博士論文

Cortical and spinal neural interactions between multiple muscles in humans

(筋間神経相互作用に関与する皮質・脊髄内神経機構)

佐々木 睦

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Abbreviations

Abbreviations	Term
ANOVA	analysis of variance
AP	anterior-posterior
BB	biceps brachii muscle
BF	biceps femoris muscle
CMEP	cervicomedullary motor evoked potential
CMS	cervicomedullary junction stimulation
CPG	central pattern generator
EMG	electromyography
ES	erector spinae muscle
FCR	flexor carpi radialis muscle
FDI	first dorsal interosseous muscle
FDS	flexor digitorum superficialis
FEBH	force expiration while breath holding
fMRI	functional magnetic resonance imaging
iEMG	integrated electromyography
LM	lateral-medial
M1	primary motor cortex
MEP	motor evoked potential
MG	medial gastrocnemius muscle
MS	masseter muscle
MT	motor threshold

MVC	maximum voluntary contraction
PA	posterior-anterior
PMd	dorsal premotor area
RA	rectus abdominis
SAI	short-latency afferent inhibition
SD	standard deviations
SE	standard error
SICI	short interval intracortical inhibition
SICF	short-interval intracortical facilitation
SMA	supplementary motor area
SOL	soleus muscle
TA	tibialis anterior
TMS	transcranial magnetic stimulation
VM	vastus medialis

Chapter 1

General introduction

1.1. Introduction

During activities of daily living such as reaching and walking, proper coordination of multiple segments, including the upper-limb, trunk, and lower-limb muscles, is essential. This interaction between multiple muscles is coordinated within the central nervous systems (CNS) and underlies important mechanisms related to human motor control (Massion 1992). For example, when reaching for an object, the CNS needs to send motor commands simultaneously to the upper limb for reaching and the trunk and lower limbs for maintaining an upright posture. In order to execute the intended movements, motor commands generated in the brain are transmitted to the muscles via the spinal cord through intricate intraneuronal circuits. Since the primary motor cortex (M1) has a role to execute motor commands by transmitting them to the spinal cord and the muscles, it is believed to be a "final common path determiner of movements" (Goldring et al. 1970). Thus, the M1 and the corticospinal systems are involved in all aspects of motor control that regulate human movements. To understand the mechanism of human motor control, it is therefore important to understand the activity of the M1 and corticospinal pathways, which are responsible for the motor outputs. Although there have been many behavioral studies examining muscle activity and motion analysis to understand how coordinated control of multiple muscles is realized, the contribution of the M1 and corticospinal systems remains largely unexplored. Therefore, the goal of my thesis is to advance the understanding of the fundamental mechanisms responsible for coordinated muscles control within the M1 and corticospinal circuits, and by doing so contribute to the development of rehabilitation methods that can assist recovery after neurophysiological injuries such as spinal cord injury and stroke.

1.2. Techniques to assess corticospinal excitability (Transcranial magnetic stimulation)

Corticospinal projection from the M1 to a muscle has an essential role in voluntary motor control (Lemon 2008). Numerous studies in humans and animals have demonstrated contributions of corticospinal activity to motor control, motor learning, as well as motor recovery after neurological injury (Lemon 2008). Transcranial magnetic stimulation (TMS) has been progressively improved as a non-invasive approach for investigating corticospinal activity in humans (Rothwell et al. 1991). TMS applied over the M1 can indirectly activate pyramidal neurons via activation of interneurons that input to the corticospinal neurons (Di Lazzaro et al. 2012). Stimulation evokes responses in the contralateral muscles, which can be measured using surface electromyography (EMG). These TMS-evoked responses are referred to as motor evoked potential (MEP). TMS can be used to assess the excitability of different neural mechanisms in the corticospinal system by varying the number of stimuli, intensity, and the current (coil) direction induced in the brain.

Single-pulse TMS on the M1 can assess corticospinal excitability between the brain and a muscle evaluated by examining the peak-to-peak amplitude of MEPs (Day et al. 1989). The TMS coil position is placed over the M1 region that represents the target muscles, and the coil direction is usually orientated to produce posterior to anterior currents. The stimulus intensity is determined based on the motor threshold which is defined as the minimum TMS intensity for which MEPs have peak-to-peak amplitudes larger than 50 μV if the target muscles are relaxed (Rossini et al. 2015). It was also known that MEPs elicited by single-pulse TMS reflect the summed corticospinal output, which could be affected by both cortical and subcortical excitability and hence cannot access either of these contributions in isolation through only single-pulse TMS-induced MEPs (McNeil et al. 2013).

Paired-pulse TMS is a technique that can assess the intracortical inhibition and facilitation circuits in the motor cortex. Using this technique, two magnetic stimuli [i.e., the conditioning stimulus (CS) and test stimulus (TS)] are provided through a coil and the MEP amplitudes are compared to those elicited by the TS alone as a reference (control) condition. Short interval intracortical inhibition (SICI) can be elicited when a subthreshold CS is followed by a suprathreshold TS with interstimulus intervals (ISIs) of 1-6 ms (Kujirai et al. 1993). Neuropharmacological studies revealed that the magnitude of SICI reflects the activity of gamma-aminobutyric acid type A ($GABA_A$) interneurons in the M1 (Ziemann et al. 1996; Di Lazzaro et al. 2007). Short interval intracortical facilitation (SICF) can be elicited by TS at a suprathreshold intensity which is followed by the CS at a suprathreshold or motor threshold level with ISIs at around 1.5, 2.9, and 4.5 ms (Tokimura et al. 1996; Ziemann et al. 1998). Neuropharmacological studies also reported that the magnitude of SICF reflects the activity of glutamatergic and $GABA_A$ interneurons within the M1 (Rossini et al. 2015).

It is also possible to selectively assess the activity of different subsets of intraneuronal circuits that input onto corticospinal neurons by using different current (coil) directions induced by TMS pulses. Specifically, TMS-induced posterior-anterior (PA) directed currents across the central sulcus preferentially evoke early indirect (I)-wave which is synaptic activities of interneurons projecting to corticospinal neurons in M1, and highly synchronized with corticospinal activity. On the other hand, anterior-posterior (AP) currents preferentially evoke late I-wave, which is less synchronized with corticospinal activity and may reflect the activity of premotor regions rather than the M1 (Aberra et al. 2020; Spampinato 2020). On the other hand, lateral-medial (LM) currents preferentially evoke direct (D)-wave, which reflects direct activation of the corticospinal axons (Day et al. 1989; Di Lazzaro et al. 2001). Therefore, using different

current orientations, it is possible to examine the activity of different subsets of interneurons that input to corticospinal neurons.

1.3. Techniques to assess spinal excitability

Since MEPs elicited by single-pulse TMS reflect the net corticospinal output affected by both cortical and subcortical excitability and therefore cannot measure either in isolation, by assessing the excitability of spinal circuits in addition to the corticospinal excitability, contributions of cortical and spinal circuits during motor control could be examined. The methods used in my thesis to assess spinal excitability are described below:

1.3.1. Transcutaneous spinal cord stimulation (tSCS)

Single- (or paired-) pulse tSCS can be used to reliably elicit spinal reflexes through the activation of monosynaptic connections between the Ia sensory fibers and motoneurons at multiple spinal levels innervating the lower-limb muscles (Courtine et al. 2007; Minassian et al. 2007; Dy et al. 2010; Roy et al. 2012; Masugi et al. 2016, 2017). To elicit responses in multiple lower-limb muscles simultaneously, a constant current electrical stimulator is used to apply single monophasic square pulses with 1-2 ms pulse widths. The cathode electrode is placed on the spine between L1 and L2 lumbar spine processes, and the anode electrode is placed on the trunk above the umbilicus (Roy et al. 2012). Computer simulations and experimental studies using animal models as well as human participants have shown compelling evidence that electric impulses induced by tSCS can primarily activate the afferent fibers in the posterior roots of the spinal cord (Minassian et al. 2007; Danner et al. 2011). Since the stimulated afferent fibers activated by tSCS have synaptic connections to the spinal interneurons and motoneurons, tSCS application can therefore be utilized to modulate spinal motor excitability. Although spinal excitability can be

assessed using the Hoffman-reflex (H-reflex), a practical limitation of this technique is that they can only be obtained consistently from one target muscle (typically soleus) (McNeil et al. 2013). Therefore, by using tSCS, the spinal reflex excitability during motor control can be assessed in multiple lower-limb muscles simultaneously.

1.3.2. Cervicomedullary junction stimulation (CMS)

Single-pulse CMS is a technique that can be used to evoke cervicomedullary motor evoked potentials (CMEP) which can be recorded using surface EMG. In the arm, leg, and trunk muscles CMEP responses can be evoked by delivering a single magnetic (or electrical) stimulus on the spinal cord to activate the axons of the descending motor pathways (Ugawa et al. 1991, 1994; Gandevia et al. 1999; Taylor and Gandevia 2004; Chiou, Strutton, et al. 2018). Specifically, to evoke CMEP responses, axons should be activated at the level of the cervicomedullary junction, near the pyramidal decussation (Taylor and Gandevia 2004). Although CMS could also possibly activate descending motor pathways other than those in the corticospinal tract, as well as the ascending pathways, there is robust evidence that CMS-evoked responses (i.e., CMEPs) primarily reflects the result of motoneuronal (subcortical pathway) activation via the descending volley elicited by excitation of the corticospinal axons (Berardelli et al. 1991; Ugawa et al. 1991; Gandevia et al. 1999; Taylor et al. 2002; McNeil et al. 2013). Specifically, CMEP responses elicited by stimulation of the corticospinal tract at the cervicomedullary junction were shown to reflect the efficacy of the corticospinal-motoneuronal synapse (Taylor and Gandevia 2004). Therefore, CMEP could be utilized to evaluate subcortical neural excitability, while MEPs elicited by TMS include the excitability of supraspinal mechanisms and reflect corticospinal excitability.

1.3.3. F-wave

F-wave can also be recorded using surface EMG and reflects backfiring of a small number of motoneurons recruited by antidromic impulse induced by supramaximal stimulation of a peripheral nerve innervating the targeted muscle (McNeil et al. 2013). When the supramaximal peripheral nerve stimulation is applied, orthodromically evoked compound muscle action potential (i.e., maximal M-wave) appears, and then antidromically evoked compound muscles action potential (i.e., F-wave) can be observed. Investigating change in the F-wave amplitude and persistence can be used to evaluate motoneuronal excitability

1.4. Background

1.4.1. Interlimb interaction in corticospinal and spinal circuits

The coordination of the muscles of the upper and lower limbs is important in various human movements. Previous studies using single-pulse TMS reported that contraction of certain upper-limb and lower-limb muscles can modulate the corticospinal excitability not only of the contracted muscle, but also of other muscles which are located in a remote segment of the body and remain at rest. This provides evidence for the existence of interlimb neural interaction in the corticospinal pathway (Kawakita et al. 1991; Pereon et al. 1995; Boroojerdi et al. 2000; Hortobágyi et al. 2003). Specifically, this phenomenon is known as "remote effect" or "cross facilitation" (Tazoe, Endoh, et al. 2007; Tazoe, Sakamoto, et al. 2007; Tazoe and Komiyama 2014; Komeilipoor et al. 2017). Therefore, by investigating whether the neural activity of the target muscle is modulated by the contraction of the remote muscle and how this is modulated, the neural interaction between different muscles can be explored. Typically, "remote effect" neural interactions have been studied between upper-limb and lower-limb segments by assessing spinal monosynaptic reflexes (i.e., H-reflex) (Kawamura and Watanabe 1975; Borroni et al. 2004; Tazoe et al. 2005). The facilitation of spinal monosynaptic reflexes elicited in lower-limb muscles by bilateral hand muscle contraction was first demonstrated over 100 years ago, using a phenomenon known as the Jendrássik maneuver (Jendrássik E 1883). Since the early 1990s, interactions between upper-limb and lower-limb muscles in the corticospinal pathway have also been studied (Kawakita et al. 1991; Pereon et al. 1995). Similar to the effect of the Jendrássik maneuver on spinal monosynaptic reflex, contraction of upper-limb or lower-limb muscles can facilitate corticospinal excitability of a muscle which is located in a distal limb (Kawakita et al. 1991; Pereon et al. 1995; Hortobágyi et al. 2003; Tazoe, Sakamoto, et al. 2007; Tazoe et al. 2009; Chiou, Wang, Liao, and Yang 2013; Chiou, Wang, Liao, Wu, et al. 2013; Komeilipoor et al. 2017). These

facilitations occur in both directions from, i.e., rostral to caudal (from upper-limb to lower-limb) and from caudal to rostral (from lower-limb to upper-limb). Moreover, the facilitation of corticospinal excitability induced by muscle contractions broadly occurs in the muscles from proximal to distal segment in the upper-limb and lower-limb muscles (Tazoe, Sakamoto, et al. 2007; Tazoe et al. 2009; Chiou, Wang, Liao, and Yang 2013; Chiou, Wang, Liao, Wu, et al. 2013). Taken together, the abovementioned studies suggest that motor functions of different body segments are not embedded in the brain (and spinal cord) as separate units; rather, they closely interact within the central nervous system. The remaining questions about the characteristics of interlimb neural interaction in the corticospinal and spinal circuits are whether the neural interaction assessed by the "remote effect" would be different between diagonal and orthogonal muscle pairs (e.g., right upper- and right lower-limb muscles vs. right upper- and left lower-limb muscles). Since the functional requirements are different for diagonal and orthogonal muscle pairs, the extent of neural interaction could be different. Furthermore, in previous investigations of remote effect facilitation, it was reported that both preparation and execution of muscle contractions led to interlimb corticospinal facilitation of upper-limb muscles during lower-limb muscle contractions (Komeilipoor et al. 2017). However, since it is widely known that the excitability of the corticospinal pathways is influenced by the excitability of both cortical and spinal circuits, such corticospinal interlimb interaction during motor preparation and execution could be attributed to cortical and/or spinal facilitation mechanisms. Moreover, although previous studies on interlimb neural interaction have focused on its characteristics, their neural mechanisms and functional implications are yet to be fully elucidated (for a review, see Tazoe and Komiyama, 2014), which can be achieved using novel neurophysiological tools and techniques. The comprehensive clarification of the characteristics of interlimb interactions would lead to the elucidation of the significance of neural interactions in human corticospinal systems,

thereby shedding light on the basic mechanisms that regulate human motor control and coordination.

1.4.2. Trunk-limb neural interaction in corticospinal and spinal circuits

In humans, most movements during activities of daily living involve simultaneous and coordinated activation of the trunk and upper- or lower-limb muscles as well as interlimb coordination. It has been reported that trunk muscles are activated before or at the same time as the voluntary-initiated arm muscle activity (Aruin and Latash 1995; Kaminski et al. 1995; Levin 1996; Hodges and Richardson 1997; Hodges et al. 1997). Although trunk and limb muscle interactions are essential for the performance of the majority of human movements, the neural mechanisms of trunk-limb interactions in corticospinal systems remain unclear and largely unexplored in the scientific literature. Based on previous studies examining kinematic and neuromuscular activations, it could be speculated that the trunk and upper- and lower-limb muscles interact closely within the CNS. Although a large number of studies using electromyographical and biomechanical analyses have reported associations between trunk and limb muscle activities (Aruin and Latash 1995; Kaminski et al. 1995; Levin 1996; Hodges and Richardson 1997; Hodges et al. 1997), there is still a lack of direct evidence for the existence of neural interaction between trunk and limb muscles in the cortical and spinal neural circuits. Moreover, if neural interaction do indeed exists between the trunk and limb muscles, the understanding of the contributions of the cortical and spinal circuits to their interactions would warrant further understanding.

1.5. Thesis objectives and hypotheses

The current body of knowledge regarding the "remote effect" neural interactions revealed that upper-limb and lower-limb muscles interact in the corticospinal and spinal circuits. These circuits contribute to upper- and lower-limb coordination during human movements. However, their characteristics, neural mechanisms, and functional implications are yet to be fully elucidated. Furthermore, although some behavioral studies suggested that trunk muscles also interacted with limb muscles during different human movements, previous studies about neural interaction of muscles have focused only on interlimb interactions (i.e., upper- and lower-limb interactions) and largely ignored the essential role of the trunk system. Therefore, the questions regarding neural interaction between different muscles, including the trunk muscles should be resolved to better understand the neural mechanisms of muscle coordination during human movements. The answer to these questions would also provide important implications for rehabilitation practice. Therefore, *this thesis aims to answer the following questions*:

- The detailed characteristics of interlimb neural interaction remain unclear, and the remaining questions about interlimb neural interaction are: (1) Are there differences in neural connectivity assessed by the "remote effect" between diagonal and orthogonal muscle pairs? (2) How does interlimb neural interaction in cortical and spinal circuits differ during different phases of the movement, including the preparation and execution for the movements?
- The existence of neural interactions between the trunk and limb muscles has not been clarified, so the question remains: Are there neural interactions between trunk and limb muscles in the corticospinal circuits?

- To better understand the control centers of the "remote effect" regulation, we should also understand the answer to the following question: Are the trunk-limb neural interaction mechanisms cortical or spinal in origin?
- To clarify the relevance of the "remote effect" to human movement coordination, the following question should be answered: What are the functional implications of such neural interactions for humans?
- Based on the evidence that at least some regulation of the "remote effect" is organized at the cortical level, the question remains: Which intracortical mechanisms at the level of interneurons contribute to such interactions?

Therefore, the main overall objective of my thesis is **to elucidate the existence, mechanisms, and function of corticospinal neural interaction between multiple muscles including the upper-limb, trunk, and lower-limb muscles**. To this end, I designed and conducted five separate studies to address the above-mentioned questions. The specific objectives of my thesis, as they relate to each of the five studies, are as follows:

- **Objective 1 (Study 1, Chapter 2):**

To investigate the effects of preparation and execution of ipsilateral, contralateral, and bilateral muscle contractions on the interlimb neural interaction in the corticospinal and spinal reflex circuits using TMS and tSCS.

***Research question*:** Does interlimb neural interaction in the corticospinal and spinal circuits change depending on the movement phase (i.e., preparation and execution) and diagonal/orthogonal muscle pairs?

***Hypotheses*:** I hypothesized that interlimb neural interactions in the corticospinal and spinal circuits would depend on the movement phase and the muscles pairs contracted.

- **Objective 2 (Study 2, Chapter 3):**

To investigate neural interactions between the trunk and limb muscles in the corticospinal circuits by examining the effects of voluntary contraction of trunk muscles on corticospinal excitability of upper- and lower-limb muscles, and vice versa, using TMS.

***Research question*:** Does neural interaction between trunk and limb muscles exist, as revealed in the upper-limb and lower-limb muscles?

***Hypotheses*:** It was hypothesized that voluntary activation of the trunk muscles would affect corticospinal circuits in the upper- and lower-limb muscles, and vice versa, which would suggest that there are neural interactions between limb and trunk muscles.

- **Objective 3 (Study 3, Chapter 4):**

To investigate the cortical and spinal contributions to neural interactions between the trunk and limb muscles using TMS and CMS.

***Research question*:** Does the neural interaction between the trunk and limb muscles occur in intracortical or subcortical circuits?

***Hypotheses*:** I hypothesized that the trunk-limb neural interaction is largely a contribution of the cortical mechanisms, rather than subcortical.

- **Objective 4 (Study 4, Chapter 5):**

To investigate the functional implication of neural interactions between multiple muscles by examining the effects of different motor tasks and contraction intensities.

***Research question*:** Does the neural interaction between upper-limb, trunk, and lower-limb muscles have a task-specific functional significance in motor control?

***Hypotheses*:** I hypothesized that the extent of neural interactions would be altered depending on the task executed and intensity (level of effort), which would suggest that neural interactions are functionally relevant to coordinate multiple muscles during motor control.

- **Objective 5 (Study 5, Chapter 6):**

To investigate the contributions of the intracortical microcircuits in the M1 to neural interactions by examining intracortical inhibitory and excitatory circuits using several different TMS cortical stimulation paradigms (i.e., single and paired-pulse TMS with different current directions).

***Research question*:** How do different interneurons and inhibitory/excitatory circuits within the M1 that project to the corticospinal neurons contribute to interlimb neural interactions?

***Hypotheses*:** I hypothesized that distinct subsets of interneurons that represent intracortical inhibitory and facilitatory networks would contribute to interlimb interaction to a different degree, which would lead to an elucidation of the neural basis for understanding interlimb interaction in the M1.

The results of the five above-mentioned studies (Study 1, Study 2, Study 3, Study 4, and Study 5) will be discussed together in the General Discussions in Chapter 7, to integrate the implications of neural control of multiple muscles coordination in the corticospinal systems, its relevance for understanding control of human movement, and the implications for rehabilitation practice.

Chapter 2 - Study 1

Interlimb neural interactions in corticospinal and spinal reflex circuits during preparation and execution of isometric elbow flexion

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***NOTE**: The content of this chapter is identical to the material presented in the publication except for the text formatting which was done according to University of Tokyo requirements.*

2.1. Introduction

During activities of daily living, such as walking and running, proper coordination of upper-limb and lower-limb muscles is essential. This interlimb neural interaction is coordinated within the central nervous system and underlies important mechanisms related to human motor control (Calancie et al. 2002; Zehr and Duysens 2004; Zehr 2005). It is well known that contraction of certain upper- and lower-limb muscles can modulate the corticospinal and spinal reflex motor circuits not only of the contracted muscle, but also of other muscles which are located in a remote segment of the body and remain at rest (Tazoe and Komiyama 2014). Specifically, this phenomenon is known as "remote effect" or "cross facilitation" (Tazoe, Endoh, et al. 2007; Tazoe, Sakamoto, et al. 2007; Tazoe and Komiyama 2014; Komeilipoor et al. 2017), while interlimb transfer (or "cross education") was generally defined as unilateral training effects of one limb on improving strength/skills in the homologous muscles pairs on the untrained side (e.g., right arm and left arm) (Yue and Cole 1992; Dragert and Zehr 2011). Such mechanisms may contribute to coordination of interlimb movements via inter-neuronal circuits within the central nervous system. Facilitation of spinal monosynaptic reflexes elicited in lower-limb muscles by bilateral hand muscle contractions was first demonstrated more than a century ago through a phenomenon known as the Jendrássik maneuver (Jendrássik E 1883). Typically, "remote effect" neural interactions have been studied between upper- and lower-limb segments by assessing spinal monosynaptic reflexes (i.e., Hoffman-reflex) (e.g., Borroni et al. 2004; Kawamura and Watanabe 1975; Tazoe et al. 2005).

Interlimb interactions have also been studied extensively in the corticospinal pathways (Kawakita et al. 1991; Pereon et al. 1995). Similar to the spinal monosynaptic reflex responses, contraction of upper- or lower-limb muscles was shown to facilitate corticospinal excitability of muscles which are located in remote limbs (Kawakita et al. 1991; Pereon et al. 1995; Hortobágyi

et al. 2003; Tazoe, Endoh, et al. 2007; Tazoe et al. 2009; Chiou, Wang, Liao, and Yang 2013; Chiou, Wang, Liao, Wu, et al. 2013; Komeilipoor et al. 2017). Such facilitation of corticospinal excitability induced by muscle contractions broadly occurs in muscles from proximal to distal segments in the upper- and lower-limbs (Tazoe, Sakamoto, et al. 2007; Tazoe et al. 2009; Chiou, Wang, Liao, and Yang 2013; Chiou, Wang, Liao, Wu, et al. 2013). Taken together, the abovementioned studies suggest that motor functions of different body segments are not embedded in the brain and the spinal cord as separate units; rather, they closely interact within the central nervous system. However, the neural mechanisms and functional implications of such interlimb facilitation is yet to be fully elucidated (for a review, see Tazoe and Komiyama 2014). Facilitation of corticospinal and spinal motor circuities of resting muscles induced by voluntary activation of remote body segments may also have implications for rehabilitation practice and understanding of impaired motor control due to neurological injury or disease. For instance, training of an unaffected or less affected limb may enhance motor recovery of the more affected / impaired limb in individuals with neurological impairments such as stroke and spinal cord injury (Klarner et al. 2016; Zhou et al. 2017, 2018; Kaupp et al. 2018). Therefore, to elucidate such mechanisms, a thorough understanding of the underlying networks that govern interlimb interactions is required.

In previous investigations of “remote effect” facilitation, it was reported that both preparation and execution of muscle contractions led to interlimb corticospinal facilitation of upper-limb muscles during lower-limb muscle contractions (Komeilipoor et al. 2017). However, since it is widely known that excitability of the corticospinal pathway is influenced by the excitability of both cortical and spinal circuits, such corticospinal interlimb facilitation during motor preparation and execution, which was demonstrated, could be attributed to cortical and/or spinal facilitation mechanisms. Moreover, it was previously reported that remote facilitation can

be induced not only during bilateral muscles contractions, but also unilateral contractions in both ipsilateral and contralateral sides of distant segmental limbs (Tazoe, Sakamoto, et al. 2007; Chiou, Wang, Liao, and Yang 2013; Chiou, Wang, Liao, Wu, et al. 2013). However, the extent of facilitation has not been directly compared across diagonal and orthogonal muscle pairs (e.g., right upper- and right lower-limb muscles vs. left upper- and right lower-limb muscles), nor during unilateral and bilateral muscles contraction. Therefore, investigating the remote effect during preparation and execution of ipsilateral, contralateral, and bilateral upper-limb muscles on the corticospinal and spinal motor circuits of the lower-limb muscles would provide new insights about neural interlimb interaction mechanisms underlining remote facilitation.

The aim of the current study was to systematically compare the effects of preparation and execution of ipsilateral, contralateral, and bilateral upper-limb muscle contractions on the excitability of corticospinal and spinal reflex circuits in the lower-limb muscles unilaterally. We hypothesized that motor preparation for the contraction of upper-limb muscles would facilitate remote corticospinal circuits of lower-limb muscles, while motor execution (contraction) of upper-limb muscles would facilitate not only remote corticospinal circuits, but also spinal reflex circuits. Moreover, it was previously reported in animal literature that intrahemispheric connectivity may contribute to spreading of neural activity to neighbouring cortical regions that represent different muscles (Capaday et al. 2009, 2011). Similarly, intrahemispheric connectivity mechanisms were proposed to exist in humans (Boroojerdi et al. 2000; Komeilipoor et al. 2017). Therefore, we hypothesized that the extent of “remote effect” facilitation on neural circuits in unilateral lower-limb muscles would be larger during ipsilateral upper-limb contractions, compared to contralateral contractions since ipsilateral muscles pairs (i.e., right upper-limb and right lower-limb muscles) may have such intrahemispheric connections (Boroojerdi et al. 2000; Capaday et al. 2009, 2011; Komeilipoor et al. 2017), while contralateral muscles pairs (i.e. left

upper-limb and right lower-limb muscles) may involve both interhemispheric inhibition (Ferbert, Priori, et al. 1992) and interhemispheric facilitation (Stippich et al. 2007) mechanisms. We also hypothesized that the extent of "remote effect" in lower-limb muscles would be larger during bilateral contractions of upper-limb muscles, compared to unilateral (ipsilateral and contralateral) upper-limb muscle contractions since ipsilateral and contralateral effects would be summed.

To test our hypotheses, we used: (1) TMS to investigate motor evoked potential (MEP), which reflect excitability of corticospinal pathway; and (2) transcutaneous spinal cord stimulation (tSCS) to investigate spinal reflex responses, which reflect excitability of spinal reflex circuits in multiple lower-limb muscles during motor preparation and execution of ipsilateral, contralateral, and bilateral upper-limb muscles contractions.

2.2. Methods

2.2.1. Participants

In Study 1, which aimed to investigate interlimb "remote effect" during early parts of remote muscle contractions, fourteen able-bodied individuals were recruited in the TMS session [26.1±3.6 years, 66.6±6.6 kg, and 173.1±5.1 cm (mean ± SD)] and another fourteen were recruited in the tSCS session [25.0±3.0 years, 69.9±6.8 kg, and 174.4±4.8 cm (mean ± SD)] (Note: nine individuals participated in both TMS and tSCS sessions in Study 1). In Study 2, which aimed to investigate interlimb "remote effect" during later steady-state part of remote muscle contractions, twelve able-bodied individuals were recruited in the TMS session [25.4±3.6 years, 68.3±7.1 kg, and 173.8±5.1 cm (mean ± SD)] and another twelve were recruited in the tSCS session [23.9±2.4 years, 69.0±6.5 kg, and 174.2±4.3 cm (mean ± SD)] (Note: nine individuals participated in both TMS and tSCS sessions in Study 2). In total, 21 individuals participated in both Study 1 and Study 2 (19 participants in Study 1 and 15 participants in Study 2, with 13 participating in both). We confirmed that all participants were right-handed in accordance with the Edinburgh Handedness Inventory (Oldfield 1971) (Note: score across participants was 0.89 ± 0.14, where 1 indicates completely right-handed, and −1 indicates completely left-handed). To examine foot preference, participants were asked which foot they would use to: kick a ball, stamp out a simulated fire, pick up a marble, and trace shapes (Chapman et al. 1987; Schneiders et al. 2010). All participants, except one, indicated that they were right-footed (Note: a score of 3 or more indicating right or left foot was used to determine preference). None of the participants had any history of neurological or musculoskeletal impairments. All participants gave written informed consent in accordance with the Declaration of Helsinki. The experimental procedures were approved by the local institutional ethics committee at The University of Tokyo.

2.2.2. Experimental procedures

2.2.2.1. General protocol

During the experiments, participants remained in the supine position. The tested (right) leg was fixed with an ankle foot orthosis and with a strap band to prevent movements during the experiments (Figure 2-1). The TMS and tSCS sessions were performed on separate days, with at least one day rest between sessions. Each session started with measuring maximum voluntary contraction (MVC) by asking the participants to perform three right and three left elbow isometric flexion trials. Force levels of the right and left hand were measured using strain gauge sensors (LCB03K025L, A&D Company Limited, Japan), which were fixed to metal frames located on the distal part of the forearm (Figure 2-1). Assessments were performed during the following experimental conditions: (a) relaxed (Baseline); (b) right / ipsilateral elbow flexion (Right); (c) left / contralateral elbow flexion (Left); and (d) bilateral elbow flexion (Bi). During the experiment, participants were instructed to relax their lower-limb muscles and EMG activities were monitored by the experimenter throughout the experiment. Prior to the experiments, the experimenter checked EMG activity and asked the participants to relax if it was necessary.

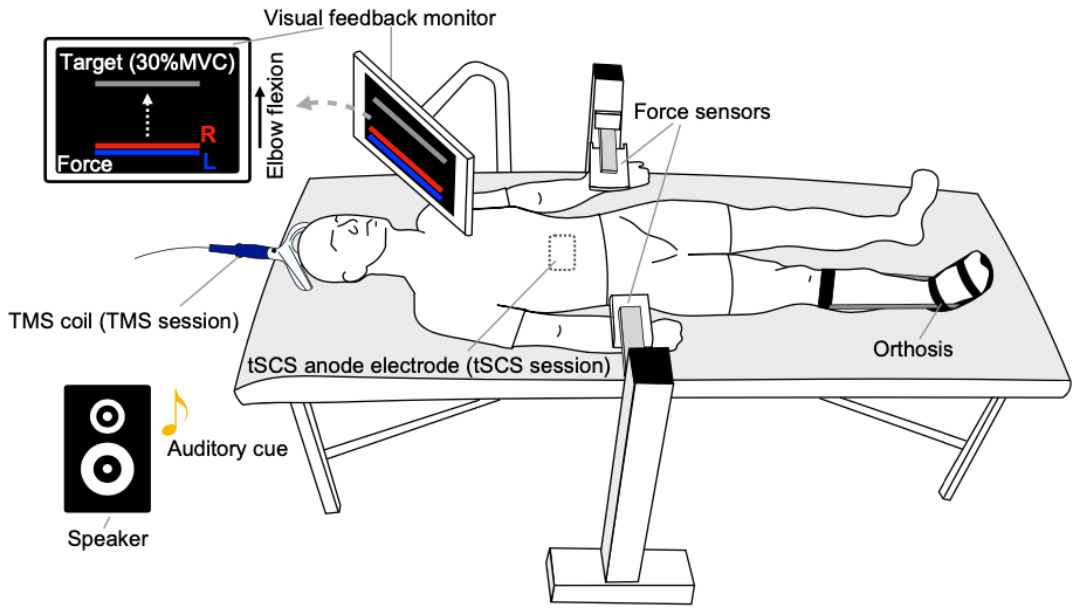

Figure 2-1: Experimental setup showing the posture of participants during the experiment, including the transcranial magnetic stimulation (TMS) setup (TMS session), transcutaneous spinal cord stimulation (tSCS) setup (tSCS session), force sensors, and visual feedback monitor. During the experiment, participants were asked to match the isometric elbow flexion forces to 30% of maximum voluntary contraction (MVC) of each arm with real-time visual feedback of force displayed on a monitor as fast as possible after an auditory cue and maintain force for 1,000 ms during presentation of the auditory cue.

2.2.2.2. Study 1

In Study 1, during the Right, Left, and Bi conditions, participants were asked to match the isometric elbow flexion forces of each arm to 30% of MVC as fast as possible after an auditory cue and maintain the force level for 1,000 ms during presentation of the auditory cue with real-time visual feedback of the force levels displayed on a monitor (Figure 2-1). TMS or tSCS stimulation in each condition was delivered either: (1) 100 ms after the presentation of the

auditory cue, when the muscles were still at rest (Preparation); or (2) 250 ms after the presentation of the auditory cue, when the muscle contractions were initiated (Motor initiation) (Figure 2-2A), since our previous study showed that EMG onset of elbow flexion was approximately 150 ms after the auditory cue (Masugi et al. 2019). Moreover, in post processing it was confirmed that EMG onset was indeed during the Motor initiation phase for each trial. However, it was well before 30% MVC force (average contraction level across conditions for the TMS session was 4.27 ± 4.76 %MVC; tSCS session was 4.97 ± 4.06 %MVC). Prior to the experiments, participants were also given time to practice the tasks in order to minimize learning effects and trial-to-trial variability. Each condition (Preparation and Motor initiation) consisted of eight trials with 10 sec interstimulus interval, which were controlled by a custom written LabVIEW program (National Instruments, USA) and delivered in a pseudorandomized order (Figure 2-2B). Baseline condition was performed by delivering TMS or tSCS stimuli 100 ms after the auditory cue, while participants were instructed to stay relaxed (i.e., not contract any muscle). Each session (i.e., TMS and tSCS session) consisted of four blocks (Figure 2-2B). Each condition (Baseline, Right, Left and Bi) was randomly set to each block (i.e., each block consisted of one of the Baseline, Right, Left or Bi conditions) (Figure 2-2B). In total, 56 trials were conducted during each session [3 contraction conditions (Right, Left and Bi) x 2 timings of stimulation (Preparation and Motor initiation) x 8 trials + 1 Baseline condition x 8 trials]. The order of experimental conditions including the Baseline condition was randomized between participants and separated by 5 min rest (Figure 2-2B). We delivered eight TMS stimuli for each condition, based on a systematic review which reported that a minimum of five stimuli is sufficient to produce reliable MEP recordings for single-site TMS investigations (Cavaleri et al. 2017).

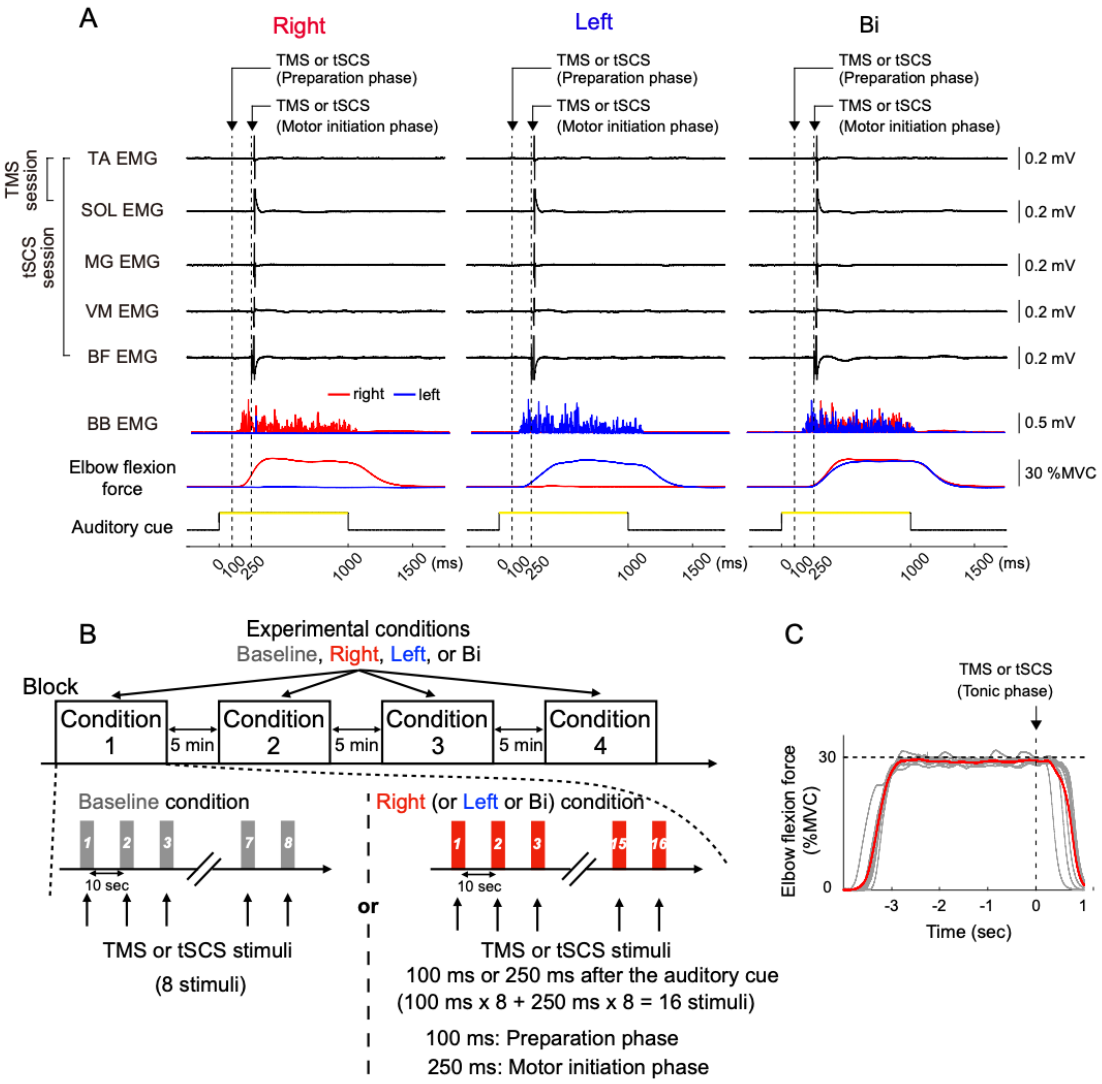

Figure 2-2: (A) Typical recording of rectified electromyographic (EMG) activities of tibialis anterior (TA), soleus (SOL), medial gastrocnemius (MG), vastus medialis (VM), biceps femoris (BF) muscles and biceps brachii (BB), and elbow flexion force in Right, Left, and Bi conditions in Study 1. Red lines indicate right arm EMG and force. Blue lines indicate left arm EMG and force. Participants were asked to match the isometric elbow flexion forces to 30% of maximum voluntary contraction (MVC) of each arm as fast as possible after an auditory cue and maintain force for 1,000 ms during presentation of the auditory cue. (B) Experiment consisted of transcranial magnetic stimulation

(TMS) and transcutaneous spinal cord stimulation (tSCS) sessions, which were performed on separate days. In each session, Baseline, Right, Left, and Bi conditions were randomized between participants and separated by 5 min rest. During Baseline conditions, TMS or tSCS stimuli were delivered 100 ms after the auditory cue and consisted of eight trials. During the Right, Left, and Bi conditions, TMS or tSCS stimuli were delivered 100 (Preparation) or 250 ms (Motor initiation phase) after the auditory cue. Each condition (100 and 250 ms) consisted of eight trials, which were delivered in a pseudorandomized order. (C) Typical recordings of elbow flexion force in Right condition in Study 2 (Tonic phase).

2.2.2.3. Study 2

In Study 2, an additional protocol was subsequently performed where TMS or tSCS stimuli were delivered after the target contraction level of 30% MVC was achieved (Tonic phase). The contraction target level in Study 2 was set such as to match Study 1. Study 2 was conducted to investigate interlimb "remote effect" during the later steady-state part of remote muscle contractions, while Study 1 was performed to investigate interlimb "remote effect" during early parts of remote muscle contractions. During Right, Left, and Bi conditions, participants were asked to match the isometric elbow flexion forces of each arm to 30% of MVC and maintain the force level with real-time visual feedback displayed on a monitor (Figure 2-1). TMS and tSCS stimuli were delivered if participants maintained the contraction with the 25-35% MVC range of isometric elbow flexion force for 3 seconds (Figure 2-2C), which were controlled by a custom written LabVIEW program (National Instruments, USA). In post processing it was confirmed that TMS/tSCS stimuli were indeed delivered during the steady-state Tonic phase for each trial (average contraction level across conditions for the TMS session was 29.1 ± 0.83 %MVC; tSCS

session was 29.0 ± 0.55 %MVC). Baseline condition was performed by delivering TMS or tSCS stimuli while participants were instructed to stay relaxed. Each condition (i.e., Baseline, Right, Left, and Bi) consisted of eight trials with 10 sec interstimulus interval. In total, 32 trials were conducted during each session [3 contraction conditions (Right, Left and Bi) x 8 trials + 1 Baseline condition x 8 trials]. The order of experimental conditions including the Baseline condition was randomized between participants and separated by 5 min rest.

2.2.3. Data acquisition

2.2.3.1. Electromyography (EMG) activity

During the TMS session, electromyographic (EMG) activity was recorded from the right tibialis anterior (TA) and soleus (SOL) to assess MEP responses (Rothwell et al. 1991). During the tSCS session, EMG activity was recorded from the right TA, SOL, medial gastrocnemius (MG), vastus medialis (VM), and biceps femoris (BF) to assess spinal reflexes / posterior root responses (Courtine et al. 2007; Minassian et al. 2007; Dy et al. 2010; Roy et al. 2012; Masugi et al. 2016, 2017). Moreover, right and left biceps brachii EMG activity was recorded to evaluate upper-limb task performance in each session. Bipolar Ag/AgCl surface electrodes (Vitrode F-150S, Nihon Kohden, Tokyo, Japan) were placed over the muscle belly with at least 1 cm separation. A ground electrode was placed around the knee. Prior to application of electrodes, skin was cleaned using alcohol to reduce skin impedance. All EMG signals were band-pass filtered (5 - 1,000 Hz) and amplified (×1,000) using a multichannel EMG amplifier (MEG-6108, Nihon Koden, Tokyo, Japan). Moreover, all data were digitized at sampling frequency of 4,000 Hz using an analog-to-digital (A/D) converter (Powerlab/16SP, AD Instruments, Castle Hill, Australia) and stored on the computer for post-processing.

2.2.3.2. Transcranial magnetic stimulation (TMS)

TMS was delivered over the primary motor cortex using a mono-phasic magnetic stimulator (Magstim 200, Magstim Co., Whitland, UK) through a double cone coil (outside diameter of 110 mm; Magstim Co., Whitland, UK). The optimal stimulation spot (i.e. "hot spot") was searched for over the left cortex where MEPs could be recorded from the right TA muscle. The motor threshold (MT) was determined while the participants remained in the supine position and relaxed since the aim of our study was to examine how neural activity of lower-limb muscles during resting state is modulated when upper-limb muscles are contracted. MT was defined as the minimum TMS intensity for which MEPs had peak-to-peak amplitudes larger than 50 μV and were evoked in at least five out of ten successive trials (Rossini et al. 2015). The stimulation intensity for experiment was set at 120% of the MT level (1.2MT) (Study 1: 64.1±8.38 % of maximal stimulator output and Study 2: 64.0±10.6 % of maximal stimulator output).

2.2.3.3. Transcutaneous spinal cord stimulation (tSCS)

To evaluate excitability of the spinal reflexes in multiple lower-limb muscles simultaneously during remote muscle contractions, a constant current electrical stimulator (DS7A, Digitimer Ltd., UK) was used to apply a single monophasic square pulse with a 1 ms pulse width. The cathode electrode (50 × 50 mm) was placed on the spine between L1–L2 lumbar spine process, and the anode electrode (100 × 75 mm) was placed on the trunk above the umbilicus (Roy et al. 2012). Prior to the experiments, the cathode was adjusted to determine the optimal stimulation location. Specifically, tSCS-evoked responses were tested when the cathode was positioned on the T12-L1, L1-L2, or L2-L3 levels using the same stimulus amplitude. The location that induced largest peak-to-peak responses in all tested muscles was chosen as the stimulation site (Study 1: T12–L1: n=1; L1–L2: n=12; L2–L3: n=1 and Study 2: T12–L1: n=0; L1–L2: n=9; L2–L3: n=3).

Electrodes were fixed with adhesive tape to prevent movement during the experiments. Next, to determine the stimulus intensity, the recruitment curves of the responses of all tested lower-limb muscles were obtained for each participant by gradually increasing the tSCS stimulation amplitude. To eliminate the ceiling effect of the evoked responses, the stimulus intensity was adjusted to evoke responses on the ascending part of the recruitment curve in all muscles (Masugi et al. 2019). The stimulus intensity was kept constant for the duration of the experiment (Study 1: 51.9±11.5 mA and Study 2: 49.3±13.0 mA). Prior to starting the experiments, a paired-pulse stimulation protocol (50 ms inter-stimulus interval) was applied to confirm whether the evoked responses were initiated in the afferent fibers to evoke reflex responses since EMG responses could potentially be contaminated by direct M-wave activation in the efferent fibers within the anterior roots (Courtine et al. 2007). The second responses were almost entirely suppressed by the first stimulus activation (Figure 2-3A). Specifically, the peak-to-peak amplitudes of the second responses were significantly smaller than those of the first response for all recorded muscles in Study 1 (Wilcoxon signed-rank test, $p<0.001$; Figure 2-3B top) and Study 2 (Wilcoxon signed-rank test, $p<0.001$; Figure 2-3B bottom). Suppression of the second response demonstrated post-activation depression, confirming that spinal reflexes were evoked by activation of the afferent roots (Courtine et al. 2007; Minassian et al. 2007; Dy et al. 2010; Roy et al. 2012; Masugi et al. 2016, 2017). Although spinal excitability can be assessed using F-wave or H-reflex, a practical limitation is that they can only be obtained consistently from one target muscle (typically SOL) (McNeil et al. 2013) and since F-waves are small and inconsistent in both size and shape, large numbers of responses are typically required for averaging (Lin and Floeter 2004). On the other hand, tSCS is a technique that can be used to constituently elicit spinal reflexes in multiple lower-limb muscles simultaneously, which has been used by our group as well as others on multiple occasions (e.g., Courtine et al. 2007; Dy et al. 2010; Masugi et al. 2016, 2017; Minassian et al.

2007; Roy et al. 2012). Therefore, tSCS was used in the current study to consistently evoke spinal reflexes in multiple lower-limb muscles simultaneously.

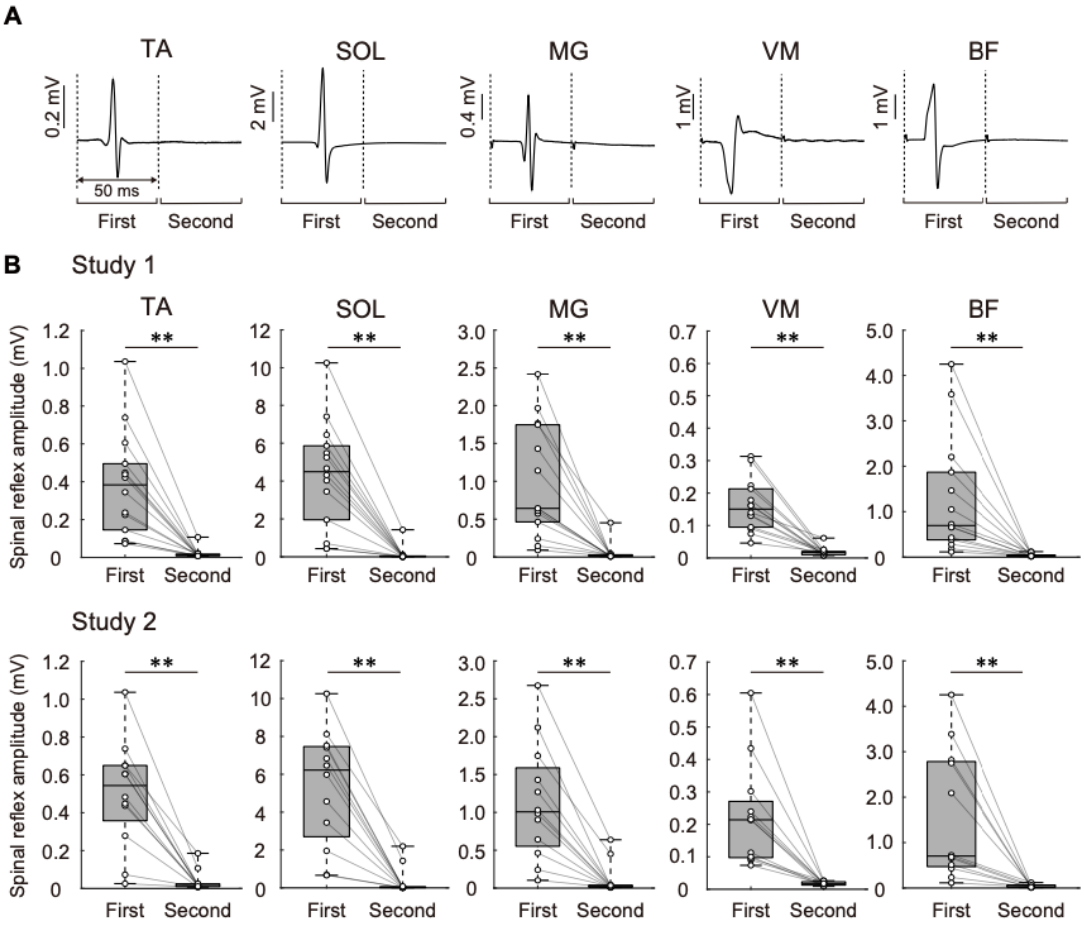

Figure 2-3: (A) Responses elicited during the paired-pulse stimulation protocol. Time series plots show mean spinally evoked motor responses of the repeated trials for one representative participant for the first and second stimulus responses, which were separated by 50 ms. (B) Group data of the first and second responses in Study1 (top) and Study 2 (bottom). Data are shown for the tibialis anterior (TA), soleus (SOL), medial gastrocnemius (MG), vastus medialis (VM), biceps femoris (BF) muscles. Legend: **p< 0.01.

2.2.4. Data analysis

Peak-to-peak amplitudes were calculated for the TMS-induced MEP and tSCS-induced spinal reflex responses of each muscle and for each trial using a custom written script in MATLAB (2017a, The MathWorks Inc., Massachusetts, USA). Moreover, background EMG activity of a 50 ms window before the TMS or tSCS stimuli was defined by calculating the root mean square value in each trial. Eight repeated trials were averaged for each contraction phase in Study 1 (i.e., Preparation and Motor initiation) and each experimental condition (i.e., Baseline, Right, Left, and Bi). Similarly, eight repeated trials were averaged for each experimental condition (i.e. Baseline, Right, Left, and Bi) in Study 2. MEP and spinal reflex amplitudes were then normalized as a percentage of the Baseline condition amplitude. Moreover, to confirm that performances of upper-limb contraction during TMS or tSCS stimulation in Study 1 were similar between each experimental condition (i.e., Right, Left and Bi), timing of TMS or tSCS stimulus delivery was evaluated based on the onset (i.e., reaction time) of biceps brachii muscle EMG activity during each task. Reaction time of right and left biceps brachii for each trial was determined as the time point at which the rectified EMG signal reached three standard deviations of rest-level EMG activity after the auditory cue (Masugi et al. 2019). Mean reaction time during each condition and each session are shown in Table 2-1. Although previous references reported that TMS can delay reaction times (e.g., Ziemann et al. 1997), these studies measured reaction time of upper-limb movement when TMS was delivered over the upper-limb muscle “hot spot” in the M1, while we measured reaction time of upper-limb movement when TMS was delivered over the lower-limb muscle representation in the M1. Therefore, in our current study, it can be assumed that TMS applied to evoke lower-limb responses did not delay reaction times of upper-limb movement. Moreover, to confirm that elbow flexion force performances during the Tonic phase in Study 2 were similar between experimental conditions (i.e., Right, Left, and Bi), mean error of the target

as a force accuracy measure and coefficient of variation [CV = (SD / mean) × 100] as a force stability measure were calculated. Mean error and CV during each condition and each session are shown in Table 2-2, suggesting that each task was performed at a relatively high level (i.e., indicated by relatively low error, CV, and standard deviations) and was not statistically significantly different between TMS and tSCS sessions ($p > 0.05$).

Table 2-1: Reaction time of the biceps brachii muscles [right: (R), left: (L)] during the Preparation and Motor initiation phase in Right, Left, and Bi conditions in Study 1.

Phase	Condition	Reaction time (ms)	
		TMS session	tSCS session
Preparation	Right	147.7 ± 38.0	146.6 ± 29.2
	Left	154.4 ± 26.7	147.2 ± 24.3
	Bi (R)	156.7 ± 47.4	150.5 ± 23.3
	Bi(L)	149.3 ± 47.5	146.6 ± 25.7
Motor initiation	Right	147.7 ± 32.4	155.7 ± 25.3
	Left	153.9 ± 33.3	146.0 ± 31.3
	Bi (R)	143.5 ± 38.7	156.2 ± 30.3
	Bi(L)	144.3 ± 37.3	150.0 ± 27.0

Values indicate means ± standard deviation.

Table 2-2: Error and coefficient of variation (CV) of the elbow flexion forces [right: (R), left: (L)] during Right, Left, and Bi conditions in Study 2.

Condition	TMS session		tSCS session	
	Error (% MVC)	CV (%)	Error (% MVC)	CV (%)
Right	1.22 ± 0.54	2.24 ± 0.73	1.15 ± 0.53	2.04 ± 0.62
Left	1.19 ± 0.63	2.05 ± 0.80	1.21 ± 0.54	2.10 ± 0.53
Bi (R)	1.34 ± 0.36	2.48 ± 0.54	1.29 ± 0.47	2.46 ± 0.95
Bi(L)	1.25 ± 0.68	2.35 ± 0.82	1.19 ± 0.37	2.28 ± 0.71

Values indicate means ± standard deviation.

2.2.5. Statistics

For each TMS and tSCS session in the Preparation phase, the Motor-initiation phase and the Tonic phase, which were analyzed separately, peak-to-peak amplitudes of MEP and spinal reflex responses were compared between the experimental conditions (i.e., Baseline, Right, Left, and Bi) using the Friedman test, a non-parametric equivalent for repeated-measure analysis of variance (ANOVA). When the Friedman test showed significant effects, post-hoc testing corrected for multiple comparisons was performed using the Wilcoxon signed-rank test with Holm correction (McLaughlin and Sainani 2014). The same analyses were performed to compare background EMG activity across experimental conditions. To ensure that the performances of upper-limb contractions (i.e., reaction time based on EMG onset) during application of TMS or tSCS stimulation were not different between experimental conditions, the Friedman test was also used to examine timing of TMS / tSCS stimuli relative to EMG onset in the Preparation phase and in the Motor initiation phase. Non-parametric tests were chosen because the sample size remains relatively small and the Shapiro-Wilk test showed that most identified measures were not normally distributed. Significance level for all tests was set at $p<0.05$.

2.3. Results

2.3.1. Study 1

2.3.1.1. Background EMG activity

The results of the background EMG activity during TMS and tSCS sessions are shown in Figure 2-4. In the TMS session, the Friedman test showed that background EMG activity was similar between all conditions (Baseline, Right, Left, and Bi) for the TA and SOL muscles in the Preparation phase [TA: $\chi^2(3)= 5.74$, $p=0.125$; SOL: $\chi^2(3)= 4.66$, $p=0.198$] and the Motor initiation phase [TA: $\chi^2(3)= 1.97$, $p=0.578$; SOL: $\chi^2(3)= 5.03$, $p=0.170$]. Similarly, in the tSCS session, the Friedman test showed that background EMG activity was similar between all conditions (Baseline, Right, Left, and Bi) for the TA, SOL, MG, and VM muscles in the Preparation phase [TA: $\chi^2(3)=7.46$, $p=0.0586$; SOL: $\chi^2(3)=3.69$, $p=0.298$; MG: $\chi^2(3)=3.51$, $p=0.319$; VM: $\chi^2(3)=0.429$, $p=0.943$; BF: $\chi^2(3)=0.600$, $p=0.896$] and the Motor initiation phase [TA: $\chi^2(3)=3.17$, $p=0.366$; SOL: $\chi^2(3)=1.71$, $p=0.634$; MG: $\chi^2(3)=0.600$, $p=0.896$; VM: $\chi^2(3)=2.31$, $p=0.510$; BF: $\chi^2(3)=1.03$, $p=0.794$].

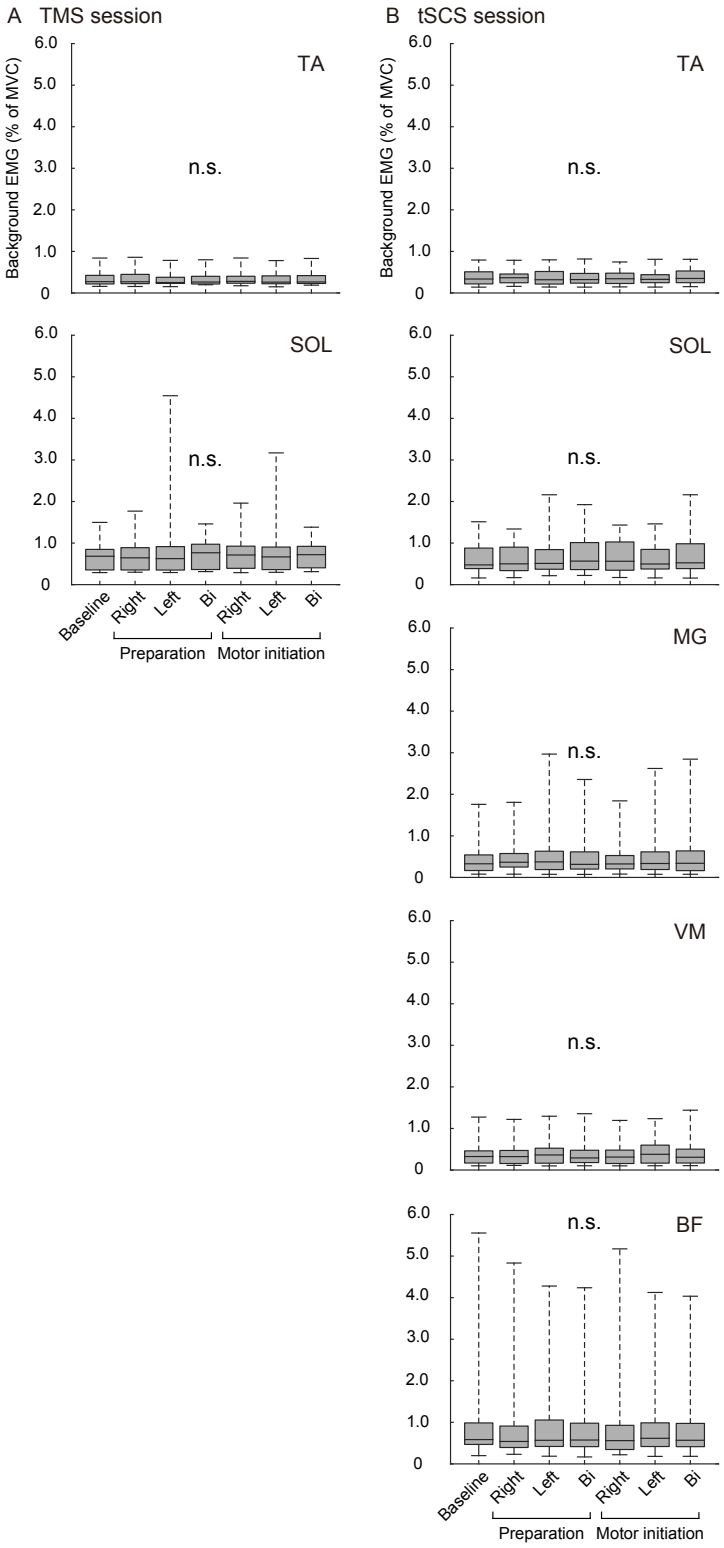

Figure 2-4: (A) Group data of background electromyographic (EMG) activity in the tibialis anterior (TA) and soleus (SOL) during rest (Baseline), right elbow flexion

(Right), left elbow flexion (Left) and bilateral elbow flexion (Bi) during the Preparation and Motor initiation phase in the transcranial magnetic stimulation (TMS) session in Study 1; (B) Groupe data of background EMG activity in the TA, SOL, medial gastrocnemius (MG), vastus medialis (VM), biceps femoris (BF) during Baseline, Right, Left, and Bi conditions during the Preparation and Motor initiation phase in the transcutaneous spinal cord stimulation (tSCS) session in Study 1. Legend: n.s. non-significant.

2.3.1.2. MEP amplitudes (TMS session)

The results of the MEP responses during the TMS session are shown in Figure 2-5. Averaged waveforms obtained from one representative subject are shown as well as the box plots, which indicate the peak-to-peak amplitudes (% of Baseline) of MEP responses during Right, Left, and Bi conditions in each stimulation phase (i.e., Preparation and Motor initiation). The Friedman test showed that MEP peak-to-peak amplitudes were statistically significantly different between the Baseline, Right, Left, and Bi conditions for the TA and SOL muscles in the Preparation phase [TA: $\chi^2(3)= 14.5$, $p<0.01$; SOL: $\chi^2(3)= 12.2$, $p<0.01$] and the Motor initiation phase [TA: $\chi^2(3)= 18.8$, $p<0.001$; SOL: $\chi^2(3)= 18.1$, $p<0.001$]. Post-hoc testing corrected for multiple comparisons for the TA muscle showed that Right, Left and Bi conditions were significantly larger compared with the Baseline condition in the Preparation phase ($p<0.05$, Wilcoxon signed-rank test) and the Motor initiation phase ($p<0.01$, Wilcoxon signed-rank test), while there were no significant differences between the Right, Left, and Bi conditions (Figure 2-5). Post-hoc testing corrected for multiple comparisons for the SOL muscle showed that the Left condition was significantly larger compared with the Baseline condition in the Preparation phase ($p<0.01$, Wilcoxon signed-rank test) and Right and Left conditions were significantly larger compared with the Baseline condition

in the Motor initiation phase ($p<0.01$, Wilcoxon signed-rank test), while there were no significant differences between the Right, Left, and Bi conditions (Figure 2-5).

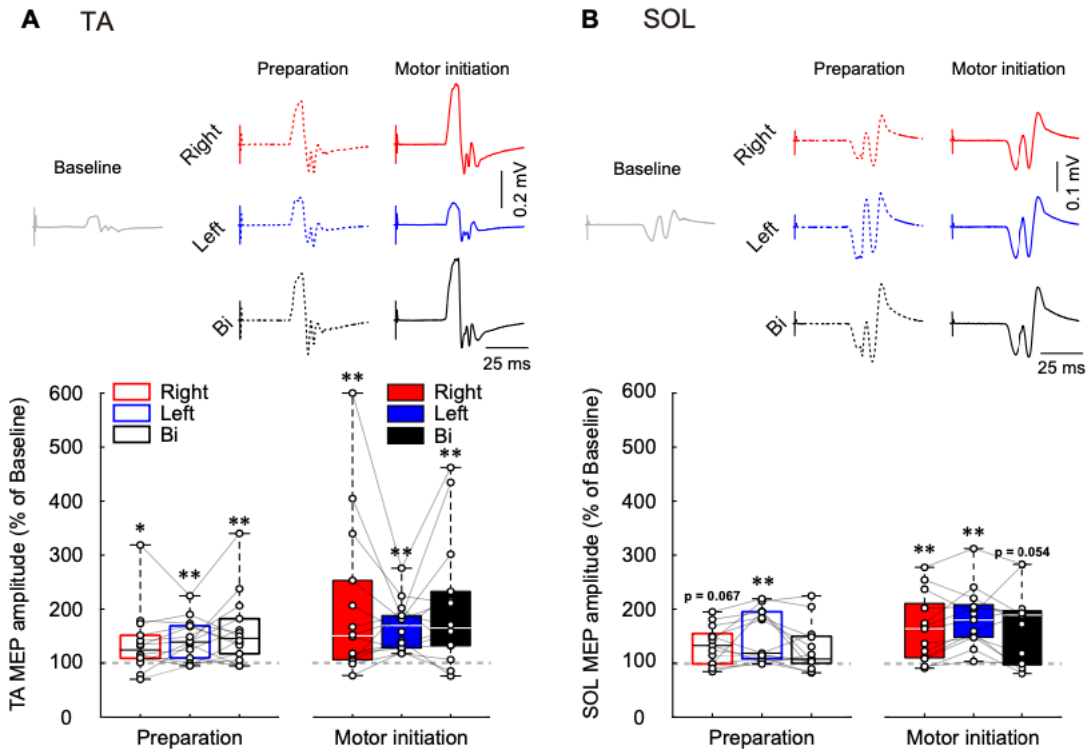

Figure 2-5: Motor evoked potentials (MEPs) in the (A) tibialis anterior (TA) and (B) soleus (SOL) muscles of one representative subject during rest (Baseline; gray trace), right elbow flexion (Right during the Preparation phase; red dashed trace and Right during the Motor initiation phase; red trace), left elbow flexion (Left during the Preparation phase; blue dash trace and Left during the Motor initiation phase; blue trace) and bilateral elbow flexion (Bi during the Preparation phase; black dash trace and Bi during the Motor initiation phase; black trace) conditions. Box plots show group data of MEPs elicited in the (A) TA and (B) SOL muscles. MEP amplitudes were normalized with respect to the MEP amplitude during the Baseline condition (% of Baseline) for each participant. Statistical analysis was used to compare the MEP amplitudes of Baseline, Right, Left, and Bi conditions during the Preparation and Motor initiation

phase. Asterisks indicate significant differences compared to the Baseline condition. Legend: *p< 0.05, **p< 0.01.

2.3.1.3. Spinal reflex amplitude (tSCS session)

The results of the spinal reflex responses during the tSCS session are shown in Figure 2-6. Average waveforms obtained from one representative subject are shown as well as the box plots, which indicate the peak-to-peak amplitudes (% of Baseline) of spinal reflex responses during Right, Left, and Bi conditions in each stimulation phase (i.e., Preparation and Motor initiation). The Friedman test showed that the spinal reflex peak-to-peak amplitudes were not statistically significantly different in any of the recorded muscles in the Preparation phase [TA: $\chi^2(3)=4.71$, $p=0.194$; SOL: $\chi^2(3)=4.03$, $p=0.258$; MG: $\chi^2(3)=4.37$, $p=0.224$; VM: $\chi^2(3)=1.37$, $p=0.712$; BF: $\chi^2(3)=3.43$, $p=0.330$], while they were statistically different between the Baseline, Right, Left, and Bi conditions for all recorded muscles in the Motor initiation phase [TA: $\chi^2(3)=25.5$, $p<0.001$; SOL: $\chi^2(3)=21.2$, $p<0.001$; MG: $\chi^2(3)=23.7$, $p<0.001$; VM: $\chi^2(3)=27.3$, $p<0.001$; BF: $\chi^2(3)=27.9$, $p<0.001$]. Post-hoc testing corrected for multiple comparisons during the Motor initiation phase showed that the spinal reflex responses for all recorded muscles during Right, Left and Bi conditions were significantly larger compared to the Baseline condition ($p<0.01$, Wilcoxon signed-rank test), while there was no significant difference between the Right, Left, and Bi conditions in the TA, SOL, MG, and VM muscles (Figure 2-6). In addition, Bi condition was significantly larger compared to Right and Left conditions ($p<0.05$, Wilcoxon signed-rank test) in the BF muscle.

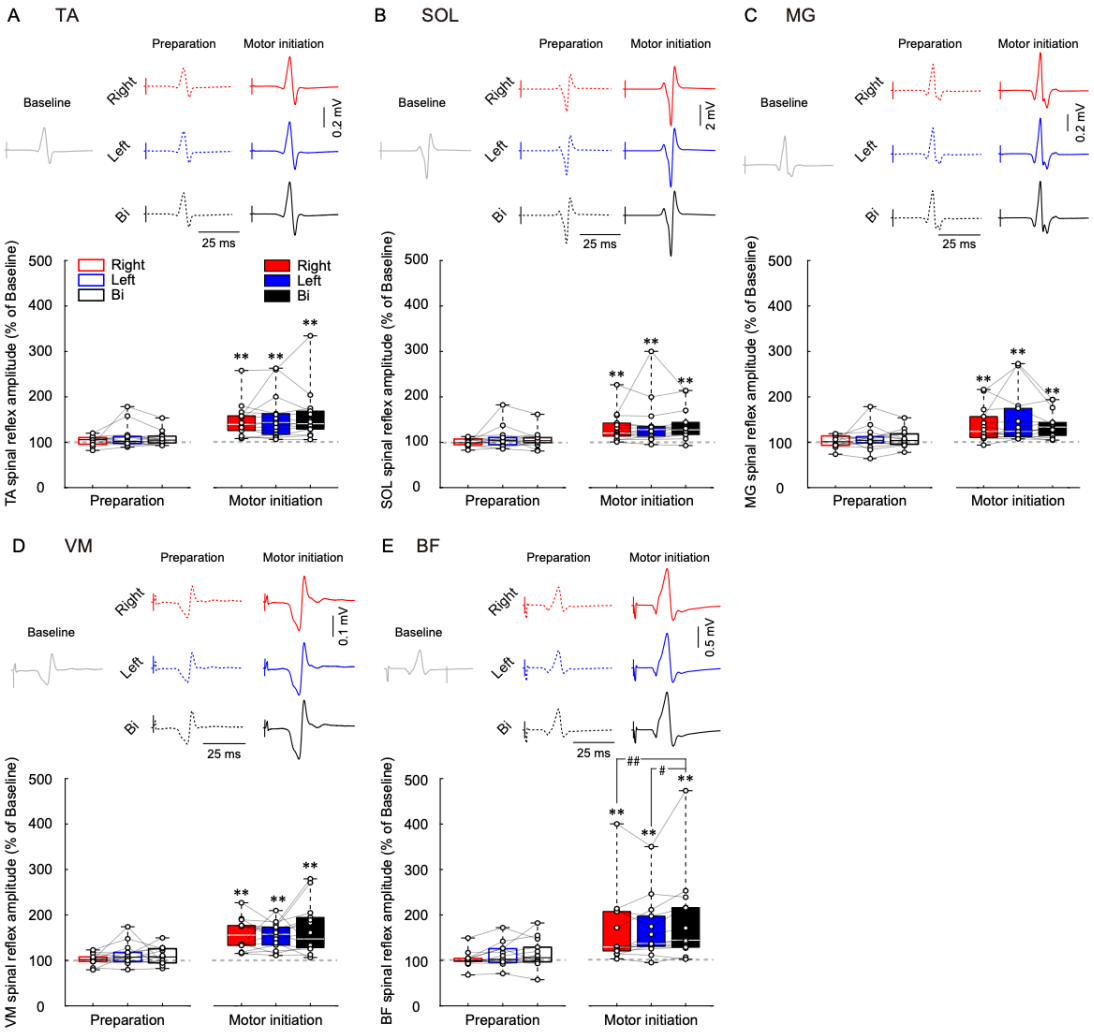

Figure 2-6: Spinal reflexes in the (A) tibialis anterior (TA), (B) soleus (SOL), (C) medial gastrocnemius (MG), (D) vastus medialis (VM), and (E) biceps femoris (BF) of a representative subject during rest (Baseline; gray trace), right elbow flexion (Right during the Preparation phase; red dash trace and Right during the Motor initiation phase; red trace), left elbow flexion (Left during the Preparation phase; blue dash trace and Left during the Motor initiation phase; blue trace) and bilateral elbow flexion (Bi during the Preparation phase; black dash trace and Bi during the Motor initiation phase; Black trace) conditions. Box plots show group data of spinal reflexes elicited in the (A) TA, (B) SOL, (C) MG, (D) VM, and (E) BF muscles. Spinal reflex amplitudes were

normalized with respect to spinal reflex amplitude during the Baseline condition (% of Baseline) for each participant. Statistical analysis compared the spinal reflex amplitudes of the Baseline, Right, Left, and Bi conditions during the Preparation and Motor initiation phase. Asterisks indicate significant differences compared to the Baseline condition. Hashtags indicate significant differences between Right, Left and Bi conditions. Legend: *, $^{\#}$ $p< 0.05$, **, $^{\#\#}$ $p< 0.01$.

2.3.1.4. Timing of TMS / tSCS relative to EMG onset

The results of the timing of TMS / tSCS relative to EMG onset during TMS and tSCS sessions are shown in Figure 2-7. In the TMS session, the Friedman test showed that the timing of TMS relative to EMG onset was similar between all conditions (Right, Left, and Bi) in the Preparation phase [$\chi^2(3)=1.36$, $p=0.715$] and the Motor initiation phase [$\chi^2(3)=3.17$, $p=0.366$] (Figure 2-7A). Similarly, in the tSCS session, the Friedman test showed that the timing of tSCS relative to EMG onset was similar between all conditions (Right, Left, and Bi) in the Preparation phase [$\chi^2(3)=3.17$, $p=0.366$] and the Motor initiation phase [$\chi^2(3)=3.77$, $p=0.287$] (Figure 2-7B). These results suggest that the performances (i.e., EMG onset) of upper-limb contraction were similar between experimental conditions (Right, Left and Bi).

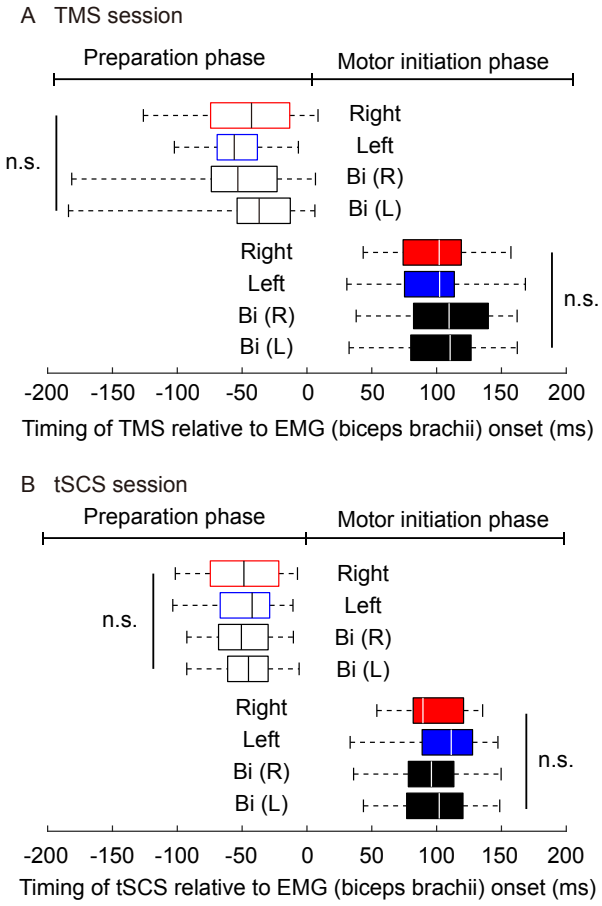

Figure 2-7: Group data of timing of: (A) transcranial magnetic stimulation (TMS) and; (B) transcutaneous spinal cord stimulation (tSCS) relative to EMG onset of the biceps brachii muscles [right: (R), left: (L)] across participants during the Preparation and Motor initiation phase in Right, Left and Bi condition. Legend: n.s. non-significant.

2.3.2. Study 2

2.3.2.1. Background EMG activity

The results of the background EMG activity during TMS and tSCS sessions are shown in Figure 2-8. In the TMS session, the Friedman test showed that background EMG activity was similar between all conditions (Baseline, Right, Left, and Bi) for the TA and SOL muscles in the

Tonic phase [TA: $\chi^2(3)= 0.900$, $p=0.825$; SOL: $\chi^2(3)= 4.10$, $p=0.251$]. Similarly, in the tSCS session, the Friedman test showed that background EMG activity was similar between all conditions (Baseline, Right, Left, and Bi) for the TA, SOL, MG, and VM muscles in the Tonic phase [TA: $\chi^2(3)=2.00$, $p=0.572$; SOL: $\chi^2(3)=0.400$, $p=0.940$; MG: $\chi^2(3)=1.30$, $p=0.729$; VM: $\chi^2(3)=1.80$, $p=0.615$; BF: $\chi^2(3)=0.300$, $p=0.960$].

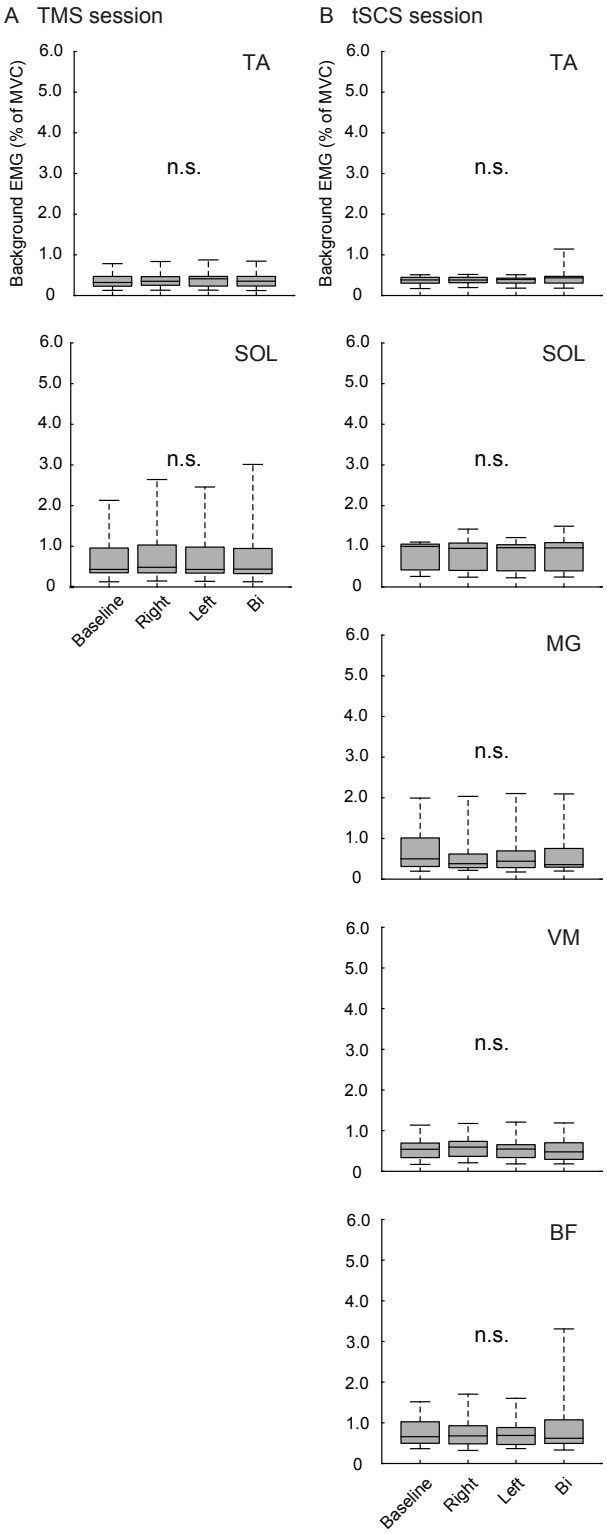

Figure 2-8: (A) Group data of background electromyographic (EMG) activity in the tibialis anterior (TA) and soleus (SOL) during rest (Baseline), right elbow flexion

(Right), left elbow flexion (Left) and bilateral elbow flexion (Bi) during the Tonic phase in the transcranial magnetic stimulation (TMS) session in Study 2; (B) Groupe data of background EMG activity in the TA, SOL, medial gastrocnemius (MG), vastus medialis (VM), biceps femoris (BF) during Baseline, Right, Left, and Bi conditions during the Tonic phase in the transcutaneous spinal cord stimulation (tSCS) session in Study 2. Legend: n.s. non-significant.

2.3.2.2. MEP amplitudes (TMS session)

The results of the MEP responses during the TMS session are shown in Figure 2-9A. The box plots indicate the peak-to-peak amplitudes (% of Baseline) of MEP responses during Right, Left, and Bi conditions in the Tonic phase. The Friedman test showed that MEP peak-to-peak amplitudes were statistically significantly different between the Baseline, Right, Left, and Bi conditions for the TA and SOL muscles [TA: $\chi^2(3)= 15.2$, $p<0.01$; SOL: $\chi^2(3)= 18.5$, $p<0.01$]. Post-hoc testing corrected for multiple comparisons for the TA and SOL muscles showed that Right, Left and Bi conditions were significantly larger compared with the Baseline condition ($p<0.05$, Wilcoxon signed-rank test), while there were no significant differences between the Right, Left, and Bi conditions (Figure 2-9A).

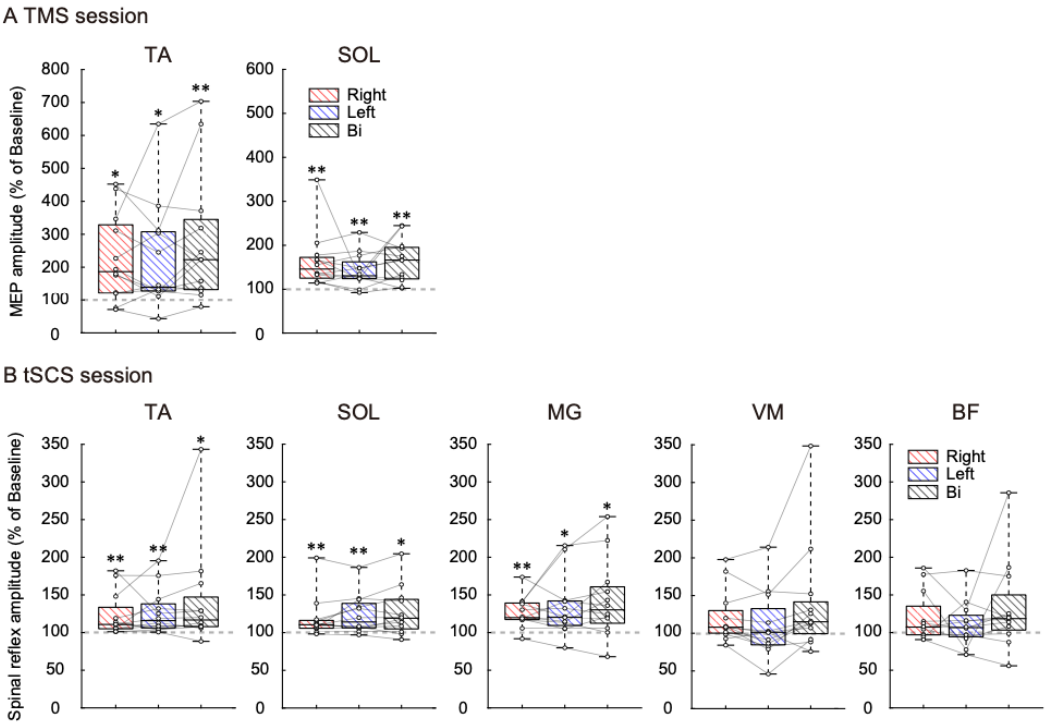

Figure 2-9: (A) Group data of motor evoked potentials (MEPs) in the tibialis anterior (TA) and soleus (SOL) muscles during right elbow flexion (Right), left elbow flexion (Left), and bilateral elbow flexion (Bi) conditions in the Tonic phase. MEP amplitudes were normalized with respect to the MEP amplitude during the Baseline condition (% of Baseline) for each participant. (B) Group data of spinal reflexes in the TA, SOL, medial gastrocnemius (MG), vastus medialis (VM), and biceps femoris (BF) during Right, Left, and Bi conditions in the Tonic phase. Spinal reflex amplitudes were normalized with respect to spinal reflex amplitude during the Baseline condition (% of Baseline) for each participant. Asterisks indicate significant differences compared to the Baseline condition. Legend: *p< 0.05, **p< 0.01.

2.3.2.3. Spinal reflex amplitude (tSCS session)

The results of the spinal reflex responses during the tSCS session are shown in Figure

2-9B. The box plots indicate the peak-to-peak amplitudes (% of Baseline) of spinal reflex responses during Right, Left, and Bi conditions in the Tonic phase. The Friedman test showed that MEP peak-to-peak amplitudes were statistically significantly different between the Baseline, Right, Left, and Bi conditions for the TA, SOL, MG and VM muscles [TA: $\chi^2(3)= 20.2$, $p<0.01$; SOL: $\chi^2(3)= 15.1$, $p<0.01$; MG: $\chi^2(3)= 15.7$, $p<0.01$; VM: $\chi^2(3)= 10.5$, $p=0.0148$] while they were not statistically different between the Baseline, Right, Left and Bi conditions for the BF muscles [BF: $\chi^2(3)= 5.20$, $p=0.158$]. Post-hoc testing corrected for multiple comparisons for the TA, SOL and MG muscles showed that Right, Left and Bi conditions were significantly larger compared with the Baseline condition ($p<0.05$, Wilcoxon signed-rank test), while there were no significant differences between the Right, Left, and Bi conditions (Figure 2-9B). Post-hoc testing corrected for multiple comparisons for the VM muscle showed no significant differences between each condition (Figure 2-9B).

2.4. Discussion

In the current study, we investigated whether MEP and spinal reflex responses of right lower-limb muscles would be modulated during motor preparation and motor execution (Motor initiation phase and Tonic phase) of ipsilateral (Right), contralateral (Left), and bilateral (Bi) remote upper-limb muscles contractions, i.e. upper-limb "remote effect" facilitation in the lower limbs. Our results showed that MEP amplitudes were significantly facilitated compared to the rest (Baseline) condition during the preparation and execution phases, while spinal reflex amplitudes were significantly facilitated compared to the rest condition only during the execution phase. Furthermore, our results showed that there were no significant differences between ipsilateral, contralateral and bilateral conditions in TMS and tSCS session (Figure 2-5, Figure 2-6, and Figure 2-9). Moreover, the results were same between different phases of motor execution (i.e., Motor initiation phase and Tonic phase) despite different levels of contraction of the elbow flexors (Motor initiation phase: TMS session was 4.27 ± 4.76 %MVC and tSCS session was 4.97 ± 4.06 %MVC; and Tonic phase: TMS session was 29.1 ± 0.83 %MVC and tSCS session was 29.0 ± 0.55 %MVC). Previous studies have shown that both tSCS-induced spinal reflex responses (Minassian et al. 2007) and MEP responses (Hallett 2007) are modulated by voluntary muscle contractions. Since the background EMG activities in all measured muscles in our current study were not significantly different between the experimental conditions (i.e., Right, Left, and Bi) and the rest state (i.e., Baseline) in each stimulation phase for both the spinal reflexes and MEP responses (see section 2.3.1.1., section 2.3.2.1., Figure 2-4, and Figure 2-8), it can be concluded that the observed modulations of the evoked responses were not affected by background EMG activities; but rather, they were an effect of "remote effect" modulation. A specific discussion of interlimb neural interactions in corticospinal and spinal reflex circuits follows in section 2.4.1.

Moreover, a key finding in our current study is that the extent of interlimb remote

facilitation in corticospinal and spinal reflex circuits: (1) was not enhanced during bilateral vs. unilateral contraction of upper limbs, and (2) was similar between ipsilateral vs. contralateral contractions, both of which are contrary to our hypothesis. Although it was reported that remote facilitation occurred during both bilateral as well as unilateral muscle contractions in both ipsilateral and contralateral sides of distal limb segments (Tazoe, Sakamoto, et al. 2007; Chiou, Wang, Liao, and Yang 2013; Chiou, Wang, Liao, Wu, et al. 2013), the extent of facilitation in multiple muscles has not been directly compared across diagonal and orthogonal muscle pairs, and during unilateral and bilateral muscles contractions. A discussion of unilateral and bilateral contraction effects on interlimb facilitation follows in section 2.4.2, while we discuss ipsilateral and contralateral contraction interlimb facilitation in section 2.4.3.

2.4.1. Interlimb facilitation of corticospinal and spinal reflex circuits during motor preparation and execution

Our results showed that spinal reflex excitability in lower-limb muscles (tSCS-evoked responses) was not modulated during preparation for upper-limb muscles contraction, while corticospinal excitability (TMS-evoked MEPs) was facilitated during both preparation and execution of upper-limb muscles contractions, as we hypothesized. MEPs elicited by single-pulse TMS over the primary motor cortex are influenced by the excitability of neurons both in the cortical and spinal motor circuits (Rothwell et al. 1991). Therefore, remote facilitation between upper-limb and lower-limb muscles in the corticospinal pathway could be attributed to cortical and/or spinal facilitation. However, excitability of spinal reflex circuits was not modulated during motor preparation for upper-limb muscles contractions, suggesting that intracortical motor circuits, rather than spinal circuits, likely contributed to interlimb remote facilitation during the preparation phase for the upper-limb contractions. Therefore, facilitation of cortical circuits

controlling lower-limb muscles by the upper-limb contractions could be induced even during motor preparation, while actual contraction (i.e., motor execution) could be required for facilitation of spinal circuits. It has previously been reported that there is some overlapping between arm and leg representations in several cortical motor-related areas, including the dorsal premotor area (PMd) and supplementary motor area (SMA) (Rijntjes et al. 1999; Henrik Ehrsson et al. 2000), which suggests that neural activations mediated by voluntary contraction of upper limbs or lower limbs interact within the cortical motor circuit and affect corticospinal excitability. Specifically, since PMd plays a major role in the selection and planning of voluntary movements (for a review, see Wise et al. 1997), it can be considered that interlimb remote facilitation during motor preparation (shown in our current study) may be induced in the motor-related cortical areas due to somatotopic overlapping of upper- and lower-limb muscle representations.

Moreover, we showed that motor execution of upper-limb muscle contractions can affect excitability of spinal reflex circuits in multiple lower-limb muscles, as well as excitability of the corticospinal tract. To test the effects of interlimb remote facilitation in spinal circuits, typically H-reflex was used to evoke responses in individual muscle such as SOL (Kawamura and Watanabe 1975; Tazoe et al. 2005). In these studies, it was reported that facilitation of SOL H-reflex was induced by the remote (upper-limb) muscle contractions (Kawamura and Watanabe 1975; Tazoe et al. 2005). Since the spinal reflexes induced by tSCS have similar characteristics as the H-reflex responses (Courtine et al. 2007; Minassian et al. 2007), our results supported previous studies that upper-limb muscles contraction can facilitate spinal reflex excitability in other ankle muscles (i.e., TA and MG) and thigh muscles (i.e., VM and BF), which were recorded simultaneously (Masugi et al. 2019). On the other hand, spinal reflexes in thigh muscles (i.e., VM and BF) were not modulated in the Tonic phase, consistent with a recent study using same tSCS methods (Kato et al. 2019). Facilitations in the Motor initiation phase and no change in the Tonic

phase in the thigh muscles may suggest that widespread remote facilitation in spinal circuits during initiation (ascending) phase of the contraction, and a more local facilitation when contraction levels are maintained for a prolonged period of time. Moreover, our results extend the current understanding by providing evidence that such modulation in spinal reflex circuits does not depend on whether contralateral or ipsilateral hands are contracted, or if they are contracted bilaterally. Specific discussion about effects of ipsilateral, contralateral, and bilateral upper-limb contraction on interlimb facilitation follows in section 2.4.2.

Remote facilitation mechanisms in spinal reflex circuits were previously attributed to: (1) an increase of fusimotor drive (Hagbarth et al. 1975; Burke et al. 1980), (2) a reduction of presynaptic inhibition to the Ia terminals on the motoneurons (Dowman and Wolpaw 1988; Zehr and Stein 1999), and (3) a direct postsynaptic facilitation of motoneurons (Boroojerdi et al. 2000; Furubayashi et al. 2003). Previous studies ruled out the contribution of fusimotor drive by examining microneurographic activities of the afferent fibers during remote muscles contractions (Hagbarth et al. 1975; Burke et al. 1980). Thus, it can be considered that presynaptic mechanisms (i.e., reduction of presynaptic inhibition onto Ia terminals) and/or the post synaptic mechanisms (i.e., spinal motoneurons) can likely explain the results in our current study. Overall, by assessing the change of excitability in both corticospinal and spinal circuits, our results present new evidence to suggest that upper-limb muscle contractions could affect intracortical motor circuits controlling lower-limb muscles even during motor preparation, while actual contraction (i.e., motor execution) is required to mediate spinal motor circuits controlling lower-limb muscles.

2.4.2. Effect of unilateral and bilateral contractions on interlimb facilitation

We hypothesized that the extent of the "remote effect" would be larger during bilateral vs. unilateral upper-limb muscle contractions due to summation effects. However, the "remote effect"

facilitation was similar between bilateral and unilateral conditions. First, it may be that excitation of neurons receiving input from remote muscles contractions could have been saturated (i.e., ceiling effect), although the contraction levels were relatively low (<30% of maximal effort). On the other hand, previous investigations of the "remote effect" facilitation during simultaneous task performance (maximum ankle dorsiflex and teeth clenching) reported that combined motor tasks led to higher excitability of hand muscle corticospinal (i.e., MEP) excitability compared to when the tasks were performed individually (Komeilipoor et al. 2017). Therefore, our results extend the current understanding of neural interactions by suggesting that combined motor task of homologous muscles pairs (e.g., ipsilateral and contralateral upper limbs) did not have a summed "remote effect". On the other hand, non-homologous muscles pairs (e.g., jaw muscles and leg muscles) were previously suggested to have a summed contributions to the "remote effect" facilitation (Komeilipoor et al. 2017). This homologous muscle effect could also in part be attributed to interhemispheric inhibition mechanisms. It has been widely accepted that neural activation (e.g., induced by upper-limb muscle contraction) in the motor cortex of one hemisphere can inhibit neural activities controlling homologous muscles (e.g., opposite upper-limb muscle) in the other hemisphere via corpus callosum pathways, which are linking both hemispheres (Ferbert, Priori, et al. 1992). Moreover, it was also reported that bilateral elbow flexion increases inhibition between hemispheres, compared to unilateral elbow flexion (Perez et al. 2014). Therefore, in our current study, it is possible that bilateral elbow flexion causes both hemispheres to inhibit each other via such interhemispheric inhibition circuits, as previously suggested (Oda and Moritani 1995; Taniguchi et al. 2001; Fling and Seidler 2012; Vieluf et al. 2017). Therefore, it can be considered that non-homologous muscles pairs (e.g., jaw muscles and leg muscles) were expressed as a summation of each contraction (Komeilipoor et al. 2017) since these tasks were not interfered by bilateral interhemispheric inhibition. On the other hand, homologous muscles

pairs (e.g., right and left upper limbs) in our current study, which induced activation of both hemispheres, did not sum due to interhemispheric inhibition. However, further work is warranted to systematically examine effects of interhemispheric inhibition on intralimb facilitation.

2.4.3. Effect of ipsilateral and contralateral contractions on interlimb facilitation

We also expected that the extent of "remote effect" on neural circuits in right lower-limb muscles would be larger during ipsilateral (right) upper-limb muscles contractions, compared when contralateral (left) upper-limb muscles were contracted. However, contrary to our hypothesis, this did not occur. In terms of remote facilitation between ipsilateral non-homologous muscles pairs (e.g., right upper-limb and right lower-limb muscles), previous studies suggested that intrahemispheric connectivity may contribute to the remote facilitation effects (Capaday et al. 2009, 2011). Specifically, these studies elegantly demonstrated that neural activity initiated at the cortical locus can spread to the nearby cortical regions representing different muscles via intrinsic horizontal connections between neurons in the motor cortex (Capaday et al. 2009, 2011). Using TMS, it was also proposed that similar neural mechanisms, including cortico-cortical connectivity, might exist within the primary motor cortex in humans (Boroojerdi et al. 2000; Komeilipoor et al. 2017; Sasaki et al. 2018). Thus, it is likely that activations of cortical motor representations by voluntary contractions of the upper limbs spread to the neighboring cortical areas representing muscles in different segments of the body (i.e., lower-limb muscles). In that case, it is likely that effects of ipsilateral upper-limb muscle contractions to lower-limb muscles could be larger compared to contraction of contralateral upper-limb muscles due to intrahemispheric connectivity. However, such intrahemispheric mechanisms cannot explain remote facilitation between contralateral muscles pairs (i.e., left upper-limb and right lower-limb muscles) as shown in our study. Neurophysiological studies in non-human primates demonstrated

that some pyramidal neurons in the primary motor cortex are activated during movements of ipsilateral upper-limb muscles, suggesting the ipsilateral and contralateral hemisphere play a role during voluntary control of unilateral movements (Tanji et al. 1988; Donchin et al. 1998; Steinberg et al. 2002). Moreover, neuroimaging studies in humans also reported that unilateral movements are associated not only with activation of the contralateral primary motor cortex but also with the co-activation of the ipsilateral primary motor cortex (Kim et al. 1993; Stippich et al. 2007). These studies suggest that there may be interhemispheric interactions during unilateral movements. Furthermore, it was demonstrated that short interval intracortical inhibition (induced by paired-pulse TMS, which reflects cortical inhibition) in lower-limb muscles was decreased during contralateral upper-limb muscle contractions (Chiou, Wang, Liao, and Yang 2013). This suggests that voluntary upper-limb muscle contractions had cortical disinhibitory effects on the contralateral lower-limb muscles. Therefore, based on the abovementioned studies, it can be considered that interhemispheric facilitatory and/or disinhibitory mechanisms could explain the results in the contralateral upper-limb muscle contraction condition in our current study. Moreover, diagonal coordinated movement between the upper-limb and lower-limb muscles has been reported in human studies (Swinnen 2002), and lower-limb muscle movements could be associated with opposite upper-limb movements (Yiou et al. 2007). It was also reported that the muscle activities and reflex responses in the lower-limb muscles were modulated during rhythmic activity of the upper-limb muscles (Zehr et al. 2009). Such functional importance of diagonal coordination between upper-limb and lower-limb muscles might also have contributed to remote facilitation between left upper-limb and right lower-limb muscles. Overall, our results suggest that net facilitatory effects, which were shown in the lower-limb muscle corticospinal and spinal circuits, were similar even during different upper-limb muscle contractions. However, the neurophysiological mechanisms and functional connectivity of these facilitation could be

different.

2.4.4. Clinical implications

The results of the present study may have clinical implications for patients with neurological injuries. Specifically, neurological injuries and/or prolonged disease of muscles are typically accompanied by reductions of central nervous system activity, which includes weakened connections between the brain and the muscle (i.e., corticospinal excitability) (Oudega and Perez 2012). First, our findings may be relevant for individuals with paraplegia or other neurological injuries. Specifically, contraction of unaffected upper limbs, which does not depend on whether contralateral/ipsilateral hands are contracted or if they are contracted bilaterally, may enhance corticospinal excitability of the affected lower limbs. Perhaps training the upper limbs may also have effects on improving the strength/skills in the remote lower-limb muscles. Second, to examine the precise pathological condition in patients with neurological injuries, evaluation of the spinal reflex arcs in multiple muscles is crucial. Indeed, tSCS-evoked spinal reflexes were previously measured in patients with spinal cord injury (Dy et al. 2010; Gerasimenko et al. 2015; Hofstoetter et al. 2019) and those with lumber nerve compression (Andriyanova 2010). Therefore, our interlimb "remote effect" facilitation paradigms may also be helpful for assessments of residual motor function and evaluation of the spinal reflexes or the extent of recovery after injury. However, to translate our current basic mechanism findings to clinical settings, future studies are warranted to examine interlimb "remote effect" facilitation in patients with neurological injuries.

2.4.5. Limitations

Our work has several limitations that should be noted. First, TMS "hotspot" and intensity were optimized for TA muscle, while we recorded both SOL and TA MEPs. Although

previous studies reported that a single TMS "hot spot" and intensity optimized for the TA muscle can also elicit MEPs from SOL muscle (e.g., Kaneko et al. 2018; Nakagawa et al. 2020), there is a possibility that modulation effects of the SOL muscle in our study were not optimal. Second, we investigated neural activities from multiple lower-limb muscles (i.e., TA, SOL, MG, VM and BF) in the tSCS session, while we investigated limited number of lower-limb muscles (i.e., TA and SOL) in the TMS session. It is well known that tSCS of the lumbar spinal cord can be used to reliably evoke spinal reflexes from multiple lower-limb muscles simultaneously, including thigh muscles (i.e., VM and BF) (Courtine et al. 2007; Minassian et al. 2007; Dy et al. 2010; Roy et al. 2012; Masugi et al. 2016, 2017) and our results in spinal reflexes showed different (segment specific) modulation between thigh and shank muscles in the Tonic phase (see Figure 2-9B). Therefore, presenting spinal reflex data from multiple muscles, including thigh muscles may offer new "remote effect" insights. However, future studies are warranted to specifically test neural activity from multiple lower-limb muscles using TMS, which is optimized for recording from shank and thigh muscles separately. Finally, in the current study we did not directly measure intracortical activity (e.g., paired-pulse TMS paradigm). By assessing corticospinal and spinal reflex excitability during same experimental conditions, we could logically speculate about cortical and spinal mechanism contributions (Kasai et al. 1997; Taube et al. 2008; Mouthon et al. 2015) to interlimb neural interactions. Nonetheless, future studies are warranted to specifically test intracortical inhibition during "remote effect" paradigms.

2.5. Conclusions

We investigated interlimb neural interaction (i.e., "remote effect" facilitation) during upper-limb contraction on the lower-limb muscles in the corticospinal and spinal reflex circuits using transcranial magnetic stimulation and transcutaneous electrical stimulation, respectively.

Our results show that preparation and execution of voluntary contractions of upper-limb muscles facilitated corticospinal excitability in lower-limb muscles, while execution of voluntary muscle contractions of upper-limb muscles facilitate the excitability of spinal reflex circuits only. Therefore, interlimb neural interactions can be attributed to cortical and/or spinal mechanisms depending on the phase of the movement such as motor preparation and execution. Specifically, it could be considered that intracortical mechanisms are likely connected via non-selective activation both upper-limb and lower-limb muscles in the motor-related areas during motor preparation. Spinal reflex results suggest that actual contraction (i.e., motor execution) is required to facilitate spinal reflex circuits in distal segmental muscles. Moreover, our results showed that the extent of such facilitation does not depend on whether contralateral or ipsilateral hands are contracted or if they are contracted bilaterally. Similar facilitatory effects during unilateral vs. bilateral upper-limb contractions suggest that ceiling effect and/or interhemispheric inhibition mechanisms could be attributed to interlimb "remote effect" facilitation, although we did not directly measure intracortical activity. Moreover, similar facilitatory effects during ipsilateral vs. contralateral upper-limb contractions suggest that neurons receiving input from remote muscles could follow an all-or-none law which does not depend contralateral or ipsilateral activations.

Chapter 3 - Study 2

Evidence for the existence of trunk-limb neural interaction in the corticospinal pathway

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NOTE*: The content of this chapter is identical to the material presented in the publication except for the text formatting which was done according to University of Tokyo requirement.*

3.1. Introduction

Corticospinal excitability between the brain and a muscle can be evaluated by examining the amplitude of the motor evoked potential (MEP) elicited through transcranial magnetic stimulation (TMS) on the primary motor cortex (M1) (Rothwell et al. 1991). Previous studies using TMS to evoke MEPs suggested the existence of a direct corticospinal pathway to the trunk muscles (Plassman and Gandevia 1991; Ferbert, Caramia, et al. 1992). Trunk muscles have an essential role in postural control during standing, sitting (e.g., Milosevic et al. 2017), and walking (e.g., Mueller et al. 2017). Therefore, it is important to understand the interaction between trunk and the upper-limb and lower-limb muscles. It has been shown that corticospinal excitability of trunk muscles can be modulated during simple voluntary movements such as flexion and extension of the trunk (Plassman and Gandevia 1991; Nowicky et al. 2001; Tunstill et al. 2001), maintenance of posture (Chiou et al. 2016), and during respiratory maneuvers (Nowicky et al. 2001; Tunstill et al. 2001; Jaberzadeh et al. 2013). Taken together, these studies suggest that trunk muscles have corticospinal drive under voluntary and involuntary conditions.

It is also known that contraction of certain upper-limb and lower-limb muscles can modulate the corticospinal excitability not only of that contracted muscle, but also of other muscles which are located in a remote segment of the body and remain at rest (Kawakita et al. 1991; Pereon et al. 1995; Boroojerdi et al. 2000; Hortobágyi et al. 2003). This phenomenon is known as “remote effect” (Tazoe, Endoh, et al. 2007; Tazoe, Sakamoto, et al. 2007; Komeilipoor et al. 2017). Previous studies have shown facilitation of MEP amplitude of upper-limb muscles during contraction of the leg muscles (Tazoe et al. 2009; Morishita et al. 2011), jaw clenching (Boroojerdi et al. 2000; Komeilipoor et al. 2017), as well as contraction of the contralateral hand muscles (Kawakita et al. 1991; Hortobágyi et al. 2003; Morishita et al. 2011). Similarly, MEP facilitation in lower-limb muscles was shown during hand contractions (Kawakita et al. 1991),

jaw clenching (Boroojerdi et al. 2000), as well as contraction of the contralateral leg muscles (Chiou, Wang, Liao, and Yang 2013). These interactions are thought to be cortically based (Tazoe, Endoh, et al. 2007; Tazoe, Sakamoto, et al. 2007; Chiou, Wang, Liao, and Yang 2013). However, previous studies examining "remote effect" have focused only on the inter-limb interaction (i.e., interaction between upper-limbs and lower-limbs). To date, no study has systematically investigated corticospinal interactions between the trunk muscles and upper-limb and lower-limb muscles. Understanding how trunk muscle contractions influence limb muscles (i.e., trunk "remote effect") is essential for understanding the underlying neural mechanics in fundamental human movements, such as postural control and walking. Moreover, such knowledge could be used for optimizing neurorehabilitation in individuals with trunk impairment, such as in people with spinal cord injury (Milosevic, Yokoyama, et al. 2017).

In the present study, we hypothesized that voluntary activation of the trunk muscles representation would affect upper-limb, lower-limb, and jaw muscle cortical and subcortical circuitry, and vice versa. The specific objectives of this study were to investigate if corticospinal excitability of: (a) trunk muscle during rest state is modulated when hands, legs, and jaw muscles are contracted and; (b) hand, legs, and jaw muscles during rest state is modulated when trunk muscles are contracted.

3.2. Methods

3.2.1. Participants

Eleven able-bodied individuals were recruited for this study. The age, body mass, and height of participants were 24.2±3.2 years, 66.7±7.6 kg, and 173.6±6.0 cm (mean±SD), respectively. None of the participants had a history of neurological and musculoskeletal impairments, including epilepsy. All participants gave written informed consent in accordance with the Declaration of Helsinki. The experimental procedures were approved by the local institutional ethic committee of the Graduate School of Arts and Science, The University of Tokyo.

3.2.2. Data acquisition

Electromyography (EMG) activities were recorded from the trunk, hand, jaw, and leg muscles unilaterally. Bipolar Ag/AgCl surface electrodes (Vitrode F-150S, Nihon Koden, Tokyo, Japan) were placed on the left side with 1 cm separation on the: (i) rectus abdominis (RA; trunk muscle); (ii) flexor digitorum superficialis (FDS; hand muscle); (iii) masseter (MS; jaw muscle); (iv) tibialis anterior (TA; leg muscle). A reference electrode was placed over the clavicle. Prior to application of electrodes, the skin was prepared using sand paper and alcohol to reduce skin impedance. The signals were band-pass filter (15 - 3,000 Hz) and amplified (×2000) using a multichannel EMG amplifier (MEG-6108, Nihon Koden, Tokyo, Japan). All data were digitized at sampling frequency of 10,000 Hz using an analog-to-digital (A/D) converter (Powerlab/16SP, AD Instruments, Castle Hill, Australia).

3.2.3. Experimental protocol

Participants were seated comfortably on the chair with no backrest and were instructed to maintain upright posture of the trunk with both arms relaxed on the side of the body. Following

a gentle warm-up and task practice, participants were asked to perform three maximal voluntary contraction (MVC) trials for each of the muscles: (i) RA muscle during force expiration while breath holding (FEBH): participants were instructed to breath out in an expiratory Valsalva maneuver as hard as possible (Jaberzadeh et al. 2013); (ii) FDS muscle during hand gripping: participants were instructed to clench their fists bilaterally as hard as possible; (iii) MS muscle during jaw clenching: participants were instructed to grit their teeth as hard as possible; and (iv) TA muscle during ankle dorsiflexion: participants were instructed to fully dorsiflex their ankles bilaterally as hard as possible, while both heels remained on the floor. Using an analog integrator (EI-601G, Nihon Koden, Tokyo, Japan) and an oscilloscope (TDS2012C, Tektronix, Oregon, USA), the integrated EMG amplitude during the 3 s epoch was measured and averaged on the three trials to define the MVCs for each muscle.

Experimental data were acquired during the following five experimental conditions: (1) while participants were relaxed (Rest); (2) during FEBH maneuver (Trunk), (3) during bilateral hand clenching (Hands); (4) during jaw clenching (Jaw); and (5) during bilateral ankle dorsiflexion (Legs). Recordings were performed on the same day, with a break of at least 5 min between experimental conditions. Moreover, the experimental conditions order was randomized between participants. The active conditions (i.e., Trunk, Hands, Jaw, Legs) were performed while participants were asked to match a visual target on an oscilloscope, which was set at 30% of MVC of the respective muscles.

3.2.4 Transcranial magnetic stimulation (TMS)

TMS was delivered over the M1 region using a mono-phasic magnetic stimulator (Magstim 200, Magxtim Co., Whitland, UK) through a double cone coil (outside diameter of 110 mm). The optimal stimulation spot (i.e. “hot spot”) was searched over the right cortex (starting

point was defined at 1.5 cm anterior and 3cm lateral to vertex) at which the MEPs could be recorded from RA muscles (Jaberzadeh et al. 2013). The MEPs in all muscles tested were evoked from the stimulation to the same location (RA "hot spot") in each experimental condition. The motor threshold (MT) was determined with the participant maintaining a voluntary contraction at 30% of MVC of RA muscle during the FEBH task. MT was defined as the minimum TMS intensity for which MEPs had a peak-to-peak amplitudes larger than 50 μV and were evoked in at least three out of five successive trials. The TMS intensities for the experiment were set to: (a) 120% of MT level (1.2MT); (b) 140% of MT level (1.4MT); and (c) 160% of the MT level (1.6MT). Five TMS stimuli were delivered for each TMS intensity (i.e., 1.2MT, 1.4MT, and 1.6MT), and for each experimental condition (i.e., Rest, Trunk, Hands, Jaw, Legs), totaling in 75 stimuli (5 stimuli x 3 TMS intensities x 5 experimental conditions). Data was recoded from 100 ms before to 300 ms after application of the TMS stimulus.

3.2.5. Data analysis

The MEP peak-to-peak amplitudes of each muscle were calculated using Matlab (2017a, The MathWorks Inc., Massachusetts, USA) for each trial. Moreover, the background EMG activity of a 50 ms window before the TMS stimulus was defined by calculating the root mean square value in each trial. Five repeated trials were averaged for each experimental condition and TMS intensity. Trials were removed from the analysis if background EMG of resting muscle exceeded three standard deviations of the mean background EMG at Rest condition.

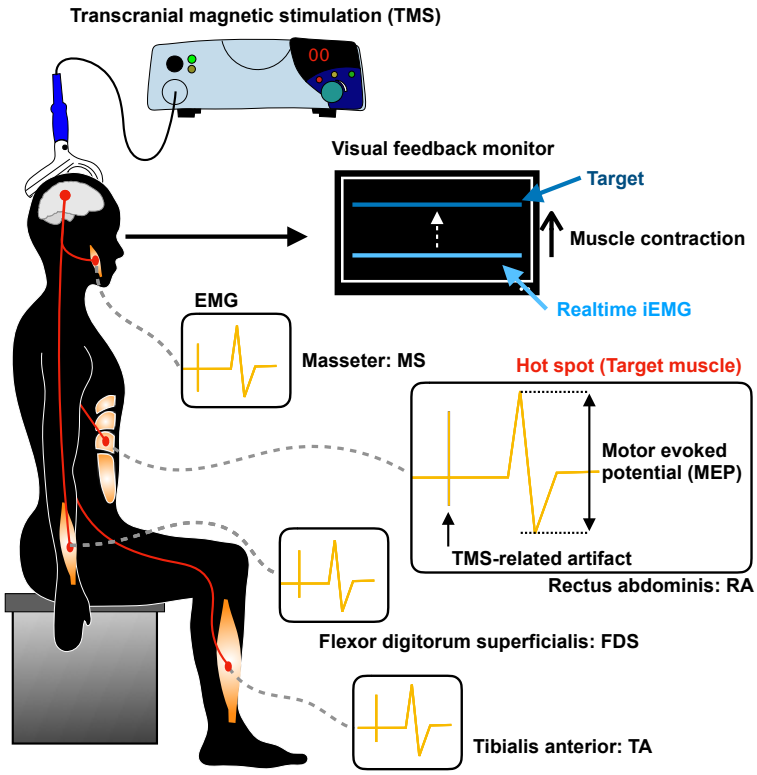

Figure 3-1. Experimental setup

3.2.6. Statistics

Comparisons were performed using a two-way repeated measures analysis of variance (ANOVA) to evaluate the effects of the experimental condition (i.e., Rest, Trunk, Hand, Teeth, and Legs) and TMS intensity (i.e., 1.2MT, 1.4MT, and 1.6MT) on the MEP amplitudes and background EMG. When significant interaction was found between the experimental conditions and TMS intensity, a one-way repeated measures ANOVA was used to confirm the effect at each TMS intensity separately. Significant results on the ANOVA were followed up with post-hoc multiple comparisons with Bonferroni corrections. Since the Shapiro-Wilk test showed that all measures were not normally distributed, a logarithmic transformation was performed to normalize the data prior to performing the ANOVAs. It was confirmed that data with a logarithmic transformation were normally distributed. Significance level on all tests was set to $p<0.05$.

3.3. Results

3.3.1. Background EMG

RA background EMG showed no interaction ($p=0.649$), but had an effect of the experimental conditions ($p=0.001$) and no effect of TMS intensity ($p=0.351$). Specific, post-hoc, comparisons showed that RA background EMG was similar across all experimental conditions, except the active contraction condition (i.e., Trunk), as expected.

FDS background EMG showed no interaction ($p=0.492$), but had an effect of the experimental conditions ($p<0.001$) and no effect of TMS intensity ($p=0.596$). Specific, post-hoc, comparisons showed that FDS background EMG was similar across all experimental conditions, except the active contraction condition (i.e., Hands), as expected.

MS background EMG showed no interaction ($p=0.887$), but had an effect of the experimental conditions ($p<0.001$) and no effect of TMS intensity ($p=0.924$). Specific, post-hoc, comparisons showed that FDS background EMG was similar across all experimental conditions, except the active contraction condition (i.e., Teeth), as expected.

TA background EMG showed no interaction ($p=0.293$), but had an effect of the experimental conditions ($p<0.001$) and no effect of TMS intensity ($p=0.415$). Specific, post-hoc, comparisons showed that TA background activity was similar across all experimental conditions, except the active contraction condition (i.e., Legs), as expected.

3.3.2. MEP amplitude

The representative data for MEPs of all muscles (i.e., RA, FDS, MS, and TA) at 1.6MT TMS intensity are shown in Figure 3-2.

RA MEP amplitudes showed an interaction ($p<0.001$), as well as an effect of the experimental conditions ($p<0.001$), and TMS intensity ($p<0.001$). Specific comparisons between

experimental conditions, shown in Figure 3-3, indicate that contracting the trunk muscles (i.e., active condition) was significantly larger than all other experimental conditions at all TMS intensities. Overall, RA MEP amplitudes were very low when trunk muscles were not contracted, with 0 out of 11 participants at 1.2MT, 3 out of 11 participants at 1.4MT, and 5 out of 11 participants at 1.6MT having any MEPs during the Rest condition. Moreover, contracting the hands increased the RA MEPs (i.e., Rest vs. Hands were different) at 1.6MT TMS intensity (Figure 3-3).

FDS MEP amplitudes showed no interaction ($p=0.151$), but had a significant effect of both the experimental conditions ($p<0.001$), and TMS intensity ($p=0.021$). Specific comparisons between experimental conditions indicate that contracting the hand muscles (i.e., active condition) significantly increased FDS MEPs (Figure 3-3). Moreover, contracting the trunk muscles increased the FDS MEPs (i.e., Rest vs. Trunk were different) at all TMS intensities (Figure 3-3).

MS MEP amplitudes showed an interaction ($p=0.017$), as well as the effect of the experimental conditions ($p=0.001$), and TMS intensity ($p=0.007$). Specific comparisons between experimental conditions indicate that contracting the jaw muscles (i.e., active condition) had some small effects on the MS MEPs at lower TMS intensities, but no experimental condition increased the MS MEPs (Figure 3-3).

Finally, TA MEP amplitudes showed an interaction ($p=0.037$), as well as the effect of the experimental conditions ($p<0.001$), and TMS intensity ($p=0.029$). Specific comparisons between experimental conditions indicate that contracting the leg muscles (i.e., active condition) had an effect on the TA MEPs at all TMS intensities (Figure 3-3). Moreover, contracting the trunk muscles increased the TA MEPs (i.e., Rest vs. Trunk were different) at all TMS intensities (Figure 3-3). Contracting the hand muscles also increased the TA MEPs (i.e., Rest vs. Hands were

different) only at 1.6MT TMS intensity.

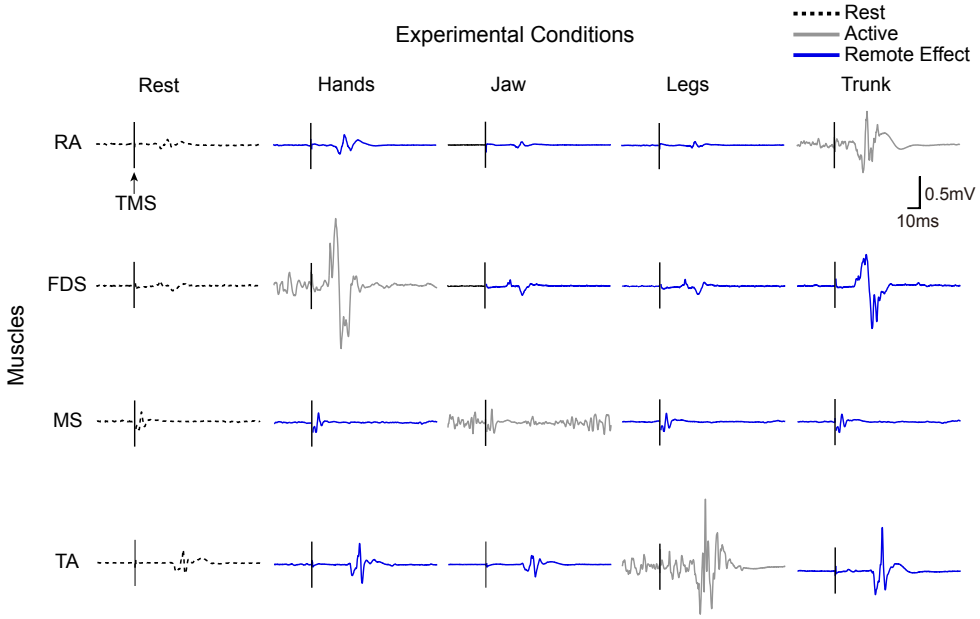

Figure 3-2. Representative plots showing motor evoked potentials (MEPs) elicited using trans cranial magnetic stimulation (TMS) at 160% of the motor threshold (1.6MT) stimulus intensity in the rectus abdominis (RA), flexor digitorum superficialis (FDS), masseter (MS) and tibialis anterior (TA) muscles during rest (Rest), hand clenching (Hands), jaw clenching (Jaw) and ankle dorsiflexion (Legs). Dashed lines indicate rest state conditions (Rest); gray lines indicate of contraction conditions of target muscles (Active); and blue lines indicate the conditions of contraction of muscles which are located in a remote segment from the target muscles (Remote Effect).

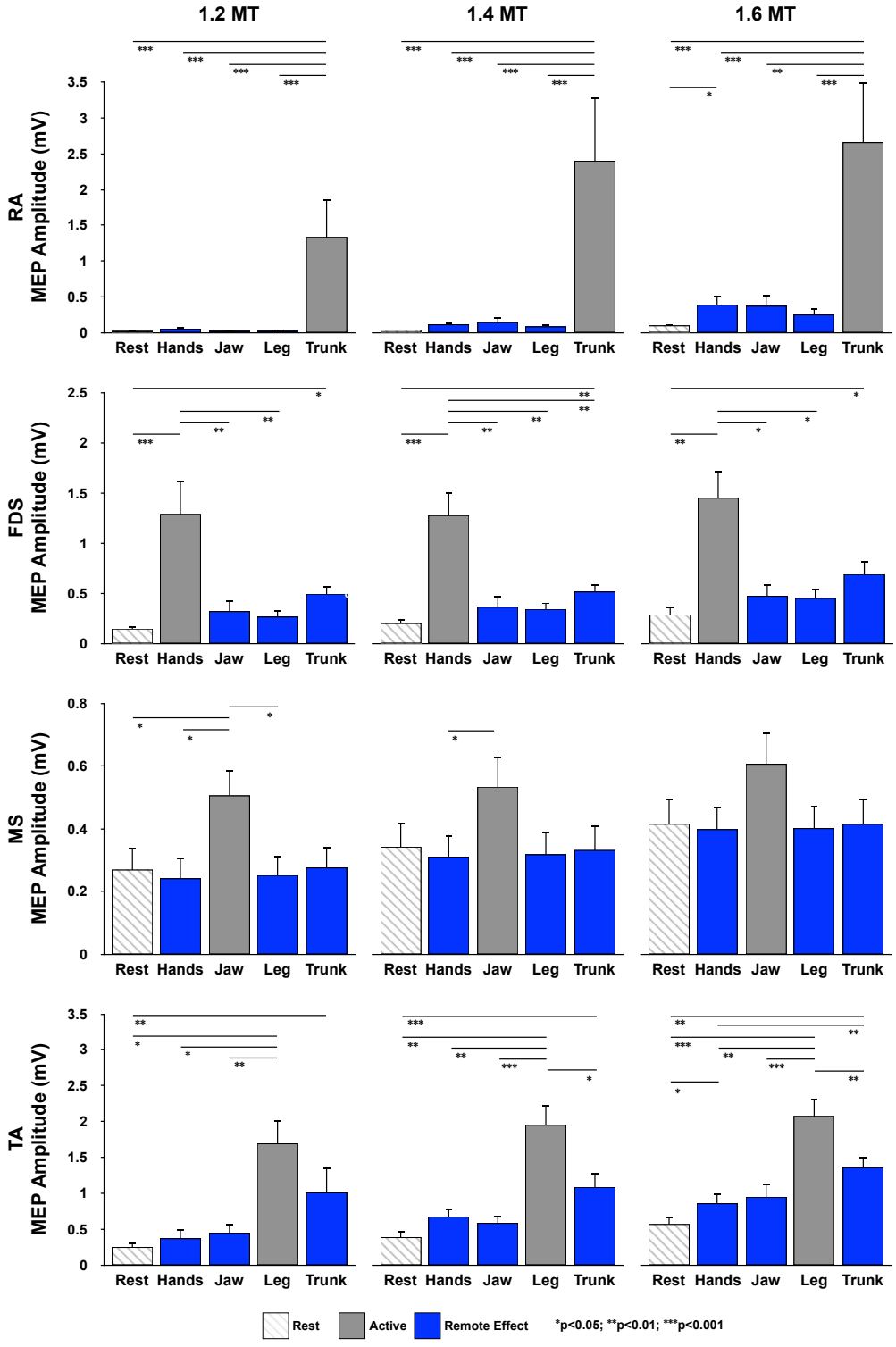

Figure 3-3. Mean amplitudes and standard error (SE) of motor evoked potentials (MEPs) elicited using transcranial magnetic stimulation (TMS) at 120% motor threshold (1.2MT); 140% motor threshold (1.4MT), and 160% motor threshold (1.6MT)

in the rectus abdominis (RA), flexor digitorum superficialis (FDS), masseter (MS) and tibialis anterior (TA) during rest (Rest), hand clenching (Hands), jaw clenching (Jaw) and ankle dorsiflexion (Legs). Dashed bars indicate rest state conditions (Rest); gray bars indicate contraction conditions of target muscles (Active); and blue bars indicate contraction conditions of muscles which are located in a remote segment from the target muscles (Remote Effect). *$p<0.05$.

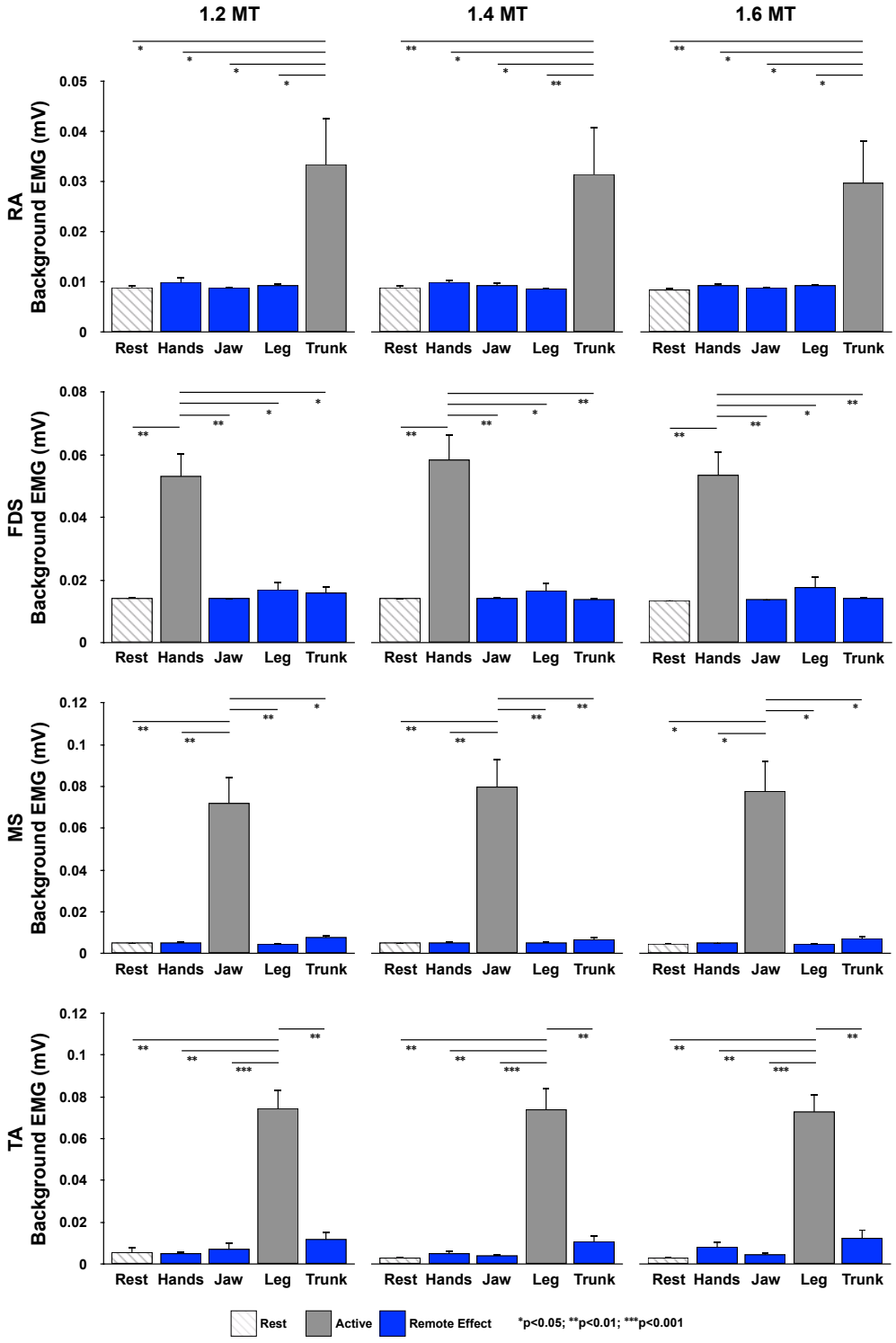

Figure 3-4. Mean root mean square value and standard error (SE) of background EMG activity of a 50 ms window before transcranial magnetic stimulation (TMS) at 120% motor threshold (1.2MT); 140% motor threshold (1.4MT), and 160% motor threshold

(1.6MT) in the rectus abdominis (RA), flexor digitorum superficialis (FDS), masseter (MS) and tibialis anterior (TA) during rest (Rest), hand clenching (Hands), jaw clenching (Jaw) and ankle dorsiflexion (Legs). Dashed bars indicate rest state conditions (Rest); gray bars indicate contraction conditions of target muscles (Active); and blue bars indicate contraction conditions of muscles which are located in a remote segment from the target muscles (Remote Effect). *$p<0.05$.

3.4. Discussion

In this study, we investigated whether the corticospinal excitability of the trunk muscles is modulated during contraction of hands, legs, and jaw muscles, as well as whether the corticospinal excitability of the hand, leg, and jaw muscles is modulated during contraction of trunk muscles. Our findings showed that voluntary contraction of hands facilitated the MEP amplitudes of rest state RA (i.e., trunk muscle), while voluntary contraction of leg muscles and jaw muscle did not facilitate the MEP amplitudes of rest state RA. Our findings also showed that voluntary contraction of the trunk facilitated the MEP amplitudes of FDS (i.e., hand muscle) and TA (i.e., leg muscle), while it did not facilitate the MEP amplitudes of MS (i.e., jaw muscle). MEPs elicited in actively contracted muscles are activated earlier and with larger amplitudes than in muscles at rest (Hallett 2007), which implies that background activity influences the evoked MEPs. Since background EMG activity was not significantly different between the experimental conditions for the muscles in the rest state in our current study (see section 3.3.1), it can be considered that MEP amplitudes facilitation was due to “remote effect”. Therefore, our results imply that an interaction between the trunk and the limbs was induced mutually, as we had hypothesized. Overall, our study is the first to demonstrate existence of trunk-limb neural interaction in the corticospinal pathway.

Our results did not show facilitation of RA MEP during jaw clenching and ankle dorsiflexion, contrary to our hypothesis and previous reports. Moreover, the “remote effect” of RA and TA muscles was predominantly seen at higher TMS intensity (Figure 3-3). Several previous studies have reported that jaw clenching (Boroojerdi et al. 2000; Komeilipoor et al. 2017) and ankle dorsiflexion (Komeilipoor et al. 2017) facilitated corticospinal excitability of different muscles, which remained in the rest state. In these studies, each task was performed at a higher contraction levels (i.e., maximum voluntary contraction), while in our present study MEPs

were elicited at 30% of MVC. The extent of the "remote effect" in upper-limb and lower-limb muscles depends on the strength of voluntary contraction of remote muscles (Tazoe and Komiyama 2014). For instance, it has been reported that the voluntary isometric knee extension facilitates MEPs of upper-limb muscle proportionally to the knee extension force (Tazoe et al. 2009). This indicates that the higher the level of muscle contractions, the larger the degree of facilitation of corticospinal excitability of resting muscles (Tazoe et al. 2009). Therefore, lack of facilitation of the MEPs of the RA during ankle dorsiflexion and jaw clenching in our study is most likely caused by low voluntary contraction levels, which did not recruit sufficiently at 30% MVC to induce facilitation. In our current study, facilitation of MEPs of the RA and TA muscles during hand contractions were observed only during high TMS intensity (1.6 MT), which is in agreement with previous findings. Therefore, the observed modulations, which were shown only at high TMS intensity may be explained by greater activation of the corticospinal neurons recruited.

In our current study, RA MEPs were very low when trunk muscles were at rest (i.e., not contracted), with 0 out of 11 participants at 1.2MT, 3 out of 11 participants at 1.4MT, and 5 out of 11 participants at 1.6MT having MEPs during the Rest condition. Low recruitment of trunk muscle MEPs during rest state is consistent to some previous studies (Ferbert, Priori, et al. 1992; Jaberzadeh et al. 2013). On the other hand, it has been reported that it was possible to elicit trunk MEPs at rest (Nowicky et al. 2001; Tunstill et al. 2001). Depending on participants, lower stimulation intensities (1.2MT or 1.4 MT) were possibly not enough to recruit trunk muscles at rest state. Therefore, higher recruitment of corticospinal neurons either through higher TMS intensity stimulation or voluntary contraction of muscles is necessary to elicit trunk muscle MEPs.

FDS MEPs under Hands condition seem to stay the same at different TMS intensities while all other muscle MEPs under contraction of target muscle condition (Active) seem to have

bigger amplitudes with increase of TMS intensities (see Figure 3-3). Some studies showed that the corticospinal facilitation pattern with increasing voluntary contraction varies according to different muscles (Kischka et al. 1993; Turton and Lemon 1999). For instance, the hand muscle has higher levels of recruitment gain at low MVC levels (20-40% MVC), possibly reflecting richness of corticospinal drive, which indicate saturation of response amplitude at low levels of voluntary contraction (Turton and Lemon 1999). On the other hand, other muscles such as upper arm and leg muscles, which do not need fine motor control have more gradual corticospinal facilitation pattern with voluntary contractions. This indicates that these muscles need high MVC levels to saturate response amplitude (Kischka et al. 1993). Therefore, in our study, only hand muscle seems to have saturation at 30% MVC level due to steeper corticospinal facilitation pattern with voluntary contraction, which could reflect richer corticospinal drive compared to other muscle.

Trunk-limb neural interactions in the corticospinal pathway, which were demonstrated in our current study, could be attributed to cortical and/or subcortical (spinal) facilitation mechanisms since the MEPs elicited using TMS over the motor cortex are influenced by the excitability of neurons both in the motor cortex and the α-motoneuron pool in the spinal pathway (Rothwell et al. 1991). Firstly, subcortical facilitation mechanism, which reflects the α-motoneuron pool excitability could play a role in explaining the results observed in our study. Previously, increased F-wave, which was elicited by electrical stimulation of ulnar nerves, and reflects the excitability of the α-motoneuron pool, was shown in the resting upper-limb muscles during jaw clenching (Boroojerdi et al. 2000). This result suggests that jaw clenching resulted in an increase in the α-motoneuron pool excitability in the upper-limb muscles (Boroojerdi et al. 2000). Moreover, increased MEPs, elicited by cervicomedullary (Bunday and Perez 2012) and cervical spinal cord (Stedman et al. 1998) electrical stimulation, reflecting the motoneuronal

excitability in subcortical motor circuity (compared to cortically induced MEPs elicited using TMS in our study, which could reflect both cortical and subcortical excitability) were shown to increase in the rest state upper-limb muscle during contraction of contralateral homologous muscle (Stedman et al. 1998; Bunday and Perez 2012). Furthermore, in previous studies using H-reflex, it was suggested that the extent of motoneuronal excitability modulated by remote muscle contraction may depend on the proximity of spinal neurons and spinal levels controlling muscles (Dowman and Wolpaw 1988; Zehr and Stein 1999). Therefore, since the spinal neurons controlling trunk muscles are located between upper-limb and lower-limb spinal neurons, there is a possibility that trunk muscles are more likely to affect upper-limb and lower-limb muscles in the spinal circuity. Overall, these studies suggest the possibility that subcortical structures (spinal mechanism) contribute to the modulation of the "remote effect". On the other hand, some previous studies reported that excitability of subcortical motor circuity was not modulated during voluntary contraction of non-homologous (remote) limb muscles (Tazoe et al. 2005, 2009). Specifically, Tazoe et al. reported that MEP of upper-limb muscle, which was evoked by cervicomedullary electrical stimulation, was not modulated by ipsilateral lower-limb contraction (Tazoe et al. 2009), contrary to the results shown by Stedman et al (Stedman et al. 1998). Thus, based on previous studies which examined the spinal circuitry modulation, it is not fully clear if subcortical structures (spinal mechanisms) contribute to the "remote effect" in non-homologous muscles, such as for trunk-limb interaction in our current study.

Moreover, it has been demonstrated that the intracortical motor circuity was modulated by remote limb (non-homologous) muscle contraction (Tazoe, Endoh, et al. 2007; Tazoe, Sakamoto, et al. 2007; Chiou, Wang, Liao, and Yang 2013; Chiou, Wang, Liao, Wu, et al. 2013). Specifically, Chiou et al showed that upper-limb and lower-limb muscle short-interval intracortical inhibition, which was induced by paired-pulse TMS and reflects cortical inhibition,

were decreased during remote limb muscle contraction (Chiou, Wang, Liao, and Yang 2013; Chiou, Wang, Liao, Wu, et al. 2013). In other studies, reduction of cortical silent period duration of upper-limb motor area, which was elicited by TMS and reflects inhibition of corticospinal neurons, was observed during lower-limb contraction (Tazoe, Endoh, et al. 2007; Tazoe, Sakamoto, et al. 2007). These studies suggested that the voluntary muscle contraction has disinhibitory effects on the motor representations of different muscle in a remote segment. Since we examined non-homologous muscle contraction conditions (trunk-limb interaction), it is implied that cortical disinhibitory mechanisms could explain the results in our current study. Furthermore, in basic studies using animal models, it has previously been shown that intracortical facilitation mechanisms may be involved in spreading of neural activity within the cortical region (Capaday et al. 2011). Capaday et al. have elegantly demonstrated that neural activity initiated at a cortical locus can spread to the nearby cortical regions representing different muscles (Capaday et al. 2011). Similarly, using TMS in human studies, it was suggested that a similar neural mechanism might exist within the M1 region (Boroojerdi et al. 2000; Komeilipoor et al. 2017). Since trunk representation in the homunculus of M1 is located between upper-limb and lower-limb representations, it is likely that voluntary activation of the trunk cortical motor representation spread bilaterally to the nearby upper-limb and lower-limb representation, in our current study. On the other hand, jaw motor representation may be too far away from the trunk motor representation in the M1 region to be activated by spreading of excitability, which is perhaps why jaw clenching did not facilitate corticospinal excitability of other muscles. Overall, although it is possible that the mechanism for facilitation of "remote effect" between the trunk and the limbs in our current study is facilitated from both subcortical and cortical influence, evidence in support of cortical mechanism is more likely to have contributed. Therefore, motor function of different muscles may be not constituted in the brain separately, but evidence suggests that cortical

activations of different motor representations interact and cross-activate within the motor cortex.

Our results imply that trunk and limb muscles interact in the corticospinal pathway. The facilitation of corticospinal excitability of resting muscles induced by remote segment muscle contraction may be helpful in neurorehabilitation. For instance, it is suggested that motor training of an unaffected (or less affected) limb may be possible to utilize for enhancing the motor recovery of the opposing paralyzed limb in neurological impairment such as stroke and spinal cord injury (Tazoe and Komiyama 2014). Similarly, based on our results, motor training of limb muscles may be beneficial for boosting corticospinal excitability of trunk muscle. Moreover, motor training of trunk muscles may also be beneficial for facilitating corticospinal excitability of arms and legs.

3.5. Conclusion

We used TMS to investigate trunk-limb interaction in the corticospinal pathway. Our results showed that voluntary contraction of trunk muscle facilitated the corticospinal excitability of the limb muscles, and vice versa. Overall, our study has demonstrated trunk-limb neural interactions in the corticospinal pathway. The present results extend the evidence suggesting that activation of cortical motor representation by voluntary contraction of trunk muscles could spread to neighboring cortical areas representing different segment muscle and that the extent of this spreading may depend on the voluntary effort level and the distance between M1 areas representing different muscles within the cortex.

Chapter 4 - Study 3

Cortical and subcortical neural interactions between the trunk and upper-limb muscles

The material presented in this chapter has been published in the article:

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NOTE*: The content of this chapter is identical to the material presented in the publication except for the text formatting which was done according to University of Tokyo requirements.*

4.1. Introduction

Corticospinal projections from the primary motor cortex (M1) to a muscle play an essential role during voluntary motor control (Lemon 2008). Strength of the corticospinal excitability between a muscle of interest and its representation in the primary cortical region can be evaluated by examining amplitude of the motor evoked potential (MEP) elicited through transcranial magnetic stimulation (TMS) (Rothwell et al. 1991). It is well known that voluntary muscle contractions can increase the excitability of the corticospinal tract, which causes facilitation of the MEP responses in the contracted muscle (Hess et al. 1987). Moreover, contraction of certain upper-limb and lower-limb muscles can modulate the corticospinal excitability not only of the contracted muscles, but also of other muscles which are located in a remote segment of the body and remain at rest. This phenomenon is known as "remote effect" or "cross facilitation" (e.g., Tazoe, Endoh, et al., 2007; Tazoe, Sakamoto, et al., 2007; Tazoe & Komiyama, 2014; Komeilipoor et al., 2017). The existence of "remote effect" facilitation provides compelling evidence that interlimb neural interactions exist in the corticospinal pathway (Kawakita et al. 1991; Pereon et al. 1995; Boroojerdi et al. 2000; Hortobágyi et al. 2003). Therefore, the abovementioned studies suggest that motor circuits of different body segments are not entirely embedded within the brain or the spinal cord as separate units; rather, they interact closely within the central nervous system to facilitate movement coordination. However, it remains unclear how such "remote effect" facilitation is modulated with the central nervous system.

Most human movements during activities of daily living, such as reaching and walking, involve simultaneous and coordinated activation of the trunk, upper-, and lower-limb muscles, which involves complex interlimb coordination and interaction. Understanding how trunk and limb muscles interact is essential for elucidating the underlying neural mechanics of fundamental

human movement such as sitting, standing, and walking. Recently, we demonstrated that voluntary contraction of trunk muscle facilitated corticospinal excitability of the upper- and lower-limb muscles, as well as that upper- and lower-limb muscle contractions facilitated corticospinal excitability of the trunk muscles (Sasaki et al. 2018). This study demonstrated evidence for existence of neural interactions between the trunk and upper- and lower-limbs (Sasaki et al. 2018). However, MEPs elicited by TMS applied over the M1 are influenced by the excitability of both in the motor cortex and the spinal pathways (Rothwell et al. 1991). Therefore, trunk-limb neural interactions in the corticospinal tract could be attributed to cortical and/or subcortical (spinal) facilitation mechanisms. It was previously suggested that cortical mechanisms are more likely to contribute to "remote effect" facilitation as spinal motor circuity of the upper-limb muscles were not modulated during contraction of lower-limb muscles (Tazoe et al. 2009). On the other hand, animal model studies showed that corticospinal axons branch to multiple motoneuron pools and could remain distributed across several levels within the spinal cord (Fetz et al. 1976; Asanuma et al. 1979; Shinoda et al. 1979, 1981; Fetz and Cheney 1980). These studies therefore suggest the possibility that subcortical motor circuits can also contribute to remote facilitation via trans-spinal branching of the descending tract, which project across different spinal segments. Proximity of spinal innervations projecting to upper-limbs in the cervical spinal cord and the trunk in the thoracic spinal cord may allow for investigation of possible trans-spinal branching of the descending tract in humans.

Cervicomedullary junction stimulation (CMS) is a technique that can be used to evoke short latency responses, termed cervicomedullary motor evoked potentials (CMEP). In the arm, leg, and trunk muscles CMEP responses can be evoked by delivering a single magnetic (or electrical) stimulus on the spinal cord to activate the axons of the descending motor pathways (Ugawa et al. 1991, 1994; Gandevia et al. 1999; Taylor and Gandevia 2004; Martin et al. 2008;

Tazoe et al. 2009; Chiou, Hurry, et al. 2018). Specifically, to evoke CMEP responses, axons should be activated at the level of the cervicomedullary junction, near the pyramidal decussation (Taylor and Gandevia 2004). On the contrary, MEP responses are elicited by stimulation over the M1 region to activate the supraspinal mechanisms. Although CMS could also possibly activate descending motor pathways other than those in the corticospinal tract, as well as the ascending pathways, there is robust evidence that CMS-evoked responses (i.e., CMEPs) primarily reflects the result of motoneuronal (subcortical pathway) activation via the descending volley elicited by excitation of the corticospinal axons (Berardelli et al. 1991; Ugawa et al. 1991; Gandevia et al. 1999; Taylor et al. 2002; McNeil et al. 2013). Subcortical neuronal excitability can also be assessed using H-reflex or F-wave; however, a practical limitation is that they can only be elicited from a few muscles at rest, typically soleus, flexor carpi radialis, and the quadriceps (McNeil et al. 2013). Assessment of subcortical neuronal excitability of trunk muscles using H-reflex or F-wave techniques is further limited due to difficulty accessing the nerves of the trunk transcutaneously. However, it has been reported that CMS can be utilized to evaluate subcortical neural excitability of the trunk muscles (Chiou, Hurry, et al. 2018; Chiou, Strutton, et al. 2018). Therefore, using the CMEP, in addition to MEP responses, it is possible to examine motoneuronal (subcortical) and cortical mechanisms in the trunk and their contributions to the trunk-limb neural interactions.

In this study, we hypothesized that trunk-limb neural interaction (i.e., “remote effect” facilitation) would be primarily facilitated in the cortical, rather than the subcortical, networks as previously suggested by interlimb “remote effect” studies in humans (e.g., Tazoe et al., 2009; Sasaki et al., 2018). Specifically, we hypothesized that contraction of the trunk muscles would not affect CMEP evoked responses in the upper-limbs, and vice versa. On the other hand, we hypothesized that MEP responses during contraction of the trunk muscles would be affected in

the upper-limbs, and vice versa, as shown previously (Sasaki et al. 2018). To test our hypothesis, we used motor cortical (i.e., TMS) and cervicomedullary (i.e., CMS) stimulation to investigate MEP and CMEP responses of: (a) trunk muscles during rest state when upper-limb muscles are contracted; and (b) upper-limb muscles during rest state when trunk muscles are contracted.

4.2. Methods

4.2.1 Participants

Twelve able-bodied individuals were recruited in this study. The age, weight, and height of the participants were 25.9±3.3 years, 68.8±8.0 kg, and 173.3±5.3 cm (mean±SD), respectively. None of the participants had a history of neurological and/or musculoskeletal impairments. The experimental procedures were approved by the local institutional ethics committee of the Graduate School of Arts and Science, The University of Tokyo. All participants gave written informed consent in accordance with the Declaration of Helsinki prior to participating in the study.

4.2.2. Data acquisition

Electromyographic (EMG) activity was recorded from the trunk and upper-limb muscles bilaterally. Specifically, bipolar Ag/AgCl surface electrodes (Vitrode F-150S, Nihon Koden, Tokyo, Japan) were placed, with 1 cm separation, on the: (i) erector spinae on the 12th thoracic vertebral level, 3 cm either side of the spinous processes (ES; trunk muscle); (ii) flexor carpi radialis on the muscle belly (FCR; upper-limb muscle). A ground electrode was placed over the right anterior superior iliac spine. Prior to application of electrodes, skin was cleaned using alcohol to reduce skin impedance. All signals were band-pass filter (5 - 1,000 Hz) and amplified (×1,000) using a multichannel EMG amplifier (MEG-6108, Nihon Koden, Tokyo, Japan). All data were digitized at the sampling frequency of 4,000 Hz using an analog-to-digital (A/D) converter (PowerLab/16SP, AD Instruments, Castle Hill, Australia) and stored on a computer for post processing.

4.2.3. Experimental protocol

During the experiment, participants were seated comfortably on the chair with no back

support and were instructed to maintain upright posture of the trunk, while keeping both arms relaxed on the side of the body. Following a gentle warm-up and task practice, participants were asked to perform three maximal voluntary contraction (MVC) trials for each muscle: (i) ES muscle during isometric contraction of trunk extensor by extending their back as hard as possible; and (ii) FCR muscle during hand gripping by clenching their fists bilaterally as hard as possible. Using a custom written LabVIEW program (National Instruments, USA), integrated EMG activity during the 3 sec epochs were measured on three trials and averaged to define the MVC level of each muscle.

Experimental data were acquired during the following conditions: (1) while participants were relaxed (Rest); (2) during 20% of MVC of trunk extension (Trunk); (3) during 20% of MVC of bilateral hand clenching (Hands). Since a previous study reported that subjects were able to maintain 20% of MVC with upper-limb muscles without eliciting additional background EMG in the ES muscles (Chiou, Strutton, et al. 2018) and in order to have a stable contractions without inducing fatigue during the experiment, target contraction level was set at 20% MVC level for each muscle. Order of experimental conditions was randomized between participants, with at least 3 min rest between tasks. The “remote effect” conditions (i.e., Trunk condition for upper-limb muscle and Hands condition for trunk muscle) were performed while participants were asked to match a visual target on a monitor for about 3-5 sec, which was set to 20% of MVC of the respective muscles. During the Trunk condition, participants were asked to arch their lower back by contracting the ES muscles bilaterally and during the Hands condition, participants were asked to perform the grip task bilaterally. Participants were asked to perform the same tasks as during MVC trials. The monitor showed EMG activity of the right side (i.e., target) muscles, while participants were instructed to exert the same effort on the right and left sides. In post-processing, it was confirmed that muscles were contracted bilaterally. Assessments during each experimental

condition were performed in two separate sessions: (a) TMS session to evoke MEPs; and (b) CMS session to evoke CMEPs. Each session was randomized such that half of the participants performed each session first, and at least 10 min rest was given between sessions (Figure 4-1).

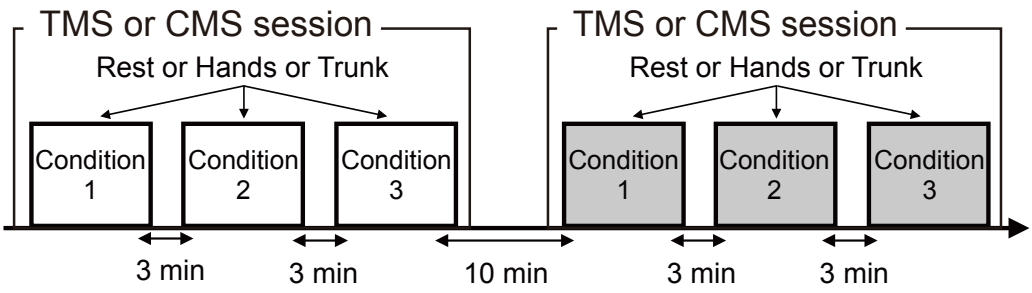

Figure 4-1: Experimental protocol - Experiments consisted of transcranial magnetic stimulation (TMS) and cervicomedullary junction stimulation (CMS) sessions, which were randomized and separated by 10 min rest. In each session, rest (Rest), hand grip (Hands), and trunk contractions (Trunk) experimental conditions were also randomized between participants and separated by 3 min rest.

4.2.4. Motor evoked potentials (MEPs)

To test "remote effect" facilitation between trunk and upper-limb muscles in the corticospinal tract, TMS was delivered over the M1 using a mono-phasic magnetic stimulator (Magstim 200, Magstim Co., Whitland, UK) through a double cone coil (outside diameter of 110 mm; Magstim Co., Whitland, UK) using a same approach outlined previously (Sasaki et al. 2018). The optimal stimulation spot (i.e., "hot spot") was searched over the right (contralateral to target muscles) cortex (starting point was defined at 1.5 cm anterior and 3 cm lateral to vertex) at which MEPs could be recorded from the left ES muscle (Jaberzadeh et al. 2013). Although the coil was positioned at the optimal site for eliciting ES responses, it was ensured that FCR responses could

also be elicited with the same coil position since the cortical areas of arm and trunk muscles are located close within the M1. The motor threshold (MT) of ES muscle was determined with participants maintaining a voluntary contraction at 20% of MVC of ES muscle during back extensions (Strutton et al. 2005; Chiou, Hurry, et al. 2018). MT was defined as the minimum TMS intensity that evoked visible MEPs in at least five out of ten successive trials (MT: 44.67 ± 11.93% of maximal stimulator output). Although some previous studies used higher TMS intensities to evoke ES MEPs during the resting state (Ferbert, Caramia, et al. 1992; Nowicky et al. 2001; Jaberzadeh et al. 2013), Strutton et al., (2005) obtained similar threshold levels as our current study during contractions (i.e., active threshold). The TMS intensity for the experiment was set to 160% of the MT, which was shown to elicit reliable ES MEPs in all subjects even at rest (Sasaki et al., 2018). Once the stimulation site and the stimulation intensity were determined, they remained consistent throughout the experiment. During Trunk and Hands conditions, TMS stimuli were manually delivered by the experimenter when participants reached the 20% of MVC of the respective muscles for approximately 3-5 sec. Five TMS stimuli were delivered with 10-15 sec interstimulus interval between consecutive trials for each experimental condition (i.e., Rest, Trunk, and Hands).

4.2.5. Cervicomedullary motor evoked potentials (CMEPs)

To test "remote effect" facilitation between trunk and upper-limb muscles in subcortical motor circuitry, CMS was applied using a mono-phasic magnetic stimulator (Magstim 200, Magstim Co., Whitland, UK) through a circular coil (diameter of 90 mm; Magstim Co., Whitland, UK) placed firmly over the left (ipsilateral to target muscles) side of the neck (ipsilateral to the ES and FCR muscles), below the inion, with current flowing downward in the coil (Taylor and Gandevia 2004; Chiou, Hurry, et al. 2018). CMEP responses have previously been used to reliably

assess subcortical (spinal) excitability in both trunk (Chiou, Hurry, et al. 2018; Chiou, Strutton, et al. 2018) and upper-limb (Butler et al. 2003; Tazoe et al. 2009; Bunday et al. 2014; Dongés et al. 2017) muscles. Specifically, since the ES muscle at the T12 level are innervated by dorsal rami of thoracic and lumbar spinal nerves (T8-L3) and the FCR muscles are innervated by cervical nerve root at C6-C7 level, the responses elicited by CMS are likely to be elicited by direct corticospinal activation below the motor cortex, rather than nerve stimulation, implying that they represent subcortical motor circuitry (Taylor and Gandevia 2004; Chiou, Hurry, et al. 2018). Moreover, to confirm that responses of FCR and ES muscles were elicited by direct activation of corticospinal axon, it was also checked that size of the responses was facilitated during voluntary contraction, suggesting an increase in excitability of the spinal motoneurons (Taylor et al. 2002; Taylor 2006). In post-processing, the size of CMEP responses was not facilitated during voluntary contractions in 2 participants. Therefore, for statistical analyses of CMEP responses, 10 of 12 participants were included. Moreover, it was ensured that the onset latency was approximately 9-10 ms after the CMS stimulus, which was previously shown as acceptable for evoking CMEP responses (Ugawa et al. 1991; Dongés et al. 2017; Chiou, Hurry, et al. 2018; Chiou, Strutton, et al. 2018). Moreover, the stimulus intensity of CMS was set to elicit ES CMEP with an amplitude of approximately 0.1-0.2 mV (Chiou, Hurry, et al. 2018; Chiou, Strutton, et al. 2018), and consequently FCR CMEP responses of approximately 0.4 mV (Tazoe et al. 2009; Dongés et al. 2017). Once the stimulation site and the stimulation intensity were determined, they remained consistent throughout the experiment. During Trunk and Hands conditions, CMS stimuli were manually delivered by the experimenter when participants reached the 20% of MVC of the respective muscles for approximately 3-5 sec. Five CMS stimuli were delivered with 10-15 sec interstimulus interval between consecutive trials for each experimental condition (i.e., Rest, Trunk, and Hands).

4.2.6. Data analysis

MEP and CMEP peak-to-peak amplitudes were calculated for each trial and each muscle. For the ES muscle, Trunk condition was defined as "active" condition and Hands condition was defined as "remote effect" condition. Similarly, for the FCR muscle, Hands condition was defined as "active" condition and Trunk condition was defined as "remote effect" condition. First, to ensure that muscles remained at rest during "remote effect" facilitation, background EMG activity of a 50 ms window before the TMS or CMS stimulus was defined by computing the root mean square (RMS) for each trial in the target (left side) FCR and ES muscles and compared to the Rest condition. Moreover, to ensure that muscles were contracted bilaterally and equally during Trunk and Hands condition, background EMG activity of both the FCR and ES muscles was computed and compared between the left and right side muscles. Finally, latencies of MEP and CMEP responses for the ES and FCR muscles were determined by visual inspection for each trial from the rectified EMG traces. Responses on five repeated trials were averaged for each experimental condition. Trials were removed from the analysis if the background EMG exceeded three standard deviations of the mean background EMG during the Rest condition. MEP and CMEP amplitudes were normalized as a percentage of the Rest condition prior to performing statistical analysis. All data analysis was performed using custom written scripts in MATLAB (2017a, The MathWorks Inc., Massachusetts, USA).

4.2.7. Statistical analysis

For each session (i.e., TMS and CMS session) and each muscle (i.e., ES and FCR muscles), peak-to-peak amplitudes of MEP and CMEP responses were compared between three experimental conditions: Rest, Hands, and Trunk using the Friedman test, a non-parametric

equivalent for repeated-measure analysis of variance (ANOVA). When Friedman test showed significant effects, multiple comparison post-hoc analyses were performed using the Wilcoxon signed-rank test with Bonferroni adjustments. Background EMG activity was also compared using the same test to ensure that remote segment muscles were not co-contracted during the "remote effect" conditions and that they were contractions bilaterally. Moreover, Wilcoxon signed-rank test was used to compared latencies of MEP and CMEP responses to check that responses of the FCR and ES muscles were elicited by direct activation of corticospinal axon. Non-parametric tests were chosen because the Shapiro-Wilk test showed that most identified measures were not normally distributed and since the sample size remains relatively small. Significance level on all tests was set to $p < 0.05$.

4.3. Results

4.3.1 Onset latencies of MEP and CMEP responses

Averaged onset latency of MEP and CMEP responses across participants in the trunk (ES) and upper-limb (FCR) muscles are shown in Figure 4-2. Comparisons showed that onset latency of CMEP responses was significantly shorter compared to MEP responses in each muscle, suggesting that the stimulation directly activated corticospinal axons distal to the motor cortex (Ugawa et al. 1991; Taylor and Gandevia 2004; Chiou, Strutton, et al. 2018) in both the trunk (ES) (MEP latency: 16.9±2.08 ms and CMEP latency: 9.45±1.80 ms; $p < 0.001$) and upper-limb (FCR) (MEP latency: 17.2±1.01 ms and CMEP latency: 9.17±0.46 ms; $p < 0.001$) muscles.

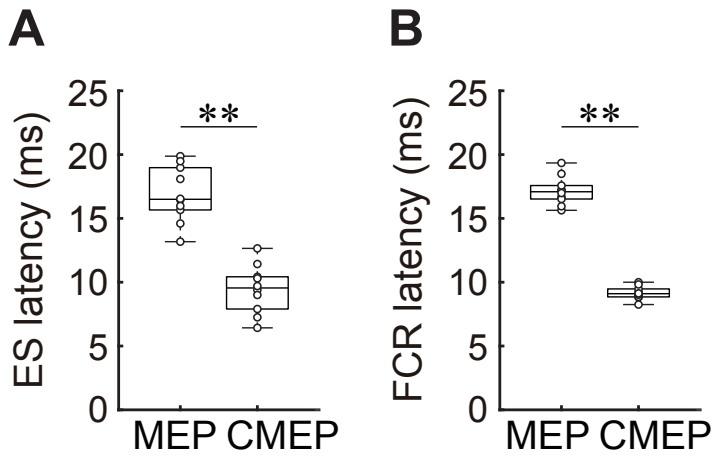

Figure 4-2: Comparisons between the onset latency of motor evoked potentials (MEP) and cervicomedullary motor evoked potentials (CMEP) in: (A) erector spinae (ES) muscle; and (B) flexor carpi radialis (FCR) muscle. Legend: **$p < 0.01$.

4.3.2. Background EMG activity

Averaged background EMG activity in each condition during the TMS and CMS sessions, are shown in Figure 4-3. During the TMS session, background EMG activity was significantly different between the Rest, Hands, and Trunk conditions for the ES (trunk) and FCR

(upper-limb) muscles (ES: $\chi^2(2) = 18.0$, $p < 0.01$; FCR: $\chi^2(2) = 17.6$, $p < 0.01$). However, multiple comparison for the ES muscle showed that the ES background EMG activity was similar between the Rest and "remote effect" (Hands) conditions, while the "active" condition (i.e., Trunk) was significantly larger compared with the Rest and "remote effect" (Hands) conditions ($p < 0.01$) (Figure 4-3A), as expected. Similarly, multiple comparison for the FCR muscle showed that the FCR background EMG activity was similar between the Rest and "remote effect" (Trunk) conditions, while the "active" condition (i.e., Hands) was significantly larger compared to the Rest and "remote effect" (Trunk) conditions ($p < 0.01$) (Figure 4-3A), as expected. Comparisons also showed that background EMG were similar between the left (target) and right side muscles during the "remote effect" conditions in the trunk (ES) muscle during the Trunk condition ($p = 0.57$) and the upper-limb (FCR) muscle during the Hands condition ($p = 0.52$) (Figure 4-3B), as expected.

During the CMS session, background EMG activity was significantly different between the Rest, Hands, and Trunk conditions for the ES (trunk) and FCR (upper-limb) muscles (ES: $\chi^2(2) = 18.2$, $p < 0.01$; FCR: $\chi^2(2) = 18.2$, $p < 0.01$). However, multiple comparison for the ES muscle showed that the ES background EMG activity was similar between the Rest and "remote effect" (Hands) conditions, while the "active" condition (i.e., Trunk) was significantly larger compared with the Rest and "remote effect" (Hands) condition ($p < 0.01$) (Figure 4-3C), as expected. Similarly, multiple comparison for FCR muscle showed that the FCR background EMG activity was similar between the Rest and "remote effect" (Trunk) conditions, while the "active" condition (i.e., Hands) was significantly larger compared with the Rest and "remote effect" (Trunk) condition ($p < 0.01$) (Figure 4-3C), as expected. Comparison also showed that background EMG activity was similar between the left (target) and right side muscles during the "remote effect" conditions in both the trunk (ES) muscle during the Trunk condition ($p = 0.32$)

and the upper-limb (FCR) muscle during the Hands condition (p = 0.23) (Figure 4-3D), as expected.

Moreover, background EMG is presented as % MVC in Table 4-1. Note that statistical analyses showed similar results as raw background EMG values.

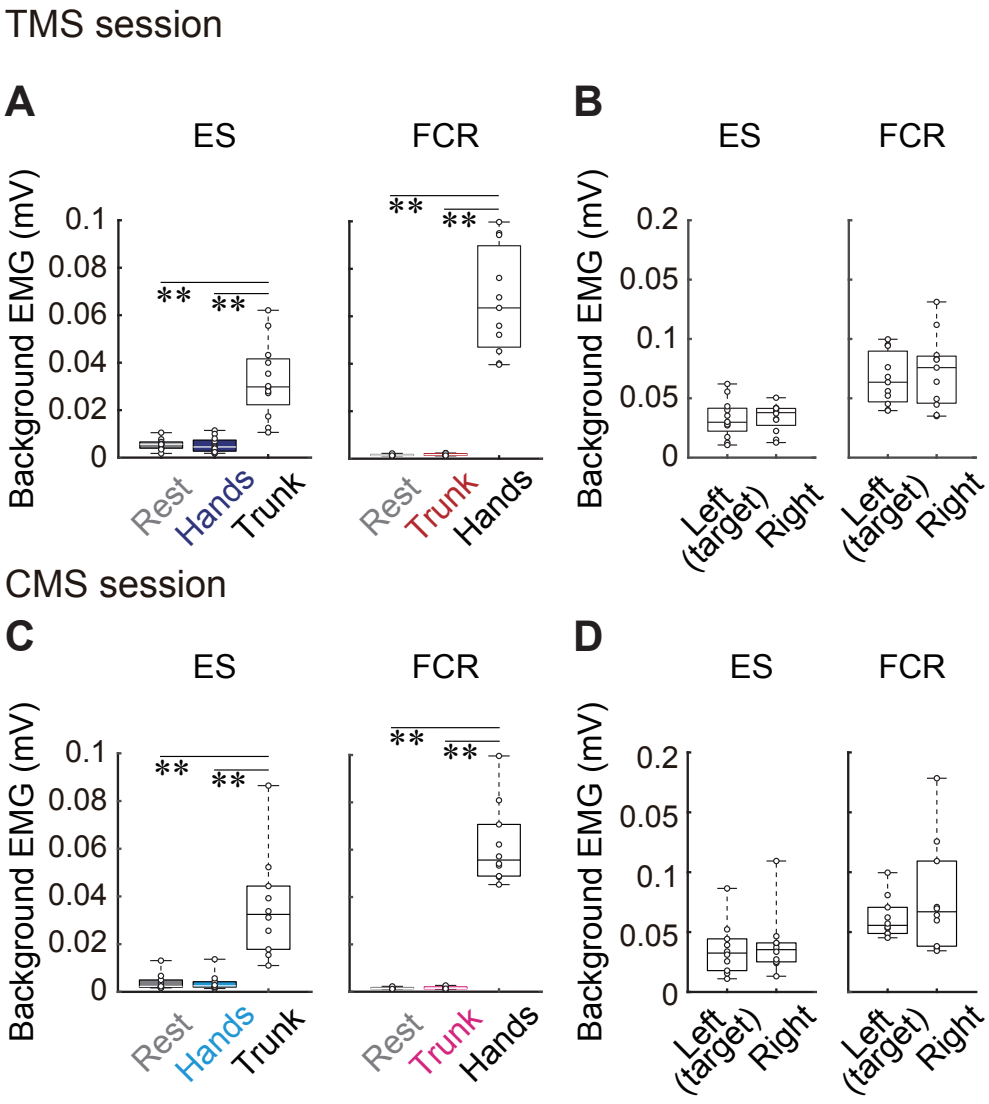

Figure 4-3: Group data of background electromyographic (EMG) activity in erector spinae (ES) during rest (Rest), "remote effect" - hand grip task (Hands), and "active" - trunk extension task (Trunk); and group data of background EMG activity in flexor carpi radialis (FCR) during Rest, "remote effect" task (Trunk) and "active" tasks (Hands) in

(A) transcranial magnetic stimulation (TMS) and (C) cervicomedullary junction magnetic stimulation (CMS) session. Group data of background EMG activity in the left (target) and right side ES during "remote effect" task (Trunk); and the FCR during "remote effect" task (Hands) in (B) TMS and (D) CMS session. Legend: **p < 0.01.

Table 4-1: Background EMG (% of MVC) of ES and FCR muscles during Rest, Hands, and Trunk conditions in TMS and CMS sessions.

Session	Condition	Background EMG (%MVC)					
		ES			FCR		
TMS	Rest	3.42	±	0.46	0.54	±	0.05
	Hands	3.48	±	0.62	22.37	±	2.25
	Trunk	19.13	±	1.65	0.60	±	0.06
CMS	Rest	2.41	±	0.63	0.54	±	0.05
	Hands	2.29	±	0.56	20.95	±	1.00
	Trunk	19.08	±	2.35	0.57	±	0.07

Values indicate means ± standard error.

4.3.3 Motor evoked potential (MEP) responses

Trunk (ES) muscle MEP responses of a representative participant during the Rest, Hands ("remote effect"), and Trunk ("active") conditions are shown in Figure 4-4A, while Figure 4-4B shows enlarged plots of the ES MEP responses during the Rest and Hands conditions to illustrate "remote effect" facilitation. Statistical comparisons of average ES responses amplitudes across all participants between the Rest, Hands, and Trunk conditions are shown in Figure 4-4C. Peak-to-peak MEP amplitudes were significantly different between the Rest, Hands, and Trunk

conditions for the ES muscle ($\chi^2(2) = 20.7$; $p < 0.01$). Multiple comparison showed that the Hands condition was significantly larger compared with the Rest condition (175.8±24.2% of Rest; $p = 0.01$), while the Trunk condition was significantly larger compared with the Rest and Hands conditions (752.4±112.8% of Rest; $p < 0.01$).

Upper-limb (FCR) muscle MEP responses of a representative participant during the Rest, Trunk ("remote effect"), and Hands ("active") conditions are shown in Figure 4-4D, while Figure 4-4E shows enlarged plots of FCR MEP responses during Rest and Hands conditions to illustrate "remote effect" facilitation. Statistical comparisons of average FCR responses amplitudes across all participants between the Rest, Trunk, and Hands conditions are shown in Figure 4-4F. Peak-to-peak MEP amplitudes were significantly different between the Rest, Trunk, and Hands conditions for FCR muscle ($\chi^2(2) = 20.2$; $p < 0.01$). Multiple comparison showed that the Trunk condition was significantly larger compared with the Rest condition (248.9±38.4% of Rest; $p < 0.01$), while the Hands condition was significantly larger compared with the Rest and Trunk conditions (1045.6±209.5% of Rest; $p < 0.01$).

ES and FCR MEP amplitudes during "active" conditions (i.e., Trunk condition for the ES muscles and Hands condition for the FCR muscle) were presented to illustrate the relative effects of "remote effect" on MEP amplitudes in relation to the well-known effect of increased background EMG on MEPs (Figure 4-4).

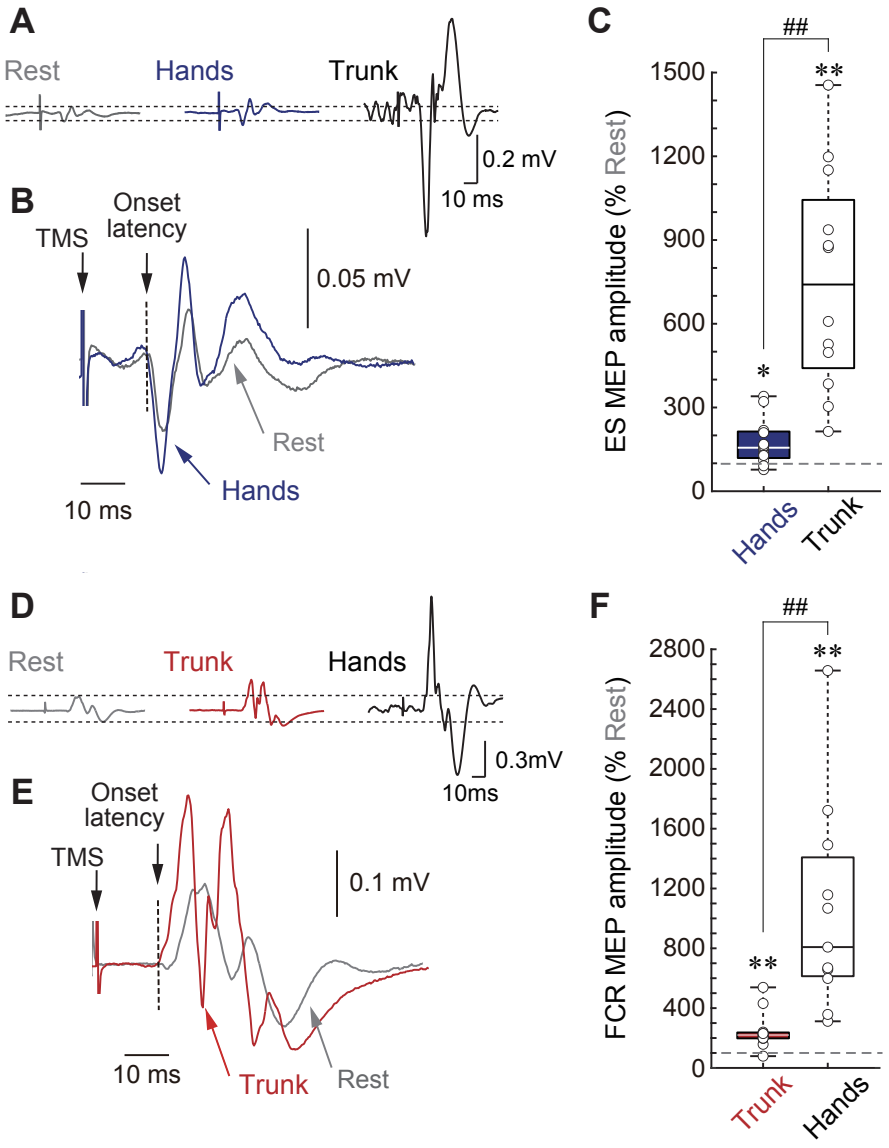

Figure 4-4: (A) Erector spinae (ES) motor evoked potentials (MEPs) of a representative participant during rest (Rest; gray trace), "remote effect" - hand grip task (Hands; blue trace), and "active" - trunk extension task (Trunk; black trace) conditions; (B) Enlarged overlay plot of ES MEPs during Rest and "remote effect" (Hands) conditions; (C) Box plots show group data of MEPs elicited in the ES during "remote effect" task (Hands) and "active" task (Trunk) conditions; (D) Flexor carpi radialis (FCR) MEP responses of a representative participant during Rest (gray trace), "remote effect" task (Trunk; red

trace), and "active" task (Hands; black trace) conditions; (E) Enlarged overlay plot of FCR MEPs during Rest and "remote effect" (Trunk) conditions; (F) Box plots show group data of MEPs elicited in the FCR during "remote effect" task (Trunk) and "active" task (Hands) conditions. MEP amplitudes were normalized with respect to the MEP amplitude during the Rest condition (% Rest) for each participant. Asterisks indicate significant differences compared to the Rest condition. Hashtags indicate significant differences between Trunk and Hands conditions. Legend: *, # $p < 0.05$, **, ## $p < 0.01$.

4.3.4. Cervicomedullary evoked potential (CMEP) responses

Trunk (ES) muscle CMEP responses of a representative participant during the Rest, Hands ("remote effect"), and Trunk ("active") conditions are shown in Figure 4-5A, while Figure 4-5B shows enlarged plots of the ES CMEP responses during the Rest and Hands conditions to illustrate "remote effect" facilitation. Statistical comparisons of average ES responses amplitudes across all participants between the Rest, Hands, and Trunk conditions are shown in Figure 4-5C. Peak-to-peak CMEP amplitudes were significantly different between the Rest, Hands, and Trunk conditions for the ES muscle ($\chi^2(2) = 12.2$, $p < 0.01$). Multiple comparison showed that the Hands condition was similar to the Rest condition (100.2±3.82% of Rest), while the Trunk condition was significantly larger compared with the Rest and Hands condition (214.0±45.3% of Rest; $p < 0.01$). Upper-limb (FCR) muscle CMEP responses of a representative participant during the Rest, Trunk ("remote effect"), and Hands ("active") conditions are shown in Figure 4-5D, while Figure 4-5E shows enlarged plots of the FCR CMEP responses during the Rest and Hands conditions to illustrate "remote effect" facilitation. Statistical comparisons of average FCR responses amplitudes across all participants between the Rest, Trunk, and Hands conditions are shown in Figure 4-5F. Peak-to-peak CMEP amplitudes were significantly different between the Rest, Trunk,

and Hands conditions for the FCR muscle ($\chi^2(2) = 12.8$, $p < 0.01$). Multiple comparison showed that the Trunk condition was significantly larger compared with the Rest condition (224.0±47.0% of Rest; $p = 0.03$), while the Hands condition was significantly larger compared with the Rest condition (310.5±69.0% of Rest; $p < 0.01$). There was no significant difference between Hands and Trunk conditions ($p = 0.70$).

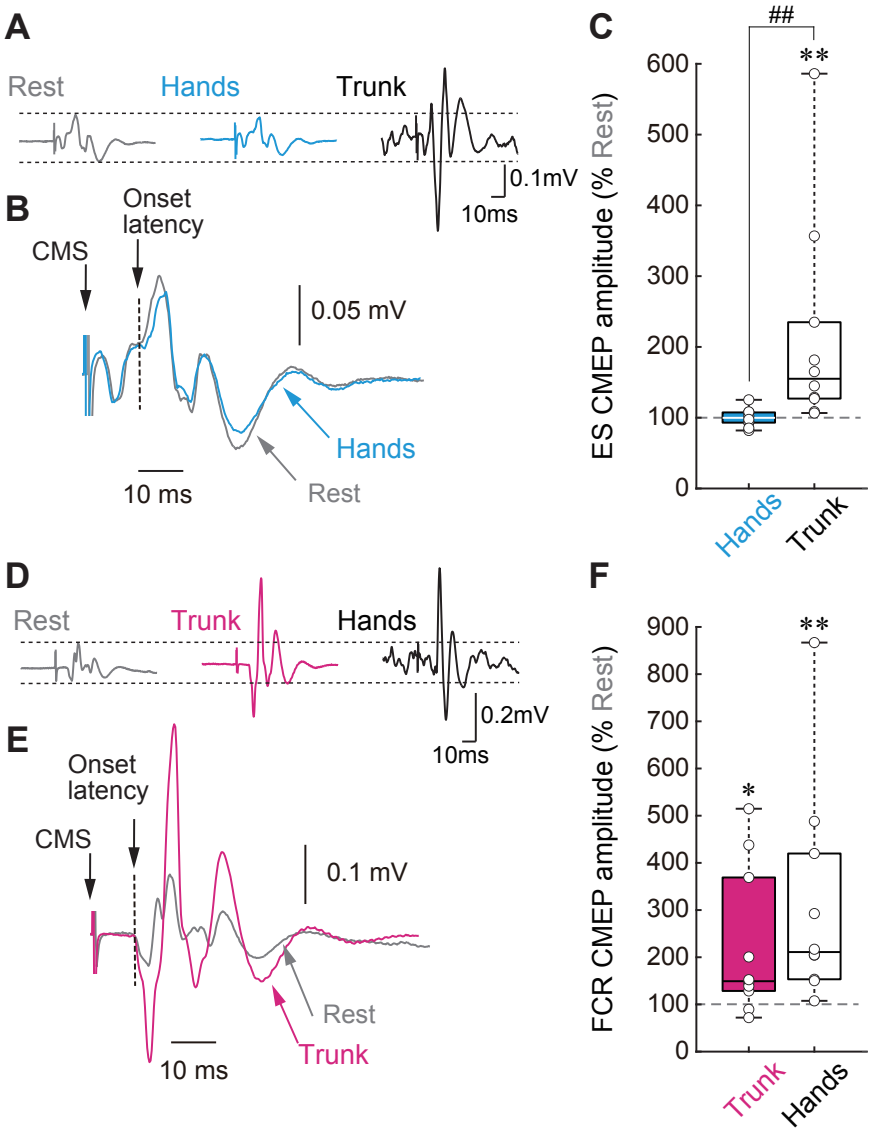

Figure 4-5: (A) Erector spinae (ES) cervicomedullary motor evoked potentials (CMEPs) of a representative participant during rest (Rest; gray trace), "remote effect" - hand grip task (Hands; light blue trace), and "active" - trunk extension task (Trunk; black trace) conditions; (B) Enlarged overlay plot of ES CMEPs during Rest and "remote effect" (Hands) conditions; (C) Box plots show group data of CMEPs elicited in the ES during "remote effect" task (Hands) and "active" task (Trunk) conditions; (D) Flexor carpi radialis (FCR) CMEP responses of a representative participant during Rest

(gray trace), “remote effect” task (Trunk; pink trace), and “active” task (Hands; black trace) conditions; (E) Enlarged overlay plot of FCR CMEPs during Rest and “remote effect” (Trunk) conditions; (F) Box plots show group data of CMEPs elicited in the FCR during “remote effect” task (Trunk) and “active” task (Hands) conditions. Asterisks indicate significant differences compared to the Rest condition. CMEP amplitudes were normalized with respect to the CMEP amplitude during the Rest condition (% Rest) for each participant. Hashtags indicate significant differences between Trunk and Hands conditions. Legend: *, $^{\#}$ $p < 0.05$, **, $^{\#\#}$ $p < 0.01$.

4.4. Discussion

In this study, we investigated whether subcortical (CMEP) and corticospinal (MEP) excitability of the trunk muscles during rest was modulated when upper-limb muscles were contracted, as well as whether upper-limb muscle excitability in the same neural structures was modulated when trunk muscles were contracted. Our results showed that subcortical (CMEP) responses of the trunk muscles were not modulated by upper-limb contractions (Figure 4-5A, B, and C), while corticospinal (MEP) responses of the trunk muscles during upper-limb contractions were modulated (Figure 4-4A, B, and C), as we hypothesized. Moreover, our results showed that subcortical (CMEP) responses of upper-limb muscles were modulated by trunk muscles contractions (Figure 4-5D, E, and F), contrary to our hypothesis, while corticospinal (MEP) response of upper-limb muscle during trunk muscles contractions were modulated (Figure 4-4D, E, and F), as we hypothesized. CMEP responses can be modulated by voluntary muscle contractions (Ugawa et al. 1994; Taylor et al. 2002; Taylor 2006), similar to MEP responses (Hallett 2007), which implies that background EMG activity affects both MEP as well as CMEP as our results confirmed during “active” conditions, ie., Trunk condition for the ES muscles and Hands condition for the FCR muscle (Figure 4-4 and Figure 4-5). However, since background EMG activity was not significantly different between the “remote effect” conditions and the rest state (Rest), i.e., ES muscle during Hands task and FCR muscle during Trunk task for both CMEP and MEP responses (Figure 4-3), it can be considered that the observed modulation was an effect of remote muscle contraction.

Overall, our current results agree with our previous study which demonstrated that corticospinal (MEP) response amplitude of the trunk muscles was facilitated during voluntary upper-limb muscles contractions, and vice versa (Sasaki et al. 2018). Moreover, the current study extended these findings to elucidate the effects of trunk-limb interaction in the subcortical

(CMEP) neural structures, which remained largely unexplored. A specific discussion about cortical and subcortical trunk-limb neural interactions follows.

4.4.1. Origins of supraspinal connectivity of the trunk-limb neural interactions

Our results showed that trunk (ES) muscle subcortical (CMEP) excitability was not facilitated during upper-limb contractions, while corticospinal (MEP) excitability was facilitated by upper-limb contractions. These findings imply that intracortical motor circuits, rather than spinal motoneuronal circuitry, likely attributed to "remote effect" facilitation of trunk muscles. It has previously been demonstrated that intracortical motor circuits were modulated by contractions of non-homologous (i.e., remote limb) muscle (Tazoe, Endoh, et al. 2007; Tazoe, Sakamoto, et al. 2007; Chiou, Wang, Liao, and Yang 2013; Chiou, Wang, Liao, Wu, et al. 2013). Specifically, it was shown that short interval intracortical inhibition (SICI) of upper-limb and lower-limb muscles was decreased during contraction of remote limbs, which suggests that remote muscles contraction could cause cortical disinhibitory effect (Chiou, Wang, Liao, and Yang 2013; Chiou, Wang, Liao, Wu, et al. 2013). Similarly, decreased cortical silent period of upper-limb primary motor cortical areas was shown during contraction of the lower-limbs, which also suggests disinhibition of cortical circuits during remote limb contractions (Tazoe, Endoh, et al. 2007; Tazoe, Sakamoto, et al. 2007). Therefore, voluntary muscle contractions could have disinhibitory effects on the cortical motor circuits of remote muscles located in a distal segment of the body to explain the trunk-limb "remote effect" facilitation in our study. Indeed, a recent study reported decrease of SICI of the trunk during upper-limb muscles contractions (Chiou, Strutton, et al. 2018). It is therefore likely that intracortical disinhibitory mechanisms could, at least in part, explain trunk-limb facilitation. Cortical basis for "remote effect" facilitation is further supported by basic animal studies, which have previously demonstrated that intracortical facilitation may be involved in

spreading of neural activity within the motor cortex (Capaday et al. 2009, 2011). Specifically, Capaday and collagues (2009, 2011) elegantly demonstrated that neural activity initiated at a cortical locus can spread to the neighboring cortical regions representing different muscles via intrinsic horizontal connections between neurons within the motor cortex. In several studies using TMS, it was also proposed that similar neural connectivity mechanisms, including cortico-cortical connectivity, might exist in humans (Boroojerdi et al. 2000; Komeilipoor et al. 2017). It is also well known that somatotopy of muscles in the same and different body segments overlap within the motor cortex (Penfield and Boldrey 1937; Brasil-Neto et al. 1992). Since the trunk representation in the homunculus of the M1 in humans is located near the upper-limb representations, there is a possibility that activation of trunk cortical motor circuits was also induced during voluntary contraction of upper-limbs due to overlapping of cortical representation.

Therefore, since subcortical excitability in the trunk muscles during upper-limb contractions was not affected, our current results suggest that the "remote effect" of upper-limb contractions on trunk facilitation is most likely modulated via intracortical connectivity (facilitatory and/or disinhibitory circuits), and/or overlapping of somatotopic representation at the supraspinal (cortical) level. However, since we did not directly quantify intracortical activity in our study, further work should systematically test activations at the cortical level during upper-limb and trunk "remote effect" tasks using methods such as functional magnetic resonance imaging (fMRI) and electroencephalography (EEG) to fully confirm our findings.

4.4.2. Evidence for selective subcortical connectivity

Our result also showed that upper-limb (FCR) subcortical (CMEP) as well as corticospinal (MEP) excitability was facilitated during trunk contraction, contrary to our hypothesis and results of the upper-limb contraction on "remote effect" facilitation of trunk

muscles. It is possible that during CMS delivery, ventral root was stimulated to evoke the M-wave. However, it was proposed that voluntary facilitation of CMS-induced responses can be used to ensure that subcortical (motoneuronal) excitability was tested since increased size of the CMEP responses reflects an increase in excitability of the spinal motoneuron pool during voluntary contraction, while the maximum M-wave elicited by nerve stimulation was unchanged (Taylor et al. 2002; Taylor 2006). Indeed, our results showed that CMEP responses during "active" conditions (i.e., Trunk condition for the ES muscles and Hands condition for the FCR muscle) were significantly facilitated compared to the Rest condition (Figure 4-5), implying that CMEP responses reflect subcortical (motoneuronal) excitability. Therefore, our findings suggest that the facilitation of corticospinal excitability observed during trunk voluntary contraction could be attributed to subcortical (spinal) facilitation mechanisms, in addition to cortical mechanisms which were discussed. Previously, anatomical and electrophysiological investigations using animal models demonstrated compelling evidence that most corticospinal axons branch extensively within the spinal cord and project to multiple motoneuron pools located at one spinal level or remain distributed across several spinal levels (Fetz et al. 1976; Asanuma et al. 1979; Shinoda et al. 1979, 1981; Fetz and Cheney 1980). This suggests the possibility that even a single corticomotoneuronal cell could have complex output patterns to multiple muscles located in distal body segments due to branching of corticospinal axons within the spinal cord (McKernan et al. 1998). Our results also suggest that corticospinal axons projecting to the trunk muscle motoneurons could also have projections to the upper-limb muscles motoneurons via trans-spinal branching, as proposed in animal models. Overall, our results provide new evidence that "remote effect" of trunk contractions on upper-limb facilitation is modulated, at least in part, within the spinal cord as well, in addition to the intracortical circuit. Moreover, branching of corticospinal axons could affect on not only trunk contraction to upper-limb "remote effect" but also the upper-

limb contraction to trunk "remote effect". However, subcortical excitability of trunk muscles was not modulated by upper-limb muscles contractions. A specific discussion about why trunk to upper-limb and upper-limb to trunk "remote effect" mechanisms may be different follows in the next section.

4.4.3. Mechanisms of neural interactions between the trunk and the upper-limb muscles

Taken together, our results suggest that cortical motor circuits contributed to upper-limb contraction to trunk facilitation, while both cortical and subcortical motor circuits contributed to trunk contraction to upper-limb facilitation. So, why are upper-limb and trunk "remote effect" interactions different? Perhaps, unique anatomical structures and the functional nature of the trunk and upper-limb muscles can provide an explanation. It is well known that M1 representations projecting to upper-limb muscles are larger compared to other muscles (Penfield and Boldrey 1937). Since upper-limb muscles contractions could induce large activations within the M1, which may contribute to spreading of neural activity within the cortical regions, intracortical facilitation mechanisms (i.e., cortico-cortical connections) are likely to be involved in upper-limb muscle contraction-induced "remote effect", rather than subcortical mechanisms. Moreover, previous anatomical evidence also suggested that upper-limb muscle motoneurons have direct monosynaptic projections from the motor cortex since they require fine motor control, unlike the trunk (Palmer and Ashby 1992; Baldissera and Cavallari 1993; Noordhout et al. 1999). Consequently, it is possible that trunk subcortical excitability was not modulated during upper-limb contractions since the descending tract controlling the upper-limbs may not have branching projections to non-homologous muscles (including the trunk). Contrary to upper-limbs, it has been reported that inputs from subcortical circuit such as the brainstem, vestibular, and spinal cord largely contribute to control of the trunk (Cottingham et al. 1988; Zedka et al. 2004; Galea

et al. 2010). Therefore, during trunk contractions, subcortical as well as cortical interlimb excitability was modulated since excitation of subcortical circuits induced by activation of the trunk muscles may have trans-spinal branching projections, as suggested in animal literature (Fetz et al. 1976; Asanuma et al. 1979; Shinoda et al. 1979, 1981; Fetz and Cheney 1980). Functional nature of such connections may be related to reaching tasks for instance, which require activation of the trunk to maintain stable sitting or standing posture before successful performance of reach and/or grasp can be achieved using upper-limb muscles (Milosevic, Gagnon, et al. 2017). Therefore, we demonstrated that trunk-limb neural interactions can be attributed to cortical and/or subcortical mechanisms, depending on whether trunk and upper-limb muscles were contracted. Overall, it is possible that corticospinal neurons of the trunk and upper-limb muscles, which project predominantly to one muscle, have complex outputs to multiple muscles in distal body segments.

4.4.4. Limitations

Our work has several limitations that should be noted. First, many previous studies investigating CMEPs used a double cone coil (e.g., Taylor, 2006) or electrical stimulation (e.g., Dongés et al., 2017), while our current study used a circular coil to elicit the CMEP responses in the ES and FCR muscles. We ensured that the responses reflect subcortical (motoneuronal) excitability using the following consideration: (1) proper placement of the coil (Taylor 2006; Chiou, Hurry, et al. 2018; Chiou, Strutton, et al. 2018); (2) voluntary facilitation of the evoked responses (Taylor et al. 2002; Taylor 2006); and (3) response latency (Ugawa et al. 1991; Dongés et al. 2017; Chiou, Hurry, et al. 2018; Chiou, Strutton, et al. 2018). However, we cannot exclude the possibility that differences in the stimulation paradigms (i.e., electrical vs. magnetic and circular vs. double cone coil) could affect our results. Future studies should therefore carefully

consider these different methodological concerns for evoking CMEP responses in upper-limb and trunk muscles. Second, although ES and FCR background EMG were not activated during "remote effect" conditions (i.e., Trunk condition for ES and Hands condition for FCR), we cannot exclude the possibility that back extension could activate other trunk group muscles even if the contraction levels were relatively low (<20%). Moreover, it is also likely that hand grip task could activate not only the agonist muscles, but also other proximal arm muscles. Finally, our results suggest that trans-spinal branching of corticospinal axons, which were proposed in animal literature (Fetz et al. 1976; Asanuma et al. 1979; Shinoda et al. 1979, 1981; Fetz and Cheney 1980), may attribute to trunk and upper-limb "remote effect" facilitation. However, since we did not directly investigate whether such corticospinal branching could attribute to trunk and limb muscles "remote effect", future studies are warranted to specifically test trunk-limb trans-spinal branching of corticospinal axons.

Chapter 5 - Study 4

Task- and intensity-dependent modulation of arm-trunk neural interactions in the corticospinal pathway in humans

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5.1. Introduction

Most human movements, even simple acts such as grasping an object, require coordinated activation of multiple muscles. Specially, interactions between arm and trunk muscles are important for performing activities of daily living. It is well known that trunk muscles are activated before the proceeding arm movements (Aruin and Latash 1995; Hodges and Richardson 1997). By assessing motor evoked potentials (MEP) using transcranial magnetic stimulation (TMS) of the primary motor cortex (M1), it was recently demonstrated that voluntary activation of upper-limb muscles can facilitate corticospinal circuits, which are responsible for controlling the trunk muscles (Chiou, Strutton, et al. 2018; Sasaki et al. 2018). Moreover, it was reported that subcortical excitability evaluated by cervicomedullary MEP was not changed by voluntary contraction of arm muscles (Chiou, Strutton, et al. 2018; Sasaki, Milosevic, et al. 2020). These results indicate that trunk corticospinal facilitation induced by arm movement may be mediated in the cortical networks. Therefore, motor control centers of trunk and arm muscles may not be embedded within the central nervous system as separate units. Rather, they seem to interact closely. Although corticospinal remote facilitation may be responsible for controlling arm-trunk coordinated movements (Chiou and Strutton 2020), its mechanisms are yet to be fully understood.

Corticospinal remote facilitation has been studied extensively between upper- and lower-limb muscles (Kawakita et al. 1991; Pereon et al. 1995; Tazoe and Komiyama 2014) and this phenomenon is known as remote effect or crossed facilitation (Tazoe and Komiyama 2014). Specifically, contraction of upper- or lower-limb muscles is known to facilitate corticospinal excitability of muscles located in different and remote segments of the body (Kawakita et al. 1991; Pereon et al. 1995; Hortobágyi et al. 2003; Tazoe, Sakamoto, et al. 2007; Tazoe et al. 2009; Chiou, Wang, Liao, and Yang 2013; Chiou, Wang, Liao, Wu, et al. 2013; Komeilipoor et al. 2017). Such interlimb corticospinal remote facilitation was achieved regardless of whether different

tasks (e.g., flexion or extension) was performed (Tazoe, Sakamoto, et al. 2007; Tazoe et al. 2009; Chiou, Wang, Liao, and Yang 2013; Chiou, Wang, Liao, Wu, et al. 2013; Tazoe and Komiyama 2014). Moreover, the extent of corticospinal remote effect facilitation between upper- and lower-limb muscles was shown to depend on the voluntary effort level of the contracted muscle (Kawakita et al. 1991; Tazoe, Sakamoto, et al. 2007; Tazoe et al. 2009). Regardless of the neurophysiological characteristics of remote effect facilitation, its functional role in human motor control is still unknown (Tazoe and Komiyama 2014). Moreover, arm-trunk neural interaction mechanisms in corticospinal tract have not been examined in detail, compared to more widely studied interlimb remote effects. Since trunk muscles are activated in a highly coordinated manner during voluntary arm movements (Aruin and Latash 1995; Hodges and Richardson 1997), characteristics of arm-trunk neural interactions may be different from those observed in interlimb remote effect facilitation (Tazoe and Komiyama 2014). Moreover, since previous studies investigating arm-trunk neural interaction used only one contraction intensity (i.e., either 20 or 30% MVC level) (Chiou, Strutton, et al. 2018; Sasaki et al. 2018; Chiou and Strutton 2020; Sasaki, Milosevic, et al. 2020), it was not clear how neural interactions between arm and trunk muscles would be modulated during different contraction intensities. Therefore, investigating arm-trunk remote effect facilitation during different exertion levels and tasks may provide new insights about underlying neural interaction mechanisms in the central nervous system. Fundamentally, this could lead to a more comprehensive understanding of the basic principles of human motor control. Moreover, it was recently reported that trunk muscle corticospinal excitability could be facilitated after short-term upper-limb training (Chiou et al. 2020). Arm-trunk corticospinal remote facilitation may also be attributed to quicker anticipatory postural adjustments of the trunk during rapid shoulder flexion in patients with spinal cord injury (Chiou and Strutton 2020). Therefore, a thorough understanding of the characteristic of arm-trunk corticospinal interactions

may also be helpful to develop new rehabilitation interventions for targeting improvements in arm-trunk interactions.

It was reported that trunk flexor (i.e., rectus abdominis: RA) and extensor (i.e., erector spinae: ES) muscle activity depends on the direction of the arm movements (e.g., flexion or extension) during various motor tasks (Aruin and Latash 1995; Hodges et al. 1997). We therefore hypothesized that arm muscle contractions would facilitate corticospinal excitability of the trunk muscles, as recently demonstrated (Chiou, Strutton, et al. 2018; Sasaki et al. 2018). Specifically, our hypothesis was that the extent of arm-trunk corticospinal remote facilitation would depend on the task performed during upper-limb muscle contractions (i.e., flexion or extension), as indicated by studies that examined muscle-level outputs (Aruin and Latash 1995; Hodges et al. 1997). We also hypothesized that muscle contraction intensity would affect the extent of arm-trunk remote effect facilitation, similar to that of interlimb facilitation (Kawakita et al. 1991; Tazoe et al. 2009). Moreover, we also expected that the abovementioned task- and intensity-dependent arm-trunk corticospinal remote facilitation profiles would change depending on the functional role of the trunk muscles (i.e., RA or ES), as suggested by a previous study that showed differences between RA and ES muscles for muscle-level outputs (Aruin and Latash 1995). Therefore, the overall objective of our study was to first confirm arm-trunk (and arm-leg) remote effect facilitation. If remote effect facilitation was indeed elicited using our current study paradigm, the second objective was to examine whether task (flexion or extension) and intensity (various exertion levels) of muscle contractions would affect remote effect facilitation. To test our hypotheses, we used TMS to investigate MEPs in the trunk extensor and flexor muscles during wrist flexion and extension tasks at the various contraction intensity levels. Lower limb MEPs were also measured under same experimental conditions to identify whether the observed modulations were specific to arm-trunk interactions, or whether co-activation of any two muscles

would produce similar facilitation patterns. Moreover, investigating remote effects of multiple body segments, including limb and trunk muscles at the same time under the same condition, could lead to a better understanding of neural interaction mechanisms of multiple muscles in human motor control.

5.2. Methods

5.2.1. Participants

Twelve healthy male volunteers were recruited for this study. The age, weight, and height of the participants were 24.8±1.5 years, 67.2±6.6 kg, and 173.5±5.3 cm (mean±SD), respectively. All participants were right-handed. None of the participants had any history of neurological or musculoskeletal impairments. Specifically, for the TMS study, we confirmed that all participants had no metal implants, cardiac pacemaker, history of epilepsy, brain injury, neurosurgery, or psychological disorders, have never had a convulsion or a seizure, and did not regularly take medications such as anti-depressants or other neuromodulatory drugs (Rossi et al. 2011a). All participants gave written informed consent in accordance with the Declaration of Helsinki. The experimental procedures were approved by the local institutional ethics committee.

5.2.2. Experimental procedures

During the experiment, participants were seated comfortably on a chair with a back support to keep their trunk muscles relaxed. Following a gentle warm-up and task practice, maximum voluntary contraction (MVC) level was first measured by asking the participants to perform three isometric wrist flexions and three isometric wrist extensions in a randomized order between participants with their right (dominant) arm. Force level of the wrist flexion and wrist extension was measured using a strain gauge sensor (LCB03K025L, A&D Company Limited, Japan), which was fixed to a metal frame located on the distal part of the forearm (Figure 5-1A). During the experiments, corticospinal excitability was assessed during: (a) wrist flexion; and (b) wrist extension. In order to control for the biomechanical effects of performing flexion and extension movements, both tasks were performed by rotating the arm such that the resultant movements were in the opposite direction to gravity (Figure 5-1A). During wrist flexion and

extension tasks, participants were asked to match the forces corresponding to a range from 0 (Rest) to 50% of MVC force level and maintain this contraction intensity by matching the force target level which was displayed on a monitor in real-time (Figure 5-1A). Each task consisted of target force (0 (Rest) to 50% MVC with steps of 5%), corresponding to 11 blocks (Figure 5-1B) which were separated by at least 3 min rest. The order of the target force levels was randomized between participants. TMS stimuli were delivered when participants maintained the corresponding contraction target level for a period of 3-5 seconds (i.e., steady-state part of the contraction). Each block consisted of eight trials, which were separated with approximately 10 sec between trials. Moreover, the order of the experimental tasks (flexion or extension) was randomized between participants, with at least 5 min rest between tasks.

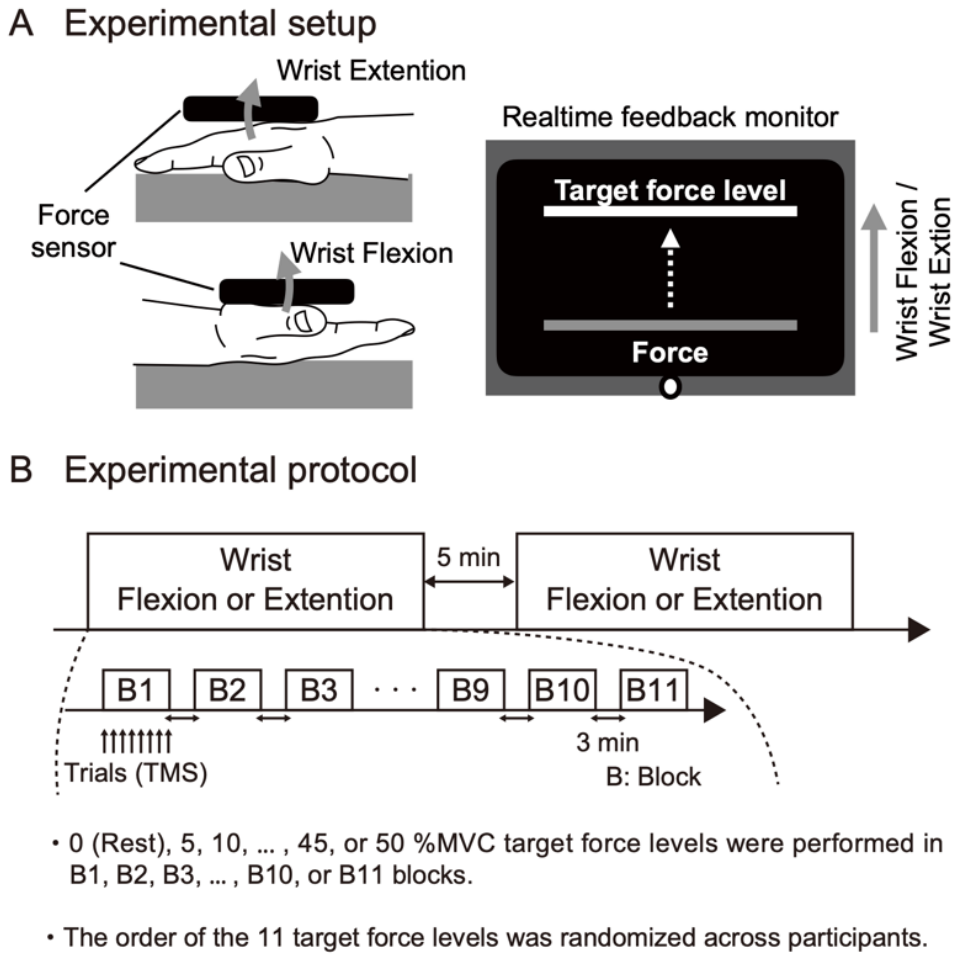

Figure 5-1: (A) Experimental setup showing the hand posture of participants during the experiment. During the experiment, participants were asked to match the isometric wrist flexion and wrist extension force 0 to 50% of maximum voluntary contraction effort using their right arm with real-time visual feedback of force displayed on a monitor. (B) Experiment consisted of wrist flexion and wrist extension conditions, which were randomized between participants and separated by 5 min rest. Each condition consisted of 11 blocks and each target force [0 (Rest), 5, 10, ..., 45, or 50% MVC] was randomly set to each block with at least 3 min rest between blocks. Each block consisted of eight trials.

5.2.3. Data acquisition

5.2.3.1 Electromyography (EMG) activity

Electromyographic (EMG) activities were recorded unilaterally from right side of: (i) erector spinae muscle on the 12th thoracic vertebral level (ES; trunk extensor muscle); (ii) rectus abdominis muscle lateral to the umbilicus (RA; trunk flexor muscle); and (iii) tibialis anterior muscle lateral to the tibia (TA; lower-limb muscle). Two bipolar Ag/AgCl surface electrodes (Vitrode F-150S, Nihon Kohden, Tokyo, Japan) were placed over the muscle belly with 1 cm separation. A ground electrode was placed over the right anterior superior iliac spine. Prior to application of electrodes, skin was cleaned using alcohol to reduce impedance. All EMG signals were band-pass filtered (5 - 1,000 Hz) and amplified (×1,000) using a multichannel amplifier (MEG-6108, Nihon Kohden, Tokyo, Japan). All data were digitized at a sampling frequency of 4,000 Hz using an analog-to-digital (A/D) converter (Powerlab/16SP, AD Instruments, Castle Hill, Australia) and stored on the computer for post-processing.

5.2.3.2 Transcranial magnetic stimulation (TMS)

TMS was delivered over the primary motor cortex using a mono-phasic magnetic stimulator (Magstim 200, Magstim Co., Whitland, UK) through a double cone coil (outside diameter of 110 mm; Magstim Co., Whitland, UK). The optimal stimulation spot ("hot spot") was searched over the left motor cortex where MEPs could be recorded from the right ES muscle. Once the "hot spot" was defined, the coil position and orientation were monitored throughout the experiment using a neuronavigation system (Brainsight, Rogue Research, Montreal, Canada) to ensure same coil placement between tasks. The motor threshold (MT) was determined while the participants remained relaxed. Specifically, the MT was defined as the minimum TMS intensity

for which ES MEPs had peak-to-peak amplitudes larger than 50 μV and were evoked in at least five out of ten consecutive trials (Rossini et al. 2015). The stimulus intensity was set at 120% of the MT level (73.3±12.3% of maximal stimulator output) and remained consistent for the duration of the experiment. Since the highest MT in the current study was 76% of maximal stimulator output, we were able to apply 120% MT level for all participants. During preliminary testing, it was confirmed that we could elicit MEP responses in RA and TA muscles when the stimulation "hot spot" and intensity were optimized for the ES muscle. However, since two participants for the RA and three participants for the TA had <0.05 mV amplitude of MEPs at rest, these muscles were excluded for MEP analysis (i.e., RA: n=10 and TA: n=9). The average MEP amplitudes with SD at rest in the ES, RA, and TA muscles were 0.08± 0.03 mV, 0.34 ± 0.25 mV, and 0.42 ± 0.27 mV, respectively.

5.2.3. Data analysis

Background EMG activity of a 50 ms window before each TMS stimulus was first defined by calculating the root mean square value in each muscle and each trial using a custom written script in MATLAB (2017a, The MathWorks Inc., Massachusetts, USA). It is well known that MEPs elicited by single pulse TMS are facilitated by background activation of the muscle (Hess et al. 1987). Therefore, if trunk and lower-limb muscles were co-activated during upper-limb muscles contraction tasks (i.e., wrist flexion and wrist extension), it would not be possible to evaluate the remote effect. Comparing background EMG activity was therefore used to ensure that remote muscles were not contracted during wrist flexion or extension tasks. If the background EMG activity in any of the experimental tasks was significantly different from Rest (0% MVC level), remote effect facilitation was not considered for these tasks (see Results section).

To analyze remote effect facilitation, MEP peak-to-peak amplitudes were calculated for

each trial and each remote muscle (i.e., ES, RA, and TA). Eight repeated trials were averaged for each task (i.e., wrist flexion and wrist extension) and each contraction intensity (i.e., % MVC contraction levels for which the remote limb muscle background EMG activity was not different from the Rest condition; see section 5.3.1). In preliminary experiments it was determined that eight trials were sufficient to obtain consistent recordings since variability was sufficiently low, consistent with previous studies (Groppa et al. 2012). MEP amplitudes were then normalized as a percentage of the amplitude of the elicited responses during the Rest condition for each participant.

5.2.4. Statistics

For each muscle (ES, RA, and TA) and each task (wrist flexion and wrist extension) separately, background EMG activities were first compared between different muscle contraction intensities (Rest and 5 to 50% MVC with steps of 5%) using the Friedman test, a non-parametric equivalent for repeated-measure analysis of variance (ANOVA). Significant results were followed up with post-hoc multiple comparisons using the Wilcoxon signed-rank test to compare Rest (0% MVC) to each remote contraction condition level (5, 10, ..., 45, and 50% MVC). Since background EMG activities of the ES muscle during wrist flexion and wrist extension at the contraction levels above 25% MVC (i.e., 30, 35, 40, 45, and 50% MVC) were significantly greater compared to that during Rest (0% MVC) (Wilcoxon signed-rank test, $p<0.05$; see section 5.3.1 and Figure 5-2A), only data below 30% MVC (5, 10, 15, 20, and 25% MVC) was used for remote effect MEP analysis for the ES muscle. Background EMG activities of the RA and TA muscle during wrist flexion and wrist extension were not significantly different compared to Rest (0% MVC) even if upper-limb muscles were contracted at 50% MVC level (Wilcoxon signed-rank test, $p<0.05$; see section 5.3.1 and Figure 5-2A). However, initially we also only included data

below 30% MVC (5, 10, ..., 20, and 25% MVC) for remote effect MEP analysis of the RA and TA muscles since it is possible that their facilitation may also have been affected by the background EMG activity of ES muscles.

For MEP analysis, we first investigated whether remote facilitation occurred in each remote contraction condition (5 to 25% MVC) using the Friedman test, a non-parametric equivalent for repeated-measure ANOVA. The Friedman test included MEP amplitudes in the Rest, 5, 10, 15, 20, and 25% MVC. Significant results were followed up with multiple comparisons using the Steel post-hoc test, which is a non-parametric equivalent for Dunnett's test, to determine whether MEPs during remote contraction conditions (5, 10, 15, 20, and 25% MVC) were significantly different from Rest for each muscle. When significant remote facilitation was shown, the Friedman test was used to compare MEP amplitudes between contraction at 5, 10, 15, 20, and 25% MVC to investigate contraction intensity effects on remote facilitation for each muscle. Significant results were followed up with post-hoc testing using the Wilcoxon signed-rank test with Holm corrections. Specifically, five remote contraction conditions (5, 10, 15, 20, and 25% MVC) were analyzed in post-hoc testing, and the statistical significance levels were adjusted using the Holm corrections, as summarized by McLaughlin and Sainani (2014).

Additional analysis was conducted to investigate remote effect contraction intensity dependence in RA and TA muscles during high intensity remote contraction (30, 35, 40, 45 and 50% MVC) since they were excluded from the main analysis due to larger ES background EMG activities (Note: RA and TA background EMG activities were not statistically different compared to Rest (0% MVC)). Specifically, since ES background EMG activities were significantly increased during 30 to 50% MVC of wrist flexion and wrist extension (see section 5.3.1.), it could be considered that activation of ES background EMG (i.e., possible remote effect facilitation from ES to RA and/or TA muscles) may have affected RA and TA corticospinal excitability. Therefore,

correlations between the remote effect in RA and TA MEPs and ES background EMG activations during 30 to 50% MVC of wrist flexion and wrist extension were first analyzed using Spearman's rank correlations. Since no significant correlations were shown (see section 5.3.3), the same statistical tests were conducted for MEP amplitudes between contraction at 30, 35, 40, 45 and 50% MVC as for those during lower contraction intensities (5 to 25% MVC).

Overall, non-parametric tests were chosen because the Shapiro-Wilk test showed that most identified measures were not normally distributed. All statistical comparisons were performed using the software package R (version 3.6.3). Significance level for all tests was set to $p<0.05$.

5.3. Results

5.3.1. Background EMG activity

The background EMG activity results are shown in Figure 5-2. The Friedman test showed that ES background EMG activities were significantly different between contraction intensities (0-50%MVC) in both wrist flexion and wrist extension tasks [wrist flexion: $\chi^2(10)=54.9$, $p<0.001$; wrist extension: $\chi^2(10)=82.2$, $p<0.001$]. Specifically, post-hoc analysis showed that the ES background EMG activities during ≥30% MVC wrist flexion and wrist extension were significantly increased, compared to Rest (0% MVC) ($p<0.05$, Wilcoxon signed-rank test; Figure 5-2A).

The Friedman test showed that RA background EMG activities were not significantly different between contraction intensities (0-50% MVC) during wrist flexion task [$\chi^2(10)=18.2$, $p=0.052$], while they were significantly different during wrist extension task [$\chi^2(10)=26.2$, $p<0.01$]. Post-hoc analysis showed no significant differences in RA background EMG activities during wrist extension between Rest (0% MVC) and remote contraction condition ($p>0.05$, Wilcoxon signed-rank test; Figure 5-2B).

Finally, the Friedman test showed that TA background EMG activities were not significantly different between contraction intensities (0-50% MVC) in both wrist flexion and wrist extension tasks [wrist flexion: $\chi^2(10)=11.1$, $p=0.354$; wrist extension: $\chi^2(10)=9.19$, $p=0.514$] (Figure 5-2C).

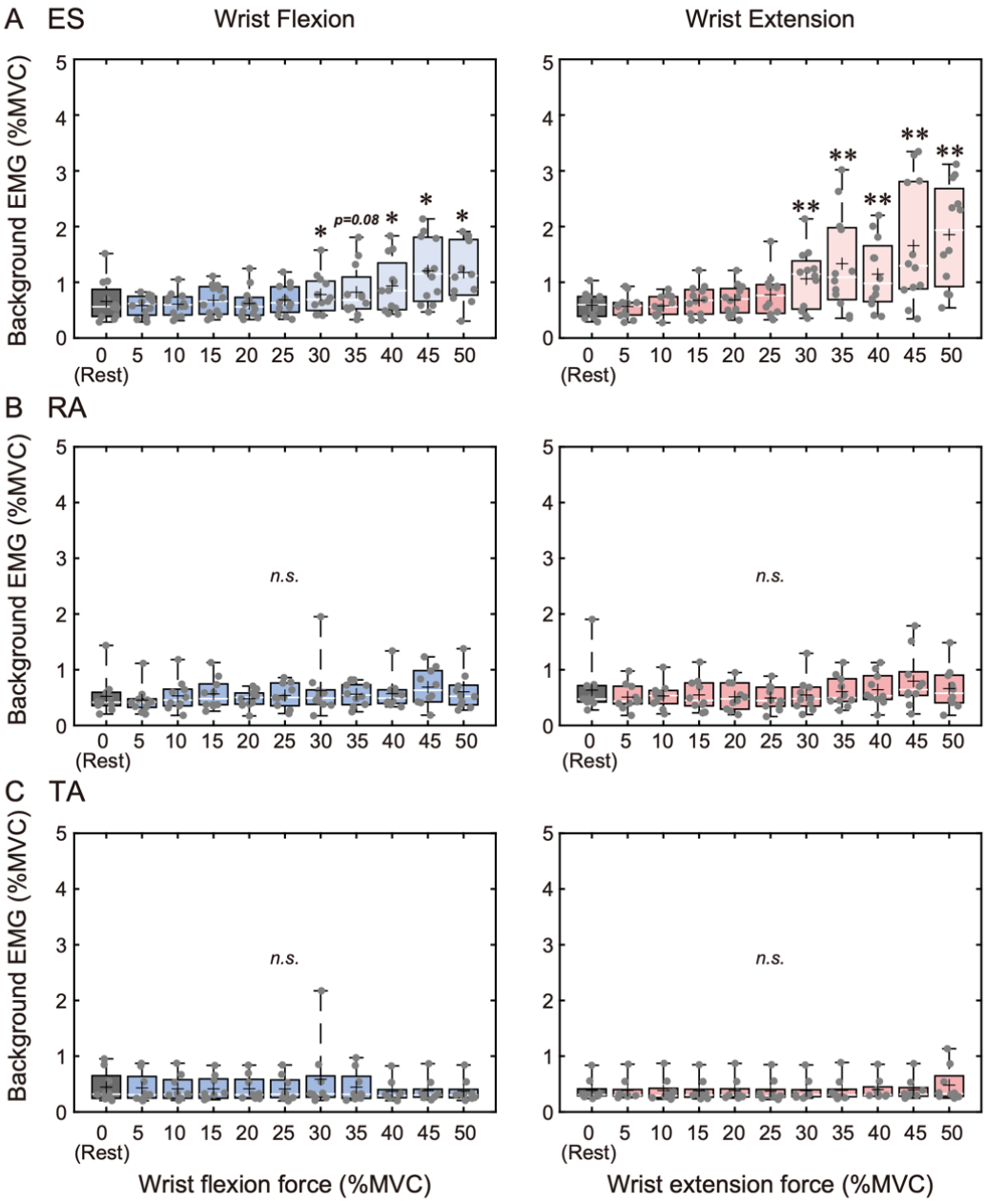

Figure 5-2: Group data for background electromyographic (EMG) activity of the: (A) erector spinae (ES); (B) rectus abdominis (RA); and (C) tibialis anterior (TA) muscles during 0 (Rest) to 50% of maximum voluntary contraction (MVC) effort of wrist flexion and wrist extension. The lines and cross marks in the box plots indicate median and mean values, respectively. The ends of the boxes represent the 25th and 75th percentiles.

The whiskers on the boxplot illustrate the minimum and maximum values. Asterisks indicate significant differences compared to 0% MVC (Rest). Legend: n.s. non-significant; $^{*}p<0.05$; $^{**}p<0.01$.

5.3.2. MEP modulation during low contraction intensities

The MEP amplitude modulation results during 5-25% MVC of wrist flexion and wrist extension are shown in Figure 5-3. For the ES muscle, the Friedman test showed that ES MEP amplitudes were significantly different between contraction intensities (0-25% MVC) in each task (i.e., wrist flexion and wrist extension) [wrist flexion: $\chi^2(5)=14.8$, $p=0.011$; wrist extension: $\chi^2(5)=40.2$, $p<0.001$]. Specifically, post-hoc analysis showed that the ES MEP amplitudes during ≥5% MVC of wrist flexion and wrist extension were significantly increased compared to Rest (0% MVC) ($p<0.05$, Steel test; Figure 5-3A and D). Moreover, the Friedman test showed no significant differences in MEP amplitudes between 5-25% MVC of wrist flexion [$\chi^2(4)=4.73$, $p=0.316$] (Figure 5-3A), while there were significant differences in MEP amplitudes between 5-25% MVC of wrist extension [$\chi^2(4)=23.3$, $p<0.001$] (Figure 5-3D). Specially, post-hoc analysis showed that MEP amplitudes during 15-25 % MVC of wrist extension were larger compared to those during 5% MVC ($p<0.05$; Figure 5-3D).

For the RA muscle, the Friedman test showed no significant difference in MEP amplitudes between 0-25% MVC of wrist flexion and wrist extension [wrist flexion: $\chi^2(5)=9.14$, $p=0.104$; wrist extension: $\chi^2(5)=5.76$, $p=0.330$] (Figure 5-3B and E).

For the TA muscle, the Friedman test showed that TA MEP amplitudes were significantly different between contraction intensities (0-25% MVC) in each task (i.e., wrist flexion and wrist extension) [wrist flexion: $\chi^2(5)=21.3$, $p<0.001$; wrist extension: $\chi^2(5)=18.2$, $p<0.01$]. Specifically, post-hoc analysis showed that the TA MEP amplitudes during ≥5% MVC

of wrist flexion and wrist extension were significantly increased compared to Rest (0% MVC) ($p<0.05$, Steel test; Figure 3C and F). Moreover, the Friedman test showed no significant difference in MEP amplitudes between 5-25% MVC of wrist flexion and wrist extension [wrist flexion: $\chi^2(4)=4.10$, $p=0.600$ (Figure 5-3C); wrist extension: $\chi^2(4)=3.38$, $p=0.497$] (Figure 5-3F).

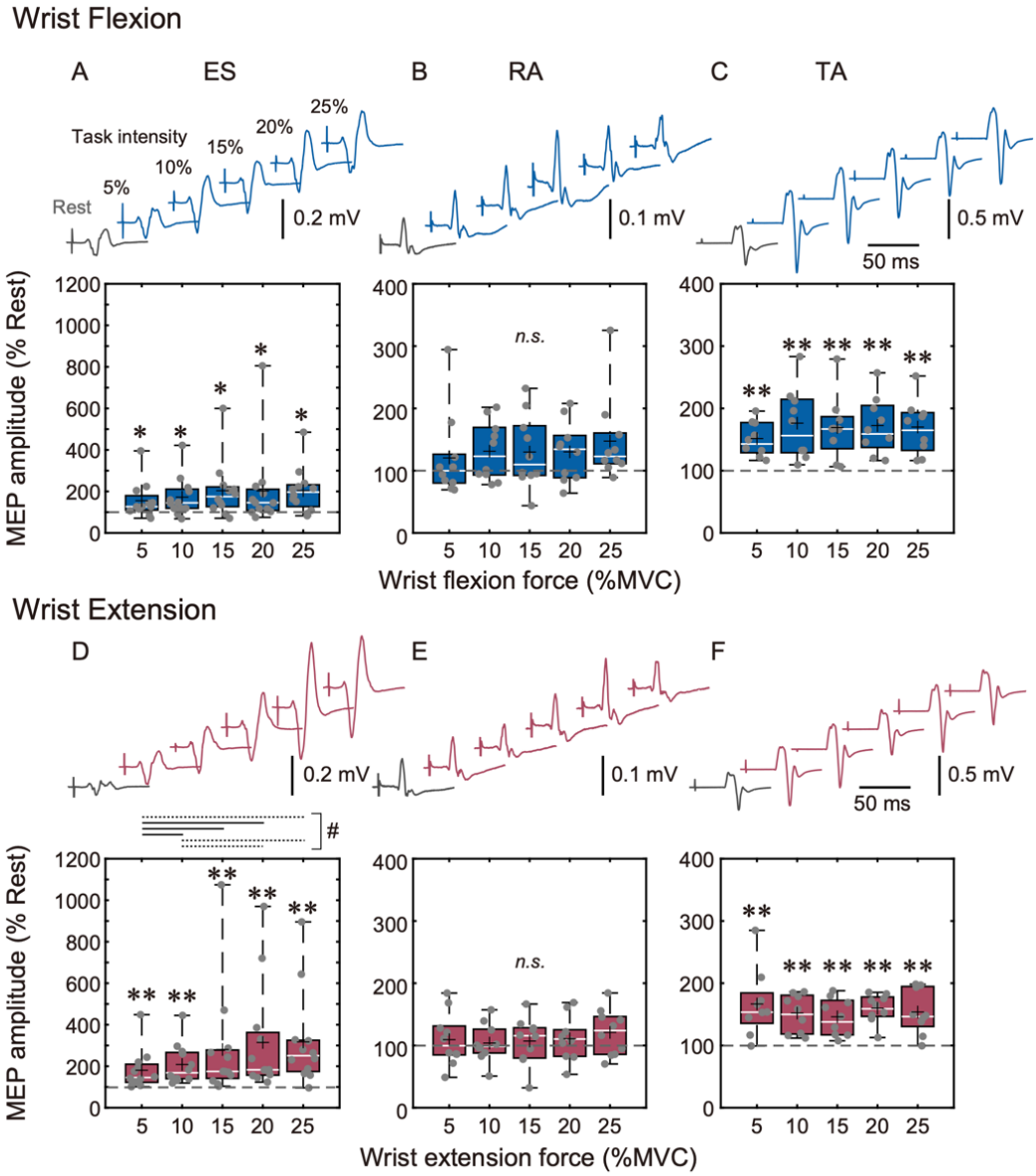

Figure 5-3: (A), (B), and (C) Wrist flexion condition: Averaged motor evoked potentials (MEPs) in the erector spinae (ES), rectus abdominis (RA), and tibialis anterior (TA) muscles of one representative subject during 0% MVC (Rest: gray traces) and 5-25% MVC (remote effect: blue traces). Box plots show group data for MEPs elicited in the ES, RA, and TA muscles. (D), (E), and (F) Wrist extension condition: Averaged MEPs in the ES, RA, and TA muscles of one representative subject during 0% MVC (Rest: gray traces) and 5-25% MVC (remote effect: red traces). Box plots show group data for

MEPs elicited in the ES, RA, and TA muscles. All MEP amplitudes were normalized with respect to the MEP amplitude at 0% MVC (Rest) for each participant. The lines and cross marks in the box plots indicate median and mean values, respectively. The ends of the boxes represent the 25th and 75th percentiles. The whiskers on the boxplot illustrate the minimum and maximum values. Asterisks indicate significant differences compared to 0% MVC (Rest). Hashtags indicate differences between 5-25% MVC of wrist extension. Legend: $^{*}p<0.05$, $^{**}p<0.01$; # and dashed line $p<0.10$, # and solid line $p<0.05$.

5.3.3. MEP modulation during larger contraction intensities

Since there was no significant correlation between ES background EMG activation and RA and TA MEP facilitation during 30-50% MVC of wrist flexion and wrist extension (all Spearman's correlations $p>0.05$), RA and TA MEP modulations during larger (30-50% MVC) contraction intensities were also compared as additional analysis.

The results of the RA and TA muscle MEP amplitudes during 30 - 50% MVC of wrist flexion and wrist extension are shown in Figure 5-4. For the RA muscle, the Friedman test showed that MEP amplitudes were significantly different between Rest (0% MVC) and each remote contraction condition (30, 35, 40, 45, and 50% MVC) in wrist flexion task [$\chi^2(5)=12.9$, $p=0.0242$] (Figure 4A), while there were no significant differences in wrist extension task [$\chi^2(5)=9.31$, $p=0.0971$] (Figure 5-4C). Specifically, post-hoc analysis showed that the RA MEP amplitudes during 40 and 45 % MVC of wrist flexion were significantly increased compared to Rest (0% MVC) ($p<0.05$, Steel test; Figure 5-4A). The Friedman test showed no significant differences in MEP amplitudes between 30-50% MVC of wrist flexion [$\chi2(4)=4.73$, $p=0.316$] (Figure 5-4A).

For the TA muscle, the Friedman test showed that MEP amplitudes were significantly different

between Rest (0% MVC) and each remote contraction condition (30, 35, 40, 45, and 50% MVC) in both wrist flexion and wrist extension tasks [wrist flexion: $\chi^2(5)=21.6$, $p<0.001$; wrist extension: $\chi^2(5)=31.2$, $p<0.001$] (Figure 5-4B and D). Specifically, post-hoc analysis showed that the TA MEP amplitudes during 30 - 50% MVC of wrist flexion and wrist extension were significantly increased compared to Rest (0%MVC) ($p<0.01$, Steel test; Figure 5-4B and D). The Friedman test showed no significant difference in MEP amplitudes between 30 - 50% MVC of wrist flexion [$\chi2(4)=3.20$, $p=0.525$] (Figure 5-4B), while there were significant differences in MEP amplitudes between 30-50% MVC of wrist extension [$\chi^2(4)=16.6$, $p<0.01$] (Figure 5-4D). Specially, post-hoc analysis showed that MEP amplitudes during 45 and 50% MVC of wrist extension were larger compared to during 30% MVC ($p<0.05$; Figure 5-4D).

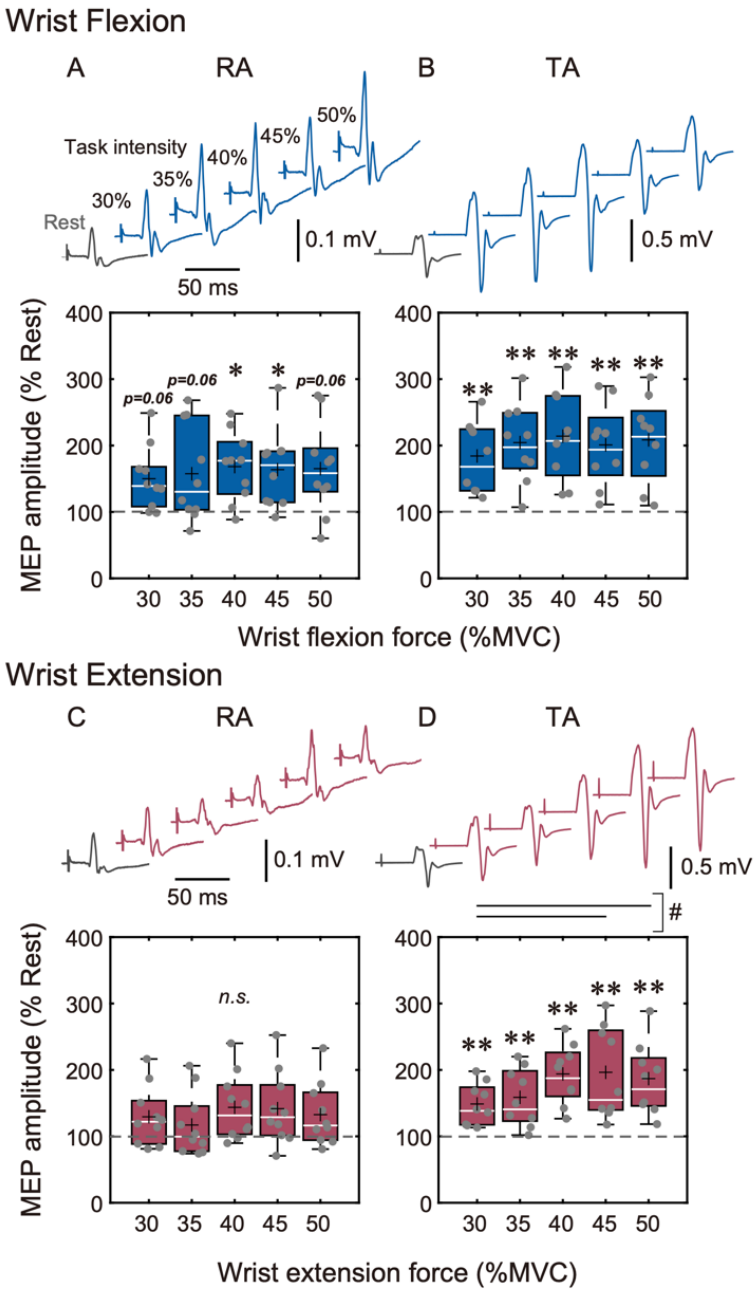

Figure 5-4: (A) and (B) Wrist flexion condition: Averaged motor evoked potentials (MEPs) in the rectus abdominis (RA) and tibialis anterior (TA) muscles of one representative subject during 0% MVC (Rest: gray traces) and 30-50% MVC (remote effect: blue traces). Box plots show group data for MEPs elicited in the RA and TA muscles. (C) and (D) Wrist extension condition: Averaged MEPs in the RA and TA

muscles of one representative subject during 0% MVC (Rest: gray traces) and 5-25% MVC (remote effect: red traces). Box plots show group data for MEPs elicited in the RA and TA muscles. All MEP amplitudes were normalized with respect to the MEP amplitude at 0% MVC (Rest) for each participant. The lines and cross marks in the box plots indicate median and mean values, respectively. The ends of the boxes represent the 25th and 75th percentiles. The whiskers on the boxplot illustrate the minimum and maximum values. Asterisks indicate significant differences compared to 0% MVC (Rest). Hashtags indicate significant differences between 30-50% MVC of wrist extension. Legend: $^{*}p<0.05$, $^{**}p<0.01$; # and solid line $p<0.05$.

5.4. Discussion

In the current study, we investigated whether the extent of corticospinal remote facilitation of the ES (trunk extensor) muscle induced by upper-limb contractions would depend on the task (wrist flexion or extension) and contraction intensity. Our results showed that corticospinal excitability of the ES was significantly facilitated even during low level (≥5% MVC) wrist flexion and wrist extension contractions (Figure 5-3A and D). However, the extent of corticospinal remote facilitation of the ES muscle during wrist flexion did not depend on contraction intensity. On the other hand, higher levels of wrist extension contractions induced greater extent of corticospinal remote facilitation in the ES, indicating contraction intensity dependence (Figure 5-3D). For the RA (trunk flexor) muscle, our results also showed significant corticospinal excitability facilitation during wrist flexion contractions above 25% MVC (Figure 5-4A), but not extension (Figure 5-4C). The extent of RA corticospinal remote facilitation was not reinforced even when wrist flexion intensity increased at 50% MVC (Figure 5-4A). Finally, for the TA (leg) muscle, corticospinal excitability was significantly facilitated during low level (≥5% MVC) wrist flexion and wrist extension contractions (Figure 5-3C and F). Specifically, the extent of corticospinal remote facilitation in the leg muscles was not changed when remote muscle contraction intensity was below 30% MVC (Figure 5-3C and F), while it was increased during higher contractions (50% MVC) during wrist extension, but not flexion (Figure 5-4B and D). Since there were statistically significant ES background EMG activations during wrist flexion and wrist extension at 30 to 50% MVC, it could be speculated that ES background EMG activation may have affected RA and TA corticospinal remote facilitation. However, it must be noted that since the ES activation levels were exceedingly low (i.e., on average 0.8-1.9% MVC, as shown in Figure 5-2A) and there was no statistically significant correlation between ES background EMG activation and RA and TA MEP facilitation during wrist flexion and wrist

extension at 30 to 50% MVC (see section 5.3.3.), it is highly unlikely that ES background EMG activation had any considerable physiological effects on the remote facilitation in the RA and TA muscles. Therefore, it can be assumed that remote facilitation observed in the RA and TA muscles is predominantly related to wrist flexion or extension task performance. A discussion about possible mechanisms of task- and intensity-dependent corticospinal remote facilitation follows.

5.4.1. Task- and intensity-dependence of arm-trunk corticospinal remote facilitation

The main findings of our current study are that: (1) corticospinal remote facilitation of the ES muscle (trunk extensor) was elicited even during low-level wrist flexion and extension contractions (≥5% MVC), while relatively strong wrist flexion and extension contractions (≥25% MVC) were required to induce remote facilitation of the RA muscle (trunk flexor); and (2) extent of corticospinal remote facilitation of the ES (trunk extensor) was proportional to the contraction intensity of wrist extension but not wrist flexion, while this was not observed for the RA (trunk flexor) muscle during both wrist extension and flexion tasks. Moreover, corticospinal excitability in the TA muscles was significantly facilitated during low level (≥5% MVC) wrist extension and wrist flexion contractions. The extent of corticospinal remote facilitation in the leg muscles was not changed when remote muscle contraction intensity was below 30% MVC, while the extent of corticospinal remote facilitation increased during higher wrist extension contractions (50% MVC), but not flexion. It has previously been reported that corticospinal remote facilitation between upper- and lower-limb muscles (interlimb facilitation) was achieved regardless of the task that was performed (Chiou, Wang, Liao, and Yang 2013; Chiou, Wang, Liao, Wu, et al. 2013; Tazoe and Komiyama 2014). Moreover, the extent of interlimb corticospinal remote facilitation was shown to depend on voluntary effort level of the contracted muscle (Kawakita et al. 1991; Tazoe, Sakamoto, et al. 2007; Tazoe et al. 2009; Tazoe and Komiyama 2014). However, it was unclear

until now if these flexion / extension task characteristics of interlimb neural interactions would remain similar during arm-trunk interactions. Based on previous studies showing that trunk flexor and extensor muscle activity depends on the direction of the arm movements (Aruin and Latash 1995; Hodges et al. 1997), we hypothesized that the extent of arm-trunk corticospinal remote facilitation would depend on the task performed during upper-limb muscle contractions (i.e., flexion or extension). We also hypothesized that muscle contraction intensity would affect the extent of arm-trunk remote effect facilitation, similar to that of interlimb facilitation (Tazoe, Sakamoto, et al. 2007; Tazoe et al. 2009; Tazoe and Komiyama 2014). Consistent with these hypotheses, our results demonstrated that the extent of corticospinal remote facilitation between arm and trunk muscles depended on the task (i.e., upper-limb flexion or extension) and the level of remote upper-limb muscle contractions. A study by Chiou *et al.*, (2016) previously reported that corticospinal excitability of the ES muscle was greater during a rapid shoulder flexion task (phasic contractions) compared to a static shoulder flexion task (tonic contractions), while the extent of remote facilitation of the RA muscle was similar between these tasks. Our results also showed that the profiles of remote facilitation during different level of contraction differed between tasks (i.e., wrist flexion and extension). Therefore, ES muscle corticospinal facilitation was affected to a different extent depending on the task during arm movements, while this was not true for the RA muscle. Specifically, our study showed that ES muscles could be facilitated more when wrist extensor (but not flexor) contraction intensities were increased, even if the effort changes were exceedingly small (i.e., 5% MVC). This may suggest that wrist extensors have a stronger connectivity with the trunk extensors (ES) compared to the trunk flexors (RA). Such functional muscle connectivity (extensor-extensor / flexor-flexor connectivity) is also supported by our results that remote facilitation of trunk flexors (RA) occurred during contractions of wrist flexors as well as that contraction intensity dependent remote facilitation of the leg extensors (TA)

was observed during contractions of the wrist extensors.

On the other hand, it has previously been reported that ES corticospinal excitability was facilitated to a larger extent by elbow flexion compared to elbow extension, which suggests that elbow flexors have the stronger interactions with trunk extensors (Chiou, Strutton, et al. 2018). Therefore, it is also possible that arm and trunk connectivity may not always depend on the flexor / extensor remote muscle pairs, but also on the functional connectivity between these muscles. In our current study, ES MEPs were facilitated during both wrist flexion and extension at contraction intensities above 5% of MVC effort, although contraction intensity dependence was only shown during wrist extension. Therefore, ES muscles could detect arm movements even at relatively small contraction intensities regardless of whether wrist extension or flexion was performed. It has been reported that activation of ES muscles has an important role for minimizing postural displacement during arm movement-induced postural perturbations (Aruin and Latash 1995; Hodges et al. 1997). Taken together, such functional connectivity of the ES and remote limb muscle pairs may perhaps explain greater sensitivity of trunk extensors to upper-limb movement and changes in corticospinal excitability to contraction intensity, as demonstrated in our current study.

5.4.2. Possible mechanisms of arm-trunk corticospinal remote facilitation

Since it is well known that the excitability of the corticospinal pathway is affected by excitation of both cortical and spinal circuits (Hess et al. 1987), arm-trunk corticospinal remote facilitation, which was demonstrated in our current study, could also be attributed to cortical and/or spinal circuits. Indeed, previous studies reported that inter-limb remote facilitation could affect both cortical (Tazoe, Endoh, et al. 2007; Chiou, Wang, Liao, and Yang 2013; Chiou, Wang, Liao, Wu, et al. 2013) and spinal (Jendrássik E 1883; Kawamura and Watanabe 1975; Borroni et

al. 2005) motor circuits. Specifically, it was demonstrated that remote limb muscle contractions decreased upper-limb and lower-limb muscle short-interval intracortical inhibition (induced by paired-pulse TMS), which implies cortical inhibition mechanisms during remote muscle contractions (Chiou, Wang, Liao, and Yang 2013; Chiou, Wang, Liao, Wu, et al. 2013). Similarly, decreased duration of the cortical silent period (induced by TMS during low levels of muscle contractions) of upper-limb muscles was shown to be elicited by contractions of lower-limb muscle, also suggesting cortical inhibition (Tazoe, Endoh, et al. 2007). These studies suggest that cortical disinhibition may contribute to corticospinal remote facilitation between upper- and lower-limb muscles. Moreover, H-reflex responses elicited by peripheral nerve stimulation (e.g., Jendrássik E, 1883; Kawamura & Watanabe, 1975; Borroni *et al.*, 2005) as well as posterior-root spinal reflex responses elicited by transcutaneous spinal cord stimulation (Kato et al. 2019; Masugi et al. 2019; Sasaki, Kaneko, et al. 2020) in the upper- or lower-limb muscles were facilitated by remote limb muscle contractions. These studies indicate spinal reflex remote modulation mechanisms also contribute to interlimb remote facilitation. Therefore, arm-leg remote facilitation observed in our current study (i.e., TA remote facilitation during wrist flexion and wrist extension) may also be caused by cortical and/or spinal mechanisms. Taken together, it may be speculated that arm-trunk corticospinal remote facilitation is also mediated in cortical and/or spinal networks. Conversely, two recent studies suggested that cortical-levels networks may primarily be attributed to arm contraction-induced trunk remote facilitation (Chiou, Strutton, et al. 2018; Sasaki, Milosevic, et al. 2020). Specifically, Chiou *et al.*, (2018) showed decreased short-interval intracortical inhibition, indicating disinhibition of intracortical circuits. However, no changes in the cervicomedurally motor evoked potentials (induced by cervicomedullary junction magnetic stimulation) of the trunk muscle during upper-limb contractions were observed, suggesting that subcortical (spinal) excitability was unaffected (Chiou, Strutton, et al. 2018).

Similarly, it was shown that cervicomedurally motor evoked potentials of the trunk muscles were not affected by upper-limb contraction, while corticospinal excitability was modulated (Sasaki, Milosevic, et al. 2020). Although it is still possible that both cortical and/or spinal networks may be involved, recent evidence suggests that arm-trunk remote facilitation is more likely mediated in the cortical-level networks. The cortical remote facilitation mechanisms hypothesis is also supported by basic animal studies which have demonstrated that intracortical facilitation may be involved in spreading of neural activity within the motor cortex (Capaday et al. 2009, 2011). Specifically, it was shown that neural activity initiated at a cortical locus can spread to the neighboring cortical regions which represent different muscles via intrinsic horizontal connections between neurons within the motor cortex (Capaday et al. 2009, 2011). In human studies using TMS, it was proposed that similar cortico-cortical connections may exist (Boroojerdi et al. 2000; Komeilipoor et al. 2017). Specifically, previous studies investigating corticospinal remote facilitation mechanisms proposed that activation of cortical motor networks by voluntary contraction of certain muscles could spread to neighboring cortical areas representing different segment muscle (Boroojerdi et al. 2000) and that the extent of this spreading may depend on the distance between M1 representations of different muscles within the cortex (Boroojerdi et al. 2000; Sasaki et al. 2018). This suggests that cortical remote facilitation mechanisms are dependent on the anatomical somatotopic representations within the primary motor cortex. Moreover, it is well known that somatotopy of muscles in the different body segments overlap within the motor cortex (Penfield and Boldrey 1937; Brasil-Neto et al. 1992). Since the trunk muscle representations in the homunculus of the primary motor cortex (M1) in humans is located near the upper-limb representations, it is likely that activation of trunk cortical motor circuits was also induced during voluntary contraction of upper limbs due to the overlapping of the cortical representations within the primary motor cortex (M1). Therefore, arm-

trunk corticospinal remote facilitations observed in our current study, are likely modulated via anatomical connections such as intracortical connectivity networks, and/or overlapping of somatotopic representations at the supraspinal level, although subcortical mechanisms cannot be fully ruled out. Indeed, previous studies reported that subcortical circuits may be more involved as contraction levels increase (Stedman et al. 1998; Muellbacher et al. 2000). Therefore, it is possible that subcortical mechanisms may also have contributed to the remote facilitation when contraction levels were higher. Moreover, the proximity of motor representations within M1 between remote muscles may be one of the possible mechanisms related to the corticospinal remote facilitation (Boroojerdi et al. 2000). However, since our current study showed that profiles of remote effect of ES and RA muscles were different, despite their proximity within M1, remote facilitation mechanisms cannot only be explained by somatotopic relationships. A specific discussion related to other possible mechanisms of corticospinal remote facilitation follows in section 5.4.3.

5.4.3. Significance of corticospinal remote facilitation

Overall, corticospinal remote facilitation relationship between certain remote muscles (e.g., arm-trunk or arm-leg) may reflect anatomical relationships with the central nervous system and/or functional connectivity between these muscles. If only the anatomical relationship between remote muscles (i.e., proximity of motor representations within M1, as discussed in section 5.4.2) were to determine the profile of remote corticospinal facilitation, remote effect between arm-trunk would simply be effective compared to that of arm-leg. However, our current results showed that even different trunk muscles (i.e., ES and RA), which are located very close with the M1 (Tsao et al. 2011), had very distinct remote facilitation responses. Moreover, the leg muscles (TA) showed lower threshold of remote facilitation, compared to the trunk flexor muscles (RA).

Therefore, our current study may suggest that functional relationships between remote muscles are also represented within the corticospinal circuits, in addition to their anatomical relationship (i.e., somatotopic representations with M1). Specifically, ES muscles have an important functional role for maintaining postural stability during arm movements (Aruin and Latash 1995; Hodges et al. 1997), which may be why ES muscle showed lower thresholds and task- and intensity- dependent modulation of corticospinal remote facilitation. On the other hand, interlimb (arm-leg) coordinated movements are functionally relevant during rhythmic movements which activate the central pattern generator, such as walking and cycling (Zehr and Duysens 2004). Indeed, it was shown that arm-leg neural interaction could be strengthened to a larger extent during rhythmic movements, compared to tonic contractions (Frigon et al. 2004; Zehr et al. 2007). Therefore, under the tonic contractions condition in the current study, intensity-dependent changes in arm-leg corticospinal remote facilitation may not be functionally required. However, intensity-dependent changes in arm-leg corticospinal remote facilitation were observed only during high levels of wrist extension intensities in the current study. It was reported that rhythmic ipsilateral hand and foot movements performed at the same time are made more reliable when they are synchronized in the same direction (Baldissera et al. 1982). A previous study investigating effects of rhythmic ankle plantar/dorsi flexion on H-reflex excitability of wrist flexors (i.e., flexor carpi radialis) also reported that modulation peak of H-reflex in the wrist flexors occurred at the same time as the contraction of the ankle plantar flexors (i.e., soleus muscle) (Baldissera and Borroni 2002). Moreover, preference of rhythmic hand and foot movements reflects spatial rather than structural constraints. When the hand was pronated, wrist flexor neural pathways were facilitated during the plantarflexion phase, while when the hand was supinated, wrist extensors were facilitated during the plantarflexion phase, and wrist flexors were facilitated during dorsiflexion (Borroni et al. 2004). Therefore, rhythmic movements eliciting

central pattern generator (CPG)-like activations were shown to strengthen arm-leg connectivity depending on the direction of the movement, rather than based on specific muscle pairs, which indicates that rhythmic interlimb interactions may reflect functional connectivity (Borroni et al. 2004). On the other hand, tonic contraction tasks, which were performed in our current study, strengthened the connectivity in specific muscle pairs (i.e., ECR and TA) during high levels of contraction. This may possibly reflect a biological (structural) characteristic, rather than a functional connection. Moreover, trunk flexors (RA) have an anatomical advantage in that the representation within the homunculus of the motor cortex is located close to the upper-limb representations. Despite this, the RA showed higher threshold of remote facilitation and no task- and intensity- dependent modulation. This may reflect weak functional connectivity between trunk flexors (RA) and arm muscles. Moreover, different neural innervations of these muscles may also contribute to their remote facilitation profiles. Specifically, the ES muscles at the T12 level are innervated by dorsal rami of thoracic and lumbar spinal nerves (T8-L3), while the RA muscles are innervated by the intercostal nerve (Pradhan and Taly 1989). Therefore, it is possible that the neural innervations may also have contributed to remote facilitation profiles in our study. Overall, multiple effect including anatomical somatotopic relationships as well as functional connectivity may attribute to task- and intensity-dependent modulation of remote facilitation between arm and trunk extensor. Our findings therefore inform a more comprehensive understanding of the basic principles of human motor control related to the arm-trunk neural interaction. Taken together, these results may also suggest that functional movement synergy oriented training is crucial in rehabilitation to strengthen arm-trunk interactions as a way for improving functional performance after neurological impairments such as spinal cord injury (Chiou and Strutton 2020; Chiou et al. 2020).

5.4.4. Limitations

Our work has several limitations that should be noted. First, some previous studies investigating interactions between trunk and upper-limb muscles have examined contralateral side trunk muscles to the contracted arm to elicit MEP responses (Davey et al. 2002; Chiou, Strutton, et al. 2018; Chiou and Strutton 2020), while we chose to investigate the ipsilateral side in the current study. Davey et al., (2002) previously reported that ES muscle activity in the contralateral side to contracted arm was increased when arm muscle contractions during shoulder abduction were increased, while that of ipsilateral side was not affected considerably. Therefore, ipsilateral side was investigated in our study with the aim to minimize co-contraction of ES muscles during wrist flexion and extension, although it is also likely that ES background EMG activations in the contralateral and ipsilateral side muscles were similar in sitting posture (Sasaki, Milosevic, et al. 2020). Nonetheless, further work is warranted to systematically examine differences in remote effect facilitation between ipsilateral and contralateral side trunk muscles during various tasks at intensities.

Second, although significant ES background EMG activations during strong wrist flexion and extension contractions (>25% MVC) were exceedingly low (i.e., on average 0.8-1.9% MVC, as shown in Figure 5-2A), and there were no significant correlations between the remote RA and TA MEPs and ES background EMG activations during 30-50% MVC of wrist flexion and wrist extension, we still cannot completely exclude a possibility that these slight ES activations could have affected the profiles of remote effect facilitation of RA and TA muscles, in additions to wrist flexion and extension contractions.

Chapter 6 - Study 5

Effect of remote muscle contractions on interneuron circuits in the human motor cortex

6.1. Introduction

Human movements require coordinated activation of the upper and lower limbs. These interlimb interactions are coordinated through complex neuroanatomical networks within the central nervous system (Zehr and Duysens 2004). It has been reported that voluntary activation of lower-limb muscles can modulate the corticospinal motor circuits not only of the contracted muscles but also upper-limb muscles that remain at rest, and vice versa (Tazoe and Komiyama 2014). This phenomenon is known as the "remote effect" and implies that motor functions of the upper- and lower-limb muscles are not embedded in the brain and/or the spinal circuits as separate units; rather, they are connected and interact within the central nervous system. The remote effect has been studied using transcranial magnetic stimulation (TMS) to understand the excitability mechanisms of the corticospinal tract. For example, the existence of neural interaction between upper- and lower-limb muscles was assessed by the motor evoked potentials (MEP) to demonstrate that modulated corticospinal activity depends on the activity of the other muscles in the remote segment of the body (Tazoe, Sakamoto, et al. 2007; Sasaki, Kaneko, et al. 2020). It was also reported that these interaction effects depend on the task and the intensity (i.e., level of effort) of the executed remote muscle contraction (Sasaki et al. 2021). Despite this, numerous aspects of the complex nature of the upper- and lower-limb interaction within the corticospinal circuits remain unclear. Elucidation and a better understanding of these mechanisms may lead to the identification of the underlying fundamental principles that govern interlimb coordinated movements and underlie human movements.

Previous studies have revealed that single-pulse TMS can elicit different descending volleys which depend on the orientation of the current flow induced across the primary motor cortex (M1) (Day et al. 1989; Di Lazzaro et al. 2001). Specifically, TMS-induced posterior-anterior (PA) directed current across the central sulcus preferentially evokes early indirect (I)-

wave which is synaptic activities of the interneurons that project to the corticospinal neurons in the M1. This reflects highly synchronized corticospinal activity (Day et al. 1989; Di Lazzaro et al. 2001). On the other hand, anterior-posterior (AP) current preferentially evokes the late I-wave, which is less synchronized with the corticospinal activity and may instead reflect the activity of premotor regions rather than the M1 (Aberra et al. 2020; Spampinato 2020). Further, lateral-medial (LM) current preferentially evokes the direct (D)-wave which reflects direct activation of corticospinal axons (Day et al. 1989; Di Lazzaro et al. 2001). Therefore, using different current orientations, it is possible to examine the activity of different subsets of interneurons that input onto the corticospinal tract neurons to understand their contributions to interlimb coordination.

Paired-pulse TMS with PA and AP currents for eliciting short-interval intracortical inhibition (SICI) and short-interval intracortical facilitation (SICF) can also respectively target the inhibitory and facilitatory cortical networks that input the interneuronal circuits activated by the PA and AP currents (Tokimura et al. 1996; Ziemann et al. 1998; Federico and Perez 2017; Long et al. 2017). It was also reported that when a TMS stimulus is preceded by afferent input elicited by peripheral nerve stimulation with an inter-stimulus interval of about 20 ms, MEP can be inhibited (Tokimura et al. 2000). This is known as short-latency afferent inhibition (SAI) and can be an assessment of afferent-induced intracortical inhibition networks in PA and AP circuits (Ni et al. 2011; Lei and Perez 2017). Since modulation of corticospinal responses elicited by single-pulse TMS is a net summation of the combined effects of the above-mentioned intracortical inhibitory and facilitatory inputs (e.g., SICI, SICF, and SAI circuits) and subcortical excitability such as spinal motoneurons, investigating the contributions of each neural mechanism during "remote effect" would lead to a more comprehensive elucidation of upper- and lower-limb interaction mechanisms in the corticospinal circuits. Thus, the purpose of the current study was to investigate the "remote effect" on the activity of different subsets of interneurons within M1

activated by PA and AP currents in addition to D-wave and spinal motoneuronal activity.

Previous studies suggested that different intraneuronal circuits in the M1 activated by the PA and AP currents have different structures and independent functions and contribute differently to individual tasks (Di Lazzaro and Ziemann 2013; Hamada et al. 2014). Based on this, we hypothesized that different subsets of interneurons would also contribute differently to the interlimb interaction. Specifically, AP circuits are more sensitive to the skilled learning task (Spampinato 2020) and possibly reflect the activity of premotor regions (Volz et al. 2015; Aberra et al. 2020) which contribute to higher motor functions. Therefore, we hypothesized that the AP circuits would be important for the interlimb neural interaction ("remote effect") since coordinated control of remote limb muscles (interlimb interaction) can be considered as a higher motor function. To test our hypothesis, we assessed the intracortical and spinal excitability of hand muscles modulated during lower-limb muscle contractions.

6.2. Methods

6.2.1. Participants

A total of nineteen healthy volunteers (7 female, 12 male) with a mean ± SD age of 27.4 ± 5.1 years were recruited for this study. None of the participants had any history of neurologic or musculoskeletal impairments. Specifically, for the TMS study, we confirmed that all participants had no metal implants, cardiac pacemaker, history of epilepsy, brain injury, neurosurgery, or psychological disorders, have never had a convulsion or a seizure, and did not regularly take medications such as anti-depressants or other neuromodulatory drugs (Rossi et al. 2011). All participants gave written informed consent in accordance with the Declaration of Helsinki. The experimental procedures were approved by the local institutional ethics committee at the University of Tokyo.

6.2.2. Electromyography (EMG) activity

Electromyographic (EMG) activity was recorded unilaterally from the right side of the first dorsal interosseous (FDI) muscle. Two bipolar Ag/AgCl surface electrodes (Vitrode F-150S, Nihon Kohden, Tokyo, Japan) were placed over the muscle belly with a 1 cm separation. A ground electrode was placed around the right olecranon. Prior to the application of electrodes, the skin was cleaned using alcohol to reduce impedance. All EMG signals were band-pass filtered (5 - 1,000 Hz) and amplified (×1,000) using a multichannel amplifier (MEG-6108, Nihon Kohden, Tokyo, Japan). All data were digitized at a sampling frequency of 4,000 Hz using an analog-to-digital (A/D) converter (Powerlab/16SP, AD Instruments, Castle Hill, Australia) and stored on the computer for post-processing.

6.2.3. Transcranial magnetic stimulation (TMS)

TMS was delivered over the primary motor cortex using a mono-phasic magnetic stimulator (Magstim 200, Magstim Co., Whitland, UK) through a figure-of-eight coil (loop diameter of 70 mm; Magstim Co., Whitland, UK). TMS was applied to activate different sets of synaptic input to corticospinal neurons by changing the current flow induced across the hand area of the M1. The following coil orientations (current directions) were used: (1) PA: coil handle 45° to the midline, (2) AP: coil handle 180° relative to PA, and (3) LM: coil handle 90° to the midline to preferentially evoke volleys with characteristics reflecting early I-wave, late I-wave, and D-wave, respectively (Sakai et al. 1997; Di Lazzaro et al. 2012; Hamada et al. 2013; Volz et al. 2015) (Figure 6-1G). The optimal stimulation spot ("hot spot") was searched by PA currents over the left motor cortex where MEPs could be recorded from the right FDI muscle since it was shown that the direction of the current does not influence the position of the hotspot (Sakai et al. 1997). Once the "hot spot" was defined, the coil position and orientation were monitored throughout the experiment using a neuronavigation system (Brainsight, Rogue Research, Montreal, Canada) to ensure the same coil placement between conditions. Above mentioned TMS settings of PA, AP, and LM coil orientations were used for experiment 1 (section 6.2.4.2), while PA and AP coil orientations were used for experiments 2 and 4 (section 6.2.4.3 and 6.2.4.5).

6.2.4. Experimental procedures

6.2.3.1. General protocol

During the experiment, participants were seated comfortably on a chair. Following a gentle warm-up and task practice, maximum voluntary contraction (MVC) level was first measured by asking the participants to perform three isometric ankle dorsiflexion with their right (dominant) leg. The force level of the dorsiflexion was measured using a strain gauge sensor

(LCB03K025L, A&D Company Limited, Japan), which was fixed to a metal frame. During each experiment, participants were asked to match the dorsiflexion forces to 30% of the MVC force level and maintain this contraction intensity which was displayed on a monitor in real-time (Figure 6-1A). Stimuli for each experiment were delivered during the following two conditions: (1) while participants were relaxed (rest); and (2) while participants maintained isometric dorsiflexion at the 30% MVC level during a random interval of 3-5 seconds (remote) (Figure 6-1B). In both conditions, participants were instructed to keep the hand muscles relaxed and FDI EMG activity was monitored by the experimenter in real-time, which was also confirmed in offline analysis.

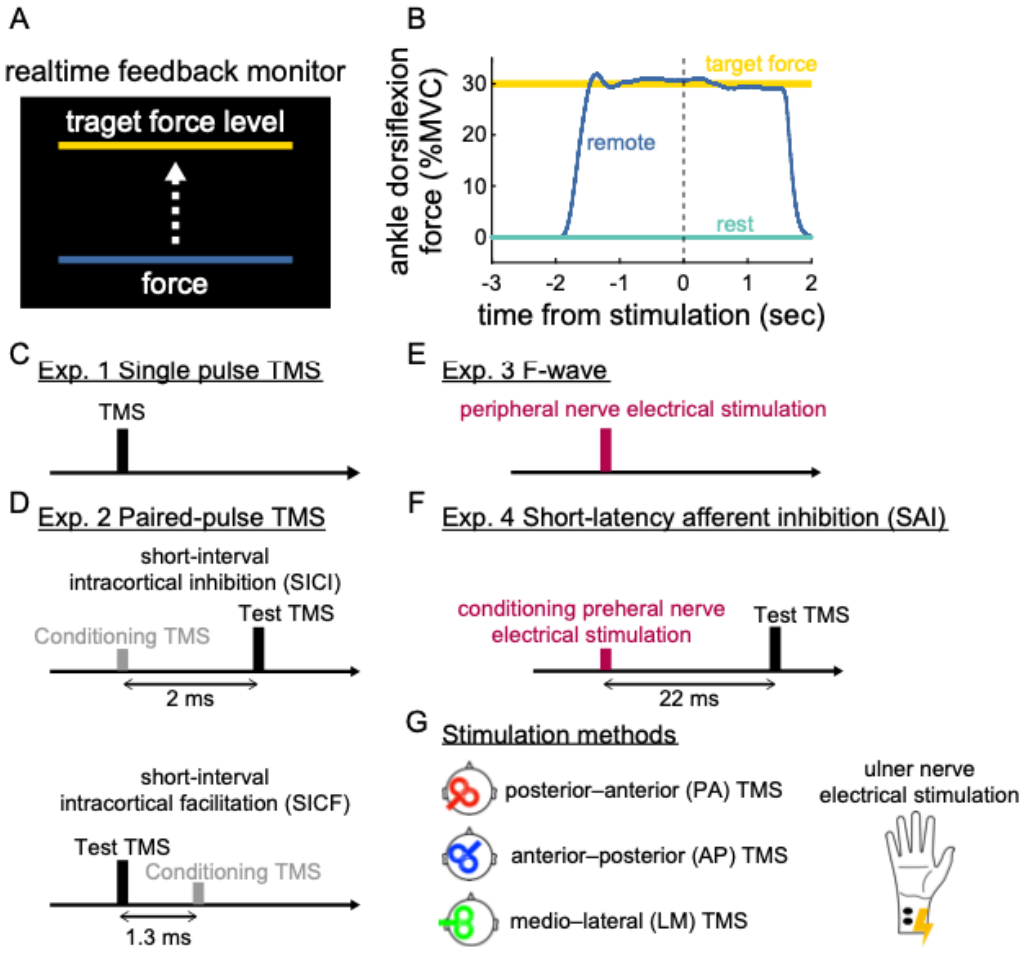

Figure 6-1: (A) During the experiment, participants were asked to match the isometric ankle dorsiflexion force to the target line displayed in the real-time feedback monitor.

(B) Ankle force behavior during rest and remote conditions from a representative participant. **(C)** Stimulation paradigm for single-pulse transcranial magnetic stimulation (TMS) in experiment 1. **(D)** Stimulation paradigm for short-interval intracortical inhibition (SICI) and short-interval intracortical facilitation (SICF) in experiment 2. **(E)** Stimulation paradigm for F-wave in experiment 3. **(F)** Stimulation paradigm for short-latency afferent inhibition (SAI) in experiment 4. **(G)** TMS coil orientation and electrode orientation for ulnar nerve electrical stimulation.

6.2.4.2. Experiment 1: Single-pulse TMS

In experiment 1, fifteen healthy volunteers (5 female, 10 male) with a mean ±SD age of 28.1 ± 5.6 years were recruited. To test activities of different subsets of interneurons during the remote condition, we recorded MEPs from FDI elicited by single-pulse TMS with three different coil orientations: (1) PA; (2) AP; and (3) LM during rest and remote conditions (Figure 6-1C). TMS intensity was adjusted to generate MEPs of approximately 0.5 mV for PA and AP and 1 mV for LM during rest (Sugawara et al. 2005; Ni et al. 2011; Ibáñez et al. 2020). Specifically, to ensure that corticospinal neurons were directly stimulated (i.e., D-wave), higher stimulus intensity was used for LM (Werhahn et al. 1994). Mean (±SD) intensities used for the measurements for each coil orientation were as follows: PA was 58.9 ± 9.8; AP was 74.3 ± 11.7; and LM was 68.3 ± 8.2 % of maximal stimulator output. Experiment 1 consisted of three coil orientation sessions (i.e., PA, AP, and LM), which were separated by at least 5 min rest. Each session includes two conditions (i.e., rest and remote), which were separated by at least 3 min rest. Each condition consisted of twelve trials, which were separated with approximately 10 s between trials. The order of the sessions was randomized between participants. Since the three coil orientation elicited MEPs with significantly different latencies [Figure 6-2A-C; $F_{(2, 28)} = 83.725$, $p < 0.001$, $\eta^2 = 0.857$;

comparisons between LM and PA, $p < 0.001$; between PA and AP, $p < 0.001$; between LM and AP, $p < 0.001$], this confirmed that PA, AP and LM stimulation preferentially evoked volleys with characteristics reflecting early I-wave, late I-wave, and D-wave, respectively (Sakai et al. 1997; Ni et al. 2011; Di Lazzaro et al. 2012; Hamada et al. 2013; Volz et al. 2015).

6.2.4.3. Experiment 2: Paired pulse TMS

In experiment 2, twelve healthy volunteers (3 female, 9 male) with a mean ±SD age of 27.4 ± 3.3 years were recruited. To investigate intracortical inhibition and facilitation mechanisms during remote muscles contractions, short-interval intracortical inhibition (SICI) and short-interval intracortical facilitation (SICF) was tested using previously established paired-pulse paradigm protocols with the coil in the PA and AP orientation (Kujirai et al. 1993; Tokimura et al. 1996; Ziemann et al. 1998; Long et al. 2017) during each condition (i.e., rest and remote).

To test SICI, a conditioning stimulus (CS) was set at an intensity of 70-80 % of resting motor threshold (PA: 71.7 ± 3.9 %; and AP: 74.6 ± 4.9 % of resting motor threshold) (Chiou, Wang, Liao, and Yang 2013; Long et al. 2017; Benavides et al. 2020). The same CS intensity was used for rest and remote conditions. The test stimulus (TS) was set to elicit MEPs of approximately 0.5 mV at rest. Since it was known that the size of a test MEP can affect the magnitude of SICI (Long et al. 2017) and we found that MEP size in the PA and AP orientation increased during remote condition compared with the rest condition in experiment 1 (Figure 6-2B and C), test MEP size during remote condition was adjusted to match MEP amplitudes produced at rest MEP (i.e., approximately 0.5 mV) (Chiou, Wang, Liao, and Yang 2013; Long et al. 2017). Specifically, the CS was delivered 2 ms before the TS for eliciting SICI (Figure 6-1D).

To test SICF, CS intensity was set at 90-100 % of resting motor threshold (PA: 90.8 ± 2.9 %; AP: 90.9 ± 2.9 % of resting motor threshold) (Cash et al. 2015; Long et al. 2017). The

same CS intensity was used for rest and remote conditions. The TS intensity was set to elicit an MEP of approximately 0.5 mV at rest. Test MEP size during remote condition was adjusted to match MEP amplitudes produced at rest MEP (i.e., approximately 0.5 mV) (Long et al. 2017). Specifically, the CS was delivered 1.3 ms after the TS for inducing SICF (Figure 6-1D).

Experiment 2 consisted of two coil orientation sessions (i.e., PA and AP), which were separated by at least 5 min rest. In each session, twelve MEPs were recorded for each stimulation paradigm [i.e., TS alone, SICI (CS+TS), and SICF (TS+CS)] during rest and remote conditions. The stimulation interval was approximately 10 s and there was at least 3 min rest between the conditions. The orders of coil orientation sessions and stimulation paradigms were randomized between participants. SICI and SICF were calculated by expressing the amplitude of conditioned MEP as a percentage of the test MEP amplitude [% = (conditioned MEP * 100) / (test MEP)].

6.2.4.4. Experiment 3: F-wave

In experiment 3, fourteen healthy volunteers (5 female, 9 male) with a mean ±SD age of 27.7 ± 5.9 years were recruited. To test spinal motoneuron excitabilities during remote muscles contractions, F-wave was measured during rest and remote conditions using supramaximal stimulus intensity to the ulnar nerve at the wrist (Figure 6-1G). A constant-current electrical stimulator (DS7A; Digitimer Ltd., United Kingdom) was used to apply a single monophasic square pulse with a 200 μs pulse width. The anode and cathode were 3 cm apart with the cathode positioned proximally. We first defined the intensity for recruitment of the maximum motor response (M max). This was defined as the minimum intensity at which an increase in current intensity would not further increase the M-wave response evoked. We then set the intensity for the experiment at 150 % of intensity for eliciting M max (Long et al. 2017). F-waves were recorded by using 150 Hz high-pass filters of an amplifier (MEG-6108, Nihon Kohden, Tokyo,

Japan) so that the tail end of M max returned to baseline by the onset of the F-wave (Tazoe and Perez 2017). Experiment 3 consisted of two conditions (i.e., rest and remote). Two sets of 15 F-waves for a total of 30 F-waves were recorded for each condition. There was at least 3 min rest between sets and between conditions. The order of the conditions was randomized between participants.

6.2.4.5. Experiment 4: TMS conditioned by peripheral electrical stimulation

In experiment 4, thirteen healthy volunteers (4 female, 9 male) with a mean ±SD age of 28.2 ± 6.0 years were recruited. To test how the corticospinal descending waves produced by the PA and AP circuits respond to sensory afferent inputs during the remote condition, we used an established peripheral and cortical paired-stimulation paradigm to test short-latency afferent inhibition (SAI) (Ni et al. 2011) during rest and remote conditions. A conditioning electrical stimulation pulse was delivered to the right ulnar nerve at the wrist while the intensity was adjusted to produce a slight thumb twitch (Ni et al. 2011) (10.7 ± 2.4 mV), which corresponded to about three times the perception threshold (i.e., the minimum intensity at which stimulation can be felt: 3.2 ± 0.8 mV). Electrode configurations were the same as in experiment 3 (Figure 6-1G). Similar to the other experiments, test TMS intensity was set to elicit an MEP of approximately 0.5 mV at rest for each coil orientation (Cash et al. 2015). Since we found that MEP size in the PA and AP orientation increased during remote condition compared with the rest condition in experiment 1 (Figure 2B and C), test MEP size during remote condition was adjusted to match MEP amplitudes produced at rest MEP (i.e., approximately 0.5 mV) (Lei and Perez 2017). The conditioning peripheral electrical stimulation was delivered 22 ms before the test TMS (Ni et al. 2011) (Figure 6-1F). Experiment 4 consisted of two coil orientation sessions (i.e., PA and AP), which were separated by at least 5 min rest. In each session, twelve MEPs were recorded

for each stimulation paradigms [i.e., Test TMS alone and SAI (conditioning peripheral electrical stimulation + Test TMS)] during rest and remote conditions. The stimulation interval was approximately 10 s and there was at least 3 min rest between the conditions. The order of coil orientation sessions was randomized between participants. SAI was calculated by expressing the amplitude of the conditioned MEP as a percentage of the test MEP amplitude [% = (conditioned MEP * 100) / (test MEP)].

6.2.5. Data analysis

Background EMG activity of a 50 ms window before each TMS or electrical stimulus was defined by calculating the root mean square value in each trial using a custom-written script in MATLAB (2017a, The MathWorks Inc., Massachusetts, USA). It is well known that MEPs elicited by single-plus TMS are facilitated by background activation of muscles (Hallett 2007). Therefore, if the FDI muscle were co-activated during ankle contraction tasks, it would not be possible to evaluate the "remote effect". Comparing background EMG activity was therefore used to ensure that FDI muscle was not contracted during remote muscle contraction task.

To analyze the remote effect facilitation, FDI MEPs or F-wave peak-to-peak amplitudes were calculated for each trial in each experiment. Moreover, in experiment 1, MEP onset latency was measured for each trial in each stimulation paradigm and condition to confirm that each coil orientation stimulated a different subset of the interneurons. The MEP onset latency was defined as the time point where rectified EMG signals exceeded 2 SD of the mean background EMG which was measured 100 ms before the TMS (Federico and Perez 2017).

For statistical analysis, background EMG activity, MEP amplitudes, F-wave amplitude, and onset latency of MEP were averaged for each stimulation paradigm and condition separately (i.e., rest and remote) in each experiment.

6.2.6. Statistics

Normal distribution was tested using the Shapiro-Wilk test. If the data failed the normality test, nonparametric tests were used.

In experiment 1, one-way repeated measure ANOVA was used to examine the effect of coil orientations (i.e., PA, AP, and LM) on the onset latency of MEPs elicited during rest condition, followed by post hoc multiple comparisons using paired t-test with Bonferroni correction. Paired t-tests were employed to compare the MEP amplitude, onset latency, and background EMG activity between the rest and remote conditions for each coil orientation.

In experiment 2, the Friedman tests, a non-parametric equivalent for repeated-measure ANOVA was used to examine the effect of condition (Test MEP vs. Conditioned MEP during the rest condition vs. Conditioned MEP during the remote condition) on SICI and SICF for each coil orientation, followed by post hoc multiple comparisons using the Wilcoxon signed-rank test with Bonferroni correction. The same analysis was used to compare the background EMG activity.
In experiment 3, the Wilcoxon signed-rank tests were used to compare F-wave amplitude and background EMG between rest and remote conditions.

In experiment 4, like the analysis of Experiment 2, the Friedman tests were performed to examine the effect of condition (Test MEP vs. Conditioned MEP during the rest condition vs. Conditioned MEP during the remote condition) on SAI for each coil orientation, followed by post hoc multiple comparisons using the Wilcoxon signed-rank test with Bonferroni correction. The same analysis was used to compare background EMG activity.

All statistical comparisons were performed using SPSS Statistics ver. 25 (IBM Corp., Chicago, Illinois, USA). The significance level for all tests was set to $p < 0.05$.

6.3. Results

6.3.1. Experiment 1

Figures 6-2A and B showed the average MEP waveforms elicited by each coil orientation stimulation during rest condition from a representative participant. One-way repeated measure ANOVA revealed an effect of coil orientation on onset latency of MEPs [$F(2, 28) = 83.725$, $p < 0.001$, $\eta^2 = 0.857$]. Post hoc test showed that the onset latency of MEP was shortest in LM, longest in AP, and middle of both in PA, with significant differences between them (Figure 6-2C; Comparisons between LM and PA, $p < 0.001$; between PA and AP, $p < 0.001$; between LM and AP, $p < 0.001$). Figure 6-2D showed the average MEP waveforms during the rest and remote conditions for PA, AP, and LM TMS. Paired t-test showed that MEP amplitudes were significantly increased during remote conditions compared with the rest condition for PA ($p = 0.001$, $r=0.73$), AP ($p = 0.015$, $r=0.60$), and LM ($p = 0.007$, $r=0.65$) stimulation (Figure 6-2E). Moreover, paired t-test showed that onset latencies of MEPs were significantly shortened during remote conditions compared with the rest condition for PA ($p < 0.001$, $r=0.85$), AP ($p = 0.022$, $r=0.57$), and LM ($p = 0.003$, $r=0.69$) stimulation (Figure 6-2F). Finally, there was no significant difference in background EMG activity between rest and remote conditions for PA ($p = 0.071$, $r=0.46$), AP ($p = 0.994$, $r=0.002$), and LM ($p = 0.25$, $r=0.31$) stimulation.

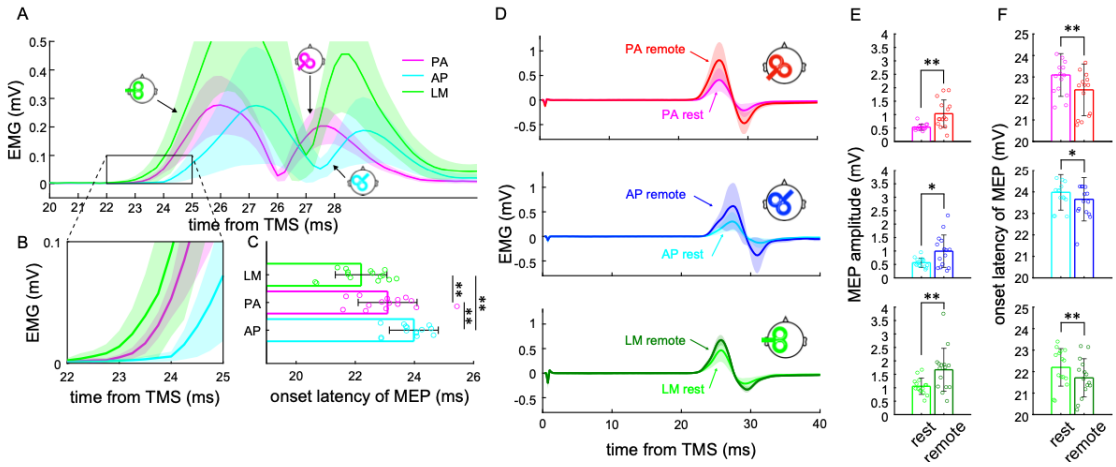

Figure 6-2: (A) Averaged rectified motor evoked potentials (MEP) traces of the first dorsal interosseous (FDI) elicited by transcranial magnetic stimulation with three coil orientations: posterior-anterior (PA, coil handle 45° to the midline), anterior-posterior (AP, coil handle 180° relative to PA), and 3) latero-medial (LM, coil handle 90° to the midline). Shadows indicate standard deviations. **(B)** Enlarged MEP traces show different onset latencies. **(C)** Group data showing mean onset latencies of MEPs (ms) elicited by PA, AP, and LM TMS during the rest condition. **(D)** Averaged MEP traces of the FDI muscles during the rest and remote conditions elicited by TMS with PA, AP, and LM coil orientations from a representative participant. **(E)** Group data showing mean MEP amplitudes (mV) during the rest and remote conditions elicited by PA, AP, and LM TMS. **(F)** Group data showing mean onset latencies of MEPs (ms) during the rest and remote conditions elicited by PA, AP, and LM TMS. Error bars indicate standard deviations. Legend: * $p < 0.05$, and ** $p < 0.01$.

6.3.2. Experiment 2

Figure 6-3A showed the average MEP waveforms from a representative participant elicited by PA stimulation during rest and remote conditions in SICI protocol. The Friedman test

revealed an effect of conditions (Test MEP vs. Conditioned MEP during the rest condition vs. Conditioned MEP during the remote condition) on SICI [χ^2 (2) = 18.67, $p < 0.001$, $\eta^2 = 0.78$] (Figure 6-3B). Post hoc test (Wilcoxon signed-rank test) showed that conditioned MEPs in PA SICI protocol were significantly inhibited during the rest ($p = 0.007$, $r = 0.62$) and remote ($p = 0.007$, $r = 0.62$) conditions compared with test MEP, while there was no significant difference between rest and remote conditions ($p = 1.16$, $r = 0.18$) (Figure 6-3B). Figure 6-3C showed that average MEP waveforms from a representative participant elicited by PA stimulation during rest and remote conditions in SICF protocol. The Friedman test revealed an effect of conditions (Test MEP vs. Conditioned MEP during the rest condition vs. Conditioned MEP during the remote condition) on SICI [χ^2 (2) = 15.17, $p < 0.001$, $\eta^2 = 0.63$] (Figure 6-3D). Post hoc test (Wilcoxon signed-rank test) showed that conditioned MEPs in SICF protocol were significantly facilitated during the rest ($p = 0.009$, $r = 0.61$) and remote ($p = 0.007$, $r = 0.62$) conditions compared with test MEP, while there was no significant difference between rest and remote conditions ($p = 1.59$, $r = 0.13$) (Figure 6-3D). There were no significant differences in background EMG activity between conditions for PA SICI [χ^2 (3) = 1.00, $p = 0.801$, $\eta^2 = 0.028$] and SICF [χ^2 (3) = 3.7, $p = 0.296$, $\eta^2 = 0.103$] protocols.

Figure 6-3E showed the average MEP waveforms from a representative participant elicited by AP stimulation during rest and remote conditions in SICI protocol. The Friedman test revealed an effect of conditions (Test MEP vs. Conditioned MEP during the rest condition vs. Conditioned MEP during the remote condition) on SICI with AP coil orientation [χ^2 (2) = 8.17, $p = 0.017$, $\eta^2 = 0.34$] (Figure 6-3F). Post hoc test (Wilcoxon signed-rank test) showed that conditioned MEPs in AP SICI protocol were significantly inhibited during the rest ($p = 0.014$, $r = 0.58$), but not remote ($p = 1.91$, $r = 0.10$), condition compared with test MEP (Figure 6-3F). Moreover, conditioned MEPs were significantly larger during remote condition compared with

the rest condition ($p = 0.029$, $r = 0.53$) (Figure 6-3F). Figure 6-3G showed the average MEP waveforms from a representative participant elicited by AP stimulation during rest and remote conditions in the SICF protocol. The Friedman test revealed an effect of conditions (Test MEP vs. Conditioned MEP during the rest condition vs. Conditioned MEP during the remote condition) on SICF with AP coil orientation [χ^2 (2) = 18.5, $p < 0.001$, $\eta^2 = 0.77$] (Figure 6-3H). Post hoc test (Wilcoxon signed-rank test) showed that conditioned MEPs in AP SICF protocol were significantly facilitated during the rest ($p = 0.009$, $r = 0.61$) and remote ($p = 0.007$, $r = 0.62$), conditions compared with test MEP (Figure 6-3H). Moreover, conditioned MEPs were significantly larger during remote condition compared with the rest condition ($p = 0.029$, $r = 0.53$) (Figure 6-3H). There was no significant difference in background EMG activity between conditions for AP SICI protocol [χ^2 (3) = 3.8, $p = 0.272$, $\eta2 = 0.106$]. Although the Friedman test showed an effect of conditions on background EMG activity in AP SICF protocol [χ^2 (3) = 8.1, $p = 0.044$, $\eta2 = 0.225$], there were no significant differences in post hoc analysis (all $p > 0.1$).

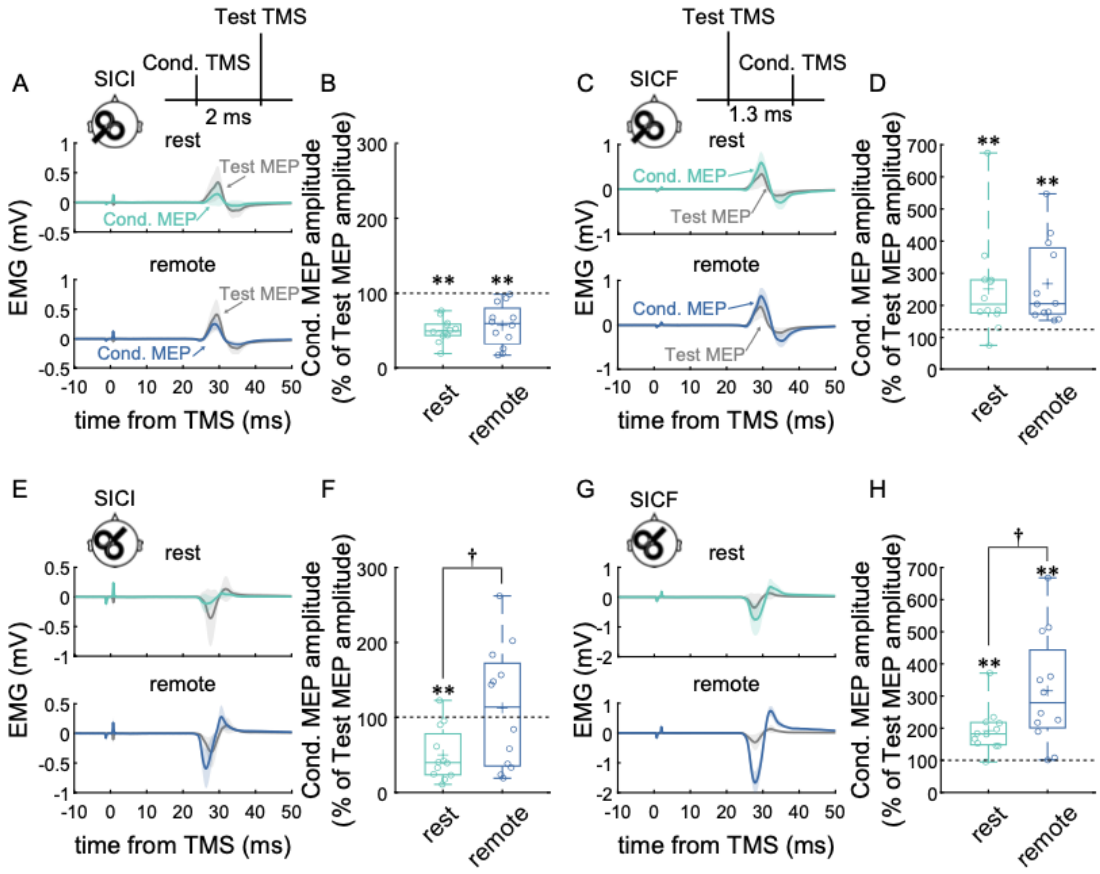

Figure 6-3: (A) Averaged motor evoked potentials (MEP) traces of the first dorsal interosseous (FDI) muscle from a representative participant elicited by transcranial magnetic stimulation (TMS) with posterior-anterior (PA, coil handle 45° to the midline) coil orientation during rest and remote conditions in short-interval intracortical inhibition (SICI) protocol. Shadows indicate standard deviations. **(B)** Boxplots showing group data of conditioned MEP amplitudes (% of Test MEP amplitude) during the rest and remote conditions in PA SICI protocol. **(C)** Averaged MEP traces of the FDI muscle from a representative participant elicited by TMS with PA coil orientation during rest and remote conditions in short-interval intracortical facilitation (SICF) protocol. **(D)** Boxplots showing group data of conditioned MEP amplitudes (% of Test MEP amplitude) during the rest and remote conditions in SICF protocol with PA coil

orientation. **(E)** Averaged MEP traces of the FDI muscle from a representative participant elicited by TMS with anterior-posterior (AP, coil handle 180° relative to PA) coil orientation during rest and remote conditions in SICI protocol. **(F)** Boxplots showing group data of conditioned MEP amplitudes (% of Test MEP amplitude) during the rest and remote conditions in SICI protocol with AP coil orientation. **(G)** Averaged MEP traces of the FDI muscle from a representative participant elicited by TMS with AP coil orientation during rest and remote conditions in SICF protocol. **(H)** Boxplots showing group data of conditioned MEP amplitudes (% of Test MEP amplitude) during the rest and remote conditions in SICF protocol with AP coil orientation. The ends of the boxes represent the 25th and 75th percentiles. The whiskers on the boxplot illustrate the minimum and maximum values. Asterisks indicate significant differences compared with Test MEP. Daggers indicate significant differences between rest and remote conditions. Legend ** $p < 0.01$, and † $p < 0.05$.

6.3.3. Experiment 3

Figure 6-4A showed the average responses including M-wave and F-wave elicited by ulnar nerve stimulation during rest and remote conditions. The Wilcoxon signed-rank showed that F-wave amplitude was significantly increased during remote conditions compared with the rest condition ($p = 0.001$, $r=0.61$) (Figure 6-4B). There was no significant difference in background EMG activity between rest and remote conditions ($p = 0.18$, $r=0.26$).

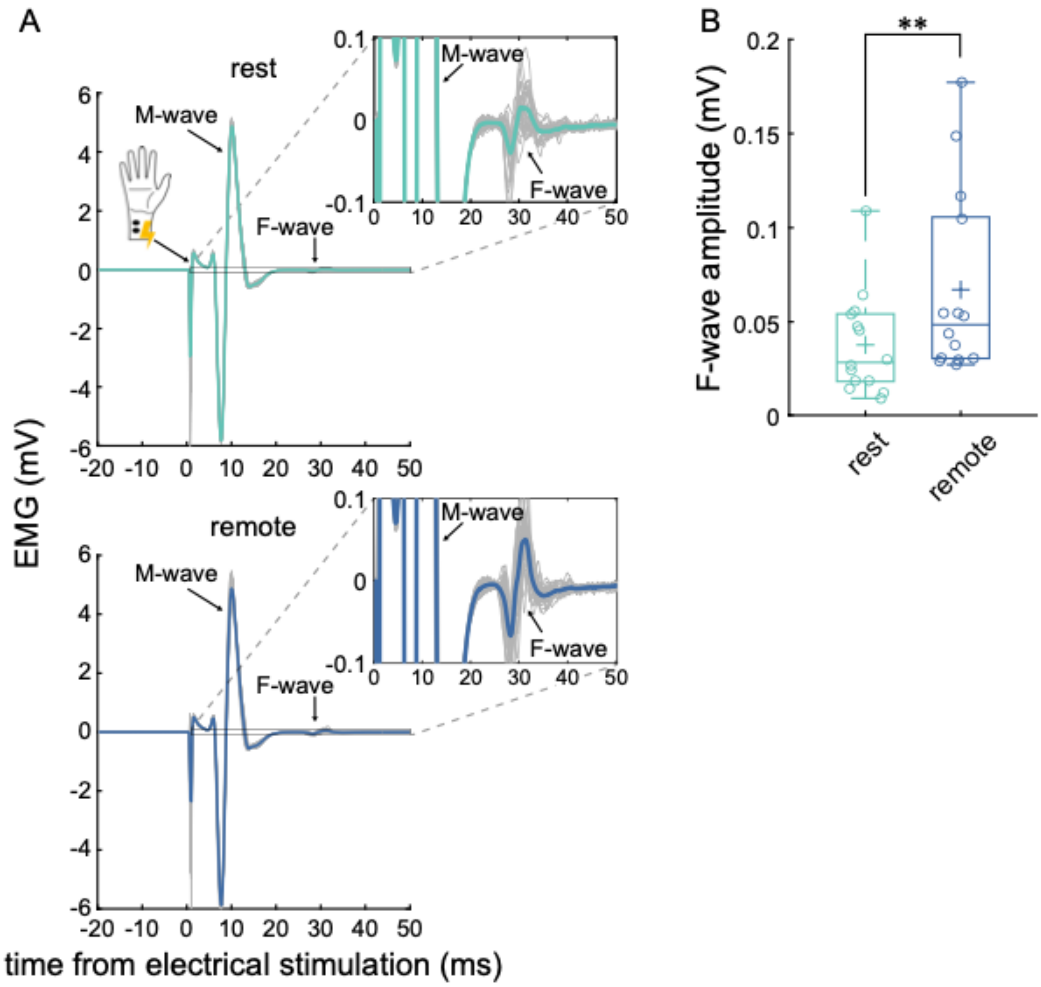

Figure 6-4: (A) Averaged responses including M-wave and F-wave in the first dorsal interosseous (FDI) muscle from a representative participant elicited by ulnar nerve stimulation during rest and remote conditions. Thick colored lines indicate average waveforms, while thin gray lines indicate individual trials. **(B)** Boxplots showing group data of F-wave amplitude (mV) during the rest and remote conditions. The ends of the boxes represent the 25th and 75th percentiles. The whiskers on the boxplot illustrate the minimum and maximum values. Asterisks indicate significant differences between rest and remote conditions.; Legend: ** $p < 0.01$.

6.3.3. Experiment 4

Figure 6-5A showed that the average MEP waveforms from a representative participant elicited by PA stimulation during rest and remote conditions in SAI protocol. The Friedman test revealed an effect of conditions (Test MEP vs. Conditioned MEP during the rest condition vs. Conditioned MEP during the remote condition) on PA SAI protocol [χ^2 (2) = 15.8, $p < 0.001$, η^2 = 0.61] (Figure 6-5B). Post hoc test (Wilcoxon signed-rank test) showed that conditioned MEPs in PA SICI protocol were significantly inhibited during the rest ($p = 0.004$, $r = 0.62$), but not remote ($p = 0.057$, $r = 0.46$), conditions compared with test MEP (Figure 6-5B). Moreover, there was no significant difference between rest and remote conditions ($p = 0.59$, $r = 0.25$) (Figure 6-5B). There were no significant differences in background EMG activity between conditions for PA SAI protocol [χ^2 (3) = 6.32, $p = 0.097$, $\eta^2 = 0.16$].

Figure 6-5C showed that the average MEP waveforms from a representative participant elicited by AP stimulation during rest and remote conditions in SAI protocol. The Friedman test revealed an effect of conditions (Test MEP vs. Conditioned MEP during the rest condition vs. Conditioned MEP during the remote condition) on AP SAI protocol [χ^2 (2) = 14.0, $p < 0.001$, η^2 = 0.54] (Figure 6-5D). Post hoc test (Wilcoxon signed-rank test) showed that conditioned MEPs in PA SICI protocol were significantly inhibited during the rest ($p = 0.004$, $r = 0.62$) and remote ($p = 0.009$, $r = 0.58$) conditions compared with test MEP, while there was no significant difference between rest and remote conditions ($p = 1.8$, $r = 0.10$) (Figure 6-5D). There were no significant differences in background EMG activity between conditions for PA SAI protocol [χ^2 (3) = 4.57, $p = 0.206$, $\eta^2 = 0.12$].

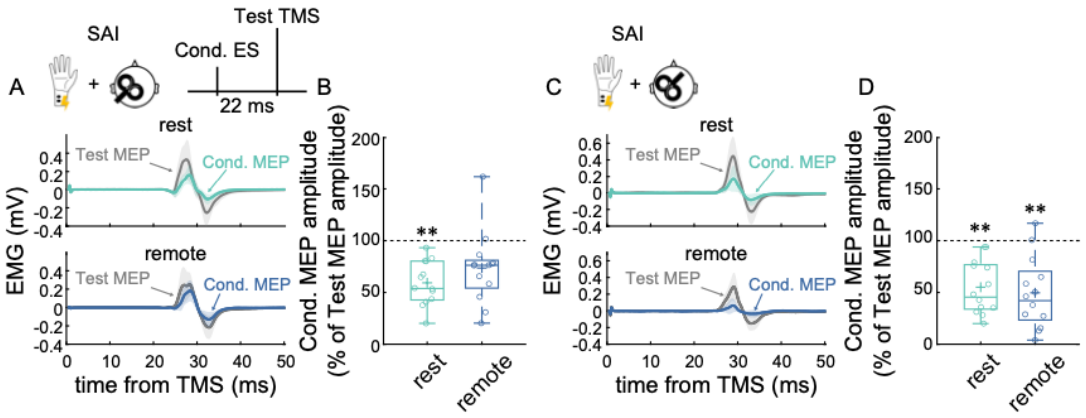

Figure 6-5: (A) Averaged motor evoked potentials (MEP) traces of the first dorsal interosseous (FDI) muscle from a representative participant elicited by transcranial magnetic stimulation (TMS) with posterior-anterior (PA, coil handle 45° to the midline) coil orientation during rest and remote conditions in short-latency afferent inhibition (SAI) protocol. Shadows indicate standard deviations. **(B)** Boxplots showing group data of conditioned MEP amplitudes (% of Test MEP amplitude) during the rest and remote conditions in PA SAI protocol. **(C)** Averaged MEP traces of the FDI muscle from a representative participant elicited by TMS with anterior-posterior (AP, coil handle 180° relative to PA) coil orientation during rest and remote conditions in AP SAI protocol. **(D)** Boxplots showing group data of conditioned MEP amplitudes (% of Test MEP amplitude) during the rest and remote conditions in AP SAI protocol. The ends of the boxes represent the 25th and 75th percentiles. The whiskers on the boxplot illustrate the minimum and maximum values. Asterisks indicate significant differences compared with Test MEP. Legend: ** $p < 0.01$.

6.4. Discussion

In the current study, we investigated the "remote effect" paradigm on the activity of different subsets of interneurons within the M1 activated by PA and AP currents as well as D-wave and spinal motoneuronal activity. Notable, we found the hand muscle MEP amplitudes were facilitated and latencies were shortened during lower-limb muscle contraction in all PA, AP, and LM current stimulation protocols (experiment 1). To clarify the detailed mechanisms of these modulations observed during experiment 1, we further measured the activity of intracortical (SICI, SICF, and SAI) and spinal (F-wave) neural circuits to examine their influence on the modulation of corticospinal circuits (experiments 2 to 4). An important novel finding is that SICI was decreased and SICF was facilitated during the remote condition only in the AP circuits, as we hypothesized. In addition, F-wave amplitudes were also facilitated during the remote condition.

It is well known that MEPs (Hess et al. 1987) and F-waves (McNeil et al. 2013) can be modulated by voluntary contraction of examined muscles. However, since background EMG activities in all our current experiments were not facilitated during the remote condition compared with the rest condition, it can be considered that the observed modulations of the evoked responses were not affected by background EMG activities, but that they were rather caused by the "remote effect" modulation. Specific discussions about selective modulation of intracortical interneurons during "remote effect" follow.

4.1. Contribution of interneuronal synaptic inputs to corticospinal neurons during remote effect

Responses elicited by PA and AP currents are thought to result from activity in distinct interneuronal circuits that have different synaptic input to corticospinal neurons (Di Lazzaro and Ziemann 2013). Moreover, responses elicited by LM currents is believed to originate from the

direct excitation of axons of the corticospinal neurons (Di Lazzaro and Ziemann 2013). Therefore, the observed modulation of MEPs evoked by each of the PA, AP, and LM currents in experiment 1 in our study suggests that the remote effect might have an impact on different subsets of interneuronal circuits in the M1 as well as subcortical (motoneuronal) neural circuits. Specifically, an increase in MEP amplitude and a decrease in MEP latency during PA and AP currents in experiment 1 (Figure 6-2E and F) likely reflects excitation of intracortical interneuronal circuits in the hand area facilitated by the "remote effect". On the other hand, since it is well known that MEPs elicited by single-pulse TMS are affected by excitation of both cortical and spinal circuits, the observed changes during "remote effect" in experiment 1 could also be attributed to subcortical mechanisms as well as intracortical mechanisms. Indeed, MEPs elicited by the LM current, which reflect direct activation of corticospinal axons (experiment 1), and the F-waves, which reflect spinal motoneuronal excitability (experiment 3) were facilitated by the "remote effect", indicating that spinal neural circuits, at least partially, contribute to single-pulse TMS induced MEP changes in the PA and AP circuits underlying "remote effect" facilitation.

The novel finding of our study is that decreased SICI and increased SICF were shown during "remote effect" in AP circuits, but not PA circuits (experiment 2). This indicates that intracortical disinhibition and facilitation mechanisms in AP circuits were activated by the "remote effect". Neuropharmacological studies suggested that SICI reflect the activity of neurotransmitter circuits mediated by gamma-aminobutyric type A ($GABA_A$) receptors, while SICF reflects excitatory glutamatergic circuits and $GABA_A$ circuits (Ziemann et al. 1996, 2015; Di Lazzaro et al. 2007; Rossini et al. 2015). Therefore, our current results suggest that the "remote effect" may influence such intracortical GABAergic and glutamatergic circuits in AP current sensitive interneurons. In the subset of PA current sensitive interneurons, intracortical inhibitory and excitatory mechanisms assessed by SICI and SICF were not affected during the "remote

effect". Therefore, increased MEPs elicited by single-pulse TMS with the PA currents during the "remote effect" (experiment 1) may be due to the contribution of spinal mechanisms, rather than intracortical mechanisms, as the increase in F-wave amplitude were also shown in experiment 4. This is consistent with results showing that excitability of spinal reflex circuits, which includes spinal motoneuron excitability, could be facilitated by the remote effect (Kato et al. 2019; Masugi et al. 2019; Sasaki, Kaneko, et al. 2020). On the other hand, SAI, which reflects afferent induced intracortical inhibition and involves cholinergic and GABAergic circuits (Di Lazzaro et al. 2000, 2005), were decreased during the "remote effect" in the PA sensitive circuits (experiment 4). Moreover, a decrease in cortical silent period elicited by TMS using the PA current was shown during the "remote effect", which also suggests disinhibition of cortical circuits (Tazoe, Endoh, et al. 2007; Tazoe, Sakamoto, et al. 2007). Taken together, intracortical mechanisms mediated by choline (SAI) or neurotransmitters other than SICI and SICF circuits may be at least partly involved in the increase of MEPs in the PA circuits during the interlimb "remote effect".

Contrary to our current results, it was also reported that upper-limb SICI elicited by the PA currents were decreased during contralateral lower-limb muscle contractions (i.e., contralateral "remote effect") (Chiou, Wang, Liao, Wu, et al. 2013). On the other hand, ipsilateral contractions were performed in our current study (i.e., ipsilateral "remote effect"), and SICI elicited by the PA currents was not affected during the "remote effect" condition. The effect from the contralateral limbs could be mediated by interhemispheric activations via the callosum motor fibers (Chiou, Wang, Liao, Wu, et al. 2013). On the other hand, the ipsilateral "remote effect" could be achieved in intrahemispheric mechanisms as demonstrated in animal models showing that spreading of neural activity via intrinsic horizontal connections between the M1 regions representing different muscles (Capaday et al. 2011). Therefore, the difference between our current results and that by Chiou et al., (2013) could be explained by different mechanisms of

ipsilateral and contralateral interlimb “remote effect”. Since we cannot confirm the effect of contralateral “remote effect” on AP-sensitive interneurons, future studies should consider differences in the ipsilateral and contralateral “remote effect” mechanisms.

4.2. Different effects on PA and AP circuits

A key question, therefore, is why intracortical circuits activated by the AP currents were mainly influenced during intralimb “remote effect”. It has been reported that interneuronal circuits activated PA and AP currents were differently activated by different voluntary motor tasks, motor learning, neuromodulatory interventions, and neurological injuries (Hamada et al. 2013, 2014; Federico and Perez 2017; Hannah and Rothwell 2017; Long et al. 2017; Jo et al. 2018). These studies indicate that the two subsets of interneurons activated by PA and AP currents have separate functions which contribute to a different degree to individual motor constraints. Specifically, it was reported that excitability changes induced by neurons activated by the AP currents depend on the cerebellar activity and can modulate model (error)-based forms of motor learning, while those induced with PA currents are independent of cerebellar activity and modulate model-free forms of motor learning (Hamada et al. 2014). Similarly, a recent study reported that cerebellar connections to PA inputs responded to motor learning of a simple motor sequence (i.e., involving mainly the M1) and cognitively demanding skill tasks (i.e., recruiting premotor areas), whereas AP inputs were sensitive to the skilled learning tasks (Spampinato et al. 2020). It was also reported that AP synaptic input to corticospinal neurons may contribute to promotor recovery of hand dexterity after spinal cord injury (Long et al. 2017). Moreover, some studies suggested that AP circuits might reflect contributions from multiple brain areas and that AP currents may recruit premotor regions (Volz et al. 2015; Aberra et al. 2020). Based on the abovementioned studies, interneurons activated by AP currents may contribute to higher motor

function compared to those activated by the PA currents. Therefore, one possible interpretation of our results is that coordinated control of remote limb muscles can be considered as a higher motor function, while AP sensitive interneurons might be selectively recruited during “remote effect”. This may be one of the neural mechanisms underlying interlimb interactions. However, future studies should consider whether selective priming of AP-sensitive interneuron excitability, which was developed in previous studies (Hamada et al. 2014; Long et al. 2017), would also affect inter-limb coordinated performance at the behavioral level.

Our results, therefore, demonstrate for the first time that lower-limb movement-induced activation of the hand M1 region (i.e., “remote effect”) is mainly mediated by late (AP currents sensitive interneurons) cortical synaptic inputs to corticospinal neurons as well as partially by spinal circuits. This also revealed the possibility that AP currents-sensitive interneurons have a role in interlimb “remote effect” interactions.

Chapter 7

General discussion and conclusions

7.1. Summary of the results

In my thesis, I asked the following research questions regarding neural interactions between muscles located in distal segments of the body:

- To understand the characteristics of interlimb neural interaction, I aimed to answer: (1) Are there differences in neural connectivity assessed by remote effects between diagonal and orthogonal muscle pairs? (2) How does interlimb neural interaction in cortical and spinal circuits differ depending on the phase of the movement, such as preparation and execution?
- To understand neural interactions between the trunk and limb muscles, I aimed to answer: Are there neural connections between trunk and limb muscles in the corticospinal circuits?
- To understand the control centers of neural interactions, I aimed to answer: Are the trunk-limb neural interaction mechanisms cortical or spinal in origin?
- To clarify the relevance of the neural interactions to human movement coordination, I aimed to answer: What are the functional implications of such neural interactions for humans?
- To understand the intracortical regulation of neural interactions, I aimed to answer: What intracortical mechanisms at the level of interneurons contribute to such interactions?

The five studies that I conducted to investigate the mechanisms of neural interactions between multiple muscles addressed each of these specific questions. A discussion of each specific study follows:

In Study 1 (Chapter 2), which was published in the Journal of Neurophysiology (Sasaki,

Kaneko, et al. 2020), I examined the effects of motor preparation and execution of ipsilateral (orthogonal) and contralateral (diagonal) remote muscle contractions on the excitability of corticospinal and spinal reflex circuits using single-pulse TMS and single-pulse lumbothoracic tSCS. My results indicate that corticospinal excitability of the lower-limb muscles was facilitated during both preparation and execution of upper-limb muscle contraction, while spinal reflexes were facilitated only during motor execution. Moreover, the extent of facilitation did not differ between the ipsilateral and contralateral contractions. Therefore, motor preparation for upper-limb muscle contractions did not affect spinal circuits but seemed to affect the supraspinal networks controlling the lower-limb muscles. However, the actual contraction (motor execution) of upper-limb muscles was required to facilitate the spinal reflex circuits that control the lower-limb muscles. Moreover, interlimb remote facilitation in corticospinal and spinal reflex circuits did not depend on whether contralateral/ipsilateral hands were contracted. Therefore, my study revealed that cortical and spinal contributions to interlimb neural interactions depend on the phase of the movement, while the extent of modulation is constant regardless of the contracted muscle pair (i.e., diagonal or orthogonal muscles).

In Study 2 (Chapter 3), which was published in the Neuroscience Letters (Sasaki et al. 2018), I used single-pulse TMS over the M1 to test whether: (a) corticospinal excitability of trunk muscles can be modulated during contraction of hand and leg muscles, and (b) corticospinal excitability of hand and leg muscles is modulated during trunk muscles contractions. My results revealed that voluntary contraction of trunk muscle facilitated corticospinal excitability of the limb muscles and vice versa. Therefore, my results suggest that consistent with the upper and lower limb, the “remote effect” neural interactions also exist between the trunk and limb muscles, which contributes to the coordinated movements between the trunk and the limbs.

In Study 3 (Chapter 4), which was published in the Neuroscience (Sasaki, Milosevic, et al. 2020), I used cervicomedullary magnetic (CMS) stimulation which can assess subcortical (spinal) excitability to test whether: (a) subcortical excitability of trunk muscles can be modulated when upper-limb muscles are contracted, and (b) subcortical excitability of limb muscles can be modulated when trunk muscles are contracted. My results showed that voluntary contraction of trunk muscles can indeed facilitate the subcortical excitability of the hand muscles, while voluntary contraction of hand muscles does not facilitate the subcortical excitability of the trunk muscles. Therefore, my results suggest that facilitation of the trunk muscle corticospinal excitability by voluntary contraction of hand muscles could be cortical in origin, while facilitation of hand muscle corticospinal excitability by voluntary contraction of trunk muscles also involves, at least in part, changes in the spinal excitability, implying that cortical and spinal contributions to neural interactions could change depending on the movement that is performed.

In Study 4 (Chapter 5), which was published in the eNeuro (Sasaki et al. 2021), I tested arm-trunk or arm-leg neural interactions in the corticospinal tract during different arm muscle contractions. Specifically, I examined the corticospinal excitability of the trunk extensor, trunk flexor, and leg muscles while participants exerted: (1) wrist flexion, and (2) wrist extension isometric contractions at various contraction intensity levels. In this study, I assessed corticospinal excitability using single-pulse TMS. My results showed that corticospinal excitability in the trunk extensor muscles was facilitated even during low contractions of wrist flexion and extension, while stronger contractions were required to facilitate corticospinal excitability of the trunk flexors. The extent of facilitation of corticospinal excitability in the trunk extensors was shown to depend on the contraction intensity of wrist extension, but not flexion. Moreover, corticospinal excitability in leg muscles was facilitated during low contraction intensity of wrist flexion and extension, but the contraction intensity dependence was only prevalent during stronger wrist

extension contractions. Therefore, my results demonstrated that the extent of neural interaction modulations depends on the task and intensity level of the contracted remote muscle, which suggests that neural interactions may contribute to the coordination of multiple muscles.

In Study 5 (Chapter 6), I examined the contributions of intracortical interneuronal circuits to interlimb neural interactions in the corticospinal circuits. Specifically, using paired-pulse TMS protocols that can assess the activity of intracortical inhibitory and excitatory circuits, and different TMS current flow configurations that can assess the activity of early and late synaptic inputs to corticospinal neurons, I examined the contributions of different subsets of intraneuronal circuits to interlimb interaction. Specifically, different subsets of interneurons activities in the hand area within the M1 were assessed during lower-limb muscles contractions. Results showed that late synaptic inputs, which might reflect the activity of the premotor cortex to the corticospinal neurons, mainly contribute to the interlimb neural interactions. The facilitation of late synaptic inputs may be mediated through a decrease in the excitability of GABAergic inhibitory circuits and an increase in the excitability of glutaminergic excitatory circuits. Moreover, in addition to such intracortical mechanisms, subcortical contributions evaluated by motoneuronal activity could also partly contribute to the interlimb neural interactions. Therefore, my study revealed the existence of specific intracortical interneurons responsible for interlimb neural interactions in the corticospinal network.

Taken together, the results obtained from the above-mentioned five studies indicate the following advancement of the scientific knowledge to answer the research questions of my thesis regarding multiple muscles coordination within the corticospinal systems:

(1) Neural interactions in the corticospinal systems depend on motor phase (i.e., motor preparation or execution) but not diagonal and orthogonal muscle pairs

(e.g., right upper- and right lower- limb muscles vs. right upper- and left lower-limb muscles) (Study 1 - Sasaki et al., 2020, Journal of Neurophysiology).

(2) There are neural interactions between trunk and limb muscles within the corticospinal networks as well as between upper- and lower-limb muscles within the same structures (Study 2 - Sasaki et al., 2018, Neuroscience Letters).

(3) Contributions of cortical and spinal mechanisms to corticospinal neural interactions depend on the anatomical and functional relationship of the contracted muscle pairs (Study 3 - Sasaki et al., 2020 Neuroscience).

(4) The extent of neural interaction regulation depends on the task and the intensity of the muscle contractions, implying that the neural interactions underly coordination of multiple muscles (Study 4 - Sasaki et al., 2021, eNeuro).

(5) Cortical late synaptic inputs to corticospinal neurons are important and responsible for initiating and regulating neural interactions in the corticospinal network (Study 5).

Specific details of the above-mentioned findings in multiple muscles control by the corticospinal systems are discussed next, in addition to the possible implications of these findings for the rehabilitation of individuals with neurological disorders, as well as the future directions of the "remote effect" research.

7.2. Scientific contributions

My findings in Study 1 (Sasaki, Kaneko, et al. 2020) demonstrated the contribution of the cortical and spinal circuits to interlimb neural interaction and that the changes depend on the motor phase (preparation to execution). Specifically, neural interactions in the cortical circuits occurred during motor preparation, indicating that the brain is preparing to send motor commands to multiple muscles that will be recruited during the movement. In addition, these interactions were shown not to depend on whether the movement was ipsilateral or contralateral, but remained constant during both types of movements. Although it has been reported that diagonal coordinations are important for some human movements such as walking (Swinnen 2002; Yiou et al. 2007; Zehr et al. 2009), it is not clear whether corticospinal interlimb neural interactions demonstrated in my current study would be involved in rhythmic movements. Therefore, further studies are warranted to better understand this. Overall, the Study 1 in my thesis expanded the understanding of neural interactions between upper- and lower-limb muscles. In the subsequent Studies 2 and 3, I extended these findings to the whole body, including the trunk muscles. The existence of neural interaction between the trunk and limb muscles in the corticospinal systems was demonstrated in Study 2 (Chapter 3) and Study 3 (Chapter 4) for the first time. Since my Study 1 and previous studies investigating neural interaction between different muscles located in distal segments of the body (Kawakita et al. 1991; Pereon et al. 1995; Boroojerdi et al. 2000; Hortobágyi et al. 2003) focused only on the upper-limb and lower-limb muscles, the understanding of mechanisms underlying neural interaction of different muscles groups (i.e., including the trunk) has been limited until now. These findings expanded our basic understanding of the underlying neural mechanisms related to the control of trunk muscles. Specifically, results in Study 2 (Chapter 3)(Sasaki et al. 2018) showed the existence of neural interactions between trunk and limb muscles and suggest that the extent of neural interaction depends on the anatomical

distance between each muscle representation in the M1. In other words, by comprehensive investigation of trunk and limb interaction, in addition to upper-limb and lower-limb interactions, we could propose the possible mechanism that regulates neural interactions in the corticospinal circuits. Specifically, my findings suggest that during voluntary contraction of a certain muscle neural activity spreads to neighboring areas within the motor cortex, which represent different muscles that remain at rest in a different segment of the body. Such spreading of neural activity may depend on the relative distances between the M1 representations of these different muscles. The cortical neural interaction mechanisms hypothesis is also supported by basic animal studies which have demonstrated that intracortical facilitation may be involved in the spreading of neural activity within the M1 (Capaday et al. 2011). In addition, Study 3 (Chapter 4) (Sasaki, Milosevic, et al. 2020) expands this knowledge by investigating subcortical mechanisms of trunk-limb neural interactions. My results showed that contribution of the cortical and spinal circuits changes depending on the movement being performed (i.e., trunk contraction or limb contraction). It is known that trunk muscles are activated in a highly coordinated manner during voluntary arm movements (Aruin and Latash 1995; Hodges and Richardson 1997). That is, in many cases, voluntary upper-limb movements drive the activation of the trunk muscles. Specifically, my results in Study 3 showed that arm contraction induced-neural interaction between trunk and limbs is mostly cortical origin since subcortical excitability was not modulated. This suggests that intracortical circuits are crucial for achieving trunk-limb neural interactions during upper-limb movement. These results are consistent with previous studies suggesting the importance of cortical control of trunk muscles during upper-limb movement (Chiou, Hurry, et al. 2018). In Study 4 (Chapter 5) (Sasaki et al. 2021), to reveal the functional implications of inter-limb and trunk-limb neural interaction demonstrated in Studies 1 to 3, I tested the effect of different tasks and intensity on the extent of neural interactions. My results showed that the neural interactions

between specific muscle pairs can change depending on tasks and the intensity of the executed remote muscle contractions. When performing coordinated movements, it is necessary to control multiple muscle activities according to the movement and the intensity that is performed. Therefore, the existence of task- and intensity-dependent changes of neural interactions shown in Study 4 indicates that neural interactions in the corticospinal system may be one of the fundamental mechanisms related to the control of coordinated muscle activities. A recent study also reported that the extent of neural interaction between the upper-limb and trunk are weakened or lost in people with spinal cord injuries and that the group with residual neural interaction has higher arm-trunk coordination performance than the group with loss of neural interactions (Chiou and Strutton 2020). Therefore, in addition to the results in Study 4, this study also supports that the neural interactions could contribute to coordinated movements between different muscles.

Taken together, Studies 1 to 4 revealed that there are neural interactions between upper-limb, trunk, and lower-limb muscles, which can be achieved in cortical and partly subcortical neural circuits. Such neural interactions may contribute to coordinated control of multiple muscles. To specify the detailed neural basis, Study 5 (Chapter 6) examined intracortical mechanisms that contribute to the neural interactions that were demonstrated in Studies 1 and 3. Specifically, I demonstrated in Study 5 that cortical late synaptic inputs from a subset of interneurons that project to the corticospinal tract are essential to achieve neural interactions in the corticospinal circuits. Moreover, these findings also suggest that these late synaptic inputs may be modulated by GABAergic and glutaminergic excitatory circuits during neural interaction. The detailed intracortical mechanisms of neural interactions have never been tested in humans, and the results of my studies may identify the possible neural basis of neural interactions for the first time ever in humans.

Overall, the series of studies in my thesis revealed the existence, elucidated the characteristics and function, as well as the mechanisms on corticospinal neural interactions between multiple muscles, which contributes to advance the understanding of basic mechanisms of human motor control as well as provides important practical implications for rehabilitation.

7.3. Implications for rehabilitation

Our results demonstrate that upper-limb, trunk, and lower-limb muscles interact in the corticospinal circuits. The facilitation of cortical and spinal excitability of resting muscles induced by remote segment muscle contractions may be helpful and provide implications for rehabilitation practice. First, motor training of an unaffected (or less affected) limb may be utilized for enhancing motor recovery of the opposing paralyzed limb in individuals with neurological impairments such as stroke and spinal cord injury (Tazoe and Komiyama 2014). Specifically, based on the results obtained in my studies, motor training of limb muscles may be beneficial for boosting corticospinal excitability of the trunk muscles. Motor training of the trunk muscles may also be utilized for the facilitation of corticospinal excitability of arm and leg muscles. Therefore, the ability to enhance neural interactions through training of different muscle groups may contribute to the development of rehabilitation paradigms requiring coordinated movements between trunk and limbs. Indeed, it was recently reported that trunk muscle corticospinal excitability in able-bodied participants could be facilitated after short-term upper-limb training (Chiou et al. 2020). Therefore, it is possible that such paradigms that aim to train the "remote effect" neural interactions could be applied to the rehabilitation after neurological impairments such as spinal cord injury to affect trunk control and improve motor function of sitting balance.

Moreover, a recent study reported that corticospinal neural interactions between trunk and limb muscles were impaired or lost by neurological injuries such as spinal cord injury, which affect postural adjustment during arm movements (i.e., arm-trunk coordinated performance) (Chiou and Strutton 2020). Therefore, it may also be possible to efficiently restore coordinated motor performance after neurological injuries by approaches aiming to restore neural interaction in the corticospinal circuits. Future studies should therefore test the effectiveness of "remote effect" based training in rehabilitation.

7.4. Perspectives and future directions

Through the series of studies in my thesis, the main focus has been on mechanisms of corticospinal neural interaction between multiple muscles. However, the plasticity mechanisms of the "remote effect" were not examined. Specifically, in my current studies, although there were large individual variations in the degree of "remote effect", the factors that cause them could not be examined. Therefore, it is still unclear how neural interaction changes with training or de-training and whether it is possible to elicit short- or long-term training after-effects. Furthermore, it is also not clear what kind of interventions can be most effective to modulate the extent of neural interaction in the corticospinal circuits. This is important for the application of the fundamental understanding of neural interaction mechanisms to rehabilitation practice.

The first possible approach to examining plasticity is to investigate the correlations between coordinated performance and the extent of neural interactions. Therefore, by examining possible differences in the extent of neural interactions between athletes, who are expected to have higher motor coordination performance, and people with disabilities, who are expected to have lower coordination performance, and general able-bodied individuals, plasticity mechanisms in neural interactions due to training and neurological impairments could be investigated. A similar approach has already been applied to patients with spinal cord injury (Chiou and Strutton 2020) and should be extended to other disorders and athletes in the future, which will lead to a more extensive understanding of "remote effect" neural interactions.

Secondly, neuromodulation techniques can be used to externally enhance neural interactions and improve performance and coordination. Specifically, the findings of Study 5 demonstrated that cortical late synaptic inputs related to a subset of interneurons in the M1 are important for interlimb neural interactions. Therefore, priming (i.e., facilitating or inhibiting) this subset of interneurons may induce improved coordinated control of multiple muscles. Selective

neuromodulation to different subsets of interneurons has been developed using repetitive TMS (rTMS) with specific current directions (i.e., AP current direction) to target the specific neural circuits (Hamada et al. 2014; Long et al. 2017). It was reported that selective activation of late synaptic input to corticospinal neurons could contribute to promotor recovery of hand dexterity after spinal cord injury (Long et al. 2017). Therefore, it is possible that using such a technique, selective activation of the subset of interneurons could also be developed into a new paradigm to enhance coordinated control of multiple muscles.

7.5. Concluding remarks

The overall objective of my thesis was to elucidate the existence, mechanisms, and functional implications of "remote effect" neural interaction between multiple muscles including upper-limb, trunk, and lower-limb muscles. Based on the five studies that were conducted, I conclude that there are neural interactions between upper-limb, trunk, and lower-limb muscles in the human corticospinal systems, which are mediated predominantly by the intracortical circuits and partially the spinal circuits. Furthermore, the neural interactions in corticospinal systems were shown to contribute to the functional coordinated movements of multiple muscles located in distal segments of the body. Overall, the knowledge related to a better understanding of neural interactions between multiple muscles and its functional implications allows for the understanding of the nature of the fundamental mechanisms of neural control of human movements and can be used to inform and develop novel rehabilitation paradigms to enhance coordinated control of multiple muscles.

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Appendix

Other scientific contributions

In addition to the work presented in Studies 1 to 5, which have been published or will be submitted in peer-reviewed journal papers, i.e., Study 1 (Chapter 2) was published in the *Journal of Neurophysiology* (**Sasaki**, Kaneko, et al. 2020) [11], Study 2 (Chapter 3) was published in the *Neuroscience Letters* (**Sasaki** et al. 2018) [1], Study 3 (Chapter 4) was published in the *Neuroscience* (**Sasaki**, Milosevic, et al. 2020) [13], Study 4 (Chapter 5) was published in the *eNeuro* (**Sasaki** et al. 2021) [20], and Study 5 (Chapter 6) will be submitted to the Journal of Neuroscience, I have published two other collaborative peer-reviewed journal papers related to the "remote effect" neural interactions. Specifically, in these papers, we revealed the time-course changes of remote effect during interlimb interaction (Masugi, **Sasaki** et al., 2019, ***Experimental Brain Research***) [6] and the effect of neuromuscular electrical stimulation (NMES) induced contraction on the "remote effect" neural interactions (Kato, **Sasaki** et al., 2019, ***Experimental Brain Research***) [8].

Beside the main research focus of my thesis, which is related to the "remote effect", I conducted other projects related to: (1) development of cervical transcutaneous spinal cord stimulation, which is a novel neuromodulation technique for upper limbs, (2) neurophysiological effect of neuromuscular electrical stimulation (NMES) to understand how it affects the CNS excitability as well as (3) other neuromodulation and basic motor control studies. A brief summary follows:

(1) Development of cervical transcutaneous spinal cord stimulation (ctSCS)

In this project, we have established a ctSCS technique for eliciting spinal reflexes from multiple upper-limb muscles and our neurophysiological investigations have confirmed that the elicited responses are spinal reflexes via spinal dorsal roots activation (Milosevic, Masugi, **Sasaki** et al., 2019, ***Journal of Neurophysiology***) [5]. We have also shown that it is possible to selectively recruit spinal reflex circuits innervating different proximal and distal upper-limb muscles by changing the placement of the cathode electrode (de Freitas, **Sasaki** et al., 2021, ***Journal of Applied Physiology***) [16]. By using these techniques, I then tested how continuous ctSCS intervention affects corticospinal and spinal circuits (**Sasaki** et al., 2021, ***Journal of Clinical Medicine***) [18]. I was also invited to publish a review article on the topic of transcutaneous spinal cord stimulation and neuromodulation in the Japanese Society for Musculoskeletal Medicine (佐々木, 中澤 2021, *運動器リハビリテーション*, 印刷中) [23].

(2) Neurophysiological effect of NMES

In this project, we investigated the neurophysiological effect of NMES to develop a rehabilitation method for patients with motor impairments induced by neurological injuries such as spinal cord injury and stroke. We examined the effect of NMES interventions on corticospinal and spinal circuits excitability in limb (Milosevic, Masugi, Obata, **Sasaki,** et al., 2018., ***Experimental Brain Research***) [3] and trunk muscles (**Sasaki** et al., Journal of Applied Physiology, in revision (R1)). Specifically, the study about NMES on trunk muscles was awarded the *Vodivnik Award* in the *International Functional Electrical Stimulation Society* [31]. Moreover, we also revealed the effects of the long-term NMES intervention on cortical reorganization and motor performance after traumatic brain injury (Milosevic, Nakanishi, **Sasaki** et al., 2021, ***Frontiers in Neuroscience***) [17].

(3) Other neuromodulation and basic motor control studies

I also investigated other neuromodulation techniques aiming to develop novel neurorehabilitation methods and interventions, which include static magnetic field stimulation (Nakagawa, **Sasaki** et al., 2019 ***Neuromodulation***) [4] and paired associative stimulation (Kaneko, **Sasaki** et al., 2021, ***Neuroscience***) [21]. Moreover, I was involved in projects related to basic motor control including studies examining corticospinal control of lower-limb muscles (Yamaguchi, **Sasaki** et al., 2019 ***Journal of Motor Behavio***r [7], 2020, ***Experimental Brain Research*** [10]), motor unit activity during voluntary muscle contractions (Yokoyama, **Sasaki** et al., 2021, ***IEEE Access***) [19], and muscles synergy analysis during various human movements (Saito, Yokoyama, **Sasaki** et al., 2021, ***Sensors***) [22].

All peer-reviewed publications during my Ph.D. (2017 to present) are listed below, including those published in my thesis [1,11,13,20]:

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1. Published Journal Papers

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2. Review Article

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3. Conferences

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4. Awards

[28] **Japan Society for the Promotion of Science (JSPS) Research Fellow (DC1),** 2019 - 2021 (¥3,400,000).

[29] **Japan Society for the Promotion of Science (JSPS) Overseas Research Program for Young Researchers,** 2020 - 2021 (¥1,400,000).

[30] **Outstanding Graduate Student Award**, Master's thesis, The University of Tokyo, 2019.

[31] **Vodivnik Award**, Conference Award, International Functional Electrical Stimulation Society, and Rehab Week, 2019**.**

[32] **Japan Society for the Promotion of Science (JSPS) Research Fellow (PD),** 2022 - 2025.