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Short-term immobilisation influences use-dependent cortical plasticity and fine motor performance

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Abbreviations

BDNF, Brain derived neurotrophic factor; EMG, electromyography; FDI, first dorsal interosseous; GABA, gamma-aminobutyric acid; ICF, intracortical facilitation; ISI, interstimulus interval; LICI, long-interval intracortical inhibition; LTD, long-term depression; LTP, long term potentiation; MEP, motor evoked potential; M_{max}, maximum M-wave; MSO, maximum stimulator output; NIBS, non-invasive brain stimulation; PAS, paired associative stimulation; RMT, resting motor threshold; SICI, short-interval intracortical inhibition; TMS, transcranial magnetic stimulation.

Abstract

Short-term immobilisation that reduces muscle use for 8-10 hours is known to influence cortical excitability and motor performance. However, the mechanisms through which this is achieved, and whether these changes can be used to modify cortical plasticity and motor skill learning, are not known. The purpose of this study was to investigate the influence of short-term immobilisation on use-dependent cortical plasticity, motor learning and retention. 21 adults were divided into control and immobilised groups, both of which underwent two experimental sessions on consecutive days. Within each session, transcranial magnetic stimulation (TMS) was used to assess motor evoked potential (MEP) amplitudes, short- (SICI) and long-interval intracortical inhibition (LICI), and intracortical facilitation (ICF) before and after a grooved pegboard task. Prior to the second training session, the immobilised group underwent 8 hrs of left hand immobilisation targeting the index finger, while control subjects were allowed normal limb use. Immobilisation produced a reduction in MEP amplitudes, but no change in SICI, LICI or ICF. While motor performance improved for both groups in each session, the level of performance was greater 24-hrs later in control, but not immobilised subjects. Furthermore, training-related MEP facilitation was greater after, compared with before, immobilisation. These results indicate that immobilisation can modulate use-dependent plasticity and the retention of motor skills. They also suggest that changes in intracortical excitability are unlikely to contribute to the immobilisation-induced modification of cortical excitability.

Keywords

Transcranial magnetic stimulation, hand immobilization, neuroplasticity, motor learning, metaplasticity, intracortical excitability

The ability of the brain to remodel its intrinsic connections, referred to as neuroplasticity, mediates the human capacity for learning (Dayan and Cohen, 2011), memory (Cooke and Bliss, 2006) and recovery from injury (Nudo et al., 2001). The mechanisms mediating neuroplasticity have been well defined in animal models, with changes in neuronal communication thought to be driven by several factors, including modifications to glutamatergic and GABAergic neurotransmission (Bliss and Collingridge, 1993). Extensive research using non-invasive brain stimulation (NIBS) techniques, such as transcranial magnetic stimulation (TMS), have suggested that similar processes occur within the human brain (Müller-Dahlhaus et al., 2010). Furthermore, NIBS techniques applied to the motor areas of the human brain have been used to identify altered cortical excitability associated with periods of motor training (Muellbacher et al., 2001, Ziemann et al., 2001), in addition to being used to induce short-lasting neuroplastic change (Nitsche and Paulus, 2000, Stefan et al., 2000, Huang et al., 2005).

The ability of NIBS to modulate cortical excitability represents a promising tool for rehabilitation in situations of abnormal cortical function, such as that seen following neurological injury (Hummel and Cohen, 2006) or in some neurological conditions (Hoffman and Cavus, 2002, Kuo et al., 2014). However, the clinical implementation of such tools is currently limited by many factors (see Ridding and Ziemann, 2010). Improving the response to NIBS has therefore been the focus of a large body of research over recent years. One approach within this literature has been the use of 'priming' protocols, in which the application of a plasticity-inducing protocol is preceded by another intervention that produces a cortical environment more amenable to the induction of plasticity, subsequently facilitating a stronger plastic response (Abraham, 2008). For example, Rosenkranz et al. (2014) measured the response to paired-associative stimulation (PAS; a plasticity inducing paradigm) following a period of short-term (8 hours) hand immobilisation, which has been previously shown to modify cortical excitability (Facchini et al., 2002, Huber et al., 2006, Ngomo et al., 2012, Burianová et al., 2014). Following immobilisation, a greater response to PAS was found, that may have been mediated by altered activity in intracortical inhibitory circuits (Rosenkranz et al., 2014). While this experiment demonstrates the effectiveness of immobilisation in improving stimulation-induced plasticity, it is not known if this potentiated response manifests as altered use-dependent plasticity and motor function.

The current study therefore aimed to determine whether short-term immobilisation influenced subsequent use-dependent plasticity and the ability to learn a fine motor task. As a secondary aim, we were interested in assessing if changes in the activity of intracortical circuitry contributed to any effects of immobilisation on motor learning. These aims were achieved by comparing changes in TMS measures of corticospinal and intracortical excitability induced by a motor learning protocol before and after 8 hours of hand immobilisation. Based on the findings of Rosenkranz et al. (2014), we expected that performance during motor training would be improved following immobilisation, and that this would be associated with a modulation of corticospinal and intracortical excitability.

Methods

21 healthy young (mean ± SD: 21.4 ± 1.4 years) subjects were recruited from the university community to participate in the current study. Exclusion criteria included a history of stroke, history of neurological or psychiatric disease, or current use of psychoactive medication (sedatives, antipsychotics, antidepressants etc.). Hand preference and laterality was assessed using the Edinburgh Handedness Inventory (Oldfield, 1971). 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

Prior to participation, a buccal swab (Isohelix, Cell Projects, Kent, UK) was obtained from each participant for later determination of BDNF genotype (for details, see; McDonnell et al., 2013). Subjects were then randomly assigned to either the immobilised or control group. Subsequently, each group attended two experimental sessions held on consecutive days, approximately 24-hrs apart. To avoid confounding effects of diurnal variations in cortisol on motor learning, these sessions were always held in the afternoon and at the same time of day (Sale et al., 2008).

The experimental time line is shown in Figure 1. During the first session, beginning at approximately 5pm, measures of corticospinal and intracortical excitability were assessed before and after a motor learning task (see below). Subjects in the immobilised group were then required to re-attend the laboratory between 8-9 am the following morning, at which

point the index finger of their left hand was immobilised, similar to that described previously (Fuglevand et al., 1995). To achieve this, the index finger was bent into the palm, with the thumb placed over the index finger between the metacarpophalangeal and proximal interphalangeal joints. Bandages were then placed around the hand and wrist in a way that limited movement of all fingers. The left arm was placed in a sling and immobilised in this way for 8 hours prior to the second experimental session. During this time, control subjects were allowed normal use of their left hand. The non-dominant left hand was chosen for immobilisation as this minimised the impact of immobilisation on activities of daily living. Furthermore, subjects were allowed to complete normal daily activities with their non-immobilised hand.

During all experimental sessions, subjects were seated in a comfortable chair with the left shoulder relaxed in a neutral position and left forearm and hand resting on a pillow placed in the lap. Surface electromyography (EMG) was recorded from the first dorsal interosseous (FDI) muscle of the left 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 100–1000) 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 being stored on a computer for later off-line analysis.

Experimental procedures

Maximal compound muscle action potential (M_{max}). 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 was applied to the right primary motor cortex using a figure-of-eight coil (external wing diameter 9 cms) with two Magstim 200^2 magnetic stimulators connected via a Bistim unit (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.

Corticospinal excitability. Single pulse TMS measures of corticospinal excitability included resting motor threshold (RMT) and MEP amplitudes with modified input-output (IO) curves. RMT was defined as the minimum stimulus intensity producing an MEP amplitude ≥ 50 μV in at least 3 out of 5 trials while the left FDI was completely relaxed. RMT was assessed at the beginning of each experimental session and expressed as a percentage of maximum stimulator output (MSO). IO curves were generated by applying 3 stimuli of increasing intensity while subjects maintained complete relaxation of FDI. These intensities, determined based on individual subject resting threshold, were 110%, 130% and 150% RMT. Within each experimental session, IO curves were recorded before and after the motor training task to assess changes in corticospinal excitability induced by training. As 10 stimuli were applied for 3 different stimulus intensities, each IO curve assessment required a total of 30 stimuli.

Intracortical inhibition and facilitation. Paired-pulse TMS was used to assess short- (SICI) and long-interval intracortical inhibition (LICI), as well as intracortical facilitation (ICF). For all paired-pulse measures, the intensity of the test stimulus was set at 120% RMT. For SICI, a 70% RMT conditioning stimulus intensity and a 2 ms interstimulus interval (ISI) were used (Kujirai et al., 1993), whereas a 120% RMT conditioning intensity and 100 ms ISI were used for the assessment of LICI (Valls-Sole et al., 1992). For ICF, while a 70% RMT conditioning intensity was also used, the ISI was extended to 10 ms (Ziemann et al., 1996b). As 30 conditioned (10 SICI, 10 LICI and 10 ICF) and 10 unconditioned stimuli (test alone MEP) were used, each assessment of activity within intracortical circuitry required a total of 40 stimuli.

Motor training. The motor training used within the current study was a grooved pegboard task, the performance of which is commonly used to assess manual dexterity (Ruff and Parker, 1993, Tremblay et al., 2003), and has been shown to induce robust increases in MEP amplitude that reflect training-dependent plasticity (Rossi et al., 1999, Garry et al., 2004, Christova et al., 2014). This task uses a test board that has a well at the top, and a series of holes located beneath the well. Subjects select small metal pegs from the well using the index

finger and thumb, and insert the pegs into the holes located beneath the well. However, as the pegs cross-section is not regular, they must be rotated between the digits to allow placement in the holes, a task requiring a high degree of fine motor control. Within each experimental session, every subject completed a total of 9 pegboard trials, during which they were given a 30-s period to place as many pegs as possible. To avoid any fatigue, these trials were separated into 3 blocks of 3 trials, with an inter-trial interval of 15 seconds and inter-block interval of 1 minute. No practice trials were given. The number of pegs placed on each day was totalled across individual blocks.

Data analysis

Data analysis was performed following visual inspection of offline EMG. Any trial with muscle activity in the 100 ms prior to stimulation with duration ≥ 5 ms or amplitude exceeding 20 μV was excluded from analysis. Individual MEP and M_{max} amplitudes were measured peak-to-peak and assessed in millivolts. For single-pulse measurements recorded during MEP assessments, individual MEP amplitudes within each stimulus state were normalised to the amplitude of M_{max}. Paired-pulse measurements of intracortical inhibition (SICI, LICI) and facilitation (ICF) were quantified by expressing the amplitude of individual conditioned MEPs as a percentage of the average unconditioned MEP amplitude. In each experimental session, grooved pegboard performance was compared between the first (trial 1) and last (trial 9) trial, and the effects of pegboard training on MEP curves, SICI, LICI and ICF were quantified by expressing the individual normalised MEP amplitudes recorded after training as a percentage of the average normalised MEP amplitude recorded before training.

Statistical analysis

Age and Handedness were compared between groups (Immobilised, Control) using unpaired students *t*-tests. The effects of group and session (Session 1, Session 2) on RMT and M_{max} amplitude were assessed using individual 2-way repeated measures analysis of variance ($ANOVA_{RM}$). Significant main effects and interactions were further investigated using Bonferroni corrected post hoc tests. Effects of immobilisation and training on all MEP (IO curves, SICI, LICI & ICF) and performance (peg board) data were investigated using linear mixed model analysis with repeated measures. For pre-training MEP amplitude and the change in MEP amplitude after training, the effects of test intensity (110%, 130% and 150% RMT) and session (day 1, day 2) were investigated, with individual models used for each

group. For pre-training paired pulse measures, test alone MEP amplitude, and the change in paired-pulse measures after training, the effects of session and group were investigated. Lastly, for pegboard data, the effect of trial (first, last) and session were investigated. For all models, subject was included as a random effect and significant interactions were further investigated using custom contrasts with Bonferroni correction. Linear regression analysis using individual subject data was used to further investigate relationships between neurophysiological and performance measures. Significance was set at $P < 0.05$ and all data are presented as mean ± standard error of the mean (SEM), unless otherwise stated.

Results

All subjects completed the experiment in full and without adverse reaction. Subject characteristics are shown in Table 1. No differences were found between groups for age (control, 21.5 ± 0.6 years; immobilised, 21.5 ± 0.5 years; $P = 0.9$) or handedness (average laterality quotient: control, 0.85 ± 0.06; immobilised, 0.85 ± 0.07; $P = 0.9$). RMT was not different between groups ($P = 0.9$) or sessions ($P = 0.4$) but a significant interaction was found between factors ($P = 0.004$). However, post-hoc analysis showed no difference in RMT between groups or sessions (all *P*-values > 0.3). M_{max} amplitude was also not different between groups ($P = 0.9$) or sessions ($P = 0.9$) and there was no interaction between factors ($P = 0.9$). DNA analysis for BDNF genotype revealed 2 Val/Val subjects, 18 Val/Met subjects and 1 Met/Met subject.

Effect of immobilisation on MEP IO curves

Baseline MEP amplitudes recorded at the beginning of each session are compared in control and immobilised subjects in Figure 2. In control subjects, MEP amplitudes were reduced in session 2 ($P = 0.01$), and increasing stimulus intensity produced significantly larger MEP amplitudes ($P < 0.0001$), but there was no interaction between factors ($P = 0.1$, Fig. 2A). In immobilised subjects, MEP amplitude was also reduced during the second session ($P < 0.0001$), increasing test stimulus intensity again produced larger test MEP amplitudes ($P < 0.0001$), and a significant interaction was found between factors ($P < 0.0001$, Fig 2B). Post hoc comparisons between sessions showed that the amplitude of the test MEP was significantly reduced during session 2 for all test intensities (all *P*-values < 0.0001).

Effect of immobilisation on paired-pulse TMS measures

Figure 3A shows the amplitude of the test alone MEP used to quantify the response to paired-pulse stimulation, which was obtained at 120% RMT, with RMT assessed at the beginning of each session. The test MEP was not different between sessions ($P = 0.8$), but was greater in control subjects ($P = 0.03$) and there was an interaction between factors ($P = 0.001$). Post hoc analysis showed that the amplitude of the test MEP was increased during the second session in control subjects ($P = 0.04$), but decreased during the second session in immobilised subjects ($P = 0.002$). Furthermore, the test MEP was not different between groups during session 1 ($P = 0.2$), but decreased in immobilised subjects during session 2 ($P = 0.004$). SICI was greater (i.e. more inhibition) at baseline in the second session ($P = 0.0002$), but there was no difference between groups ($P = 0.1$), and no interaction between factors ($P = 0.1$; Fig. 3B). LICI was increased during session 2 ($P = 0.008$), but was not different between groups ($P = 0.1$) and there was no interaction between factors ($P = 0.8$; Fig. 3C). ICF was increased in immobilised subjects ($P = 0.009$), but not different between sessions ($P = 0.07$), and there was no interaction between factors ($P = 0.8$; Fig. 3D).

Effect of immobilisation on grooved peg-board performance

Performance during motor training is compared between sessions for control and immobilised subjects in Figure 4. In control subjects, a greater number of pegs were placed during the last trial ($P < 0.0001$), more pegs were placed during the second session ($P = 0.0002$) and there was an interaction between factors ($P = 0.04$; Fig 4A). Post hoc analysis showed that performance during session 2 was greater than session 1 for the first trial ($P = 0.0002$), but not different between sessions for the last trial ($P = 0.2$). In immobilised subjects, more pegs were placed in the last trial ($P < 0.0001$), but this was not different between sessions ($P = 0.8$) and there was no interaction between factors ($P = 0.8$; Fig 4B).

Effect of grooved peg-board training on MEP Amplitudes

Changes in MEP amplitudes after motor training are compared between sessions in control and immobilised subjects in Figure 5, with values above 100% representing training-related increases in MEP amplitude. For control subjects, changes in MEP amplitude differed between test stimulus intensities ($P = 0.007$) and sessions ($P < 0.0001$), and there was an interaction between factors ($P < 0.0001$; Fig. 5A). Comparisons between sessions showed that the change in MEP amplitude after motor training was significantly reduced during

session 2 at the 110% RMT ($P < 0.0001$) and 150% RMT ($P = 0.04$) intensities, but not different between sessions for the 130% RMT intensity ($P = 0.7$; Fig. 5A). For immobilised subjects, training-related changes in MEP amplitude again differed between sessions ($P = 0.0001$) and stimulus intensities ($P = 0.03$), and there was an interaction between factors ($P = 0.009$; Fig. 5B). Comparisons between sessions showed a greater training-related increase in MEP amplitude during the second session for the 110% RMT ($P = 0.05$) and 150% RMT ($P < 0.0001$) intensities, whereas there was no difference between sessions for the 130% intensity ($P = 0.2$; Fig 5B).

Effect of grooved peg-board training on paired-pulse TMS measures

Changes in the response to paired-pulse TMS after motor training are shown for each session in Figure 6. For SICI, the training-related change in inhibition was not different between sessions ($P = 0.09$), or groups ($P = 0.2$), and there was no interaction between factors ($P = 0.8$; Fig. 6A). For LICI, the training-related change in inhibition was greater during the second session ($P = 0.02$), but this was not different between groups ($P = 0.8$), and there was no interaction between factors ($P = 0.1$; Fig. 6B). For ICF, training-related changes in facilitation were not different between sessions ($P = 0.5$), or groups ($P = 0.9$), and there was no interaction between factors ($P = 0.07$; Fig. 6C).

Linear regression analysis

Linear regression analysis of individual subject data was used to compare training-related changes in motor performance with training-related changes in RMT, MEP amplitude, SICI, LICI and ICF in each session. However, results of these comparisons failed to show any significant correlations between behavioural and neurophysiological measurements (all *P*-values > 0.05).

Discussion

The current study assessed if a period of short-term hand immobilisation influenced use-dependent plasticity and motor skill performance. This was accomplished by comparing changes in TMS measures of corticospinal and intracortical excitability induced by a pegboard task before and after 8 hours of immobilisation or normal hand use. At least 3 main findings can be reported from this experimental approach. First, short-term (8 hrs) immobilisation produced a reduction in cortical excitability that was not related to changes in

SICI, LICI or ICF. Second, performance of the motor task was impaired by immobilisation, although motor skill learning was not affected. Third, changes in cortical excitability induced by training were greater following immobilisation.

Cortical excitability is modified after 8 hours of hand immobilisation

The effects of immobilisation on MEP amplitude have been investigated by several previous studies, with findings suggesting that the outcome depends on the duration of immobilisation. Previous work has reported reduced MEPs following short-term immobilisation (i.e., < 4 days; Facchini et al., 2002, Huber et al., 2006, Avanzino et al., 2011, Ngomo et al., 2012, Avanzino et al., 2014, Bassolino et al., 2014, Rosenkranz et al., 2014), but increased MEPs following long-term immobilisation (i.e., > 10 days; Zanette et al., 1997, Zanette et al., 2004, Roberts et al., 2007, Clark et al., 2008). Within the current study, immobilisation caused a reduction in MEP amplitude without a change in RMT. As RMT is associated with a corticospinal descending volley consisting primarily of the early I_1 wave, whereas the generation of suprathreshold MEPs is associated with a descending volley including both early and late I waves (Di Lazzaro et al., 2001), the differential change in these measures suggest that the reduced cortical excitability observed within the current study following immobilisation was driven by a modulation of the cortical elements responsible for generation of the late I-waves. Nonetheless, when combined with the findings of a previous study (Rosenkranz et al., 2014), our results confirm that a reduction in cortical excitability can be obtained from as little as 8 hours of reduced use due to immobilisation.

The current study failed to find any change in the magnitude of SICI following 8 hours of immobilisation, suggesting that alterations to $GABA_A$-mediated intracortical inhibition (Ziemann et al., 1996a) did not contribute to the observed reductions in cortical excitability. Previous studies utilising several weeks of immobilisation have reported decreased (Zanette et al., 2004) and no change (Clark et al., 2010) in SICI. Decreased SICI has also been previously reported after 8 hours of immobilisation (Rosenkranz et al., 2014), which contradicts the findings in the current study. Methodological variations between studies (e.g., ISI and conditioning intensities) may have contributed to this discrepancy. In particular, our decision to utilise a constant intensity test stimulus resulted in a slightly reduced (by ~ 0.4 mV) test MEP amplitude after immobilization. However, this small difference is unlikely to have influenced SICI, as we have previously shown that measurements of SICI are not significantly modified when test MEP amplitude varies between 0.5 – 2 mV (Opie and

Semmler, 2014), which encompasses the range seen in the present study. We therefore suggest that other factors, such as the effectiveness of the immobilisation procedure in different target muscles (thumb *vs.* index finger), resulting in differential changes to proprioceptive feedback (Avanzino et al., 2014), may have contributed to these divergent effects of immobilisation on intracortical inhibition.

Measures of ICF were unaffected by immobilisation, which both supports (Clark et al., 2010) and contradicts (Zanette et al., 2004) the findings of previous work using longer periods of immobilisation. Interestingly though, subjects in the immobilised group showed elevated ICF that was consistent between session (Fig 3D). While the reason for this is currently unclear, the induction of use-dependent plasticity is not associated with changes in ICF (Perez et al., 2004, Smyth et al., 2010, Lee et al., 2013), suggesting that these variations between groups (but not sessions) would have been unlikely to have any physiological impact on the outcomes of the current study. In addition to ICF, measures of LICI were also unaffected by immobilisation, supporting the findings of a previous study using longer periods of immobilisation (Clark et al., 2010). However, changes in both ICF and LICI have been investigated after more prolonged interventions (Zanette et al., 2004, Clark et al., 2010), with results suggesting that immobilisation either does not change (Clark et al., 2010) or increases (Zanette et al., 2004) ICF, but has no effect on LICI (Clark et al., 2010). However, as previous studies have reported an immobilisation-induced increase in LICI in active muscle (Clark et al., 2010), as well as an increased EMG silent period duration (Clark et al., 2008), the effects of reduced use on $GABA_B$-mediated intracortical inhibition may only be apparent during muscle activation. In addition, a previous study has suggested that interhemispheric inhibition (IHI), which is also thought to involve activation of the $GABA_B$ receptor (Irlbacher et al., 2007), is modified by short-term immobilisation. In particular, over-use of the non-immobilised hand is thought to potentiate IHI from the cortex ipsilateral to immobilisation to the cortex contralateral to immobilisation. As subjects within the current study were allowed normal use of the non-immobilised hand to minimise the impact of immobilisation on daily activities, this may suggest that an increased inhibitory tone from left to right primary motor cortex may have contributed to our observed reductions in corticospinal excitability.

Motor performance is impaired following immobilisation

The effects of reduced limb use on motor performance have been previously demonstrated using several different motor tasks. Huber et al. (2006) and Moisello et al. (2008) both

reported that 12 hours of left arm immobilisation produces increased normalised hand-path area and variability during out-and-back movements. Furthermore, Weibull et al. (2011) reported that 3 days of right hand immobilisation produces deficits in fine motor dexterity (assessed using the Purdue pegboard) and Ngomo et al. (2012) showed that 4 days of immobilisation of the non-dominant hand significantly impedes the ability to acquire a novel motor task. Within the current study, both groups demonstrated motor learning by increasing the number of pegs placed within each session, suggesting that motor performance improved with training irrespective of immobilisation. However, at the beginning of the second session, control subjects demonstrated a level of performance comparable to that achieved at the end of the first session, whereas immobilised subjects reverted to performance levels comparable to baseline (start of session 1). While these findings suggest that 8 hours of immobilisation may not be enough to affect the ability to learn a novel pegboard task, they do suggest that it is sufficient to impede access to the previously learned neural commands for that task. Alternatively, it could be suggested that stiffness within the immobilised joint may have contributed to the reduced performance within the first training block. While we cannot exclude this possibility, immobilisation was removed prior to baseline TMS measures (which lasted > 1 hr) and movement within the immobilised hand was not restricted. As subjects were therefore able to move the hand, it seems likely that any stiffness would have resolved by the time the peg board task was performed.

Use-dependent plasticity is modified after immobilisation

It has been well established in humans that both motor training and non-invasive brain stimulation protocols can be used to modulate motor cortical excitability (Muellbacher et al., 2001, Garry et al., 2004, Ziemann et al., 2004, Rogasch et al., 2009, Cirillo et al., 2011, Cirillo et al., 2012), with several lines of evidence supporting long term potentiation (LTP)-like and long-term depression (LTD)-like changes in synaptic communication within sensorimotor cortex as mediating factors (Stefan et al., 2002, Nitsche et al., 2003, Ziemann et al., 2004, Huang et al., 2007). Furthermore, a growing body of evidence suggests that these neuroplastic changes are subject to regulation by homeostatic mechanisms (see Müller-Dahlhaus and Ziemann, 2015). For example, a major determinant of the response to a plasticity-inducing protocol is the history of activity within the postsynaptic neuron, where reduced activity is thought to produce predisposition toward LTP-like modification, and increased activity is thought to produce predisposition toward LTD-like modification. This

process has been described as metaplasticity and formalised by the Bienenstock-Cooper-Munro theory (Bienenstock et al., 1982). This ability to modulate the induction of synaptic plasticity has led to concepts of metaplasticity being utilised in research aiming to improve the response to a given plasticity protocol by first 'priming' the cortex with an initial intervention favouring the induction of LTP-like or LTD-like modification.

One recent example of this approach can be seen in a study by Rosenkranz et al. (2014), who used 8 hours of hand immobilisation as a priming tool prior to application of paired-associative stimulation (PAS), a NIBS paradigm able to induce neuroplastic change within motor cortex (Stefan et al., 2000). In line with metaplasticity theory, this previous study observed an increased response to PAS25 (LTP-like synaptic modification) following an immobilisation-induced decrease in MEP amplitude (LTD-like synaptic modification). As an extension of this study, we sought to assess whether an immobilisation-induced decrease in cortical excitability increased subsequent use-dependent plasticity following training on a fine motor task. The training protocol used for this purpose was a grooved pegboard task, which has been previously shown to produce a robust increase in cortical excitability (Rossi et al., 1999, Garry et al., 2004, Christova et al., 2014). However, we only observed relatively small changes in MEP amplitude after training with this task (Session 1, Figure 5). The most likely reason for this is the unusually high proportion of subjects carrying the BDNF Val66Met polymorphism in our study (85% vs. 30-50% in the general population; Bath and Lee, 2006), the presence of which has been associated with an diminished use-dependent plasticity response in motor cortex (Kleim et al., 2006). Nonetheless, our results, although relatively modest, showed that the effects of training on cortical excitability were greater following the second training session in immobilised (but not control) subjects, reflecting increased use-dependent plasticity following immobilisation.

We expected to see an association between the magnitude of use-dependent plasticity and motor learning, given that several studies have demonstrated that TMS-induced and use-dependent plasticity share similar mechanisms (Ziemann and Siebner, 2008). However, in this study the increased use-dependent plasticity after immobilisation was not accompanied by improved motor learning. This dissociation is not unique to our study, as others have also shown no relation between NIBS-induced plasticity and motor learning (Voti et al., 2011, Vallence et al., 2013, López-Alonso et al., 2015). This lack of association may occur because the neural networks used during voluntary motor actions are more diverse than those

activated by TMS, and changes in the MEP may have no causal relevance to specific measures of motor performance or learning (see Bestmann and Krakauer, 2015). Furthermore, this dissociation could also occur due to factors that influence within-subject variability in cortical plasticity, such as day-to-day variations in attention, prior synaptic history, or levels of various hormones (Ridding and Ziemann, 2010), which could be exacerbated by immobilisation, but is unlikely to affect motor learning in a similar way.

Conclusions

In conclusion, our findings suggest that 8 hours of immobilisation is sufficient to modulate motor cortical excitability, and that this modulation of cortical excitability appears to increase the plastic response to subsequent motor training. However, the increased use-dependent plasticity after immobilisation did not translate to improved motor performance during a pegboard task, possibly due to a reduction in motor skill retention after immobilisation. Further work is therefore needed to determine whether short-term immobilisation could be used as a rehabilitation tool to optimise plasticity and improve motor function in selected clinical populations.

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References

Abraham WC (2008) Metaplasticity: tuning synapses and networks for plasticity. *Nat Rev Neurosci* 9:387.

Avanzino L, Bassolino M, Pozzo T, Bove M (2011) Use-dependent hemispheric balance. *J Neurosci* 31:3423-3428.

Avanzino L, Pelosin E, Abbruzzese G, Bassolino M, Pozzo T, Bove M (2014) Shaping motor cortex plasticity through proprioception. *Cereb Cortex* 24:2807-2814.

Bassolino M, Campanella M, Bove M, Pozzo T, Fadiga L (2014) Training the motor cortex by observing the actions of others during immobilization. *Cereb Cortex* 24:3268-3276.

Bath KG, Lee FS (2006) Variant BDNF (Val66Met) impact on brain structure and function. *Cogn Affect Behav Neurosci* 6:79-85.

Bestmann S, Krakauer JW (2015) The uses and interpretations of the motor-evoked potential for understanding behaviour. *Exp Brain Res* 233:679-689.

Bienenstock EL, Cooper LN, Munro PW (1982) Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. *J Neurosci* 2:32-48.

Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361:31-39.

Burianová H, Sowman PF, Marstaller L, Rich AN, Williams MA, Savage G, Al-Janabi S, de Lissa P, Johnson BW (2014) Adaptive motor imagery: a multimodal study of immobilization-induced brain plasticity. *Cereb Cortex* 26:1072-1080.

Christova M, Rafolt D, Golaszewski S, Nardone R, Gallasch E (2014) Electrical stimulation during skill training with a therapeutic glove enhances the induction of cortical plasticity and has a positive effect on motor memory. *Behav Brain Res* 270:171-178.

Cirillo J, Hughes J, Ridding M, Thomas PQ, Semmler JG (2012) Differential modulation of motor cortex excitability in BDNF Met allele carriers following experimentally induced and use-dependent plasticity. *Eur J Neurosci* 36:2640-2649.

Cirillo J, Todd G, Semmler JG (2011) Corticomotor excitability and plasticity following complex visuomotor training in young and old adults. *Eur J Neurosci* 34:1847-1856.

Clark BC, Issac LC, Lane JL, Damron LA, Hoffman RL (2008) Neuromuscular plasticity during and following 3 wk of human forearm cast immobilization. *J Appl Physiol* 105:868-878.

Clark BC, Taylor JL, Hoffman RL, Dearth DJ, Thomas JS (2010) Cast immobilization increases long - interval intracortical inhibition. *Muscle & nerve* 42:363-372.

Cooke S, Bliss T (2006) Plasticity in the human central nervous system. *Brain* 129:1659-1673.

Dayan E, Cohen Leonardo G (2011) Neuroplasticity Subserving Motor Skill Learning. *Neuron* 72:443-454.

Di Lazzaro V, Oliviero A, Saturno E, Pilato F, Insola A, Mazzone P, Profice P, Tonali P, Rothwell JC (2001) The effect on corticospinal volleys of reversing the direction of current induced in the motor cortex by transcranial magnetic stimulation. *Exp Brain Res* 138:268-273.

Facchini S, Romani M, Tinazzi M, Aglioti SM (2002) Time-related changes of excitability of the human motor system contingent upon immobilisation of the ring and little fingers. *Clin Neurophysiol* 113:367-375.

Fuglevand AJ, Bilodeau M, Enoka RM (1995) Short-term immobilization has a minimal effect on the strength and fatigability of a human hand muscle. *J Appl Physiol* 78:847-855.

Garry MI, Kamen G, Nordstrom MA (2004) Hemispheric differences in the relationship between corticomotor excitability changes following a fine-motor task and motor learning. *J Neurophysiol* 91:1570-1578.

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

Huang YZ, Chen RS, Rothwell JC, Wen HY (2007) The after-effect of human theta burst stimulation is NMDA receptor dependent. *Clin Neurophysiol* 118:1028-1032.

Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC (2005) Theta burst stimulation of the human motor cortex. *Neuron* 45:201-206.

Huber R, Ghilardi MF, Massimini M, Ferrarelli F, Riedner BA, Peterson MJ, Tononi G (2006) Arm immobilization causes cortical plastic changes and locally decreases sleep slow wave activity. *Nat Neurosci* 9:1169-1176.

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

Irlbacher K, Brocke J, Mechow J, Brandt S (2007) Effects of GABA A and GABA B agonists on interhemispheric inhibition in man. *Clin Neurophysiol* 118:308-316.

Kleim JA, Chan S, Pringle E, Schallert K, Procaccio V, Jimenez R, Cramer SC (2006) BDNF val66met polymorphism is associated with modified experience-dependent plasticity in human motor cortex. *Nat Neurosci* 9:735-737.

Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, Wroe S, Asselman P, Marsden CD (1993) Corticocortical inhibition in human motor cortex. *J Physiol* 471:501-519.

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

Lee M, Kim SE, Kim WS, Lee J, Yoo HK, Park K-D, Choi K-G, Jeong S-Y, Kim BG, Lee HW (2013) Interaction of motor training and intermittent theta burst stimulation in modulating motor cortical plasticity: influence of BDNF Val66Met polymorphism. *PLoS One* 8:e57690.

López-Alonso V, Cheeran B, Fernández-del-Olmo M (2015) Relationship Between Non-invasive Brain Stimulation-induced Plasticity and Capacity for Motor Learning. *Brain Stimul* 8:1209-1219.

McDonnell MN, Buckley JD, Opie GM, Ridding MC, Semmler JG (2013) A single bout of aerobic exercise promotes motor cortical neuroplasticity. *J Appl Physiol* 114:1174-1182.

Moisello C, Bove M, Huber R, Abbruzzese G, Battaglia F, Tononi G, Ghilardi MF (2008) Short-term limb immobilization affects motor performance. *J Mot Behav* 40:165-176.

Muellbacher W, Ziemann U, Boroojerdi B, Cohen L, Hallett M (2001) Role of the human motor cortex in rapid motor learning. *Exp Brain Res* 136:431-438.

Müller-Dahlhaus F, Ziemann U (2015) Metaplasticity in human cortex. *Neuroscientist* 21:185-202.

Müller-Dahlhaus F, Ziemann U, Classen J (2010) Plasticity resembling spike-timing dependent synaptic plasticity: the evidence in human cortex. *Front Synaptic Neurosci* 2:10.3389/fnsyn.2010.00034.

Ngomo S, Leonard G, Mercier C (2012) Influence of the amount of use on hand motor cortex representation: Effects of immobilization and motor training. *Neuroscience* 220:208-214.

Nitsche M, Fricke K, Henschke U, Schlitterlau A, Liebetanz D, Lang N, Henning S, Tergau F, Paulus W (2003) Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. *J Physiol* 553:293-301.

Nitsche M, Paulus W (2000) Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J Physiol* 527:633-639.

Nudo RJ, Plautz EJ, Frost SB (2001) Role of adaptive plasticity in recovery of function after damage to motor cortex. *Muscle & nerve* 24:1000-1019.

Oldfield RC (1971) The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* 9:97-113.

Opie GM, Semmler JG (2014) Modulation of short- and long-interval intracortical inhibition with increasing motor evoked potential amplitude in a human hand muscle. *Clin Neurophysiol* 125:1440-1450.

Perez MA, Lungholt BK, Nyborg K, Nielsen JB (2004) Motor skill training induces changes in the excitability of the leg cortical area in healthy humans. *Exp Brain Res* 159:197-205.

Ridding MC, Ziemann U (2010) Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects. *J Physiol* 588:2291-2304.

Roberts DR, Ricci R, Funke FW, Ramsey P, Kelley W, Carroll JS, Ramsey D, Borckardt JJ, Johnson K, George MS (2007) Lower limb immobilization is associated with increased corticospinal excitability. *Exp Brain Res* 181:213-220.

Rogasch NC, Dartnall TJ, Cirillo J, Nordstrom MA, Semmler JG (2009) Corticomotor plasticity and learning of a ballistic thumb training task are diminished in older adults. *J Appl Physiol* 107:1874-1883.

Rosenkranz K, Seibel J, Kacar A, Rothwell J (2014) Sensorimotor deprivation induces interdependent changes in excitability and plasticity of the human hand motor cortex. *J Neurosci* 34:7375-7382.

Rossi F, Triggs WJ, Eisenschenk S (1999) Topographic differences in task-dependent facilitation of magnetic motor evoked potentials. *Neurology* 52:537-537.

Ruff RM, Parker SB (1993) Gender-and age-specific changes in motor speed and eye-hand coordination in adults: normative values for the Finger Tapping and Grooved Pegboard Tests. *Percept Mot Skills* 76:1219-1230.

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

Smyth C, Summers JJ, Garry MI (2010) Differences in motor learning success are associated with differences in M1 excitability. *Hum Mov Sci* 29:618-630.

Stefan K, Kunesch E, Benecke R, Cohen LG, Classen J (2002) Mechanisms of enhancement of human motor cortex excitability induced by interventional paired associative stimulation. *J Physiol* 543:699-708.

Stefan K, Kunesch E, Cohen LG, Benecke R, Classen J (2000) Induction of plasticity in the human motor cortex by paired associative stimulation. *Brain* 123:572-584.

Tremblay F, Wong K, Sanderson R, Coté L (2003) Tactile spatial acuity in elderly persons: assessment with grating domes and relationship with manual dexterity. *Somatosens Mot Res* 20:127-132.

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

Valls-Sole J, Pascual-Leone A, Wassermann EM, Hallett M (1992) Human motor evoked responses to paired transcranial magnetic stimuli. *Electroencephalogr Clin Neurophysiol* 85:355-364.

Voti PL, Conte A, Suppa A, Iezzi E, Bologna M, Aniello M, Defazio G, Rothwell J, Berardelli A (2011) Correlation between cortical plasticity, motor learning and BDNF genotype in healthy subjects. *Exp Brain Res* 212:91-99.

Weibull A, Flondell M, Rosén B, Björkman A (2011) Cerebral and clinical effects of short - term hand immobilisation. *Eur J Neurosci* 33:699-704.

Zanette G, Manganotti P, Fiaschi A, Tamburin S (2004) Modulation of motor cortex excitability after upper limb immobilization. *Clin Neurophysiol* 115:1264-1275.

Zanette G, Tinazzi M, Bonato C, di Summa A, Manganotti P, Polo A, Fiaschi A (1997) Reversible changes of motor cortical outputs following immobilization of the upper limb. *Electroencephalogr Clin Neurophysiol* 105:269-279.

Ziemann U, Ilic TV, Pauli C, Meintzschel F, Ruge D (2004) Learning modifies subsequent induction of long-term potentiation-like and long-term depression-like plasticity in human motor cortex. *J Neurosci* 24:1666-1672.

Ziemann U, Lonnecker S, Steinhoff BJ, Paulus W (1996a) The effect of lorazepam on the motor cortical excitability in man. *Exp Brain Res* 109:127-135.

Ziemann U, Muellbacher W, Hallett M, Cohen LG (2001) Modulation of practice-dependent plasticity in human motor cortex. *Brain* 124:1171-1181.

Ziemann U, Rothwell JC, Ridding MC (1996b) Interaction between intracortical inhibition and facilitation in human motor cortex. *J Physiol-London* 496:873-881.

Ziemann U, Siebner HR (2008) Modifying motor learning through gating and homeostatic metaplasticity. *Brain Stimul* 1:60-66.

Table 1. Subject Characteristics

	Control (*n* = 10)	Immobilised (*n* = 11)
Age (years)	21.5 ± 1.8	21.5 ± 1.5
Handedness (LQ)	0.85 ± 0.2	0.85 ± 0.2
RMT (%MSO)		
Session 1	44.0 ± 4.8	42.3 ± 4.3
Session 2	43.0 ± 4.3	44.0 ± 5.2
M_{max} (mV)		
Session 1	18.9 ± 4.2	18.9 ± 2.6
Session 2	18.9 ± 3.5	19.1 ± 3.5
BDNF Genotype (*n*)		
Val / Val	-	2
Val / Met	10	8
Met / Met	-	1

Data are shown as mean ± SD. Abbreviations: LQ laterality quotient; RMT, resting motor threshold; MSO, maximum stimulator output; M_{max}, maximum M-wave; BDNF, brain-derived neurotrophic factor

Figure 1. Experimental protocol. Abbreviations; Mmax, maximum compoud muscle action potential; RMT, resting motor threshold; MEP, motor evoked potential; SICI, short-interval intracortical inhibition; LICI, long-interval intracortical inhibition; ICF, intracortical faciliatation.

Figure 2. Effects of immobilisation on corticospinal excitability. Data show the MEP curves produced by three test stimulus intensities at the beginning of session 1 *(filled circles)* and session 2 *(unfilled circles)* for control *(A)* and immobilised *(B)* subjects. Abbreviations: Mmax, maximum M-wave; RMT, resting motor threshold. $^{*}P < 0.05$ between sessions.

Figure 3. Effects of immobilisation on intracortical excitability. Data show variations in the amplitude of the test alone MEP *(A)*, SICI *(B)*, LICI *(C)* and ICF *(D)* at the beginning of session 1 *(filled bars)* and session 2 *(unfilled bars)* in control and immobilised subjects. The dotted horizontal line shows no change in MEP amplitude, with values greater than 100% representing facilitation of the test MEP. Abbreviations: MEP, motor evoked potenial. $^{\#}P < 0.05$ when compared to control subjects; $^{*}P < 0.05$ between sessions.

Figure 4. Effect of immobilisation on motor training. The number of pegs places during the first and last motor training blocks during session 1 *(filled circles)* and session 2 *(unfilled circles)* is compared in control *(A)* and immobilised *(B)* subjects. $^{\#}P < 0.05$ when compared to the first training block; $^{*}P < 0.05$ between sessions.

Figure 5. Effect of motor training on corticospinal excitability. The change in MEP amplitude at each TMS intensity is shown for control (*A*) and immobilised (*B*) subjects in each experimental session. The dotted horizontal line shows pre-training MEP amplitudes, with values above 100% showing an increase in amplitude. Abbreviations: RMT, resting motor threshold; MEP, motor evoked potential. $^{\#}P < 0.05$ when compared to 130% RMT; $^{\dagger}P < 0.05$ when compared 130% RMT and 150% RMT; $^{*}P < 0.05$ between sessions.

Figure 6. Effect of motor training on intracortical excitability. Data show the change in SICI *(A)*, LICI *(B)* and ICF *(C)* produced by motor training during session 1 (*filled bars*) and session 2 (*unfilled bars*) in control and immobilised subjects. The dotted horizontal line shows the pre-training response to paired-pulse stimulation, with values above 100% showing a decrease in inhibition (A, B) or increase in facilitation (C) of the test alone MEP amplitude.