, producing an anteriorly directed current flow in the brain. The coil was positioned on the scalp over the location producing an optimum response in the relaxed FDI muscle of the right hand. This location was marked on the scalp for reference and continually checked throughout the experiment. TMS was delivered at 0.2 Hz with a 10% jitter to avoid anticipation of the stimulus.

Resting motor threshold (RMT) was defined as the minimum stimulus intensity producing a motor evoked potential amplitude ≥50 μV in at least 5 of 10 trials while the right FDI was relaxed. RMT, expressed as a percentage of maximum stimulator output, was assessed at both the baseline and 24 hours retention time points. Corticospinal excitability was assessed using MEPs recorded while FDI was relaxed. At the baseline, the stimulus intensity was set to evoke an MEP with peak-to-peak amplitude of ~1 mV (MEP_{1mV}) when averaged over 20 trials. This intensity was then used for all subsequent blocks of MEPs. Following baseline measurements, 20 MEPs were recorded between PAS and motor training, and then 0, 20, and 40 minutes after motor training. The 1 mV intensity was also reassessed at the beginning of the 24 hours retention session.

2.2.2. Paired associative stimulation

PAS consisted of percutaneous electrical stimulation of the ulnar nerve at the wrist, followed by TMS applied to the hand area of the left primary motor cortex. Each block of PAS included 200 pairs of stimuli applied at a rate of 0.25 Hz (Ziemann et al., 2004). Electrical stimuli (square wave, 1 ms duration) were applied at an intensity of 3 × perceptual threshold using a constant current stimulator (DS7AH; Digitimer, UK) and bipolar surface electrodes (cathode proximal), while TMS was set at the 1 mV intensity. Three interstimulus intervals (ISIs) between peripheral and central stimuli were used across the 3 sessions. These corresponded to the latency of the N20 response after ulnar nerve stimulation plus 5 ms (PAS_{LTP}), the latency of the N20 response minus 10 ms (PAS_{LTD}), or a fixed ISI of 100 ms ($PAS_{Control}$). An interval of 5 ms was used for PAS_{LTP} to approximate an ISI of 25 ms (allowing for intraindividual variations in afferent conduction), which has been shown to be effective for modifying model-based motor learning tasks such as the one used in the present study (Hamada et al., 2014). By contrast, an interval of 10 ms for PAS_{LTD} approximated a 10 ms ISI that has been previously reported as effective in reducing corticospinal excitability (Weise et al., 2006; Wolters et al., 2003). For assessment of the N20, 500 percutaneous electrical stimuli were applied to the ulnar nerve at the wrist (3 × perceptual threshold), with the

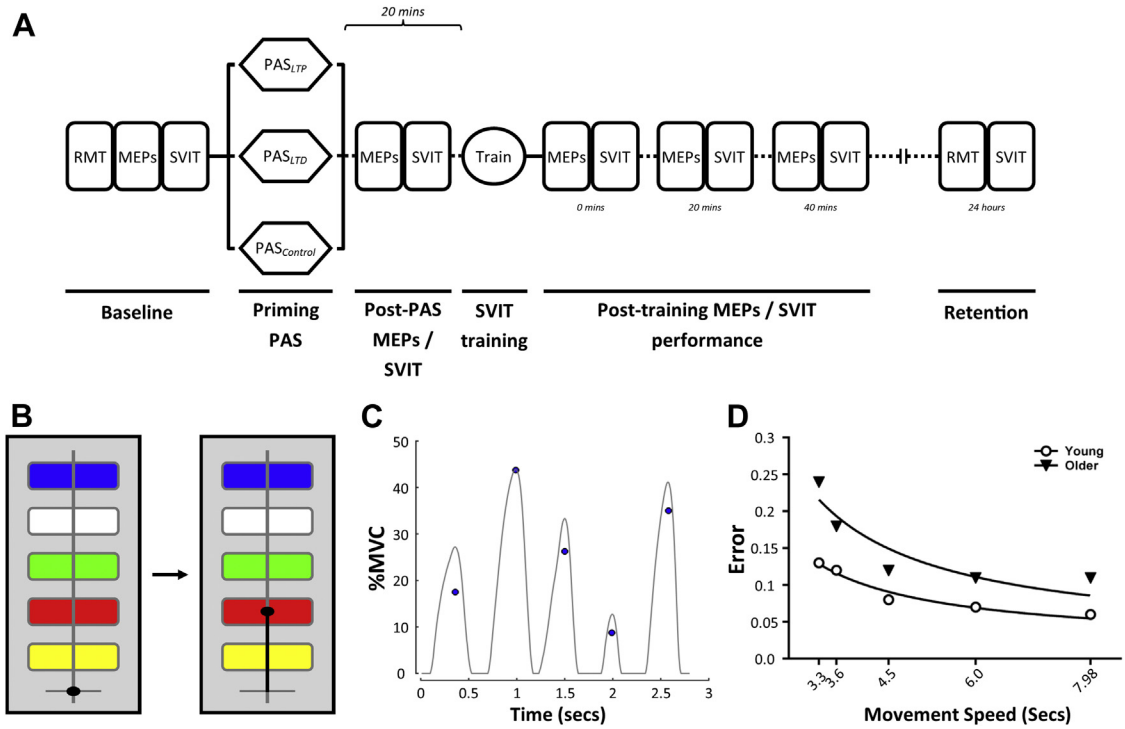

Fig. 1. Experimental protocol and SVIT task. (A) Timeline of events during each experimental session. (B) Example of the visual feedback displayed to participants during the SVIT task, showing movement of the cursor from the baseline *(left)* to the first target box *(right)*. (C) Raw force data from an individual participant showing the pulses generated during a single trial. Blue circles identify the force required to meet the center of the target box. (D) Examples of the speed-accuracy trade-off functions generated in young and older adults by plotting error against total movement time. Abbreviations: SVIT, sequential visual isometric task. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

resulting SEP recorded using electrodes located at FC3 and 2 cm posterior to C3 (presumed hand area of sensory cortex; Enomoto et al., 2001). N20 was recorded at the beginning of the first session and each PAS protocol was tested in separate sessions in a randomized, crossover design. For all sessions, the time interval between PAS and motor training was set at 20 minutes (Goldsworthy et al., 2014a, b, 2012, ; Müller-Dahlhaus et al., 2015). As the level of attention may influence the response to PAS (Stefan et al., 2004), participants were instructed to count additional electrical stimuli that were applied to the index finger of the right hand during the PAS block. Participants received 15–25 stimuli (varied randomly between sessions and subjects) at an intensity of 2 × perceptual threshold (Stefan et al., 2004).

2.2.3. Sequential visual isometric task

Motor learning and performance was assessed using a modified version of the sequential visual isometric pinch task originally described by Reis et al. (Reis et al., 2009). The right hand was placed in a plastic cast that separated the index finger from the other digits. Participants abducted the distal interphalangeal joint of the index finger against a force transducer (MLP-25; Transducer Techniques, USA) that controlled a cursor displayed on a monitor placed in front of them. When force was applied, the cursor moved vertically past 5 colored boxes that were presented as targets, with more forceful contractions required to meet targets further from the origin (Fig. 1B). The targets were displayed so that the furthest always represented 45% of the individual subject's maximum voluntary contraction force. This was established at the beginning of each session, as the greatest force produced from 3 consecutive contractions. During a single trial, the participant was required to optimize accuracy as they moved the cursor from a baseline "home" location to the center of each target (Fig. 1C). The color of the boxes determined the target order, and participants were required to return the cursor to the home location between each target (e.g., *Home* - Red Box - *Home* - Blue Box - *Home* - Green Box - *Home* - Yellow Box - *Home* - White Box).

Task difficulty was increased by utilizing a nonlinear transformation of force application to cursor movement, requiring the participant to learn the relationship between force production and cursor movement to improve their motor skill. To minimize carryover of sequential visual isometric task (SVIT) performance between each PAS treatment, a different transformation of force application to cursor movement was used in each session. Specifically, $PAS_{Control}$ used a logarithmic transform, PAS_{LTD} used an exponential transform, and PAS_{LTP} used a sigmoidal transform. In addition, while the order in which participants were required to reach each colored box was consistent between sessions (i.e., red-blue-green-yellow-white), the order in which they were displayed on screen was different between sessions, although the forces required to reach the 5 targets was the same for each session.

Motor performance was assessed by using SVIT to construct a speed-accuracy function (SAF). This was achieved by completing the task at 6 different speeds (0.5, 0.75, 1, 1.33, 1.67, and 1.83 Hz), with force production to each target paced by a metronome. Participants were instructed to reach each target force as the metronome sounded. Three trials were completed for each speed. SAFs were recorded at the baseline, post-PAS, 0, 20, and 40 minutes after training, and during the 24 hours retention session.

Motor training included 12 blocks of the SVIT task, each of which contained 8 trials. Participants were allowed to complete each trial at their own pace, but were periodically encouraged to improve their speed and accuracy. On-screen feedback was provided at the end of each block by plotting the participants "skill" score (see below) for that block against all previous blocks.

2.3. Data analysis

2.3.1. Neurophysiological measures

During offline analysis of EMG data, trials containing EMG activity >20 μV (peak-to-peak amplitude) in the 100 ms before TMS application were discarded. MEPs were measured peak to peak and expressed in mV. Isolated changes in corticospinal excitability after PAS (referred to as "PAS" in figures), and cumulative changes in excitability after both PAS and training (referred to as "PAS + Training" in figures), were quantified by normalizing individual MEPs within post-PAS and post-training time points to the average MEP amplitude recorded at the baseline. In addition, the specific influence of motor training was quantified by expressing the amplitude of the individual post-training MEPs within each block as a percentage of the average post-PAS MEP amplitude (referred to as "Training" in figures).

2.3.2. Behavioral measures

For each force peak, accuracy was recorded as the Euclidean distance between the applied force and the force necessary to reach the center of the target box (Stavrinos and Coxon, 2017). An error score was then calculated for each trial by summing the accuracy values for each of the 5 peaks included within a trial. For the assessment of motor performance, we quantified changes in the SAF using the skill measure proposed by Reis et al., (Reis et al., 2009), calculated as follows:

$$\text{Skill} = \frac{(1 - accuracy)}{accuracy\ (\ln(movement\ time)^{b})}$$

In contrast to Reis et al., who used a binary measure of accuracy, our accuracy measure was the summed Euclidean distance defined previously (Stavrinos and Coxon, 2017). Movement time was the average time taken to complete a trial. Skill values recorded for each movement speed were transformed with a logarithmic function to homogenize variance (Reis et al., 2009) and averaged within a time point, providing a functional measure sensitive to changes in performance at different movement speeds.

The dimensionless free parameter b has previously been shown to be relatively insensitive to changes in performance after training (Reis et al., 2009), and is therefore usually set at a constant value. However, given our use of different force transforms and comparison between age groups (i.e., factors that may affect task difficulty), we first compared b values from individual subjects between PAS conditions and age groups. These were calculated according to the approach of Reis et al., using error and movement values that were averaged over all time points, within each PAS condition and age group. Statistical comparison of these values showed no significant difference between groups ($p = 0.9$) or PAS conditions ($p = 0.4$), and no significant interaction between factors ($p = 0.9$). A single b parameter of 1.16 was therefore calculated based on all subject data.

Skill improvement post-PAS was quantified by normalizing the individual skill values recorded for each trial post-PAS, to the average skill value recorded across trials at the baseline (referred to as "PAS" in figures). Skill improvement during SVIT training was quantified by normalizing the average level of skill acquired within each training block, to the level of skill acquired during the first block of training. Skill improvement after training was quantified by normalizing the individual skill values recorded for each trial, at each time point after training, to average skill values recorded across trials after PAS (referred to as "Training" in figures). Finally, cumulative skill improvement over the course of each main session (referred to as "PAS + Training" in figures) was quantified by normalizing the individual skill values recorded for each trial, at each time point post training, to average skill values recorded across trials at the baseline.

2.4. Statistical analysis

All statistical analysis was performed using SPSS for Windows (version 24; SPSS Inc, Chicago, IL, USA). Normality of data was assessed using Kolmogorov-Smirnov tests, with log transformations applied where necessary to meet assumptions of normality. For clarity, data are displayed in nontransformed form. Unpaired students t-tests were used to compare age and N20 latencies between young and old groups. A three-factor repeated measures analysis of variance was used to compare the number of reported attentional stimuli between groups (young, old) and PAS conditions (PAS_{LTP}, PAS_{LTD}, $PAS_{Control}$). Significant main effects and interactions were further investigated using Bonferroni corrected post hoc tests.

A three-factor linear mixed model analysis with repeated measures (LMM_{RM}) was used to compare RMT between groups, PAS conditions, and sessions (day 1, day 2). A two-factor LMM_{RM} was used to compare MEP_{1mV} between groups, and PAS conditions. A three-factor LMM_{RM} was used to compare baseline SVIT error between groups, PAS conditions, and movement times (3.3, 3.6, 4.5, 6, 7.98 seconds for the metronome rate used). A three-factor LMM_{RM} was used to compare skill improvement during training between age groups, PAS conditions, and training blocks. Three-factor LMM_{RM}'s were also used to compare normalized MEP amplitude and SVIT skill improvement between groups, PAS conditions, and time points (MEP data: post 0, post 20, post 40; SVIT data: post 0, post 20, post 40, retention). Data normalized to the baseline and to the post-PAS time points were analyzed using individual models. Finally, a three-factor LMM_{RM} comparing SVIT skill improvement between groups, PAS conditions, and time points (post-PAS, post 40, retention) was used to investigate retention of SVIT skill. 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. As the aims of this study were specifically concerned with age-dependent changes in the effects of different priming conditions on the neurophysiological and behavioral response to training, it was decided a priori to focus on statistical effects or interactions including factors age group or PAS condition. Unless otherwise stated, all data are shown as mean ± standard error of the mean.

3. Results

All subjects completed the experiment in full and without adverse reaction. Table 1 shows subject characteristics, baseline neurophysiological values, and reported attentional stimuli compared between groups and PAS conditions. RMT was increased in older adults ($F_{1, 25} = 14.3$, $p = 0.0001$), varied between PAS conditions ($F_{2, 67} = 3.2$, $p < 0.05$), and there was interaction between group and session ($F_{1, 55} = 10.3$, $p = 0.002$; see Table 1 for post hoc comparisons). Although MEP_{1mV} was not different between groups ($F_{1, 25} = 0.3$, $p = 0.09$) or PAS conditions ($F_{2, 446} = 2.4$, $p = 0.6$), there was an interaction between factors ($F_{2, 446} = 5.0$, $p = 0.007$; see Table 1 for post hoc comparisons). For attentional stimuli applied during PAS, 4 younger subjects reported large values that far exceeded the actual number of stimuli (e.g., >60 stimuli in excess of the applied values), suggesting that these participants were not counting the correct stimuli applied to the hand area. These data were therefore removed from this analysis, which showed that the reported number of attentional stimuli were not different between age groups ($F_{1, 21} = 2.3$, $p = 0.1$) or PAS conditions ($F_{2, 42} = 0.4$, $p = 0.6$), and there was no interaction between factors ($F_{2, 42} = 2.3$, $p = 0.7$). Inclusion of these subjects did not influence these outcomes (age group effect, $F_{1, 25} = 2.3$, $p = 0.1$; PAS effect, $F_{2, 50} = 1.4$, $p = 0.3$;

Table 1
Subject characteristics

Dependent variable	Young	Older
Age (y)	20.4 ± 0.6	69.0 ± 1.6[a]
N20 latency (ms)	19.7 ± 0.4	21.2 ± 0.5[a]
RMT (%MSO)		
Session 1	41.1 ± 0.5	49.8 ± 1.0[a]
Session 2	42.6 ± 0.6[b]	49.4 ± 1.2[a]
MEP_{1mV} (mV)		
PAS_{LTP}	0.9 ± 0.05[c]	1.0 ± 0.04
PAS_{LTD}	1.1 ± 0.05	1.0 ± 0.05
$PAS_{Control}$	1.1 ± 0.05	1.0 ± 0.04
Attentional stimulus error[d]		
PAS_{LTP}	0.4 ± 0.3	0.9 ± 0.3
PAS_{LTD}	0.6 ± 0.4	1.2 ± 0.3
$PAS_{Control}$	0.3 ± 0.3	1.2 ± 0.3

[a] $p < 0.05$ when compared with young subjects.
[b] $p < 0.05$ when compared with session 1.
[c] $p < 0.05$ compared with PAS_{LTD} and $PAS_{Control}$.
[d] Calculated as the absolute deviation from the correct number of attentional stimuli applied.

interaction, $F_{2, 50} = 1.5$, $p = 0.2$), and therefore all subjects were included in the subsequent analyses of MEP and skill data.

3.1. Effects of PAS and training on MEP amplitude

Fig. 2 shows changes in MEP amplitude after priming PAS and training in young and older adults, with data collapsed over time for post-training MEPs. After PAS, normalized MEP amplitude was not different between groups ($F_{1, 25} = 0.3$, $p = 0.6$), but varied between PAS conditions ($F_{2, 376} = 5.9$, $p = 0.003$) and there was an interaction between factors ($F_{2, 376} = 3.2$, $p = 0.04$; Fig. 2A). For young subjects, normalized MEPs were not different between PAS conditions (all p-values > 0.5). For older subjects, normalized MEPs after PAS_{LTD} were reduced relative to both PAS_{LTP} ($p = 0.009$) and $PAS_{Control}$ ($p = 0.001$). Between-group comparisons showed that the response in young and older adults was not different for any PAS condition (all p-values > 0.2). After training, normalized MEPs (relative to post-PAS) varied between PAS conditions ($F_{2, 949} = 4.4$, $p = 0.01$), with the response after PAS_{LTD} being significantly greater than $PAS_{Control}$ ($p = 0.01$), but no difference between PAS_{LTD} and PAS_{LTP} ($p = 0.1$), or between PAS_{LTP} and $PAS_{Control}$ ($p > 0.9$). However, there was no difference between groups ($F_{1, 25} = 1.0$, $p = 0.3$) and no interaction between factors ($F_{2, 949} = 1.0$, $p = 0.4$; Fig. 2B). For the cumulative effect of both PAS and training, while there was no difference between groups ($F_{1, 25} = 2.1$, $p = 0.2$), normalized MEPs (relative to baseline) varied between PAS conditions ($F_{2, 951} = 3.3$, $p = 0.04$) and there was an interaction between group and PAS condition ($F_{2, 951} = 5.7$, $p = 0.004$; Fig. 2C). For young subjects, there was no difference in normalized MEP amplitude between PAS conditions (all p-values > 0.07). For older subjects, normalized MEPs after $PAS_{Control}$ were significantly larger than PAS_{LTD} ($p = 0.001$). In addition, between-group comparisons within each PAS condition showed that the response to $PAS_{Control}$ was greater in the older group ($p = 0.04$).

3.2. Baseline SVIT performance

Fig. 3 shows SVIT error values recorded at the start (baseline) of each PAS condition, in young (Fig. 3A) and older (Fig. 3B) adults. These data varied between age groups ($F_{1, 30} = 8.7$, $p = 0.006$), PAS conditions ($F_{2, 314} = 15.7$, $p < 0.0001$), and movement times ($F_{4, 641} = 55.8$, $p < 0.0001$). In addition, interactions between PAS condition and age group ($F_{2, 316} = 4.9$, $p = 0.008$; Fig. 3C) and movement time and age group ($F_{4, 644} = 3.0$, $p = 0.02$; Fig. 3D) were also found. While there were no differences in the baseline error between PAS conditions for older adults, error within the $PAS_{Control}$ condition was significantly reduced relative to both PAS_{LTP} and PAS_{LTD} (both p-values < 0.0001) in young subjects. In addition, between-group comparisons showed that error was reduced in young subjects at the baseline for both PAS_{LTD} ($p = 0.04$) and $PAS_{Control}$ ($p = 0.0003$). Furthermore, error was significantly reduced at the baseline in young subjects at slower movement speeds ($p < 0.003$).

3.3. Effects of PAS on SVIT training

During training, skill improvement was not different between age groups ($F_{1, 25} = 1.1$, $p = 0.3$). However, it did vary between PAS conditions ($F_{2, 1733} = 31.2$, $p < 0.0001$; Fig. 4A) and training blocks ($F_{10, 2252} = 3.4$, $p = 0.0002$), and there was an interaction between these factors ($F_{20, 2246} = 1.6$, $p = 0.04$; Fig. 4B). Post hoc analysis showed that skill did not improve as much after PAS_{LTP}, relative to both PAS_{LTD} (blocks 4–7 and 12; all p-values < 0.04) and $PAS_{Control}$ (blocks 6–7 and 10–12; all p-values < 0.03). In addition, skill improvement only increased over training following $PAS_{Control}$, with improvement during blocks 10 and 11 being greater than block 2 (both p-values < 0.05).

3.4. Effects of PAS on SVIT performance

Fig. 5 shows changes in normalized SVIT performance in young and older adults, providing an index of skill improvement (absolute skill values recorded at each time point in each PAS condition have

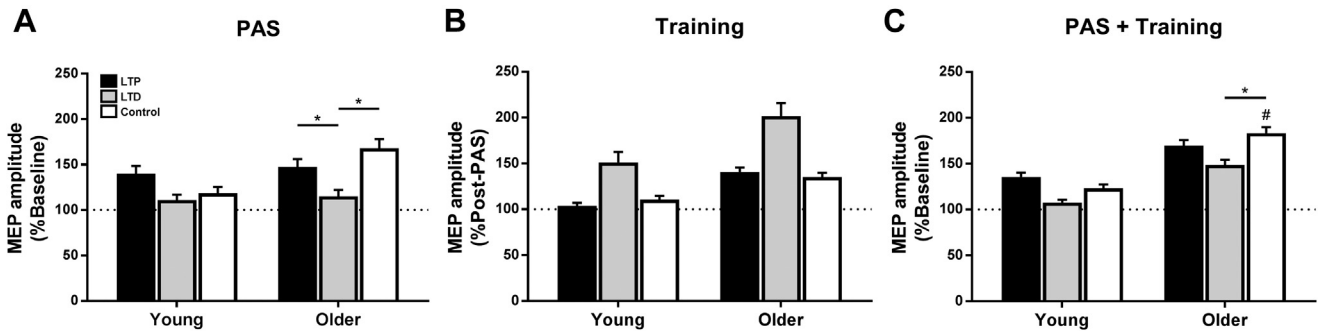

Fig. 2. Effect of PAS and training on MEP amplitude. Data show mean post-PAS MEP amplitude normalized to baseline (A), post-training MEP amplitude normalized to post-PAS MEP amplitude (B) and post-training MEP amplitude normalized to baseline (C). Post-training MEP data are collapsed over time point. The dotted horizontal line represents no change in MEP amplitude relative to baseline *(A, C)* or post PAS *(B)*. Plotted values include responses from 14 young (7 female) and 13 older (6 female) adults, which were compared using linear mixed model analysis. $^{\#}p < 0.05$ when compared with the same condition in older adults; $^{*}p < 0.05$. Abbreviations: PAS, paired associative stimulation; MEP, motor evoked potential.

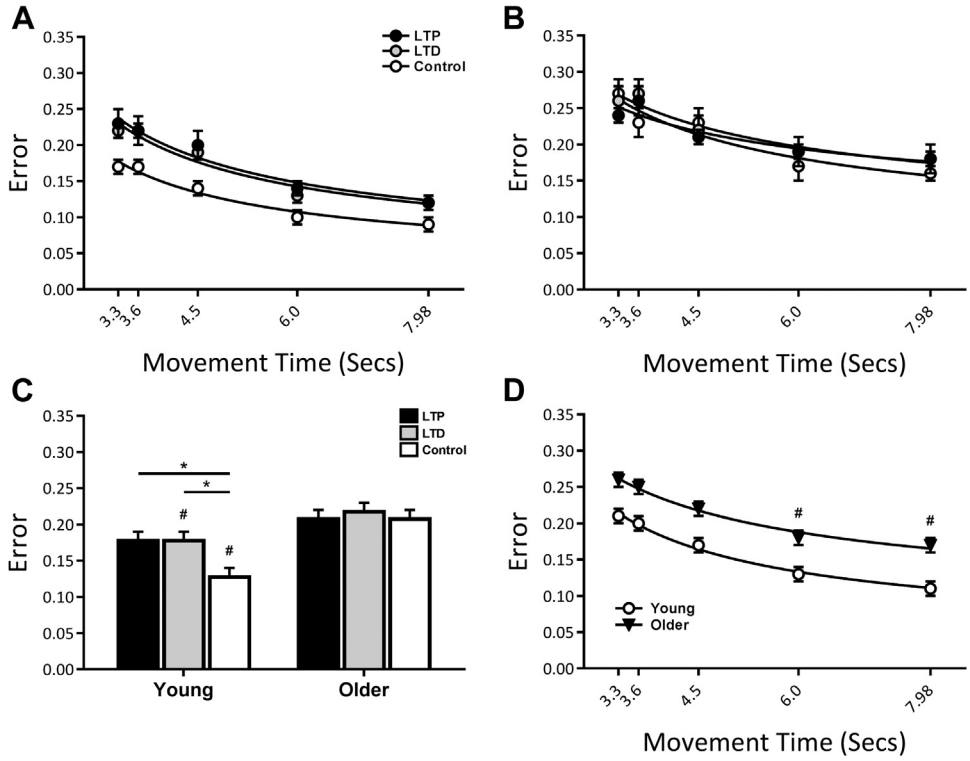

Fig. 3. Baseline SVIT performance in young and older adults. (A, B) SAF functions for young *(A)* and older *(B)* adults recorded at baseline during PAS_{LTP} *(black circles)*, PAS_{LTD} *(gray circles)* and $PAS_{Control}$ *(white circles)* sessions. (C) Baseline error values compared between groups and PAS conditions, collapsed over movement speed. (D) Baseline error values compared between groups and movement speeds, collapsed over PAS condition. Plotted values include responses from 14 young (7 female) and 13 older (6 female) adults, which were compared using linear mixed model analysis. $^{\#}p < 0.05$ when compared to the same condition in older adults; $^{*}p < 0.05$. Abbreviations: SVIT, sequential visual isometric task; PAS, paired associative stimulation; SAF, speed-accuracy function.

been provided in Supplementary Fig. 1). After PAS, skill improvement was not different between age groups ($F_{1, 25} = 1.1$, $p = 0.3$) or PAS conditions ($F_{2, 313} = 0.5$, $p = 0.6$) and there was no interaction between factors ($F_{2, 313} = 1.1$, $p = 0.3$; Fig. 5A). However, after training, skill improvement varied between PAS conditions ($F_{2, 1110} = 7.5$, $p = 0.001$) and there was an interaction between PAS condition and group ($F_{2, 1110} = 3.1$, $p = 0.04$; Fig. 5B). For young subjects, post-training skill improvement was not different between PAS conditions (all p-values > 0.2). For older subjects, post-training skill improvement after $PAS_{Control}$ was greater than both PAS_{LTP} ($p = 0.02$) and PAS_{LTD} ($p = 0.0001$). Within each PAS condition, there was no difference in skill improvement between age groups (all p-values > 0.06). For the cumulative effect of both PAS and training (Fig. 5C), while skill improvement varied between PAS conditions ($F_{2, 1169} = 3.1$, $p = 0.04$), post hoc analysis failed to identify any significant differences (all p-values > 0.05). Furthermore, no differences were found between groups ($F_{1, 25} = 0.05$, $p = 0.8$) and there was no interaction between factors ($F_{2, 1169} = 0.0002$, $p > 0.9$).

For the assessment of skill retention, no differences were found between groups ($F_{1, 25} = 0.1$, $p = 0.8$) or PAS conditions ($F_{2, 862} = 0.8$,

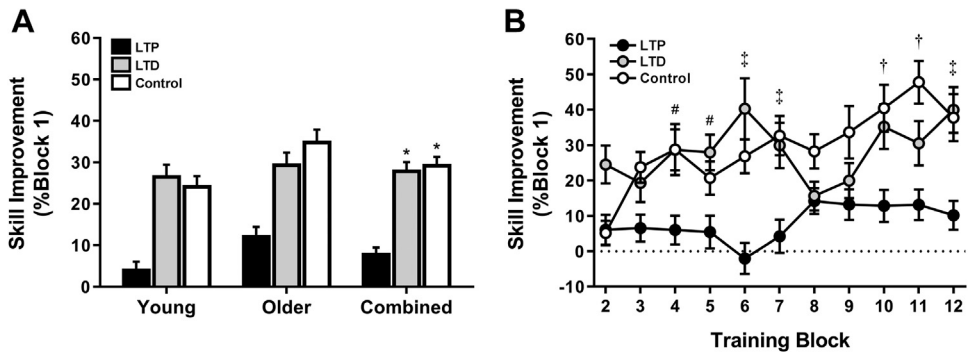

Fig. 4. Effects of PAS on SVIT training. (A) Skill improvement during the training period compared between PAS_{LTP} *(black bars)*, PAS_{LTD} *(gray bars)*, and $PAS_{Control}$ *(white bars)*, shown for young subjects, older subjects, and collapsed over age group. (B) Skill improvement within each block of the training period, compared between PAS conditions. Data have been normalized to the average level of skill recorded during the first block of training. The dotted horizontal line represents no change in skill relative to block 1. Plotted values include responses from 14 young (7 female) and 13 older (6 female) adults, which were compared using linear mixed model analysis. $^{*}p < 0.05$ compared with PAS_{LTP}; $^{\#}p < 0.05$ between PAS_{LTP} and PAS_{LTD}; $^{\dagger}p < 0.05$ between PAS_{LTP} and $PAS_{Control}$; $^{\ddagger}p < 0.05$ between PAS_{LTP} and both PAS_{LTD}/$PAS_{Control}$. Abbreviations: SVIT, sequential visual isometric task; PAS, paired associative stimulation.

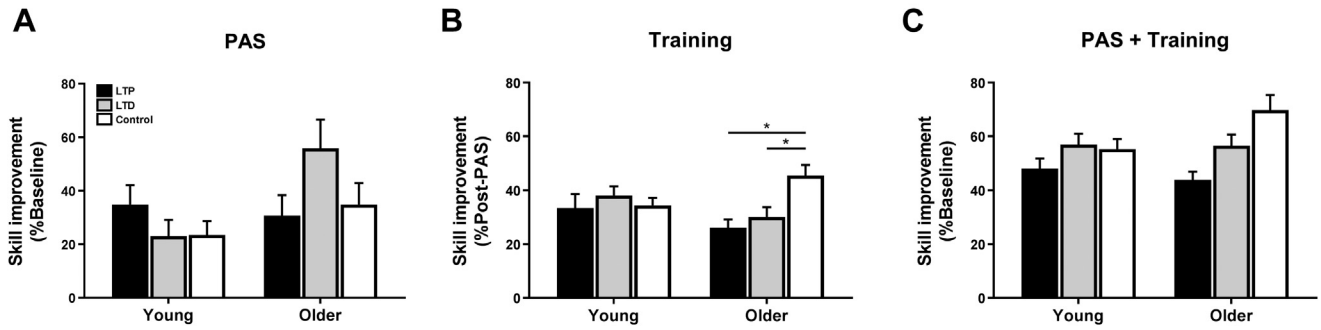

Fig. 5. Effect of PAS and training on SVIT acquisition. Data show SVIT skill improvement post-PAS normalized to baseline (A), post-training normalized to post-PAS (B) and post-training normalized to baseline (C). Data have been collapsed over time point and movement time. Plotted values include responses from 14 young (7 female) and 13 older (6 female) adults, which were compared using linear mixed model analysis. $^{*}p < 0.05$. Abbreviations: SVIT, sequential visual isometric task; PAS, paired associative stimulation.

$p = 0.4$). However, there was an interaction between age group and time point ($F_{2,\ 1073} = 3.2$, $p = 0.04$). For young subjects, skill improvement after 40 minutes was greater than after PAS ($p < 0.0001$) and remained elevated relative to post PAS at the 24 hours time point ($p = 0.008$). For older subjects, skill improvement at post 40 minutes was elevated relative to post PAS ($p < 0.0001$), but was not different between 24 hours retention and post PAS ($p > 0.9$) time points. Within each time point, there was no difference between age groups (all p-values > 0.1).

4. Discussion

This study used PAS in conjunction with a novel motor task to investigate the potential of priming stimulation for improving the response to motor training in older adults. For both young and older groups, we found that priming with PAS_{LTP} resulted in reduced skill acquisition during the training period. Furthermore, although post-training performance was unaffected by priming in young subjects, both PAS_{LTD} and PAS_{LTP} priming resulted in reduced post-training performance in older subjects. In addition, priming stimulation failed to affect the retention of SVIT performance, irrespective of age.

4.1. Effect of PAS and training on motor learning and performance

Consistent with a previous study using an SVIT task (Fujiyama et al., 2017), and in addition to an extensive literature using other motor tasks (for review: Seidler et al., 2010; Voelcker-Rehage, 2008), motor performance was reduced in older adults at the baseline. However, the magnitude of skill improvement during training was not different between age groups, suggesting that the ability of older adults to improve their performance was comparable with that of young adults. This is consistent with a growing literature showing that the elderly maintain a high capacity for learning new motor tasks (Cirillo et al., 2011; Fujiyama et al., 2017; Monteiro et al., 2017; Solesio-Jofre et al., 2018). Despite this, skill acquisition during training was significantly reduced in the PAS_{LTP} condition, relative to both PAS_{LTD} and $PAS_{Control}$. Consequently, LTP-priming appeared to have a negative influence on motor skill learning in both groups. This observation suggests that priming stimulation was able to regulate the acquisition of a visuomotor skill in a metaplastic manner, and that this effect was maintained in older adults. While a homeostatic effect of LTP-priming has been reported previously (Amadi et al., 2015; Hamada et al., 2014; Kang et al., 2010), other work has also reported beneficial effects (Hamada et al., 2014; Jung and Ziemann, 2009; Teo et al., 2010) or no effect (Kuo et al., 2008) on subsequent motor learning. Although the cause of these differential outcomes is currently unclear, variations in the utilized task may contribute. For example, beneficial effects of LTP-priming are associated with the performance of ballistic abduction tasks, for which a potentiation of corticospinal excitability may facilitate more forceful and synchronized activation of task-relevant muscles (Hamada et al., 2014). By contrast, such increases in excitability may be detrimental to the generation of coordinated patterns of muscle activation required for precise force control and more complex motor task acquisition (Hamada et al., 2014), as would be expected during the task utilized within the present study. However, other neurophysiological factors may also be important. For example, variations in the occlusion of LTP-like plasticity mechanisms (Cantarero et al., 2013) may result in differential (i.e., homeostatic *vs.* nonhomeostatic) interactions; groups having greater occlusion after initial LTP induction are more likely to demonstrate a reduced/absent response to subsequent LTP-inducing interventions.

Despite the metaplastic effects of PAS_{LTP} during the training period, there was no effect of PAS_{LTD} on motor skill learning during training, suggesting that our results can only be considered partially homeostatic in nature. This occurred despite significant increases in MEP amplitude after training when primed with PAS_{LTD}, supporting the emerging view that changes in the MEP may not be directly relevant to many forms of motor behavior (Bestmann and Krakauer, 2015). Nonetheless, one possible explanation for the ineffectiveness of LTD-priming on motor skill learning may have been that both groups were training at the upper limit of their capacity for the SVIT task, resulting in a ceiling effect that prevented further homeostatic potentiation after PAS_{LTD}. However, the beneficial effects of NIBS on SVIT training may occur at least partially offline (Reis et al., 2009), suggesting that PAS_{LTD} may have resulted in improved training performance on subsequent days. As the retention session of the present study did not include a second training block, this possibility will require investigation in future studies.

After training, the improvement in motor performance observed in young subjects was not different between PAS conditions, suggesting that priming stimulation failed to effect post-training performance in this group. Although contradictory to some previous work (Jung and Ziemann, 2009; Kang et al., 2010), this outcome is in keeping with recent studies reporting no effect of PAS_{LTP} (Hamada et al., 2014) or PAS_{LTD} (Sasaki et al., 2018) on motor performance after training. In contrast to the young group, post-training performance after active priming was significantly reduced in older adults relative to $PAS_{Control}$, and this effect was not different between PAS_{LTP} and PAS_{LTD}. This outcome is in contrast to the recent findings of Fujiyama et al (Fujiyama et al., 2017), who reported that SVIT performance after the concurrent application of anodal tDCS (i.e., an LTP-inducing NIBS paradigm; Nitsche et al., 2003; Nitsche and Paulus, 2000) and training was significantly greater in older adults

when primed by cathodal tDCS (i.e., an LTD-inducing NIBS paradigm; Nitsche et al., 2003; Nitsche and Paulus, 2000). Importantly, the training task utilized by Fujiyama et al was almost identical to that of the present study, differing only in the number of force gates (9 in Fujiyama et al., 5 in the present study) and the structure of the training block (9 blocks of 2 minutes each in Fujiyama et al., 12 blocks of 8 trials in the present study). These differential effects of priming on post-training performance are therefore unlikely to stem from variations in the training task, instead possibly reflecting the alternative NIBS approaches used in each study. A major difference in this regard is the application of stimulation during training by the previous study. This additional, concurrent stimulation may facilitate stronger activation of cortical motor networks that are endogenously activated during task performance (Miniussi et al., 2013), possibly acting as a "gating" mechanism that could enhance the response to priming (Ziemann and Siebner, 2008). In addition, whereas tDCS may have nonfocal effects (Datta et al., 2009; Thielscher et al., 2011) that may activate a number of brain areas involved in task performance, the stimulation provided by PAS is more specific in nature, both spatially and temporally (for review: Polanía et al., 2018; Suppa et al., 2017). We could therefore speculate that beneficial functional effects require priming to modulate the relevant motor network more broadly, as opposed to affecting specific components within the activated network. This concept requires further investigation in future studies.

Within the present study, a number of factors may have contributed to the differential effects of priming on post-training performance in each group. For example, recent work from our group has shown that the timing-dependent interaction between 2 plasticity-inducing paradigms (Müller-Dahlhaus et al., 2015) is modified by age, with the strongest interaction occurring in older adults when using an interval of 30 minutes (Sidhu et al., 2017). Consequently, a longer interval between priming and training may have provided more consistent effects between groups, or alternatively, the most effective interval may be different in young and older adults. In addition, the efficacy of the applied PAS paradigm was developed in healthy young populations. Given that direct evidence in animal models suggests that the aging process modifies the threshold for plasticity induction (Barnes et al., 2000; Deupree et al., 1993), in addition to the stability of induced plasticity (Barnes and McNaughton, 1980), whereas work in humans reports reduced effectiveness of NIBS-based plasticity interventions (see Section 1), it seems likely that interventions developed in young subjects may not be optimal for use in older adults (Freitas et al., 2013). Although we were able to partially mitigate this factor by adjusting the timing of the afferent stimulus to allow for age-related reductions in sensory conduction, additional optimization of stimulus intensities may be necessary. In particular, recent work suggests that temporal characteristics of the intracortical circuits activated by TMS, which are modulated by PAS (Di Lazzaro et al., 2009a; Di Lazzaro et al., 2009b), are different in older adults (Opie et al., 2018), and that adjusting an NIBS intervention to allow for these differences may result in a significantly enhanced plasticity response (Opie et al., 2018). Priming interventions that are individualized based on the recruitment of intracortical circuits may therefore be a more effective approach in older adults.

4.2. Effects of PAS and training on corticospinal excitability

In young subjects, the MEP response to PAS was not different between priming conditions, suggesting that priming stimulation failed to induce overt changes in corticospinal excitability in this group. For older subjects, although the response to PAS_{LTD} was significantly reduced relative to both PAS_{LTP} and $PAS_{Control}$, MEP amplitude remained above baseline values (i.e., 115% of baseline). Furthermore, while PAS_{LTP} resulted in a relatively large increase in MEP amplitude (147% of baseline), interpretation of this response is complicated by a greater response to $PAS_{Control}$ (168% of baseline). Taken together, these observations demonstrate that priming stimulation failed to induce the previously observed excitatory response after PAS_{LTP}, inhibitory response after PAS_{LTD}, and lack of response after $PAS_{Control}$ (Stefan et al., 2002, 2000; Wolters et al., 2003). This is despite having controlled for important methodological factors, including the level of subject attention (Stefan et al., 2004), time-of-day effects (Sale et al., 2007, 2008), and interindividual variations in the conduction time of the afferent stimulus (Ziemann et al., 2004). While it is currently unclear why priming stimulation failed to induce the expected effects, the high variability associated with both PAS (Lahr et al., 2016; López-Alonso et al., 2014) and MEP measures of corticospinal excitability (Goldsworthy et al., 2016) is likely to be an important factor. This is no doubt compounded by the effectiveness of PAS only being assessed at a single time point directly after stimulation, whereas conventional effects may have been apparent at later time points.

After training, there was a large increase in MEP amplitude within the PAS_{LTD} condition that was significantly greater than the response observed after both PAS_{LTP} and $PAS_{Control}$. These observations suggest that the application of PAS_{LTD} led to a significant potentiation of the neuroplastic response to motor training, despite a lack of MEP suppression after PAS_{LTD} priming. This outcome is consistent with the induction of homeostatic metaplasticity, in which a period of reduced post-synaptic activation lowers the threshold for LTP induction, making an excitatory response to subsequent input more likely (Abraham, 2008; Hulme et al., 2013)—a result that supports findings of several previous studies (Müller-Dahlhaus and Ziemann, 2015). However, given that changes in MEP amplitude after training were not different between PAS_{LTP} and $PAS_{Control}$ (Fig. 2B), whereas a reduction in excitability would be expected in a homeostatic interaction (Müller et al., 2007), these results can, again, only be considered partially homeostatic in nature.

The effects of PAS_{LTD} on MEP amplitude after training was not different between age groups (and in fact was numerically greater in older adults), suggesting that the homeostatic response to LTD-priming is maintained in the elderly. This is in contrast to previous findings from our group, where the response to paired blocks of PAS (Opie et al., 2017a) and theta burst stimulation (Opie et al., 2017b) was reduced in older adults. Given that recent work suggests that metaplastic interactions are temporally sensitive (Müller-Dahlhaus et al., 2015), one factor that may have contributed to these contradictory findings is that priming stimulation and training were separated by a 20 minutes period in the present study, whereas the paired interventions of the previous studies were separated by a 10 minute period. Alternatively, it may be that while older adults are less responsive to the experimental techniques used to test metaplastic interactions in the previous studies (i.e., PAS and theta burst stimulation), they remain responsive to the more behaviorally relevant cortical activation associated with the performance of a motor task. For example, although TMS has been shown to activate both local and distributed aspects of the motor network (Bestmann et al., 2008), it is unlikely that this pattern of activation is identical to that underlying performance of a complex learning task. It seems possible that there may be variations in the specific elements involved, in addition to the order and timing of their activation.

It is necessary to acknowledge some limitations within the present study. First, RMT was increased in older adults, and this was consistent across PAS conditions. Although there have been mixed effects of age reported by previous studies, this finding is consistent with a recent meta-analysis including data from 453 young and 485 older adults (Bhandari et al., 2016). Despite this, as RMT was not

used to calibrate other stimulus intensities within the present study, the differences between groups could not have affected our findings. Second, MEP_{1mV} in young subjects was significantly reduced at the beginning of the PAS_{LTP} session. However, the small difference (0.1 mV) was unlikely to be experimentally relevant, and amplitudes were still comparable between age groups for all PAS conditions. Furthermore, analysis of MEP data within the present study was based on normalized values, reducing the influence of baseline variance. Third, several young subjects incorrectly reported large numbers of attentional stimuli during the PAS protocol, suggesting they were not attending to the correct stimulus. However, this did not influence the findings in the present study, as removal of these young subjects did not influence any of the main outcomes for the MEP or skill analysis. Fourth, baseline skill levels were reduced in older adults. As data were normalized to baseline, this difference makes it difficult to separate age-related deficits in motor learning from baseline differences in performance (Berghuis et al., 2016; Centeno et al., 2018; Vallence and Goldsworthy, 2013), and between-group comparisons of motor learning may therefore be confounded within the present study. However, baseline performance was not different between PAS conditions in the older group. Within-group comparisons of motor performance between PAS conditions, which directly address the main aims of our study, are therefore not affected by this limitation. Finally, as TMS was applied without neuronavigation, we cannot exclude the possibility that small inconsistencies in the location of stimulation may have contributed some variability to our data.

In conclusion, the present study found differential, partially homeostatic effects of priming stimulation on the neurophysiological and behavioral response to motor training. In particular, we found that PAS_{LTD} resulted in a greater potentiation of corticospinal excitability after training, whereas PAS_{LTP} was detrimental to performance during the training period. Furthermore, these outcomes were not different between young and older adults, suggesting that homeostatic metaplasticity may be maintained in the elderly. By contrast, post-training performance was unaffected by priming stimulation in young participants, whereas active priming was detrimental to post-training performance in older participants. While it is therefore possible to homeostatically regulate the motor system of older adults using PAS, developing the functional utility of this approach requires further investigation, possibly involving extended training interventions and individualized priming approaches.

Disclosure

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neurobiolaging.2019.05.017.

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