George M. Opie, Eleni Vosnakis, Michael C. Ridding, Ulf Ziemann, John G. Semmler
Priming theta burst stimulation enhances motor cortex plasticity in young but not old adults
Brain Stimulation, 2017; 10(2):298-304

Priming theta burst stimulation enhances motor cortex plasticity in young but not old adults

George M Opie[1], Eleni Vosnakis[1], Michael C Ridding[2], Ulf Ziemann[3] and John G Semmler[1]

1. Discipline of Physiology, School of Medicine, The University of Adelaide, Adelaide, Australia
2. Robinson Research Institute, School of Medicine, The University of Adelaide, Adelaide, Australia
3. Department of Neurology & Stroke, and Hertie-Institute for Clinical Brain Research, Eberhard Karls University of Tübingen, Tübingen, Germany

Correspondence: John G. Semmler, Ph.D.
School of Medicine
The University of Adelaide
Adelaide, South Australia 5005
Australia
Telephone: Int + 61 8 8313 7192
FAX: Int + 61 8 8313 4398
E-mail: john.semmler@adelaide.edu.au

Abstract

Background: Primary motor cortex neuroplasticity is reduced in old adults, which may contribute to the motor deficits commonly observed in the elderly. Previous research in young subjects suggests that the neuroplastic response can be enhanced using non-invasive brain stimulation (NIBS), with a larger plastic response observed following priming with both long-term potentiation (LTP) and depression (LTD)-like protocols. However, it is not known if priming stimulation can also modulate plasticity in older adults.

Objective: To investigate if priming NIBS can be used to modulate motor cortical plasticity in old subjects.

Methods: In 16 young (22.3 ± 1.0 years) and 16 old (70.2 ± 1.7 years) subjects, we investigated the response to intermittent theta burst stimulation (iTBS; LTP-like) when applied 10 mins after sham stimulation, continuous TBS (cTBS; LTD-like) or an identical block of iTBS. Corticospinal plasticity was assessed by recording changes in motor evoked potential (MEP) amplitude.

Results: In young subjects, priming with cTBS (cTBS+iTBS) resulted in larger MEPs than priming with either iTBS (iTBS+iTBS; $P = 0.001$) or sham (sham+iTBS; $P < 0.0001$), while larger MEPs were seen following iTBS+iTBS than sham+iTBS ($P < 0.0001$). In old subjects, the response to iTBS + iTBS was not different to sham + iTBS ($P > 0.9$), whereas the response to cTBS + iTBS was reduced relative to iTBS + iTBS ($P = 0.02$) and sham + iTBS ($P = 0.04$).

Conclusions: Priming TBS is ineffective for modifying M1 plasticity in older adults, which may limit the therapeutic use of priming stimulation in neurological conditions common in the elderly.

Keywords: Ageing, Transcranial Magnetic Stimulation, Theta Burst Stimulation, Metaplasticity, Priming

Abbreviations: cTBS, continuous TBS; EMG, electromyography; FDI, first dorsal interosseous; iTBS, intermittent TBS; LTD, long-term depression; LTP, long-term potentiation; MEP, motor evoked potential; mGluR, metabotropic glutamate receptor; M_{max}, maximum M-wave; MMSE, mini-mental state examination; MSO, maximum stimulator output; NIBS, non-invasive brain stimulation; NMDA, N-methyl-D-aspartate; RMT, resting motor threshold; rTMS, repetitive TMS; TBS, theta burst stimulation; TMS, transcranial magnetic stimulation; PAS, paired associative stimulation.

Introduction

It is now well recognised that the neural architecture of the human brain is not static, but instead demonstrates extensive and remarkable flexibility. This flexibility, referred to as neuroplasticity, has been shown to represent a fundamental component of learning and memory [1, 2], in addition to being important for recovery from brain injury or damage [3]. While the mechanisms contributing to neuroplasticity are not fully understood, an extensive body of literature has identified several contributing factors, including alterations to inhibitory neurotransmission [4] and unmasking of latent neuronal pathways [5]. However, animal research has shown that long-term potentiation (LTP) or depression (LTD) of synaptic strength is particularly important [see; 6]. These findings have been supported in humans by studies using non-invasive brain stimulation (NIBS), a technique able to induce and measure LTP- and LTD-like changes within the human brain [7].

Some of the best evidence for the functional importance of neuroplasticity is seen in situations where plasticity is altered. While such changes are often associated with central nervous system damage or pathology [8-10], they may also be observed in otherwise healthy individuals. For example, several lines of evidence suggest that neuroplastic capacity is reduced by healthy ageing. This includes reports that older adults demonstrate a reduced potentiation of corticospinal excitability following the application of plasticity-inducing NIBS paradigms [11-14], as well as following a period of motor training [15, 16]. The functional importance of neuroplasticity suggests that this reduced response in older adults may contribute to the motor deficits commonly associated with the ageing process. An improved understanding of age-related reductions in plasticity, as well as the development of interventions able to ameliorate this deficiency, therefore represents an important area of neuroscience research.

The response to a plasticity-inducing paradigm is known to be affected by a number of factors, including time of day, attentional focus and genetics [see; 17]. However, one major influence on plasticity induction is the level of previous activity within the area targeted by the intervention [18]. A history of increased synaptic activity within the target area can reduce or even reverse the expected response to a plasticity inducing NIBS paradigm. This type of interaction is referred to as metaplasticity and has been suggested to represent a means of homeostatically moderating changes in synaptic excitability in order to avoid the potentially destabilising influence of run-away potentiation/depression that LTP and LTD are inherently capable of producing [see; 19]. However, this mechanism has also formed the basis for interventions aiming to manipulate the plasticity response by first 'priming' synapses of the target area. This approach has been studied in young subjects using a number of different NIBS techniques, with the findings suggesting that the resulting neuroplastic modifications are stronger, longer lasting and more stable [20]. However, it is currently unknown if priming stimulation can be used to compensate for age-related reductions in the plasticity response to NIBS interventions.

Therefore, the aim of the current study was to investigate the efficacy of priming stimulation in healthy elderly adults. This was accomplished by comparing the response to paired blocks of a NIBS protocol [theta burst stimulation, TBS; 21], separated by a 10 min rest period, between young and old adults. In keeping with homeostatic metaplasticity mechanisms, we hypothesised an increase in LTP-like plasticity when the induction protocol was primed by a prior LTD-like plasticity protocol. However, based on previous observations of age-related declines in the response to TBS [13], we also expected that this effect would be reduced in elderly adults.

Methods

16 young (mean ± SD, 22.3 ± 1.0 years; 11 females) and 16 old (mean ± SD, 70.2 ± 1.7 years; 9 females) subjects were recruited from the university and wider community to participate in the current study. Exclusion criteria included a history of neurological or psychiatric disease, or current use of psychoactive medication (sedatives, antipsychotics, antidepressants etc.). Hand preference and laterality were assessed using the Edinburgh Handedness Inventory [22], while cognitive impairment was assessed using the mini-mental state examination [MMSE; 23]. 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 prior to participation.

Experimental Arrangement

Subjects were required to attend the laboratory on 3 occasions separated by at least 1 week. To avoid the confounding influence of diurnal variations in cortisol on the induction of cortical plasticity [24], all experiments were conducted between 11 am and 4 pm, with repeat sessions within each subject always occurring at the same time of day. During each experimental session, subjects sat in a chair with their right arm abducted approximately 45° at the shoulder, and right forearm and hand resting on a cushion placed next to them. Surface electromyography (EMG) was recorded from the first dorsal interosseous (FDI) muscle of the right hand using two Ag–AgCl electrodes placed approximately 2 cm apart in a belly-tendon montage and a strap placed around the wrist to ground the electrodes. EMG signals were amplified (x 1000) and band-pass filtered (20 Hz–1 kHz) using a CED 1902 signal conditioner (Cambridge Electronic Design Co. Ltd, Cambridge, UK), before being digitized

at 2 kHz using a CED 1401 analogue-to-digital converter (Cambridge Electronic Design Co. Ltd, Cambridge, UK) and stored on a computer for later off-line analysis.

Experimental Procedures

The experimental protocol is shown in Figure 1. Within each session, all baseline and post-test TBS measures were the same. However, the type of intervention differed between sessions.

Maximal compound muscle action potential (M_{max}). In a subset of subjects (13 young, 13 old), electrical stimulation applied at the wrist was used to stimulate the ulnar nerve, generating maximal compound muscle action potentials within FDI. Stimuli were applied using a constant-current stimulator (DS7AH, Digitimer, UK) and bipolar surface electrodes with the cathode positioned distally. Each stimulus was a square wave pulse of 100 μs duration and intensity set at 120% of that required to produce a maximal response in FDI (i.e. 120% M_{max}). M_{max} was obtained by averaging the responses to 5 stimuli delivered at the beginning of each experimental session.

Transcranial magnetic stimulation (TMS). 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. This location was marked on the scalp for reference and continually checked throughout the experiment. TMS was delivered at 0.2 Hz for all measurements.

Resting motor threshold (RMT) was defined as the minimum stimulus intensity producing an MEP amplitude ≥ 50 μV in at least 3 out of 5 trials while the right FDI was completely relaxed. RMT was assessed at the beginning of each experimental session and expressed as a percentage of maximum stimulator output (MSO). Corticospinal excitability was assessed by investigating changes in the amplitude of the motor evoked potential (MEP) recorded during complete relaxation of FDI. At baseline, the stimulus intensity was set at the level producing an MEP with peak-to-peak amplitude of ~ 1 mV when averaged over 20 trials. This intensity was then used to record all subsequent blocks of MEPs. Following baseline measurements, 10 MEPs were recorded between the first (priming TBS) and second (test TBS) blocks of TBS (referred to as post-priming TBS MEPs), and then every 10 minutes for the 60 minutes following test TBS (referred to as post-test TBS MEPs).

Theta Burst Stimulation (TBS). Theta burst stimulation was applied to the hand area of the left primary motor cortex using a Super Rapid magnetic stimulator (Magstim, Dyfed, UK) connected to an air-cooled figure-of-eight coil. The stimulation protocol was the same as that originally described by Huang *et al.*, [21], consisting of TMS triplets applied at 50 Hz and repeated at 5 Hz. When applied continuously (cTBS) for 40 seconds (i.e., 600 pulses), this pattern of stimulation can temporarily reduce cortical excitability [21]. However, when applied for 190 seconds in an intermittent pattern (iTBS; 2 seconds on, 8 seconds off), cortical excitability is temporarily increased [21]. Within the current study, priming and test TBS protocols were separated by a 10-minute interval [20, 25, 26]. While test TBS was always iTBS, priming TBS was either cTBS, iTBS or sham stimulation, with each paradigm applied within a separate session in a randomised, single-blinded, crossover design. Sham stimulation was achieved by applying iTBS through a sham rTMS coil (placebo coil PN 3285-00; Magstim, Dyfed, UK) that generates the audible click associated with TMS, but without applying any stimulation to the brain. For all TBS blocks, stimulation was applied at

an intensity of 70% RMT [27], the value of which was determined immediately prior to application of the first block.

Data Analysis

Data analysis was completed manually by visual inspection of offline EMG data. Traces showing any muscle activity prior to the stimulus were removed from the analysis. Across conditions, this resulted in the removal of 88/3360 (~ 3%) trials in young subjects and 206/3360 (~ 6%) trials in old subjects. MEP and M_{max} amplitudes were measured peak-to-peak and expressed in mV. Isolated changes in corticospinal excitability following priming TBS (post-priming TBS MEPs), and cumulative changes following test TBS (post-test TBS MEPs) were quantified by expressing individual MEP amplitudes within each block as a percentage of the average MEP amplitude recorded at baseline. Furthermore, the specific influence of test TBS was also quantified by expressing the amplitude of the individual post-test TBS MEPs within each block as a percentage of the average post-priming TBS MEP amplitude (Figure 1).

Statistical Analysis

Unpaired students *t*-tests were used to compare age, handedness and MMSE scores between young and old groups. Individual two-way repeated measures analysis of variance ($ANOVA_{RM}$) were used to compare RMT and M_{max} amplitude between age groups (young, old) and sessions, with significant main effects and interactions further investigated using Bonferroni corrected post hoc tests. Violations of sphericity were assessed using Mauchly's test, but correction was not necessary as all variables met this assumption. Effects of age group, time (mid-intervention, post 10, post 20, post 30, post 40, post 50 & post 60) and intervention (cTBS + iTBS, iTBS + iTBS, sham + iTBS) on MEP amplitude were assessed using linear mixed model analyses with repeated measures. Data normalised to baseline and

to the mid-intervention time point were analysed using individual models. 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. Unless otherwise stated, all data are shown as mean ± standard error of the mean (SEM).

Results

All subjects completed the experiment in full and without adverse reaction. With the exception of age, no differences in subject characteristics, RMT or M_{max} amplitude were found between groups or sessions (Table 1). Furthermore, the amplitude of the baseline MEP was not different between groups ($F_{1, 30} = 1.1$, $P = 0.3$) or sessions ($F_{2, 1334} = 1.4$, $P = 0.3$), and there was no interaction between factors ($F_{2, 1334} = 1.7$, $P = 0.2$; Table 2).

Isolated and cumulative effects of TBS intervention in young and old adults

Figure 2A – 2C shows the main effects of each priming condition on the response to test TBS, collapsed over time, in young and old adults. After priming TBS, MEP amplitude was not different between priming conditions ($F_{2, 670} = 3.4$, $P = 0.9$) or age groups ($F_{1, 30} = 0.7$, $P = 0.4$), and there was no interaction between factors ($F_{2, 670} = 0.8$, $P = 0.5$; Fig 2A).

After test TBS, there was no difference between age groups ($F_{1, 30} = 0.03$, $P = 0.9$) or priming conditions ($F_{2, 3976} = 0.8$, $P = 0.5$), but the interaction between factors was significant ($F_{2, 3976} = 4.1$, $P = 0.02$; Fig 2B). In young subjects, the response to iTBS-primed iTBS was not different to sham-primed iTBS ($P > 0.9$) or cTBS-primed iTBS ($P = 0.1$). However, the response to cTBS-primed iTBS was greater than sham-primed iTBS ($P = 0.01$). In old subjects, no significant differences for iTBS were found between priming conditions (all P-values > 0.8). In addition, the iTBS response to each priming condition was not different between age groups.

The cumulative effect of priming + test TBS was again not different between age groups ($F_{1, 30} = 1.2$, $P = 0.3$), but varied between priming conditions ($F_{2, 4436} = 14.7$, $P < 0.0001$), and there was an interaction between factors ($F_{2, 4436} = 32.7$, $P < 0.0001$; Fig 2C). In young subjects, the response to both iTBS + iTBS and cTBS + iTBS was greater than the response to sham + iTBS (both *P*-values < 0.0001), but there was no difference in response between iTBS + iTBS and cTBS + iTBS ($P = 0.08$). In old subjects, the response to iTBS + iTBS was not different to sham + iTBS ($P > 0.9$), but was greater than cTBS + iTBS. Furthermore, the response to sham + iTBS was also greater than cTBS + iTBS. In addition, cTBS + iTBS produced a greater response in young than old subjects, whereas the response to both iTBS + iTBS and sham + iTBS was not different between age groups (both *P*-values > 0.2).

Given that variations in the menstrual cycle may affect the response to a plasticity inducing paradigm [28], and the current study utilised a higher proportion of female subjects, we ran additional analyses to compare the response of male and female subjects. While this analysis did not produce a significant effect of gender ($F_{1, 28} = 0.7$, $P = 0.4$), there was an interaction between gender, age and priming condition ($F_{2, 4405} = 4.8$, $P = 0.008$). However, all post hoc comparisons between genders failed to reach significance. Subsequently, the slightly higher proportion of females within our sample did not influence our findings.

Time-related variations in the effect of priming condition in young and old adults

Figure 3 shows time-related variations in the response to each priming condition in young and old adults. For post-test TBS responses normalised to the post-priming response (effect of test TBS), changes in MEP amplitude varied over time ($F_{5, 3344} = 4.4$, $P = 0.001$) and this effect was different between priming conditions ($F_{10, 4422} = 3.4$, $P = 0.0002$) and age groups ($F_{5, 3344} = 3.8$, $P = 0.002$), and the interaction between time, age group and priming condition was significant ($F_{10, 4422} = 1.9$, $P = 0.04$; Fig 3A & 3C). In young subjects, post hoc

comparisons showed that the response to both iTBS-primed iTBS and cTBS-primed iTBS was greater than sham-primed iTBS at the 50 min and 60 min time points (all P-values < 0.05). In old subjects, no consistent time related differences between priming conditions were found.

For post-intervention responses normalised to baseline (effect of priming + test TBS), changes in MEP amplitude varied over time ($F_{6, 3677} = 5.3$, $P < 0.0001$), and this effect differed between priming conditions ($F_{12, 5076} = 2.7$, $P = 0.001$) but not age groups ($F_{6, 3677} = 1.5$, $P = 0.2$). However, the interaction between age group, priming condition and time was significant ($F_{12, 5076} = 1.8$, $P = 0.04$). Post hoc comparisons in young subjects showed that the response to both iTBS + iTBS and cTBS + iTBS was greater than sham + iTBS at 30 min, 50 min and 60 min following the intervention (all *P*-values < 0.0003), while differences between cTBS + iTBS and sham + iTBS also reached significance at the 20 min time point ($P = 0.002$). However, there was no difference between cTBS + iTBS and iTBS + iTBS at any time point (Fig 3B). In old subjects, no consistent time-related variation in MEP amplitude was seen in any of the priming conditions (Fig 3D).

Discussion

The current study investigated the ability of priming stimulation to modify the induction of motor cortical neuroplasticity in young and old adults. Our main finding was that priming stimulation was ineffective in old adults, whereas an increased plasticity response was observed following priming in young adults. Furthermore, in both groups, these effects were not dependent on the type of priming protocol.

Corticospinal excitability was unchanged following priming TBS

In both age groups, MEP amplitude following priming stimulation was unchanged relative to sham, suggesting that corticospinal excitability was not modified by priming. In young subjects, this finding is at first sight contrary to the reported excitatory and inhibitory effects of cTBS and iTBS, respectively [29]. Furthermore, while the response to cTBS has been previously reported to be reduced in older subjects [13], the response to iTBS is thought to be unaffected by age [30, 31]. Despite this, the current study is not the first to report reduced efficacy of TBS, with several previous studies having failed to observe changes in MEP amplitude [26, 32-34], or finding variability in the direction of excitability change [35]. As the effects of priming stimulation could only be assessed at a single time point 5 mins post priming TBS, and changes in MEP amplitude vary over time [36], it may be that this single time point was not optimal for observing the effects of priming within this subject cohort. In addition, the high inter-individual variability of response to TBS is often viewed as an important factor contributing to the contradictory outcomes reported by many studies. A number of elements have been suggested to contribute to this, including stimulation parameters, the presence of genetic polymorphisms and variations in the preferential recruitment of the early and late Indirect (I) waves [35, 37, 38], all of which may have contributed to our observations. Nonetheless, priming by itself does not need to alter synaptic efficacy to induce metaplasticity [18], and the lack of change in corticospinal excitability following priming stimulation removes the potential confounding influence of altered MEP amplitude on the subsequent response to test TBS.

Metaplastic interactions are reduced in older adults

Left unchecked, the mechanisms of neuroplasticity can produce run-away potentiation or depression of synaptic activity, subsequently destabilising neural function. For these reasons, mechanisms exist which are thought to govern the induction of neuroplasticity. Broadly

referred to as metaplasticity [18, 39], these mechanisms have been extensively investigated in young subjects using a range of NIBS priming protocols. Many studies within this literature report a homeostatic interaction between stimulation protocols, during which the use of priming and test protocols that are similar in nature (i.e. both increasing/decreasing excitability), produces a response that is ***reduced*** in comparison to the response seen when the test protocol is applied in isolation [for review, see; 19]. However, non-homeostatic metaplasticity has also been reported, during which the application of priming and test protocols that are similar in nature produces a response that is ***increased*** in comparison to the response seen when the test protocol is applied in isolation [20, 25, 26]. A recent study has reported that interactions between two blocks of paired-associative stimulation (PAS) are non-homeostatic when separated by short periods, but homeostatic when separated by longer periods [40], suggesting that the inter-block interval is an important determinant of the metaplastic response to priming stimulation.

In young subjects, the response to test TBS (iTBS) was enhanced following priming with cTBS, and the combined effects of priming + test TBS were greatest within the cTBS + iTBS condition. These observations are consistent with the induction of homeostatic metaplasticity, and support the findings of Murakami *et al.*, [41], who observed a potentiated response to iTBS when primed by cTBS. However, we also observed an enhanced response for the iTBS + iTBS condition in young subjects, suggesting activation of non-homeostatic mechanisms following priming with iTBS. This result is in contrast to the findings of Murakami *et al.*, [41] and Gamboa *et al.*, [42], both of whom observed a negative interaction between paired blocks of iTBS. One factor that may have contributed to these contrasting results is the time between priming and test TBS, as this interval differed between the current (10 min) and previous (Murakami *et al.*, 15 mins; Gamboa *et al.*, 2, 5 & 20 min) studies. In addition, while the previous studies set TBS intensity based on active motor threshold, requiring a period of

muscle activation prior to the intervention, the current study utilised resting motor threshold, therefore negating the need to activate the muscle. This may be a particularly important difference, as voluntary contraction has been shown to modify interactions between paired blocks of cTBS [26]. Despite this, our findings in young subjects suggest that we were able to effectively augment plasticity induction using a priming intervention involving either cTBS or iTBS.

In old subjects, the response to test TBS was not different between priming conditions. Furthermore, the response to priming + test TBS was reduced following cTBS + iTBS, suggesting that priming with cTBS prevented an excitatory response to subsequent iTBS. Given that previous studies have reported age-related reductions in the response to TBS [13], it could be suggested that these findings were driven by a reduced effectiveness of priming stimulation in the old group. As the response to priming TBS was not different between groups (Fig. 2A), it seems unlikely that this would be the case. However, as no overt change in MEPs was seen in either group following priming, we cannot exclude subliminal differences in priming effectiveness between groups, where there could be a change in the readiness of synapses to generate subsequent LTP or LTD without a change in excitability. Despite this, time-dependent variations in the response to the intervention were not different between priming conditions in old subjects, suggesting that differential temporal effects in each age group did not contribute to our findings. We therefore suggest that our findings are indicative of a reduced metaplastic response in the elderly. This has important implications for the clinical utilisation of priming stimulation, not just in healthy old adults, but also in pathological situations which are common in the elderly, such as stroke, Alzheimer's disease and Parkinson's disease.

The candidate mechanism for the changes in corticospinal excitability induced by TBS involves LTP/LTD-like modifications to synaptic communication that are thought to be dependent on activation of post-synaptic N-methyl-D-aspartate (NMDA) receptors, and subsequent influx of Ca^{2+} into the post-synaptic neuron [21, 43]. As work in animal models has suggested that the number of NMDA receptors is reduced by age [44], and age-related alterations to Ca^{2+} regulation have been well documented [45], changes to both of these crucial processes may have contributed to the reduced metaplastic response in our old cohort. However, metaplastic modifications to plasticity induction can also be induced via non-NMDA dependent mechanisms, instead utilising activation of the metabotropic glutamate receptor (mGluR; [18]). While the role of the mGluR in mediating the response to plasticity inducing NIBS is currently unknown, it has been recently suggested to contribute to the response to dual blocks of paired-associative stimulation [PAS; 40]. Furthermore, work in animal models suggests that mGluR-mediated pathways are altered by ageing, and that deficits within these pathways are related to cognitive decline [for review, see; 46, 47]. Age-related alterations to mGluR mediated metaplasticity may therefore also have contributed to our findings.

In addition to the numerous neurophysiological alterations that may have contributed to our findings, age-related changes in the stimulation parameters required to induce metaplastic interactions may also be an important factor. For example, the time interval between priming and test blocks can modulate metaplastic interactions [40, 42, 48, 49]. The lack of metaplastic interaction observed in the older adults could reflect an age-related shift in this window of associativity. Alternatively, older adults may require more prolonged or different stimulation intensities to induce a metaplastic response.

In conclusion, the current study found that priming stimulation could be used to increase the plasticity response in young but not old subjects, suggesting that metaplastic interactions may be altered in healthy elderly adults. While the functional ramifications of these finding are not clear, they suggest that the priming strategy used in this study may not be useful for enhancing the plasticity response in elderly adults. This may represent a significant limitation to the therapeutic utilisation of priming stimulation in neurological disorders which predominantly affect the elderly, such as Parkinson's disease and Alzheimer's disease.

Acknowledgements

This study was funded by an Australian Research Council Discovery Projects Grant (grant number DP150100930).

Bibliography

[1.] Dayan E, Cohen Leonardo G. Neuroplasticity Subserving Motor Skill Learning. *Neuron* 2011; 72: 443-454

[2.] Cooke S, Bliss T. Plasticity in the human central nervous system. *Brain* 2006; 129: 1659-1673

[3.] Nudo RJ, Plautz EJ, Frost SB. Role of adaptive plasticity in recovery of function after damage to motor cortex. *Muscle Nerve* 2001; 24: 1000-1019

[4.] Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 1993; 361: 31-9

[5.] Jacobs KM, Donoghue JP. Reshaping the cortical motor map by unmasking latent intracortical connections. *Science* 1991; 251: 944-947

[6.] Sanes JN, Donoghue JP. Plasticity and primary motor cortex. *Annu Rev Neurosci* 2000; 23: 393-415

[7.] Müller-Dahlhaus F, Ziemann U, Classen J. Plasticity resembling spike-timing dependent synaptic plasticity: the evidence in human cortex. *Front Synaptic Neurosci* 2010; 2: 178-188

[8.] Hummel FC, Cohen LG. Non-invasive brain stimulation: a new strategy to improve neurorehabilitation after stroke? *Lancet Neurol* 2006; 5: 708-712

[9.] Kuo M-F, Paulus W, Nitsche MA. Therapeutic effects of non-invasive brain stimulation with direct currents (tDCS) in neuropsychiatric diseases. *Neuroimage* 2014; 85: 948-960

[10.] Hoffman RE, Cavus I. Slow transcranial magnetic stimulation, long-term depotentiation, and brain hyperexcitability disorders. *Am J Psychiatry* 2002; 159: 1093-1102

[11.] Müller-Dahlhaus JFM, Orekhov Y, Liu Y, Ziemann U. Interindividual variability and age-dependency of motor cortical plasticity induced by paired associative stimulation. *Exp Brain Res* 2008; 187: 467-475

[12.] Todd G, Kimber TE, Ridding MC, Semmler JG. Reduced motor cortex plasticity following inhibitory rTMS in older adults. *Clin Neurophysiol* 2010; 121: 441-7

[13.] Freitas C, Perez J, Knobel M, Tormos JM, Oberman LM, Eldaief M *et al.* Changes in cortical plasticity across the lifespan. *Front Aging Neurosci* 2011; 3: 5

[14.] Fathi D, Ueki Y, Mima T, Koganemaru S, Nagamine T, Tawfik A *et al.* Effects of aging on the human motor cortical plasticity studied by paired associative stimulation. *Clin Neurophysiol* 2010; 121: 90-93

[15.] Sawaki L, Yaseen Z, Kopylev L, Cohen LG. Age-dependent changes in the ability to encode a novel elementary motor memory. *Ann Neurol* 2003; 53: 521-524

[16.] Rogasch NC, Dartnall TJ, Cirillo J, Nordstrom MA, Semmler JG. Corticomotor plasticity and learning of a ballistic thumb training task are diminished in older adults. *J Appl Physiol* 2009; 107: 1874-83

[17.] Ridding MC, Ziemann U. Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects. *J Physiol* 2010; 588: 2291-304

[18.] Abraham WC. Metaplasticity: tuning synapses and networks for plasticity. *Nat Rev Neurosci* 2008; 9: 387

[19.] Müller-Dahlhaus F, Ziemann U. Metaplasticity in human cortex. *Neuroscientist* 2015; 21: 185-202

[20.] Goldsworthy MR, Pitcher JB, Ridding MC. Spaced Noninvasive Brain Stimulation Prospects for Inducing Long-Lasting Human Cortical Plasticity. *Neurorehabil Neural Repair* 2014; 29: 714-721

[21.] Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. Theta burst stimulation of the human motor cortex. *Neuron* 2005; 45: 201-6

[22.] Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* 1971; 9: 97-113

[23.] Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; 12: 189-98

[24.] Sale MV, Ridding MC, Nordstrom MA. Cortisol inhibits neuroplasticity induction in human motor cortex. *J Neurosci* 2008; 28: 8285-8293

[25.] Goldsworthy MR, Müller-Dahlhaus F, Ridding MC, Ziemann U. Resistant against de-depression: LTD-like plasticity in the human motor cortex induced by spaced cTBS. *Cereb Cortex* 2014; 25: 1724-1734

[26.] Goldsworthy MR, Pitcher JB, Ridding MC. The application of spaced theta burst protocols induces long-lasting neuroplastic changes in the human motor cortex. *Eur J Neurosci* 2012; 35: 125-134

[27.] Goldsworthy MR, Vallence A-M, Hodyl NA, Semmler JG, Pitcher JB, Ridding MC. Probing changes in corticospinal excitability following theta burst stimulation of the human primary motor cortex. *Clin Neurophysiol* 2016; 127: 740-747

[28.] Inghilleri M, Conte A, Curra A, Frasca V, Lorenzano C, Berardelli A. Ovarian hormones and cortical excitability. An rTMS study in humans. *Clin Neurophysiol* 2004; 115: 1063-1068

[29.] Chung SW, Hill AT, Rogasch NC, Hoy KE, Fitzgerald PB. Use of theta-burst stimulation in changing excitability of motor cortex: A systematic review and meta-analysis. *Neurosci Biobehav Rev* 2016; 63: 43-64

[30.] Young-Bernier M, Tanguay AN, Davidson PS, Tremblay F. Short-latency afferent inhibition is a poor predictor of individual susceptibility to rTMS-induced plasticity in the motor cortex of young and older adults. *Front Aging Neurosci* 2014; 6: 182

[31.] Di Lazzaro V, Pilato F, Dileone M, Profice P, Oliviero A, Mazzone P *et al.* The physiological basis of the effects of intermittent theta burst stimulation of the human motor cortex. *J Physiol* 2008; 586: 3871-3879

[32.] López-Alonso V, Cheeran B, Río-Rodríguez D, Fernández-del-Olmo M. Inter-individual variability in response to non-invasive brain stimulation paradigms. *Brain stimul* 2014; 7: 372-380

[33.] Vallence A-M, Kurylowicz L, Ridding MC. A comparison of neuroplastic responses to non-invasive brain stimulation protocols and motor learning in healthy adults. *Neurosci Lett* 2013; 549: 151-156

[34.] Player MJ, Taylor JL, Alonzo A, Loo CK. Paired associative stimulation increases motor cortex excitability more effectively than theta-burst stimulation. *Clin Neurophysiol* 2012; 123: 2220-6

[35.] Hamada M, Murase N, Hasan A, Balaratnam M, Rothwell JC. The role of interneuron networks in driving human motor cortical plasticity. *Cereb Cortex* 2013; 23: 1593-605

[36.] Wischnewski M, Schutter DJ. Efficacy and Time Course of Theta Burst Stimulation in Healthy Humans. *Brain Stimul* 2015; 8: 685-92

[37.] Suppa A, Huang YZ, Funke K, Ridding MC, Cheeran B, Di Lazzaro V *et al.* Ten Years of Theta Burst Stimulation in Humans: Established Knowledge, Unknowns and Prospects. *Brain Stimul* 2016; 9: 323-35

[38.] Brownjohn PW, Reynolds JN, Matheson N, Fox J, Shemmell JB. The effects of individualized theta burst stimulation on the excitability of the human motor system. *Brain Stimul* 2014; 7: 260-268

[39.] Abraham WC, Bear MF. Metaplasticity: the plasticity of synaptic plasticity. *Trends Neurosci* 1996; 19: 126-30

[40.] Müller-Dahlhaus F, Lücke C, Lu M-K, Arai N, Fuhl A, Herrmann E *et al.* Augmenting LTP-Like Plasticity in Human Motor Cortex by Spaced Paired Associative Stimulation. *PloS one* 2015; DOI: http://dx.doi.org/10.1371/journal.pone.0131020.

[41.] Murakami T, Müller-Dahlhaus F, Lu MK, Ziemann U. Homeostatic metaplasticity of corticospinal excitatory and intracortical inhibitory neural circuits in human motor cortex. *J Physiol* 2012; 590: 5765-5781

[42.] Gamboa OL, Antal A, Laczo B, Moliadze V, Nitsche MA, Paulus W. Impact of repetitive theta burst stimulation on motor cortex excitability. *Brain Stimul* 2011; 4: 145-151

[43.] Huang YZ, Chen RS, Rothwell JC, Wen HY. The after-effect of human theta burst stimulation is NMDA receptor dependent. *Clin Neurophysiol* 2007; 118: 1028-32

[44.] Clayton DA, Grosshans DR, Browning MD. Aging and surface expression of hippocampal NMDA receptors. *J Biol Chem* 2002; 277: 14367-14369

[45.] Foster TC, Kumar A. Calcium dysregulation in the aging brain. *The Neuroscientist* 2002; 8: 297-301

[46.] Ménard C, Quirion R. Group 1 metabotropic glutamate receptor function and its regulation of learning and memory in the aging brain. *Front Pharmacol* 2012; 3: 182

[47.] Hullinger R, Puglielli L. Molecular and cellular aspects of age-related cognitive decline and Alzheimer's disease. *Behav Brain Res* 2016;

[48.] Monte-Silva K, Kuo MF, Liebetanz D, Paulus W, Nitsche MA. Shaping the optimal repetition interval for cathodal transcranial direct current stimulation (tDCS). *J Neurophysiol* 2010; 103: 1735-40

[49.] Monte-Silva K, Kuo MF, Hessenthaler S, Fresnoza S, Liebetanz D, Paulus W *et al.* Induction of late LTP-like plasticity in the human motor cortex by repeated non-invasive brain stimulation. *Brain Stimul* 2013; 6: 424-32

Table 1. Subject Characteristics

	Young	Old
Age (yrs)	22.3 ± 0.1	70.2 ± 1.7*
Handedness (Laterality Index)	0.9 ± 0.02	0.9 ± 0.04
MMSE	29.6 ± 0.2	29.5 ± 0.2
RMT (%MSO)		
iTBS + iTBS	47.1 ± 1.9	48.3 ± 2.5
cTBS + iTBS	46.6 ± 1.8	48.2 ± 2.3
Sham + iTBS	47.6 ± 1.8	48.1 ± 2.6
M_{max} (mV)		
iTBS + iTBS	17.5 ± 1.1	15.2 ± 1.1
cTBS + iTBS	19.1 ± 1.0	17.5 ± 1.5
Sham + iTBS	19.0 ± 1.1	14.7 ± 1.3

Data shown as mean ± SEM. *P < 0.05 compared to young subjects;

Table 2. Baseline MEP amplitudes

	iTBS + iTBS	cTBS + iTBS	Sham + iTBS
Young (mV)	1.0 ± 0.1	1.1 ± 0.1	1.2 ± 0.1
Old (mV)	1.4 ± 0.1	1.3 ± 0.1	1.3 ± 0.1

Data shown as mean ± SEM

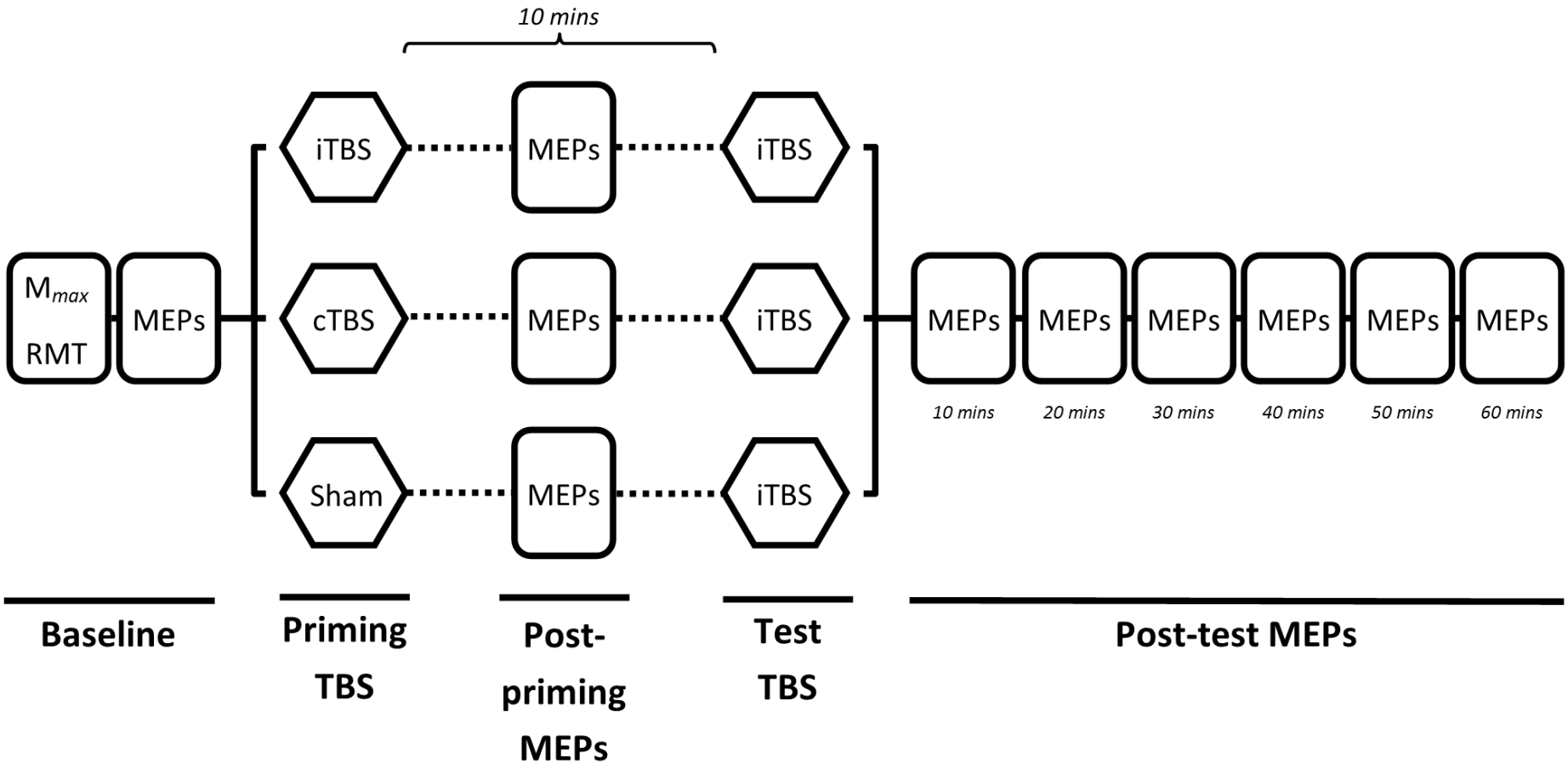

Figure 1. Experimental protocol

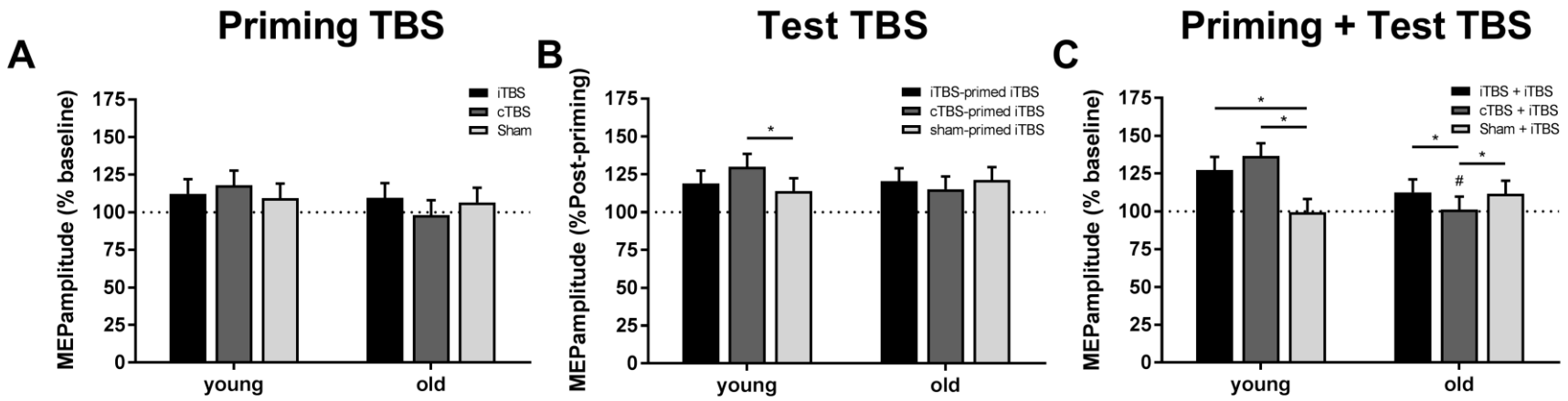

Figure 2. Effects of priming and test TBS in young and old adults. *A)* MEP amplitude recorded 5 mins after priming TBS (i.e., post-priming MEPs) is shown normalised to baseline MEP amplitude *(effect of priming TBS). B)* MEP amplitude at each time point post-test TBS was normalised to the post-priming TBS MEP, then collapsed over time *(effect of test TBS*). *C)* MEP amplitude at each time point post-test TBS was normalised to the baseline MEP, then collapsed over time *(cumulative effect of priming + test TBS)*. The dotted horizontal line represents MEP amplitude at baseline *(A, C)* and post-priming TBS *(B)* time points, with values above 100% representing an increase in MEP amplitude. *P < 0.05 between priming conditions; $^{\#}$P < 0.05 between age groups.

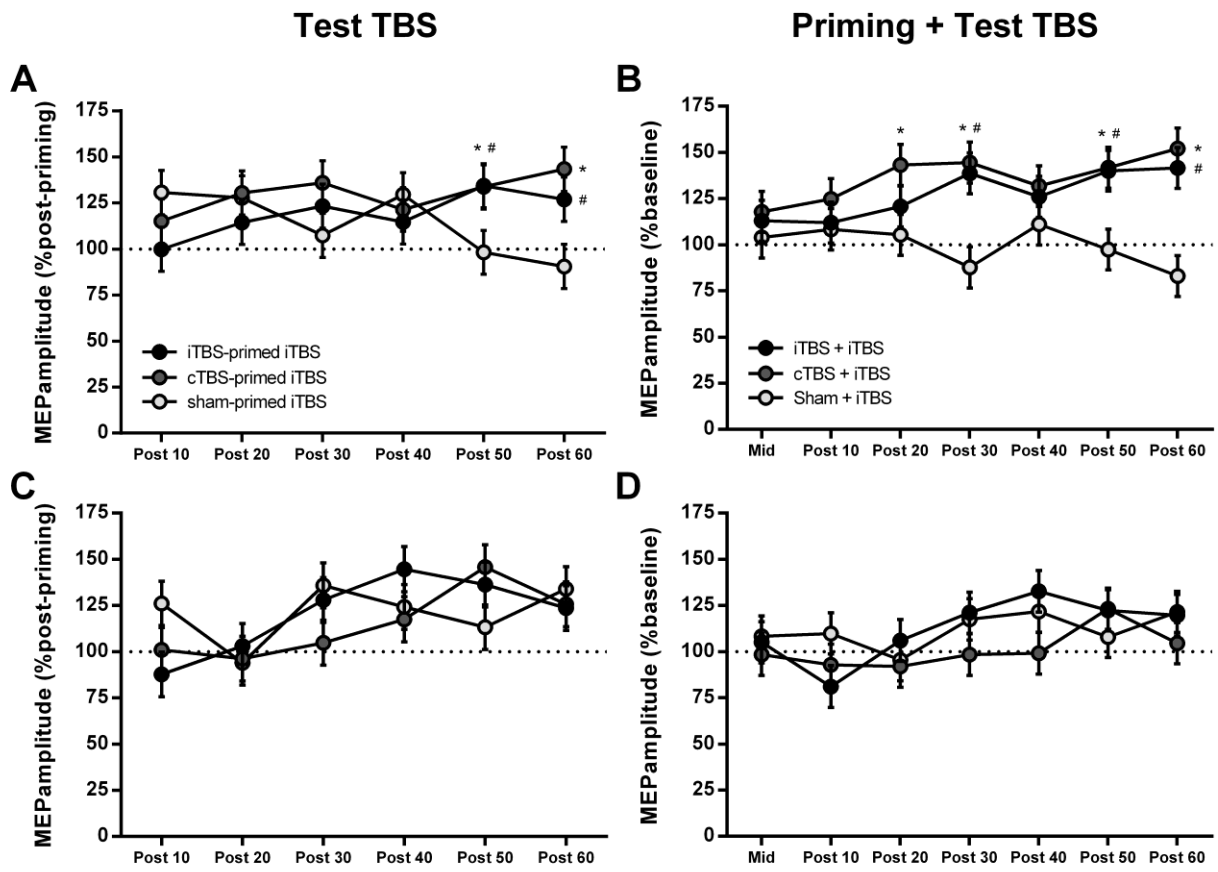

Figure 3. Effect of time on the response to paired TBS in young and old adults. Data show MEP amplitude at each post-intervention time point normalised to the post-priming TBS *(effect of test TBS; A, C)* and baseline *(cumulative effect of priming + test TBS; B, D)* MEP amplitude in young *(top row)* and old *(bottom row)* adults. The dotted horizontal line represents MEP amplitude at baseline *(right column)* and post-priming TBS *(left column)* time points, with values above 100% representing an increase in MEP amplitude. $^{*}P < 0.05$ when comparing cTBS priming to sham priming; $^{\#}P < 0.05$ when comparing iTBS priming to sham priming.