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Effects of sensorimotor deprivation on human cortical excitability and motor learning

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Effects of sensorimotor deprivation on human cortical excitability and motor learning

By

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B.S., The University of North Carolina at Chapel Hill, 2015

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Abstract

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Synaptic strengthening, specifically long-term potentiation- (LTP)-like plasticity in primary motor cortex (M1) underlies the ability to learn complex motor skills. Understanding how to enhance the capacity for LTP-like plasticity in M1 is necessary to improve recovery for individuals with motor deficits, such as after stroke. While long-term disuse of a limb has been shown to have maladaptive structural and functional consequences, short-term immobilization of the arm has shown promise as a low-cost method to enhance the capacity for LTP-like plasticity in M1. However, the specific synaptic changes that underlie this enhanced capacity for plasticity after immobilization, as well as its behavioral effects, remain poorly understood. This dissertation investigates the changes in cortical excitability that occur after short-term immobilization and the effect of immobilization on acquisition and consolidation of a novel sequence-specific motor skill. We replicated previous findings by demonstrating that immobilization decreased corticospinal excitability (CSE) in, and increased interhemispheric inhibition (IHI) onto, the hemisphere associated with the immobilized limb, but a significant change in intracortical inhibition was not observed at the group level. However, our results revealed that decreased CSE was significantly correlated with decreased $GABA_A$-ergic intracortical inhibition only in the immobilization group. Previous research has demonstrated that decreases in $GABA_A$-ergic inhibition are necessary for induction of LTP-like plasticity in M1; therefore, decreased intracortical inhibition after short-term arm immobilization may provide a novel mechanism to enhance the capacity for LTP-like plasticity within M1. Additionally, we examined the effect of short-term immobilization on two stages of motor learning, skill acquisition and consolidation, using a modified version of the Serial Reaction Time Task (SRTT). While task performance improved during training across participants, skill improvement was not sequence-specific, and there was not a significant effect of immobilization on acquisition or consolidation of SRTT skill. However, we hypothesize that immobilization is more likely to influence performance and learning on a task that requires proprioceptive feedback or multi-joint coordination. Taken together, our results suggest that while short-term immobilization of the arm modulates neurophysiological markers of plasticity in M1, it does not influence performance or learning on a motor task that involves individuated, sequenced finger movements.

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Dedication

This dissertation is dedicated to my grandma, Carol Friedlander, who uprooted her life and gave up her retirement years to help raise me and make me the person I am today.

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Chapter 1: General Introduction

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Edwards L*, **King E***, Buetefisch C, Borich M; "Putting the 'Sensory' into Sensorimotor Control: The Role of Sensorimotor Integration in Goal-Directed Hand Movements after Stroke" *Frontiers in Integrative Neuroscience,* 2019. doi: 10.3389/fnint.2019.00016

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1.1 Abstract

Integration of sensory and motor information is one step, among others, that underlies the successful production of goal-directed hand movements necessary for interacting with our environment. Disruption of sensorimotor integration is prevalent in many neurologic disorders, including stroke. In most stroke survivors, persistent paresis of the hand reduces function and overall quality of life. Current rehabilitative methods are based on neuroplastic principles to promote motor learning that focus on regaining motor function lost due to paresis, but the sensory contributions to motor control and learning are often overlooked and currently understudied. There is a need to evaluate and understand the contribution of both sensory and motor function in the rehabilitation of skilled hand movements after stroke. Here, we will highlight the importance of integration of sensory and motor information to produce skilled hand movements in healthy individuals and individuals after stroke. We will then discuss how compromised sensorimotor integration influences relearning of skilled hand movements after stroke. Finally, we will propose an approach to target sensorimotor integration through manipulation of sensory input and motor output that may have therapeutic implications.

1.2 Introduction

Goal-directed movements of the hand are required to perform most tasks of daily living, such as tying a shoe, buttoning a shirt, and typing, among others. These highly coordinated voluntary movements involve interacting with and manipulating objects in the environment and rely on sensorimotor integration. Sensorimotor integration is the ability to incorporate sensory inputs that provide information about one's body and the external environment to inform and shape motor output (Wolpert, Goodbody et al. 1998). More specifically, sensory inputs for goal-directed hand movements provide information in an egocentric reference frame detailing location, size, weight, and shape of an object. In addition, kinematic information about the hand and upper extremity, including the trajectory needed to interact with the object, is provided. Successful integration of information contributes to generating the most efficient motor plan to execute a given task. Additionally, ongoing sensory feedback during motor performance refines the motor plan to optimize current and future performance. This process of sensorimotor integration is often disrupted in neurological disorders, such as stroke.

Stroke is defined as infarction of central nervous system tissue attributable to ischemia, based on neuropathological, neuroimaging, and/or clinical evidence of permanent injury (Sacco, Kasner et al. 2013). Stroke is the fourth leading cause of death and remains the number one leading cause of long-term adult disability (Benjamin, Blaha et al. 2017). Furthermore, the loss of productivity after stroke currently costs the United States an average of $33.9 billion per year and is expected to reach $56 billion by 2030 (Ovbiagele, Goldstein et al. 2013), making stroke a public health crisis. A primary contributor to persistent disability after stroke is incomplete motor recovery (Lai,

Studenski et al. 2002). Spontaneous biological recovery of motor function occurs during the first months after stroke (Cramer 2008), underlying a current emphasis on intensive early intervention, although results are often mixed and complex (Bernhardt, Godecke et al. 2017). Despite intensive therapy, upper extremity impairment resolves up to 70% of baseline function for a given patient with some patients showing even less recovery than predicted (Winters, van Wegen et al. 2015). Most stroke survivors are left with a limited ability to perform skilled hand movements necessary for daily functioning (Lee, Lim et al. 2015). To reduce disability after stroke, there is a need to improve our understanding of the neuronal network physiology necessary to regain skilled functional hand use.

In the following brief review, we will highlight the importance of processing and integrating sensory and motor information that underlies skill performance and learning with an emphasis on skilled hand movements in stroke. We will focus primarily on three cortical regions: primary motor cortex (M1), posterior parietal cortex (PPC) and primary somatosensory cortex (S1) while briefly mentioning other cortical and subcortical brain areas also involved in sensorimotor integration. These brain regions are highlighted due to our focus on the integration of sensory and motor information at the level of the cortex, but also because these cortical areas receive blood supply from the middle cerebral artery (MCA), which is the most common type of stroke (Walcott, Miller et al. 2014). It should be noted that strokes occur in other brain regions but usually have less of an impact on sensorimotor integration underlying goal-directed, skilled hand movements and are outside the primary scope of this review.

In the first section of this review, we will discuss the role of sensorimotor integration via M1, PPC, and S1 in normal, skilled hand movements. We will then discuss how

sensorimotor integration is affected by stroke and how impaired sensorimotor integration can impact relearning of skilled hand movements. Lastly, we propose an approach to target sensorimotor integration by manipulating sensory input and restricting motor output that may have therapeutic implications for stroke recovery.

1.3 The Role of M1 in Goal-Directed Hand Movements

1.3.1 M1 Involvement in Movement Execution

The M1 has a critical role in the execution of voluntary movements. Upper extremity movement execution is particularly dependent on descending output from M1 through the spinal cord to upper limb muscles. Pyramidal neurons in layer 5 have axons that are bundled together as a significant portion of the CST, where 85-90% of the fibers decussate in the pyramids to provide control to the hand contralateral to the hemisphere of the M1 (Rosenzweig, Brock et al. 2009). The remaining fibers, approximately 10-15%, maintain ipsilateral projections that have a minor role in distal extremity motor control (Zaaimi, Edgley et al. 2012). Of the neurons terminating in the spinal cord, some neurons will indirectly influence movements by synapsing onto interneurons in the intermediate zone (Rathelot and Strick 2009) whereas direct control arises from the cortico-motoneuronal (CM) cells that terminate monosynaptically on α-motoneurons in the ventral horn of the spinal cord (Lemon, Mantel et al. 1986). These α-motoneurons innervate skeletal muscle to control contralateral muscle contractions, and subsequently, voluntary movements (Rathelot and Strick 2009, Schieber 2011). The most abundant projections from M1 are to motor neurons that innervate hand muscles allowing for direct and individualized control of fingers required for complex and skilled

hand movements (Dum and Strick 1996). A lesion to these CST axonal fibers is the leading cause of motor disability and specifically causes loss in individualized finger function (Lawrence and Kuypers 1968, Lemon 2008), reiterating the importance of this connection from M1 to the α-motoneurons innervating muscles of the hand. While CST is the largest contributor to skilled hand movement, there are other pathways, such as the reticulospinal tract, that offer additional contributions to certain aspects of hand function (see (Baker 2011) for review). The topographical organization of M1 demonstrates a large spatial representation for the hand compared to other segments of the limb, reflecting the relative importance of the output from CM cells to the hand (Penfield and Boldrey 1937). The populations of CM cells in M1 fire differentially to allow for a variety of functional uses of the hand (Griffin, Hoffman et al. 2015). Within these populations, individual neurons can be tuned to preferentially code for single or multiple fingers or more proximal joints (Kirsch, Rivlis et al. 2014), and the kinematics of a movement, such as direction, force, and speed are also encoded (Georgopoulos, Kalaska et al. 1982, Georgopoulos, Ashe et al. 1992, Mahan and Georgopoulos 2013). This level of specification in M1 neuronal tuning allows for the execution of an extensive repertoire of complex hand movements.

As mentioned previously, the execution of skilled hand movements by M1 requires sensory information. Representations of the external environment must be generated from visual, proprioceptive, and tactile input (Makino, Hwang et al. 2016), and these representations are combined with internal representations of the motor system, such as hand position, to create an internal model (Blakemore, Goodbody et al. 1998). Both external and internal representations have inherent variability that can be reduced by incorporating input from multiple sensory modalities (Kording and Wolpert 2004).

Successful multisensory integration contributes to execution of a motor command that results in the desired movement outcome. For instance, if the goal is to button a shirt, the internal model should include the position of the button and buttonhole and starting position of the hand. These positions are determined by visual, proprioceptive, and tactile information that will be processed through PPC (visual (Kaas, Gharbawie et al. 2011)) and S1 (proprioceptive, tactile (Kim, Gomez-Ramirez et al. 2015), and nociceptive (Liang, Mouraux et al. 2011)). Sensory information associated with manipulation of the button will also be provided. The relevant sensory information is then relayed to M1, where a motor command is generated. This internal model will also be influenced by prior motor execution that contributes to development of an efference copy of the motor output (von Holst and Mittelstaedt 1950). Using this information, an internal model includes predictions about expected sensory feedback resulting from the generated movement (Flanagan, Bowman et al. 2006). In this example, if the button is not at the correct angle required for it to go through the button hole, or if the hand is in the incorrect starting position, the sensory reafferent information occurring in response to movement will not align with the predicted feedback generated from the efference copy (von Holst and Mittelstaedt 1950). Therefore, the predicted sensory consequence will be updated, the model adapted, and subsequently, the error will be corrected by adjusting the motor command (Shadmehr, Smith et al. 2010).

There are several brain regions involved in sensorimotor integration for goal-directed hand movements (**Figure 1-1**). Non-cortical structures contributing to sensorimotor integration include the: basal ganglia (Nagy, Eordegh et al. 2006), cerebellum (Proville, Spolidoro et al. 2014), and thalamus (Mo and Sherman 2019). In rodents and primates, it has been shown that distinct subdivisions of the thalamus

receive input from the basal ganglia and cerebellar nuclei and project to M1 (Bosch-Bouju, Hyland et al. 2013, Bopp, Holler-Rickauer et al. 2017). The ventroanterior and ventromedial nuclei receive information from the basal ganglia, typically through GABAergic projections. The ventrolateral nucleus receives glutamatergic projections from cerebellar nuclei. In addition to these motor thalamic regions, there has been evidence from rodent models to suggest that sensory thalamic regions, such as the posterior medial nucleus, project directly to M1 (Ohno, Kuramoto et al. 2012, Hooks, Mao et al. 2013, Hooks, Lin et al. 2015). However, it is unclear whether these specific regions are present in humans and non-human primates.

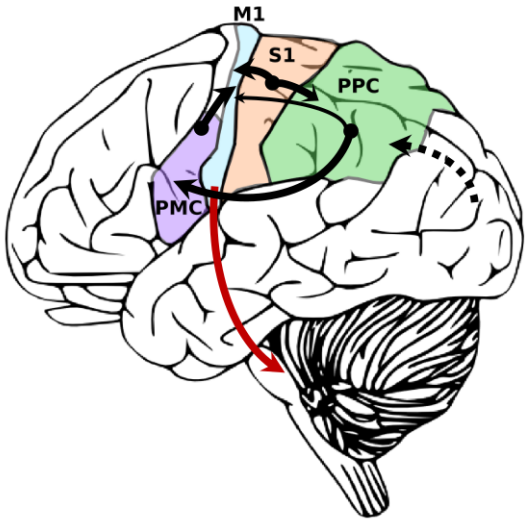

Figure 1-1. Simplified diagram demonstrating primary sensory inputs to primary motor cortex. Cortico-cortical connections are black. Cortico-fugal projections from M1 are red. Width of arrow denotes strength of connection. Dotted line denotes primary visual input from visual cortex into PPC for multimodal integration.

The ability to transform visual and proprioceptive information about location and space of the internal and external world is important to inform motor commands

(Burnod, Grandguillaume et al. 1992). M1 neurons fire in response to both visual and proprioceptive stimuli (see for review (Hatsopoulos and Suminski 2011)). The M1 hand area is separated into caudal (M1c) and rostral (M1r) subregions: CM cells primarily arise from M1c and provide direct control of movements of the hand and distal forearm, whereas neurons in M1r influence motor control indirectly using interneurons in the spinal cord (Rathelot and Strick 2009). Recent work suggests that this rostral and caudal subdivision of the M1 hand area also exists in humans and maintains differences in function (Vigano, Fornia et al. 2019). S1 has strong reciprocal connections with M1c, whereas PPC has comparatively weaker connections to M1r (Stepniewska, Preuss et al. 1993). Lesions made independently to M1c and M1r in adult squirrel monkeys produced different deficits, where M1c lesions resulted in cutaneous sensory deficits, and M1r lesions produced errors in aiming of the hand (Friel, Barbay et al. 2005). These results are not only consistent with the sensory inputs that are expected to arise from PPC and S1 but show the importance of sensorimotor integration such that different regions of M1 specialize in integrating the unique sensory information provided by PPC and S1. Furthermore, proprioceptive and visual inputs to input to M1 will be weighted differently depending on the goal of the task (Sober and Sabes 2003) further attesting to the dynamic nature of sensorimotor integration in M1.

1.3.2 M1 Plasticity and Sensorimotor Learning

In addition to the role of M1 in the production of movement, M1 also undergoes substantial plasticity, which has a critical role for learning skilled movements. Here we define 'motor learning' as an improvement in motor skill beyond baseline performance leading to a reduction in performance error that is retained over time (Shmuelof,

Krakauer et al. 2012). Given that an error signal is inherently tied to sensory feedback and therefore needed for learning of motor skills guided by sensory information (see (Seidler, Kwak et al. 2013) for review), we refer to motor learning as sensorimotor learning. Sensorimotor learning has been shown to induce functional and structural changes in M1 in rodents (Kleim, Barbay et al. 1998) and non-human primates (Nudo, Milliken et al. 1996). In rodents, compared to practicing an unskilled lever-pressing task, practicing a skilled task that required specific paw manipulations to retrieve food pellets resulted in larger changes in M1 motor map representation of the forelimb, demonstrating that sensorimotor learning induces M1 plasticity (Kleim, Barbay et al. 1998). M1 plasticity is defined as lasting changes in the morphological and/or functional properties of M1 (Sanes and Donoghue 2000); experience-dependent plasticity is when these changes occur in response to life experiences, such as stroke (Kleim and Jones 2008). In the rodent M1, plasticity underlying sensorimotor learning occurs through mechanisms of synaptic long-term potentiation (LTP) and long-term depression (LTD) (Rioult-Pedotti, Friedman et al. 2000). Similar to these results from rodent studies, the involvement of LTP-like mechanisms has been also demonstrated in plastic changes of M1 when adult humans practice ballistic thumb movements (Butefisch, Davis et al. 2000). Importantly, in non-human primates, changes in M1 motor map representation of the distal forelimb were specific to skilled motor learning, whereas performing repetitive unskilled movements alone was not sufficient to induce changes in motor representations (Plautz, Milliken et al. 2000). Additionally, disrupting M1 activity in humans with transcranial magnetic stimulation (TMS) immediately after motor practice can disrupt memory consolidation for that skill (Muellbacher, Ziemann et al. 2002, Robertson 2004) resulting in reduced learning, indicating the importance of M1 in the

early consolidation of motor learning. The role of M1 plasticity in sensorimotor learning has also been demonstrated in the orofacial representations in humans (Arima, Yanagi et al. 2011) and nonhuman primates (Arce-McShane, Hatsopoulos et al. 2014).

LTP in M1 is considered a primary synaptic process involved in the experience-dependent plasticity that underlies sensorimotor learning (Kleim, Barbay et al. 1998, Butefisch, Davis et al. 2000, Sanes and Donoghue 2000, Ziemann, Ilic et al. 2004, Nudo 2013). At the synaptic level, a bidirectional range of dynamic modifiability exists, such that a synapse experiences a limited amount of synaptic strengthening (LTP) or reduction in strength (LTD) (Rioult-Pedotti, Friedman et al. 2000). The ability of a synapse to maintain a target range of modifiability to prevent over- or under-excitation of the neural circuit is referred to as homeostatic metaplasticity (Whitt, Petrus et al. 2014). Evidence of synaptic metaplasticity suggests that prior history of synaptic plasticity influences the degree of future synaptic modification (Abraham and Bear 1996). For instance, a synapse that is close to the upper limit of synaptic modifiability would not experience the same degree of LTP induction as a synapse farther away from its upper limit (**Figure 1-2**). Previous electrophysiological evidence from in vitro studies suggests that inducing LTD at a synapse, bringing it farther from its upper limit of modifiability, enhances the capacity for subsequent LTP induction (Rioult-Pedotti, Friedman et al. 2000). This same principle has been demonstrated at the systems level (Ziemann, Ilic et al. 2004). It was shown that sensorimotor learning reduced the capacity for subsequent LTP but enhanced the capacity for LTD in human M1. Additionally, the degree to which further LTP is blocked has been correlated with the magnitude of motor memory retention after sensorimotor learning (Cantarero, Lloyd et

al. 2013, Cantarero, Tang et al. 2013). Taken together, these results highlight the importance of experience-dependent plasticity in sensorimotor learning.

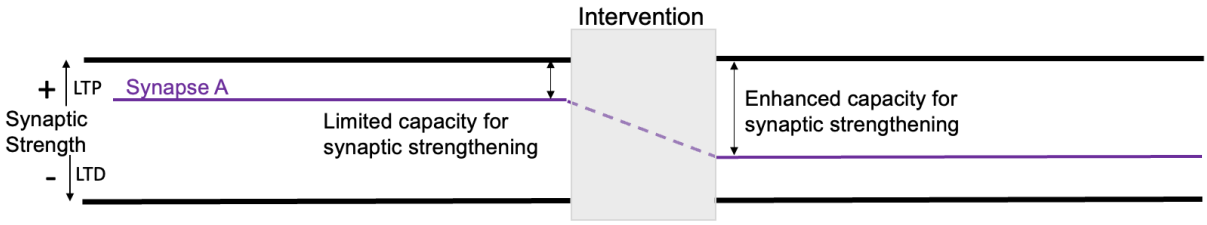

Figure 1-2. Homeostatic range of synaptic modifiability. In the illustration, *Synapse A* begins closer to its upper limit of modifiability (top black line) and has limited capacity for synaptic strengthening. An intervention that reduces synaptic strength (dotted line), bringing it further from its upper limit of modifiability, has the potential to increase the capacity for synaptic strengthening.

LTP is largely mediated by glutamate, the primary excitatory neurotransmitter in the brain, and its interaction with the N-methyl-D-aspartate (NMDA) receptor throughout the cortex (Luscher and Malenka 2012). Functional inactivation of the NMDA receptor in M1 abolished the capacity for LTP induction in vivo, suggesting that these glutamatergic receptors are necessary for LTP to occur (Hasan, Hernandez-Gonzalez et al. 2013). In addition to glutamatergic synapse contributions to experience-dependent plasticity, gamma-aminobuytric acid (GABA) synaptic modifiability is another important contributor to plasticity. GABA is the main inhibitory neurotransmitter in the brain (Blicher, Near et al. 2015), and transient reductions in GABAergic inhibition have been shown to be necessary for LTP induction in M1 (Hess, Aizenman et al. 1996, Blicher, Near et al. 2015, Kida, Tsuda et al. 2016).

In subsequent sections, we will review the importance of sensory inputs in shaping experience-dependent plasticity underlying sensorimotor learning under normal conditions and after stroke.

1.4 The Role of Sensory Regions in Goal-Directed Hand Movements

1.4.1 Posterior Parietal Cortex (PPC) as a Sensorimotor Integration Hub

The PPC is comprised of Brodmann Area (BA) 5, 7, 39 and 40 in the human brain and is anatomically connected to motor areas M1 and premotor cortex (PMC) via the superior longitudinal fasciculus (SLF) (Makris, Kennedy et al. 2005, Koch, Cercignani et al. 2010). Although the PPC is not traditionally considered a primary part of the cortical motor network, it is involved in motor execution with populations of neurons that are motor dominant, in addition to populations that are visual dominant, or a combination of the two (Sakata, Taira et al. 1995). Non-human primate studies have demonstrated dense reciprocal PPC-M1 connections between the rostral strip of PPC and the medial lateral portion of M1 (Fang, Stepniewska et al. 2005). Furthermore, regions of the PPC have distinct and direct pathways and networks with prefrontal motor cortical regions organized in functional zones (Gharbawie, Stepniewska et al. 2011), which demonstrates the level of specific information the PPC can provide to the motor network. While PPC has been speculated to primarily influence M1 through polysynaptic connections with the PMC (Chao, Karabanov et al. 2015), support has been shown for monosynaptic projections from PPC to M1 (Karabanov, Jin et al. 2012). Additionally, in non-human primates, it has been shown that PPC has disynaptic connections with hand motoneurons in the dorsal horn and intermediate zone of the spinal cord (Rathelot, Dum et al. 2017), further suggesting potential contributions of PPC to the control of hand movements via the motor and sensory information PPC provides.

The PPC is a multisensory association area functioning to integrate different sensory modalities from visual, somatosensory, prefrontal and auditory inputs (Whitlock 2017). The PPC has abundant reciprocal connections with sensory areas and is functionally parcellated such that the rostral portion of PPC is connected to somatosensory and motor regions, and the caudal portion of PPC has connections with visual and auditory regions (Stepniewska, Cerkevich et al. 2009). The necessary inputs to PPC for sensorimotor processing needed for skilled hand movements include direct reciprocal inputs from the dorsomedial visual area that allows for continuous visual motion analysis necessary for interacting with the environment (Beck and Kaas 1998, Kaskan and Kaas 2007, Rosa, Palmer et al. 2009) (see (Kaas, Gharbawie et al. 2011) for review). Sensory inputs to BA 5 primarily come from somatosensory area S2 and the parietal ventral area, along with weaker inputs from S1 (Stepniewska, Cerkevich et al. 2009). All three regions provide pertinent sensory information to PPC about proprioceptive and tactile activity of hand movements (Cohen, Prud'homme et al. 1994, Prud'homme and Kalaska 1994) that are important for sensorimotor integration used in hand exploration and object discrimination (Hinkley, Krubitzer et al. 2007). Inputs to BA 5 are important as BA 5 is responsible for visuomotor transformations (Kalaska 1996), making the PPC-M1 connection important for visuomotor control and visual spatial processing (Binkofski, Dohle et al. 1998, Mutha, Sainburg et al. 2011). PPC combines sensory signals about visual and kinematic reference frames into complex sensorimotor representations that are relayed to M1 to optimize motor commands (Sabes 2011). PPC neurons are not only involved in control and error correction of a movement once initiated, but are important for movement planning to achieve a motor goal (Mulliken, Musallam et al. 2008, Aflalo, Kellis et al. 2015), as neuronal firing also encodes

movement intention (Snyder, Batista et al. 1997). Lesions in the rostral portion of PPC result in difficulty with shaping the fingers prior to grasping an object (Binkofski, Dohle et al. 1998), further demonstrating an important role for PPC during the sensorimotor integration required for successfully performing goal-directed hand movements.

1.4.2 Primary Somatosensory Cortex Involvement in Sensorimotor Integration

In the human brain, S1 is comprised of BA 3a, 3b, 1, and 2 and receives direct somatosensory input from thalamus (Kaneko, Caria et al. 1994). Somatosensory information is relayed from the periphery to the thalamus from the medial lemniscus (Boivie 1978) via the spinothalamic tract (Boivie 1979). Additionally, the posterior medial nucleus of the thalamus connects to inhibitory neurons in layer 1 (L1) of S1 that synapse onto the apical dendrites of neurons from other cortical layers (Castejon, Barros-Zulaica et al. 2016). Peripheral sensory information that is task-irrelevant can be filtered out through inhibition of afferent pathways via a process known as sensory gating (Eguibar, Quevedo et al. 1994). The thalamic relay nuclei are important for sensory gating, and lesions to the thalamus cause sensory gating impairments (Staines, Black et al. 2002). This ascending sensory information can be modulated or gated by corticofugal descending projections from S1 to the dorsal column nuclei (Jabbur and Towe 1961, Martinez-Lorenzana, Machin et al. 2001). Both S1 and M1 demonstrate somatotopic organization with representation of body regions localized to specific cortical cell columns (Kuehn, Dinse et al. 2017). Furthermore, while M1 was previously thought to be agranular, it is now known that M1 shares the same structure as other primary cortical areas (Barbas and Garcia-Cabezas 2015). The L4 in M1 is not cytoarchitecturally distinguishable, but electrophysiological studies have demonstrated

it has traditional input/output proprieties: L4 receives excitatory input from the thalamus, has excitatory unidirectional outputs to L2/3, and weaker long-range corticortical connections (Yamawaki, Borges et al. 2014). However, there are distinct differences in that M1 has approximately half the amount of synapses that are exclusively excitatory whereas in S1, there are more synapses formed with both excitatory and inhibitory neurons (Bopp, Holler-Rickauer et al. 2017). It is proposed that M1 likely receives its feedforward inhibition through thalamacortical projections to L1 instead of L4 (Kuramoto, Furuta et al. 2009, Bopp, Holler-Rickauer et al. 2017). In addition to connections from the thalamus, S1 also has direct projections to M1 that are important for the integration of somatosensory and motor information (Cash, Isayama et al. 2015). In rodents, reciprocal projections connect the sensory representation in S1 to the corresponding motor representation in M1, creating a glutamatergic M1-S1 loop that connects L2/3 and 5a in S1 with L2/3 and 5a in M1 (**Figure 1-3**) (Mao, Kusefoglu et al. 2011, Hooks, Mao et al. 2013). S1 relays somatosensory information through monosynaptic and polysynaptic connections to M1 (Kaneko, Caria et al. 1994), and ongoing sensory input is used to refine and update descending motor commands (Rosenkranz and Rothwell 2012). L2/3 neurons in M1 are able to directly excite pyramidal output neurons within the same cortical area (Kaneko, Caria et al. 1994). At the network level, S1 activity has both excitatory (Rocco-Donovan, Ramos et al. 2011) and inhibitory (Borich, Brodie et al. 2015) effects on M1 at the network level. However, only excitatory projections from S1 to M1 have been characterized at the synaptic level (Papale and Hooks 2018). The connectivity of inhibitory interneurons within M1 and how they are affected by sensory input have not been well studied. These S1-M1

connections provide an infrastructure for highly complex information integration that has the potential to be shaped and targeted for sensorimotor control and learning.

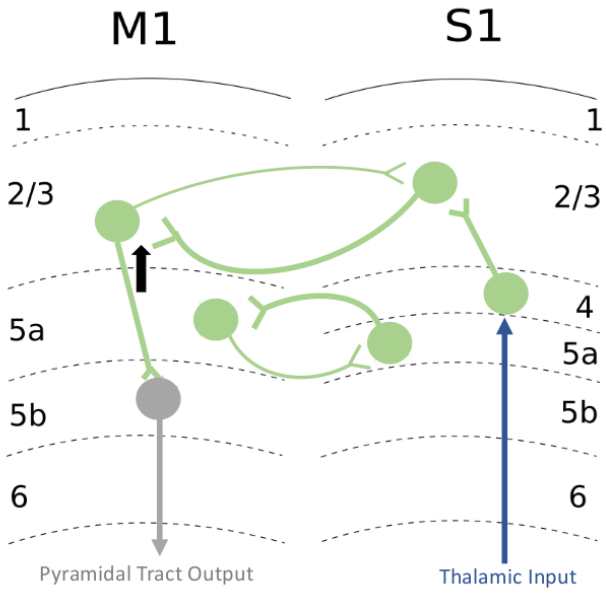

Figure 1-3. Excitatory M1-S1 connections. Sensory input from thalamus is relayed to layer 4 (L4) then to L2/3 of S1. S1 sends glutamatergic projections onto excitatory neurons in L2/3 of M1, and these synapses are sites of LTP and LTD plasticity of connections involved in sensorimotor integration (denoted with a black arrow). Reciprocal connections of the M1-S1 loop are also shown. Pyramidal neurons from L2/3 of M1 project to output neurons in L5b of M1. Afferent inputs are shown in blue, intracortical connections are in green, and efferent outputs are shown in gray. Circles denote populations of neurons. Additional inputs and outputs are not shown. Refer to text for additional detail regarding M1-S1 connections.

The ability of S1 to influence synaptic plasticity in M1 depends on sensorimotor synapses in L2/3 of M1. Synapses between S1 and M1 undergo plasticity that is driven by sensory input and results in the alteration of motor output (Kaneko, Caria et al. 1994, Kaneko, Caria et al. 1994). These synapses are a main site of LTP and LTD in M1 (Kaneko, Caria et al. 1994) and send excitatory projections to the pyramidal output neurons of M1 (Kaneko, Caria et al. 1994, Huber, Gutnisky et al. 2012). These

connections allow for sensory feedback to shape motor output both in the short-term (immediate to minutes) and long-term (hours or longer). The ability for sensory input to influence motor output is specific to the connections between primary sensorimotor areas. Tetanic stimulation of S1, but not the ventrolateral nucleus of the thalamus, has been shown to produce LTP in L2/3 synapses of M1 (Iriki, Pavlides et al. 1989, Kaneko, Caria et al. 1994). Tetanic stimulation of sensory thalamus only resulted in LTP in thalamocortical synapses with concurrent stimulation of S1 (Kaneko, Caria et al. 1994). The S1-M1 connection has also been implicated in sensorimotor learning in vivo and are thought to be a main site of synaptic modifiability in response to motor skill learning (Papale and Hooks 2018). These direct projections have been hypothesized to be a site of integration of sensory input and motor output and have an important role in guiding motor activity in response to sensory input (Hasan, Hernandez-Gonzalez et al. 2013). One study in non-human primates demonstrated that ablation of S1 impaired the acquisition of motor skill but did not impair performance of the particular motor skill that had been learned previously, possibly due to intact thalamo-cortical connections that had been strengthened during skill training (Pavlides, Miyashita et al. 1993). Additionally, temporary inhibition of S1 in rodents has been shown to impair the ability to adapt motor performance based on changes in sensory input; however, basic motor patterns and motor commands that had learned previously were not affected (Mathis, Mathis et al. 2017). Therefore, there is evidence to suggest that S1 is important for the ability to learn skilled movement and adjust motor plans to sensory input but may be less important for performance of overlearned or stereotyped movements in the upper limb. It should be noted, however, that ablation of other areas of S1, such as the face area, can lead to deficits in basic motor function, and previously learned motor tasks

(Lin, Murray et al. 1993, Hiraba, Yamaguchi et al. 2000, Yao, Yamamura et al. 2002). In addition to connections between S1 and the ipsilateral M1, interhemispheric inhibitory connections between S1s exist in humans (Coleman, Ragert et al. 2011) and have been shown to influence plasticity in M1. For example, Conde, Vollmann et al. (2013) demonstrated that LTP-like plasticity in M1 induced by paired TMS and peripheral stimulation of the contralateral upper extremity switched to LTD-like plasticity when peripheral stimulation was applied to the upper limb ipsilateral to the TMS. These results demonstrate that the cortical sensorimotor circuitry that contributes to plasticity is not limited to one hemisphere, and interhemispheric network connectivity likely influences sensorimotor learning. However, the specific involvement of S1 in motor performance will depend on the characteristics of the task including the importance of sensory information for skilled performance.

1.5 Impact of Stroke on Sensorimotor Integration and Learning

1.5.1 Sensorimotor Deficits after Stroke

The impact of stroke on sensorimotor integration depends on the location of the stroke. Because the MCA supplies both the motor and sensory regions and is the most common type of stroke (Walcott, Miller et al. 2014), stroke in this vascular territory has a great likelihood of affecting sensorimotor integration. Therefore, our discussion is primarily focused on MCA strokes affecting the sensorimotor cortex although strokes in other vascular territories may also impact sensorimotor integration (Staines, Black et al. 2002). There are dynamic processes post-stroke that change as a function of time and affect the neurophysiology of sensorimotor integration. Time post-stroke is defined in

phases: hyper-acute (0-24 hours); acute (1-7 days); early subacute (7 days-3months); late subacute (3-6 months); and chronic (>6 months) (Bernhardt, Hayward et al. 2017). Initial neuronal cell death in the lesion core leads to both structural and functional disconnection with brain regions outside the primary area of infarct (Carrera and Tononi 2014). Motor recovery occurs in part from spontaneous biological repair (SBR) that transitions from a state of cell death and inflammation to a state of increased neuronal excitability and experience-dependent plasticity lasting ~3 months post-stroke (Cramer 2008). Most recovery post-stroke occurs rapidly in the early sub-acute phase and the magnitude of improvement slows down in the late sub-acute phase (Lee, Lim et al. 2015). In the chronic phase post-stroke, patients have reached a stable, though modifiable plateau in motor recovery (Jorgensen, Nakayama et al. 1995) with less than 20% of patients experiencing full recovery of upper extremity motor function (Kwakkel, Kollen et al. 2003).

Upper extremity paresis is the most predominant motor impairment after MCA stroke, which results from a lesion involving the CST that is also necessary for skilled hand movements (Lang and Schieber 2003). Paresis can contribute to deficits in both the initiation and termination of voluntary movement of the wrist (Chae, Yang et al. 2002). Other motor deficits include spasticity and impaired motor control (Raghavan 2015), with 85% of patients in the chronic phase post-stroke still possessing residual motor deficits (Lee, Lim et al. 2015).

Common somatosensory modalities affected after stroke are tactile sensation, proprioception, and stereognosis (Connell, Lincoln et al. 2008). It has been recently reported that 62% of acute stroke patients demonstrated deficits in their ability to locate their hand and arm in space (Findlater, Desai et al. 2016). Deficits in proprioception

have direct implications as information about the arm and hand are necessary for proper movement and important for improving sensorimotor function after stroke (Aman, Elangovan et al. 2014). Due to the reliance of the motor system on sensory information for movement optimization, sensory impairment is expected to have motor repercussions. Similarly, sensory deficits can occur even when there are ischemic lesions specifically in the M1 motor pathway and not in somatosensory afferents (Nudo, Friel et al. 2000), suggesting that sensory integration can be disrupted even in the absence of a lesion present in sensory afferent pathways.

1.5.2 Plasticity and Sensorimotor Learning after Stroke

Many cellular and synaptic processes contribute to plasticity after stroke. In the acute phase after stroke, LTP is facilitated in the perilesional areas, suggesting an amplification of network plasticity that influences cortical reorganization (Hagemann, Redecker et al. 1998). Neuroplasticity is enhanced through processes such as axonal sprouting and GABA receptor downregulation (Carmichael 2016). Additionally, functional recovery is most rapid during this early time period, occurring in the first three months for humans and roughly one month for rodents (Caleo 2015). Plasticity subsequently plateaus in the chronic stage of recovery (Hendricks, van Limbeek et al. 2002, Hara 2015). Rehabilitative interventions have been shown to be most effective when initiated early after stroke and become less effective with increasing time post-stroke (Biernaskie, Chernenko et al. 2004). Despite the plateau in neuroplasticity during the chronic phase of recovery, it is currently unclear whether this level differs from that of matched healthy controls. In a study by Zeiler, Hubbard et al. (2016) in a rodent model of chronic stroke, the induction of a second stroke enhanced plasticity and

response to skilled motor training, indicating that it is possible to reopen this window of enhanced plasticity during the later stages of recovery. Increasing the capacity for neuroplasticity during the chronic stage of recovery has the potential to enhance recovery of function for stroke survivors with persistent motor-related disability.

As mentioned previously, GABAergic activity is strongly related to synaptic plasticity in healthy individuals. In rodent models of cerebral ischemia, GABAergic inhibition has been shown to be elevated within minutes (Globus, Busto et al. 1991), a potentially neuroprotective mechanism to counteract excitotoxicity caused by excess glutamate release (Pellegrini-Giampietro 2003). GABA levels return to baseline within an hour of reoxygenation (Schwartz-Bloom and Sah 2001). Reductions in GABAergic inhibition continue during the acute phase after stroke, and this process has been related to functional motor recovery in mice (Clarkson, Huang et al. 2010). It has been suggested that this reduction in GABAergic activity serves to facilitate neuroplasticity in M1 through unmasking of existing, inactive synaptic connections (Paik and Yang 2014), the development of new connections (Murphy and Corbett 2009), or the induction of LTP (Hess, Aizenman et al. 1996, Sanes and Donoghue 2000). While GABAergic activity has been shown to be an important contributor to plasticity after stroke, other mechanisms, such as brain derived neurotrophic factor (BDNF) and neuromodulin signaling, have been implicated as well. For in-depth reviews of cellular and synaptic mechanisms of plasticity after stroke, see Murphy and Corbett (2009) and Alia, Spalletti et al. (2017). Given that similar mechanisms are thought to underlie neuroplasticity and functional recovery after stroke (Kleim and Jones 2008), therapeutic strategies that optimally promote neuroplasticity hold promise for improving the rate and magnitude of functional recovery after stroke.

As discussed earlier, motor skill learning has been shown to induce structural and functional changes in M1 that underpin sensorimotor learning in rodents, (Kleim, Barbay et al. 1998), non-human primates (Nudo 2013), and healthy humans (Butefisch, Davis et al. 2000, Sanes and Donoghue 2000, Ziemann, Ilic et al. 2004). It has also been shown that motor skill learning underlies recovery of function after stroke in humans (Krakauer 2006) and non-human primates (Nudo, Wise et al. 1996). One mechanism underlying recovery is the preservation or expansion of the M1 representation of the affected hand. Skilled motor training after stroke in non-human primates prevented the reduction of the affected distal upper extremity representation in M1 that occurred after an equivalent period of no training (Nudo, Wise et al. 1996). In some cases, the hand representation expanded into representations for adjacent body parts after training, and this reorganization of M1 corresponded to better recovery of skilled hand function. It has also been shown that S1 activity contributes to sensorimotor learning and recovery after stroke in humans and non-human primates. Nudo, Friel et al. (2000) demonstrated that impairments in sensory inputs to M1 after stroke in non-human primates contributed to motor deficits in a task that required skilled hand movements. In humans, continuous theta burst stimulation (cTBS), a TMS paradigm that can decrease excitability of the stimulated area, delivered over contralesional S1 in order to reduce transcallosal inhibition on ipsilesional S1 was shown to enhance motor recovery after stroke (Meehan, Dao et al. 2011). Another study by Brodie et al. demonstrated that excitatory rTMS to the ipsilesional S1 paired with motor skill training increased sensorimotor learning compared to stimulation or skill training in isolation (Brodie, Meehan et al. 2014). Therefore, attempting to enhance S1

excitability and/or sensorimotor integration may offer an effective approach to improve sensorimotor learning and functional recovery after stroke.

1.6 Strategies to Modulate Sensorimotor Integration and Potential Therapeutic Effects after Stroke

1.6.1 Current Therapeutic Interventions

Sensorimotor integration occurs across the neuraxis and therefore provides multiple potential targets for therapeutic intervention. Several experimental procedures have been developed to modulate afferent input to M1, and therefore sensorimotor integration, in humans. Peripheral vibration is a neuromodulation approach that increases afferent input that is thought to modulate M1 excitability by regulating activity of cortical inhibitory interneurons that are involved in motor output (Rosenkranz and Rothwell 2006). This increase in afferent input is thought to change the response of M1 to sensory input and therefore influence sensorimotor integration in the cortex. Both focal (Celletti, Sinibaldi et al. 2017) and whole-body vibration (Boo, Moon et al. 2016) have shown promise in improving upper extremity function in individuals with stroke. Additionally, whole-body vibration has shown promise for improving lower extremity and walking function in individuals with spinal cord injury (Ness and Field-Fote 2009). However, across studies, the effectiveness of vibration to improve post-stroke motor function remains unclear (Liao, Huang et al. 2014, Park, Park et al. 2018).

In contrast to increasing afferent input to M1, models of temporary deafferentation have shown promise in targeting sensorimotor integration by reducing sensory input to modulate motor output. In rodents, transection of the facial nerve leads to a rapid

expansion of the adjacent forelimb representation in M1, likely due to rapid removal of GABAergic inhibition (Sanes, Suner et al. 1988, Huntley 1997). This concept has been applied non-invasively in humans by temporarily reducing afferent input from a portion of the upper extremity to M1 with the goal of reducing GABAergic inhibition to adjacent areas of the limb. It is thought that rapid unmasking of horizontal connections leads to an expansion of the cortical representation. Targeting this mechanism, several temporary deafferentation strategies have been studied in humans with the goal of increasing M1 representation of the affected limb to improve functional outcomes after stroke. Ischemic nerve block (INB) of the arm is one method that serves as a model of transient segmental deafferentation in humans. Using a pneumatic tourniquet at the elbow, afferent sensory inputs from the distal forearm to the sensorimotor cortex are restricted, leading to an increase in excitability of cortical representations of muscles immediately proximal to the deafferented forearm (Brasil-Neto, Valls-Sole et al. 1993). However, this form of INB may be less applicable for individuals with stroke, as a main goal of stroke rehabilitation is to improve hand function, and it appears that INB affects more proximal parts of the arm (Lang, Bland et al. 2013). A different approach that has been shown to increase motor function after stroke is the application of anesthesia to areas proximal to the hand, such as the brachial plexus (Muellbacher, Richards et al. 2002) or forearm (Sens, Teschner et al. 2012, Sens, Knorr et al. 2013), simulating deafferentation of the upper or lower arm, respectively. After applying anesthesia to the brachial plexus of the affected arm, Muellbacher, Richards et al. (2002) demonstrated an improvement in motor skill after training in individuals with chronic stroke compared to training without anesthesia. Additionally, there was an increase in motor output in response to TMS application with no change in motor threshold, suggesting a

rapid cortical reorganization and reduction in inhibition. Application of anesthetic cream to the forearm, another region proximal to the hand, improved somatosensory and motor function distal to the site of application in individuals with chronic stroke (Sens, Knorr et al. 2013). Blood flow restriction (BFR) is another technique that uses a pneumatic cuff applied to the arm to reduce blood flow to a target level that is maintained during exercise (Yasuda, Fukumura et al. 2014). Brandner, Warmington et al. (2015) showed that BFR during resistance exercise increases corticomotor excitability, and this effect is thought to be mediated by the reduction in cortical afferent input. A primary concern for the use of INB and BFR in a rehabilitation setting is that the use of a tourniquet or arm cuff poses a risk for individuals with sensory impairments and/or cardiovascular irregularities, such as individuals with stroke (Spranger, Krishnan et al. 2015). Therefore, individuals with stroke may benefit from a method of temporary deafferentation with fewer potential risks.

1.6.2 Future Directions for Therapeutic Interventions

Short-term immobilization of the arm is a safe, low-cost approach for the transient modulation of sensorimotor cortical function in healthy individuals (Huber, Ghilardi et al. 2006, Avanzino, Bassolino et al. 2011, Rosenkranz, Seibel et al. 2014). In humans and animals, prolonged immobilization or disuse of a limb can occur after neurological insult that induces maladaptive plasticity, such as reduction in cortical representations of the limb (Pons, Garraghty et al. 1991, Langer, Hanggi et al. 2012, Milliken, Plautz et al. 2013, Viaro, Budri et al. 2014), which can contribute to “learned nonuse” and a compensatory reliance on the unaffected limb (Wolf 2007). While learned nonuse and its effects on cortical organization have been examined, short-term immobilization has

been less well-studied. Short-term arm immobilization (typically 8 hours) reduces sensory input to, and motor output from, the contralateral sensorimotor cortex resulting in transiently decreased M1 and S1 cortical excitability following immobilization in healthy individuals (Huber, Ghilardi et al. 2006, Rosenkranz, Seibel et al. 2014). This decrease in excitability is thought to be driven by LTD-like processes (Huber, Ghilardi et al. 2006). Allen, Celikel et al. (2003) demonstrated that whisker deprivation in rodents induced LTD-like effects in sensorimotor areas that occluded further LTD induction but enhanced LTP induction in slice preparations, consistent with the model of homeostatic metaplasticity (**Figure 1-2**). Short-term immobilization of the arm has been proposed as a strategy to induce LTD-like plasticity and enhance the capacity for LTP induction in the human motor cortex. Indeed, a single short bout (8hrs) of immobilization temporarily reduced TMS-based measures of cortical excitability; however, the capacity for synaptic strengthening was significantly enhanced (Rosenkranz, Seibel et al. 2014). Currently, the behavioral effects of this enhanced synaptic strengthening are poorly understood.

Given that short-term immobilization modulates excitability of S1 and M1, it is likely that immobilization impacts the integration of sensory and motor information that underlies experience-dependent plasticity. Therefore, short-term immobilization could potentially modulate neural processes underlying sensorimotor learning. However, the effects of immobilization on sensorimotor learning have not been well studied in humans. To our knowledge, only one study has examined sensorimotor learning after short-term arm immobilization (Opie, Evans et al. 2016) and did not show a clear effect of immobilization on sensorimotor learning. The lack of effect could be due, in part, to the high number of individuals with the BDNF Val66Met polymorphism that is

associated with reduced use-dependent plasticity in sensorimotor areas (Kleim, Chan et al. 2006). Given the relationship between neural plasticity and sensorimotor learning, further examination of the effect of short-term arm immobilization on sensorimotor learning is warranted. Short-term arm immobilization could show promise as a rehabilitative intervention to increase post-stroke sensorimotor recovery by enhancing the capacity for neuroplasticity leading to better training-related increases in motor function. More broadly, given its demonstrated role in motor control, promotion of sensorimotor integration plasticity has potential as a therapeutic strategy post-stroke.

1.7 Gap in Knowledge

Previous research has determined that short-term arm immobilization has the potential to enhance the capacity for LTP-like plasticity in M1 (Rosenkranz, Seibel et al. 2014). This enhanced capacity for plasticity in M1 has been shown to be related to a decrease in corticospinal excitability (Rosenkranz, Seibel et al. 2014), which is thought to be reflective of a decrease in synaptic strength (Huber, Ghilardi et al. 2006). Additionally, an increase in interhemispheric inhibition (IHI) onto the immobilized hemisphere has been demonstrated after short-term immobilization (Avanzino, Bassolino et al. 2011, Avanzino, Pelosin et al. 2014). However, it is unclear whether reduced corticospinal excitability and increased IHI are associated with changes in intracortical inhibition, which has been shown to be important for regulating neuroplasticity in human M1 (Floyer-Lea, Wylezinska et al. 2006, Bachtiar and Stagg 2014, Kim, Stephenson et al. 2014). It is possible that an increase in intracortical inhibition underlies the increase in IHI and contributes to the decrease in corticospinal excitability seen after short-term immobilization. Alternatively, immobilization may

lead to global downscaling of synaptic strength within the contralateral M1, which would be reflected in a decrease in both corticospinal excitability as well as intracortical inhibition. Currently, no studies have assessed intracortical and interhemispheric inhibition and their relation to decreased corticospinal excitability. Understanding the effect of experimental manipulations of neural activity in humans, such as immobilization, on inhibitory circuit activity is important for identifying mechanisms for enhancing experience-dependent plasticity that underpins sensorimotor learning.

Given that plasticity within human M1 underlies sensorimotor skill learning (Butefisch, Davis et al. 2000, Sanes and Donoghue 2000, Ziemann, Ilic et al. 2004), an intervention that has the potential to enhance the capacity for LTP-like plasticity in M1 may influence skill learning. Although short-term arm immobilization has been demonstrated to induce cortical plasticity in humans (Huber, Ghilardi et al. 2006, Rosenkranz, Seibel et al. 2014), the effects on learning are poorly understood. Initial learning of complex motor skill involves two steps: acquisition and consolidation. The acquisition of complex motor skill involves task improvement that occurs through repetitive practice (Krakauer and Mazzoni 2011). After the period of practice ends, the memory for the acquired skill can be maintained and strengthened through the process of consolidation (Karni, Meyer et al. 1998, Censor, Sagi et al. 2012). Two different behavioral phenotypes can result from consolidation of skill: stabilization and enhancement (Robertson, Pascual-Leone et al. 2004, Walker 2005, Robertson 2012). Stabilization involves maintenance and reduction of fragility of the memory, while enhancement leads to performance improvements in the absence of additional practice. Neuroplastic mechanisms within M1 have been implicated in both acquisition (Butefisch, Davis et al. 2000) and consolidation (Krakauer and Shadmehr 2006) of

motor skill. It is thought that different neural processes underlie skill acquisition and consolidation as a means to balance the flexibility required to acquire new skills in a variable environment with the stability required to retain those skills with reduced cognitive demands (Clark and Ivry 2010). Currently, no studies have examined the effect of short-term immobilization on these steps of skill learning independently. It is important to do so in order to determine whether short-term immobilization may benefit skill learning and also to determine the proper time to apply immobilization relative to training. Overall, this dissertation aims to address the gap in knowledge regarding the neurophysiological and behavioral effects of short-term arm immobilization. By understanding these effects, we can more comprehensively understand the potential of short-term arm immobilization to benefit sensorimotor learning in healthy individuals as well as the potential clinical translations to populations with motor deficits, such as after a stroke.

1.8 Dissertation Overview

Chapter 1 has introduced the relationship between neural plasticity and sensorimotor learning, as well as methods of sensorimotor deprivation that have been used to enhance the capacity for neural plasticity and subsequent motor learning. Short-term immobilization is a non-invasive method that has been shown to lead to greater capacity for LTP-like plasticity in M1. However, the specific circuit changes that underlie this enhanced capacity for plasticity, as well as its behavioral effects, remain poorly understood (**Figure 1-4**).

While decreased corticospinal excitability has been consistently observed after immobilization, it is unclear to what extent changes in corticospinal excitability are

associated with, and possibly caused by, changes in intracortical and interhemispheric inhibition. **Chapter 2** investigates whether changes in excitability of inhibitory circuits within and between M1s may explain changes in corticospinal excitability after immobilization. In order to investigate the effects of short-term immobilization on motor learning, **Chapter 3** assesses the effect of short-term arm immobilization on the acquisition of motor skill, while the effect of immobilization on consolidation of motor skill is examined in **Chapter 4**. **Chapter 5** includes a summary discussion of the findings from these studies in the context of current literature as well as suggestions for future studies based on our results.

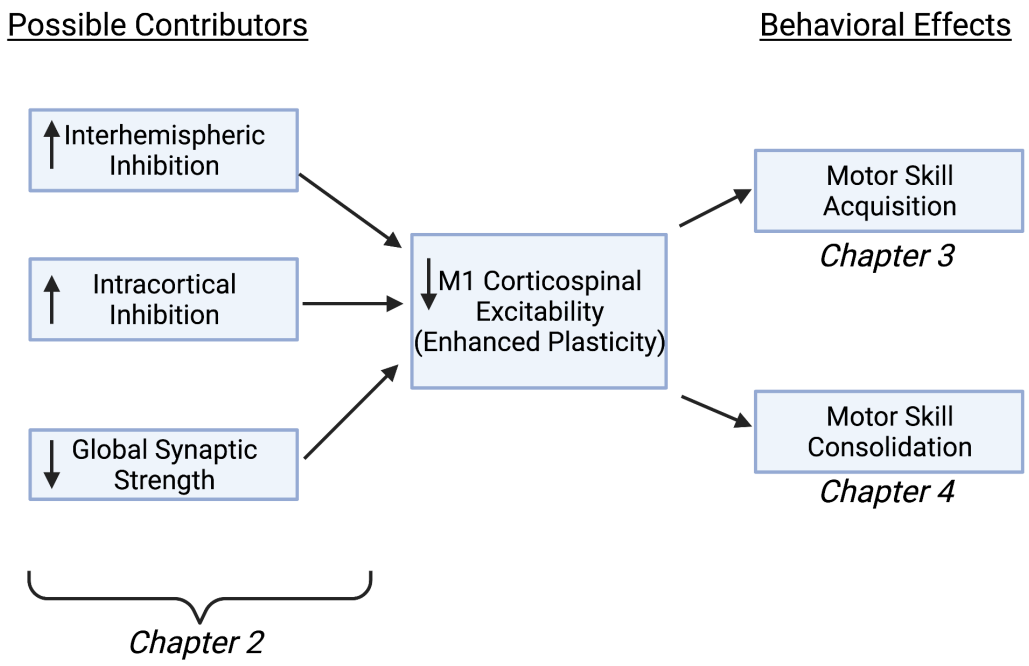

Figure 1-4. Dissertation overview. Chapter 2 examines the possible neurophysiological contributors to enhanced M1 plasticity seen after short-term immobilization. Chapters 3 and 4 investigate the effects of short-term immobilization on motor skill acquisition and consolidation, respectively. Figure created with BioRender.com.

Chapter 2: Short-term arm immobilization modulates excitability of inhibitory circuits within, and between, primary motor cortices

This chapter is reproduced with minor edits from:

King, E; Edwards, L; Borich, M. "*Short-term arm immobilization modulates excitability of inhibitory circuits within, and between, primary motor cortices"* (*in preparation*).

2.1 Abstract

Previous research has suggested that short-term immobilization of the arm may be a low-cost, non-invasive strategy to enhance the capacity for long-term potentiation (LTP)-like plasticity in primary motor cortex (M1). Short-term immobilization reduces corticospinal excitability (CSE) in the contralateral M1, and interhemispheric inhibition (IHI) from ipsi- onto contralateral M1 is increased. However, it is unclear whether reduced CSE and increased IHI are associated with changes in intracortical inhibition, which has been shown to be important for regulating neuroplasticity in M1. The current study used transcranial magnetic stimulation (TMS) to evaluate the effects of short-term (6 hours) arm immobilization on CSE, IHI, and intracortical inhibition measured bilaterally in 43 neurotypical young adults. We replicated previous findings demonstrating that immobilization decreased CSE in, and increased IHI onto, the immobilized hemisphere, but a significant change in intracortical inhibition was not observed at the group level. Across individuals, decreased CSE was associated with a decreased $GABA_A$-ergic intracortical inhibition within the immobilized hemisphere only in the immobilization group. Previous research has demonstrated that decreases in $GABA_A$-ergic inhibition are necessary for the induction of LTP-like plasticity in M1; therefore, decreased intracortical inhibition after short-term arm immobilization may provide a novel mechanism to enhance the capacity for LTP-like plasticity within M1 and may be a potential target for strategies to augment plasticity capacity to enhance motor learning in health and disease.

2.2 Introduction

Experience-dependent plasticity is a fundamental property of the brain and refers to the ability to undergo structural and functional change in response to experiences (Fu and Zuo 2011). In sensorimotor networks, experience-dependent plasticity allows organisms to adjust their behavior in order to successfully interact with their environment over time. Mechanisms of neuroplasticity have been shown to underpin experience-dependent learning of new motor skills in rodents (Rioult-Pedotti, Friedman et al. 2000) and humans (Butefisch, Davis et al. 2000). Additionally, recovery of motor function after neural insult, such as stroke, is influenced by experience-dependent plasticity (Allred, Kim et al. 2014). Given the fundamental importance of experience-dependent plasticity for brain function and learning, understanding mechanisms of plasticity and developing strategies to enhance these mechanisms is important. Synaptic strengthening through long-term potentiation (LTP) is a main contributor to experience-dependent plasticity in human motor cortex (Butefisch, Davis et al. 2000). However, synaptic strength must be maintained within a target range to prevent over- or under-excitation of the circuit (Abbott and Nelson 2000). This model of homeostatic metaplasticity suggests that the recent history of synaptic strength influences the degree to which further synaptic strengthening can occur (Bienenstock, Cooper et al. 1982, Park, Jung et al. 2014, Whitt, Petrus et al. 2014). This concept has been demonstrated in rodent primary motor cortex (M1), where in order to maintain neural activity within a physiologically stable dynamic range, the ability to induce LTP or long-term depression (LTD) was adjusted by the prior history of synaptic activity associated with motor skill training (Rioult-Pedotti, Friedman et al. 2000). Additionally, similar homeostatic mechanisms have been

demonstrated in human M1 using noninvasive brain stimulation (Ziemann, Ilic et al. 2004, Muller, Orekhov et al. 2007, Karabanov, Ziemann et al. 2015).

A noninvasive approach to experimentally induce experience-dependent plasticity in M1 is short-term limb immobilization. Short bouts (<12 hours) of arm immobilization reduce input to, and output from, the contralateral sensorimotor cortex, resulting in a transient decrease in corticospinal excitability (CSE) in M1 as measured with transcranial magnetic stimulation (TMS) (Facchini, Romani et al. 2002, Crews and Kamen 2006, Huber, Ghilardi et al. 2006, Clark, Taylor et al. 2010, Opie, Evans et al. 2016, Karita, Matsuura et al. 2017). Reduced M1 CSE is thought to reflect a decrease in synaptic strength through LTD-like mechanisms (Huber, Ghilardi et al. 2006). Subsequently, the capacity for synaptic strengthening in M1 is enhanced immediately after immobilization in healthy humans, and the degree of enhanced capacity for plasticity is correlated with the degree of decrease in CSE (Rosenkranz, Seibel et al. 2014). CSE is influenced by multiple circuits, made up of several intracortical and interhemispheric inputs onto pyramidal output neurons. Currently, changes in inhibitory circuitry that may underlie the increase in markers of plasticity within M1 after immobilization have not been fully characterized in humans. Previous research has demonstrated that excitability in both excitatory and inhibitory circuits in M1 can be modulated in a homeostatic manner (Murakami, Muller-Dahlhaus et al. 2012); however, it is unclear whether intracortical inhibition contributes to the possible metaplastic effects of short-term immobilization.

When a limb is immobilized, cortical excitability can be modulated bilaterally and is likely mediated to an extent by direct transcallosal projections between homologous cortical regions. Transcallosally-mediated interhemispheric inhibition

(IHI) is generated by excitatory transcallosal projections that synapse onto inhibitory interneurons in the opposite hemisphere (Ferbert, Priori et al. 1992, Chen 2004) (**Figure 2-1**). Avanzino, Bassolino et al. (2011) demonstrated that IHI from the non-immobilized M1 onto the immobilized M1 significantly increased after short-term arm immobilization and IHI from the immobilized M1 onto the non-immobilized M1 was reduced. Since the IHI circuit is, at least, di-synaptic, consisting of both excitatory and inhibitory components, it remains unclear whether immobilization modulates activity of the excitatory transcallosal projections or the inhibitory interneurons receiving transcallosal input.

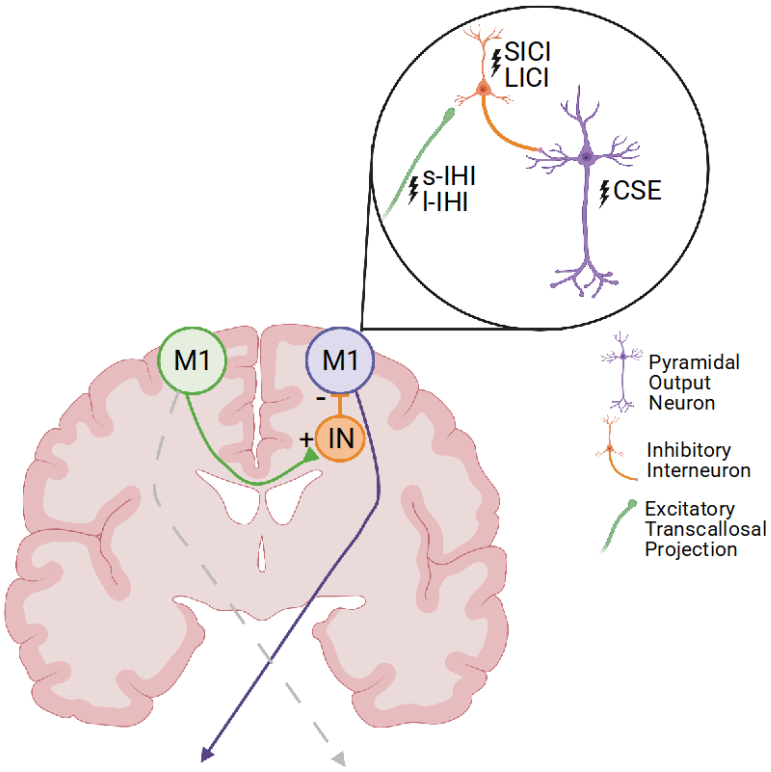

Figure 2-1. Cortical circuits of interest to evaluate the neuromodulatory effects of short-term arm immobilization. Activation of neurons in M1 (green) leads to inhibition of contralateral M1 (purple) through excitatory transcallosal projections synapsing onto inhibitory interneurons (orange). Transcranial magnetic

stimulation (TMS) can be used to probe different components of this circuit. Figure created with Biorender.com.

Several studies have measured intracortical inhibition after a period of immobilization, but results have been inconsistent between studies (Clark, Taylor et al. 2010, Rosenkranz, Seibel et al. 2014, Opie, Evans et al. 2016). Short-interval intracortical inhibition (SICI), an assessment reflective of $GABA_A$-ergic processes and indexed with paired pulse TMS (inter-pulse interval range: 1-5 ms), has been found to be decreased (Rosenkranz, Seibel et al. 2014) or unchanged (Clark, Taylor et al. 2010, Opie, Evans et al. 2016) after immobilization. Long-interval intracortical inhibition (LICI, inter-pulse interval range: 50-200 ms), reflective of $GABA_B$-ergic processes, has been shown to be increased after three weeks of immobilization when measured during muscle contraction (Clark, Taylor et al. 2010) but unchanged when measured at rest (Clark, Taylor et al. 2010, Opie, Evans et al. 2016).

Understanding the effect of experimental manipulations of neural activity in humans, such as immobilization, on inhibitory circuit activity is important for identifying mechanisms of enhanced plasticity. In particular, GABA-mediated neuroplasticity is a fundamental contributor to motor skill learning and can alter functioning of entire neural circuits (Butefisch, Davis et al. 2000, Kolasinski, Hinson et al. 2019, King, Rumpf et al. 2020). Modulation of GABAergic inhibition is essential for synaptic plasticity in primary sensorimotor cortex in humans (Floyer-Lea, Wylezinska et al. 2006, Kim, Stephenson et al. 2014) and rodents (Kida, Tsuda et al. 2016). However, it remains unclear how inter- and intra-cortical inhibitory circuit activity are independently and/or co-modulated by experience-dependent plasticity induction.

The purpose of the current study was to characterize the neuromodulatory effects of short-term (6 hours) arm immobilization on intra- and inter-cortical circuit excitability compared to an equivalent period without immobilization. We used a battery of TMS-based assessments to evaluate the neurophysiologic effects of immobilization on cortical excitability within and between M1s in neurotypical healthy adults randomly assigned to undergo immobilization or no immobilization. Due to the inherent interindividual variability in human neuromodulation studies (Muller-Dahlhaus, Orekhov et al. 2008, Schilberg, Schuhmann et al. 2017), we also examined associations between neurophysiologic outcomes across individuals.

2.3 Materials and Methods

2.3.1 Participants

Forty-five healthy participants (13 male) aged 18-35 years old (mean=24.5 ±4.7y) participated in this study. Inclusion criteria included (1) no history of movement impairment or neurodegenerative disease, (2) right handedness according to the Edinburgh Handedness Scale (Oldfield 1971), and (3) no contraindication to transcranial magnetic stimulation (TMS) (Rossi, Hallett et al. 2009) or magnetic resonance imaging (MRI). All study procedures were approved by the Emory University Institutional Review Board in accordance with the Declaration of Helsinki. According to the Edinburgh Handedness Scale, one participant had mixed handedness, and data from that participant was excluded from analysis. Additionally, one participant reported taking a nap in between TMS sessions. Due to the potential confounding effect of sleep

on neural plasticity (Tononi and Cirelli 2014), data from this participant were also excluded from analysis.

2.3.2 TMS Assessments of Intra- and Inter-cortical Excitability

Transcranial magnetic stimulation (TMS) was used to evaluate CSE, IHI, and intracortical inhibition in M1 bilaterally both before and after 6 hours of immobilization (**Figure 2-1**). The **target** hemisphere for all participants was the right M1 corresponding to the left, non-dominant arm that was immobilized in the Immobilization group. The **non-target** hemisphere was left M1; corresponding to the right, dominant arm that was not immobilized in either group. A standard magnetic resonance template brain was used in BrainSight software (Rogue Research Inc.) to localize stimulation over the motor representation associated with the first dorsal interosseous (FDI) muscle of the hand bilaterally. Guided real-time stereotactic navigation was used throughout the sessions to ensure consistent TMS targeting of the FDI representation in M1. Surface electromyography (EMG) was used to measure TMS-motor evoked potentials (MEPs) of the FDI muscles during all TMS assessments.

The resting motor threshold (RMT) was determined for the M1 in each hemisphere using the ML-PEST method (Awiszus 2003). CSE was measured by the average peak-to-peak amplitude of the MEP in response to 20 unpaired TMS pulses at 120% of RMT (SP120). Paired pulse (PP) paradigms involved a conditioning stimulus followed by a test stimulus at a given interstimulus interval (ISI). Conditioning and test pulses were either applied to the FDI representation of the same hemisphere (intrahemispheric) or FDI representations in opposite hemispheres (interhemispheric).

The average peak-to-peak amplitude of the conditioned MEP response across 20 trials was divided by the average MEP response during the SP120 condition (PP/SP120) to measure the degree of inhibition caused by the conditioning pulse. TMS assessments, summarized in **Table 2-1**, were performed in both the target and non-target hemispheres in a randomized order (**Figure 2-1**).

Table 2-1. Summary of TMS-based outcome measures to evaluate immobilization effects on cortical excitability

TMS Condition	Neural Process(es) Measured	Paired/Unpaired	Conditioning Pulse Intensity	Test Pulse Intensity	Interstimulus Interval (ISI)
Resting Motor Threshold (RMT)	Neuronal Membrane Excitability (Ziemann, Reis et al. 2015)	N/A	N/A	N/A	N/A
Corticospinal Excitability (CSE)	Corticospinal Excitability (Amassian, Stewart et al. 1987)	Unpaired	N/A	120% RMT	N/A
Short-Interval Intracortical Inhibition (SICI)	Synaptic $GABA_A$ (Ziemann, Reis et al. 2015)	Paired-intrahemispheric	80% RMT	120% RMT	2 ms
Long-Interval Intracortical Inhibition (LICI)	Synaptic $GABA_B$ (Werhahn, Kunesch et al. 1999, Ziemann, Reis et al. 2015)	Paired-intrahemispheric	120% RMT	120% RMT	60 ms
Short Interhemispheric Inhibition (s-IHI)	Interhemispheric activation of $GABA_A$ (Kawaguchi 1992)	Paired-interhemispheric	120% RMT	120% RMT	10 ms
Long Interhemispheric Inhibition (l-IHI)	Interhemispheric activation of $GABA_B$ (Irlbacher, Brocke et al. 2007)	Paired-interhemispheric	120% RMT	120% RMT	50 ms

2.3.3 Arm Immobilization

After completion of the morning testing session, 23 out of the 43 included participants were randomly assigned to undergo arm immobilization. The

other 20 participants were randomly assigned to be in the No Immobilization control group. The immobilized individuals were instructed to wear a finger control mitt on the left (non-dominant) hand that secured positioning of the fingers in a padded mitt to restrict finger movement and sensory input. In addition to the hand mitt, the arm was placed in a sling to reduce movement of the wrist, elbow, and shoulder joints. Participants were instructed to move their left arm as little as possible during the immobilization period but to use the non-immobilized arm as they would normally. The non-immobilized individuals did not wear a mitt or a sling and were told to use their arms normally throughout the day. Activity monitors (wGT3x-BT, ActiGraph) were worn on both wrists by all participants to measure bilateral arm movements between sessions and determine compliance with the immobilization procedure. A mixed-effects analysis was performed to determine the effects of arm (target or non-target) and group (control or immobilized) on activity counts.

2.3.4 MEP Analysis and Statistical Approach

A custom Matlab (2016a) script was used to automatically identify the minimum and maximum peak amplitudes of the TMS-evoked response in the FDI to calculate the peak-to-peak amplitude for all MEPs. Each MEP was also confirmed visually. Mean MEP amplitude for each TMS assessment was analyzed before and after immobilization, and between-group analyses were performed to examine changes in excitability between those who were immobilized compared to those who were not immobilized. Mixed-effects analyses, with within-subject factors of time (AM or PM) and hemisphere (target or nontarget) and between-subject factor of group (non-immobilized control or immobilization group), were performed to evaluate main effects

of immobilization and time on all TMS assessments. Follow-up mixed-effects analyses with within-subject factors of time and hemisphere were completed for control and immobilized groups separately in order to evaluate within-group effects of immobilization. To assess associations between immobilization effects on different outcome measures within individuals, we performed bivariate correlation analyses between change in CSE with changes in interhemspheric and intracortical inhibition, adjusted for multiple comparisons. Prism GraphPad 8 statistical packages were used for all statistical analyses. Significance level was set as $\alpha<0.05$.

2.4 Results

2.4.1 Activity Monitoring

A mixed-effects analysis showed significant effects of arm ($F=163.7$, $p<.0001$) and group ($F=26.8$, $p<.0001$), and there was a significant Group x Arm interaction ($F=110.9$, $p<.0001$) on wrist activity counts (**Figure 2-2a**). Sidak's multiple comparisons test demonstrated that the immobilized individuals used their target (immobilized) arm significantly less than their nontarget (non-immobilized) arm ($t=17.2$, $p<.0001$).

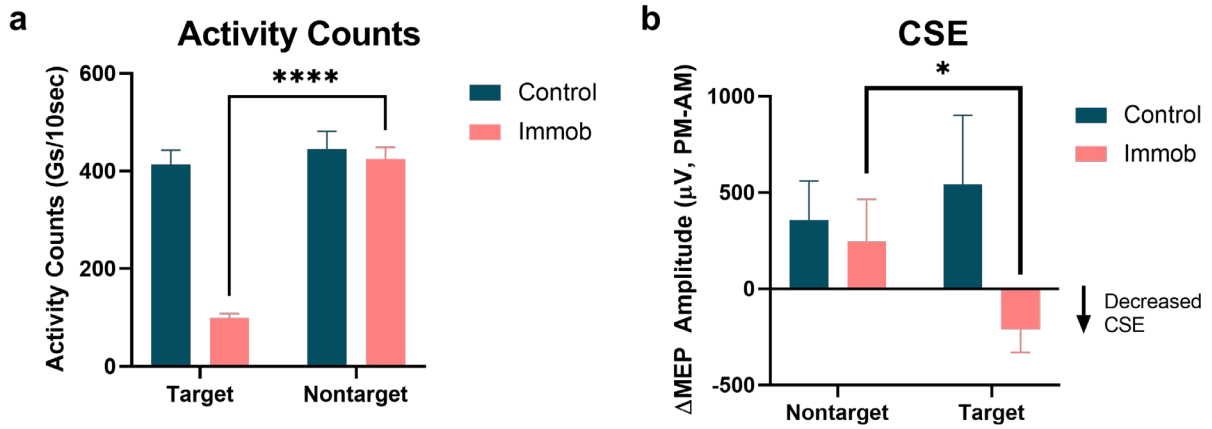

Figure 2-2. (a) Immobilized participants demonstrated significantly reduced activity in their immobilized (target) arm during the six-hour immobilization period. (****p<.0001). **(b)** Corticospinal excitability (CSE) was significantly reduced following immobilization (*p<.05). Error bars represent standard error.

2.4.2 Resting Motor Threshold (RMT)

A mixed-effects analysis showed a trend for an effect of hemisphere (F=3.9, p=.054) on RMT, where RMT was higher in the target hemisphere in both groups across testing sessions. Additionally, there was a trend for an effect of time (F=3.5, p=.07), where RMT increased from the AM to the PM session. There was no effect of group (F=1.3, p=.26) on RMT.

2.4.3 Corticospinal Excitability (CSE)

Mixed-effects analysis showed lower cortical excitability in the immobilized group (Group: F=4.4, p=.04). Additionally, there was a trend for CSE increase over the day (Time: F=3.6, p=.067), preferentially for the non-immobilized control group (Group x Time: F=3.5, p=.07) (**Figure 2-2b**). Mixed-effects analyses separated by group demonstrated that CSE significantly increased from AM to PM in the non-immobilized control group (Time: F=4.7, p=.037), and there was a significant effect of

immobilization on the change in CSE in the immobilized group (Group x Time: F=4.4, p=.04).

2.3.4 Interhemispheric Inhibition (s-IHI & l-IHI)

In both IHI conditions, control participants showed a bilateral release of interhemispheric inhibition over the course of the day, with less IHI observed at the PM session compared to the AM session. However, immobilization led to an increase in inhibition from the nontarget hemisphere onto the target hemisphere. Additionally, the decrease in inhibition from target to nontarget hemisphere observed in non-immobilized controls was blocked for both s-IHI and l-IHI (**Figure 2-3**). A mixed-effects analysis showed a trend for a decrease in s-IHI over the course of the day (Time: F=3.6, p=.06), significantly mediated by group (Group x Time: F=5.3, p=.03). Additionally, s-IHI was greater from the target onto the nontarget hemisphere (Hemisphere: F=23.1, p<.0001) (**Figure 2-3a**). Analyses separated by group demonstrated that there was a significant decrease in s-IHI in the non-immobilized control group (Time: F=6.8, p=.01), and s-IHI was greater onto the nontarget hemisphere (Hemisphere: F=7.01, p=.01). In the immobilized group, there was a significant effect of hemisphere with higher IHI onto the non-target hemisphere across test sessions (Hemisphere: F=19.2, p=.0001).

For l-IHI, a mixed-effects analysis demonstrated greater IHI onto the nontarget hemisphere (Hemisphere: F=25.2, p<.0001). Additionally, immobilization significantly modulated the change in l-IHI over the course of the day (Group x Time: F=8.8, p=.006) (**Figure 2-3b**). Analyses separated by group indicated that l-IHI significantly decreased over the course of the day (Time: F=12.3, p=.001) and

was greater onto the nontarget hemisphere (Hemisphere: F=8.1, p=.007) in non-immobilized control participants. In the immobilized participants, l-IHI was also greater onto the nontarget hemisphere (Hemisphere: F=17.6, p=.0002).

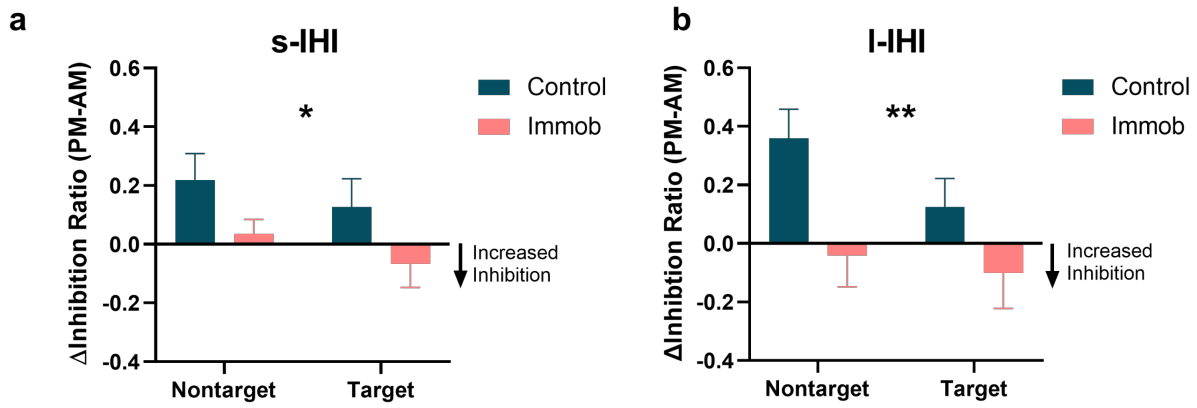

Figure 2-3. Immobilization led to an increase in (a) short interhemispheric inhibition (*p<.05) and (b) long interhemispheric inhibition (l-IHI) (**p<.01) from the nontarget hemisphere to the target hemisphere. Error bars represent standard error.

2.3.5 Intracortical Inhibition (SICI & LICI)

A mixed-effects analysis showed trends for differences in SICI between hemispheres (Hemisphere: F=3.7, p=.06) and an interaction between time and hemisphere (Hemisphere x Time: F= 3.2, p=.08) on SICI (**Figure 2-4a**). Within groups, mixed-effects analyses demonstrated that SICI was not significantly different between hemispheres (Hemisphere: F= .22, p= .64) and did not change throughout the day (Time: F= 2.5, p= .12) in non-immobilized control participants. In immobilized participants, SICI was not significantly different between hemispheres (Time: F=.08, p=.78), but there was a trend for lower SICI in the target hemisphere at both time points (Hemisphere: F=3.4, p=.07).

A mixed-effects analysis showed that the left, nontarget hemisphere had increased LICI (Hemisphere: F=7.5, p=.009), and there was a trend for an interaction between time and hemisphere (Hemisphere x Time: F=2.9, p=.09) on LICI (**Figure 2-4b**). Mixed-effects analysis for the control group and the immobilization group separately demonstrated no significant effects of time or hemisphere, as well as no interaction effect, in either group.

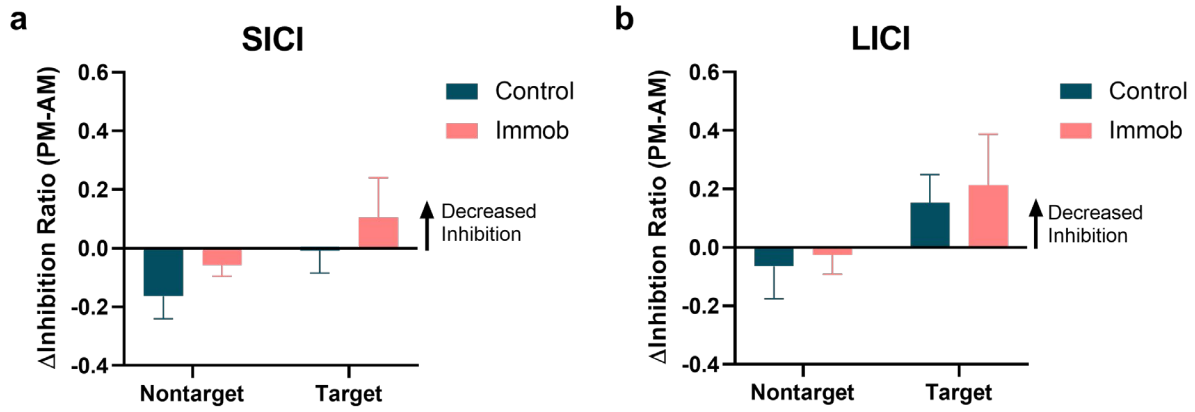

Figure 2-4. Intracortical inhibitory circuits were not significantly modulated by immobilization. Mixed effects analyses showed no significant effect of immobilization on **(a)** SICI or **(b)** LICI. Measures of intracortical inhibition showed a non-significant reduction in inhibition in the target hemisphere following immobilization. Error bars represent standard error.

Table 2-2. Summary of TMS Results.

TMS Condition	Neural Process(es) Measured	Target (Immobilized) Hemisphere	Nontarget (Non-Immobilized) Hemisphere

RMT	Neuronal Membrane Excitability		
CSE	Corticospinal Excitability		
SICI	Synaptic $GABA_A$		
LICI	Synaptic $GABA_B$		
s-IHI	Interhemispheric activation of $GABA_A$		
l-IHI	Interhemispheric activation of $GABA_B$		

 *= no change*

 *=increased*

 *=decreased*

 *=lack of typical increase*

Compared to data from control participants, the effects of immobilization on TMS measures are described in Table 2-2. With measures of interhemispheric inhibition, the hemisphere in the table corresponds to the hemisphere that is being inhibited. Arrows indicate the direction of change after short-term arm immobilization. Horizontal bars represent no change (dashed bars represent a lack of a typical increase in IHI that occurs without short-term immobilization).

2.3.6 *Bivariate Correlations*

Our results demonstrated that change in SICI was negatively correlated with change in CS excitability (r=-.44, p=.035) (**Figure 2-5**). This relationship indicates that a decreased CSE was associated with decreased $GABA_A$-ergic inhibition in the target hemisphere in the immobilization group. There were no significant correlations between change in CSE and change in LICI (r=.10, p=.65), IHI10 (r=-.06, p=.83), or IHI50 (r=-.04, p=.86) in the target hemisphere in immobilized individuals. While CSE increased across the 6-hour immobilization period in the non-immobilized control group, change in CSE was not significantly correlated with change in any measure of inhibition (all p > .05).

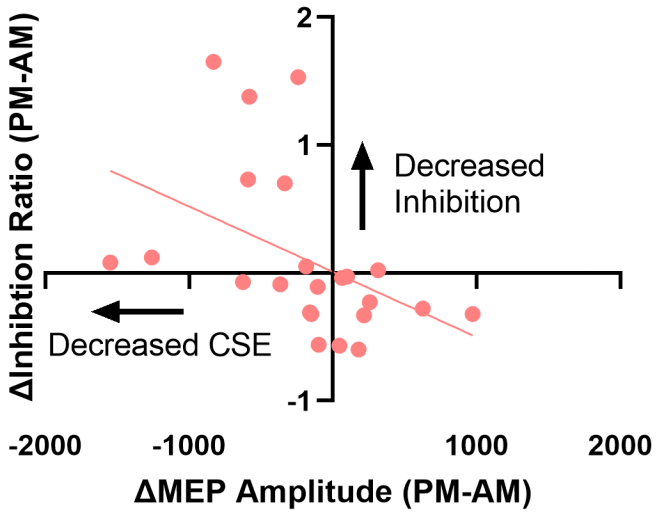

Figure 2-5. Decreased CSE was significantly associated with decreased SICI in the target hemisphere of participants in the immobilization group (r=-.44, p=.035)**.** Change in CSE did not correlate with changes in other measures of intracortical or interhemispheric inhibition in either group.

2.5 Discussion

Six hours of immobilization decreased CSE in the target hemisphere and increased s-IHI from the non-target to the target hemisphere, in line with previous studies. We also observed that l-IHI was increased onto the target hemisphere following immobilization, suggesting that immobilization modulates both $GABA_A$ and $GABA_B$-ergic interhemispheric circuits. However, changes in IHI and CSE were not associated, implying that increased IHI is unlikely the primary driver behind reduced CSE following immobilization. Additionally, our results indicate that immobilization did not significantly modulate intracortical inhibitory circuit activity in the target M1 at the group level. However, reductions in CSE were uniquely associated with reduced intracortical inhibition following immobilization. Taken together, our results demonstrate that increased IHI following short-term immobilization is likely due to an increase in activity of the excitatory transcallosal projections from the non-target onto the target hemisphere and may suggest that modulation of intracortical inhibition contributes to homeostatic plasticity effects associated with immobilization.

We observed a reduction in CSE due to immobilization of the arm, consistent with other studies (Facchini, Romani et al. 2002, Crews and Kamen 2006, Huber, Ghilardi et al. 2006, Clark, Taylor et al. 2010, Opie, Evans et al. 2016, Karita, Matsuura et al. 2017). In the current study, immobilization-induced decrease in CSE was observed in the largest cohort of participants studied to date and with a duration of immobilization (6 hours) shorter than most previous studies, supporting a robust effect of immobilization on reducing CSE. Decreased CSE after immobilization is thought to be due to LTD-like processes (Huber, Ghilardi et al. 2006) and is associated with an enhanced capacity for LTP-like plasticity after immobilization (Rosenkranz, Seibel et al.

2014), consistent with the model of homeostatic metaplasticity. TMS measures of CSE are influenced by inhibitory and excitatory circuits in M1 (Ziemann, Reis et al. 2015); therefore, characterization of CSE in isolation provides limited insight into circuit-specific changes in cortical neurophysiology after immobilization.

Characterizing immobilization effects on IHI circuits revealed increased inhibition from the non-target onto the target hemisphere. Previous studies have found increased s-IHI, mediated by $GABA_A$, from the non-target to the target hemisphere after a 10-hour period of immobilization (Avanzino, Bassolino et al. 2011, Avanzino, Pelosin et al. 2014). Here, were observed a similar effect after a 6-hour period of immobilization. Additionally, we extended previous findings to show that l-IHI, primarily thought to be mediated by $GABA_B$ receptor activity, also increases onto the target hemisphere. While there was an effect of immobilization on IHI at the group level, the change in IHI after immobilization was not correlated with the change in CSE in the target hemisphere of immobilized participants. This suggests that decreased CSE after immobilization is not primarily the result of increased IHI from the contralateral hemisphere. The ability of short-term immobilization to modulate IHI could be leveraged in individuals where a shift in IHI balance would be beneficial. For example, one model of stroke recovery posits that an imbalance in IHI may contribute to persistent motor dysfunction (Murase, Duque et al. 2004, Di Pino, Pellegrino et al. 2014, Di Pino and Di Lazzaro 2020). Thus, immobilization may hold promise as a noninvasive neuromodulation approach to rebalance activity between hemispheres to facilitate post-stroke recovery in some patients.

Group-level analyses showed no effect of immobilization on $GABA_A$- or $GABA_B$-ergic intracortical inhibition. However, in the target hemisphere in immobilized

participants, reduced CSE was associated with reduced SICI, reflective of intracortical $GABA_A$-ergic inhibition (Ziemann, Reis et al. 2015). Importantly, an association between the modulation of CSE and SICI was not present in the target hemisphere of the non-immobilized control participants, suggesting that the association between reduced CSE and reduced intracortical inhibition was specific to immobilization. The observed relationship with reduced CSE may implicate decreased intracortical inhibition as a potential mechanism for the increase in the capacity for LTP-like plasticity following immobilization. Previous research has shown that reductions in intracortical inhibition in M1 can facilitate LTP-like plasticity (Ziemann, Muellbacher et al. 2001), while increases in $GABA_A$-ergic inhibition block LTP-like plasticity in human M1 (Butefisch, Davis et al. 2000, Ziemann, Muellbacher et al. 2001). In rodent M1, reductions in $GABA_A$-ergic inhibition are required to induce LTP (Hess, Aizenman et al. 1996). Despite the established role of intracortical inhibition in neuroplastic changes, it is less well-characterized in the context of mechanisms of neuroplasticity in the human brain and is less often considered in studies of plasticity induction using neuromodulation techniques.

Taken together, we interpret a reduction in CSE to be due, in part, to enhanced IHI onto the immobilized hemisphere and not a local increase in intracortical inhibition. However, the lack of correlation between changes in IHI and changes in CSE imply that increased IHI is not the sole contributor to changes in CSE. The significant relationship between reduced CSE and reduced SICI may indicate a local reduction in the excitability of intracortical circuits within M1. Our results support the possibility of global downscaling of activity with immobilization, but it should be noted that intracortical inhibition was not significantly modulated by immobilization at the group level. Due to

the large inter-individual variability in neuromodulatory response to short-term immobilization, further investigation is required in order to determine the cause of this variability. Additionally, future studies should also include evaluation of intracortical faciliatory pathways (e.g., TMS measures of intracortical facilitation (ICF)) to assess whether the downscaling of excitability within M1 is specific to inhibitory circuits or if it is indeed a more global phenomenon.

Changes in the activity of other regions that are interconnected with M1 may also contribute to the decrease in CSE following short-term immobilization. Given the observation of profoundly reduced movement in the immobilized arm, which necessarily reduces both motor output and sensory input, one potential cortical region of interest is the primary somatosensory cortex (S1). Previous research has demonstrated that M1 and S1 are highly interconnected, and these connections strongly influence motor behavior (Edwards, King et al. 2019). Only a few studies have examined the effect of immobilization on S1 activity, measuring somatosensory-evoked potentials (SEPs) in response to peripheral nerve stimulation. Huber, Ghilardi et al. (2006) found that after 12 hours of arm immobilization, the P45 component of the SEP, thought to reflect activation of S1 (Allison, McCarthy et al. 1992, Sasaki, Tsuiki et al. 2018), was reduced in the S1 corresponding to the immobilized arm compared to the contralateral S1. Another study reported an increase in the N9 component, which corresponds to excitability of the peripheral nerve, after 10 hours of arm immobilization (Ikeda, Oka et al. 2019). This finding potentially reflects a sensitization of the ascending sensory pathway after short-term immobilization. However, additional research is necessary to assess if and to what extent these results would be observed following only six hours of immobilization. The effects of immobilization on other brain regions in the

sensorimotor network that have been shown to influence plasticity in human M1, such as premotor cortex (Huang, Chen et al. 2018) and supplementary motor area (Hamada, Hanajima et al. 2009), have been less well-studied but could also influence the targeted neurophysiologic effects observed with TMS-based measures of M1 excitability.

Measures of spinal excitability were not collected in the current study that may also have influenced TMS-based assessments of CSE. It has been demonstrated that longer durations of immobilization (e.g., one week) led to an increase in spinal excitability (Lundbye-Jensen and Nielsen 2008). In contrast, it has been suggested that shorter periods of immobilization, such as hours to days, do not modulate spinal excitability (Facchini, Romani et al. 2002, Bassolino, Bove et al. 2012). Although participants were provided standardized instructions for the immobilization period and wrist actigraphy demonstrated that the amount of limb activity was substantially reduced during immobilization, individuals were not directly monitored during this period. Given this point, it is possible that the specific activities they engaged in, and the level of engagement, contributed to inter-individual differences in the neurophysiologic effects of immobilization. It has previously been demonstrated that the amount of use of the non-immobilized arm can influence changes in intracortical and interhemispheric excitability (Avanzino, Bassolino et al. 2011). Several intraindividual traits, such as history of migraine with aura (Antal, Lang et al. 2008) and BDNF val66met polymorphism (Kleim, Chan et al. 2006), have been shown to influence the capacity for neuroplasticity in human M1 but were not assessed. Therefore, it is possible that these or other factors may have also contributed to inter-individual variability of the neuromodulatory effects of immobilization.

2.6 Conclusions

Overall, our results demonstrate that immobilization reduces CSE and increases IHI, consistent with our hypotheses and previous findings. Interestingly, decreased CSE was significantly associated with a decrease in $GABA_A$-ergic intracortical inhibition, suggesting that short-term arm immobilization may lead to a global downscaling of synaptic strength in M1. Reduced intracortical inhibition may provide a novel mechanism by which short-term immobilization may enhance the capacity for LTP-like plasticity within M1 and may be a potential target for strategies to augment plasticity capacity to enhance motor learning in health and disease.

Chapter 3: Effects of short-term immobilization on motor skill acquisition

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King, E; Edwards, L; Borich, M. "*Effects of short-term immobilization on motor skill acquisition"* (Under Review, *PloS One*).

3.1 Abstract

Learning to sequence movements is necessary for skillful interaction with the environment. Neuroplasticity, particularly long-term potentiation (LTP), within sensorimotor networks underlies the acquisition of motor skill. Short-term immobilization of the arm, even less than 12 hours, can reduce corticospinal excitability and increase the capacity for LTP-like plasticity within the contralateral primary motor cortex. However, it is still unclear whether short-term immobilization influences motor skill acquisition. The current study aimed to evaluate the effect of short-term arm immobilization on sequence-specific motor skill acquisition using a modified Serial Reaction Time Task (SRTT). Twenty younger neurotypical adults underwent a single SRTT training session after six hours of immobilization of the non-dominant arm or an equivalent period of no immobilization. Our results demonstrated that participants improved SRTT performance overall after training, but improvements were not influenced by immobilization prior to task training. Further, improvements on the modified SRTT were not sequence-specific. Taken together, our results suggest that immobilization does not significantly augment motor skill acquisition for sequential, individuated finger movements.

3.2 Introduction

Learning to coordinate motor sequences is essential for completing tasks necessary for daily living, such as tying a shoe or typing. Experience is a potent driver of neuroplasticity throughout the cortex (Whitt, Petrus et al. 2014), and even short-term experiences, such as motor skill practice, have been shown to induce structural and functional changes within the human motor cortex and to underlie motor learning

(Butefisch, Davis et al. 2000, Gryga, Taubert et al. 2012). Synaptic processes such as long-term potentiation (LTP) and long-term depression (LTD) (Luscher and Malenka 2012), alterations of dendritic spine density and morphology (Kida, Tsuda et al. 2016), and changes in inhibitory neurotransmission (Butefisch, Davis et al. 2000, Paille, Fino et al. 2013) have all been shown to contribute to the training-induced plasticity that underlies motor skill acquisition.

Previous research in humans has demonstrated that experience-dependent synaptic strengthening through LTP-like plasticity in sensorimotor circuits is necessary for acquisition of motor skill (Butefisch, Davis et al. 2000). However, in order to maintain stable levels of activity in these circuits, it is necessary to regulate synaptic strength at the level of the individual synapse (Abbott and Nelson 2000) to maintain synaptic homeostasis. The model of homeostatic plasticity suggests that the degree of experience-dependent strengthening or weakening that can occur in a given synapse is influenced by the recent history of synaptic activity (Rioult-Pedotti, Friedman et al. 2000, Ziemann, Ilic et al. 2004), which prevents the circuit from becoming over- or under-excited (Abraham and Bear 1996, Abraham 2008, Whitt, Petrus et al. 2014, Edwards, King et al. 2019). For example, in a synapse that has recently undergone a period of synaptic strengthening, additional synaptic strengthening becomes less likely to occur, and synaptic weakening becomes more likely. This principle has been demonstrated in both excitatory and inhibitory circuits within M1 using noninvasive brain stimulation (NIBS), where increasing activity of these circuits prior to plasticity-induction protocols resulted in reduced potential for further LTP-like plasticity (Potter-Nerger, Fischer et al. 2009, Murakami, Muller-Dahlhaus et al. 2012). Since LTP is a primary contributor to experience-dependent plasticity, inducing LTD-like plasticity

within M1 prior to training may leverage homeostatic mechanisms to enhance the capacity for task-specific synaptic strengthening and performance improvement in humans.

Short-term (≤12hr) limb immobilization transiently reduces sensory input to and motor output from the contralateral sensorimotor cortex, resulting in a temporary decrease in corticospinal excitability (Rosenkranz, Seibel et al. 2014, Karita, Matsuura et al. 2017), thought to reflect a decrease in synaptic strength through LTD-like processes (Huber, Ghilardi et al. 2006). Further, the capacity for LTP-like plasticity is enhanced immediately after short-term immobilization in human primary motor cortex (Rosenkranz, Seibel et al. 2014). Although short-term limb immobilization modifies systems-level indices of synaptic strength in humans, the effect on motor skill training-induced plasticity is unclear. The purpose of the current study was to evaluate the effects of short-term limb immobilization on motor skill acquisition in young, healthy individuals. In this study, participants completed a single motor skill training session after a period of 6 hours with or without immobilization of the non-dominant arm. We hypothesized that if short-term limb immobilization increases the capacity for activity-dependent synaptic strengthening in the corresponding contralateral M1 representation, then greater motor skill acquisition would be observed with training that followed immobilization compared to training following an equivalent period of no immobilization.

3.3 Materials and Methods

3.3.1 Study Participants

Twenty-one healthy individuals (6 male) aged 18-35 (24.8 ± 4.7 years) participated in the current study spanning the morning and evening of one day. The age range was selected in order to reduce the influence of age on motor skill learning (Meissner, Keitel et al. 2016). Inclusion criteria included (1) no history of movement impairment or neurodegenerative disease, (2) right handedness according to the Edinburgh Handedness Scale (Oldfield 1971), and (3) no contraindications to transcranial magnetic stimulation (TMS) (Rossi, Hallett et al. 2009). All study procedures were approved by the Emory University Institutional Review Board in accordance with the Declaration of Helsinki. Written consent was obtained from all participants prior to testing procedures. Behavioral data from one participant were excluded due to equipment malfunction.

3.3.2 Motor Task Paradigm

A version of the Serial Reaction Time Task (SRTT) (Nissen and Bullemer 1987, Robertson 2007) modified from Clos, Sommer et al. (2018) was used in this study. Participants placed the fingers of their left (non-dominant) hand on a custom-made button box. The buttons on the box corresponded to the top four colored squares on a computer display placed at eye-level in front of the participant (**Figure 3-1**). Participants were instructed to press the button corresponding to the top square that matched the color of the bottom (target) square as quickly and accurately as possible. An example trial and overall study design are shown in **Figure 3-1.** In the morning baseline (BL) session, participants completed one test block that consisted of 280 button presses (50 random, 180 repeated, 50 random) during the session prior to the

immobilization period to assess motor performance. Repeated button presses consisted of 15 repeats of a 12-item sequence, and the participants were instructed there was a repeating sequence.

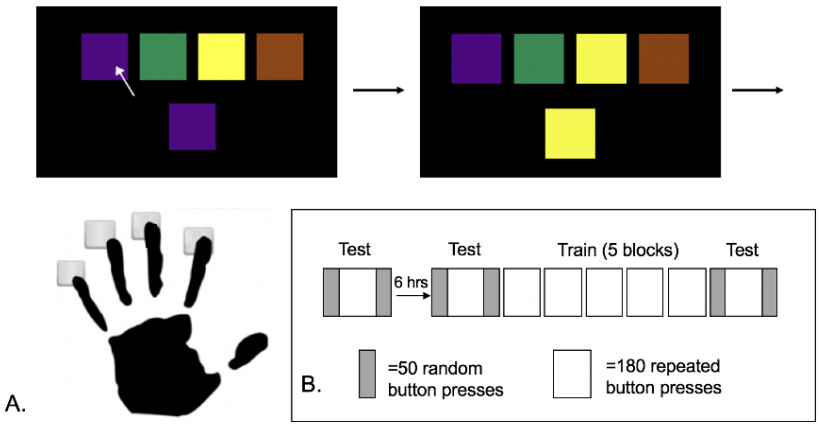

Figure 3-1. Modified Serial Reaction Time Task (SRTT). (A) Participants were instructed to press the key on a custom button box that corresponded to the top square that matched the target (bottom) square (B) Sequence-specific skill (SKILL) was calculated as the mean difference in response time (RT) between repeated (white) and random (gray) sequences for test blocks at each time point.

3.3.3 Arm Immobilization

After completion of the BL session, 10 participants were randomly assigned to undergo arm immobilization. These individuals were instructed to wear a finger control mitt on the left (non-dominant) hand, which secured positioning of the fingers in a padded mitt to restrict finger movement. In addition to the hand mitt, the arm was placed in a sling to reduce movement of the wrist, elbow, and shoulder joints. Participants were instructed to move their left arm as little as possible during the immobilization period but to use the non-immobilized right (dominant) hand and arm

normally. Individuals in the control group (n=10) were instructed to use both arms normally between sessions.

3.3.4 Motor Skill Acquisition Paradigm

After the 6-hour immobilization period, skill acquisition was assessed in the evening using a pre-train test block (PRE), five training blocks consisting of 180 repeated button presses, and a post-train test block (POST). Colors changed each trial in order to mask the sequence and prevent explicit awareness of the sequence. At the end of the evening session, the degree of explicit awareness of the presence of a sequence was assessed by asking if the participants noticed any pattern of button presses during the task. If they indicated that they noticed a pattern during training, they were asked to freely recall the sequence. The ability to freely recall ≥4 consecutive items of the 12-item sequence was considered explicit awareness (Robertson, Pascual-Leone et al. 2004, Cohen, Pascual-Leone et al. 2005).

3.3.5 Behavioral Outcome Measures

During the six-hour immobilization period, activity monitors (wGT3x-BT, ActiGraph) were worn on both arms by all participants to determine compliance with the immobilization procedure since participants were allowed to leave the lab between test sessions. Movement of each arm, measured in units of Gs, was collected bilaterally (left/target and right/non-target arms) throughout the six-hour immobilization period for both groups. A two-way ANOVA was performed to examine the effect of immobilization on activity counts, with within-subject factor of hand (two levels: target

and non-target) and between-subject factor of group (two levels: immobilization or control group).

3.3.6 Assessment of general motor performance

Response time for each button press was acquired during task performance with a custom Java script. Outlier response times, defined as a response time three standard deviations greater than the mean response time within each block for each participant, were removed from analysis.

General motor performance was assessed by calculating the response times for button presses across task exposure. Response times were then normalized to the average response time for the first 50 random button presses in order to account for variations in baseline motor performance. Normalized response times for random sequence in the test blocks and repeated sequence in the training blocks were analyzed separately. A two-way ANOVA was used to assess the effect of immobilization on general motor performance, as measured by the normalized response time for the first 50 random sequence button presses during each of the three test blocks, with within-subject factor of time (three levels: baseline (BL), pre-training (PRE), and post-training (POST)) and between-subject factor of group (two levels: immobilization or control group). A separate two-way ANOVA was used to assess normalized response time for repeated sequence performance across the training blocks (five levels: Training blocks 1-5) with between-subject factor of group (two levels: immobilization or control group).

3.3.7 Assessment of sequence-specific skill

To assess sequence-specific skill, two outcome measures were calculated: Skill Score (SS) and Interference Score (IS). The Skill Score (SS) was calculated as the average response time for the last 50 random button presses minus the average response time for the last four repeated sequences in the test block (48 repeated button presses) (Palmer, Halter et al. 2019). A larger SS indicates greater sequence-specific skill, such that when trained, participants' repeated sequence performance is faster than random sequence performance. Skill Scores were calculated for BL (SS_{BL}), PRE (SS_{PRE}), and POST (SS_{POST}) test blocks.

The Interference Score (IS) was calculated to assess potential interference caused by an abrupt transition from repeated sequence to random sequence (Rep-Rand) button presses, which leads to an increase in response time (Robertson 2007). Rather than assessing the relative response times of repeated sequence and random sequence button presses averaged over many button presses, the IS examines the impact of disrupting the trained motor sequence with a random sequence by calculating the average response time for the first 12 random button presses immediately following a transition minus the average response time for the 12 repeated button presses immediately preceding the transition. Twelve button presses were selected in order to include one full 12-item sequence for the repeated button presses and an equivalent number of presses for the random button presses. A larger IS represents a larger increase in response time at the transition from random to repeat and therefore greater interference. Interference Score (Rep-Rand) was calculated for each test block (IS_{BL}, IS_{PRE}, IS_{POST}).

3.3.8 Associations between behavioral and neurophysiological measures

To assess associations between immobilization effects on immobilization-induced decreases in corticospinal excitability (CSE) (see **Chapter 2.3.2** for methodological details) and training-related changes in motor skill at an individual level, bivariate correlation analyses between change in CSE with changes in general motor skill and sequence-specific skill were performed.

All statistical analyses were performed with Prism GraphPad 8, and a critical α was set at 0.05 corrected for multiple comparisons as appropriate.

3.4. Results

A two-way ANOVA demonstrated that there were significant effects of time ($F=60.1$, $p<.0001$) and of group ($F=14.3$, $p<.01$), as well as a time X group interaction ($F=40.5$, $p<.0001$) on average activity counts (**Figure 3-2**). Post-hoc t-tests indicated that activity in the immobilized (target) arm were significantly reduced in immobilized individuals ($t=9.98$, $p<.0001$) confirming participants complied with the immobilization procedure.

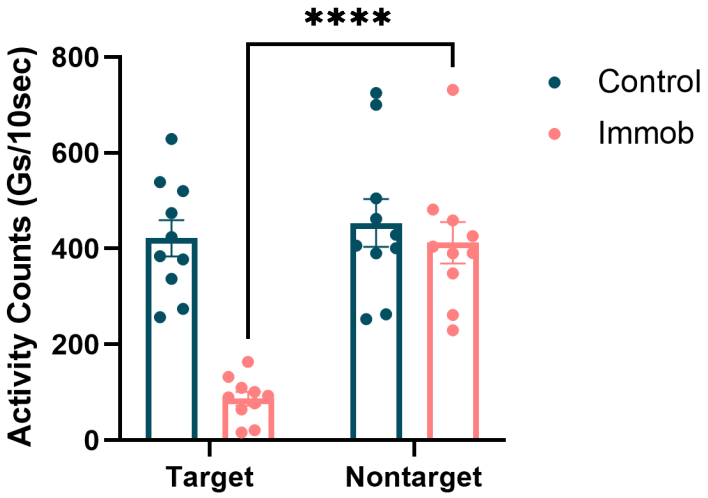

Figure 3-2. Immobilized participants complied with the immobilization procedure. Participants wore activity monitors on both wrists throughout the six-hour immobilization period. Activity counts in the immobilized (target) arm were significantly reduced compared to the non-immobilized (nontarget) limb in immobilized participants (t=9.98, p<.0001). Error bars represent standard error.

3.4.1 Assessment of general motor performance

Our results demonstrate that general motor performance improved across task exposure, measured by a decrease in response time for both random and repeated button presses with task exposure. Normalized response times for each group across training are shown in **Figure 3-3**.

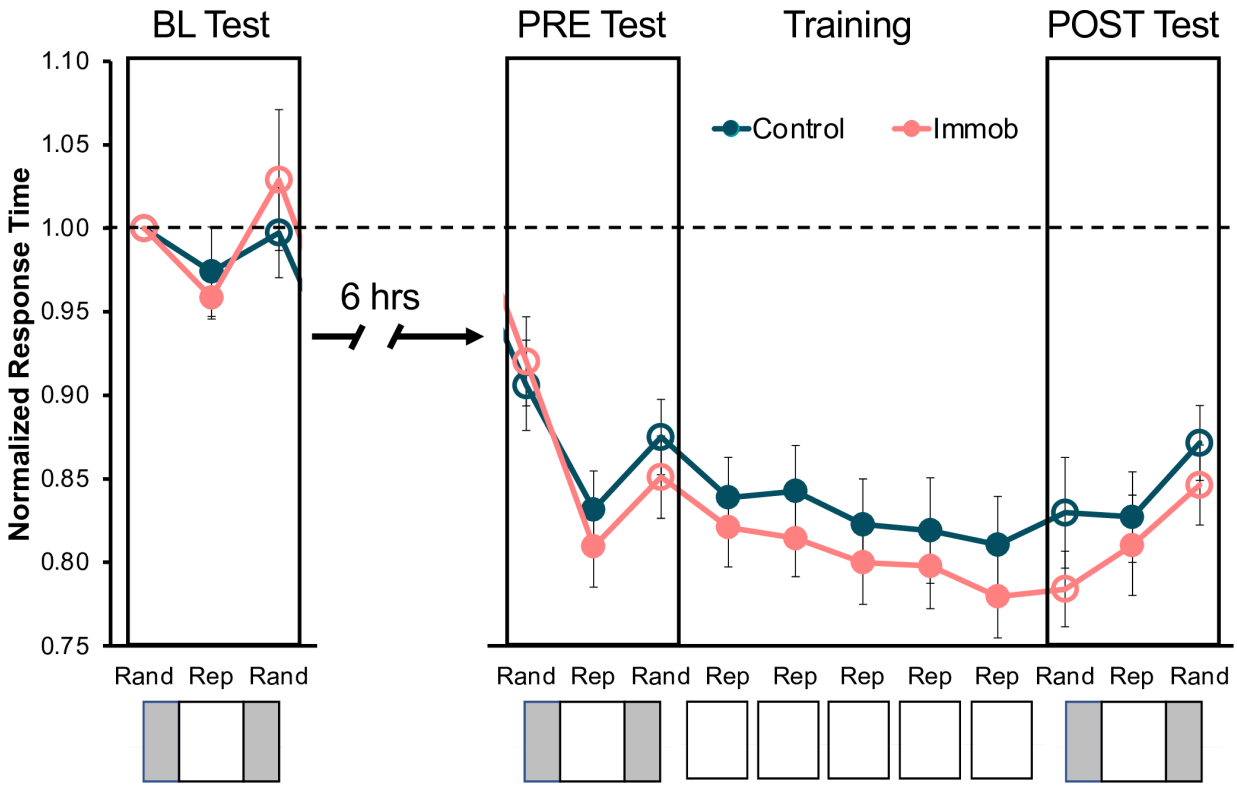

Figure 3-3. Normalized SRTT response time across performance assessment timepoints. Values less than one (black dashed line) indicate faster than baseline performance. Open circles represent 50 random button presses, and closed circles represent 180 sequenced button presses. One test block occurred in the morning (BL Test) to assess baseline motor performance. The evening session consisted of five

training blocks (Training) with test blocks before (PRE Test) and after (POST Test). Error bars represent standard error.

Random sequence performance increased across test blocks, as measured by a decrease in response time, for both control and immobilized participants. A two-way ANOVA indicated that there was a significant main effect of time ($F=57.5$, $p<.0001$) (**Figure 3-4A**) on normalized response time; however, there was not a main effect of group nor a time X group interaction. Tukey's post-hoc tests showed that there was a significant increase in performance for the control group from BL to PRE ($q=4.9$, $p=.02$) and PRE to POST ($q=5.2$, $p=.013$) test blocks. Performance for the POST test block was also significantly increased compared to the BL test block in the control group ($q=7.4$, $p=.001$). Similarly, for the immobilized individuals, random sequence performance was significantly improved from BL to PRE ($q=4.2$, $p=.04$), PRE to POST test blocks ($q=9.1$, $p<.001$), and from BL to POST test blocks ($q=13.4$, $p<.0001$).

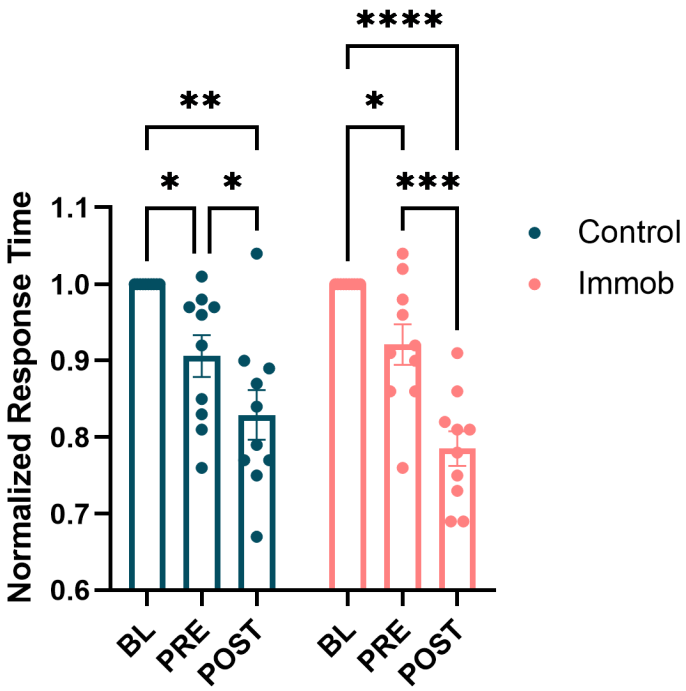

Figure 3-4. General motor performance increased with task exposure in both groups. Normalized response time significantly decreased across training regardless of immobilization condition (F=57.5, p<.0001). *p<.05, **p<.01, ***p<.001, ****p<.0001. Error bars represent standard error

A two-way ANOVA showed a significant main effect of time (F=6.3, p=.0008) on normalized response time for repeated sequence performance across training blocks 1-5 (**Figure 3-3**). There was no effect of group nor a time X group interaction on response time throughout training blocks. Post-hoc t-tests showed that the normalized response time for training block 5 was significantly faster than training block 1 in the immobilized participants (t=4.2, p=.024) but not the control participants (t=2.5, p=.30).

3.4.2 Assessment of sequence-specific skill

Both groups showed an average increase from SS_{BL} to SS_{PRE}, but a two-way ANOVA showed no effect of group or time on skill score and no time X group interaction (**Figure 3-5A**) across all three timepoints. Similarly, a two-way ANOVA showed no effect of group or time nor a time X group interaction on interference scores. **Figure 3-5B** shows the interference scores for the transition from repeated to random for BL, PRE, and POST test blocks. **Table 3-1** summarizes the results of each ANOVA for the SRTT-based outcome measures**.**

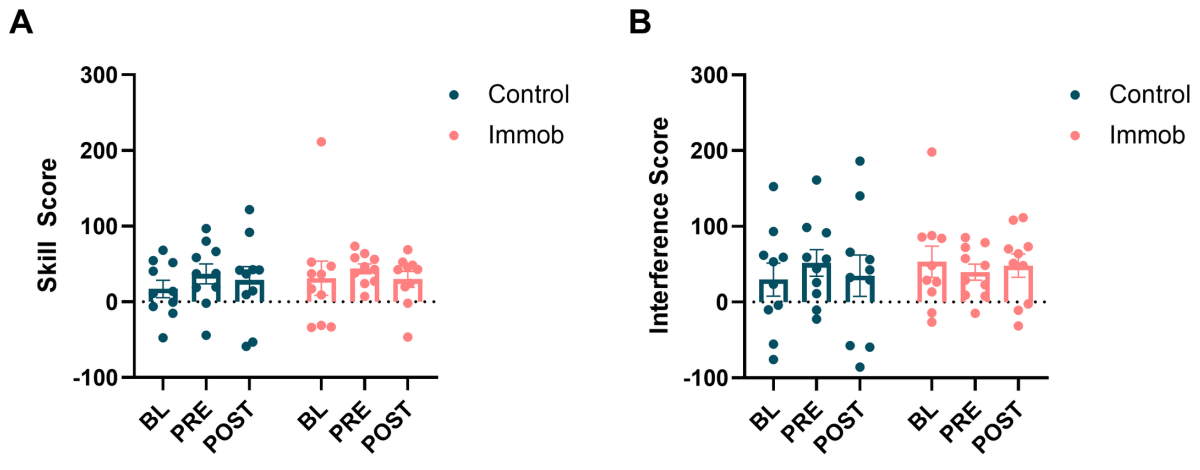

Fig 3-5. Sequence-specific skill did not change after training and was not influenced by immobilization. Neither **(A)** Skill Score nor **(B)** Interference score significantly changed across task exposure for either group. Error bars represent standard error.

Table 3-1. SRTT ANOVA Results. Significant p-values are in bold.

Measure	**Source**	**df**	**F Statistic**	**p-value**
Random Sequence Performance	Time	2	57.5	**<.0001**
	Group	1	0.16	.69
	Time X Group	2	1.4	.25
Repeated Sequence Performance	Time	4	6.3	**.0008**
	Group	1	0.44	.51
	Time X Group	4	0.19	.95
Skill Score	Time	2	0.73	.46
	Group	1	0.30	.59
	Time X Group	2	0.12	.89
Interference Score	Time	2	0.03	.91

Group	1	0.24	.63
Time X Group	2	0.46	.64

3.4.3 Associations between behavioral and neurophysiological measures

Bivariate correlation analyses demonstrated that change in CSE across the immobilization period was not significantly correlated with training-related changes in general motor skill (r^2=.02, p=.71) or sequence-specific skill (r^2=.07, p=.48) (**Figure 3-6**).

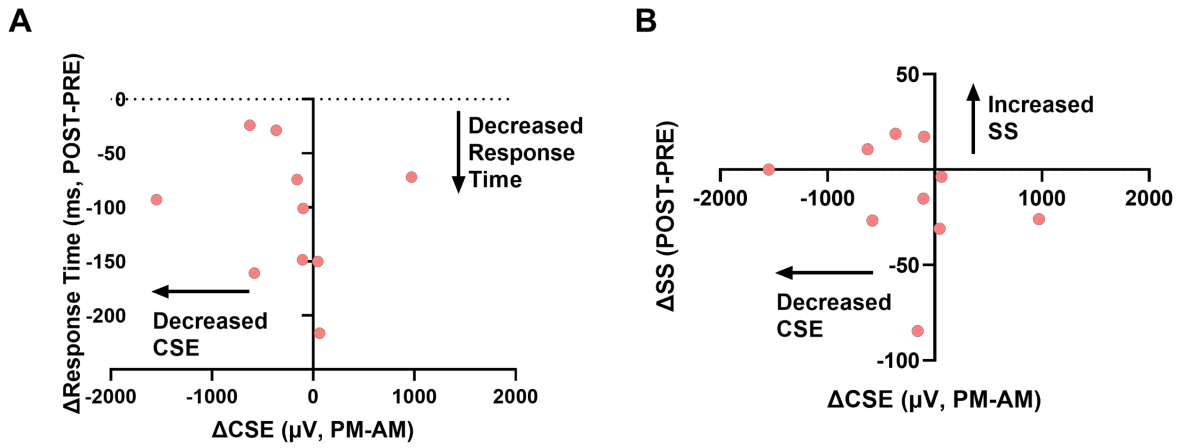

Figure 3-6. Changes in CSE across the immobilization period did not significantly correlate with change in **(A)** general motor skill or **(B)** skill score across training.

3.5 Discussion

In the current study, we investigated the effect of short-term arm immobilization on implicit motor sequence acquisition. General motor performance improved with task exposure in both groups; however, improvement was not sequence-specific. Despite confirming that the immobilization protocol was followed by individuals in the

immobilization group, no group differences were found in general motor performance or sequence-specific skill. Additionally, changes in immobilization-induced markers of enhanced plasticity within M1 were not associated with changes in general motor skill or sequence-specific skill after training at an individual level. Overall, our results suggest that immobilization did not significantly augment sequence-specific skill acquisition or online improvements in general motor performance on the SRTT.

3.5.1 Immobilization does not influence general motor performance on the SRTT

Contrary to the results of previous studies utilizing short-term immobilization (Huber, Ghilardi et al. 2006, Moisello, Bove et al. 2008, Weibull, Flondell et al. 2011, Bassolino, Bove et al. 2012, Bolzoni, Bruttini et al. 2012, Opie, Evans et al. 2016, Karita, Matsuura et al. 2017, Scotto, Meugnot et al. 2020), we did not observe a decrement in motor performance after a period of limb immobilization. In fact, general motor performance increased from BL to PRE test blocks in both groups, although the performance increase between those two timepoints was not significant in the immobilized group. One potential explanation for the difference in our results could be the duration of immobilization. While changes in measures of corticospinal excitability have been shown in as little as 3 hours after onset of immobilization (Karita, Matsuura et al. 2017), most of the previous studies that found impaired motor performance after immobilization used immobilization periods of at least 8 hours. In the current study, arm immobilization occurred for a period of six hours. The idea that duration of immobilization could influence motor performance is supported by a study by Moisello, Bove et al. (2008), which found that motor performance on an out-and-back cursor task decreased after 12, but not 6 hours, of immobilization. Therefore, it is possible that six

hours of immobilization may not have been sufficient to influence motor performance or motor skill acquisition.

3.5.2 Acquisition of sequential, individuated finger movements is not preferentially enhanced after immobilization

Despite the potential for short-term immobilization to enhance plasticity within M1, our results demonstrated that motor skill acquisition on a task that requires coordination of sequential movements was not influenced by immobilization. While M1 has been shown to be involved in the execution of sequential movements (Karni, Meyer et al. 1998, Bhattacharjee, Kashyap et al. 2021), it is unclear whether M1 has a role in acquisition of the sequenced motor skill itself (Hardwick, Rottschy et al. 2013). Therefore, increasing the capacity for LTP-like plasticity in M1 may not be sufficient to influence acquisition of an implicit, sequenced motor skill. Outside of M1, short-term immobilization has been shown to influence markers of plasticity in primary somatosensory cortex (Huber, Ghilardi et al. 2006), but it is unclear whether immobilization influences plasticity in other brain areas that may be involved in acquisition of sequence-specific skill, such as premotor cortex or supplementary motor area (Hardwick, Rottschy et al. 2013).

3.5.3 Task characteristics may influence the effects of immobilization

One unique aspect of the current study was that this was the first study to examine the effect of immobilization on a sequence learning task that relies on individuated finger movements. Therefore, the different characteristics of the task itself as well as the outcome measures could have contributed to inconsistent results between

this study and others. Several previous studies that observed a decrease in motor performance after a period of immobilization used tasks that required control of more proximal portions of the upper extremity, such as reaching, while the current study used a modified SRTT that utilized fine control of the distal upper extremity. Previous research has demonstrated that the composition of descending projections to the distal and proximal upper extremity are different in primates (Palmer and Ashby 1992, McKiernan, Marcario et al. 1998, Aune, Loras et al. 2020), and it is possible that immobilization differentially modulates these pathways. Bolzoni, Bruttini et al. (2012) suggested that function of the proximal muscles responsible for postural control is more likely to be influenced by a period of immobilization, even when only distal hand muscles are immobilized. Similarly, in a task requiring participants to pick up and put down a pencil repeatedly, immobilization of the dominant arm increased reach duration and changed acceleration and deceleration of movement but did not influence grip aperture (Bassolino, Bove et al. 2012). In the current study, outcome measures to assess general motor performance and sequence-specific skill were calculated using response time, which are not able detect changes in joint kinematics that may occur after a period of immobilization. Future studies can quantify joint coordination during task performance with kinematic data (Hirano, Kubota et al. 2018) and/or separating response time into reaction time and movement time to examine central nervous system contributions to changes in SRTT performance with training and immobilization (Du and Clark 2017).

Another potential explanation for the observed findings is that immobilization impairs proprioceptive processing, and tasks that require proprioceptive information to complete will be impacted by a period of immobilization. This idea is supported by

Avanzino, Pelosin et al. (2014) that showed that neurophysiological changes normally seen after immobilization were blocked when proprioceptive receptors were selectively activated during the immobilization period. This could explain why performance on the modified SRTT, which required small amplitude, individuated finger movements that should not have been influenced by postural control or proprioceptive deficits, was not negatively impacted by immobilization. Taken together, our findings support prior literature suggesting that multi-joint coordination of arm movements is primarily impacted by immobilization. It remains unclear if immobilization can modulate skill acquisition for tasks requiring multi-joint coordination of the arm.

3.5.4 Skill improvements across groups were not sequence-specific

An unexpected result from the current study was that sequence-specific skill did not significantly improve after training in either group. One possible explanation for the lack of change in skill score and interference score in both groups across training is that time of day influenced sequence-specific skill acquisition, since all motor training was performed in the evening session for the current group of participants in our study. Previous research has suggested that skill improvement on a sequence learning task is greater in the morning compared to the evening (Kvint, Bassiri et al. 2011), which does not seem to be the case with acquisition of skill in a repetitive ballistic motor training task (Sale, Ridding et al. 2013). Interestingly, Keisler, Ashe et al. (2007) suggested that motor sequence learning itself may not be impaired in the evening relative to morning, but factors such as motivation, attention, and fatigue may lead to the impairment of the expression of learning (in the form of task performance). Additionally, previous research has shown that performance during a skill acquisition task cannot be equated

with skill learning. In fact, certain features of a motor task itself, such as task difficulty or practice structure, can lead to a decrease in performance during the acquisition phase of learning but subsequently enhance retention of skill during a follow-up assessment (Kantak and Winstein 2012, Soderstrom and Bjork 2015). Including follow-up retention testing would assess the effect of immobilization on motor sequence learning when training occurs in the evening.

3.5.5 Study limitations

There are several limitations to the current study. The current study assessed skill acquisition rather than skill learning. Skill retention should be assessed in future studies to evaluate the effects of immobilization on motor skill learning. Additionally, it is possible that the color-matching component of the task may have masked the sequence, making sequence-specific acquisition, even implicitly, more difficult. This could have contributed to the lack of change in sequence-specific skill after a single training session. Future studies could employ different versions of the SRTT to address this limitation or investigate other tasks more closely aligned with the effects of arm immobilization (e.g., skilled reaching movements).

3.6 Conclusions

Overall, our results suggest that short-term immobilization of the arm does not significantly augment acquisition of skill on a task that requires individuated, sequenced finger movements at a group level. However, it is possible that characteristics of the modified version of the SRTT used in the current study influenced our results. These results provide evidence for the specificity of behavioral effects of short-term

immobilization. Future studies should assess the effects of immobilization on skill acquisition using tasks that require proprioceptive feedback or more proximal control of the upper extremity.

Chapter 4: Short-term immobilization of the arm does not modify consolidation of skilled finger movements

4.1 Abstract

Motor skill learning requires memory consolidation that typically involves neuroplasticity induction within the primary motor cortex (M1). Previous research has shown that short-term immobilization of the arm has the potential to enhance the capacity for neuroplasticity within M1. The current study aimed to assess the effect of short-term arm immobilization on consolidation of sequence-specific skill using a modified version of the Serial Reaction Time Task (SRTT). Twenty-one neurotypical adults underwent a single SRTT training session prior to a six-hour period of arm immobilization or an equivalent period of no immobilization. Skill retention was assessed after the immobilization period. Results indicated that SRTT skill improved with training, but was not sequence-specific, and skill was retained regardless of immobilization condition. Findings suggest short-term arm immobilization-induced plasticity does not significantly modify the consolidation of skilled finger movements in young adults.

4.2 Introduction

The acquisition of complex motor skill occurs through repetitive task practice. However, consolidation is necessary for the memory of the acquired skill to be stabilized and strengthened after the period of practice ends (Karni, Meyer et al. 1998, Censor, Sagi et al. 2012). Different neural processes underlie skill acquisition and skill consolidation likely to balance the flexibility required to acquire new skills in a variable environment with the stability necessary to retain skills over time (Clark and Ivry 2010). Early consolidation (within the first few hours after training) of skilled finger movements involves experience-dependent plasticity of the primary motor cortex (M1)

in humans (Classen, Liepert et al. 1998, Krakauer and Shadmehr 2006, Pollok, Latz et al. 2014). Disrupting M1 activity with repetitive transcranial magnetic stimulation (rTMS) immediately after training on an implicit sequence-learning task blocked offline performance improvements during the consolidation period (Robertson, Press et al. 2005). Additionally, learning a second motor skill immediately after training on the first skill disrupts consolidation of the first skill, but interference does not occur when training for two tasks occur four hours apart (Brashers-Krug, Shadmehr et al. 1996). Given that information processing in M1 for recently acquired motor skill is present for a time period following training, it may be possible to modify skill consolidation by manipulating M1 activity immediately after training.

Since skill consolidation has been shown to involve neuroplastic mechanisms in M1 (Classen, Liepert et al. 1998, Krakauer and Shadmehr 2006), increasing the capacity for neural plasticity in M1 during the early consolidation period may enhance skill consolidation. Short-term immobilization of the arm has been previously shown to lead to a reduction in corticospinal excitability, thought to be driven by a reduction in synaptic strength in M1 (Huber, Ghilardi et al. 2006). Further, short bouts of immobilization have been shown to enhance the capacity for long-term potentiation (LTP)-like plasticity in M1 (Rosenkranz, Seibel et al. 2014). It is currently unclear whether plasticity induced with short-term arm immobilization interacts with processes of consolidation following motor skill training.

To study the effects of short-term arm immobilization on motor skill consolidation, we asked participants to perform a modified version of the serial reaction time task (SRTT) (Nissen and Bullemer 1987), a well-validated motor learning task (Hardwick, Rottschy et al. 2013) that depends on M1 for skill consolidation during

wakefulness (Robertson, Press et al. 2005). We hypothesized that if short-term immobilization enhanced consolidation of motor skill after training, then same-day skill retention would be higher for a group of individuals that underwent arm immobilization after training during the skill consolidation period compared to a group that did not undergo immobilization.

4.3 Methods

4.3.1 Study Participants

Twenty-four young, healthy individuals (7 male) between the ages of 18 and 35 years (Average = 24.3 years, SD = 4.7 years) participated in the current study. The age range was selected in order to reduce the influence of age on motor skill learning (Meissner, Keitel et al. 2016). Inclusion criteria included no history of movement impairment or neurodegenerative disease and right handedness according to the Edinburgh Handedness Scale (Oldfield 1971). Additionally, participants were only included if they had no contraindication to transcranial magnetic stimulation (TMS) (Rossi, Hallett et al. 2009) as part of another experiment aimed at understanding immobilization effects on cortical excitability (**Chapter 2**). Data from four participants were excluded from analysis due to the following reasons: one participant had mixed-handedness according to the Edinburgh Handedness Scale; two participants gained explicit awareness of the sequenced button presses (more details below); and one participant took a nap in between sessions. Due to the well-documented effect of sleep on motor memory consolidation (King, Hoedlmoser et al. 2017), data from this participant were also excluded from analysis.

4.3.2 Motor Task Paradigm

The modified version SRTT used in the current study to assess skilled finger movements was described in **Chapter 3.3.2**. Briefly, the SRTT consists of repeated individuated finger movements that have an embedded 12-item sequence of button presses, unbeknownst to the participant. Test blocks consisted of both random sequence and repeated sequence button presses in order to assess the relative response times of the repeated (trained) sequence and the random (untrained) sequence. The training session, consisting of a PRE training test block, five training blocks, and one POST training block, took place during the AM session. A follow-up test block (RET) was completed after the six-hour immobilization period to assess retention of skill. The overall study design is illustrated in **Figure 4-1**. At the end of the evening session, the degree of explicit awareness for the sequence was assessed by asking if the participants noticed any pattern of button presses during the task. Participants that indicated that they noticed a pattern during training were asked to freely recall the sequence. The ability to freely recall 4 or more consecutive items of the 12-item sequence was considered explicit awareness of the presence of the repeating sequence (Robertson, Pascual-Leone et al. 2004, Cohen, Pascual-Leone et al. 2005).

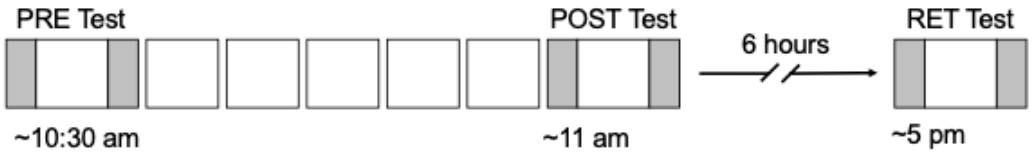

Figure 4-1. Study Design. Participants completed a session of training on a modified version of the Serial Reaction Time Task (SRTT) before a six-hour period of immobilization or an equivalent period of no immobilization. Participants completed a

follow-up retention test block of the SRTT six hours later in order to assess consolidation of skill. Gray blocks represent 50 random sequence button presses, and white blocks represent 180 repeated sequence button presses (15 repeats of a 12-item sequence).

4.3.3 Arm Immobilization

After the training session in the morning, participants were randomly assigned to undergo six hours of immobilization ('immobilization') (N=13) or an equivalent period of no immobilization ('control') (N=11). The immobilization protocol, described fully in **Chapter 2.3.3**, involved immobilization of the hand, wrist, elbow, and shoulder or the non-dominant (left) arm. Activity monitors (wGT3x-BT, ActiGraph) were worn on both wrists by participants in both groups in order to measure arm movements during the immobilization period.

4.3.4 General Motor Performance

Measures of general motor performance were calculated to assess skill improvement across task exposure. Response time was normalized to the average response time for the first 50 random button presses during the PRE test block in order to account for the between-subject variability in baseline motor performance. The first measure of general motor skill involved calculating the normalized response time for the first 50 random button presses of each test block. A two-way ANVOA, with within-subject factor of time (three levels: baseline (BL), pre-training (PRE), and post-training (POST)) and between-subject factor of group (two levels: immobilization or control group). Additionally, the average response time for the 180 repeated sequence button presses in each training block (Trains 1-5) was normalized to the first 50 button presses of the PRE test block. A two-way ANOVA was used to assess normalized response time

for repeated sequence performance across the training blocks (five levels: Training blocks 1-5) with between-subject factor of group (two levels: immobilization or control group).

4.3.5 Sequence-specific skill

Two measures of sequence-specific skill, Skill Score (SS) and Interference Score (IS), were calculated for each test block. Full details for the calculations of SS and IS can be found in **Chapter 3.3.7**. A two-way ANOVA was performed for each SS and IS with within-subject factor of time (three levels: PRE, POST, and RET) and between-subject factor of group (immobilization or control group).

4.3.6 Associations between behavioral and neurophysiological measures

To assess whether markers of immobilization-induced neuroplasticity in M1 influenced consolidation of skill on an individual level, we evaluated associations between changes in corticospinal excitability (CSE) (see **Chapter 2.3.2** for methodological details) and change in skill across the consolidation period. Correlation analyses were performed between change in CSE and change in general motor skill and also between change in CSE and change in SS across the consolidation period.

4.4 Results

Upon being asked if they noticed any pattern of button pushes, 2 participants (out of the 24 total participants) said they noticed a pattern. When asked if they could verbally repeat the sequence, they were correctly able to recall more than four items of

the sequence, so their data were excluded from further analyses. Therefore, data for 9 control participants and 11 immobilized participants were analyzed.

4.4.1 Activity Monitoring

Activity monitor-based activity counts showed that immobilized participants complied with the immobilization procedure (**Figure 4-2**). A two-way ANOVA showed significant effects of arm (F=24.7, p<.0001) and group (F=17.5, p=.0002), as well as a Group x Arm interaction (F=17.1, p=.0002). Sidak's multiple comparisons test demonstrated that immobilized participants used their immobilized (target) arm significantly less than their non-immobilized (nontarget) arm (t=6.7, p<.0001) and significantly less than the control participants used their target arm (t=5.9, p<.0001).

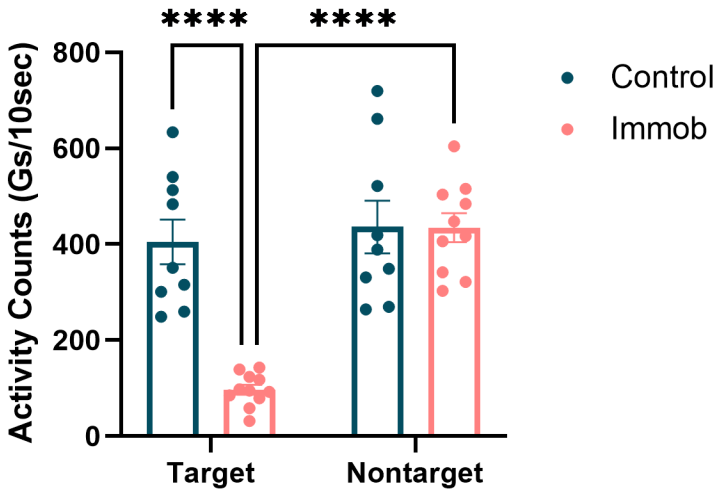

Figure 4-2. Immobilized participants complied with the immobilization protocol. Immobilized participants used their immobilized (target) arm significantly less than their non-immobilized (nontarget) arm and significantly less than the control participants used their target arm (****p<.0001). Error bars represent standard error.

4.4.2 General Motor Performance

Normalized response time decreased across participants with task exposure (**Figure 4-3**). General motor performance on the SRTT increased in both groups. A two-way ANOVA demonstrated that normalized response time significantly decreased, indicative of improved SRTT performance, for both random sequence ($F=74.0$, $p<.0001$) (**Figure 4-4**) and repeated sequence ($F=13.9$, $p<.0001$) (**Figure 4-3**). Sidak's multiple comparisons test demonstrated that in the control group, normalized response time for the random sequence was significantly reduced from PRE to POST ($t=9.5$, $p<.0001$) and from PRE to RET ($t=12.1$, $p<.0001$) test blocks. Normalized response time remained stable across the consolidation period, and there was not a significant difference from POST to RET test blocks ($t=2.3$, $p=.15$). The same pattern was observed in the immobilized group, where there was a significant reduction in response time from PRE to POST ($t=16.8$, $p<.0001$) and from PRE to RET ($t=4.5$, $p<.01$), while there was not a significant difference between POST and RET test blocks ($t=.71$, $p=.87$). There was not an effect of group nor a Group x Time interaction for either of these measures. Full ANOVA results are summarized in **Table 4-1**.

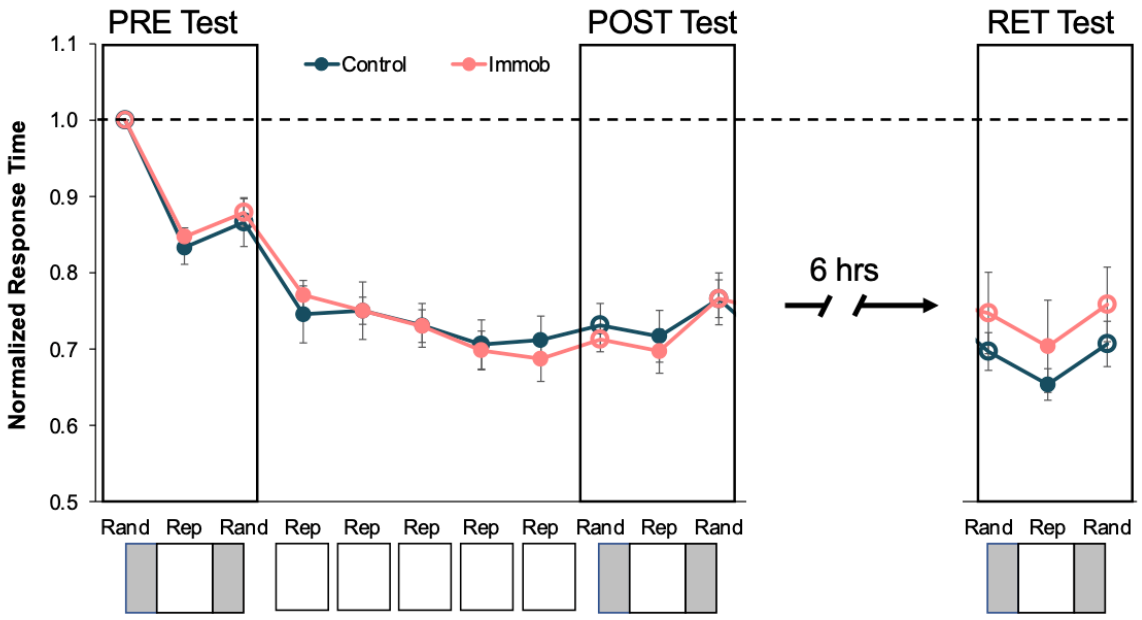

Figure 4-3. Normalized response time decreases with task exposure. Response times were normalized to the average response time for the first 50 random button presses of the baseline (PRE) test block in order to account for baseline variability in performance. Values less than one (dashed line) indicate faster than baseline performance. Open circles represent 50 random button presses, and closed circles represent 180 sequenced button presses. The three test blocks (PRE, POST, RET) are in the black rectangles. Error bars represent standard error.

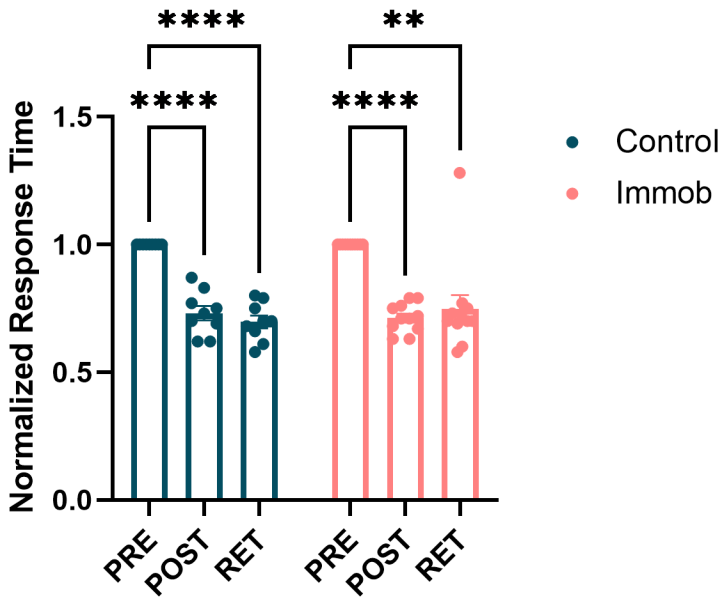

Figure 4-4. General motor performance increased with task exposure. Normalized response time significantly decreased over the training period, and this reduction was maintained after the consolidation period. There were no group differences in general motor performance **(****p<.01, ****p<.0001). Error bars represent standard error.

4.4.3 Sequence-specific skill

Despite improvements in general motor performance, sequence-specific skill did not significantly improve with task exposure (**Figure 4-5**). Additionally, there were not group differences in consolidation of sequence-specific skill. A two-way ANOVA showed no significant effect of time, group, nor a Group x Time interaction on skill score or interference score. Full ANOVA results are summarized in **Table 4-1**.

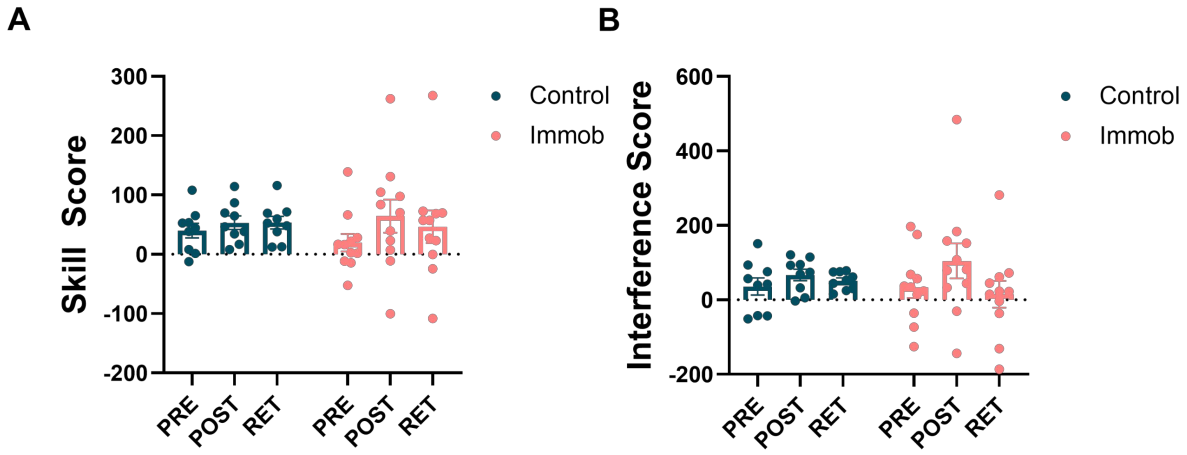

Figure 4-5. Sequence-specific skill did not significantly change with task exposure in either group. Measures of **(A)** Skill Score and **(B)** Interference Score did not significantly change across training nor across the consolidation period. Error bars represent standard error.

Table 4-1. SRTT ANOVA Results. Significant p-values are in bold.

Measure	Source	df	F Statistic	p-value
Random Sequence Performance	Time	2	74.0	**<.0001**
	Group	1	.13	.73
	Time X Group	2	.89	.42
Repeated Sequence Performance	Time	4	13.9	**<.0001**
	Group	1	.003	.95
	Time X Group	4	1.5	.21
Skill Score	Time	2	1.6	.22
	Group	1	.07	.80
	Time X Group	2	.44	.65
Interference Score	Time	2	2.6	.09
	Group	1	8.6e-006	.998
	Time X Group	2	.996	.38

4.4.4 Associations between behavioral and neurophysiological measures

Change in CSE was not significantly associated with change in general motor performance (r^2=.11, p=.31) nor change in sequence-specific skill (r^2=.03, p=.62) across the consolidation period (**Figure 4-6**).

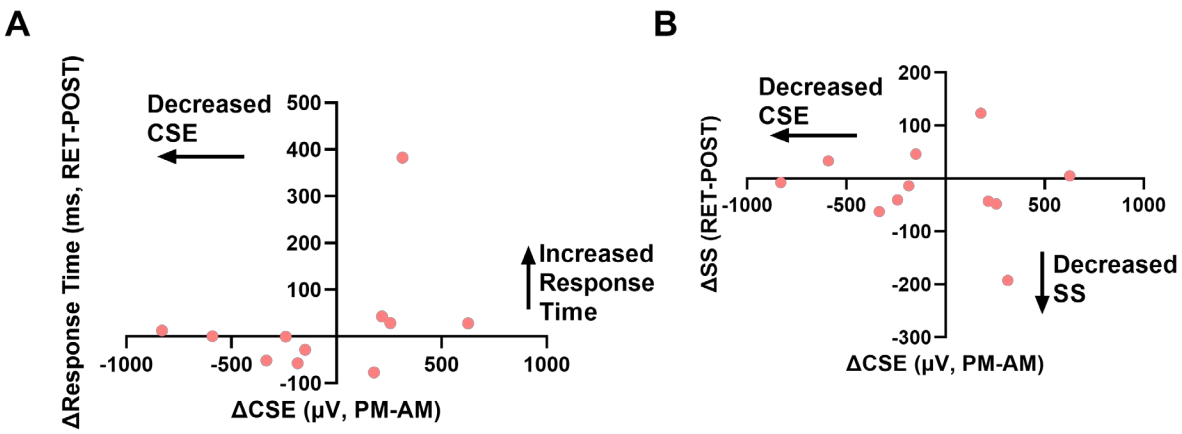

Figure 4-6. Changes in CSE across the immobilization period did not significantly correlate with change in **(A)** general motor skill or **(B)** skill score across the consolidation period.

4.5 Discussion

The current study aimed to examine the effect of short-term arm immobilization on consolidation of motor skill. Our results demonstrated that participants had improvements in skilled finger movements during training, and this skill improvement was retained after a six-hour consolidation period, suggesting that the motor memory was stabilized. One surprising finding was that sequence-specific skill did not significantly improve after training, and there was also no change in sequence-specific skill after the consolidation period. Additionally, there was no effect of immobilization on general motor performance nor sequence-specific skill at a group level, and neurophysiological markers of immobilization-induced plasticity were not associated with consolidation of skill at an individual level. Taken together, our results demonstrate that short-term immobilization does not significantly modify skill consolidation for individuated finger movements.

4.5.1 Consolidation of sequential, individuated finger movements is not enhanced after immobilization

Neuroplastic mechanisms in M1 contribute to consolidation of motor skills (Krakauer and Shadmehr 2006). Therefore, we hypothesized that the ability of short-term immobilization to enhance the capacity for LTP-like plasticity in M1 (Rosenkranz, Seibel et al. 2014) would lead to enhanced skill consolidation in M1. Participants completed a training session of the SRTT in the morning, followed by a six-hour period of immobilization or an equivalent period of no immobilization. A follow-up SRTT test block was performed in the evening session to assess retention of skill. Our results indicate that immobilization did not influence consolidation of motor skill on a task that required sequential finger movements. While this is the first study that examined the effect of short-term arm immobilization on consolidation of motor skill, other studies have utilized other neuromodulatory and behavioral approaches to alter consolidation of motor skill. For instance, disrupting M1 activity with TMS immediately after the end of training blocked consolidation of skill (Robertson, Press et al. 2005, Hotermans, Peigneux et al. 2008); however, this behavioral effect was not observed when disruption of M1 activity occurs two (Robertson, Press et al. 2005) or four (Hotermans, Peigneux et al. 2008) hours after the end of training. Additionally, learning a second motor skill immediately after training on the first skill disrupts consolidation of the first skill, but when the two tasks are spaced out by four hours, interference does not occur (Brashers-Krug, Shadmehr et al. 1996). Previous research has demonstrated that immobilization influences M1 excitability as early as three hours after the onset of immobilization (Karita, Matsuura et al. 2017), but whether immobilization influences M1 excitability earlier than three hours is still unclear. Therefore, it is possible that stabilization of the

trained motor skill mediated by M1 is largely complete by the time that immobilization influences cortical excitability and neuroplasticity.

Sleep is another method of neuromodulation that influences the consolidation of motor skill (Fischer, Hallschmid et al. 2002, King, Hoedlmoser et al. 2017). Research has shown that sleep serves as a homeostatic regulator of synaptic strength and plays a crucial role in the process of learning (Tononi and Cirelli 2014). Over the course of wakefulness, synapses are potentiated as an individual interacts with their environment (Vyazovskiy, Cirelli et al. 2008). Changes in synaptic strength throughout the day are reflected in an increase in cortical excitability in human frontal cortex (Huber, Maki et al. 2013) as well as resting gray matter cerebral blood flow in human sensorimotor cortices (Elvsashagen, Mutsaerts et al. 2019), both of which return to baseline levels after a period of sleep. While the process of synaptic potentiation during wakefulness is important for learning and behavioral adaptation, unregulated synaptic potentiation has the potential to lead to maladaptive over-excitation of neural circuitry and saturation of plastic capacity (Tononi and Cirelli 2014, Tononi and Cirelli 2020). Therefore, global downscaling of neural activity and synaptic strength during sleep may be necessary to restore homeostatic balance of synaptic strength and allow for future synaptic potentiation (Tononi and Cirelli 2014). Previous research has demonstrated that sleep after training enhances skill on a finger movement task regardless of the time of day of training (Fischer, Hallschmid et al. 2002). However, it has also been shown that consolidation of sequential finger movements is time-dependent and occurs over the course of the day, and is not sleep-dependent (Robertson, Pascual-Leone et al. 2004). For a full review of the effect of sleep on motor memory consolidation, see King, Hoedlmoser et al. (2017). It is still unclear whether short-term immobilization mimics

the effect of sleep, but both are thought to induce synaptic depression and enhance the capacity for plasticity within M1. Thus, it is possible that immobilization would be most likely to modify consolidation of motor skills that are normally influenced by sleep, rather than skills that may be consolidated over the course of the day.

While our original hypothesis that immobilization would enhance consolidation of motor skill was not supported by our results, it is important to note that immobilization did not negatively affect consolidation of motor skill either. This finding is in contrast to results from previous studies utilizing short-term immobilization that observed a decrement in motor performance after a period of arm immobilization (Huber, Ghilardi et al. 2006, Moisello, Bove et al. 2008, Weibull, Flondell et al. 2011, Bassolino, Bove et al. 2012, Bolzoni, Bruttini et al. 2012, Opie, Evans et al. 2016, Karita, Matsuura et al. 2017, Scotto, Meugnot et al. 2020). As discussed in **Chapter 3.5.3**, immobilization has been shown to influence motor performance on tasks that require proprioceptive information and control of proximal musculature of the upper extremity for performance. In contrast, the current study utilized a task that prioritized visual feedback over proprioceptive feedback, and fine control of distal musculature was needed rather than control of more proximal muscles. For these reasons, further studies utilizing different motor tasks would be required in order to fully elucidate whether immobilization influences consolidation of motor skill.

While short-term immobilization has been shown to enhance the capacity for neuroplasticity in M1, the effect of immobilization on other brain regions involved in consolidation of skilled finger movements has not been established. For example, posterior parietal cortex (PPC) (Pollok, Keitel et al. 2020) and supplementary motor area (SMA) (Tanaka, Honda et al. 2010) have been implicated in the consolidation of

sequenced motor skill. In addition, previous research suggests that the specific brain regions responsible for consolidation of motor skill can depend on whether the practice structure is continuous or variable (Kantak, Sullivan et al. 2010). Specifically, M1 has been implicated in consolidation when the motor skill was practiced continuously, as in the current study, but not when motor practice is interleaved with practice of other motor tasks.

4.5.2 Sequence-specific skill did not improve after training and was not influenced by immobilization

In the current study, training on the repeating sequence did not preferentially benefit response time for the repeating sequence relative to the random sequence. Participants became more skilled at pressing the buttons over time, as indicated by the decrease in response time, but skill improvement during training and the stabilization of skill over the consolidation period was not sequence-specific, despite training blocks consisting of only the repeated sequence of button presses. It is possible that features of the task itself, such as the color-matching component, impaired the ability to acquire and consolidate implicit sequence-specific skill. Matching the colored squares to the target square may have masked spatial information about the sequence that made the sequence more difficult to learn, even implicitly. Additionally, it is possible that the colors changing every trial was distracting to participants and interfered with the acquisition and consolidation of sequence-specific skill.

Neither group demonstrating sequence-specific learning could explain why our results showed no effect of immobilization on skill learning. If a single training session was not sufficient to induce sequence-specific learning in non-immobilized control

participants, then it may not be surprising that immobilization did not influence sequence-specific skill in the current study. It would be valuable to follow up using a task known to show single-session learning or look at the effect of short-term immobilization on repeated bouts of training. Examining the effect of short-term immobilization on multiple training sessions would be more translatable to application in clinical populations, where rehabilitative interventions occur over the course of multiple sessions.

4.5.3 Study limitations

One limitation of the current study, mentioned in **Chapter 3.5.5**, is that the color-matching design of the task itself may have influenced the degree to which sequence-specific skill was acquired. Since sequence-specific skill did not increase in either the non-immobilized control group or the immobilized group, the effect of immobilization on sequence-specific skill cannot be clearly determined from the current study findings. Future studies should assess the effects of immobilization on skill consolidation on other tasks more closely aligned with the effects of arm immobilization (e.g., skilled reaching movements requiring multi-joint coordination). Additionally, while activity monitors were worn bilaterally by participants, the specific activities that they engaged in during the consolidation period were not monitored. Therefore, it is possible that these specific activities contributed to the degree of consolidation of skill that was acquired prior to the consolidation period. Greater documentation of activities during the immobilization period or standardized activities should be employed to address this limitation in future studies.

4.6 Conclusions

Our results demonstrate that, at a group level, consolidation of skill on a task that requires individuated, sequenced finger movements is not influenced by short-term immobilization of the arm after training. It is possible that skill consolidation is largely complete by the time immobilization influences plasticity in M1. These results provide evidence for the specificity of the effects of short-term immobilization on motor behavior. Future studies should assess the effects of immobilization on skill acquisition using tasks that require proprioceptive feedback and/or multi-joint control of the upper extremity.

Chapter 5: Discussion

5.1 Summary of Results

The purpose of this dissertation was to identify the mechanisms underlying an enhanced capacity for neural plasticity in M1 after short-term immobilization and whether that enhanced plasticity influenced different stages of motor skill learning. The previous chapters described the effect of short-term arm immobilization TMS markers of cortical excitability, replicating previous findings that short-term immobilization influences changes in cortical excitability and interhemispheric interactions between primary motor cortices (M1s). Additionally, we demonstrated a novel finding that decreased corticospinal excitability (CSE) was associated with decreased intracortical inhibition, providing a novel mechanism by which short-term immobilization may enhance the capacity for neural plasticity in M1. Further experiments explored the possibility of utilizing the enhanced capacity for neuroplasticity after immobilization to benefit motor sequence learning. It was demonstrated that short-term arm immobilization did not influence acquisition or consolidation of sequenced motor skill. Below is a summary of key findings from each chapter (**Figure 5-1**).

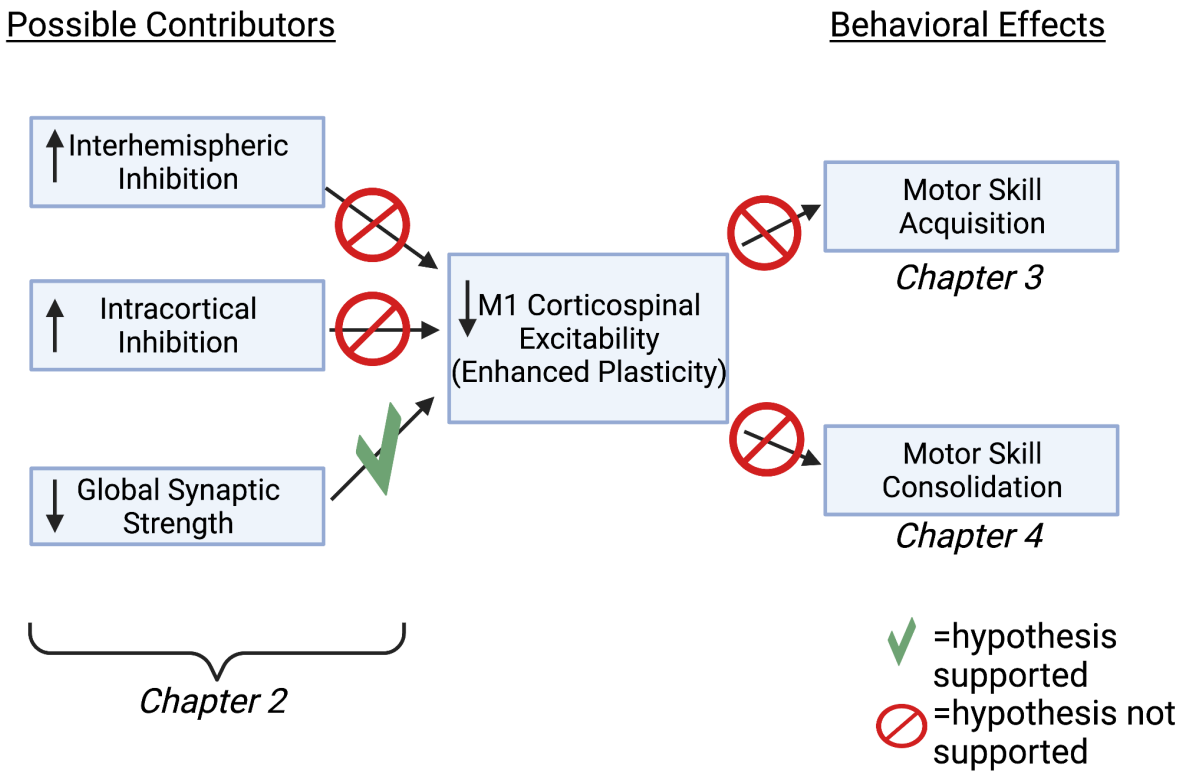

Figure 5-1. Summary of dissertation findings. The significant correlation between decreased intracortical inhibition and decreased corticospinal excitability (CSE) indicates that short-term immobilization may lead to a global decrease in synaptic strength, which may lead to an enhanced capacity for synaptic strengthening. While interhemispheric inhibition (IHI) increased onto the immobilized hemisphere, this increase did not correlate with decreased CSE, suggesting that immobilization may influence these two circuits independently. Additionally, short-term immobilization did not influence motor skill acquisition nor consolidation, providing no evidence to support the hypothesis that the enhanced capacity for synaptic strengthening after immobilization would enhance motor skill learning. However, characteristics of the motor task used in the current study could have contributed to these results. Each arrow represents a hypothesis that was tested.

Chapter 1 provided background on how integration of sensory and motor information contributes to skilled hand movements in healthy individuals as well as after stroke. It is also discussed how neuroplasticity within sensorimotor regions of the brain is necessary for learning skilled hand movements in healthy individuals as well as

for recovering hand motor function in stroke survivors. Finally, literature regarding methods to enhance the capacity for neuroplasticity within sensorimotor networks and the potential translation to stroke survivors was reviewed.

Chapter 2 examined the effect of short-term arm immobilization on CSE, interhemispheric inhibition, and intracortical inhibition in M1. We were able to replicate results from previous studies showing a decrease in CSE in the M1 corresponding to the immobilized arm (target M1) and an increase in interhemispheric inhibition from the opposite M1 to the target M1. While our results showed no effect of immobilization at the group level, decrease in CSE was significantly correlated with a decrease in $GABA_A$-ergic intracortical inhibition in the target hemisphere of immobilized participants. Our results indicate that the effect of immobilization on interhemispheric inhibition is likely due to increased activity of the excitatory transcallosal projections rather than an increase in intracortical inhibition. Additionally, the relationship between change in CSE and change in intracortical inhibition suggests that global downscaling in excitability may underlie the enhanced capacity for LTP-like plasticity that is seen after immobilization.

Chapter 3 presented data on the effect of short-term arm immobilization on motor skill acquisition, where participants were either immobilized or not immobilized for six hours prior to a training session of the Serial Reaction Time Task (SRTT). The SRTT includes both random and repeated sequence button presses, allowing for the assessment of improvement of both general motor skill and sequence-specific skill. Participants all completed a test block of the task prior to the immobilization period in order to assess and control for baseline motor performance. Interestingly, contrary to previous research using immobilization, no detriment in motor performance was seen

immediately after the immobilization period. Additionally, there was no effect of immobilization on acquisition of general motor skill on the SRTT.

SRTT data showed that motor performance increased, as measured by a decrease in response time, over the training period. However, on average, performance improvement was not preferential for the trained repeated sequence relative to the untrained random sequence button presses. This indicates that participants acquired general motor skill but did not acquire sequence-specific skill.

Chapter 4 investigated whether short-term arm immobilization influenced consolidation of sequenced motor skill. In this chapter, participants underwent a training session in the morning prior to a six-hour period of arm immobilization or an equivalent period of no immobilization. A follow-up retention test block after the immobilization period was used to assess skill consolidation. Results demonstrated that immobilization did not influence consolidation of motor skill. Similar to Chapter 3, skill improvement over the training period was not sequence-specific, and immobilization did not negatively impact general motor performance.

Taken together, our results suggest that while short-term immobilization of the arm modulates neurophysiological markers of plasticity in M1, it does not influence performance or learning on a motor task that involves individuated, sequenced finger movements. Further, our results demonstrated that neurophysiological changes indicative of enhanced plasticity capacity were not significantly associated with SRTT skill acquisition or consolidation, supporting the idea that neurophysiological changes after immobilization did not significantly contribute to behavioral changes on the motor task employed. The remainder of this discussion will focus on the potential of

immobilization to enhance motor learning, considerations of the SRTT in the context of this research, future directions, and limitations.

5.2 Immobilization as a method to enhance motor learning

Previous research studying the effects of short-term immobilization has indicated that markers of neural plasticity within M1 are altered after immobilization. For example, the decrease in corticospinal excitability seen after immobilization is thought to be due to synaptic depression (Huber, Ghilardi et al. 2006), and the capacity for LTP-like plasticity induction, was enhanced after immobilization of the upper limb (Rosenkranz, Seibel et al. 2014). These studies suggest that immobilization acts in a homeostatic manner to induce LTD-like plasticity, which in turn increases the capacity for LTP-like plasticity. Since our results replicated the findings of changes in markers of neuroplasticity within M1, we anticipated that enhanced capacity for LTP-like plasticity after immobilization would benefit task-specific synaptic strengthening. We hypothesized that immobilization would lead to greater skill acquisition and skill consolidation of the SRTT, a well-validated motor learning paradigm. Our results did not support this hypothesis, and immobilization did not benefit learning of skilled, individuated finger movements.

Rather than an increase in the general capacity for plasticity within M1, it is possible that short-term immobilization enhances the capacity for neuroplasticity in a circuit-specific manner. The method that was previously used to probe the capacity for LTP-like plasticity after immobilization was paired associative stimulation (PAS) (Rosenkranz, Seibel et al. 2014). PAS is a technique that repeatedly pairs peripheral nerve stimulation and TMS to the contralateral M1 at an interstimulus interval,

leveraging spike-timing dependent plasticity to strengthen the connections between primary somatosensory cortex (S1) and M1 (Classen, Wolters et al. 2004). After immobilization, the degree of enhanced capacity for plasticity, as measured by amount of PAS induction, was correlated with the degree of decrease in CSE (Rosenkranz, Seibel et al. 2014). Together, these results implicate the neural circuitry including S1-M1 connections as a potential driver behind the neuroplastic effects of short-term immobilization. It is, therefore, possible that only performance on motor tasks that utilize these S1-M1 connections would be influenced by a period of immobilization. Performance on the modified version of the SRTT may require some degree of tactile feedback from pressing the buttons, but since the position of the button box and hand are fixed, other types of sensory feedback, such as proprioceptive feedback, are less relevant. This hypothesis is supported by the finding that a decrease in CSE, a marker of enhanced capacity for plasticity within M1 after immobilization, was not associated with acquisition or consolidation of skill using the modified SRTT. Indeed, motor function that requires inter-joint coordination and proprioceptive feedback has been shown to be altered after immobilization (Huber, Ghilardi et al. 2006, Bassolino, Bove et al. 2012, Bolzoni, Bruttini et al. 2012, Opie, Evans et al. 2016, Scotto, Meugnot et al. 2020). Reductions in inter-joint coordination were associated with the degree of reduction in somatosensory evoked potentials (SEPs) in S1 after immobilization (Huber, Ghilardi et al. 2006), demonstrating that the changes in cortical excitability after immobilization are behaviorally relevant. Interestingly, delivering vibration that preferentially activates proprioceptive receptors during immobilization reduces the effect of immobilization on corticospinal excitability (Avanzino, Pelosin et al. 2014). Our results finding no effect of immobilization on motor performance on a task requiring individuated, sequenced

finger movements may indicate that immobilization more specifically modulates activity in the S1-M1 circuit, and tasks that are less dependent on this circuit may not be influenced. More research needs to be done utilizing other task designs to understand whether immobilization holds promise for enhancing motor learning in tasks that require proprioceptive feedback.

5.3 Implications for clinical translation

The current research was conducted with healthy, young participants with the intention of understanding ways to noninvasively enhance the capacity for use-dependent plasticity in M1 and eventually translating these findings to benefit motor recovery of individuals with motor deficits, such as stroke survivors. The results of the current study showing no effect of immobilization on acquisition or consolidation of motor skill do not provide support for the translation of immobilization to clinical populations at this point. However, our results demonstrating an increase of both short- and long-interhemispheric inhibition onto the immobilized hemisphere provide an interesting possible avenue for further consideration. The interhemispheric imbalance model (Murase, Duque et al. 2004) suggests that, after stroke, a decrease in inhibition from ipsilesional motor cortex (iM1) to contralesional motor cortex (cM1) leads to hyperexcitability of cM1 and subsequent increase in inhibition of iM1 (Murase, Duque et al. 2004). Previous research has demonstrated that greater IHI imbalance correlates with poorer motor functioning and may contribute to poorer motor recovery (Murase, Duque et al. 2004). However, recent research has suggested that IHI imbalance may not occur in all stroke survivors and may be influenced by factors such as movement phase and time post-stroke (Boddington and Reynolds 2017, Xu, Branscheidt et al. 2019).

Therefore, it is possible that utilizing short-term immobilization may help rebalance IHI in some, but not all, stroke survivors.

Additionally, the significant association between decreased CSE and decreased intracortical inhibition may indicate that immobilization can be used to decrease GABA-ergic inhibition in some individuals. As mentioned in Chapter 1.5.2, reductions in GABAergic inhibition have been related to functional motor recovery in mice (Clarkson, Huang et al. 2010) and humans (Blicher, Near et al. 2015) and may serve to facilitate neuroplasticity in M1 (Hess, Aizenman et al. 1996, Sanes and Donoghue 2000, Murphy and Corbett 2009, Paik and Yang 2014). Therefore, if short-term immobilization can be used to decrease intracortical inhibition, it is possible that it could have utility in the motor recovery process after stroke. However, reduction in intracortical inhibition was not consistent across all participants; therefore, it is crucial to understand the individual factors that influence the change in intracortical inhibition before considering immobilization as a potential therapeutic intervention.

In order to translate a therapeutic intervention most effectively, such as short-term arm immobilization, into clinical practice, it is necessary to establish the dosing of immobilization required to lead to lasting effects on motor function as well as the motor skill that would be most likely to be benefitted by immobilization. The current study only examined a single training session and a single bout of short-term immobilization. While previous research has found that a single bout of short-term immobilization is sufficient to influence motor behavior (Huber, Ghilardi et al. 2006, Bassolino, Bove et al. 2012, Bolzoni, Bruttini et al. 2012, Opie, Evans et al. 2016), the effects of repeated bouts of short-term immobilization are less well understood. Typically, rehabilitative interventions after stroke require multiple training sessions to result in skill learning

and improved motor function (Lohse, Lang et al. 2014). Therefore, it would be necessary to understand the effects of repeated bouts of short-term immobilization in order to evaluate the possibility of integrating short-term immobilization into plans for rehabilitation. In addition to understanding dosing effects, translation of short-term immobilization to clinical practice would need further investigation regarding which motor skills would benefit most from this intervention. The results from **Chapters 3 and 4** suggest that motor tasks requiring individuated finger movements are not influenced by a single bout of short-term immobilization. Therefore, a single session of immobilization may be less useful to incorporate into a rehabilitation plan with the goal of improving individuated finger movements. If results from future studies indicate that enhanced plasticity after immobilization benefits training on tasks that require inter-joint coordination or control of more proximal musculature, immobilization may be more relevant in cases where improving those aspects are the goal. Overall, further research regarding dosing and behavioral effects would be necessary before clinical translation of short-term immobilization could occur.

5.4 Methodological considerations for short-term immobilization

One difficulty in comparing existing studies utilizing short-term immobilization of the upper limb is that the method and duration of immobilization is not consistent across studies. Various immobilization protocols involve immobilizing the whole arm (Avanzino, Bassolino et al. 2011, Langer, Hanggi et al. 2012, Avanzino, Pelosin et al. 2014, Opie, Evans et al. 2016, Ikeda, Oka et al. 2019), only the hand and wrist (Crews and Kamen 2006, Huber, Ghilardi et al. 2006, Clark, Taylor et al. 2010, Karita, Matsuura et al. 2017), or only a few fingers (Facchini, Romani et al. 2002). These

differences are likely to influence the activities that participants are able to perform during the immobilization period and therefore the amount of tactile and proprioceptive information that is received in the corresponding sensorimotor cortical areas. In the current study, we chose to immobilize the entire arm in order to limit as much sensory stimulation to the target sensorimotor cortical areas as possible.

Additionally, while the current study involved six hours of immobilization, other studies have used immobilization periods of eight (Rosenkranz, Seibel et al. 2014, Opie, Evans et al. 2016), ten (Avanzino, Bassolino et al. 2011, Ikeda, Oka et al. 2019), or twelve hours (Huber, Ghilardi et al. 2006). Longer time periods of 48 hours (Meugnot and Toussaint 2015), one week (Lundbye-Jensen and Nielsen 2008), two weeks (Langer, Hanggi et al. 2012, Newbold, Laumann et al. 2020), and three weeks (Clark, Taylor et al. 2010) have also been used. One study by Karita, Matsuura et al. (2017) demonstrated that CSE in the target M1 decreased after three hours of immobilization and continued to decrease throughout a 24-hour immobilization period and returned to baseline levels three hours after removal of the cast. No excitability measurements were taken before three hours after immobilization onset, so it is unclear whether changes in excitability begin even earlier. These results demonstrate that the duration of immobilization is likely to influence the results of the TMS assessments. Therefore, it is important to assess a comprehensive battery of TMS measures in individuals that underwent a consistent period of immobilization in order to fully characterize the changes in M1 excitability that occur after immobilization.

It is important to note that the results from the current study are specific to upper limb immobilization. It is unclear whether the results from this study would translate to immobilization of the lower limb, since immobilization to upper and lower

extremities may have different effects due to the differing levels of descending cortical control (Campbell, Varley-Campbell et al. 2019, Campbell, Varley-Campbell et al. 2019). Additionally, immobilizing one of the lower limbs would be more likely to limit the mobility that participants would have during the immobilization period and influence neurophysiological or behavioral outcome measures.

5.5 Utilization of the SRTT to assess behavioral effects of short-term immobilization

The Serial Reaction Time Task (SRTT) (Nissen and Bullemer 1987) is a widely used motor learning paradigm (Hardwick, Rottschy et al. 2013) that involves participants learning to coordinate a series of individuated finger movements. This task design has several advantages. The first advantage is that the task setup is easy to establish, needing only a keyboard or button box and a computer monitor. Additionally, the calculation of outcome measures is straightforward and easy to interpret across studies. Implicit learning on the SRTT increases the cortical representation of the trained fingers in M1 (Pascual-Leone, Grafman et al. 1994), and training in skilled hand movements has been shown to improve hand function, at least in older adults (Ranganathan, Siemionow et al. 2001). These results provide support for utilizing the simple SRTT paradigm to assess overall motor functioning.

One consideration when using the SRTT is that the outcome measure, response time, may be less sensitive to potential kinematic changes that can occur with improved task performance. Response time is the time from visual stimulus presentation to the button press that the participant makes. Response time is composed of two components: (1) reaction time, which is the time from visual stimulus presentation to

the onset of movement, and (2) movement time, which is time from movement onset to the button press. Our results showed that response time decreases across training, but it is unclear whether that is due to a decrease in reaction time, movement time, or a combination of both. Electromyography (EMG) could be used in future studies to determine movement onset time in order to calculate both reaction time and movement time in addition to response time.

The version of the SRTT used in the current study was different from the standard SRTT version that is commonly used, and it is possible that these modifications impacted the ability of participants to acquire sequence-specific skill. Our version of the SRTT, modified from Clos, Sommer et al. (2018), utilizes colors that change randomly in order to mask the repeated sequence of button presses and prevent the participants from becoming explicitly aware of the repeating sequence. Additionally, the color-matching component of the task minimizes the ability of the participants to learn the repeated sequence based on the location of the screen in which the stimulus appears. One possibility is that the randomly changing colors is distracting enough to prevent the sequence from being acquired, even implicitly. Another possibility is that having the participants match the color of the correct square with a target square that has a fixed position reduces or eliminates the amount of spatial information about the sequence that may be necessary for sequence-specific skill to be acquired. The necessity of spatial information for acquiring sequenced finger movements is currently unclear but could be explored in future studies.

5.6 Limitations

One limitation to the current set of experiments is that we did not control for the specific activities that participants completed during the six-hour break between the morning and evening testing sessions. Although the activity tracker data demonstrated that participants in the immobilization group had markedly decreased movement in their immobilized arm, we did not ask participants to record specific information about what they did during the immobilization period. Many of the participants in our study were students and may have used the time between sessions to study or work on assignments, while others mentioned playing tennis or doing yoga. It is possible that varying degrees of physical and mental energy expended during the immobilization period influenced the large inter-individual variability in our neurophysiological or behavioral outcome measures. It is also possible that whether a participant was in the immobilization or control group influenced the specific activities they chose to perform during the break. For instance, it would be difficult for a person to play tennis while wearing the immobilization setup. Future studies should ask participants to complete a log of activities that they completed during the break or standardize activities for all participants.

While results from **Chapter 3** demonstrate that immobilization did not influence acquisition of skill on the modified SRTT, it is not possible to determine whether there were group differences in learning of the skill acquired. A follow-up delayed retention test after the acquisition period would allow us to fully assess the effects of short-term immobilization on motor learning when immobilization occurs before training. It is possible that the effects of immobilization on motor skill learning would be better reflected in the consolidation of skill acquired after a period of

immobilization, but those data were not collected within the current experimental design. Additionally, based on results from **Chapters 3 and 4** that showed no effect of immobilization on different stages of motor memory formation, there may have been a mismatch between immobilization involving the whole arm and the modified SRTT that required individuated finger movements. Outcome measures for this task may have been less sensitive to kinematic or proprioceptive changes that occurred after short-term arm immobilization. If we had employed a task that utilized proprioceptive feedback and/or multi-joint coordination, it is possible that immobilization would have influenced skill acquisition or consolidation and is an avenue for future investigation.

Another limitation is that we did not have sufficient power to evaluate potential sex differences in our neurophysiological and behavioral measures. It has previously been demonstrated that sex does not influence corticospinal excitability at rest (Livingston, Goodkin et al. 2010), and changes in corticospinal excitability and intracortical inhibition seen after aerobic exercise are not influenced by biological sex nor ovarian hormones (El-Sayes, Turco et al. 2019). However, sex has been shown to influence IHI (De Gennaro, Bertini et al. 2004), and fluctuations in estrogen and progesterone over the course of the menstrual cycle are associated with changes in IHI (Hausmann, Tegenthoff et al. 2006). We did not collect information about menstrual cycle, so it is not possible to rule out the effect of sex hormones on our measures of interhemispheric inhibition. Additionally, research has indicated that biological sex influences the acquisition of skilled hand movements, possibly due to differences in anatomical and functional organization of the neural systems required for production of these movements (Cohen, Pomplun et al. 2010). In the current study, we only categorized participants as male or female and did collect information about gender

identity. In order to fully assess the effects of sex and gender on outcome measures, it would be helpful to ask participants to indicate sex assigned at birth as well as gender identity. However, researchers would need to ensure that the sample size is large enough to have the statistical power required to assess group differences in outcome measures.

5.7 Conclusions

Chapter 2 aimed to identify neurophysiological effects of short-term arm immobilization that would contribute to an enhanced capacity for synaptic strengthening. Results from this chapter demonstrate that immobilization reduces CSE and increases IHI, consistent with our hypotheses and previous findings. Interestingly, decreased CSE was significantly associated with a decrease in $GABA_A$-ergic intracortical inhibition, suggesting that short-term arm immobilization may lead to a global downscaling of synaptic strength in M1. Reduced intracortical inhibition may provide a novel mechanism by which short-term immobilization may enhance the capacity for LTP-like plasticity within M1 and may be a potential target for strategies to augment plasticity capacity to enhance motor learning in health and disease (**Figure 5-1**). Due to inter-individual variability in response to the immobilization protocol, as measured by change in CSE, further research could be done to identify predictors of whether immobilization would influence CSE in a given individual. It is also still unclear whether neurophysiological changes in regions interconnected with M1 are associated with the markers of enhanced capacity for neuroplasticity identified in Chapter 2.

Our results from Chapters 3 and 4 suggest that short-term immobilization of the arm does not significantly augment acquisition or consolidation of skill on a task that

requires individuated, sequenced finger movements (**Figure 5-1**). However, it is possible that characteristics of the modified version of the SRTT used in the current study influenced our results. Additionally, future studies should aim to understand the timing of the neurophysiological effects of short-term immobilization and their interaction with potential behavioral effects. Overall, our results provide evidence for the specificity of behavioral effects of short-term immobilization. Future studies should assess the effects of immobilization on skill acquisition using tasks that require proprioceptive feedback or more proximal control of the upper extremity.

Taken together, results from this dissertation indicate that while neurophysiological markers of plasticity capacity were enhanced after a period of short-term arm immobilization, that enhanced capacity for plasticity did not translate into greater acquisition or consolidation of a motor skill involving individuated finger movements. Based on these results, there is not clear evidence that immobilization augments skill learning; however, the effect of immobilization on neurophysiological and behavioral measures utilizing other circuits within the sensorimotor network should be assessed. Specifically, measures and tasks that assess functioning of the connection between primary somatosensory cortex (S1) and M1 are most likely to be influenced by immobilization. To provide a more comprehensive understanding of how immobilization influences circuitry involved in sensorimotor learning, the effects of immobilization on other brain regions, such as posterior parietal cortex (PPC), premotor cortex (PMC), and supplementary motor cortex (SMA), should also be investigated Future studies are needed to investigate ways to leverage the markers of enhanced capacity for plasticity seen after short-term immobilization to benefit motor skill learning. Additionally, neurophysiological changes after immobilization, such as

increased IHI and decreased intracortical inhibition, could be investigated as a potential method to improve motor function in stroke survivors or other neurologic conditions.

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