Time course of disuse-induced corticomotor plasticity in individual human brains: a precision TMS study

by

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Submitted to the Graduate Faculty of the

School of Health and Rehabilitation Sciences in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2022

UNIVERSITY OF PITTSBURGH

SCHOOL OF HEALTH AND REHABILITATION SCIENCES

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University of Pittsburgh, 2022

The organization of the human brain can be modified by behavior. Disuse reduces skeletomotor and corticomotor function, but the exclusive use of endpoint measures in the disused limb without consideration of spinal or peripheral influences has left the time course, origin and extent of disuse-induced corticomotor adaptations unclear.

PURPOSE: 1) to determine the time course, origin and extent of corticomotor plasticity in response to skeletal muscle disuse, 2) to assess the relationship between disuse-induced changes in corticomotor and skeletomotor function and 3) to examine whether mental imagery (MI) can counteract the disuse-induced skeletomotor and corticomotor loss of function.

METHODS: Six (3W, age: 22.7yrs, BMI: 24.4kg/m^2) healthy young adults performed daily assessments of upper- (casted and un-casted first dorsal interosseus) and lower-extremity (non-dominant tibialis anterior) skeletomotor function, corticospinal, spinal and peripheral excitability over the course of twenty-one days. To induce disuse, three participants completed a 7-day immobilization intervention (*Cast)* after seven days of baseline testing (*Pre*), which was followed by another seven days of recovery testing (*Post*). The remaining participants performed a 5-day MI counter-intervention that started 48h after the onset of immobilization. Changes in corticomotor white matter microstructure were assessed with differential tractography between diffusion scans obtained before the first day of testing, three times throughout the intervention and at the end of the study. Changes in skeletomotor and corticomotor function were determined

within-subject using ANOVAs (*Pre, Cast, Post)* with Benjamini-Hochberg corrections for multiple comparisons.

RESULTS: Immobilization markedly reduced casted hand use, strength and fine motor skill. Skeletomotor deficits coincided with reduced white matter microstructure in smaller corticomotor regions and rapid homotopic reductions in corticospinal excitability (CSE) that occurred independent of changes at the spinal or peripheral level and reversed with the recovery of function after cast removal. MI preserved skeletomotor function when CSE was maintained, but had no beneficial effects when CSE decreased.

DISCUSSION: Our results indicate that disuse-induced corticomotor plasticity is homotopic, that the skeletomotor consequences of such adaptations depend on the interplay between supraspinal and peripheral excitability, and that MI may attenuate the loss of skeletomotor function by preserving CSE.

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1.0 Introduction

In 1949, Donald Hebb hypothesized that the brain is capable of adapting its structure and function to environmental constraints based on the synchronization of pre- and post-synaptic neuronal activity, summarized by the adage *"neurons that fire together wire together"* (Hebb, 1949). Decades later, this conception use-dependent synaptic plasticity was confirmed in animal models based on hippocampal long-term potentiation (LTP) (Bliss & Gardner-Medwin, 1973; Bliss & Lomo, 1973) and depression (LTD) (Ito, 1989; Dudek & Bear, 1995) in response to repeated stimulation (Bramham & Srebro, 1987). Further evidence from *in vivo* stimulation of the human motor system (Pascual-Leone *et al.*, 1994; Muellbacher *et al.*, 2000; Stefan *et al.*, 2000; Huang *et al.*, 2005) followed courtesy of the advent of non-invasive brain stimulation techniques such as transcranial magnetic stimulation (TMS) (Barker et al., 1985). Combining neuroimaging and neuromodulatory techniques, neuroplasticity is now recognized as a key component of the acquisition or loss of skeletomotor function, as changes in brain structure (e.g. synaptogenesis, changes in expression of post-synaptic receptors, myelination, or neurotransmitter release) and function (e.g. activation of previously silent synapses, changes in neuronal synchronicity, corticospinal excitability (CSE), or functional connectivity) coincide with changes in task performance (Bütefisch *et al.*, 2000).

Much of our current understanding of *use-dependent* plasticity (Bütefisch *et al.*, 2000) is based on learning studies. For instance, repeated execution of a motor task biases movements evoked by transcranial magnetic stimulation (TMS) towards the practiced direction (Classen *et al.*, 1998) and increases the area of the motor cortex representation(s) of the involved muscles (Pascual-Leone *et al.*, 1995a). Thus, neural manifestations of use-dependent plasticity are evident

in people with unique sensorimotor skills, such as those proficient in Braille (Pascual-Leone *et al.*, 1993; Pascual-Leone & Torres, 1993; Pascual-Leone *et al.*, 1995b) and musicians (Elbert *et al.*, 1995). However, even though learning-induced sensorimotor and skeletomotor adaptations provide striking examples of use-dependent plasticity, the bidirectional relationship between the brain and body is also exemplified during reduced use (i.e. disuse).

1.1 Use-Dependent Plasticity: Disuse

Skeletal muscle disuse is a hallmark of the nearly 100 million cases of neurological disorders and skeletomotor injuries that occur every year in the United States (Yelin *et al.*, 2016; Gooch *et al.*, 2017). These disorders frequently involve temporary movement restriction, immobilization, avoidance, or in severe cases, the chronic absence of movement. It is well established that disuse negatively impacts muscle size (Seki *et al.*, 2001b) and strength (Lundbye-Jensen & Nielsen, 2008; Newbold *et al.*, 2020). However, because strength declines exceed reductions in muscle size (Campbell *et al.*, 2019), disuse-induced loss of function cannot be explained solely by skeletomotor structural adaptations (Campbell *et al.*, 2019).

Indeed, disuse is associated with a plethora of neuromuscular adaptations, including changes in central activation, motor unit firing rate (Seki *et al.*, 2001b) and peripheral excitability (Lundbye-Jensen & Nielsen, 2008). Given that skeletomotor function is mediated by cortical signals, it is no surprise that disuse also leads to corticomotor reorganization (Clark *et al.*, 2008; Campbell *et al.*, 2019; Newbold *et al.*, 2020): disuse reduces functional connectivity within sensorimotor networks (Newbold *et al.*, 2020), shrinks the size of the motor cortex representation(s) of disused (i.e. homotopic) muscle(s) (Liepert *et al.*, 1995), reduces CSE (Huber

et al., 2006; Clark *et al.*, 2008; Gaffney *et al.*, 2021) and decreases corticomotor thickness and corticospinal tract fractional anisotropy (Langer *et al.*, 2012). Use-dependent corticomotor plasticity is evident across a wide range of neuromuscular disorders, where prolonged periods of disuse is common (e.g., Parkinson's disease (Morgante *et al.*, 2006), stroke (Liepert *et al.*, 2000b), nerve injury (Ziemann *et al.*, 1998), amputation (Cohen *et al.*, 1991; Flor *et al.*, 1995), anterior cruciate ligament rupture (Flanagan *et al.*, 2021) or fractures (Zanette *et al.*, 2004)). Thus, changes in skeletomotor behavior may represent an important mechanism of corticomotor plasticity in the event of neuromuscular pathology, yet we are only beginning to understand the nature, origin and extent of such adaptations.

1.2 Definition of the Problem

Although the prevalence and cost of skeletomotor injuries and neurological disorders have risen, rehabilitation outcomes remain unsatisfactory, as re-injury and lower quality of life are common (Fältström *et al.*, 2013; Yelin *et al.*, 2016). Disuse-induced corticomotor adaptations may contribute to skeletomotor re-injury, if modifications in behavior due to an initial injury (e.g. pain avoidance, swelling, movement restriction or immobilization) result in long-lasting changes in movement strategies that exacerbate the subsequent likelihood for injury. For instance, three years after anterior cruciate ligament rupture, normative leg strength is accomplished via increased contribution from antagonist muscles and cortical thickness of the motor cortex leg representation is reduced (Flanagan *et al.*, 2021).

Disuse-induced corticomotor adaptations may further complicate recovery processes if changes in brain structure/function expand beyond the homotopic cortical regions. Even simple

unilateral movements involve bilateral cortical inputs (Chettouf *et al.*, 2020) and restricted use of one limb is frequently accompanied by increased use of the other limb (Newbold *et al.*, 2020). As such, *bilateral* corticomotor adaptations to *unilateral* disuse are possible. After stroke for instance, constraint-induced movement therapy (CIMT) restricts unilateral movements to force the use of the paretic limb (Wolf, 2007). Despite frequent increases in motor function in the paretic limb (Liepert *et al.*, 2000a), CIMT can induce structural brain changes in the non-lesioned hemisphere (Kozlowski *et al.*, 1996; Sterr *et al.*, 2013) or slow recovery compared with traditional occupational therapy (Dromerick *et al.*, 2009). Because unilateral immobilization can alter bilateral sensorimotor cortical function (Weibull *et al.*, 2011), there is increasing concern that immobilization may negatively affect the non-paretic limb and perhaps more importantly, attenuate recovery processes mediated by interhemispheric interactions (Wolf, 2007; Weibull *et al.*, 2011; Langer *et al.*, 2012). Given growing awareness that the brain is best characterized as a complex network of integrated and segregated nodes (Bullmore & Sporns, 2009; Rubinov & Sporns, 2010), changes in corticomotor organization (Newbold *et al.*, 2020) may also have downstream effects on distant, but interconnected networks. Unfortunately, prior work has emphasized the effects of unilateral immobilization on homotopic areas of the contralateral hemisphere in isolation.

Besides changes in brain function, recent evidence suggests that the time-scale of *structural* neuroplasticity is much shorter than once believed; activity-dependent changes in grey matter volume and white matter integrity can occur in less than 2h (Sagi *et al.*, 2012; Jung & Lambon Ralph, 2021). Only two studies investigated the effects of disuse on structural brain adaptations, in which immobilization decreased corticospinal white matter integrity (Langer *et al.*, 2012) and increased cortical thickness (Sterr *et al.*, 2013). As these studies involved patients with upper

extremity injuries or stroke, respectively, changes in brain structure cannot be attributed to behavior alone. Thus, the independent effects of disuse on brain structure are largely unknown.

In addition to an incomplete understanding of the extent (i.e., corticomotor vs. network-wide) and nature (i.e., structural vs. functional) of adaptation, the time course of disuse-induced corticomotor plasticity remains unclear. Few studies assessed corticomotor function early (<7 days) or *during* immobilization, which is particularly unfortunate when considering that the greatest rate of skeletomotor decline occurs during the initial days of immobilization (Campbell *et al.*, 2019). Instead, almost all studies included endpoint measurements (i.e., before and after immobilization), but the intervention duration varies extensively (range: 7-35d) (Campbell *et al.*, 2019). Although the majority of work suggests that disuse decreases CSE (Huber *et al.*, 2006; Raffin & Siebner, 2019; Gaffney *et al.*, 2021), other studies found no change (Clark *et al.*, 2008; Clark *et al.*, 2010) or even an increase (Zanette *et al.*, 2004; Roberts *et al.*, 2007). Such discrepancies could be attributed to differences in immobilization methods (e.g., upper vs. lower limb, sling vs. cast) (Campbell *et al.*, 2019), or alternatively, are tied to differences in the timing of corticomotor assessment, if disuse-induced corticomotor function increases *and* decreases at various intervals from the onset of immobilization (Wenger *et al.*, 2016). Indeed, activity-dependent changes in CSE are volatile (Gruet *et al.*, 2014), and can include transient increases *during* immobilization (Clark *et al.*, 2008).

Given the widespread use of pre-post study designs, there is also limited knowledge about the rate of corticomotor and skeletomotor recovery *after* immobilization. For example, full recovery of corticomotor and skeletomotor function can be achieved seven days after immobilization (Clark *et al.*, 2008), but the use of a single timepoint obscures the timing of such recovery. Considering that disuse can induce corticomotor and skeletomotor deficits in as little as

12h (Huber *et al.*, 2006) and that minutes of training or repeated stimulation can induce use-dependent plasticity (Pascual-Leone *et al.*, 1995a; Classen *et al.*, 1998; Huang *et al.*, 2005), it is plausible that recovery can be initiated in a similar time-frame. This has important clinical implications when an accelerated resumption to baseline activities is desirable, such as return to sports after injury or return to duty after spaceflight. Therefore, establishing the time course of corticomotor and skeletomotor function before, during, and after immobilization would not only clarify the relationship between corticomotor and skeletomotor adaptations during disuse, but provide important context about recovery in clinical or rehabilitative settings.

One avenue to determine the time course of corticomotor and skeletomotor adaptations to disuse is provided by study designs that densely sample individuals over time (Poldrack *et al.*, 2015; Gordon *et al.*, 2017b; Newbold *et al.*, 2020). In contrast to traditional approaches (e.g. pre- vs. post), such "*precision neuroimaging*" studies examine fewer participants at multiple (often daily) timepoints to allow for within-subject comparisons; in this case, each participant is treated as an experimental replicate (Newbold *et al.*, 2020). This *precision neuroimaging* approach can alleviate common issues with group-level analyses such as high inter-individual variability and preprocessing or co-registration pipelines that align individuals at the expense of spatial resolution. Moreover, precision neuroimaging provides the temporal resolution necessary to capture the interplay between disuse-induced skeletomotor and corticomotor adaptations (Campbell *et al.*, 2019). Thus, precision neuroimaging could minimize methodological confounds and advance our understanding of the progression of use-dependent corticomotor plasticity.

The feasibility of combining a precision neuroimaging approach with an immobilization intervention has been confirmed. A recent study used a 14-day immobilization intervention with daily neuroimaging and demonstrated reductions in functional connectivity between homotopic

areas of the sensorimotor cortices within 48h of disuse (Newbold *et al.*, 2020). Assessments during the intervention were restricted to behavioral use and functional magnetic resonance imaging (fMRI), with fMRI measurements limited to supraspinal brain regions. In the context of immobilization, no precision neuroimaging study has employed TMS or peripheral nerve stimulation (PNS), even though the two techniques could further elucidate the nature of neural-skeletomotor interactions at the cortical, spinal, and peripheral level (Bestmann & Krakauer, 2015) and thereby extend previous work limited to supraspinal adaptations (Newbold *et al.*, 2020).

1.2.1 Rehabilitation strategies for disuse-induced loss of function

Disuse-induced loss of function is common in neurological disorders, after skeletomotor injury, and during spaceflight. As such, strategies to mitigate skeletomotor dysfunction are needed. Minimizing disuse is an obvious first choice as physical training can counteract disuse-induced reductions in skeletal muscle protein synthesis (Ferrando *et al.*, 1997). However, in many circumstances, some degree of disuse is inevitable due to physical restrictions imposed by injury (e.g. immobilization) or pathophysiological processes (e.g. peripheral nerve damage). Thus, alternative approaches that activate shared motor neural circuits but do not require the contraction of skeletal muscle are desirable. Mental imagery (MI; i.e. visualization of movements in the absence of muscle activity) is a practical and cost-effective therapeutic technique that can modulate corticomotor activity (Mulder, 2007; Neuper *et al.*, 2009; Maranesi *et al.*, 2014). For instance, in addition to the primary motor cortex (M1), imagery activates the supplementary and premotor areas (Malouin *et al.*, 2003), all of which contain corticospinal neurons (Dum & Strick, 1996). Thus, *in the absence of skeletomotor activity,* MI can produce considerable increases in strength

(13-35%) and cortical activity (Ranganathan *et al.*, 2004) and attenuate disuse-induced loss of function (Clark *et al.*, 2014).

Although there is growing evidence that MI mitigates skeleto- and corticomotor loss of function based on assessments *before* and *after* immobilization (Clark *et al.*, 2006b; Clark *et al.*, 2014), little is known about the impact of MI on cortico- and skeletomotor function *during* disuse. Because the largest change in neuromuscular function occurs during the early days of immobilization (Wenger *et al.*, 2016; Campbell *et al.*, 2019; Gaffney *et al.*, 2021), increased understanding about the immediate impact of MI would provide important information for clinical scenarios in which an immediate preservation of immobilization-induced loss of function is important. Mental imagery appears to exert some of its skeletomotor benefits via functional neuroplasticity (e.g. maintaining inhibitory activity) (Clark *et al.*, 2014), but it is unknown if such plasticity includes detectable changes in brain structure.

1.3 Purpose

The primary purpose of this study is to investigate the origin, extent and time course of corticomotor and skeletomotor adaptations to disuse and the rate of recovery thereafter in healthy young adults. A secondary purpose is to determine whether a MI counter-intervention can mitigate disuse-induced corticomotor and skeletomotor loss of function.

1.4 Specific Aims and Hypotheses

1.4.1 Aim 1

To determine the nature of corticomotor adaptations to skeletomotor disuse.

Hypothesis 1A: Upper arm immobilization will result in use-dependent decreases in corticospinal excitability (CSE) as well as white matter integrity in corticomotor pathways specific to the casted hand (Huber *et al.*, 2006; Langer *et al.*, 2012; Opie *et al.*, 2016; Gaffney *et al.*, 2021) but will not influence peripheral excitability.

Hypothesis 1B: Disuse-induced plasticity will extend to both hemispheres, as indicated by concurrent increases in motor-evoked potential amplitude and white matter integrity in corticomotor pathways specific to the non-casted hand, as well as greater path lengths and reduced clustering coefficients of the entire sensorimotor network.

1.4.2 Aim 2

To determine the time course and relationship between corticomotor and skeletomotor adaptations to disuse.

Hypothesis 2A: Disuse-induced reductions in CSE will be evident within the first 48h of immobilization and coincide with skeletomotor loss of function, as indicated by reductions in strength and cross-sectional area.

Hypothesis 2B: Corticomotor and skeletomotor recovery will be achieved within 48h, as indicated by an increase in CSE and normalization of strength, muscle activity and cross-sectional area.

1.4.3 Aim 3

To determine the effects of mental imagery (MI) on disuse-induced corticomotor plasticity and skeletomotor loss of function.

Hypothesis 3A: MI will attenuate disuse-induced corticomotor adaptations, indicated by the maintenance of CSE and white matter integrity.

Hypothesis 3B: MI will mitigate disuse-induced skeletomotor deficits, as indicated by the maintenance of strength, muscle activity and cross-sectional area.

1.5 Study Significance

Immobilization is common in neurological and skeletomotor disorders and results in the loss of muscle strength, size, and neuromuscular function, but the nature of the effects on the nervous system remains unclear (Campbell *et al.*, 2019). For example, it is unknown whether corticomotor adaptations are restricted to the motor cortex representation of the immobilized limb, expand to both hemispheres or involve the entire sensorimotor network. Rehabilitation strategies such as CIMT encourage the preferential use of the affected limb to enhance motor recovery after stroke, yet unilateral immobilization can induce *bilateral* corticomotor adaptations. Accordingly, there is a need to better understand if unilateral disuse produces bilateral sensorimotor adaptations that may result in unintended maladaptive behavioral responses to therapies such as CIMT.

In addition, the time course and relationship between disuse-induced corticomotor and skeletomotor adaptations are not well established. For instance, disuse-induced alterations in CSE are not uncommon (Zanette *et al.*, 2004; Roberts *et al.*, 2007; Clark *et al.*, 2008), but because of

methodological discrepancies (e.g. timing and frequency of assessments, immobilization type (e.g. cast, sling), or duration (7 – 35d)), information on the direction (i.e. increase vs. decrease) of change is contradictory. Even though the largest neuromuscular changes occur within days of disuse, few studies to date have assessed the time course of skeleto- and corticomotor adaptations during short-term immobilization, let alone distinguished the extent of peripheral and central neural contributions. Similarly, there is a paucity of work on the time course of skeletomotor and corticomotor recovery *after* immobilization, an important consideration for clinical settings.

Thus, this study will follow the recommendations of the most recent systematic review to examine mechanisms underlying skeletomotor loss of function during short-term immobilization (Campbell *et al.*, 2019) and perform daily assessments of corticomotor and skeletomotor function before, during, and after seven days of immobilization. Using a precision neuroimaging design, this project will advance our understanding of the nature and time course of disuse-induced skeleto- and corticomotor adaptations, and their relationship during immobilization and recovery.

Finally, because immobilization is frequently used in clinical settings despite negative skeletomotor and corticomotor consequences, there is a need to examine counter-interventions that mitigate loss of function when disuse is inevitable. In the absence of skeletomotor physiological activity, MI produces favorable skeletomotor *and* corticomotor adaptations (Ranganathan *et al.*, 2004; Clark *et al.*, 2014), but its effect(s) on skeletomotor loss of function and corticomotor plasticity during short-term immobilization are understudied. MI is free, well tolerated, and can be translated into clinical settings with minimal logistical challenge. Moreover, because MI exerts its effects onto motor circuits alone (Ranganathan *et al.*, 2004), the mitigation of disuse-induced changes in skeleto- and corticomotor function during immobilization would *directly* confirm cortical involvement in loss of function. These results would facilitate the development of cost-

effective and practical rehabilitation protocols, which could benefit numerous populations (Campbell *et al.*, 2014; Yelin *et al.*, 2016; Gooch *et al.*, 2017).

2.0 Literature Review

Over the past few decades, it has become increasingly clear that the relationship between the brain and body is bidirectional. While the brain mediates behavior, behavioral experiences act as potent determinants of brain structure and function. Such *use-dependent plasticity* (UDP) is frequently viewed through the lens of learning but is also exemplified by the sensorimotor (i.e. primary motor, somatosensory, premotor cortex and supplementary motor area) and skeletomotor adaptations that accompany skeletomotor injuries (MSI), neurological disorders, and experimental skeletomotor disuse models. In this review, we first summarize the principles underlying UDP and then discuss how the progression from initial lesion mapping studies to complex combinations of behavioral interventions has informed current conceptions of UDP. Drawing on insight from neuroimaging, neuromodulatory and electrophysiological efforts, we showcase the overlap of several principles of sensorimotor and skeletomotor adaptations but remind the reader that metaplastic principles may govern the extent of such plasticity. We conclude by examining how the quantity, quality and timing of behavioral experiences modulates UDP and explain how interventions inducing metaplastic adaptations could provide insight into the functional relevance of use-dependent sensorimotor plasticity in healthy and clinical adult populations.

2.1 Principles of use-dependent plasticity

It has long been known that two neurons can change the strength of their connection if one persistently takes part in the firing of the other (Hebb, 1949). Use-dependent plasticity (UDP) is

one form of such Hebbian plasticity, as repeated efferent and afferent activity – common to all behavior – facilitates the creation and reinforcement of synapses that are active during fragmented but coincident movements and somatosensation (Bütefisch et al., 2000). Thus, UDP provides a flexible mechanism to encode kinematic details that are important for motor and sensory performance (Classen *et al.*, 1998) and is often considered a primary mechanism of sensorimotor learning (Muellbacher *et al.*, 2001).

UDP can be transient and occur within minutes or persist over days and years. Crucially, the frequency and duration of synaptic activation influences the direction of UDP: whereas short bursts of high-frequency stimulation induce a fast post-synaptic rise in Ca^{2+} and long-term potentiation (LTP), prolonged low-frequency stimulation induces a slow post-synaptic rise in Ca^{2+} and long-term depression (LTD) (Yang *et al.*, 1999; Malenka & Bear, 2004). UDP is also associative and temporally asymmetric, as presynaptic stimulation prior to postsynaptic activation induces LTP, but the opposite pattern produces LTD (Magee & Johnston, 1997; Bi & Poo, 1998). This spike-timing dependence of UDP is likely related to the arrival of back-propagated action potentials in the post-synaptic dendrite following presynaptic stimulation (Stuart & Sakmann, 1994) and implies that the causality of neuronal activity is yet another important feature of UDP (Caporale & Dan, 2008).

Non-mutually exclusive, UDP involves the functional reinforcement (or weakening) of existing connections and the *de novo* structural formation (or elimination) of synapses within sensorimotor regions of cortex. Existing synapses can be modified by gamma aminobutyric acid (GABA) (dis)inhibition-induced (un)masking of horizontal excitatory connections (Jacobs & Donoghue, 1991; Chen *et al.*, 1998; Liepert *et al.*, 2000b). Accordingly, during pharmacological blockade of inhibition, movements typically associated with neighboring areas can be evoked by

the stimulation of adjacent brain regions (Jacobs & Donoghue, 1991). At the same time, new synapses can be formed via axonal or dendritic arborization: dendritic spines – major recipients of excitatory synaptic signaling – grow and appear during LTP, but shrink and disappear during LTD (Hess & Donoghue, 1994; Rema *et al.*, 1998; Allen *et al.*, 2003) (Trachtenberg *et al.*, 2002). Finally, UDP may also extend beyond synapses, as oligodendrocyte progenitor cells modify axonal myelination status in an activity-dependent manner, which alters information transmission synchronization patterns (Fields, 2015; Noori *et al.*, 2020). Thus, changes in behavior can facilitate the gain or loss of sensorimotor structure and function across a variable time scale, depending on the intricacies of the experience.

Although most of our understanding of UDP is based on cellular evidence, the advent of non-invasive brain stimulation and neuroimaging techniques has enabled the study of UDP at the systems level in humans *in vivo.* For instance, transcranial magnetic stimulation (TMS) (Barker *et al.*, 1985) can be used to quantify changes in excitatory and inhibitory interactions within the corticospinal tract (i.e. corticospinal excitability, CSE) which are frequently involved in UDP. Together with functional magnetic resonance imaging (fMRI), TMS can capture practice-related changes in the activation of trained motor cortex representations (Karni *et al.*, 1995; Pascual-Leone *et al.*, 1995a; Pascual-Leone *et al.*, 1995b). In addition to the diagnostic applications of single pulse TMS, repetitive TMS (rTMS) can induce UDP and thereby provide causal information about the behavioral consequences of LTP-like or LTD-like processes. Paired-pulse TMS can be used to assess changes in intracortical facilitation and inhibition (Kujirai *et al.*, 1993; Liepert *et al.*, 2000b; Weier *et al.*, 2012) that resemble cellular aspects of GABAergic disinhibition inherent to UDP (Stefan *et al.*, 2002). In addition, paired associative stimulation (PAS) (Stefan *et al.*, 2000), a specialized rTMS technique that combines peripheral (somatosensory) and central (motor) nerve

stimulation, has been used to confirm the spike-timing-dependent properties of UDP (Wolters *et al.*, 2003). Finally, voxel and surface-based measures of grey matter volume or thickness based on structural magnetic resonance imaging (MRI) provide a proxy for the use-dependent formation and elimination of synapses, while diffusion MRI (dMRI) can provide information about the changes in the integrity or myelination of white matter (Langer *et al.*, 2012). Thus, courtesy of an ever-increasing arsenal of neuroimaging tools, greater efforts are being made to clarify the relationship between sensorimotor and skeletomotor function in humans *in vivo*.

2.2 Use-dependent interplay between sensorimotor and skeletomotor function: insight from learning studies, skeletomotor injuries and neurological disorders

The interplay between skeletomotor behavior and sensorimotor plasticity is often highlighted by learning studies. Use-dependent increases in CSE are fundamental to the consolidation of movements, since learning-induced enhancements in skeletomtotor function coincide with increases in CSE and the acute attenuation of CSE abolishes such behavioral improvements (Muellbacher *et al.*, 2002). For instance, the application of low-frequency rTMS over the primary motor cortex (M1), but not the dorsolateral prefrontal or occipital cortex, eliminates skeletomotor improvements otherwise seen with training (Muellbacher *et al.*, 2002; Hortobágyi *et al.*, 2009). In accordance, individuals with unique sensorimotor skills, such as those proficient in Braille (Pascual-Leone *et al.*, 1993; Pascual-Leone & Torres, 1993; Pascual-Leone *et al.*, 1995b; Cohen *et al.*, 1997), musicians (Elbert *et al.*, 1995; Gaser & Schlaug, 2003; Rosenkranz *et al.*, 2007; Herholz & Zatorre, 2012) and elite athletes (Nielsen & Cohen, 2008) display unique sensorimotor properties compared with controls. Thus, learning studies suggest that the repeated

execution of a motor task influences sensorimotor organization and that such modifications in return improve skeletomotor behavior.

Even though learning provides an obvious example of UDP, the bidirectional relationship between sensorimotor and skeletomotor function also extends to reduced behavior (i.e. disuse). Early evidence for disuse-induced UDP was based on lesion mapping (Bates *et al.*, 2003), an approach that links sensorimotor and skeletomotor function based on the overlap of lesions across patients with shared symptomatology. After stroke, for instance, lesions are often focal and the size as well as location typically coincide with impairment(s) (Marchina *et al.*, 2011). Most famously, this simple, but powerful reasoning shaped an entire field of study when a bilateral medial temporal lobe resection resulted in memory impairments in patient H.M. (Scoville & Milner, 1957). Yet, the logic inherent to lesion mapping is also restricted by its simplicity, which assumes that behavior is mediated by isolated brain areas when most behaviors instead involve complex sensorimotor integration across hemispheres and functionally connected, but segregated networks (Cisek & Kalaska, 2010; Bullmore & Sporns, 2012). Lesions can downregulate GABAergic activity in the contralateral hemisphere (Shimizu *et al.*, 2002), facilitate the induction of LTP in perilesional areas (Feeney & Baron, 1986; Hagemann *et al.*, 1998), or alter cortico-cortical connections that shape whole brain architecture (Honey & Sporns, 2008). Thus, two identical lesions can manifest as distinct skeletomotor gains or losses of function (Carrera & Tononi, 2014) and additional sensorimotor deficits can arise in distant but functionally connected brain areas (Bütefisch *et al.*, 2003). Because such findings were incompatible with the principles of lesion mapping, hypotheses emerged that changes in sensorimotor functional or structural connectivity may contribute to the skeletomotor deficits after brain injury (Nudo, 1999).

The idea that behavior could alter such sensorimotor connectivity and contribute to UDP gained traction with emerging evidence of immobilization-induced decreases in the size of the immobilized motor cortex leg representation following fracture (Liepert *et al.*, 1995). Damage to the brain, therefore, was not a pre-requisite for sensorimotor reorganization. Other forms of injury-related disuse, such as amputation, also result in altered sensorimotor reorganization (Chen *et al.*, 1998) while rehabilitation can reverse existing sensorimotor and skeletomotor deficits post stroke (Bütefisch *et al.*, 1995). Collectively, these findings provide compelling evidence that the sensorimotor system is modified by use and that such use-dependent adaptations have important implications for skeletomotor function in health and disease. Because CSE and motor cortex representations increase with use (i.e. training) (Pascual-Leone *et al.*, 1995a), but decrease with disuse (i.e. immobilization) (Liepert *et al.*, 1995), these observations also suggest that sensorimotor and skeletomotor adaptations are subject to similar principles, mneumonically summarized as *"use it or lose it"*. Consequently, there is increasing interest in efforts to leverage behavioral strategies to bias sensorimotor reorganization in a manner that optimizes recovery following injury or neurological insult.

One of the first approaches to bias sensorimotor reorganization was constraint-induced movement therapy (CIMT). CIMT is a popular therapeutic strategy after stroke and involves the restriction of movement in the non-paretic limb to force the use of the paretic limb and thereby improve adaptive recovery of motor function (Wolf *et al.*, 2006; Kwakkel *et al.*, 2015). In addition to enhancements in skeletomotor function, CIMT increases CSE and the size of the motor cortex representation of the paretic limb (Liepert *et al.*, 1998), reiterating that changes in behavior influence sensorimotor function and highlighting that UDP can be induced late in adulthood. Yet, CIMT may also decrease the size of the motor cortex representation of the non-paretic (disused)

limb (Liepert *et al.*, 1998), suggesting that UDP involves interhemispheric dynamics. Neural mechanisms, including contralateral strength effects, were hypothesized to contribute to strength increases more than a century ago (Sale, 1988) (Scripture *et al.*, 1894), but were questioned due to concerns that such improvements were biased by issues related to (a lack of) familiarity to testing protocols (Housh *et al.*, 1992). Nevertheless, accumulating evidence of *bilateral* improvements in skeletomotor function after *unilateral* resistance training (Carolan & Cafarelli, 1992; Munn *et al.*, 2004; Carroll *et al.*, 2006) reinstated interest in cross-education. Indeed, when training only the non-paretic limb after stroke, muscle function and strength of the paretic limb improves (Dragert & Zehr, 2013). Bilateral strength improvements further coincide with increases in CSE of the trained limb and reductions in inhibition of the untrained leg (Goodwill *et al.*, 2012; Latella *et al.*, 2012). Accordingly, when combined with immobilization, cross-education mitigates the immobilization-induced reduction in strength and corticospinal excitability (Farthing *et al.*, 2009; Pearce *et al.*, 2013). Thus, CIMT and cross-education strengthened hypotheses that already emerged in lesion mapping studies: UDP is not necessarily restricted to the (dis)used circuits and instead involves functionally connected networks.

2.3 Use-dependent interplay between sensorimotor and skeletomotor function: insight from experimental models of disuse

Although UDP is exemplified by the sensorimotor and skeletomotor adaptations that accompany neurological disorders, MSI and recovery thereafter, the presence of pathophysiological process complicates the interpretation of the behavioral contributions to UDP. For instance, inflammation, pain, and edema are all common after stroke and alter afferent

signaling. Similarly, deafferentation, common to MSI, can induce disinhibitory effects that alter CSE and motor cortex representation topography (Brasil-Neto *et al.*, 1992; Brasil-Neto *et al.*, 1993). Thus, to study the distinct contributions of behavior to sensorimotor reorganization, UDP must be induced via experimental models that do not involve peripheral or neuronal injury.

Because early work already demonstrated its efficacy after peripheral injury (Liepert *et al.*, 1995), immobilization presented a promising intervention to clarify distinguish the contributions of injury and behavior to UDP. In the absence of injury, immobilization decreased strength, muscle size, CSE and the size of the motor cortex representations of the casted limb (Facchini *et al.*, 2002; Huber *et al.*, 2006; Opie *et al.*, 2016; Raffin & Siebner, 2019), suggesting that behavior alone can influence sensorimotor organization. Whereas immobilization demonstrated that skeletomotor behavior can modify sensorimotor organization, mental imagery (MI) indicated that sensorimotor behavior can modify the skeletomotor system. MI involves the rehearsal of movements in the absence of neuromuscular activity and activates movement-related brain regions, including the motor, somatosensory and premotor cortices, as well as the supplementary motor area (Grèzes & Decety, 2001; Facchini *et al.*, 2002; Hanakawa *et al.*, 2008; Raffin *et al.*, 2012). MI increases CSE (Rossi *et al.*, 1998; Rossini *et al.*, 1999) and strength (Ranganathan *et al.*, 2004), and when combined with immobilization, mitigates disuse-induced changes in corticospinal inhibition, voluntary activation and strength (Clark *et al.*, 2014). Thus, immobilization and MI provided bi-directional evidence to strengthen the concept that behavior independently contributes to UDP.

2.4 Factors influencing the magnitude and extent of use-dependent plasticity

2.4.1 Quantity and timing of behavioral experiences

With the growing awareness of behavior-induced UDP, there was a surge of interest to identify how UDP could be optimized. A single session of rTMS was known to induce UDP (Muellbacher *et al.,* 2000), so an intriguing possibility was that consecutive sessions would amplify such adaptations. Although some early findings suggested that the effects of low-frequency rTMS could be primed by subthreshold, inhibitory stimulation of the motor cortex (Iyer *et al.*, 2003), as it turned out, more (especially too soon) stimulation was not always better and could reverse the effects of UDP via metaplasticity (i.e. the plasticity of synaptic plasticity) (Abraham & Bear, 1996). Whereas LTP is easier (more difficult) to induce following inhibitory (facilitative) preconditioning (Müller *et al.*, 2007), LTD is more pronounced (diminished or reversed) following facilitative (inhibitory) priming (Siebner *et al.,* 2004). Because metaplastic adaptations favor the stability of excitability, are most pronounced immediately after stimulation and vanish with increasing latency between UDP-inducing events (Fricke *et al.*, 2011), homeostatic mechanisms seem to control the ease and direction of UDP. Such regulation is not provided by the positive feedback loop inherent to Hebbian plasticity, as continuous increases in excitation would increase the risk of runaway excitation (Pozo & Goda, 2010). Therefore, as put forth in the Bienenstock-Cooper-Munro rule (Bienenstock *et al.*, 1982) UDP is regulated by a time-variable induction threshold for LTP/LTD, such that the history and temporal proximity of activity prior to LTP/LTD induction (e.g. sleep, physical exertion, nutritional intake) shapes the capacity for UDP. Such regulation is also reminiscent of the skeletomotor hypertrophy in response to

resistance training, but muscle breakdown with overtraining, providing yet another common element of skeletomotor and sensorimotor adaptation.

2.4.2 Quality of behavioral experiences

In addition to the quantity and timing of behavior, there are several other factors that modulate UDP. Pharmacological studies suggest that UDP can be modified by circulating levels of cortisol (Sale *et al.*, 2008), in addition to dopaminergic and cholinergic inputs (Sawaki *et al.*, 2002a; Sawaki *et al.*, 2002b). Moreover, the quality of behavioral experiences modulates UDP, as enriched sensorimotor stimuli boost UDP. For instance, during MI, participants are frequently relayed first-person videos of muscular contractions. Such action observation increases CSE (Fadiga *et al.*, 1995) and activates overlapping but distinct cortical areas compared with movement execution and MI (Grèzes & Decety, 2001). Thus, when combining action observation with MI, increases in CSE are greater than after MI or action observation alone (Roosink & Zijdewind, 2010).

Importantly, rather than the addition of visual stimuli in general, action-observation induced modulation of CSE is driven by the processing and insight gained from movement-specific information (Stinear *et al.*, 2006). When observing grasping actions of different weights, for instance, CSE is increased during heavy- versus light weights (Alaerts *et al.*, 2010a; Alaerts *et al.*, 2010b; Senot *et al.*, 2011). Such weight-dependent modulation is sensitive to intrinsic cues, such as the contraction state of the hand or the kinematic details of the task, but insensitive to external information, such as object characteristics (Alaerts *et al.*, 2010b). Similar findings have been reported for the processing of auditory information (Buccino *et al.*, 2005), which reiterates that

behavior involves complex interplay across multiple brain networks and suggests that UDP may be enhanced when experiences are enriched by behaviorally-relevant multisensory inputs.

2.4.3 Combination of behavioral experiences: synergistic or competing interactions?

One important detail about multisensory enhancement of UDP is that each behavioral component induces similar UDP when performed in isolation (e.g. action observation and MI each increase CSE and motor cortex representation size). This similarity may be crucial, as earlier work demonstrated that the combination of immobilization and MI, which elicit opposing effects (i.e. increase vs. decrease in strength, CSE and motor cortex representation size), result in the net maintenance of sensorimotor and skeletomotor function (Clark *et al.*, 2014). Similar competition is evident when attention is focused on or diverted away from a muscle targeted by PAS (Stefan *et al.*, 2004), which suggests that, for a given experience and muscle, the quality of sensorimotor input can strongly influence the extent of UDP.

Because these findings imply that UDP reflects the sum of individual behavioral effects, it appears that disuse models could be strategically applied to neighboring muscles to facilitate training-induced UDP in the targeted muscle(s). Such surround inhibition has been described in the visual system (Blakemore *et al.*, 1970), but appears as an important mechanism to increase the spatial and temporal acuity of inputs during fine sensorimotor tasks (Sohn & Hallett, 2004; Beck & Hallett, 2011). For example, if upregulation of GABAergic inhibitory inputs to antagonistic muscles facilitates the focal disinhibition of agonistic muscles, improvements in motor function are likely. Indeed, isolated training of the index or little finger during concurrent immobilization of all other fingers more focally up-regulates CSE, disinhibits the trained muscle and accelerates learning compared with training alone (Raffin & Siebner, 2019). Therefore, the extent and

magnitude of UDP may also be modified when behavioral strategies with opposing (i.e. LTD vs. LTP) effects on sensorimotor organization are applied to neighboring muscles with distinct involvement in the trained task.

Ironically, the synergistic interplay between immobilization and training across neighboring muscles (Raffin & Siebner, 2019) is reminiscent of early lesion, cross-education and CIMT studies which suggested that UDP is not restricted to the involved brain area (Feeney & Baron, 1986). The spatial extent of UDP is an evolving topic of study, as immobilization disconnects the disused sub-circuit from the remainder of the sensorimotor network, while emergent pulses of spontaneous activity maintain the functional connectivity within the disused network (Newbold *et al.*, 2020). Thus, the spatial extent may be similarly governed by homeostatic mechanisms and intervention potency as the magnitude of UDP, which raises important questions about the functional consequences of homeostatic plasticity. Does the prohibition of LTP after an initial increase in neuronal activity limit skeletomotor adaptation? Conversely, can the preferential facilitation of LTP after LTD induction enhance skeletomotor performance? Skeletomotor and sensorimotor adaptations often coincide (Ziemann *et al.*, 2001), but causal evidence is surprisingly sparse (Muellbacher *et al.*, 2002; Hortobágyi *et al.*, 2009). Similarly, compensatory behavioral alterations are common after rehabilitative interventions and injuries, but it remains unclear if sensorimotor adaptations mediate such changes. Thus, contrasting the skeletomotor consequences of UDP with those elicited by homeostatically-induced LTD/LTP may provide one avenue to clarify the causal interplay between sensorimotor and skeletomotor systems.

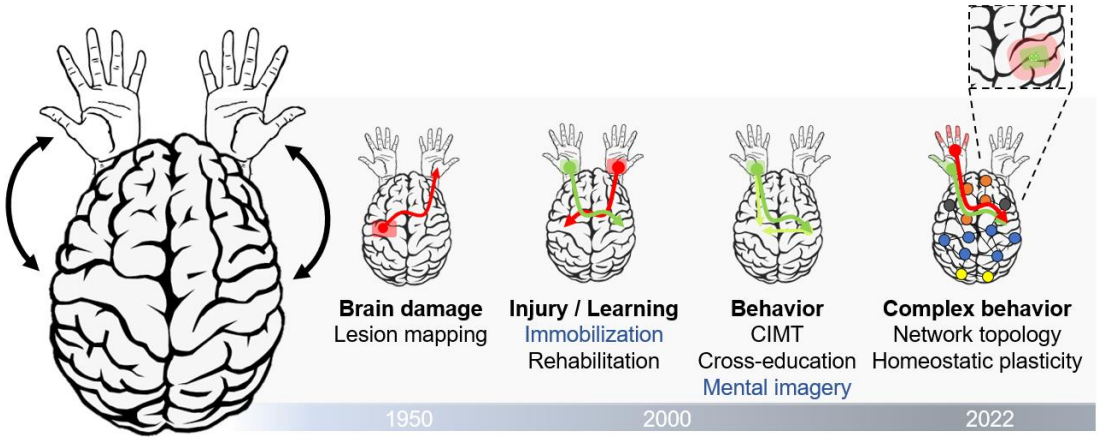


Figure 1. The growing understanding of the bidirectional relationship between brain and body.

2.5 Concluding remarks

As indicated by a plethora of work in motor learning, neurological disorders, skeletomotor injuries and experimental models of UDP, there is a bidirectional relationship between brain and body structure and function. The tight interplay between sensorimotor and skeletomotor systems is highlighted by several shared principles of adaptation: both 1) requirement for perturbation (i.e. overload principle), 2) enhancement with increased use and degradation with reduced use (i.e. *'use it or lose it'*), 3) specificity (i.e. (dis)used muscle(s)) 4) sensitivity to the timing, quantity and quality of perturbation and 5) the ability to functionally support or interfere with connected, but distant regions. Following early lesion mapping studies, which demonstrated correlational reciprocity between the sensorimotor and skeletomotor systems, there is now growing interest in efforts to selectively bias sensorimotor adaptations with behavioral and neuromodulatory techniques. The brain's predisposition for plasticity and stability may govern the extent of such adaptations, as the timing, quantity and quality of environmental experiences modulate the

magnitude of UDP. Thus, homeostatic mechanisms may become an increasingly important area focus to clarify the functional consequences of sensorimotor adaptations and determine how to optimize the bidirectional relations between the brain and body.

3.0 Methods

3.1 Study Design

The study followed recent precision neuroimaging approaches that densely sample individual participants over time and treat each participant as an experimental replicate (Poldrack *et al.*, 2015; Gordon *et al.*, 2017b; Fair & Yeo, 2020; Newbold *et al.*, 2020). Six (N=6) individuals completed the study, and each participant was tested for twenty-one consecutive days After seven days of baseline testing, each participant completed a seven-day upper extremity casting intervention, followed by seven days of recovery testing. Three (N = 3) randomly selected participants performed a five-day MI counter-intervention beginning 48h after the commencement of immobilization. All statistical comparisons were made within-subject. Daily assessments were based on accelerometry, questionnaires, transcranial magnetic stimulation (TMS), peripheral nerve stimulation (PNS) and behavioral assays of skeletomotor function. Five times throughout the study, participants completed magnetic resonance imaging (MRI) to improve the accuracy and consistency of TMS targeting and to assess changes in white matter microstructural integrity.

The study followed the recommendations of a recent systematic review on immobilization interventions that include: 1) use of fixed-joint immobilization; 2) multiple assessments of neuromuscular function during the early stages of immobilization; and 3) preferential use of shorter immobilization periods (≤7 days) (Campbell *et al.*, 2019). The feasibility of this approach was confirmed by a recent study that combined immobilization with daily neuroimaging over the course of 42-61 days (Newbold *et al.*, 2020).

3.1.1 Casting Intervention

During the immobilization intervention, participants were fitted with a non-removeable upper extremity cast from the shoulder to fingertips of the non-dominant arm (Clark *et al.*, 2014; Newbold *et al.*, 2020). Hand dominance was determined via the Edinburgh Handedness Inventory (Oldfield, 1971) and casts were customized to enable electromyography (EMG), ultrasound (hand), as well as peripheral (ulnar) nerve stimulation.

3.1.2 Mental Imagery Counter-intervention

Three (N=3) participants completed a five-day MI counter-intervention to attenuate immobilization-induced strength loss (Clark *et al.*, 2014). Randomization was completed using a random number generator (R Studio Team, 2020). Given that the biggest changes in neuromuscular function occur within the initial days of immobilization (Campbell *et al.*, 2019), the delayed onset of the MI counter-intervention allowed for within-subject determination whether MI can attenuate immobilization-induced declines in neuromuscular function. Mental imagery was performed daily and included one hundred 5s imagined maximal contractions of the casted hand separated into four sets of 25 repetitions, with 30s rest between sets (total duration ~25 min). Importantly, the regular completion of 52 5s mental repetitions (four sets of 13 repetitions, with one minute of rest between sets) repeated over four weeks can reduce immobilization-induced muscle weakening (Clark *et al.*, 2014).

Due to the shorter time-course of immobilization in this study, an increased number of mental repetitions was used. In addition, as the combination of action observation and MI induces greater brain plasticity than passive MI alone (Bisio *et al.*, 2018), videos of maximum contractions

(recorded during baseline testing using a chest- or head-mounted camera to obtain a first person perspective) were relayed to each participant during MI (GoPro Hero, GoPro, Inc. San Mateo, CA, USA). Participants received standardized instructions to imagine abducting their index finger against a force transducer (as shown on a video screen in front of them) while keeping the target muscles rested (Ranganathan *et al.*, 2004; Clark *et al.*, 2014). Raw EMG signals were monitored in real-time to confirm the absence of muscle activity.

3.2 Participants

3.2.1 Recruitment

Six (N=6; 3 women) healthy young adults, between the ages of 18 and 45 participated in this study, but seven individuals were enrolled, since one participant did not respond to TMS. Participants were recruited from the local area via recruitment flyers. Prospective participants contacted the research team for screening and to determine eligibility.

3.2.2 Inclusion Criteria

To be eligible for the study, participants needed to be: 1) comfortable with transcranial magnetic stimulation (TMS), electroencephalography (EEG), magnetic resonance imaging (MRI) and peripheral nerve stimulation (PNS), 2) between 18 and 45 years of age, 3) complete at least 150 minutes of physical activity per week and 4) have normal or corrected normal vision.

3.2.3 Exclusion Criteria

Individuals were excluded based on the following criteria:

- History of mental imagery training
- History of epilepsy, seizure, or sleep disorders
- History of neurological, cardiovascular, psychiatric, mental health of other major disorders
- History of alcohol or substance abuse
- No medical clearance for physical activity
- Contraindication(s) to transcranial magnetic stimulation (TMS) or magnetic resonance imaging (MRI) such as metal implants, shrapnel in the body, dental retainers, copper IUDs
- Limiting skeletomotor injury
- Current use of CNS-active, seizure-threshold-lowering, anti-inflammatory, ototoxic, or anabolic hormonal substances
- Pregnancy
- Claustrophobia
- Inability to avoid caffeine (e.g. energy drinks, coffee, supplements) 6h prior to testing
- Current brain injury, psychiatric or mental health disorder(s)
- Inability to produce a response to single-pulse TMS at ≤86% of maximum stimulator output
- Weight ≥300lbs due to MRI scanner restrictions

3.3 Instrumentation and Experimental Procedures

3.3.1 Accelerometry

Wrist-worn accelerometers provide valid quantifications of real-world upper extremity movement (Hoyt *et al.*, 2019) and have been used to confirm disuse (Newbold *et al.*, 2020). Thus, participants wore tri-axial accelerometers (wGT3X-BT, Actigraph, Pensacola, FL, USA) on both wrists throughout the study to confirm requisite use and disuse patterns. Every day, participants returned the accelerometers to the laboratory. Acquired data was downloaded and batteries were recharged prior to the next day's use.

3.3.2 Experimental Controls

At the beginning of each visit, participants completed electronic surveys on RedCap (Patridge & Bardyn, 2018) to confirm adherence to experimental controls and account for potential confounds. Sleep quality and soreness were assessed via Likert scales and affective state was assessed using the Profile of Mood States short form (Curran *et al.*, 1995). During the casting intervention, an additional Likert scale was used to determine cast comfort. Participants were instructed to maintain regular medication use, diet, exercise and sleep throughout the study, which was confirmed every day. Prior to each visit, behavioral restrictions included heavy exercise (2h), caffeine (6h) and alcohol or drugs/analgesics (12h). To minimize the influence of circadian rhythmicity, participants were tested at a similar time of day (±2h) each visit. As the safety of TMS for fetuses is unknown, pregnancy resulted in exclusion.

3.3.3 Sensor Placement

After completion of questionnaires and return of accelerometers, all experimental procedures began with calibration and sensor placement. Electromyography (EMG) signals were recorded from both hands (first dorsal interosseus; FDI) and the non-dominant leg (control; tibialis anterior; TA) using wireless active Ag differential parallel-bar sensors (Trigno, Delsys, Natick, MA). Skin preparation included light exfoliation with adhesive tape and cleansing with alcohol swabs. EMG sensor positioning conformed with SENIAM guidelines (Hermens *et al.*, 2000). Signal quality was verified before testing and sensor location marked with indelible ink for consistent positioning throughout the study.

3.3.4 Transcranial Magnetic Stimulation

Transcranial magnetic stimulation (SuperRapid^2, The Magstim Company Ltd., Carmarthernshire, UK) was used to assess daily changes in corticospinal excitability (CSE) for the hands (FDI) and the non-dominant leg (TA). Biphasic single TMS pulses were delivered using a figure-of-eight coil with ~1cm^3 focality (D70^2, The Magstim Company, Ltd. Carmarthernshire, UK; hands) or a curved coil (96mm, Jaltron LLC, Waltham, MA, USA;leg). During all test procedures, participants were resting in a seated position in a comfortable chair with the hands and legs completely relaxed (confirmed by EMG). Individual structural MRI images (see MRI section below) were uploaded into a neuronavigation system (see *Magnetic Resonance Imaging* section below) that uses frameless stereotactic neuronavigation system (Brainsight v2.4, Rogue Research, Inc., Montreal, Quebec, Canada) and used to maximize stimulation accuracy and precision during the study.

For all TMS procedures, the coil was positioned at a 45-degree angle (FDI) or parallel to the longitudinal fissure (TA) to induce a posterior-anterior/anterior-posterior current along the precentral gyrus. The stimulation site (motor hotspot) was determined on the first day of testing and maintained for all other visits. To locate the motor hotspots, suprathreshold single-pulse TMS were delivered with coil positioning slightly adjusted in 1-2cm increments (Groppa *et al.*, 2012), starting at the region of maximal functional MRI activity until the location that consistently produced the largest MEPs was found. All subsequent TMS was referenced to these hotspots, with coil location- and angle error minimized in real-time and recorded for subsequent analysis.

Resting motor thresholds (RMT) were determined in accordance with current consensus guidelines (Groppa *et al.*, 2012). Starting at subthreshold stimulation intensities, the minimum stimulation intensity required to elicit an MEP in each target muscle was determined using the adaptive parameter estimation by sequential testing procedure (Mishory *et al.*, 2004). Testing order (hands vs. the non-dominant leg) was randomized. Participants then received 120 single TMS pulses to each hotspot (N = 360) at 115% of *baseline* RMT and an interstimulus interval of ~0.2Hz (Premoli *et al.*, 2014; Zrenner *et al.*, 2020). Motor-evoked-potentials (MEP) were quantified as the peak-to-peak amplitude of the 15-65ms post stimulus for each trial.

3.3.5 Peripheral Nerve Stimulation

To assess peripheral responses to disuse, compound motor action potentials (M_{MAX}) were recorded in accordance with consensus guidelines (Rossini *et al.*, 2015) and published experience (Giroux *et al.*, 2018) (Brownstein *et al.*, 2018b). Monophasic rectangular pulses (200μs pulse width) were applied to each ulnar nerve (FDI) and the non-dominant peroneal nerve (TA) using a gold-plated bar electrode and a constant current stimulator (DS7HA, Digitimer LLC, Ft.

Lauderdale, FL) with an interstimulus interval of 0.1–0.2Hz. Stimulation intensity was increased in 20mA steps until an initial M_{MAX} (I_{MAX}) was consistently produced. Three test stimuli were then delivered at 130% I_{MAX} to ensure that stimulation was supramaximal (Brownstein *et al.*, 2018b). M_{MAX} was used to normalize MEPs and to assess the possibility of changes in peripheral excitability.

The use of a cast represented a unique sensorimotor experience during the immobilization when compared with baseline and recovery testing. Thus, cast-related changes in sensory input could influence sensorimotor organization and electrophysiological measurements independent of disuse. To confirm that changes intervention effects reflected responses to immobilization and sensorimotor aspects of casting, peripheral and central nerve stimulation procedures were performed twice (with and without cast) on the first day of the casting intervention.

3.3.6 Ultrasonography

Ultrasonography (SonoSite X-Porte, Bothell, WA, USA) was used to quantify disuse-induced changes in bilateral FDI and non-dominant TA muscle cross-sectional area and to confirm skeletomotor structural recovery after the immobilization intervention. Conductive gel was used to maximize image quality. Probe positioning was marked with indelible ink and framed by the cast (non-dominant FDI) for consistent placement throughout the study.

3.3.7 Skeletomotor Function

At the end of each visit participants completed a battery of skeletomotor functional tests. All procedures were completed with both hands and the non-dominant leg, except during the casting

intervention, when only the dominant (non-casted) hand and non-dominant leg were tested (because the cast prohibited assessments in the casted hand).

Strength was determined based on maximum voluntary isometric contractions (MVC). For the FDI, participants were seated and instructed to maximally abduct their index finger against a load cell (MB-100, Interface Inc.). During TA MVCs, participants were instructed to maximally dorsiflex their ankle while seated, with hip-, knee- and ankle at 90º (Biodex, Biodex Medical Systems). Four 3-5s trials were performed and all trials were retained for analysis.

Force steadiness was assessed unilaterally with a trapezoidal ramp protocol. Participants were shown visual guidelines equal to 10, 30 and 50% MVC (10,40,70%MVC for TA) and instructed to match force to these guidelines. Force was increased linearly from rest (0%MVC) at 10% MVC per second until target force was reached, followed by a 10s plateau and a decrease back to rest at 10%MVC per second (~20s per trial). After unilateral trials, the hands additionally performed two bilateral trials at each intensity, during which one force guideline (equivalent to the sum of forces of the unilateral trials) was shown.

Finally, to determine fine motor skills, participants completed the grooved pegboard test with each hand. Participants placed twenty-five small metal pegs (~3cm) into small holes as fast as possible using one hand and one peg at a time. Participants completed two trials per side, with time to completion as the primary experimental outcome and and all trials retained for analysis.

3.3.8 Magnetic Resonance Imaging

Structural and functional brain images were acquired at five time points: 1) prior to the first day of testing; 2) the start of the casting intervention; 3) the second day of casting; 4) the end of casting; and 5) at the end of the study. Brain images will be used to: 1) facilitate TMS navigation

and 2) examine changes in structural connectivity (diffusion MRI; [dMRI]) and 3) sensorimotor network organization. During all procedures, participants laid flat inside the MRI bore and focus their attention on a white fixation cross or instructions (black background) while remaining still. A standard magnetization-prepared rapid gradient-echo sequence (voxel size = 1 × 1 × 1mm, TR = 2300ms, TE = 2.03ms) was completed first. Second, to investigate the possibility of changes in white matter microstructure or structural connectivity between sensorimotor regions over time, a diffusion sequence (dMRI) was performed. A second dMRI scan was obtained at baseline to calculate false-discovery rates for differential tractography analysis (Yeh *et al.*, 2019b). Finally, on the first day of MRI only, participants completed a 10-min task-based functional MRI procedure (fMRI; voxel size = 2 × 2 × 2mm, TR = 2000, TE = 30ms) to identify functional activation maps for import into neuronavigation software. The task comprised alternating unilateral and bilateral isometric contractions of the FDI and TA separated by 10s of rest (total ~ 7min) All imaging was performed at the Carnegie Mellon University (CMU)-Pitt Brain Imaging Data Generation and Education (BRIDGE) center.

Table 1. Daily Visit Summary

Task	*Device*	*Coil*	*Site*	*Intensity*	*Pulses*	Measure	*Action*	*Time (min)*
Intake forms	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	N/A	*N/A*	10
Sensor placement	*N/A*	*N/A*	*2x FDI, 1x VL*	*N/A*	*N/A*	N/A	Rest	0
Hotspot*	Biphasic TMS	F8 / CC	2x FDI, 1x VL	45-75%	~60	Hotspot	Rest	15
RMT	Biphasic TMS	F8 / CC	2x FDI, 1x VL	N/A	~90	RMT (%)	Rest	15
CSE	Biphasic TMS	F8 / CC	2x FDI, 1x VL	115%RMT	360	MEP/TEP (μV)	Rest	30
M_{MAX}	Digitimer	N/A	2 x Ulnar, 1x femoral	Supramax	~18	M_{MAX} (μV)	Rest	15
Ultrasound	Ultrasound	N/A	2x FDI, 1x VL	N/A	N/A	CSA (mm^2)	Rest	10
Skeletomotor function	Load cells / EMG	N/A	2x FDI, 1x VL	N/A	N/A	MVC (N)	Active	20
Mental imagery[$]	N/A	N/A	1x FDI	N/A	N/A	N/A	Rest	20

3.4 Data Analysis

Accelerometry data was transferred from devices during the experimental visit and downsampled from 30Hz to 1Hz. Use counts were quantified as the number of seconds per day with the root mean square (RMS) above a noise threshold of 10 accelerometer units (1 unit = $0.016 m/s^2$) and expressed as the ratio between the casted and un-casted hand (Newbold *et al.*, 2020).

All electrophysiological processing procedures followed recent guidelines and current best practices (Rossini *et al.*, 1994; Groppa *et al.*, 2012; Rossini *et al.*, 2015; Brownstein *et al.*, 2018b). Corticospinal (peripheral) excitability was assessed as motor-evoked-potential (compound muscle action potentials, M_{MAX}), quantified as the peak-to-peak EMG amplitude from 15-65ms (0-15ms) post TMS (PNS). Given the large intersession variability of MEPs (Kiers *et al.*, 1993), outliers will be removed prior to statistical analysis.

For MVCs, force steadiness and bimanual force contributions, all trials were used in the analysis. Force steadiness at each contraction intensity was expressed as the coefficient of variation (CV) of force (Almuklass *et al.*, 2018; Mani *et al.*, 2018). Force contributions of each hand were quantified as the percentage of force generated during bilateral trials, relative to the respective unilateral trials.

3.5 Statistical Analysis

In alignment with recent precision neuroimaging studies, each participant was treated as a separate (replicate) experiment. As such, statistical comparisons were made within-subject using one-way ANOVAs when possible (Newbold *et al.*, 2020). The Benjamini-Hochberg (Benjamini & Hochberg, 1995) procedure was used to correct for multiple comparisons based on false-discovery rates ($q < 0.05$). Normality was assessed using Shapiro-Wilk's test. In the event of non-normality, data was log-transformed.

4.0 Time course of disuse-induced corticomotor plasticity in individual human brains: a precision TMS study

Abstract

Introduction: Behavior can modify the organization of the human brain. Disuse reduces skeletomotor function and changes corticomotor activity, but the exclusive use of endpoint measures in the disused limb without consideration of spinal or peripheral influences has left the origin, extent and time course of disuse-induced central neural adaptations unclear.

***Purpose*:** to determine the origin, extent and time course of corticomotor plasticity in response to skeletal muscle disuse.

***Methods*:** Six (3W, age: 22.7yrs, BMI: 24.4kg/m^2) healthy young adults performed daily assessments of upper- (casted and un-casted first dorsal interosseus) and lower-extremity (non-dominant tibialis anterior) skeletomotor function, corticospinal, spinal and peripheral excitability over the course of twenty-one days. To induce disuse, three participants completed seven days of baseline testing (*Pre*) followed by a seven-day immobilization intervention (*Cast)* and seven days of recovery testing (*Post*). The remaining participants additionally performed a five-day mental imagery (MI) counter-intervention that started 48h after the onset of immobilization. Changes in corticomotor white matter microstructure were assessed with differential tractography between diffusion scans obtained at the beginning of the study, three times throughout the intervention and at the end of the study. Changes in skeletomotor and corticomotor function were determined within-subject with ANOVAs (*Pre, Cast, Post)* and Benjamini-Hochberg pairwise-comparisons.

***Results*:** Immobilization markedly reduced the use, strength and fine motor skill of the casted hand. Skeletomotor loss of function coincided with rapid reductions in corticospinal

excitability (CSE) in the casted- but not un-casted hand or leg, and these adaptations occurred independent of changes at the spinal or peripheral level. MI maintained strength when CSE was maintained, but did not preserve fine motor skill.

***Discussion*:** These results confirm that disuse-induced corticomotor deficits primarily occur supraspinal, that MI may attenuate the loss of skeletomotor function by increasing CSE, and that the skeletomotor benefits of MI are likely task-specific.

4.1 Introduction

The structural and functional organization of the brain can be modified by changes in use (Wiesel & Hubel, 1965; Wong, 1995). Such use-dependent plasticity (UDP) plays a critical role in learning (Pascual-Leone *et al.*, 1995a; Muellbacher *et al.*, 2001; Muellbacher *et al.*, 2002) and recovery from injury (Liepert *et al.*, 1995; Liepert *et al.*, 1998; Liepert *et al.*, 2000b), since changes in sensorimotor function encode kinematic details important for behavioral improvements (Classen *et al.*, 1998). One powerful model to study UDP in the human sensorimotor system is provided by immobilization: disuse decreases corticospinal excitability (CSE) (Facchini *et al.*, 2002; Huber *et al.*, 2006; Opie *et al.*, 2016), reorganizes sensorimotor circuits (Langer *et al.*, 2012; Newbold *et al.*, 2020; Newbold *et al.*, 2021) and reduces strength, muscle activation patterns and fine motor skill (Seki *et al.*, 2001b, a).

Previous disuse studies revealed that the relationship between the brain and body is bidirectional, but the exclusive use of endpoint measurements (i.e, *pre-post* comparisons) has left the time course of corticomotor adaptations unclear (Clark *et al.*, 2014). Even though the preponderance of skeletomotor loss of function occurs during the early phase of immobilization (Campbell *et al.*, 2019), little is known about disuse-induced corticomotor adaptations during this time, which is particularly unfortunate considering the popularity of immobilization remains as a therapeutic strategy following injury. Moreover, few studies have investigated the consequences of disuse for the non-immobilized limb or distant but functionally connected cortical regions even though early evidence from rehabilitative studies (Liepert *et al.*, 1998) and more recent graph theoretical analyses confirm that the consequences of disuse may extend beyond the disused cortical circuits (Newbold *et al.*, 2021).

Precision neuroimaging densely samples individual brains over time (Laumann *et al.*, 2015; Poldrack *et al.*, 2015; Gordon *et al.*, 2017a; Gordon *et al.*, 2017b; Greene *et al.*, 2020; Laumann *et al.*, 2021) and can be used to trace the time course of disuse-induced corticomotor plasticity. Using daily resting-state functional magnetic resonance imaging (rs-fMRI) during the course of ~42-64 days, two-weeks of upper-extremity immobilization rapidly (~48h) disconnected the disused- from the remaining sensorimotor circuits, but increased functional connectivity to the cingulo-opercular network (Newbold *et al.*, 2020; Newbold *et al.*, 2021). Changes in functional connectivity coincided with weakening of the casted hand, but because rs-fMRI is inherently restricted to supraspinal areas, disuse-induced adaptations at the spinal and peripheral level, which also mediate brain-body interactions, remained uninvestigated. Characterization of spinal and peripheral adaptations to disuse could clarify the origin of disuse-induced reductions in CSE, but current evidence for such contributions based on endpoint measurements is contradictory (Clark *et al.*, 2006b; Clark *et al.*, 2008).

We performed daily transcranial magnetic stimulation (TMS) and peripheral nerve stimulation (PNS) over the course of 21 consecutive days to monitor disuse-induced skeletomotor and corticomotor adaptations at distinct corticospinal levels before, during and after seven days of unilateral upper extremity immobilization. To determine the extent of disuse-induced corticomotor plasticity, CSE was assessed in the casted and un-casted hand, as well as the non-dominant leg. If disuse-induced changes in CSE mirror reductions in functional connectivity between homologous sensorimotor cortical areas (Newbold *et al.*, 2020), immobilization should decrease motor-evoked potentials (MEP) independent of changes in spinal or peripheral excitability. Given the use of immobilization as a rehabilitative strategy, cost-effective and practical interventions to mitigate disuse-induced corticomotor adaptations may improve clinical outcomes. In the absence of

muscular contractions, mental imagery (MI) activates motor circuits in and has the potential to attenuate the disuse-induced loss of skeletomotor and corticomotor function. Given that little is known about the immediacy of MI effects., we also examined whether a five-day MI counter-intervention could mitigate disuse-induced corticomotor deficits and clarify the nature of corticomotor contributions to skeletomotor (loss of) function.

4.2 Methods

4.2.1 Participants and experimental design

Three healthy, young adults (ages 21-23, 1W, 1 left-handed and left-footed, **Table 2**) without recent skeletomotor injury or contraindications to TMS (Rossi *et al.*, 2011) and magnetic resonance imaging (MRI) completed daily TMS and PNS along with skeletomotor structural and functional assessments for twenty-one consecutive days (D1-D21). After seven days of baseline testing (*Pre*), participants underwent a seven-day immobilization intervention (*Cast*), followed by seven days of recovery testing (*Post*). Prior to the beginning of the study, 24h and 48h after cast construction, the day of cast removal, and completion of the study, participants also completed structural, functional and diffusion magnetic resonance imaging (**Fig. 1A**). Similar to previous precision neuroimaging efforts, each participant was analyzed as a distinct experimental replicate. All study procedures were performed by the same member of the research team and testing time was standardized (±2h).

Table 2. Participant characteristics.

ID	Gender	Age (years)	Height (cm)	Weight (kg)	Handedness	Footedness
DIS1	M	23	188	85.6	Right	Right
DIS2	W	21	170	64.8	Right	Right
DIS3	M	23	193	87.5	Left	Left

Heavy physical exercise, caffeine intake and alcohol were prohibited 2h, 6h and 12h prior to each visit, respectively. Participants were also instructed to maintain similar dietary and sleep habits throughout the study. Stability of sleep quality and quantity, mood as well as adherence to the experimental restrictions were confirmed every day (**Sup. Table 1**). In addition, adequate hydration was ensured using urine refractometry (Master URC, Atago Co, Ltd., Tokyo, Japan). In the event that participants were dehydrated (urine-specific gravity (USG) $\leq$ 1.025), water was provided.

Written informed consent was obtained for all study procedures and participants were familiarized to the protocol. Data collection was completed from January-April 2022, and the study was approved by the University of Pittsburgh Institutional Review Board (IRB#: STUDY21020173).

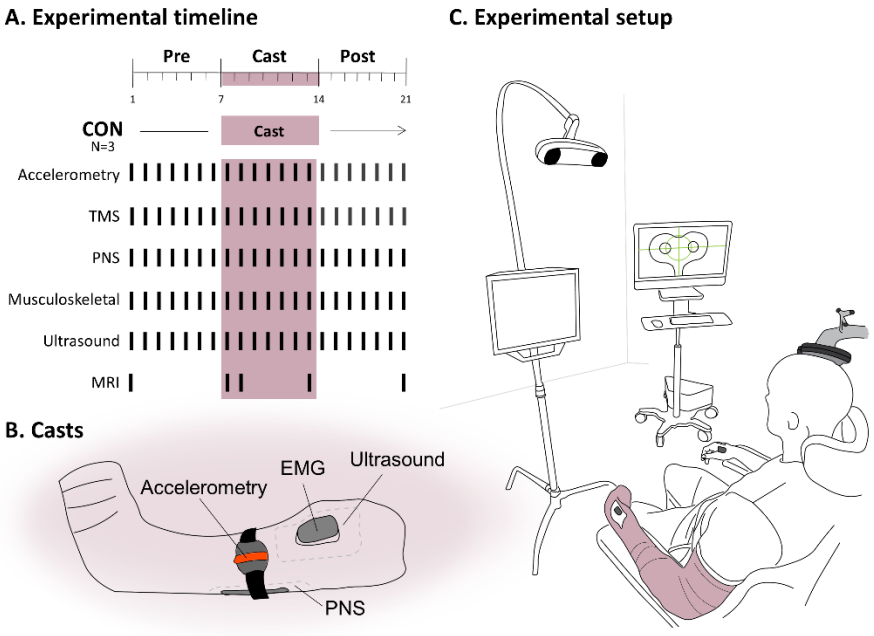


Figure 2. Experimental conditions.

Three participants completed daily assessments of skeletomotor and corticomotor function over the course of 21 consecutive days, with seven days of unilateral immobilization (A). Casts were customized to allow for peripheral nerve stimulation (PNS), electromyography and ultrasound measurements (B). Setup for transcranial magnetic stimulation and PNS (C).

4.2.2 Casting intervention

Disuse was induced via an immobilization intervention. Participants were fitted with a cast on the non-dominant arm, from the shoulder to beyond the fingertips. The casts were constructed immediately after D7 using a stockinette, an inner layer of cotton undercast padding (Webril^{TM}, Covidien, Medtronic Inc., Minneapolis, MN, USA) and an outer layer of fiberglass tape (Scotchcast^{TM}, 3M Company, Maplewood, MN, USA). To minimize discomfort, casts were constructed with hands positioned in *'intrinsic-plus'*: slight extension at the wrist, the

metacarpophalangeal joints at ~90º and full extension of the proximal interphalangeal joints. The elbow joint was bent naturally, at ~90-100º flexion. An oscillating blade saw was used to customize the casts with cutous that would allow for ulnar nerve stimulation, as well as electromyography (EMG) and ultrasound measurements of the first dorsal interosseus (FDI; **Fig 1B**). Outside of data collection, the sensor cutouts were carefully placed back into the frame of the cast and secured using Coban. All casts were worn for the entire time during the casting intervention, and no participant reported discomfort or pain due to the cast.

4.2.3 Accelerometry

To quantify activity of daily living outside of the laboratory and confirm unilateral disuse participants wore tri-axial accelerometers on each wrist (wGT3X-BT, Actigraph, Pensacola, FL, USA); these sensors provide valid estimates of upper extremity movement (Hoyt *et al.*, 2019). The accelerometers were worn throughout the study, except during testing when batteries were recharged and data was downloaded. Data was sampled at 30Hz and wear time was validated with non-wear time removed prior to analysis (ActiLife V6.13.4, Actigraph, Pensacola, FL, USA). To quantify daily hand use, we summed the three-dimensional use counts and calculated the ratio between the casted and un-casted hand (Newbold *et al.*, 2020).

4.2.4 Electromyography

Participants completed the same battery of transcranial magnetic stimulation (TMS), peripheral nerve stimulation (PNS) and skeletomotor functional tests each day of the study. Wireless electromyography (EMG) sensors (Trigno Galileo, Delsys, Inc., Natick, MA, USA; inter-

electrode spacing = 5mm, bandwidth filter = 20-450Hz) were placed over each first dorsal interosseus (FDI) and the non-dominant tibialis anterior (TA) muscle according to SENIAM guidelines (Hermens *et al.*, 2000). Skin preparation included shaving hair, exfoliating skin using adhesive tape and applying isopropyl alcohol. Adequate signal to noise ratio were confirmed prior to data collection. Sensor placement was marked with a surgical pen to ensure consistency across visits. Data was sampled at 2kHz, with sensor input range set to 22mV (TMS & PNS) or 11mV (skeletomotor function).

4.2.5 Transcranial magnetic stimulation

Biphasic single pulse TMS (Super Rapid^2, The Magstim Company Ltd., Carmarthernshire, UK) was applied to the motor cortex representation of each hand and the non-dominant leg in randomized order using a figure-of-eight (hands; The Magstim Company Ltd., Carmarthernshire, UK) or a custom curved coil (leg; Jaltron, LLC, Boston, MA, USA) and following current best practices (Rossi *et al.*, 2009; Groppa *et al.*, 2012; Rossi *et al.*, 2020). TMS assessments were performed using frameless stereotactic neuronavigation (Brainsight v2.4, Rogue Research, Inc., Montreal, Quebec, Canada) and an infrared camera system (Polaris Vicra, Northern Digital Inc., Waterloo, Ontario, Canada), which incorporated individual images of brain structure and functional activation during contractions of the target muscles (see *magnetic resonance imaging* section below). Participants and structural brain images were co-registered in Montreal Neurological Institute (MNI) space using reflective markers and cranial landmarks (nasion, left and right periauricular area, inion).

Hotspot identification started with the coil positioned over the peak functional activation area of the respective target muscle. Coil positioning was adjusted in 1-2mm increments until the

site that consistently produced the largest motor-evoked potential (MEP) was identified. Hotspots were determined on D1 and used for all subsequent visits. Resting motor thresholds (RMTs) were then assessed using maximum likelihood regression as implemented by the adaptive parameter estimation through sequential testing (Mishory *et al.*, 2004). RMTs were re-assessed every day, but D1 RMT stimulation intensity was maintained throughout the study. Finally, 120 biphasic TMS pulses were delivered to the hotspot of each hand and the non-dominant leg to quantify CSE. Simulation intensity was set to 115% RMT and the interstimulus interval was 0.3-0.4Hz. Individual MEPs were quantified as the peak-to-peak EMG amplitude 15-65ms after stimulation.

During all procedures, participants rested comfortably in a seated position in a therapeutic chair (The Magstim Company Ltd., Carmarthernshire, UK) and each coil was oriented to induce a posterior-anterior/anterior-posterior current across the precentral gyrus/paracentral lobule (45º for FDI, parallel to longitudinal fissure for TA). For each trial, coil placement was referenced to the hotspot, with target and angle error recorded for analysis (Proessl *et al.*, 2021).

4.2.6 Peripheral nerve stimulation

Peripheral and spinal excitability were determined via transcutaneous electrical stimulation of each ulnar (FDI) and the peroneal (TA) nerve. The anode was placed distal. Starting at 20mA, square wave pulses (200μs pulse width) were applied using a constant current stimulator and a bar stimulating electrode (DS7A, Digitimer Ltd, Welwyn Garden City, UK). Stimulation intensity was incrementally increased until a maximal response (I_{MAX}) consistently appeared in the target muscle at a latency 5-25ms post-stimulation. Then, ten stimuli were delivered at 130%I_{MAX} to derive maximal muscle compound action potentials (M_{MAX}) (Brownstein *et al.*, 2018a; Ansdell *et al.*, 2019). Supramaximal stimulation can elicit late responses that reflect the antidromic activation of

motor neurons in the spinal cord. Thus, F-waves (Mesrati & Vecchierini, 2004) were also interrogated in order to better understand the effect of disuse on spinal excitability. M_{MAX} and F-waves were defined as the peak-to-peak amplitude in EMG activity from 5-25ms (M_{MAX}) and 25-40ms (F-wave) after stimulation, respectively.

4.2.7 Skeletomotor function

Skeletomotor function was assayed based on maximum voluntary isometric force (MVC), submaximal isometric force steadiness, unimanual force contributions during bimanual force steadiness tasks, as well as pegboard test completion time. For MVCs, four 3-5s trials (30s rest between) of maximum index finger abduction or ankle dorsiflexion were performed against load cells (MB-100, Interface Inc., Scottsdale, AZ, USA; 500 gain) or an isokinetic dynamometer (Biodex System 4, Biodex, Shirley, NY, USA), respectively. For the hands, participants were seated with elbows at 90º and wrists slightly extended. For the leg, participants were seated, with the knee and ankle at approximately 90º and the tibia oriented parallel to the ground. Body position was recorded on D1 and kept constant for the remaining visits. Verbal encouragement was given. For each trial, peak force (torque) was retained for analysis.

After MVCs, participants completed submaximal isometric contractions with force matched to visual guidelines set to 10, 30 and 50% (hands) or 10, 40, and 70% (leg) of D1 MVC values. One participant performed non-compliant MVCs on D1, so force guidelines were determined based on MVC obtained on D2. Two trials were obtained at each %MVC and target muscle. The force (torque) ramp phases for each trapezoidal contraction were standardized at ~10%MVC·s^{-1} using a metronome. Target force (torque) was maintained for 10s. During baseline and recovery, contractions were performed with each hand and the non-dominant leg. During the

intervention, contractions were performed with the un-casted hand and the non-dominant leg only. Each task was performed unilaterally and force steadiness was quantified as the coefficient of variation (CV) during the 10s maintenance of the target force.

To determine the force contributions of each hand during bimanual tasks, participants performed two additional bilateral submaximal isometric contractions at each %MVC. For these trials, participants were asked to match one visual guideline equivalent to the sum of unilateral forces. The contributions of the casted and un-casted hand were then quantified as the percentage of force during the bilateral contraction relative to their unilateral contraction.

Fine motor skill was assessed based on the grooved pegboard test completion time (Lafayette Instruments, Lafayette, IN, USA). Briefly, participants took pegs from a basket and placed them into twenty-five holes with randomly positioned grooves as fast as possible. The pegs were placed one at a time in a standardized order as quickly as possible. Two trials were completed for each hand with time to completion recorded using a stopwatch.

4.2.8 Skeletomotor structure

Bilateral FDI muscle cross-sectional area (CSA) was assessed with ultrasonography (X-Porte, FUJIFILM SonoSite, Inc., Bothwell, WA, USA) (Miller *et al.*, 2018; Miller *et al.*, 2019). Images were obtained with participants seated, hands pronated, and the thumb and index finger positioned at a ~80-90º angle. On D1, the midpoint between origin and insertion was identified and marked with a surgical pen. EMG sensor placement overlapped with the midpoint of the probe position. Transverse cross-sectional images were captured with generous amounts of ultrasound gel and the transducer (HFL50 15-6MHz, Fujifilm SonoSite Inc., Bothwell, WA, USA) oriented perpendicular to the 2nd metacarpal. Images were obtained in duplicate and exported to ImageJ

(National Institutes of Health, Bethesda, Maryland, USA) for analysis. After scaling images from pixels to cm using the straight-line function, CSA was derived by outlining the perimeter of the FDI using the polygon function.

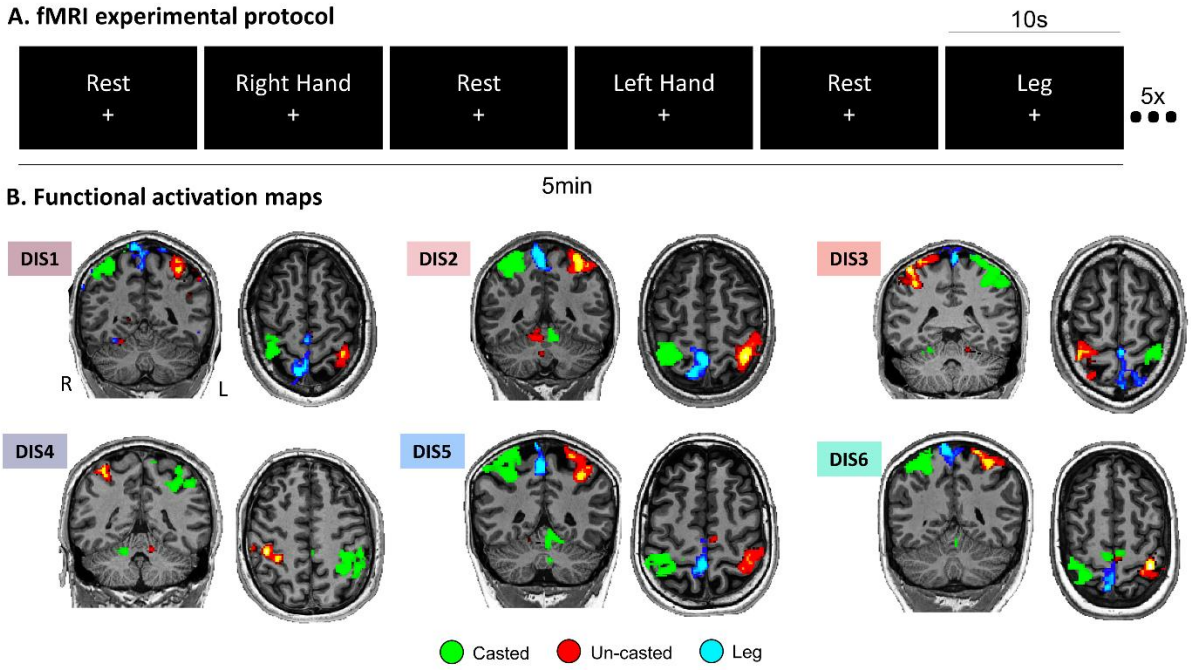
4.2.9 Magnetic resonance imaging

Brain structure and function were assessed via magnetic resonance imaging (MRI; 3T Tim Trio System, Siemens, Erlangen, Germany) on five separate days: prior to the first day of testing, 24h and 48h after the start of immobilization, upon completion of the intervention and one the last day of testing. Each day, participants completed a T1-weighted magnetization-prepared rapid gradient echo scan (MP-RAGE; TR = 2300ms, TE = 2.03ms, flip angle=9º, voxel size=1mm^3), and a diffusion-weighted scan (dMRI: TR = 2500ms, TE = 99.6ms, multi-band acceleration factor = 4, flip angle = 90º, in-plane resolution and slice thickness = 2mm, b-value range: 0-4000 s/mm^2, 258 diffusion directions). To better handle susceptibility artifact and current distortion, which is common in diffusion MRI, a reverse phase encoding scan was acquired (TR = 2490ms, TE = 999.2ms, multi-band acceleration factor = 4, flip angle = 90º, in-plane resolution and slice thickness = 2mm). To estimate false discovery rates during differential tractography, a second diffusion scan (i.e. sham scan) was obtained at baseline, which had identical parameters to the first sequence. Throughout all procedures, participants were instructed to minimize head movement, relax and focus their attention onto a white fixation cross. Finally, participants performed intermittent contractions of each hand and the non-dominant leg interleaved by 10s of rest during a task-based functional scan (fMRI; TR = 2000ms, TE = 30ms, voxel size = 2mm isovoxel; **Sup. Fig. 1A)**. Contractions were performed based on written cues presented on a screen, which was synchronized to the MR sequence using E-Prime and a trigger interface (Chronos Adapter,

Psychology Software Tools, Inc., Sharpsburg, PA, USA). For all scans, a 64-channel coil and a head stabilizer were used to minimize head movement artifacts.

4.2.10 Functional and structural MRI

Functional images were analyzed with established preprocessing pipelines in the fMRI Expert Analysis Tool in FMRIB's Software Library (FSL V.6.00; www.fmrib.ox.ac.uk/fsl). Preprocessing included motion correction (Jenkinson *et al.*, 2002), removal of non-brain tissue with the brain extraction tool (Smith, 2002), spatial smoothing (5mm full-width at half maximum Gaussian kernel), intensity normalization via single multiplicative factors, and high-pass temporal filtering (sigma = 50.0s). Independent component analysis was used to remove unexpected artefacts or activation when present (Beckmann & Smith, 2004). Structural and functional images were co-registered with FLIRT and refined into standard space with FNIRT nonlinear registration (Jenkinson & Smith, 2001; Jenkinson *et al.*, 2002). Statistical analyses of time-series data was performed using FILM with local autocorrelation correction (Woolrich *et al.*, 2001) and gaussianized Z-statistic images were thresholded using a cluster and corrected significance threshold of $Z > 3.1$ and $p < 0.05$, respectively. Functional activation maps (**Sup. Fig. 1B**) were then imported into neuronavigation software to facilitate TMS targeting.



Supplementary Figure 1. Motor cortex representations of the hands and leg at baseline.

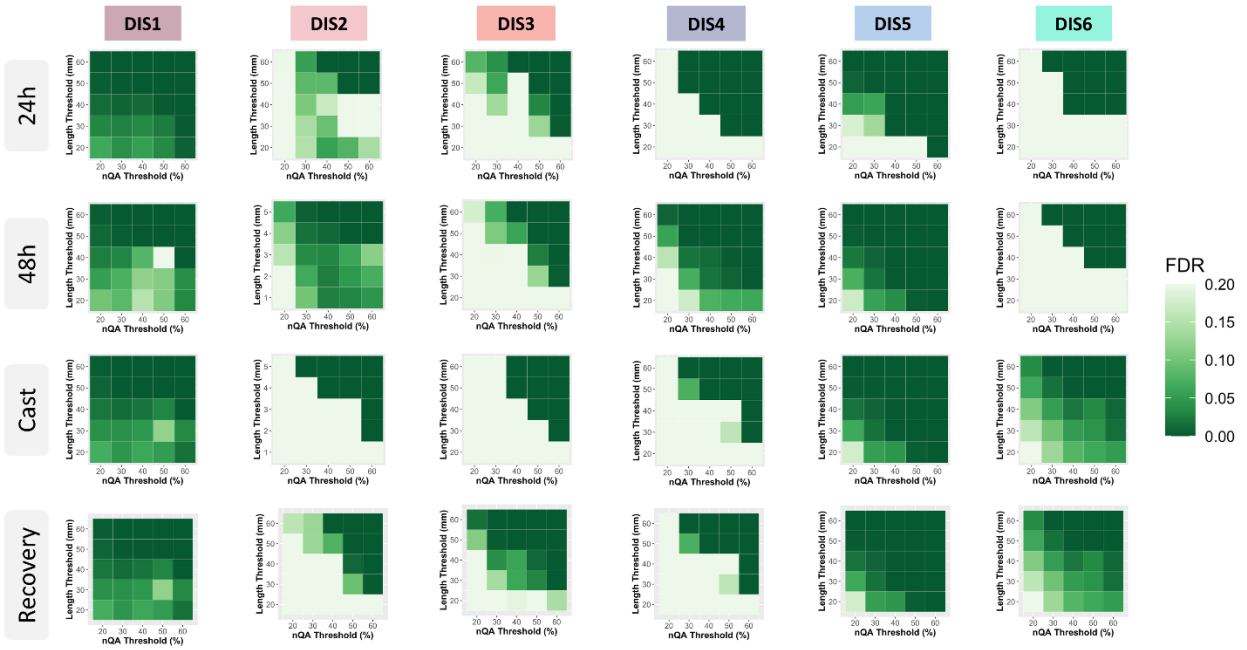
Participants performed a five-minute task-based functional magnetic resonance imaging (fMRI) sequence with interleaved 10s contractions of the casted (green) hand, un-casted hand (red), or leg (blue) and 10s of rest between contractions (A). Each contraction was repeated five times to derive functional activation maps (B). Functional activation maps were imported into neuronavigation software to facilitate TMS targeting. Color intensity in (B) is proportional to activation magnitude (i.e., z-score), which was thresholded to 50% of maximum activation for visualization purposes. DIS3 and DIS4 were left-handed, all other participants were right-handed. DIS3 was left-footed. R: right hemisphere, L: left hemisphere.

4.2.11 Differential tractography

Diffusion images were analyzed in DSI studio (Version "Chen", dsi-studio.labsolver.org). The quality of b-table accuracy was verified by comparing fiber orientations to a population-averaged template (Yeh *et al.*, 2018). Susceptibility artifact was estimated using reversed phase-encoding scans via DSI Studio's implementation of FSL's EDDY and TOPUP (Andersson *et al.*,

2003; Smith *et al.*, 2004). Restricted diffusion was quantified (Yeh *et al.*, 2017) and reconstruction was performed using generalized q-sampling imaging (Yeh *et al.*, 2010). Whenever necessary, masks were smoothed, eroded, dilated or defragmented to ensure accurate coverage prior to reconstruction. Differential tractography (Yeh *et al.*, 2019b) was used to explore changes in normalized quantitative anisotropy (nQA) (Yeh *et al.*, 2013) at each timepoint relative to baseline. Whole brain tractograms were obtained by placing 5,000,000 seeds throughout the brain, with angular threshold and step size randomly selected from 15-90º and 0.5-1.5 voxels, respectively. Tracts shorter than 30 or longer than 200mm were discarded. Two iterations of topology-informed pruning (Yeh *et al.*, 2019a) were performed to remove false positives. In the event that topology-informed pruning could not resolve obvious false positives, manual pruning was performed.

Pairwise (i.e., scan-to-scan) differential tracking was performed after the removal of false positives. To maximize sensitivity and specificity, differential tractography was performed across a range of length (20-60mm, 10mm increments) and nQA thresholds (20-60%, 10% increments) (Yeh *et al.*, 2019b). Whereas higher nQA and length thresholds minimize false positives and improve specificity, lower nQA and length thresholds minimize false negatives and improve sensitivity, especially in shorter segments. After inspection of the length-nQA-threshold matrices for each participant, we used an nQA threshold of 50% and a length threshold of 50mm for primary analysis. The number of tracts that differed in each pairwise comparison was further divided by the difference between the baseline and a sham scan in order to calculate the false discovery rate (FDR) (Yeh *et al.*, 2019b). Between-day changes were considered statistically significant when $q \leq 0.05$. Pairwise FDR statistics across all length and nQA thresholds can be found in **Sup. Fig. 2**.



Supplementary Figure 2. Pairwise false discovery rates (FDR) for varying length and normalized quantitative anisotropy (nQA) change thresholds . Matrices denote participant- and day-specific FDRs across varying length (20-60mm) and nQA (20-60% decrease) thresholds. Color coding is proportional to FDR with darker shades of green indicating q < 0.05.

4.2.12 Graph theoretical analysis

The impact of disuse on sensorimotor network architecture was explored using graph theory. First, the hand, leg and face sensorimotor representations, the premotor cortex, and supplementary motor area were parcellated into thirty (15 per hemisphere) regions of interest (ROI) using the Brainnetome atlas. Detailed descriptions of the Brainnetome labels and their corresponding anatomical region are shown in (**Sup. Table 2**). Connectivity matrices were computed with edges defined as the mean nQA of tracts between each node. To remove false positives, connectivity matrices were thresholded by 0.001 of the matrix sum (default). Global graph theoretical outcomes (i.e., characteristic path length, global efficiency) were derived using

the Brain Connectivity Toolbox (Rubinov & Sporns, 2010). To determine the influence of disuse on global sensorimotor network architecture, graphs were additionally generated using a region of avoidance (ROA) that removed tracts which previously demonstrated disuse-induced decreases in nQA using differential tractography. After the tracts that were affected by disuse were removed, graph theoretical outcomes were quantified again and compared to baseline. Given the exploratory nature of this analysis, differences in network topology were not assessed statistically, but expressed as percentage changes.

4.2.13 Data analysis and preprocessing

Data processing, visualization and analysis was performed in Rstudio (Version 1.2.5019) (R Studio Team, 2020). Daily median MEP estimates were generated after outliers (i.e., interquartile rule) and trials with poor TMS targeting (i.e., >1mm target or >2º angle error) were removed (<5% for each participant). Time courses of M_{MAX} and F-wave amplitudes were similarly determined as the median amplitude for each day after outliers were removed (Mesrati & Vecchierini, 2004). To minimize the potential of technical and peripheral confounds (e.g., subtle differences in sensor placement) MEP amplitudes were normalized to M_{MAX}.

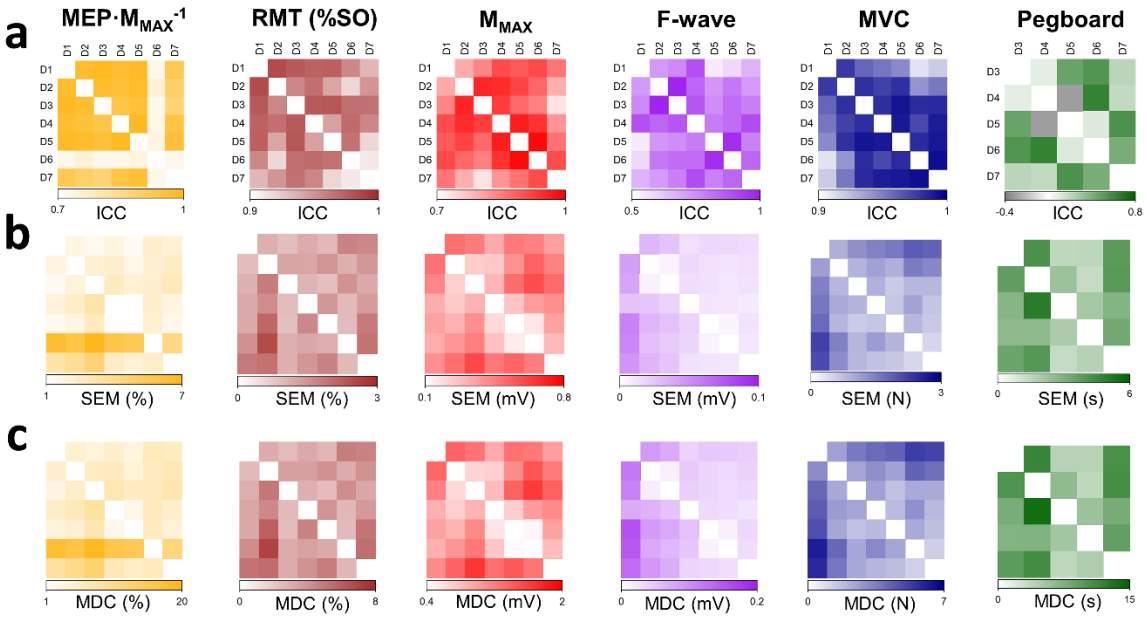
4.2.14 Statistical analysis

In accordance with previous precision neuroimaging efforts, each participant was treated as a separate replicate and analyzed using within-subject statistics. The influence of disuse on skeletomotor function (strength, force steadiness, bimanual control, pegboard time) was assessed based on differences across *Pre* (D1-D7), *Cast* (D8-14) and *Post* (D15-D21) using separate one-

way ANOVAs for each measure and muscle (casted and un-casted hand, or leg). Because casted hand skeletomotor function was not assessed during D8-D13, statistical comparisons of the casted hand only consisted of trials recorded on D14, whereas all analyses involving the un-casted hand or leg included all individual trials of D8-D14. Given the apparent learning effect in fine motor skill early in the study, the comparison of pegboard completion time was restricted to the three days before immobilization.

Corticospinal, spinal and peripheral excitability, as well as skeletomotor structure (FDI CSA) were compared between *Pre, Cast, and Post* with separate one-way ANOVAs for each muscle. To avoid F-statistic inflation, ANOVAs were performed on the daily medians, rather than individual trials (as done with skeletomotor function). Time courses were visualized using LOESS regression.

For all ANOVAs, Benjamini-Hochberg corrections were made for pairwise comparisons (**Sup. Tables 3&4**). When statistically significant differences were indicated, the compatibility of the findings was determined by comparing change scores with the standard error of measurement (SEM) and minimum detectable change (MDC). For each measure, SEM was calculated as the average intraclass correlation coefficient (ICC) of all pairwise ICCs between each of the seven days of baseline testing (e.g. D1-D2, D1-D3…). Because disuse primarily influenced corticomotor and skeletomotor function of the casted hand, ICC, SEM and MDC were calculated based on the casted hand only. Average test-retest reliability was good to excellent ($MEP \cdot M_{MAX}^{-1}$ ICC = 0.89, SEM = 2.70%, MDC = 7.60%; M_{MAX} ICC = 0.89, SEM = 0.38mV, MDC = 1.06mV; F-wave: ICC = 0.79, SEM = 0.02mV, MDC = 0.06mV; RMT ICC = 0.96, SEM = 1.37%, MDC = 3.81%; detailed results in **Sup. Fig. 3**).



Supplementary Figure 3. Pairwise test-retest reliability statistics for primary study outcomes . **Correlation matrices illustrate the day-to-day intraclass correlation coefficient (A), standard error of measurement (B) and minimum detectable change scores (C) for corticospinal excitability (MEP·M_{MAX}^{-1}), resting motor thresholds (RMT), peripheral excitability (M_{MAX}), spinal excitability (F-wave), maximum voluntary contraction forces (MVC) and fine motor skill (Pegboard). Color intensity is directly proportional to measurement score.**

4.3 Results

4.3.1 Effects of disuse on skeletomotor function and structure

Immobilization markedly reduced the casted upper extremity use (DIS1 -73%, DIS2 -59%, DIS3 -62%), strength (DIS1 -18%: $F_{2,57} = 33.58$, $p < 0.001$, DIS2 -21%: $F_{2,57} = 29.97$, $p < 0.001$, DIS3 -22%: $F_{2,57} = 31.77$, $p < 0.001$) and fine motor skill (DIS1 +5%: $F_{2,19} = 14.92$, $p < 0.001$, DIS2 +21%: $F_{2,19} = 11.5$, $p < 0.001$, DIS3 +19%: $F_{2,19} = 3.62$, $p = 0.047$, **Fig. 3A-C**). Unsurprisingly, disuse did not affect strength (DIS1 un-casted $F_{2,81} = 7.02$, $p < 0.001$, leg: $F_{2,81} = 2.26$, $p = 0.11$, DIS2 un-casted: $F_{2,81} = 30.27$, $p < 0.001$, leg: $F_{2,81} = 2.71$, $p = 0.07$, DIS3: $F_{2,81} = 2.019$, $p = 0.14$, leg: $F_{2,81} = 2.84$, $p = 0.06$) or fine motor skill (DIS1 $F_{2,31} = 2.67$, $p < 0.09$, DIS2 $F_{2,31} = 11.46$, $p < 0.001$, DIS3 $F_{2,31} = 2.41$, $p = 0.11$) in the un-casted hand and leg. Immediately after cast removal, unimanual force steadiness was similar (DIS1 casted $F_{2,87} = 0.28$, $p = 0.76$, un-casted: $F_{2,87} = 0.47$, $p = 0.63$; DIS2 casted*:* $F_{2,87} = 1.19$, $p = 0.31$, un-casted*:* $F_{2,87} = 1.81$, $p = 0.31$; DIS3 casted $F_{2,87} = 6.15$, $p = 0.003$, un-casted: $F_{2,87} = 1.76$, $p = 0.18$), but disused hand contributions during bimanual contractions decreased (DIS1: $F_{2,87} = 6.98$, $p = 0.002$, DIS2: $F_{2,87} = 6.29$, $p = 0.003$, DIS3: $F_{2,87} = 9.59$, $p < 0.001$; **Fig 4A&B**).

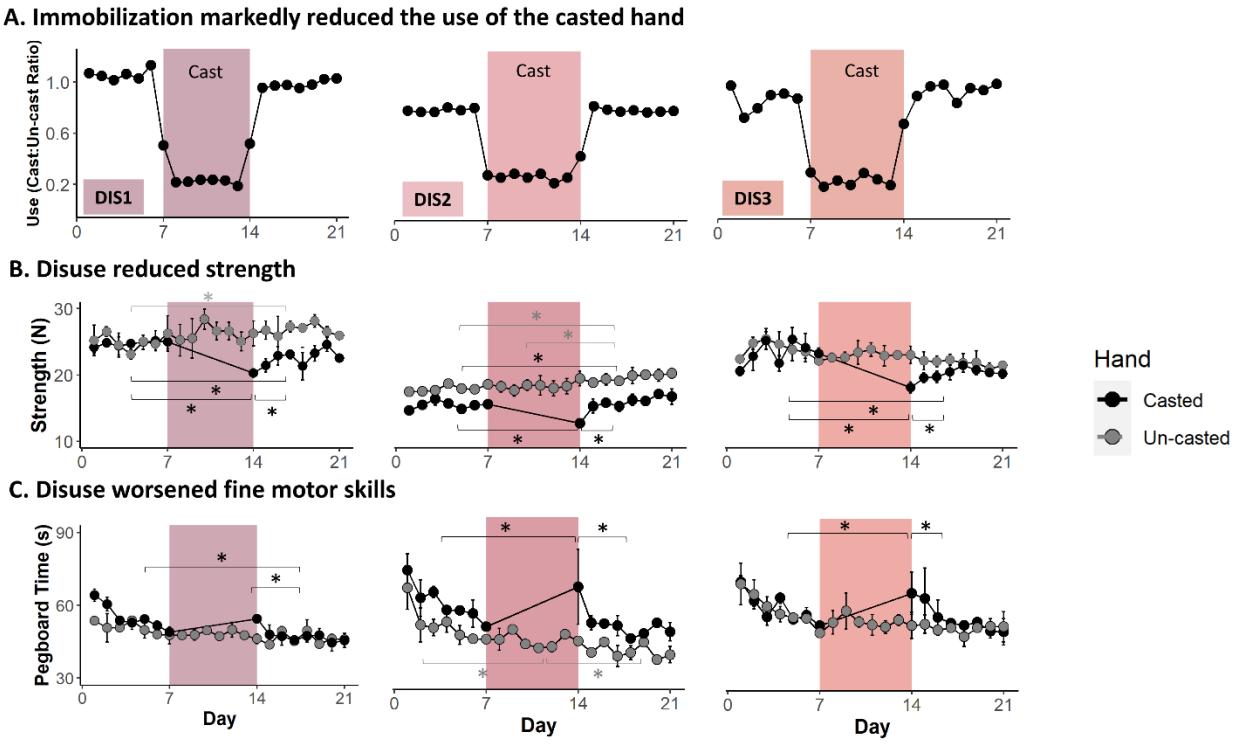


Figure 3. Influence of disuse on behavioral outcomes. Immobilization reduced the use of the casted hand (A), which resulted in worsening of manual dexterity (B) and weakening (C). Grey (black) colored points in (B) and (C) denote the un-casted (casted) hand. Use in (A) was derived as the ratio of use in the casted and un-casted hand, with lower values reflecting disuse. Shaded rectangles indicate the casting intervention from day 7-14. Data are means ± standard deviation.

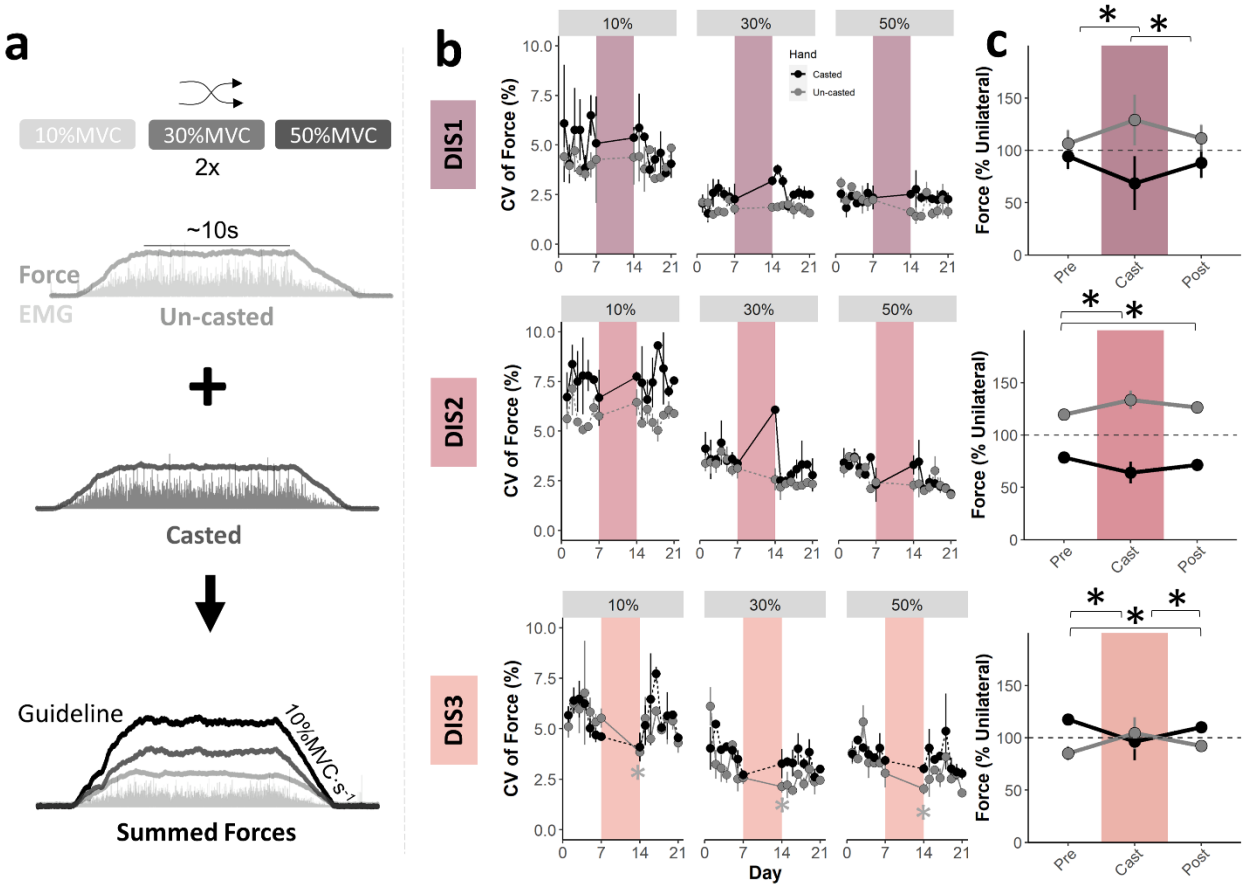
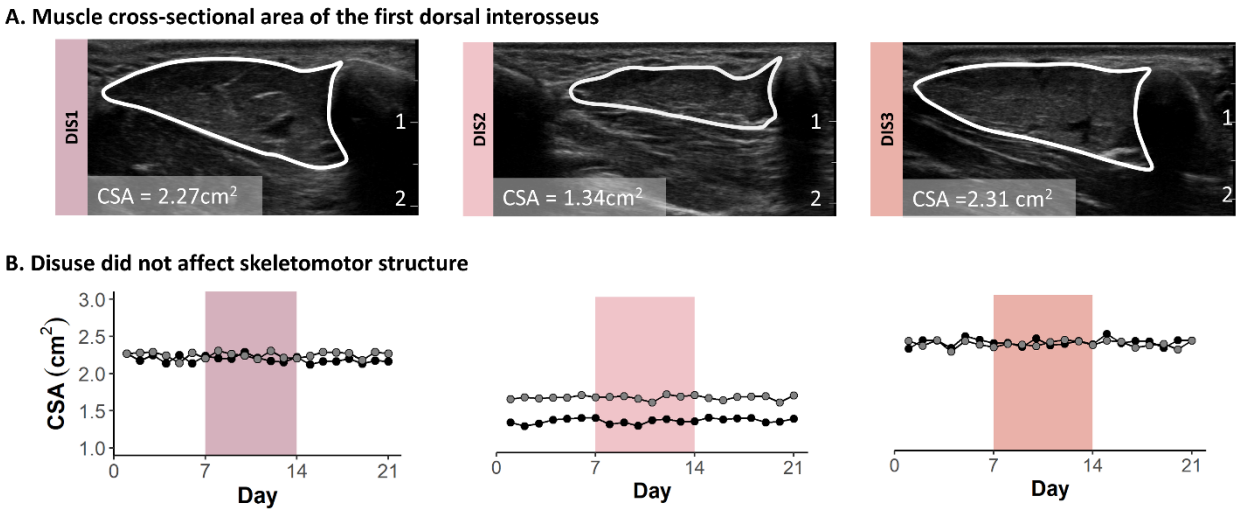


Figure 4. Influence of disuse on unimanual and bimanual force . Participants completed two unilateral trials of submaximal isometric index finger abduction at 10, 30 and 50%MVC in randomized order (a). Afterwards, participants used both hands to match one visual guideline equal to the sm of unilateral forces. Disuse did not affect unilateral force steadiness (b), but reduced the contributions of the casted hand during bilateral contractions (c). MVC: maximum voluntary contraction, EMG: electromyography, CV: coefficient of variation. Time courses are means ± standard deviation. Horizontal lines in (c) indicate point of equal unimanual contribution to total bimanual force.

Despite loss of function in the casted hand, disuse did not affect skeletomotor structure (**Sup. Fig. 4;** all $p > 0.05$).



Supplementary Figure 4. Stable skeletomotor structure during disuse . Muscle cross sectional area of the casted- and un-casted first dorsal interosseus (A) was unaffected by immobilization (B). Data shown are means. CSA: cross-sectional area

4.3.2 Effects of disuse on corticomotor function

Disuse reduced CSE in the casted FDI but did not change MEP amplitudes in the un-casted FDI, or non-dominant TA (DIS1 casted -53%: $F_{2,18} = 4.30$, $p = 0.03$; un-casted: $F_{2,18} = 2.07$, $p = 0.16$, leg: $F_{2,18} = 0.91$, $p = 0.42$; DIS2 casted -39%: $F_{2,18} = 13.11$, $p < 0.001$; un-casted: $F_{2,18} = 1.85$, $p = 0.18$, leg: $F_{2,18} = 1.01$, $p = 0.38$; DIS3 casted -41%: $F_{2,18} = 30.05$, $p < 0.001$, un-casted: $F_{2,18} = 0.14$, $p = 0.87$, leg: $F_{2,18} = 5.89$, $p = 0.01$; **Fig. 4**). Corresponding increases in RMTs were also evident in the casted hand (DIS1 casted +13%: $F_{2,18} = 5.96$, $p = 0.01$, un-casted: $F_{2,18} = 4.52$, $p = 0.03$; leg: $F_{2,18} = 2.64$, $p = 0.10$; DIS2 casted +7%: $F_{2,18} = 5.08$, $p = 0.02$, un-casted: $F_{2,18} = 1.73$, $p = 0.21$, leg: $F_{2,18} = 4.12$, $p = 0.03$; DIS3 casted +8%: $F_{2,18} = 20.49$, $p < 0.001$; un-casted: $F_{2,18} = 14.14$, $p < 0.001$; leg: $F_{2,18} = 14.14$, $p < 0.53$).

To confirm that changes in CSE were not the result of cast-related sensorimotor activity, we compared MEPs obtained immediately before and after casting using paired t-tests. Casting

status did not influence CSE (t(5) = 0.77, p = 0.48, **Sup. Fig 5A&B**). Moreover, follow-on analysis of neuronavigation-derived targeting measures confirmed high TMS targeting consistency and stable coil orientation (**Sup. Fig 6**). Accordingly, fewer than 5% of trials were removed (on average) due to unacceptable targeting accuracy (≥1mm distance error, ≥2° angle error).

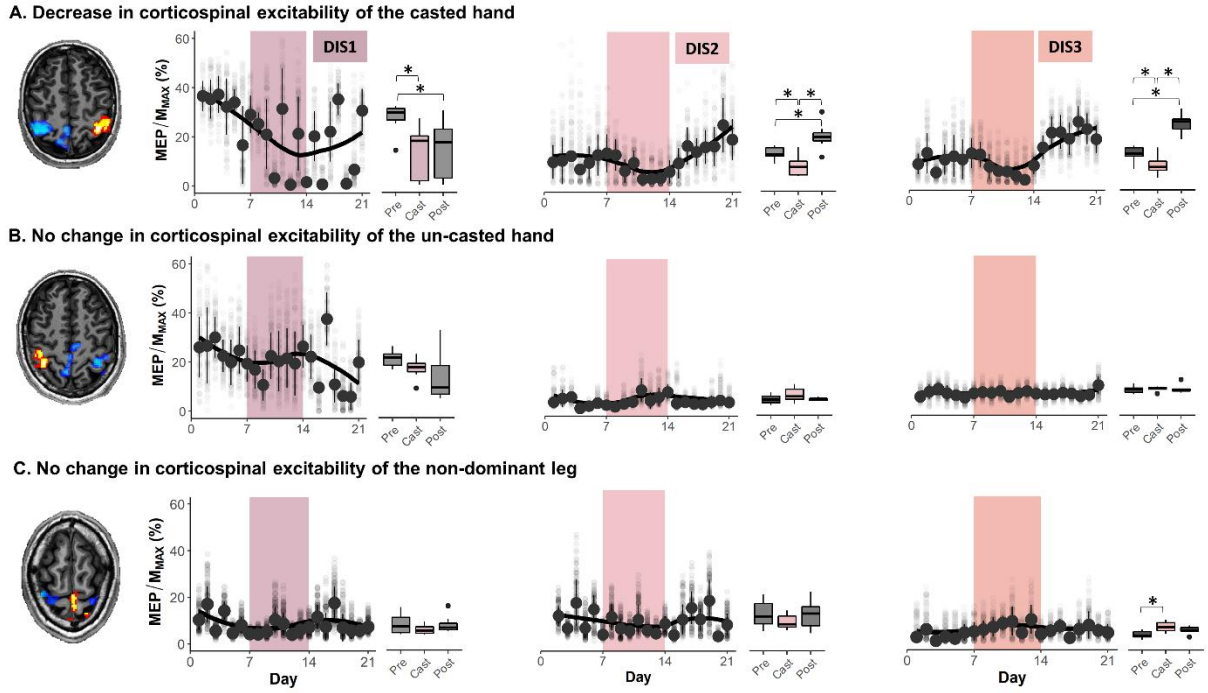
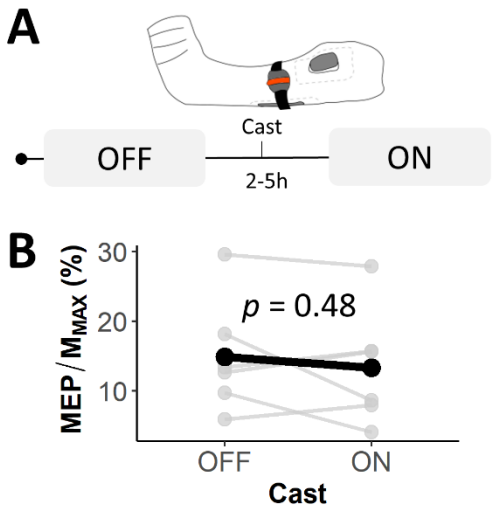
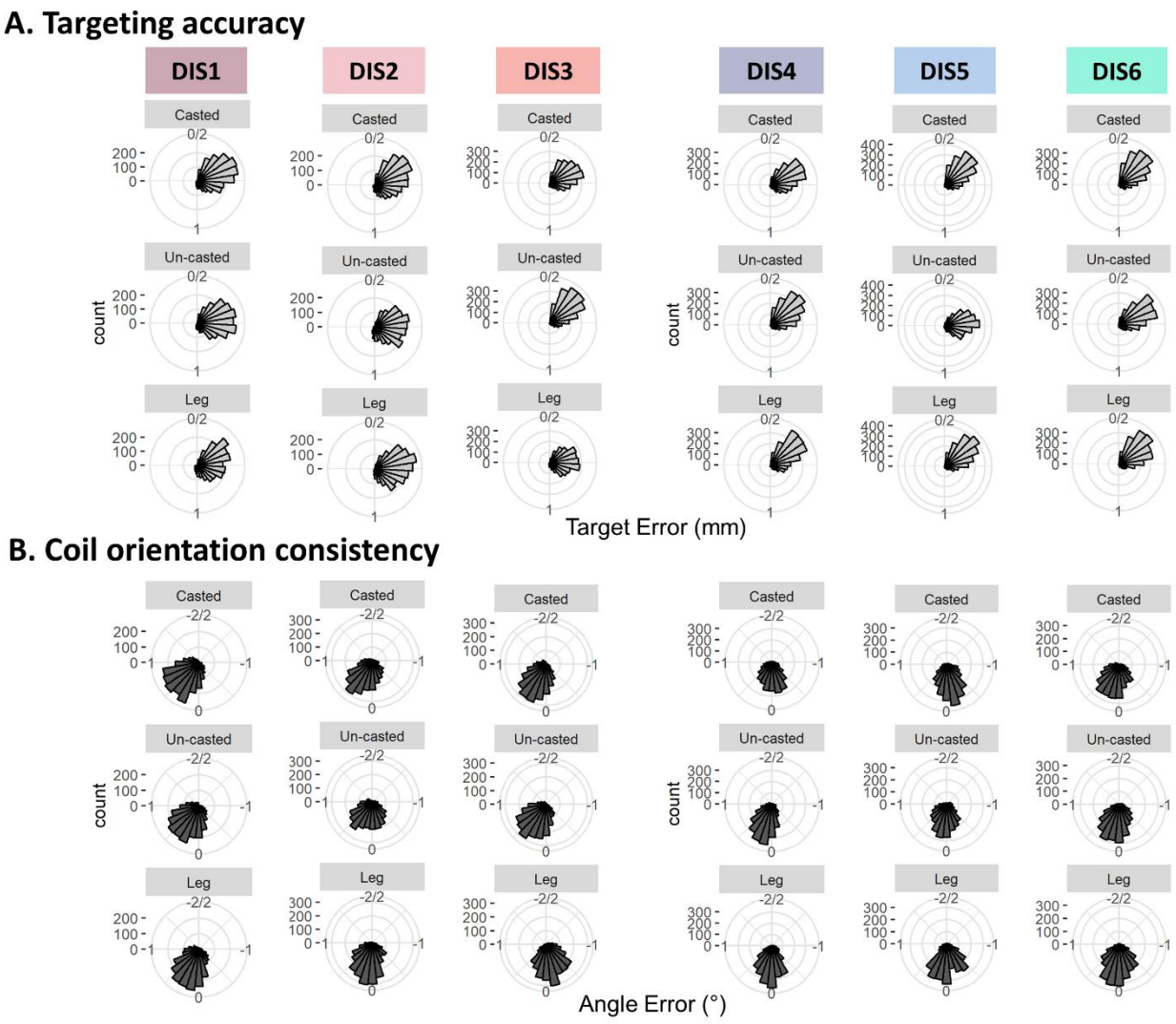


Figure 5. Disuse-induced reductions in corticospinal excitability in the casted hand . Disuse decreased corticospinal excitability in the casted (A), but not un-casted hand (B) or leg (C). Data are presented as medians, with individual trials shown in transparent and error bars reflecting the standard deviation. Asterisks (*) indicate statistical significance (Pre, Cast, Post) after Benjamini-Hochberg correction for multiple comparisons.



Supplementary Figure 5. Casting setup does not directly influence corticospinal excitability. Corticospinal excitability was assessed before and immediately after casting to determine if casting alters corticospinal excitability independently of subsequent disuse (A). When assessed immediately before and after casting, corticospinal excitability did not differ (B).



Supplementary Figure 6. High TMS targeting accuracy and consistency during 21 consecutvie days of assessments. Circular plots show individual histograms of target (A) and angle error (B) specific to the motor cortex representations of the casted hand, un-casted hand, and non-dominant leg. Data shown is after removal of bad trials (>1mm target error, >2°angle error; <5% of trials).

Compared to disuse-induced reductions in CSE, spinal and peripheral adaptations were less consistent: DIS1 did not display any changes in F-wave or M_{MAX} amplitude in the casted hand (F-wave $F_{2,18} = 0.41$, $p = 0.67$, M_{MAX} $F_{2,18} = 0.37$, $p = 0.70$), but these measures gradually decreased in the un-casted hand (F-wave $F_{2,18} = 5.64$, $p = 0.01$, M_{MAX} $F_{2,18} = 16.67$, $p < 0.001$). DIS2

demonstrated a gradual increase in F-wave amplitude in the casted, but not un-casted hand (Casted $F_{2,18}$ = 5.85, p = 0.01, Un-casted $F_{2,18}$ = 0.014, p = 0.99), but with disuse-induced increases in M_{MAX} in the un-casted, but not casted hand (Casted $F_{2,18}$ = 2.66, p = 0.10, Un-casted $F_{2,18}$ = 16.23, p < 0.001). In DIS3, disuse decreased spinal and peripheral excitability in the casted hand (F-wave $F_{2,18}$ = 6.45, p = 0.008, M_{MAX} $F_{2,18}$ = 26.03, p < 0.001) with no detectable effect on the un-casted hand (F-wave $F_{2,18}$ = 0.36, p = 0.70, M_{MAX} $F_{2,18}$ = 1.52, p = 0.25; **Fig 5C&D**).

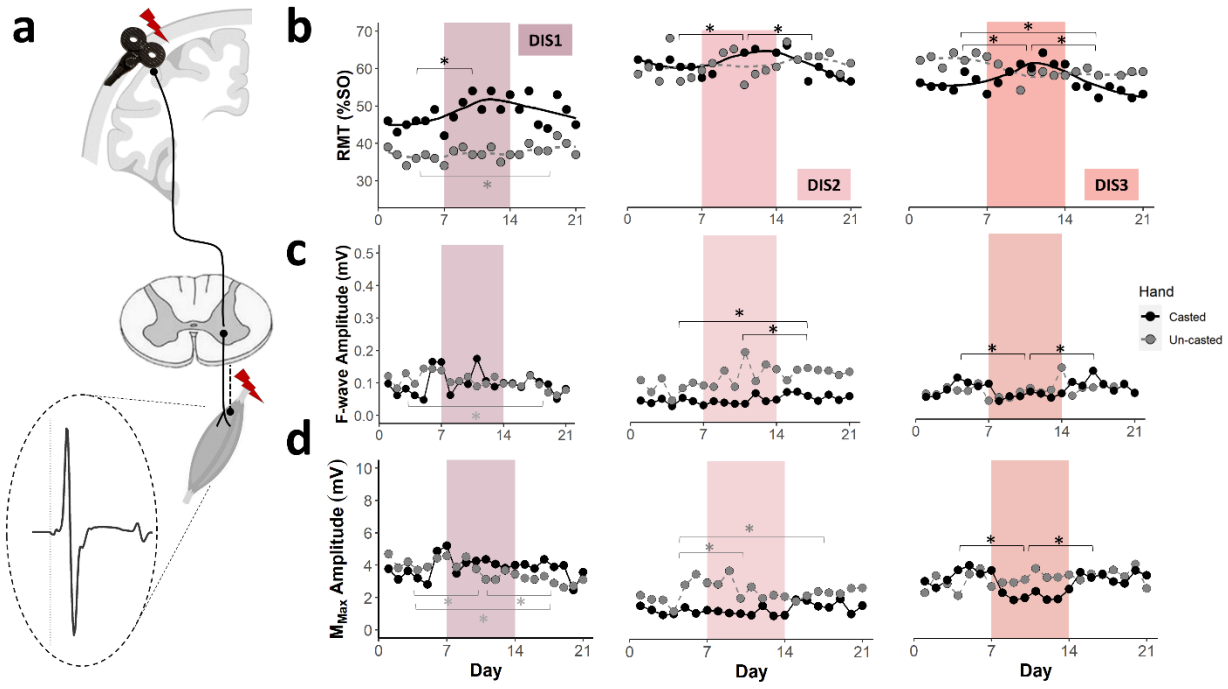


Figure 6. The effects of disuse on supraspinal, spinal and peripheral excitability. Excitability at different levels of the corticospinal tract was determined based on motor cortex (TMS) and ulnar nerve (electrical) stimulation stimulation (a). Disuse increased resting motor thresholds (RMT) in the casted, but not un-casted hand (b), whereas the spinal (c) and peripheral adaptations (d) were more variable. A representative example of an ulnar nerve muscle compound action potential and F-wave is shown in (a). SO: stimulator output, M_{MAX}: muscle compound action potential.

4.3.3 Mental imagery counter-intervention

Given converging evidence that disuse induces functional neuroplasticity in corticomotor circuits, an additional three (N = 3) individuals were recruited to test the possibility that mental imagery (MI) may restore endogenous corticomotor physiological activity and mitigate disuse-induced neuroplasticity and skeletomotor loss of function. Mental imagery started 48h after casting and ended on the same day as cast removal (**Fig. 6A**).

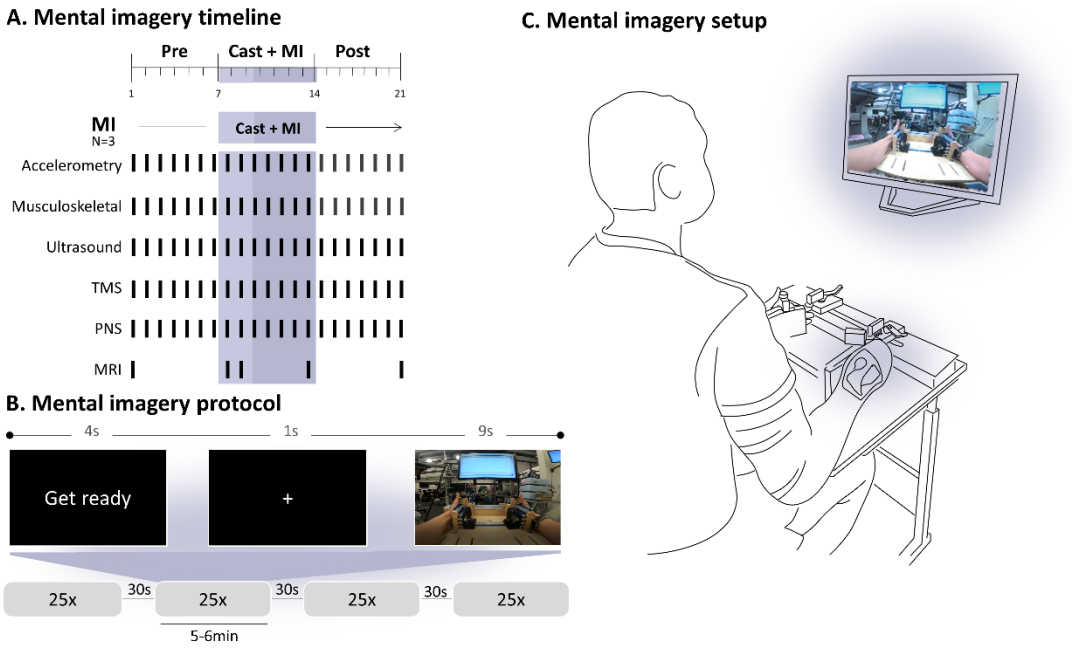


Figure 7. Mental imagery timeline, protocol and setup. Mental imagery training was performed for five days, beginning two days after the start of the immobilization intervention (A). During mental imagery, participants completed imagined maximum voluntary contractions (MVC) (B) with instructions relayed on a screen. Each trial consisted of a four-second preparation slide, followed by a 1s fixation cross and ~9s first-person video of MVC performance during Pre visits. 100 trials were performed as four sets of 24 repetitions with 30s rest between each set. Electromyography was used to confirm an absence of muscle activation. To maximize the kinesthetic experience, the setup during was otherwise identical to the one used for MVC contractions during Pre (C).

MI involves the imagination of movement and activates similar brain circuits as movement execution, including the primary motor cortex and somatosensory cortex, and the supplementary motor area (Hardwick *et al.*, 2018). Accordingly, MI can increase strength in the absence of skeletomotor contractile activity (Ranganathan *et al.*, 2004). This observation raises the possibility that MI can also attenuate disuse-induced corticomotor adaptations and mitigate skeletomotor loss of function. In this study, MI consisted of one hundred imagined MVCs separated into four sets of 25 repetitions, with 30s of rest between sets (**Fig 6B**). While seated in the same position and setup as *Pre* MVC FDI contractions, participants were asked to imagine themselves maximally abducting their index finger against a force transducer while EMG activity was monitored to confirm the absence of skeletomotor activity (**Fig. 6C**).

To ensure a kinesthetic experience (Stinear *et al.*, 2006) and enhance beneficial neuroplastic effects (Bisio *et al.*, 2018), MI was performed while participants watched videos of their MVCs trials that were filmed during *Pre* using a first-person perspective (GoPro Hero 8 Black, GoPro, Inc. San Mateo, CA, USA; Superlab V6.0, Cedrus Corporation, San Pedro, CA, USA). Each imagined contraction repetition consisted of a 4s preparation slide, a 1s fixation cross and ~9s long videos of the MVC (total duration each day ~23-25 minutes). Relative to previous MI protocols (Clark *et al.*, 2014), more trials were performed each session to account for the relatively brief time frame of MI exposure (5 days). Still, care was taken not to exceed a recommended protocol duration limit of~20 minutes (Malouin *et al.*, 2013) (Driskell *et al.*, 1994). To avoid any influences of MI on CSE (Grosprêtre *et al.*, 2016), MI was performed after TMS and PNS, but prior to all skeletomotor assessments.

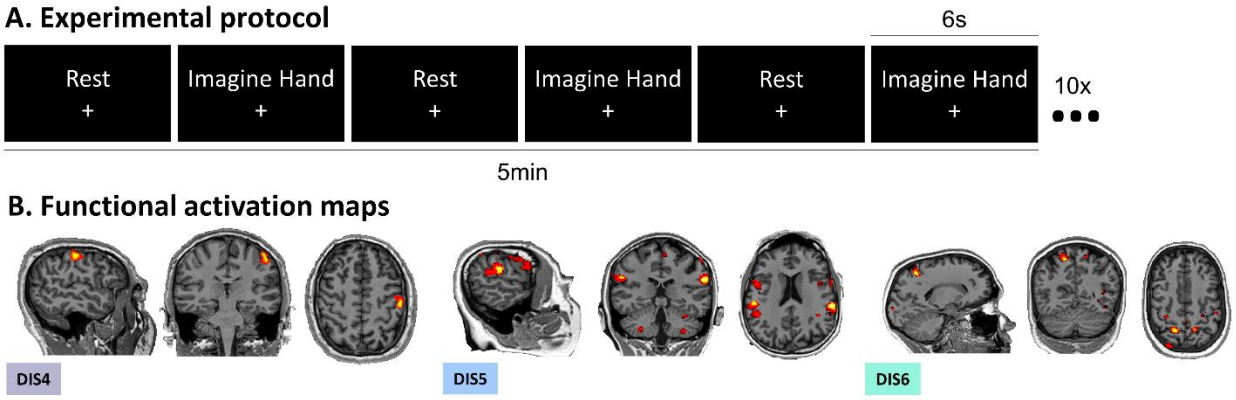
To ensure that all participants could activate homotopic areas of sensorimotor cortex during MI, a task-based functional magnetic resonance imaging (fMRI) experiment was performed, in

which twenty-five 6s blocks of imagined contractions of the casted hand were interleaved with 6s rest periods. An MRI-compatible EMG system confirmed the absence of skeletomotor physiological activity during MI (MP150, BIOPAC Systems, Inc., Goleta, CA, USA). In all participants, MI increased functional activity in the dorsal stream, the ventral streams, and homotopic regions of sensorimotor cortex (**Sup. Fig. 7**).

Table 3. Participant characteristics of individuals performing mental imagery.

ID	Gender	Age (years)	Height (cm)	Weight (kg)	Handedness	Footedness	VVIQ
DIS4	M	22	188	87.2	Left	Right	3.27
DIS5	W	24	163	69.7	Right	Right	3.07
DIS6	W	23	160	63.5	Right	Right	4.27

VVIQ: Vividness of visual imagery questionnaire.



Supplementary Figure 7. Mental imagery activates motor circuits. Each participant performing mental imagery completed a task-based functional magnetic resonance imaging scan to ensure that motor circuits could be activated during imagination. Briefly, the protocol interleaved 6s blocks of imagined contractions of the casted hand with 6s of rest, for a total of 30 trials (A). The corresponding functional activation maps are shown in (B), demonstrating variable, but consistent activation in motor circuits. MRI-compatible electromyography sensors were used to confirm an absence of muscle activity during the scan.

4.3.4 Effects of mental imagery on disuse-induced skeletomotor deficits

Even though the immobilization reduced the use of the casted hand (DIS4 -54%, DIS5 -52%, DIS6 -69%; **Fig. 7A**), mental imagery prevented disuse-induced weakening in DIS4 and DIS5 (DIS4 casted: $F_{2,57} = 15.28$, $p < 0.001$, un-casted: $F_{2,81} = 8.53$, $p < 0.001$, leg: $F_{2,81} = 2.80$, $p = 0.07$; DIS5 casted: $F_{2,57} = 49.9$, $p < 0.001$, un-casted: $F_{2,81} = 0.10$, $p = 0.91$, leg: $F_{2,81} = 2.67$, $p = 0.08$). In DIS6, a small, but statistically significant decrease in strength remained evident (DIS6 casted -15%: $F_{2,53} = 3.37$, $p = 0.04$, un-casted: $F_{2,81} = 1.17$, $p = 0.32$, leg: $F_{2,81} = 1.00$, $p = 0.37$; **Fig 7B**). MI did not mitigate the loss of fine motor skill still, apparent in all participants immediately after cast removal (DIS4 +19% $F_{2,19} = 4.35$, $p = 0.03$, DIS5 +20% $F_{2,19} = 4.20$, $p = 0.03$, DIS6 +19% $F_{2,19} = 10.32$, $p < 0.001$). In contrast, fine motor skill did not change, or slightly improved in the un-casted hand (DIS4 $F_{2,31} = 2.48$, $p = 0.10$, DIS5 $F_{2,31} = 2.73$, $p = 0.08$, DIS6 $F_{2,31} = 8.79$, $p < 0.001$; **Fig 7C**).

Similar to disuse, there were no changes in unilateral force steadiness in the casted or un-casted hand in individuals who performed MI (DIS4 casted: $F_{2,87} = 1.65$, $p = 0.20$, un-casted: $F_{2,87} = 1.11$, $p = 0.33$, DIS5 casted: $F_{2,87} = 0.15$, $p = 0.86$, un-casted: $F_{2,87} = 1.38$, $p = 0.26$, DIS6 casted: $F_{2,87} = 1.14$, $p = 0.32$, un-casted: $F_{2,87} = 1.28$, $p = 0.28$; **Fig. 8A**). The contributions of the casted hand during bimanual contractions decreased in DIS6 ($F_{2,87} = 11.48$, $p < 0.001$; **Fig. 8B**). Yet, in accordance with the maintenance in strength, DIS4 and DIS5 maintained similar unimanual force contributions during bilateral submaximal contractions immediately after cast removal (DIS4 $F_{2,87} = 1.71$, $p = 0.19$, DIS5 $F_{2,87} = 2.59$, $p = 0.08$). Again, skeletomotor structure remained stable throughout the study (**Sup. Fig. 8**).

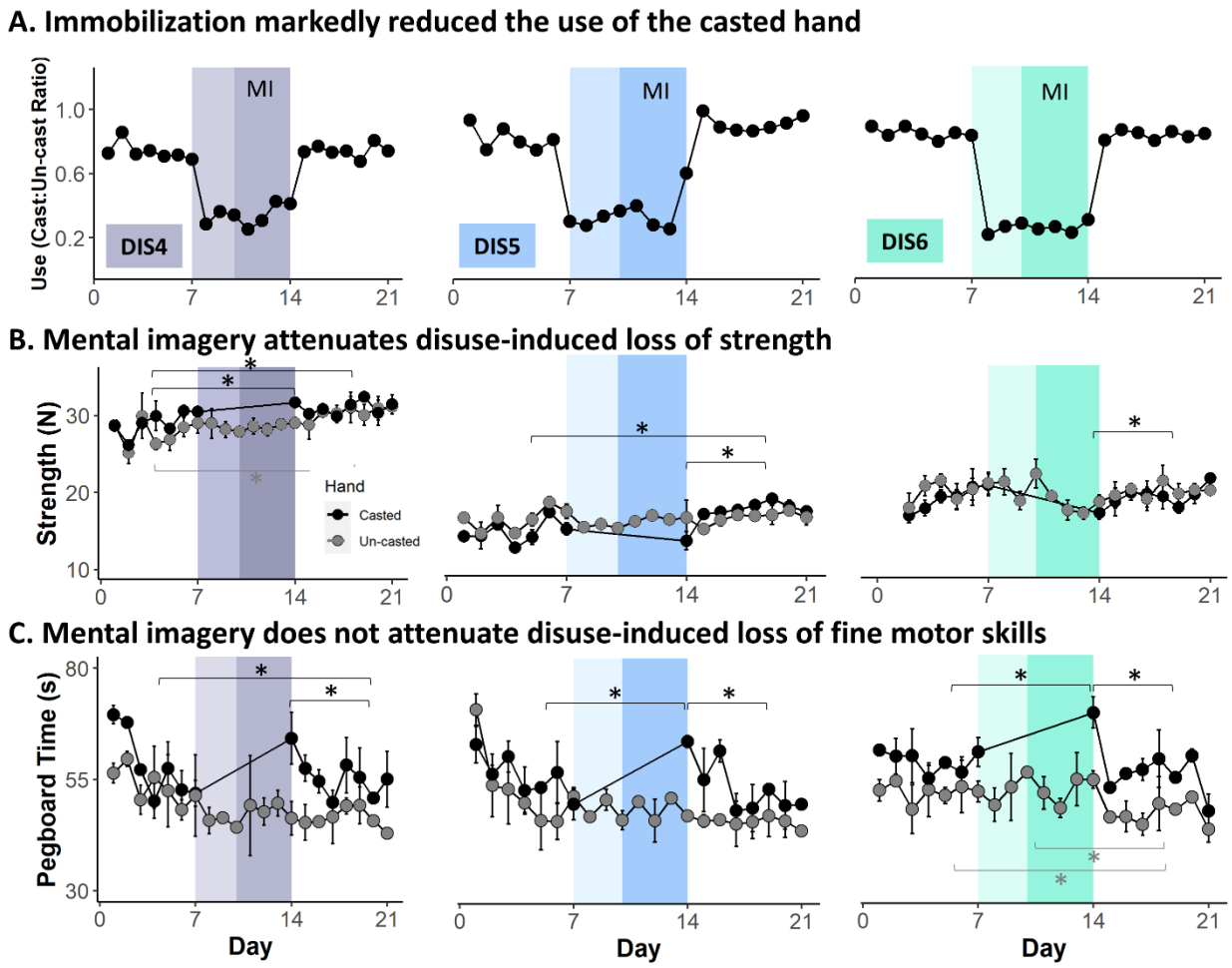
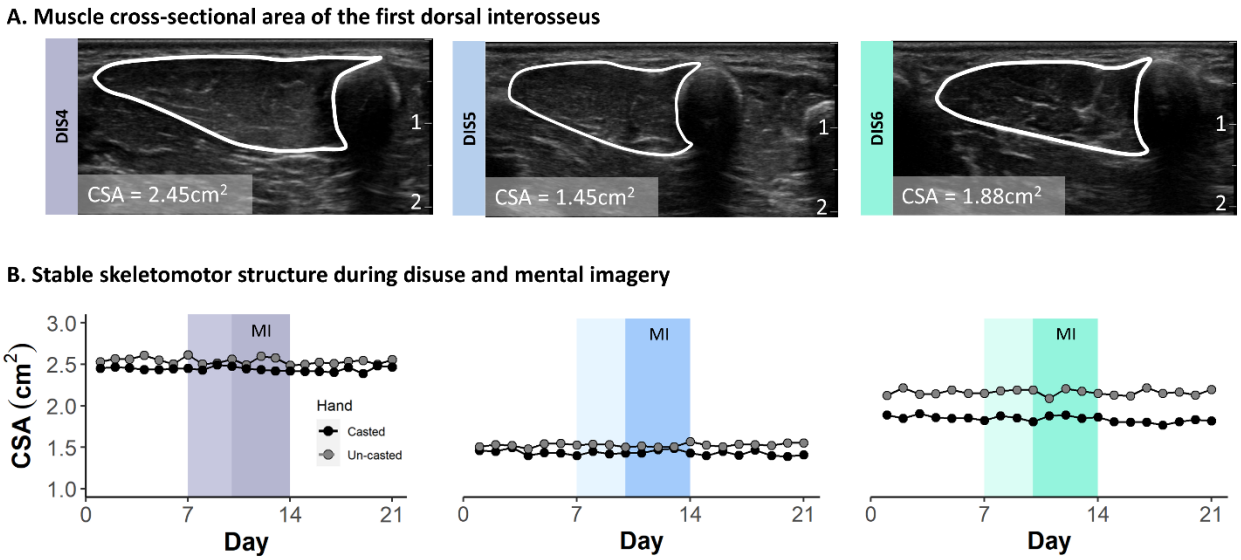


Figure 8. Mental imagery mitigates disuse-induced reductions in strength, but not fine motor skill. **Although immobilization decreased use of the casted hand (A), mental imagery mitigated disuse-induced strength loss in DIS4 and DIS5 (B). Nonetheless, disuse-induced reductions in fine motor skill were still evident (C). Data shown are means ± standard deviations. Asterisks indicate significant between-period (*Pre, Cast, Post*) difference, based on one-way ANOVAs and Benjamini-Hochberg correction for multiple pairwise comparisons.**



Supplementary Figure 8. No changes in skeletomotor structure with disuse and mental imagery. **Representative ultrasound images of the non-dominant first dorsal interosseus muscles for each participant undergoing mental imagery are shown in (A). Despite disuse and mental imagery, muscle-cross sectional area (CSA) remained stable throughout the study. MI: mental imagery.**

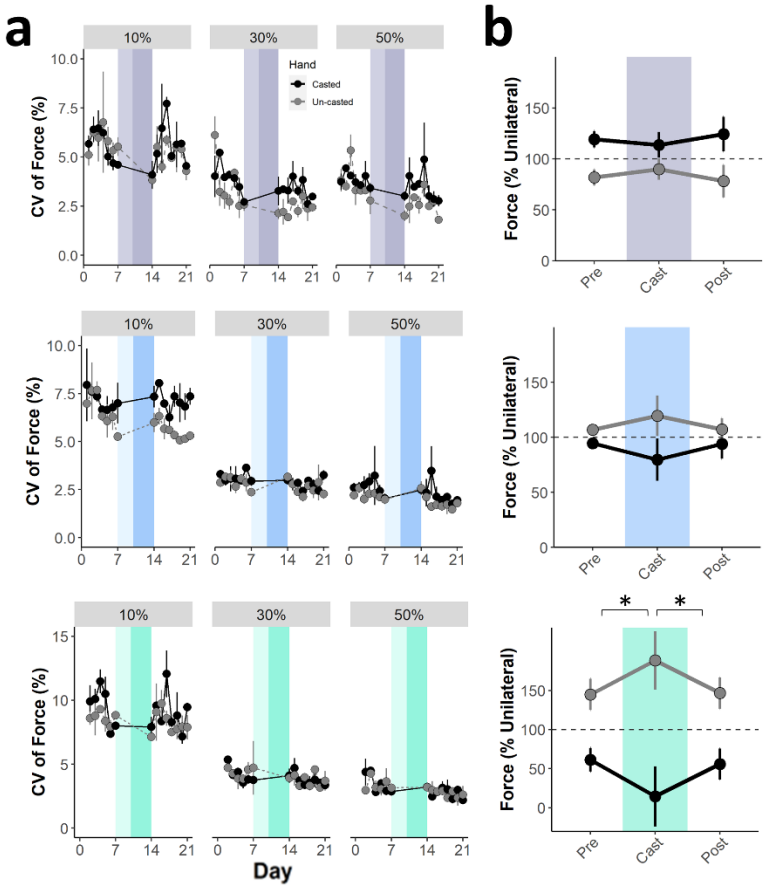


Figure 9. Mental imagery attenuates disuse-induced reductions in unilateral force during bimanual tasks. Similar to disuse, unimanual force steadiness was not influenced by the intervention (a). However, mental imagery attenuated the reduced contributions of the casted hand during bimanual tasks in DIS4 and DIS5. DIS6 presented with similar skeletomotor deficits as DIS1-3 (b). Time courses in (a) show means ± standard deviations. Shaded regions indicate the onset of disuse and mental imagery. Asterisks in (b) indicate significant between-period (Pre, Cast, Post) difference after significant one-way ANOVA with Benjamini-Hochberg correction for multiple pairwise comparisons. CV: coefficient of variation.

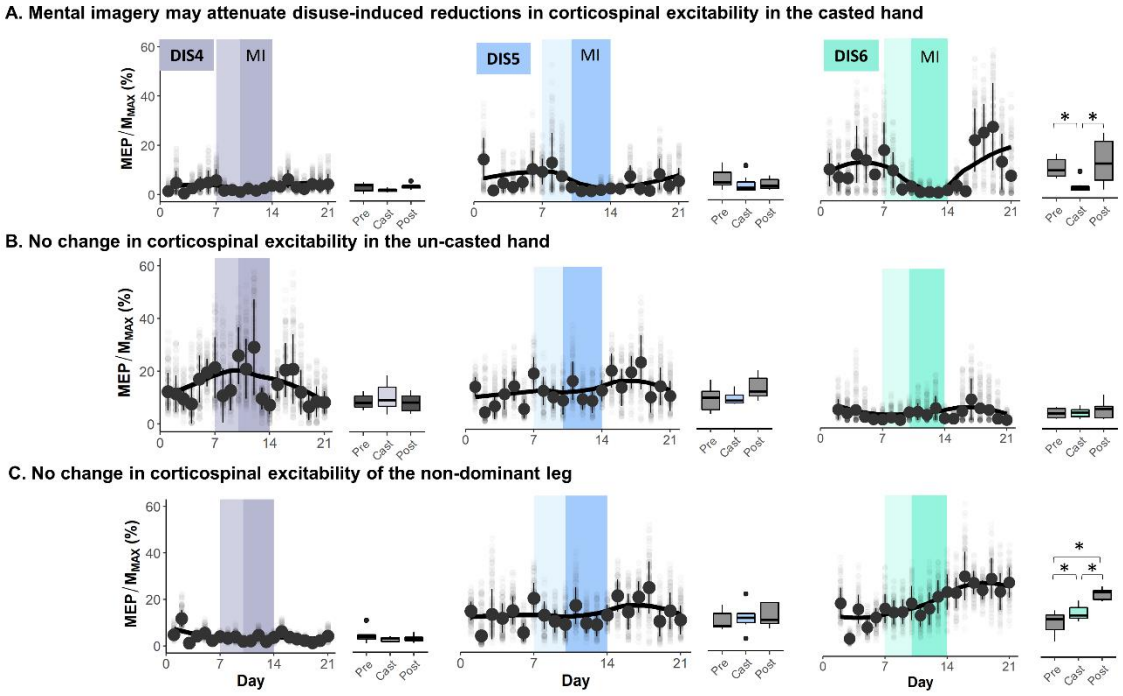


Figure 10. Mental imagery attenuates disuse-induced reductions in corticospinal excitability. Five days of mental imagery training mitigated disuse-induced reductions in corticospinal excitability in the casted hand (A) in DIS4 and DIS5. A decline in MEP/M_{MAX} was still evident in DIS6. Similar to disuse alone, no changes in corticospinal excitability were evident in the un-casted hand (B) or leg (C). Data show the median and standard deviation of 120 daily trials, with individual responses in the background. Time courses are visualized with LOESS regression. Shaded regions indicate immobilization (light) and mental imagery (dark) interventions. Boxplots summarize corticospinal excitability during each period (Pre, Cast and Post). Asterisks (*) denote between-period differences, following one-way ANOVA and Benjamini-Hochberg pairwise comparisons.

4.3.5 Effects of mental imagery on disuse-induced corticomotor deficits

Similar to strength, MI counteracted disuse-induced reductions in CSE in DIS4 (casted: $F_{2,18}$ = 2.77, p = 0.09, un-casted: $F_{2,18}$ = 0.51, p = 0.61, leg: $F_{2,18}$ = 1.31, p = 0.29) and DIS5 (casted: $F_{2,18}$ = 0.96, p = 0.40, un-casted: $F_{2,18}$ = 2.41, p = 0.12, leg: $F_{2,18}$ = 0.19, p = 0.83), but not DIS6

(casted -78%: $F_{2,18} = 5.21$, $p = 0.02$, un-casted: $F_{2,18} = 0.29$, $p = 0.75$, leg: $F_{2,18} = 21.36$, $p < 0.01$; **Fig. 9**). RMTs were unaffected by disuse in DIS4 (casted: $F_{2,18} = 1.04$, $p = 0.37$, un-casted: $F_{2,18} = 1.44$, $p = 0.26$, leg: $F_{2,18} = 1.81$, $p = 0.20$), but increased in the casted hand of DIS5 (casted +14%: $F_{2,18} = 8.36$, $p = 0.003$, un-casted: $F_{2,18} = 0.99$, $p = 0.39$; leg: $F_{2,18} = 0.92$, $p = 0.42$) and DIS6 (casted +9%: $F_{2,18} = 8.12$, $p = 0.003$, un-casted: $F_{2,18} = 0.58$, $p = 0.57$, leg: $F_{2,18} = 2.73$, $p = 0.09$; **Fig 10A**).

The maintenance of CSE in DIS4 coincided with stable spinal (casted: $F_{2,18} = 0.89$, $p = 0.43$, un-casted: $F_{2,18} = 1.18$, $p = 0.33$) and peripheral excitability (casted: $F_{2,18} = 1.67$, $p = 0.22$, un-casted: $F_{2,18} = 0.31$, $p = 0.74$). Yet, in DIS5, F-wave amplitude increased towards the end of the study in the un-casted only (casted: $F_{2,18} = 0.89$, $p = 0.43$; un-casted: $F_{2,18} = 5.16$, $p = 0.02$), whereas M_{MAX} decreased in the casted hand only (casted: $F_{2,18} = 4.69$, $p = 0.02$, un-casted: $F_{2,18} = 0.14$, $p = 0.87$). In DIS6, spinal excitability of the un-casted hand increased (casted: $F_{2,18} = 3.25$, $p = 0.06$; un-casted: $F_{2,18} = 6.29$, $p = 0.01$), whereas peripheral excitability increased bilaterally (casted: $F_{2,18} = 3.25$, $p = 0.06$; un-casted: $F_{2,18} = 6.29$, $p = 0.009$; **Fig. 10B&C**)

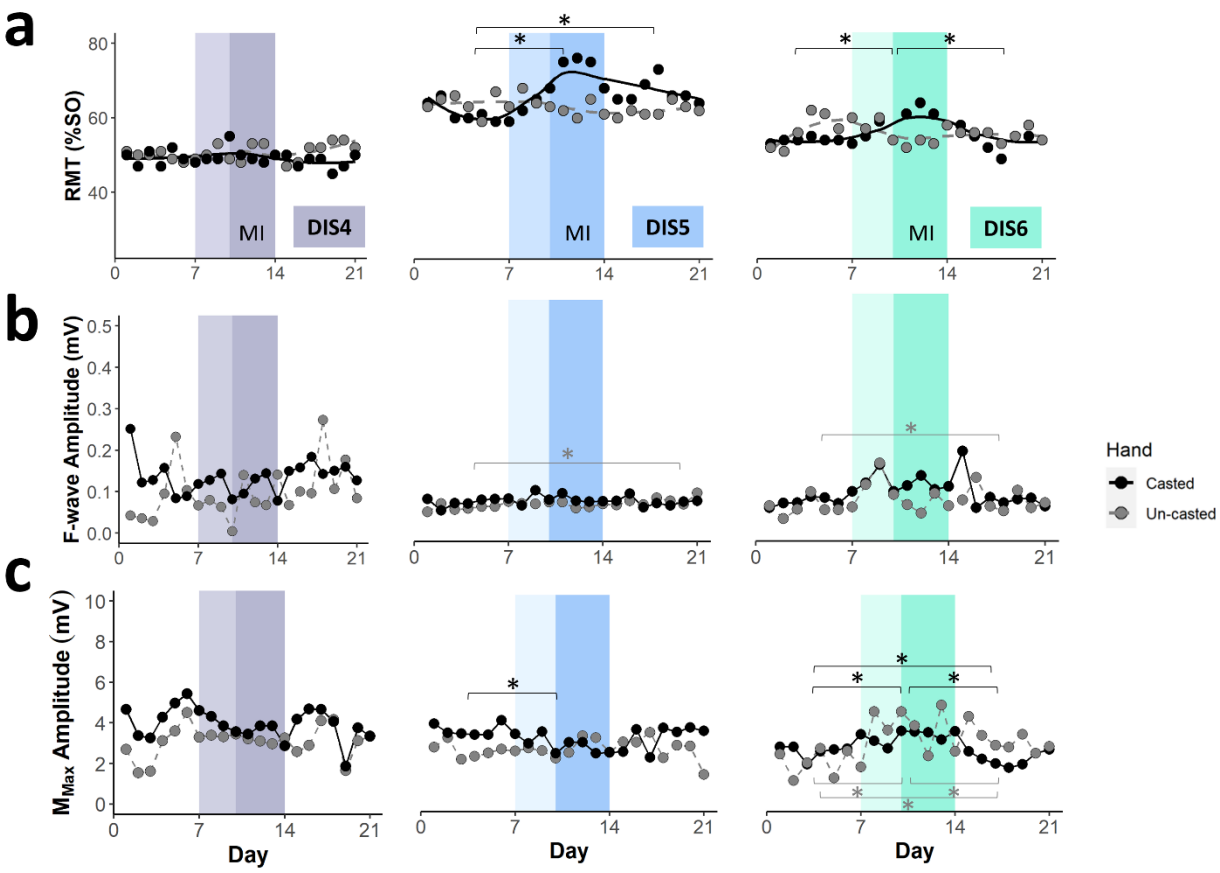


Figure 11. Resting motor thresholds and spinal and peripheral excitability with mental imagery training during immobilization. Disuse increased resting motor thresholds (RMT) in the casted, but not un-casted hand of DIS5 and DIS6 with no changes in DIS4 (a). Spinal (b) and peripheral responses (c) were less consistent. SO: stimulator output, M_{MAX}: muscle compound action potential

4.3.6 Effects of disuse and mental imagery on corticomotor structure

Since disuse consistently decreased CSE in the casted hand and mental imagery tended to attenuate such deficits, we explored whether brain functional deficits were accompanied by changed in white matter microstructure. Disuse reduced the normalized quantitative anisotropy (nQA) of homotopic (disused) sensorimotor tracts (i.e., within pre- and post-central gyrus) and cingulum. These same tracts demonstrated increased nQA seven days after cast removal (**Fig. 12**

A&B). In individuals who performed MI, nQA differed predominantly in the cingulum, but not in sensorimotor circuits. Graph theoretical analysis revealed that disuse decreased (increased) sensorimotor global efficiency (path length) and that MI training mitigated these changes in white matter network topology (**Fig. 12C-E**).

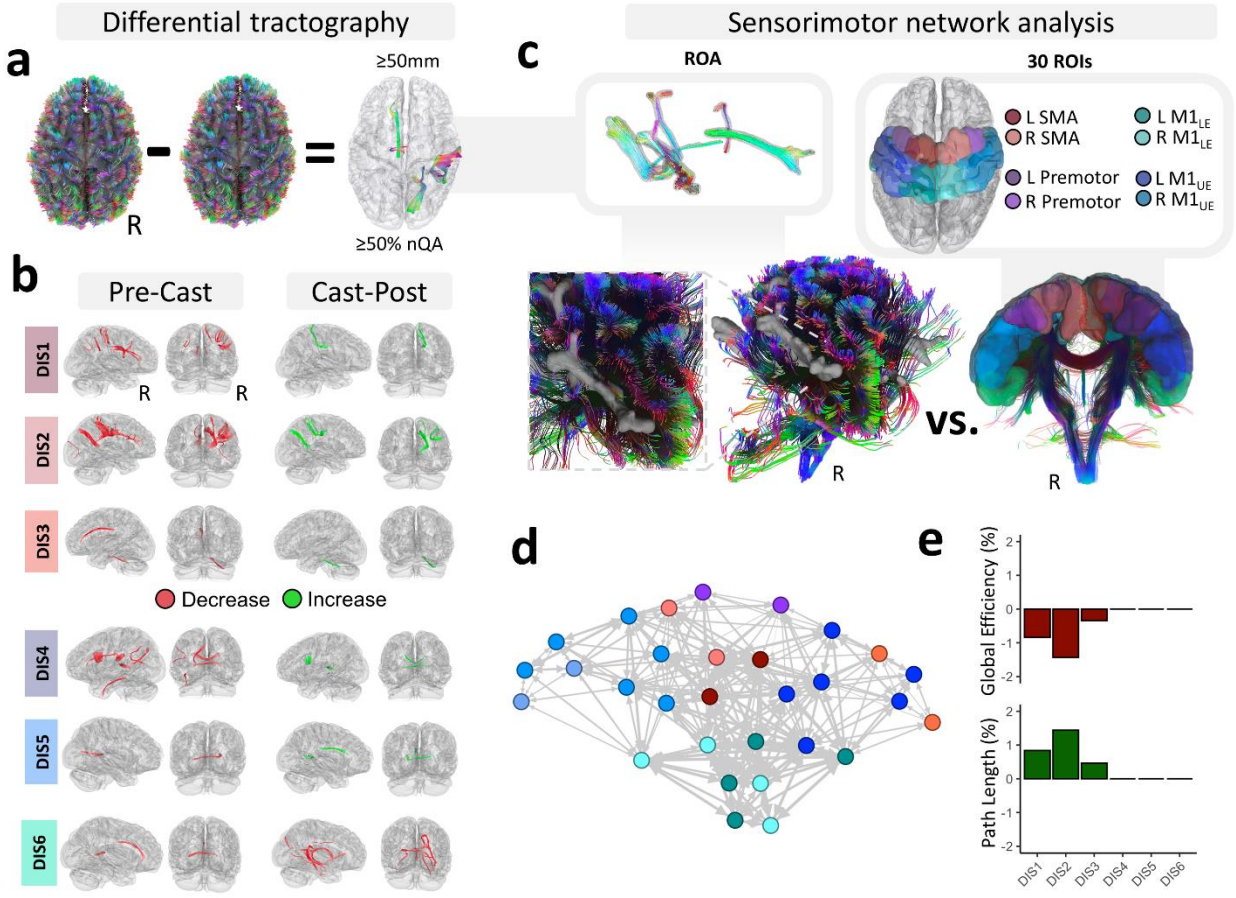


Figure 12. Influence of disuse and mental imagery on sensorimotor white matter microstructure and network topology. Differential tractography (a) was used to identify disuse-induced changes in normalized quantitative anisotropy (nQA) in sensorimotor circuits (b). Areas with reduced nQA were used to construct a region of avoidance (ROA) and thus examine the effect of disuse-induced reductions in nQA on sensorimotor network topology (c). Graphs were constructed by parcellating the supplementary motor area, premotor cortex, hand, leg and face sensorimotor representations into 30 regions of interest (ROI) from the Brainnetome atlas. Connectivity properties were determined based on the mean nQA of tracts between ROIs (d). Graph theory was used to quantify changes in global efficiency and characteristic path length (e).

4.4 Discussion

The aim of this precision neuroimaging study was to determine the origin, extent and time course of disuse-induced sensorimotor cortical plasticity. Over the course of twenty-one consecutive days, we used daily TMS, peripheral nerve stimulation and a comprehensive battery of skeletomotor assessments to monitor individual changes in corticomotor and skeletomotor function and structure before, during and after seven days of immobilization. Disuse reduced strength and fine motor skill, and such losses of function coincided with reduced corticospinal excitability in the casted. Reductions in CSE were evident after ~72 hours, specific to the casted hand, greater than measurement error, occurred regardless of peripheral and spinal alterations and returned to baseline levels within seven days of cast removal in parallel with skeletomotor function. Mental imagery mitigated disuse-induced skeletomotor loss of function when CSE was maintained but did not prevent loss of function when CSE decreased. Together, these findings provide novel insight into the link between supraspinal corticomotor excitability and skeletomotor function and provide direct evidence for a bidirectional relationship between the brain and body.

4.4.1 Origin of disuse-induced corticomotor deficits

Disuse reduced strength (**Fig. 2**) but did not influence muscle cross-sectional area (**Sup. Fig. 1**). Thus, even though muscle atrophy and changes in contractile properties are common following prolonged immobilization (Seki *et al.*, 2001b, a; Clark *et al.*, 2006a), the imaging techniques used in this study provide no evidence that changes in skeletomotor structure are responsible for loss of function during the initial days of disuse. Instead, our results indicate that corticomotor mechanisms likely mediate reductions in strength: CSE markedly (mean relative

change: -52.4%; absolute change: -8.5%, SEM = 2.70%, MDC = 7.60%) decreased within ~72h of immobilization (**Fig 2&3**) and mimicked the time course of skeletomotor loss of function, similar to recent evidence of rapid disuse-induced reductions in functional connectivity (Newbold *et al.*, 2020; Newbold *et al.*, 2021). Importantly, CSE increased in parallel with the recovery of skeletomotor function after cast removal (DIS2, DIS3, DIS6), which suggests a direct interplay between corticomotor and skeletomotor function.

As normalized MEPs decreased with (DIS1 and DIS2) *and* without (DIS3) changes in spinal/peripheral excitability, the origin of use-dependent corticomotor alterations appears predominantly supraspinal. Moreover, consistent increases in resting motor thresholds imply that disuse reduces the intrinsic membrane excitability of cortico-cortical axons or their downstream excitatory synapses onto corticospinal neurons (Ziemann, 2004; Ziemann *et al.*, 2015). Exploratory analysis using differential tractography indicated that disuse decreased the integrity of white matter microstructure in homotopic regions of sensorimotor cortex, which did not extend beyond supraspinal levels. Thus, use-dependent loss and recovery of corticomotor function appears to reflect supraspinal adaptations that are accompanied but not mediated by alterations in peripheral and spinal excitability.

4.4.2 Extent of disuse-induced corticomotor deficits

The spatial extent of disuse-induced corticomotor adaptations is currently unclear. Data from constraint-induced movement therapy studies (Liepert *et al.*, 1998) suggest that use-dependent changes in corticomotor function extend bilaterally. Yet, the few studies assessing bilateral corticospinal adaptations to disuse suggest that decreases in CSE are most pronounced or restricted to the casted hand (Burianová *et al.*, 2016; Debarnot *et al.*, 2021; Gaffney *et al.*, 2021).

Given that learning (i.e., greater use) increases CSE (Rogasch *et al.*, 2009; Cirillo *et al.*, 2011), we hypothesized that immobilization would reduce MEPs in the casted hand and lead to increases in MEPs in the un-casted hand. Contrary to our hypothesis, CSE decreased in the casted hand, but was unaffected in the un-casted hand or leg.

Our results most likely reflect that disuse only influences the disused sensorimotor circuits or that the intervention had little impact on the behavior of the un-casted hand. Disuse-induced reductions in white matter microstructure were restricted to homotopic sensorimotor areas and the cingulum, with only marginal effects on global sensorimotor network architecture (**Fig. 12**). Even though strength increased towards the end of the study in DIS1 and DIS2, there was no indication of *disuse-induced* behavioral improvements in the un-casted hand (**Fig. 3**). In contrast, constraint-induced movement therapy not only decreases the use of the non-paretic limb, but also *increases* the use and skeletomotor function of the paretic limb (Kwakkel *et al.*, 2015). Thus, differences between the bihemispheric corticomotor plasticity during CIMT, yet homotopic changes during immobilization in healthy individuals may reflect different adaptive behavioral responses (Liepert *et al.*, 1998).

Moreover, the spatial extent of corticomotor plasticity likely depends on the severity and dosage of the behavioral intervention. Training and immobilization-induced changes in CSE require varying amounts of (dis)use (Ngomo *et al.*, 2012), demonstrating that bilateral changes in skeletomotor behavior (such as the increased use of the un-casted hand but reduced use of the casted hand) may not necessarily involve bilateral corticomotor adaptations. Whereas the immobilization intervention markedly reduced the use of the casted hand (**Fig. 3**), the constant yet unstructured exposure to a variety of tasks may be insufficient to induce learning in healthy individuals during seven days of immobilization. Indeed, learning-induced increases in CSE

typically occur in concert with task-specific improvements in motor performance due to prolonged training (Beck *et al.*, 2007), which were not evident in the un-casted hand in this study. As such, the homotopic reductions in CSE need to be interpreted within the specific context of the selected immobilization intervention.

4.4.3 Effects of mental imagery on disuse-induced corticomotor and skeletomotor deficits

Given that disuse affects brain structure and function and that the brain mediates skeletomotor function, the use of a mental imagery counter-intervention during immobilization allowed us to directly assess the influence of corticomotor activity on disuse-induced changes in skeletomotor function and structure. MI activates movement-related brain areas (Decety, 1996), such as the sensorimotor cortex, premotor cortex and the supplementary motor area (Malouin *et al.*, 2003; Hanakawa *et al.*, 2008; Raffin *et al.*, 2012), increases CSE (Fadiga *et al.*, 1995; Fadiga *et al.*, 1999; Grosprêtre *et al.*, 2016; Ruffino *et al.*, 2019) and improves motor performance (Ranganathan *et al.*, 2004; Gentili *et al.*, 2010) in the absence of muscular contraction. In accordance with previous observations, five-days of imagined MVC task performance attenuated disuse-induced corticomotor adaptations and skeletomotor loss of function in DIS4 and DIS5 (Bassolino *et al.*, 2014; Clark *et al.*, 2014; Debarnot *et al.*, 2021). Although fMRI-derived functional activation maps suggest that all participants were capable of activating motor circuits during MI (**Sup. Fig. 4**), DIS6 displayed disuse-induced corticomotor and skeletomotor deficits that resembled those of participants who did not perform MI (DIS1-3; **Fig. 5 and Fig. 10**). Because the disuse-induced decreases in skeletomotor function were only mitigated when CSE was maintained (DIS4 and DIS5 vs. DIS6), our results suggest that the skeletomotor benefits of MI

were related to increases in CSE, which resulted in a net maintenance during immobilization (Ruffino *et al.*, 2019).

4.4.4 Precision neuroimaging: a novel approach to study corticomotor plasticity via TMS

Following a recent paradigm shift in functional magnetic resonance imaging (Laumann *et al.*, 2015; Poldrack *et al.*, 2015; Gordon *et al.*, 2017b; Greene *et al.*, 2020; Newbold *et al.*, 2020; Newbold *et al.*, 2021), this is the first precision TMS study. While our findings of disuse-induced reductions in CSE in the casted hand are consistent with traditional group-level designs (Huber *et al.*, 2006; Burianová *et al.*, 2016; Raffin & Siebner, 2019; Debarnot *et al.*, 2021; Gaffney *et al.*, 2021), the dense sampling of corticomotor and skeletomotor function during twenty-one consecutive days provided additional insight into the time course of use-dependent plasticity in *individual* human brains. Moreover, within-subject comparisons allowed us to replicate study results across multiple participants and, unlike group-level averages, enabled the interpretation of treatment efficacy while considering individual nuances, as is typical in medical practice. For example, MI attenuated disuse-induced skeletomotor deficits in DIS4 and DIS5, but not DIS6, and the time course of CSE in DIS6 strongly resembled that of DIS1-3, who did not perform MI. Whereas the observed corticomotor adaptations suggests minimal efficacy of the MI training for DIS6, the corresponding loss of skeletomotor function strengthened the idea that corticomotor and skeletomotor function are directly related. With reemerging skepticism about the reproducibility of *correlational* neuroimaging approaches in smaller (N<50) samples (Vul *et al.*, 2009; Marek *et al.*, 2022), within-subject analyses may overcome technical hurdles and provide an economic alternative to derive strong causal inferences in individual humans. Moreover, as a proof-of-concept study, the application of TMS in a precision neuroimaging framework may be particularly

insightful for rare clinical cases, where recruitment is challenging but longitudinal assessments are possible, as recently showcased by the functional brain network remapping after bilateral perinatal stroke using fMRI (Laumann *et al.*, 2021).

4.4.5 Practical applications of immobilization and mental imagery

Finally, given the popularity of immobilization and MI as rehabilitation strategies (Malouin *et al.*, 2013) following injury, our findings have several important practical implications. Disuse changed corticomotor function in the absence of injury. Growing evidence suggest that pathophysiological processes associated with skeletomotor injuries such as deafferentation (Brasil-Neto *et al.*, 1992; Brasil-Neto *et al.*, 1993) or pain (Neige *et al.*, 2021; Rohel *et al.*, 2021) influence corticomotor properties and that such adaptations can affect neuromuscular function even after successful return to sports. For instance, more than three years after surgical reconstruction and full medical clearance, individuals with unilateral anterior cruciate ligament rupture demonstrate aberrant corticomotor structure and function compared with healthy controls (Flanagan *et al.*, 2021) and only 40-55% of patients return to the same level of activity after surgery (Ardern *et al.*, 2014; Musahl & Karlsson, 2019), with re-injury rates as high as 30% (Paterno *et al.*, 2014; Webster *et al.*, 2014). In this study, corticomotor deficits emerged simply because of reductions in use, cautioning that immobilization could also contribute to maladaptive corticomotor plasticity and aberrant changes in skeletomotor function.

In alignment with previous work, MI mitigated disuse-induced skeletomotor and corticomotor adaptations in two out of three participants, highlighting its potential as a cost-effective rehabilitation method (Clark *et al.*, 2014; Debarnot *et al.*, 2021). Importantly, even though MI was performed *after* TMS, no disuse-induced decrease in CSE was evident in DIS4 and

DIS5. Thus, when effective, the corticomotor benefits of MI are likely immediate, and persist for at least 24h (i.e., until the next day of testing). Finally, the behavioral effects of mental imagery are likely task-specific. Whereas regular imagined maximum voluntary contractions mitigated the disuse-induced loss of strength and bimanual task muscle synergies, MI (of MVCs) did not preserve fine motor skill. Thus, optimization of the skeletomotor benefits induced by MI may require careful consideration of the somatosensory demands of the skeletomotor task(s) (Malouin *et al.*, 2013).

4.5 Limitations

Mental imagery commenced two days after disuse 1) to increase generalizability to real-life scenarios (i.e. seeking care provider after injury) and 2) because disuse-induced reductions in CSE may not arise until 48h (Gaffney *et al.*, 2021), in the absence of circadian influences (Huber *et al.*, 2006; Huber *et al.*, 2013; Debarnot *et al.*, 2021; Truong *et al.*, 2022). Indeed, no change in CSE was evident until ~72h of disuse, but the conservative delay and the seven days of immobilization limited the total duration of the MI counter-intervention to five days. The efficacy of short-term MI interventions has been confirmed (Di Rienzo *et al.*, 2015), but it remains unclear if more prolonged exposure would have facilitated corticomotor responses in DIS6. In this study, interventional blinding was not possible. To prevent changes in performance due to knowledge of results, participant feedback was minimized until study completion. Finally, we holistically assessed efferent aspects of disuse-induced corticomotor plasticity, but additional knowledge might be gained from the assessment of changes in somatosensory afference (Huber *et al.*, 2008; Lissek *et al.*, 2009). Concurrent neuroimaging techniques, such as TMS-EEG may also provide

further insight into the spatial specificity of disuse-induced corticomotor plasticity and clarify individual responses to MI.

4.6 Conclusion

We combined a novel precision neuroimaging approach with an established immobilization intervention to determine the origin, extent and time course of skeletomotor disuse-induced corticomotor plasticity. In addition to changes in skeletomotor function, corticospinal, spinal and peripheral excitability were assessed every day for twenty-one consecutive days. Immobilization markedly reduced the use of the casted hand and worsened strength as well as fine motor skill. Loss of skeletomotor function coincided with rapid (~72h) reductions in CSE specific to the casted hand. Skeletomotor and corticomotor function recovered within seven days of cast removal. Disuse-induced corticomotor deficits occurred independently of changes at the spinal or peripheral level, suggesting that disuse primarily affected supraspinal circuits. Mental imagery attenuated disuse-induced loss of skeletomotor function when CSE was maintained but did not sustain skeletomotor function when CSE decreased. Regardless of changes in CSE, MI of maximum voluntary contractions did not preserve fine motor skill. Thus, the skeletomotor benefits of MI likely involve increases in CSE and are specific to the imagined task. Together, these findings confirm use-dependent interdependencies between the corticomotor and skeletomotor systems in healthy humans and provide causal evidence for a bidirectional relationship between the brain and body.

Appendix A Abbreviations

ANOVA	Analysis of variance
CIMT	Constraint-induced movement therapy
CNS	Central nervous system
CSE	Corticospinal excitability
CV	Coefficient of variation
dMRI	Diffusion magnetic resonance imaging
EEG	Electroencephalography
EMG	Electromyography
FDI	First dorsal interosseus
FDR	False discovery rate
FEAT	fMRI expert analysis tool
fMRI	Functional magnetic resonance imaging
FWHM	Full-width at half maximum
GABA	Gamma aminobutyric acid
ICA	Independent component analysis
ICC	Intraclass correlation coefficient
I_{MAX}	Initial maximal response during peripheral nerve stimulation
LTD	Long-term depression
LTP	Long-term potentiation
M1	Primary motor cortex
MDC	Minimum detectable change
MEP	Motor-evoked potential
MI	Mental imagery
M_{MAX}	Compound muscle action potential
MNI	Montreal Neurological Institute
MRI	Magnetic resonance imaging
MSI	Skeletomotor injury
MVC	Maximum voluntary contraction
nQA	Normalized quantitative anisotropy
PAS	Paired-associative stimulation
PEST	Parameter estimation through sequential testing
PNS	Peripheral nerve stimulation
QA	Quantitative anisotropy
RMT	Resting motor threshold
ROA	Region of avoidance
ROI	Region of interest
SEM	Standard error of measurement

SO	Stimulator output
TA	Tibialis anterior
TMS	Transcranial magnetic stimulation
UDP	Use-dependent plasticity
USG	Urine-specific gravity

Appendix B Supplementary Tables

Supplementary Table 1. Stability of confounding variables throughout the study.

	Pre	Cast	Post	*F*	*p*
Hydration (USG)	1.018 ± 0.008	1.018 ± 0.008	1.019 ± 0.007	0.24	0.79
Sleep quantity (h)	6.93 ± 1.06	6.73 ± 1.12	6.73 ± 0.88	0.34	0.71
Sleep quality (0-5)	3.36 ± 0.49	3.07 ± 0.60	3.25 ± 0.44	2.19	0.12
Soreness (0-100)	29.52 ± 27.08	19.76 ± 20.35	31.19 ± 27.08	2.36	0.10
Total mood disturbance	27.14 ± 3.88	26.46 ± 2.86	27.00 ± 2.35	0.55	0.58

USG: urine-specific gravity

Supplementary Table 2. Regions-of-interest (ROI) used in the graph theoretical analysis.

Brainnetome Label	Area	Brain Region
PrG_L_6_5	Face	M1
PoG_L_4_2	Face	S1
PoG_R_4_2	Face	S1
PrG_R_6_5	Face	M1
PoG_L_4_3	Hand	S1
PrG_L_6_1	Hand	M1
PrG_L_6_2	Hand	M1
PrG_L_6_3	Hand	M1
PrG_L_6_6	Hand	M1
PrG_R_6_2	Hand	M1
PrG_R_6_6	Hand	M1
PoG_R_4_1	Hand	S1
PrG_R_6_1	Hand	M1
PrG_R_6_3	Hand	M1
PoG_L_4_1	Hand	S1
PoG_R_4_3	Hand	S1
PrG_L_6_4	Leg	M1
PrG_R_6_4	Leg	M1
PCL_L_2_1	Leg	M1
PCL_L_2_2	Leg	M1
PCL_R_2_1	Leg	M1
PCL_R_2_2	Leg	M1
PoG_L_4_4	Leg	S1
PoG_R_4_4	Leg	S1
MFG_L_7_6	Premotor	Premotor
MFG_R_7_6	Premotor	Premotor
SFG_L_7_4	SMA	SMA
SFG_R_7_4	SMA	SMA
SFG_R_7_5	SMA	SMA
SFG_L_7_5	SMA	SMA

Supplementary Table 3. Pairwise comparisons for individuals completing the immobilization intervention

		DIS1			DIS2			DIS3		
	Target	Pre-Cast	Cast-Post	Pre-Post	Pre-Cast	Cast-Post	Pre-Post	Pre-Cast	Cast-Post	Pre-Post
Strength	Casted	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.006**	**<0.001**	**0.004**	**<0.001**
	Un-casted	0.85	0.23	**0.001**	0.77	**0.004**	**<0.001**			
	Leg									
Pegboard Time	Casted	0.19	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.06	**0.045**	**0.045**	0.84
	Un-casted				0.448	**0.001**	**0.001**			
Force contribution	Casted	**0.001**	**0.011**	0.08	**0.01**	0.158	**0.01**	**<0.001**	**0.013**	**0.009**
CV Force	Casted							**0.019**	0.230	**0.015**
	Un-casted									
MEP/M_{MAX}	Casted	**0.042**	0.785	**0.042**	**0.036**	**<0.001**	**0.014**	**0.035**	**<0.001**	**<0.001**
	Un-casted									
	Leg							**0.009**	0.168	0.094
RMT	Casted	**0.009**	0.138	0.111	**0.023**	**0.039**	0.566	**<0.001**	**<0.001**	0.963
	Un-casted	0.172	0.172	**0.023**				**<0.001**	>0.999	**<0.001**
	Leg				**0.037**	0.091	0.444			
F-wave amplitude	Casted				0.558	**0.026**	**0.014**	**0.008**	**0.033**	0.345
	Un-casted	0.12	0.12	**0.01**						
M_{MAX} amplitude	Casted							**<0.001**	**<0.001**	0.78
	Un-casted	**0.017**	**0.008**	**<0.001**	**<0.001**	0.205	**<0.001**			

Supplementary Table 4. Pairwise comparisons for individuals completing the immobilization and mental imagery interventions.

		DIS4			DIS5			DIS6		
	Target	Pre-Cast	Cast-Post	Pre-Post	Pre-Cast	Cast-Post	Pre-Post	Pre-Cast	Cast-Post	Pre-Post
Strength	Casted	**0.001**	0.334	**<0.001**	0.095	**<0.001**	**<0.001**	0.06	**0.04**	0.38
	Un-casted	0.276	0.630	**<0.001**						
	Leg									
Pegboard Time	Casted	**0.031**	0.82	**0.031**	**0.049**	**0.027**	0.687	**<0.001**	**<0.001**	0.148
	Un-casted							0.804	**0.001**	**0.017**
Force contribution	Casted							**<0.001**	**<0.001**	0.29
CV Force	Casted									
	Un-casted									
MEP/M_{MAX}	Casted							**0.048**	0.444	**0.018**
	Un-casted									
	Leg							**0.02**	**<0.001**	**0.014**
RMT	Casted				**0.002**	0.176	**0.026**	**0.004**	**0.004**	0.921
	Un-casted									
	Leg									
F-wave amplitude	Casted									
	Un-casted				0.179	0.131	**0.015**	**0.103**	**0.103**	**0.007**
M_{MAX} amplitude	Casted				**0.021**	0.135	0.225	**0.010**	**<0.001**	**0.030**
	Un-casted							**<0.001**	**0.041**	**0.041**

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Appendix C Consent Form

CONSENT TO ACT AS A PARTICIPANT IN A RESEARCH STUDY

TITLE: The effects of mental imagery on corticomotor plasticity during skeletal muscle disuse

PRINCIPAL INVESTIGATOR

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Key Considerations

1. This is a consent form for research participation. It contains important information about this study and what to expect if you decide to participate. Please consider the information carefully. Feel free to discuss the study with your friends and family and to ask questions before you decide whether to participate.
2. Your participation is voluntary. You may refuse to participate in this study. If you decide to take part in the study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your usual benefits.
3. You may or may not benefit as a result of participating in this study. Your participation may result in unintended or harmful effects for you that may be minor or serious depending on the nature of the research.
4. You will be provided with any new information that develops during the study that may affect your decision whether to continue to participate. If you decide to participate, you will be asked to sign this form and will receive a copy of the form. You are being asked to consider participating in this study for the reasons explained below.

1. Why is this research being done?

You are invited to participate in a study to determine how not using your arm influences your brain. Arm inactivity is common during casting and has been shown to change how your brain communicates with the muscles that are casted. Little is known about the effects of casting on the muscles of your non-casted arm, and how widespread the changes in brain structure and function are. Previous work has shown that mental imagery can counteract muscular and brain changes that happen during immobilization, but it is unclear how quickly this occurs. The purpose of this study is to 1) determine the influence of arm casting on brain structure and function, 2) examine the influence of arm casting on function of the casted and non-casted arm, and 3) determine the effects of mental imagery during arm casting. If you decide to participate in this study, you will complete an experimental visit every day for 21 consecutive days. Each visit will include non-invasive brain stimulation, brain imaging, electrical nerve stimulation, and tests of muscle structure and function. Each visit will take three (3) to four (4) hours and take place at the same time of day. Five (5) times throughout the study, we will also take pictures of your brain with MRI; each of these visits will take one hour. The total time commitment for this study will be between 70 and 90 hours, and the study will be completed in less than one month.

2. Who is being asked to participate in the research study?

We will enroll six people. Participants will be healthy, right-handed/legged men and women aged 18 to 45 years with normal or corrected-normal vision. We are looking for people who are comfortable with maximal strength testing, electrical nerve stimulation, transcranial magnetic stimulation (TMS) and magnetic resonance imaging (MRI). Due to MRI restrictions, individuals must weigh less than 300lb.

3. What will happen if I take part in this study?

All tests will occur at the University of Pittsburgh Neuromuscular Research Laboratory (NMRL) and Brain Imaging Data Generation and Education (BRIDGE) Center:

NMRL	BRIDGE Center – Mellon Institute
3860 South Water St	4400 Fifth Ave
Pittsburgh, PA 15203	Pittsburgh, PA 15213
412-240-0460	412-268-7140

We will use transcranial magnetic stimulation (TMS) and electroencephalography (EEG) to stimulate and record your brain's activity. For TMS, a coil (**Figure 1**) is placed on your head above the areas of the brain we intend to stimulate. When turned on, the coil produces a strong but brief magnetic field (i.e. pulse) that activates small areas of your brain. When we deliver a TMS pulse, we can measure how your brain responds with EEG. To do so, you will receive approximately 450 magnetic pulses (150 to each of three muscles) spread out over the course of approximately 60-90 minutes every day. During each stimulus you will feel a light tapping sensation on your head and your muscles will respond, similar to knee reflex examinations at your doctor's.

In addition, we will also stimulate nerves that control muscles of your hands and your non-dominant leg to see how muscle and nerve function change during the intervention. Every day, we will measure the size of your muscles via ultrasound, we will test the strength of your muscles and you will receive up to ~3100 electrical pulses (~1000 to each of three nerves: two in your hands and one by your leg). Electrical pulses feel similar to a light poke with a push pin, without actually breaking your skin, but they will be repeated very rapidly (5 times per second), so you will receive stimulation at each nerve for about 5 minutes. Two times during the study, you will complete this procedure back to back (before and after you start wearing the cast or we remove it). All of these tests will be performed daily over the course of 21 days. In the event that you have to miss a testing visit, we will make up the missed session the next day and move all other visits to one day later than originally planned, so that your enrollment will expand by one additional day and you can complete the full 21 days of testing.

Figure. Set-up with TMS in the reclining chair.

In this study, the goal is to see if inactivity in one arm changes brain activity and muscular performance in both arms. Since we expect changes in brain activity and muscular performance in both arms, we will compare all outcomes to your nondominant leg, which will not be casted. You are encouraged to ask any questions you have about the study procedures. The study you are about to review will take approximately 4 hours (brain imaging included) per visit.

When you first visit the NMRL, before you volunteer to participate in this study, you will be asked to complete forms for: 1) TMS safety, 2) MRI safety, 3) medical history, 4) physical activity readiness, 5) physical activity, and 6) handedness and 7) footedness. The forms will help us confirm your eligibility and determine if there are any issues that might make it unsafe for you to participate.

You will not be selected to participate if you have:

- History of mental imagery training
- History of epileptic events, seizures, or sleep disorders
- No medical clearance for physical activity
- Implanted medical device, shrapnel/metal object in body, dental retainer, copper IUD
- History of neurological, cardiovascular, or other major disorder(s)
- Injuries or physical limits that prevent or make maximal exercise (e.g. a maximum contraction of your legs or hands) uncomfortable/unsafe
- History of alcohol or substance abuse
- Currently use any medication with the following properties ○ Acts on the central nervous system ○ Lowers seizure threshold ○ Can damage hearing (ototoxic)
- Pregnancy or attempting pregnancy
- Claustrophobia
- Inability to refrain from caffeine (e.g. energy drinks, coffee) for 6h before each visit
- Current brain injury, psychiatric, or mental health disorder(s)
- Do not respond to or are uncomfortable with TMS, nerve stimulation, MRI or EEG - Weigh more than 300lbs

If you are still interested and eligible, we will explain or demonstrate the study procedures and give you the opportunity to ask questions and review the consent form. If you would like to review the study procedures privately or with others, you are encouraged to take the consent form with you and contact us with any other questions. If you provide informed consent, you will receive TMS to confirm that you respond and are comfortable with the procedures. You will also complete the strength tests. We will shave and mark areas of the skin on your hands and the non-dominant leg so that we can place sensors that measure muscle activity more quickly during the remaining visits, which we will then schedule.

Throughout the study you will be asked to maintain a similar diet and maintain your normal activity levels outside of the study. Female participants must be using a contraceptive that

stabilizes circulating hormone concentrations. These requests are made to minimize possible effects of nutrition, soreness, menstrual cycle or fatigue on performance or responses to TMS. Between and prior to each visit, you will also be asked (and reminded) to:

1. Avoid changes in medication use
2. Maintain a similar diet and exercise regimen
3. No alcohol, drug, or analgesic use within 12hr
4. Similar amount of sleep the evening before
5. No heavy exercise within 2hr
6. No caffeine within 6hr

Throughout the study, you will also be asked to wear a sensor (accelerometer) on each wrist at all times. These sensors will allow us to measure how you use your hands throughout the day when you are not in the laboratory. Every day you will bring the accelerometer to the laboratory, we will download the data, recharge the batteries, and hand them back to you at the end of testing.

At the beginning of each visit, you will fill out forms about your sleep quality, ability to imagine different life scenarios, mood, stress, and soreness. We will test a urine sample to confirm you are not dehydrated and administer a pregnancy test (if applicable). If you are dehydrated, we will ask you to drink water that we will provide. When you wear the cast, we will also ask questions about the comfort of the cast to make sure you are comfortable. Each visit will be scheduled at the same time of day (±2hr).

During the first visit, you will travel to the BRIDGE center so that we can take pictures of your brain with magnetic resonance imaging (MRI). We will reconfirm your responses to the MRI safety form to reduce any inconvenience if you are not eligible. Before the MRI procedure, we will explain the experimental procedures, which will involve you lying still on a bed for several minutes. During the MRI session, you will be exposed to magnetic fields and radio waves. You will hear repetitive tapping sounds and be required to wear earplugs to reduce the noise. The visit will take about one hour and will be repeated on the 8^{th}, 9^{th}, 14^{th}, and 21^{st} day of the study.

Every day, you will have testing at the NMRL to measure how your brain communicates with your muscles normally and during casting; and to measure how responsive your hands and legs are to electrical stimulation. On the first day of testing, you will complete a quick hearing test. During the hearing test, you will tell us whether you can hear TMS pulses while white noise is played through earplugs, and we will adjust the loudness of the volume until you can no longer hear the TMS pulses. This sound will be played during all visits whenever we use TMS. We will use hypoallergenic double-sided tape to place sensors on your skin so that we can measure signals produced by the muscles. Electrical stimulation will involve brief, transiently painful stimuli delivered through an electrode placed close to your outer wrist and shin. An EEG cap will be placed on your head to measure how your brain responds to stimulation. Sensors will also be placed above and below your eye to measure blinks. The device looks like a mesh cap, but has

small electrodes coming out of it. After this, you will lay down comfortably for 10 minutes and focus your attention to a black screen with a white fixation cross while minimizing movement.

Next, we will locate the areas of the brain we want to stimulate using TMS. During TMS, a magnetic field passes through your skull and activates neurons (brain cells) that are connected to your hands or legs, depending on where we stimulate. When we will deliver TMS pulses at different intensities while you rest in a seated position, your hands or legs may therefore start to twitch. When stimulating once at a time, we only assess how your brain communicates with your muscles and we do not change what your brain is doing. Immediately after TMS, we will deliver electrical stimuli to each wrist and the non-dominant lower leg at different intensities. Each electric pulse feels like a light poke (without actually breaking the skin) using a push pin. Finally, you will complete tests of your finger and leg muscles. During all these procedures, you will be in a seated position.

Whenever TMS is used, you will wear in-ear headphones that play whitenoise at an intensity recorded on day 1. When we stimulate areas of the brain that control movement, it often makes the target muscles twitch. Also, because we stimulate the scalp, each pulse creates a tapping sensation and may produce responses in the face, including twitches and eye blinks. Twitches are normal and painless, but can cause scalp soreness and headaches, which are not the result of brain stimulation, but tightness in scalp muscles not used to being activated this way. In addition, responses depend on stimulation intensity: at the lowest intensities, you may feel nothing. As intensity increases, the machine will become louder, tapping sensations on your scalp will grow stronger, and twitches in your muscles will get larger and possibly include other muscles.

In addition to measures of brain activity, we will also conduct daily tests of the structure and function of your muscles. For instance, you will be asked to perform maximal and submaximal contractions of your hands and legs, while we record your strength and muscle signals. We will place an ultrasound probe on your muscle to measure their size. Finally, you will perform a pegboard test, during which you have to move small little pegs from one board to another.
After seven days of testing, an orthopaedic specialist or the PI of the study who was trained by the specialist will cast your non-dominant arm and you will wear this nonremoveable cast for one week (7 days). We will do several things to make the cast as comfortable as possible; you will be able to bathe and shower. You will not be allowed to take the cast off. As such, the cast will severely restrict the use of your non-dominant hand in activities of daily living. For instance, you may have to find alternative forms of transportation, won't be able to use this arm to perform heavy lifting, performance of household chores or even call of work depending on your job. The same tests will be performed each day during the 7-day casting period, with the exception of some muscle function tests on your casted hand, since the cast will prohibit us from doing so.

If you are randomized into the mental imagery group, you will receive twenty minutes of mental imagery training for five days beginning 48h after casting (days 3-7 of casting). During this time, you will imagine yourself contracting your casted hand and we will also show you a video of your contractions from the first seven days of testing to help you envision yourself contracting your hand muscles.

After the conclusion of the 7-day casting period, there will be another 7 days of testing with otherwise identical procedures and no cast (like the first 7 days).

4. How long will I be in the study?

Each visit will last 3-4 hours and brain imaging will add another hour as described below:

Name	Visit (#)	Time (Hr)	Total Time (Hr)
Test Visit	21	4	84
Brain imaging	5	1	5
		Total	**89**

5. What are the risks, side effects, and discomforts of this research study?

There may be adverse events or side effects that are unknown and some of these risks could be permanent, severe or life-threatening. If you experience a life-threatening event, a medical doctor will not be in the facility; 911 will be called. To ensure your safety, the investigators will take every precaution to prevent adverse events and minimize risks. These precautions include adequate screening, familiarization, proper instructions, safe and validated protocols, optimized testing procedures, and close supervision.

Risks of Transcranial Magnetic Stimulation (TMS)

Transcranial Magnetic Stimulation (TMS) is non-invasive and there are conservative guidelines for the use of TMS in research. The known serious risks of TMS are few and the procedures follow guidelines to minimize risk. The safety of chronic TMS for brain tissue was confirmed in animals. In the few human case studies performed, no undesirable changes were found in the brain. Nevertheless, *TMS carries several risks you should carefully consider.*

TMS and Metal Objects or Devices: If you have metallic objects or implanted devices in close contact with TMS coils, we cannot perform TMS. Examples of such devices include cochlear implants and deep brain stimulators. Of lower risk but still excluded are cardiac pacemakers, vagus nerve stimulators, or spinal cord stimulators. It is very important that you notify us of any metal objects, devices, or implants in your body before TMS is used.

TMS and Seizure: There is a small risk of experiencing a seizure during TMS. We will lessen this risk by following all current safety guidelines for TMS application. Sleep deprivation and proconvulsant medication may increase this risk. In the rare instance when seizure or fainting occurs, it is usually of short duration, does not require drug treatment, causes no durable effects, and does not increase your risk of such events in the future. If a seizure occurs, you may lose driving privileges for up to one year.

TMS and Scalp Soreness/Headaches: Stimulation can cause brief local pain below the sites of stimulation due to soreness from neck or head muscle contraction. Mild headaches, local pain, neck pain, and toothache may occur. These effects are brief and typically limited to the first TMS session.

TMS Noise: TMS produces loud clicking sounds. As a precautionary measure, TMS will not be performed if you use drugs that can damage the ears.

TMS and Pregnancy: If you are or are trying to get pregnant, the effects of TMS on a fetus are unknown and, therefore, we will not perform the examination at this time.
You may have the stimulation stopped at any time if you feel uncomfortable. We will work closely with you and carefully monitor your responses during all TMS tests.

By consenting, you agree to:

- Answer the TMS safety and study intake forms accurately
- Communicate the experience of any discomfort during TMS tests

Risks of Electrical Nerve Stimulation
Similar to TMS, we follow conservative guidelines to minimize risk associated with electrical nerve stimulation. Electrical stimulation generates a twitch in the stimulated muscle(s) and produces a brief painful (but harmless) pinching sensation. Every day, you will receive approximately 3,100 stimuli, so there is a moderate risk of red skin or itching and very small risk of burn or symptomatic electrical shock. Each nerve of your wrist and the non-dominant lower leg will be stimulated for about 5 minutes and each stimulus will feel similar to a light poke with a push pin, without actually breaking your skin. We will ask for your feedback during the procedure to make sure you are comfortable.

Risks of Magnetic Resonance Imaging (MRI)
There are no known significant risks with MRI because the magnetic field strengths used are believed to be without harm. There are conservative guidelines for radio frequency magnetic field exposure and our examinations fall within them. We believe these are safe and less hazardous than an x-ray computed tomography examination (CT scan). A call button and an intercom are provided so that you may have the scan stopped at any time during the study.
MRI and Metallic objects: If a person has a cardiac pacemaker or a certain type of metallic clip or prostheses in their body (i.e., an aneurysm clip in the brain); if a person has worked with metal or had a piece of metal removed from the eye(s); or if a person has shrapnel, bullets, or buckshot in their body. As metallic objects may be strongly attracted to the magnet, *it is very important that you notify us of any metal objects, devices or implants in or on your body before you enter the magnet room.*
All other metallic objects must also be removed from you prior to entering the magnet room or approaching the magnet, to prevent them from becoming a projectile or being pulled by the magnet. This includes keys, jewelry, pocketknives, money clips, paper clips, safety pins, hairpins, and barrettes. In addition, objects such as watches, credit cards, and hearing aids could be damaged in the presence of the magnetic field. A locker will be provided for you to secure your valuables.
MRI and Pregnancy: If you are or are trying to get pregnant, the effects of the scan on a fetus are unknown and, therefore, you will be excluded from the study.
MRI and Heating: There is a risk of heating of metal objects such as wires from exposure to radio waves. Please report any heating/burning sensation immediately. You may have the scan stopped at any time if this occurs using the call button.

MRI and Muscle Twitches: There is a possibility that you will experience a localized twitching sensation due to the magnetic field changes during the scan. This is not unexpected and should not be painful. However, you may have the scan stopped at any time if this occurs using the call button.
MRI and Dizziness: Dizziness and nausea may occur momentarily when your head is moved in or out of the tunnel of the magnet. The sensation should disappear quickly. If not, you may discontinue the scan at any time.
MRI and Claustrophobia: You may experience claustrophobia, i.e. the fear of having no escape and being closed in. You may discontinue the scan at any time.
MRI and Incidental Findings: The MRI you will receive during this study is for research purposes only. It is not a clinical scan intended for diagnostic or therapeutic purposes. The BRIDGE Center is a research institute; it is NOT a Clinical MRI facility in a hospital. There are no neuroradiologists at the BRIDGE Center. Therefore, staff are unable to make any medical comments about your scan. Should you want to know if your scan is normal or abnormal, the staff will not be able to tell you.
Only in the event that one of the study team members incidentally recognizes a severe abnormality in your brain scan a neuroradiologist will be consulted. If the radiologist suggests you obtain further diagnostic tests, the Principal Investigator of the study will attempt to contact you with this recommendation. You will be responsible for following up with your physician, and if you or your physician requests copies of your brain images from this study, they will be provided to you.
At the investigator's discretion, you may view your brain images and receive copies of them. However, you should be aware that brain structures within the normal population are highly variable, and that it is difficult to draw any conclusions from your images. You should also be aware there is a potential you could experience some distress or discomfort from viewing your own images.

By consenting, you agree to:

- Answer the MRI safety form accurately; tell investigators about any metal in your body
- Not bring any metal devices into the scanning room without staff approval

Risks of Body Sensors / Ultrasound
Sensors will be placed on your skin with temporary adhesives. The electrodes can cause discomfort when pressed tightly. Sensor application involves the use of scrubbing to remove dead skin, adhesive tape, and alcohol swabs. An ultrasound probe will be placed on your hand and legs which requires the use of conductive gel. Each of these steps may cause temporary discomfort and skin itchiness or redness.

Risks of arm casting
The intervention will involve arm casting, which will significantly limit your ability to use your arm in activities of daily living, such as driving, writing, typing, etc. Arm casting may cause you to be

less independent or unable to drive for the duration of the intervention. As such, you may have to find alternative forms of transportation, won't be able to use this arm to perform heavy lifting and household chores or even have to call off work depending on your job. The cast may also cause skin irritation, itchiness, discomfort, and the loss of muscle strength and size. The loss of muscle strength and size due to immobilization typically recovers within 7 days of cast removal. After cast removal, we will monitor your performance to confirm recovery. If you have not fully recovered 7 days after cast removal, we will provide you with a strength training program and the opportunity to come back to the lab for additional tests to confirm recovery.

Risks of Internet Communication and Breach of Confidentiality

Internet Communication: Although every reasonable effort has been taken, confidentiality during Internet communication activities cannot be guaranteed and it is possible that additional information beyond that collected for research purposes may be captured and used by others not associated with this study.

Breach of Confidentiality: Breach of confidentiality is a risk of any research study. To minimize this risk, all data is kept in locked cabinets and password protected computers without any information that could link you to your data. Any information that could identify you will be destroyed after 7 years. Any remaining data will be anonymous.

Risks of physical activity

When performed correctly, physical activity is a low-risk activity in healthy individuals. However, risks exist, including fatigue, soreness, dizziness, lightheadedness, fainting, nausea, and vomiting. Additional risks associated with physical activity include the possibility of falls, muscle strains or pulls of the involved musculature, muscle spasms or strains, and in extremely rare instances, muscle, ligament, or tendon damage. There is also a slight risk of cardiopulmonary overexertion. We will make all exercises as safe as possible through screening, familiarization, instruction, practice, and supervision by experienced testing personnel. All lab personnel are CPR and automated external defibrillator (AED) certified, and there is an AED in the laboratory. However, in the case of a life-threatening event, a medical doctor will not be in the facility; 911 will be called.

Risks of Repeated Visits

The study requires multiple visits, which could cause financial or time-related inconveniences.

6. What benefits can I expect from being in the study?

There are no direct benefits of participation. This investigation will strengthen our understanding of how inactivity affects your brain and if we can use mental imagery to attenuate these changes. You will learn about modern neurophysiological techniques. At your request, we will review your data with you after testing and explain our observations.

7. If I agree to take part in this research study, will I be told of any new risks that may be found during the course of the study?

You will be notified if any new information we learn during this research study may cause you to change your mind about continuing to participate in the study.

8. Will my insurance provider or I be charged for the costs of any procedures performed as part of this research study?

None of the services or procedures you receive during this research study will be billed to you or your health insurance. If you receive a bill or believe your health insurance has been billed for something that is part of the study, notify a member of the research team or UPMC Patient Billing Services.

9. Will I be paid if I take part in this research study?

You will receive $15 for every day of testing ($15 x 21 visits = $315 total). In the event that you started, but are unable to complete the entire visit, you will still be compensated the full $15 for that day. Moreover, after completing the first week of testing, you will receive an additional $100, after the second week of testing an additional $150 and when completing the entire study an extra $250, for a total of $815 ($315 + $100 + $150 + $250).
Payment to participants is considered taxable income regardless of the amount. If a participant receives $600 or more in a calendar year from one organization, that organization is required by law to file a "Form 1099 – Miscellaneous" with the IRS and provide a copy to the taxpayer. We are required to give your name and social security number to the Accounting Office. Participants who do not provide a social security number may still participate in the research, but the IRS requires that 26% of the payment be sent by the institution to the IRS for 'backup withholding;' thus you would only receive 74% of the expected payment

10.Who will pay if I am injured as a result of taking part in this study?

If you believe that the research procedures have resulted in an injury to you, immediately contact the Principal Investigator who is listed on the first page of this form. Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by the hospitals of UPMC. Your insurance provider may be billed for the costs of this emergency treatment, but none of those costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the costs of this follow-up care. At this time, there is no plan for any additional financial compensation. You do not, however, waive any legal rights by signing this form. The study does not provide compensation for care of injuries sustained at the BRIDGE MRI center.

11.Who will know about my participation in this research study?

Per University of Pittsburgh policy all research records must be maintained for at least 7 years following final reporting or publication of a project. All records related to your involvement in this research study will be stored in a locked file cabinet or passwordprotected computer and any information about you will be kept as private as possible. Your identity on these records will be indicated by a case number rather than by your name, and the information linking these numbers

with your identity will be kept separate from the research records. You will not be identified by name in any publication of research results unless you sign a separate form giving your permission.

12.Who will have access to identifiable information related to my participation in this research study?

In addition to the investigators listed on the first page of this authorization (consent) form and their research staff, the following individuals will or may have access to identifiable information related to your participation in this research study:
Authorized representatives of the University of Pittsburgh Office of Research Protections may review your identifiable research information (which may include your identifiable medical information) for the purpose of monitoring the appropriate conduct of this research study. In unusual cases, the investigators may be required to release identifiable information related to your participation in this research study in response to an order from a court of law. If the investigators learn that you or someone with whom you are involved is in serious danger or potential harm, they will need to inform, as required by Pennsylvania law, the appropriate agencies.

13.Is my participation in this research study voluntary?

Your participation in this research study is entirely voluntary. You may want to discuss this study with your family and friends and your personal physician before agreeing to participate. If there are any words you do not understand, feel free to ask us. The investigators will be available to answer your current and future questions. Whether or not you provide your consent for participation in this research study will have no effect on your current or future relationship with the University of Pittsburgh. Whether or not you provide your consent for participation in this research study will have no effect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider. To formally withdraw your consent for participation in this research study you should provide a written and dated notice of this decision to the principal investigator of this research study at the address listed on the first page of this form.

14.May I withdraw my consent for participation in this research study?

You can, at any time withdraw from this research study; you can also withdraw your authorization for us to use your identifiable medical information for the purposes described above. This means that you will also be withdrawn from further participation in this research study. Any identifiable research or medical information obtained as part of this study prior to the date that you withdrew your consent will continue to be used and disclosed by the investigators for the purposes described above. To formally withdraw from this research study, you should provide a written and dated notice of this decision to the principal investigator of this research study at the address listed on the first page of this form. Your decision to withdraw from this study will have no effect on your current or future relationship with the University of Pittsburgh.

15. If I agree to participate in this research study, can I be removed from the study without my consent?

It is possible that you may be removed from the research study by the researchers if, for example, you are unable or unwilling to perform the required tasks or upon the unlikely development of a neurological or cardiovascular disorder. The investigators have the right to withdraw you from this study if you develop a muscular, ligament or bone injury. Any injury will be determined by the investigators through questioning and physical examination. You may be removed for signs of intolerance to TMS, including severe headaches, dizziness, nausea, or hearing loss, or an inability to respond to TMS pulses.

****

VOLUNTARY CONSENT

The above information has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions, voice concerns or complaints about any aspect of this research study during the course of this study, and that such future questions, concerns or complaints will be answered by a qualified individual or by the investigator(s) listed on the first page of this consent document at the telephone number(s) given.

I understand that I may always request that my questions, concerns or complaints be addressed by a listed investigator. I understand that I may contact the Human Subjects Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668) to discuss problems, concerns, and questions; obtain information; offer input; or discuss situations that occurred during my participation. By signing this form I agree to participate in this research study. A copy of this consent form will be given to me.

_____

Participant's Name (Print)

_____ _____

Participant's Signature Date

CERTIFICATION OF INFORMED CONSENT

I certify that I have explained the nature and purpose of this research study to the above-named individual(s), and I have discussed the potential benefits and possible risks of study participation. Any questions the individual(s) have about this study have been answered, and we will always be available to address future questions, concerns or complaints as they arise. I further certify that no research component of this protocol was begun until after this consent form was signed.

_____ _____

Printed Name of Person Obtaining Consent | Role in Research Study

_____ | _____

Signature of Person Obtaining Consent | Date

Appendix D Screening Form

In-Person/Phone Script for Recruitment/Screening-Corticomotor Plasticity During Disuse

Thank you for calling to find out more about our research study.
OR I am returning your call to provide more information about our research study.
OR We recently talked about the study and are calling to tell you more about the study procedures.

My name is [Insert Name] and I am a researcher at the University of Pittsburgh Neuromuscular Research Lab.

The purpose of this study is to determine how an upper arm cast influences your brain's structure and function and to examine if mental imagery (i.e. imaging and yourself using your muscles) can counteract such changes. If you participate in the study you will complete daily visits for 21 days with non-invasive brain stimulation and imaging, electrical nerve stimulation, physiological monitoring (i.e., skin conductance, heart rate, respiration, skin temperature, blood pressure), and muscle tests. Five times throughout the study, we will obtain additional brain images with an MRI machine.

At first, we will measure your brain and muscles for seven days in order to get a good understanding of how your brain and muscles communicate. Then, for one week, you will be asked to wear a non-removeable cast on your left hand/arm and we will measure what happens to the communication between your brain and your muscles during that time and for the seven days after we take it off. The cast will significantly interfere with activities of your daily life and will make you less independent. For instance, you may have to find alternative forms of transportation, you won't be able to use this arm to perform heavy lifting or household chores and you may even have to call off work, depending on your job. To measure how frequently you use your hand, you will be asked to wear an accelerometer (similar to an Apple Watch) on both wrist for 21 days. You will receive more than 3,000 stimuli every day (~450 magnetic and ~3000 electric stimuli) and each testing session will take approximately 4h. Each magnetic stimulus will feel like a light tapping sensation on your head and your muscles will respond, similar to knee reflex examinations at your doctor's. Each electrical stimulus will feel like a light poke using a push pin (without actually breaking the skin). When we take pictures of your brain with MRI, this will cost another hour of your time. Testing will occur at the Neuromuscular Research Lab in Southside and the Bridge Center in Oakland. You will be compensated $15 for every visit ($315 total) and if you complete the following milestones you can earn an additional $300 (after first 7 days of testing - $100; after 14 days of testing - $150; after 21 days of testing - $250), for a total of $815.

Do you have any questions or concerns?

Now that you have a basic understanding of the study, do you think you might be interested in participating?

If no: Thank you for your time, can you tell me why you are no longer interested in participating?

If yes: Before enrolling people in this study, we need to determine if you may be eligible to participate. I would now like to ask you a series of questions about your health and physical activity. It will take approximately 20 minutes of your time. There is a possibility that some of these questions may make you uncomfortable or distressed; if so, please let me know. You can skip questions you do not wish to answer. I will keep all the information I receive from you by phone, including your name and any other identifying information confidential. The purpose of these questions is to determine whether you may be eligible to participate in the study. Additional screening at a later time may be necessary beyond answering these questions. Remember, your participation is voluntary; you do not have to complete these questions. Please feel free to stop me at any time if you have any questions or concerns. Do I have your permission to ask you these questions?

If yes: Begin phone screening questionnaire

Phone Screening Consent Date ____/____/_____ Staff Initials _____

If no: Thank you for your time, can you tell me why you are no longer interested in participating?

Continue on next page

1. Demographics
   Sex
   M / F
   Height _____
   Weight _____ (Exclude if >300lbs)

2. Physical Activity
   a. How many days per week do you engage in physical activity? _____
   b. How many minutes do you spend engaged in physical activity on these days? _____
      Total minutes of exercise per week _____
      (Total of days per week x minutes/day less than 150 minutes Exclude)

3. Females only
   a. Are you pregnant?
      ☐ No
      ☐ Yes (If Yes Exclude)
   b. Do you have an IUD?
      ☐ No

☐ Yes (if yes, please list below which one) (3T compatible IUDs are: Mirena and Liletta)

4. Experimental controls

a. Are you willing and able to avoid consuming caffeine (e.g. coffee, energy drinks, certain soft drinks) within 6hr of every test visit over the course of 34 days?

☐ No (If No Exclude)

☐ Yes

b. Do you have previous experience with mental imagery training?

☐ No

☐ Yes (If Yes Exclude)

5. MRI/TMS Safety Screening

a. Have you had a prior surgery or operation?

☐ No

☐ Yes (if yes, please list below)

_____

_____

b. Do you have non-removable electronic, mechanical or magnetic implants (e.g., metal screws, etc.) anywhere in your body?

☐ No

☐ Yes (if yes, please list below) (If yes Exclude)

_____

_____

c. Have you been injured by a metallic object or foreign body (e.g., BB, bullet, shrapnel, etc.?

☐ No

☐ Yes (if yes, please list below) (If yes review with PI prior to enrollment)

_____

_____

d. Do you have any intravascular stents, filters, aneurism clips or shunts?

☐ No

☐ Yes (if yes, please list below) (If yes Exclude)

_____

_____

e. Do you have a cardiac pacemaker?

☐ No

☐ Yes (If yes Exclude)

f. Do you have a cochlear implant?

☐ No

☐ Yes (If yes Exclude)

g. Do you have any non-removable body piercings?

☐ No

☐ Yes (If yes Exclude)

h. Do you have any large tattoos?

☐ No

☐ Yes (if yes, please list locations below) (If yes review with PI)

_____

_____

i. Have you ever done any welding, grinding, or cutting of metal in your lifetime?

☐ No

☐ Yes (if yes, please describe how much, if you wore eye protection, and every had an injury while working with metal) (If yes review with PI)

_____

_____

_____

_____

j. Do you have a medical history of developing seizures?

☐ No

☐ Yes (Exclude if Yes)

k. Do you have a history of claustrophobia or discomfort with confined spaces?

☐ No

☐ Yes (Exclude if Yes)

l. Are you able to tolerate loud noises for sustained periods of time?

☐ No (explain) (If No review with PI)

☐ Yes

_____

m. Have you experienced any problem related to a previous MRI examination or MR procedure?

☐ No

☐ Yes (if yes, please describe below) (If yes review with PI)

n. Do you currently have an ear infection or any ear pain?

☐ No

☐ Yes

If yes, wait for enrollment until condition is resolved.

o. Do you currently wear hair extensions, a weave or wig?

☐ No

☐ Yes

If yes, are your extensions/weave/wig held in place with metallic clips, threads, or another metallic item that cannot be removed for the experiment?)

☐ Can be removed

☐ Cannot be removed (If cannot be removed Exclude)

_____

a. Do you currently have braces?

☐ No

☐ Yes (please indicate below) (If yes exclude)

_____

b. Do you have a permanent retainer?

☐ No

☐ Yes (If yes exclude)

_____

c. Do you wear glasses?

☐ No

☐ Yes

If YES: Is your corrected vision normal (20/20)?

☐ No (If No Exclude)

☐ Yes

d. Do you wear contacts?

☐ No

☐ Yes

e. Are you able to hold still for over an hour?

☐ No (If no Exclude)

☐ Yes

f. Do you currently have a cold or allergies that result in sneezing or coughing?

☐ No

☐ Yes

If yes, wait for enrollment until condition is resolved.

5. Other medical history

a. Do you have a history of epilepsy, seizure or sleep disorders?

☐ No

☐ Yes (If Yes Exclude)

b. Do you have a history of any other major disorder (e.g, cardiovascular or neurological disorder)?

☐ No

☐ Yes (If Yes Exclude)

c. Do you currently have any skeletomotor injuries or physical limitations?

☐ No

☐ Yes (If Yes Exclude)

6. Age and contact information

How old are you (years)? _____

Best way to contact you:

- Phone _____
- Email _____

If inclusion criteria are met: Based on your answers, it appears you may be eligible to participate in this study. Would you like to schedule a time to come to the NMRL to complete the screening/enrollment process?
Date Scheduled ____/_____/_____ Time ___:____
If you have any questions or concerns, please feel free to contact me. My name is [name] and I can be reached at [phone number] and/or [email address].

If PI review is needed: Based on your answers it appears you may be eligible to participate in this study. However, our PI will need to review some of your answers prior to continuing. We'll call you back in 24-48 hours to let you know if you are eligible.
If you have any questions or concerns, please feel free to contact me. My name is [name] and I can be reached at [phone number] and/or [email address].

If any exclusion criteria are met: Based on your answers, it appears that you are not eligible to participate in this study at this time. Could we contact you again if we have other study opportunities?

☐ No ☐ Yes

If you have any questions or concerns, please feel free to contact me. My name is [name] and I can be reached at [phone number] and/or [email address].

For Staff Completion:

Date of phone screening: ____/_____/_____

Screening Result: ☐ Eligible Enrollment Scheduled

☐ Eligible-Declined participation

Reason for declining _____

☐ Excluded

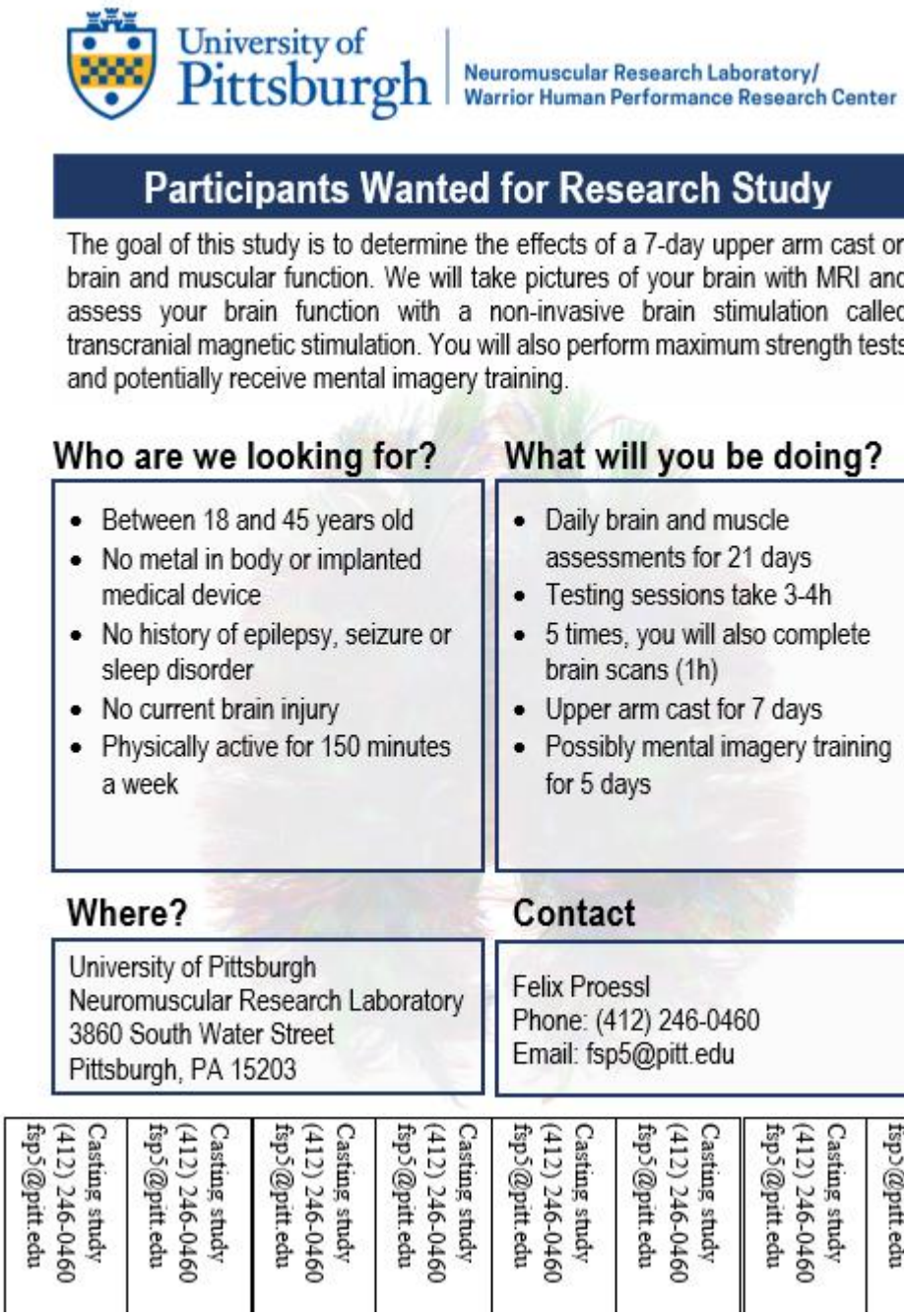
Reason for exclusion _____

Staff completing phone screening:

Print Name _____

Signature _____

Appendix E Recruitment Flyer



Appendix F Questionnaires

Edinburgh Handedness Inventory

Please mark the box that best describes which hand you use for the activity in question

	Always Left	Usually Left	No Preference	Always Right	Usually Right
Writing					
Throwing					
Scissors					
Toothbrush					
Knife (without fork)					
Spoon					
Match (when striking)					
Computer mouse					

Waterloo Footedness Questionnaire

Answer each of the following question as best you can. Please do not simply check one box for all questions, but imagine yourself performing each activity in turn, and then mark the appropriate answer. If necessary, stop and simulate the activity.

	Always Left	Usually Left	Equal	Always Right	Usually Right
1. Which foot would you use to kick a stationary ball at a target straight in front of you?					
2. If you had to stand on one foot, which foot would it be?					
3. Which foot would you use to smooth sand at the beach?					
4. If you had to step up onto a chair, which foot would you place on the chair first?					
5. Which foot would you use to stomp on a fast-moving bug?					
6. If you were to balance on one foot on a railway track, which foot would you use?					
7. If you wanted to pick up a marble with your toes, which foot would you use?					
8. If you had to hop on one foot, which foot would you use?					
9. Which foot would you use to help push a shovel into the ground?					
10. During relaxed standing, people usually put most of their weight on one foot, leaving the other leg slightly bent. Which foot do you put most of your weight on first?					
11. Is there any reason (i.e. injury) why you have changed your foot preference for any of the above activities?	YES	NO	(circle one)		
12. Have you ever been given special training or encouragement to use a particular foot for certain activities?	YES	NO	(circle one)		

13. If you answered YES for question 11 or 12, please explain:

Subject Number: _____ Date: _____

Shortened Profile of Mood States

Below is a list of words that describe feelings people have. Please read each one carefully; then mark ONE circle under the answer to the right which best describes HOW YOU FEEL RIGHT NOW.

The numbers refer to these phrases:

0 = Not at all
1 = A little
2 = Moderately
3 = Quite a bit
4 = Extremely

	0	1	2	3	4
Tense					
Angry					
Worn Out					
Unhappy					
Lively					
Confused					
Peeved					
Sad					
Active					
On edge					
Grouchy					
Blue					
Energetic					
Hopeless					
Uneasy					
Restless					
Unable to Concentrate					
Fatigued					
Annoyed					

	0	1	2	3	4
Discouraged					
Resentful					
Nervous					
Miserable					
Cheerful					
Bitter					
Exhausted					
Anxious					
Helpless					
Weary					
Bewildered					
Furious					
Full of Pep					
Worthless					
Forgetful					
Vigorous					
Uncertain about things					
Bushed					

Subject_____ Date_____ Visit_____ Time Point _____

Muscle Pain/Soreness Data Sheet

Draw a vertical line corresponding to the pain/soreness that you have as a result of the exercise protocol.

No pain/ soreness

Pain/soreness as bad as it could be

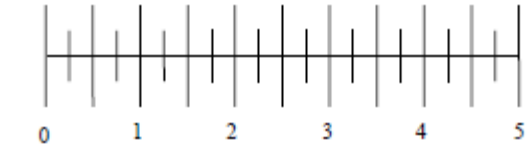
Screening Questionnaire for TMS Candidates

Please answer each question by checking the yes or no box. Provide any additional details where requested.

Question	Yes	No	Details
1. Have you ever received TMS?	☐	☐	If yes, were there any problems?
2. Do you have a cochlear implant?	☐	☐	
3. Do you have a neurostimulator implanted in your body?	☐	☐	If yes, of what type?
4. Do you have an implanted device for drug delivery?	☐	☐	
5. Do you have a cardiac pacemaker?	☐	☐	
6. Do you have any metal particles in the brain or skull (such as shrapnel, surgical clips, or fragments from metal work)?	☐	☐	If yes, what type?
7. Have you ever had a seizure?	☐	☐	If yes, can you describe the occasion?
8. Does anyone in your family have a history of epilepsy?	☐	☐	
9. Have you ever had syncope?	☐	☐	If yes, can you describe the occasion?
10. Do you have hearing problems?	☐	☐	If yes, what type?
11. Do you suffer from frequent or severe headaches?	☐	☐	
12. Have you ever had a stroke?	☐	☐	
13. Have you undergone MRI examination in the past?	☐	☐	If yes, were there any problems?
14. Have you ever had a severe head injury, where you lost consciousness?	☐	☐	
15. Are you pregnant or could you be pregnant?	☐	☐	
16. Do you take medications?	☐	☐	If yes, please list them

Subject Number: _____ **Date:** _____

Sleep Quality



No sleep Best sleep

1- Very light sleep
2- Poor sleep quality
3- Moderate quality sleep
4- Very good sleep quality

Hours of sleep last night: _____

Where did you sleep? _____

What Kind of Surface did you sleep on?

CIRCLE:

BED

SOFA

OUTDOORS

CARPET

HARDWOOD

WATERBED

Appendix G Funding

This work was supported by a National Strength and Conditioning Association Doctoral Research Grant, a Freddie Fu Grant and a SHRS Doctoral Student Grant (all FP).

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