Doctoral Thesis

Modulation of Spinal Neural Circuits Induced by Corticospinal Descending and Peripheral Afferent Inputs

Shinji Kubota

Division of Integrated Arts and Sciences

Graduate School of Integrated Arts and Sciences

Hiroshima University

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This thesis consists of four studies concerning activity-dependent spinal cord neuroplasticity. This work was carried out while the author was a doctoral student of the Graduate School of Integrated Arts and Sciences at Hiroshima University, Japan from April 2012 to September 2016.

Shinji Kubota

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CONTENTS

CHAPTER 1

GENERAL INTRODUCTION ······ 1

1.1 Spinal neural circuits ······ 3

1.2 Hoffmann reflex ······ 9

1.3 Transcranial magnetic stimulation ······ 14

1.4 Summary of the aim of the thesis ······ 21

CHAPTER 2

EFFECTS OF CORTICOSPINAL DESCENDING INPUTS ON THE ACTIVITY OF RECIPROCAL IA INHIBITION

2.1 Introduction ······ 24

2.2 Methods

2.2.1 Subjects ······ 25

2.2.2 Electromyography recording ······ 25

2.2.3 H-reflex ······ 26

2.2.4 Conditioning stimulation ······ 28

2.2.5 Study design ······ 30

2.2.6 Data analysis ······ 32

2.3 Results ······ 32

2.4 Discussion ······ 36

2.5 Conclusion ······ 38

CHAPTER 3

THE MECHANISMS OF CHANGES IN THE IA PRESYNAPTIC INHIBITION FOLLOWING SKILLED MOTOR TASK

3.1 Introduction ········· 40

3.2 Methods

3.2.1 Subjects ········· 41

3.2.2 Electromyography recording ········· 41

3.2.3 Presynaptic inhibition ········· 43

3.2.4 Transcranial magnetic stimulation ········· 44

3.2.5 Visuomotor task ········· 45

3.2.6 Experimental procedures ········· 48

3.2.7 Statistical analysis ········· 48

3.3 Results ········· 49

3.3.1 Changes in the task performance ········· 49

3.3.2 Effect of corticospinal descending inputs on the presynaptic inhibitory pathway ········· 50

3.4 Discussion ········· 60

3.4.1 Methodological consideration associated with TMS conditioning techniques ········· 60

3.4.2 Consideration of the muscle fatigue effects ········· 61

3.4.3 Effects of corticospinal descending inputs on Ia presynaptic inhibition ········· 62

3.5 Conclusion ········· 64

CHAPTER 4

CHANGES IN THE SPINAL NEURAL CIRCUITS ARE DEPENDENT ON THE MOVEMENT SPEED OF THE VISUOMOTOR TASK

4.1 Introduction ········· 65

4.2 Methods

4.2.1 Subjects ········· 66

4.2.2 Electromyography recording ········· 66

4.2.3 Ia presynaptic inhibition and reciprocal Ia inhibition ········· 66

4.2.4 Visuomotor task ········· 68

4.2.5 Experimental Procedure ········· 70

4.2.6 Statistical analysis ········· 71

4.3 Results ········· 71

4.3.1 Changes in the task performance ········· 71

4.3.2 Effects of task movement speed on Ia presynaptic inhibition and reciprocal Ia inhibition ········· 72

4.3.3 Visuomotor task ········· 75

4.3.4 Control task ········· 77

4.4 Discussion ········· 77

4.4.1 Effects of task movement speed on Ia presynaptic inhibition ········· 79

4.4.2 Effects of task movement speed on reciprocal Ia inhibition ········· 81

4.4.3 Time course effects of the visuomotor task on spinal neural circuits ········· 83

4.5 Conclusion ········· 83

CHAPTER 5

SENSORY NERVE STIMULATION ENHANCES THE REACTIVITY OF SPINAL IA INHIBITORY INTERNEURONS

5.1 Introduction ········· 84

5.2 Methods

5.2.1 Subjects ········· 85

5.2.2 Electromyography recording ········· 86

5.2.3 Experimental procedures ········· 86

5.2.4 Conditioning stimulation ········· 86

5.2.5 Statistical analysis ········· 89

5.3 Results ········· 89

5.4 Discussion ········· 90

5.5 Conclusion ········· 97

CHAPTER 6

General Discussion and Conclusion ········· 98

References ········· 105

Published papers associated with this dissertation ········· 119

Acknowledgements ········· 120

Abbreviations

A/D: analog/digital

ANOVA: analysis of variance

CPN: common peroneal nerve

CS: conditioning stimulation

C-T: conditioning-test

EMG: electromyography

EPSPs: excitatory postsynaptic potentials

GABA: gamma aminobutyric acid

H-reflex: Hoffmann reflex

Hmax: maximum amplitude of H-reflex

iEMG; integrated electromyography

INs: interneurons

IPSPs: inhibitory postsynaptic potentials

K^+: potassium ion

M-wave: motor response

M1: primary motor cortex

MEP: motor evoked potential

Mmax: maximum amplitude of M-wave

MNs: motor neurons

MT: motor threshold

MVC: maximum voluntary contraction

Na^+: sodium ion

nVM: non-visuomotor

PAD: primary afferent depolarization

PC: personal computer

PES: patterned electrical stimulation

PI: presynaptic inhibition

RI: reciprocal inhibition

SD: standard deviation

SEM: standard error of mean

SO: stimulator output

SOL: soleus

TA: tibialis anterior

TMS: transcranial magnetic stimulation

TS: test stimulation

VM: visuomotor

CHAPTER 1
GENERAL INTRODUCTION

Human movement is controlled by the central nervous system consisting of the brain and spinal cord. The central nervous system is a complex, sophisticated system that regulates our body movements and has the ability to change itself during development and throughout life to support the acquisition and maintenance of motor behaviors. This ability to change is called activity-dependent neuroplasticity. Previously, it was believed that this plasticity occurs in only a few very specific locations, such as the cortex, cerebellum, and closely related brain regions, and that the spinal cord is a hard-wired organ without plastic properties (Wolpaw, 2007). However, recent evidence suggests that activity-dependent neuroplasticity can occur throughout the central nervous system from the cortex to the spinal cord, and that spinal cord neuroplasticity is likely to contribute to the mastery of motor skills (Wolpaw, 2010; Thompson & Wolpaw, 2014). In support of this concept, several studies have reported that spinal reflex responses (e.g., stretch reflex and cutaneous reflex) are modulated by motor learning and physical training (Nielsen *et al.*, 1993a; Nadler *et al.*, 2000; Meunier *et al.*, 2007). Nielsen *et al.*, (1993) showed that the excitability of the H-reflex, an electrical analogous of spinal stretch reflex, is different in athletes and non-athletes and among different kinds of athletes. Also, Nadler *et al.*, (2000) demonstrated that learning a new motor skill produces changes in the cutaneous reflex response. These findings suggest that the changes in spinal cord function are an important process in the acquisition of new motor skills.

Automatic movements, such as reflex responses and locomotion, are generally considered to be governed by neural circuits in the brain stem and spinal cord, while voluntary movements, such as skilled reaching and grasping, are considered to be governed by neural circuits in the primary motor cortex (M1) (Alstermark & Isa, 2012). Although the skilled movement is mainly controlled by the motor areas in the cerebral cortex, the output from these areas eventually reaches the spinal cord. Therefore, it is conceivable that spinal cord neuroplasticity could affect all motor behaviors, and exploring this plasticity will lead to a better understanding of the functional role of the central nervous system in motor control. Moreover, recent studies have shown that promoting spinal cord neuroplasticity might be a useful strategy for inducing motor recovery after spinal cord injury (Edgerton *et al.*, 2004; Bunday & Perez, 2012; Thompson *et al.*, 2013b; McPherson *et al.*, 2015). For these reasons, understanding the mechanisms underlying spinal cord neuroplasticity will likely be vital in developing effective rehabilitation approaches for people with central nervous system disorders.

Activity-dependent spinal cord neuroplasticity has been shown to be driven by supraspinal descending and peripheral ascending inputs associated with physical activities (Wolpaw, 2007). The neuroplastic changes in the spinal cord are postulated to be the result of altered synaptic strength, axonal sprouting, altered motor neuron property, or increase in the number of spinal inhibitory interneurons due to the repetitive inputs from descending and ascending pathways (Wolpaw, 2010; Carmel & Matrin, 2011; Wang *et al.*, 2012; Ueno *et al.*, 2012; Ethier *et al.*, 2015). Moreover, it is suggested that no matter what factors lead to spinal cord neuroplasticity, ongoing descending input from corticospinal tracts is essential both for producing and

maintaining plasticity (Chen & Wolpaw, 2002; Chen *et al.*, 2006a; Chen *et al.*, 2006c). Although there are several studies relating to activity-dependent spinal cord neuroplasticity in humans (Crone *et al.*, 1985; Nielsen *et al.*, 1993a; Thompson *et al.*, 2009, 2013a; Thompson *et al.*, 2013b), the mechanisms by which corticospinal descending inputs influence the activity of spinal neural circuits are not well understood. In addition, although several studies reported the effects of motor skill training on changes in spinal neural circuits (Perez *et al.*, 2005a; Roche *et al.*, 2011a), the factors that influence the induction of changes in spinal neural circuits are not clarified. To answer these questions, in this thesis, I carried out several experiments involving healthy subjects. The function of neural circuits in the spinal cord involving limb movement and the main experimental techniques used in the present study (i.e., Hoffmann reflex, transcranial magnetic stimulation) will be outlined in the following sections of this chapter.

1.1 Spinal neural circuits

The spinal cord receives descending inputs from the supraspinal centers and ascending inputs from peripheral sensory receptors. Movement-related signals generated in the brain converge on the M1, located in Brodmann area 4, and are conveyed to the spinal cord via descending pathways (Porter & Lemon, 1992). Additionally, movement related signals generated by peripheral sensory receptors are conveyed to the spinal cord via sensory nerves. This incoming information is coordinated by spinal neural circuits and then ultimately transmitted to the alpha motor neurons, located in the ventral horn of the spinal cord. Therefore spinal neural circuits are linked directly to the activation of muscles, the effectors of movement, because the axons of alpha motor neurons project

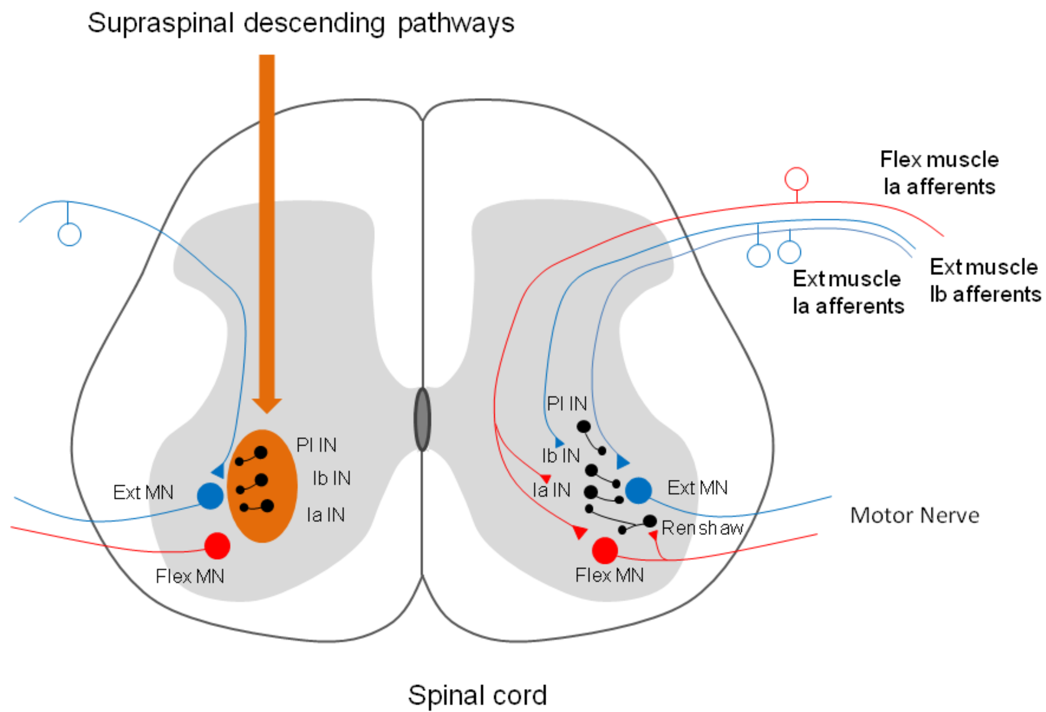

Figure 1. Illustration of a circuit diagram of the spinal cord. The right side shows neural circuits within the spinal cord. The left side indicates the influence of supraspinal descending pathways on spinal interneurons. Closed black circles indicate spinal interneurons and their terminals. Closed triangles indicate excitatory synaptic terminals. Ext, extensor; Flex, flexor; IN, interneuron; MN, motor neuron; PI, presynaptic inhibition.

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to the muscles of the body. A circuit diagram of the spinal cord is shown in Figure 1.

Moving our limbs produces time-varying sensory signals arising from cutaneous and proprioceptive receptors. Some sensory nerve fibers form synapses with neurons in the spinal cord and constitute a reflex arc that contribute to coordinated patterns of muscle contraction, while others continue up to the brain and provide feedback information needed for management of the state of muscle contraction and limb positions in order to execute controlled limb movements (Gardner & Johnson, 2012). Because sensory inputs to the spinal cord vary depending on motor tasks (e.g., movement type, task difficulty level, and movement speed) (Poppele & Bowman, 1970; Kakuda *et al.*, 1997; Bosco & Poppele, 1999; Jones *et al.*, 2001), and are likely to influence the activity level of muscle during voluntary movement (Nielsen & Sinkjaer, 2002; Seki *et al*., 2003), modification of sensory signals at the spinal level appears to be a critical factor in executing skilled motor tasks (Doemges & Rack, 1992; Dun *et al*., 2007). Presynaptic inhibition is a neural mechanism for controlling these sensory signals. Presynaptic inhibition is induced by gamma aminobutyric acid (GABA)ergic interneurons forming axo-axonic contacts with sensory afferent terminals. Activation of GABAergic interneurons produces primary afferent depolarization (PAD) of sensory afferent fibers, which leads to a reduction in the release of neurotransmitters from sensory afferents (Rudomin, 2009). Interneurons constituting Ia presynaptic inhibitory circuit are activated by inputs from sensory receptors or supraspinal centers (Jankowska, 1992). The reflex responses produced by sensory inputs, such as the stretch reflex and/or cutaneous reflex, might interfere with active voluntary movements due to the unpredictable activation of agonist muscles. Therefore, changes in the sensory inputs at

preneuron level contribute to the control of these spinal reflexes and have a potential effect on the control of limb movement and sensory perception (Sinkjaer & Hayashi, 1989; Bawa & Sinkjaer, 1999; Seki *et al.*, 2003). In addition to the presynaptic inhibitory circuit, the activity of muscles is also controlled by other spinal neural circuits exerting excitatory or inhibitory synaptic inputs on alpha motor neurons. This is accomplished through the interaction of excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) at the level of the motor neurons. Reciprocal Ia inhibition is one of the neural mechanisms providing inhibitory synaptic inputs to motor neurons. The reciprocal Ia inhibitory pathway is composed of glycinergic Ia inhibitory interneurons projecting monosynaptically onto antagonistic muscle motor neurons (Fyffe, 1991). The Ia inhibitory interneurons are activated by Ia afferent inputs from agonist muscle spindles, and the activation of Ia interneurons contributes to the hyperpolarization of target motor neurons (Eccles *et al.*, 1956; Geertsen *et al.*, 2011). This inhibition prevents the activation of antagonist muscles during voluntary contraction of agonist muscles. This is why, reciprocal Ia inhibition is an important neural mechanism for controlling the coordinated contraction of opposing muscle groups (Geertsen *et al.*, 2011). Recurrent inhibition and Ib inhibition/facilitation are also important spinal neural mechanisms for regulating the activity of the alpha motor neurons (Knikou, 2008). Recurrent inhibition is provided by Renshaw cells that are excited by axon collaterals from motor neurons and make inhibitory synaptic connections with several populations of spinal neurons, including the motor neurons that excite them and Ia inhibitory interneurons (Pearson & Gordon, 2012). This inhibitory mechanism acts to stabilize the firing rate of motor neurons and to regulate the strength of inhibition of antagonist motor neurons (Katz & Pierrot-Deseilligny,

1999; Baret *et al.*, 2003). Golgi tendon organs are force-sensitive receptors that respond to muscle force, and group Ib afferents arising from this receptors make synaptic connections with inhibitory interneurons projecting to motor neurons of synergistic muscles (Ib inhibition) (Knikou, 2008). This inhibitory effect is usually observed in a resting condition and switches from inhibition to excitation during walking (Ib facilitation) (Pearson & Gordon, 2012). These inhibitory or facilitatory mechanisms are an integral part of the regulation of motor neuron activity during walking (Knikou, 2008). Although there are many spinal neural circuits that control the excitability of alpha motor neurons, this thesis is focused on Ia presynaptic inhibitory and reciprocal Ia inhibitory circuits, because these inhibitory circuits are speculated to play important roles in the control of joint movement (Geertsen *et al*., 2011; Fink *et al*., 2014).

The supraspinal descending pathways involved in motor control influence the activity levels of interneurons constituting these spinal neural circuits (Fig.1) (Jankowska, 1992). Previous studies have shown that presynaptic inhibition of soleus (SOL) muscle Ia afferents is increased before and during contraction of ankle dorsiflexor muscles (Nielsen & Kagamihara, 1993), and that blockade of afferent inputs does not affect Ia presynaptic inhibition during ankle dorsiflexion (Nielsen *et al.*, 1992). In a cat study, Rudomin *et al.,* (1983) reported that presynaptic inhibition of Ia afferents is tonically controlled by several descending pathways, such as the reticulospinal, rubrospinal, and corticospinal pathways, by acting on interneurons mediating Ia presynaptic inhibition. The same study also indicated that in the hind limbs, the main descending control on these interneurons is depression. Meunier and Pierrot-Deseilligny (1998) showed descending control of presynaptic inhibition of Ia afferents in humans,

as revealed by increase or decrease in Ia presynaptic inhibition following stimulation of the motor cortex. Moreover, long-term stimulation of the sensorimotor cortex has been shown to produce increase in GABAergic interneurons in the ventral horn and decrease in GABAergic terminals of alpha motor neurons (Wang *et al.*, 2012). Likewise, during Ia presynaptic inhibition, central facilitation of reciprocal Ia inhibition was observed before the contractions of agonist muscles (Crone *et al.*, 1987; Kasai & Komiyama, 1988). Jankowska *et al.*, (1976) reported that neurons in the motor cortex make monosynaptic connections not only with alpha motor neurons, but also with Ia inhibitory interneurons, and that descending inputs from corticospinal tracts activate Ia inhibitory interneurons that project to antagonist alpha motor neurons, as well as agonist motor neurons. Additionally, in a rat study, Chen *et al.*, (2006) showed that reciprocal inhibition could be changed by operant conditioning training, and that the corticospinal tract was fundamental to these changes. These findings strongly support the concept that changes in Ia presynaptic inhibition and reciprocal Ia inhibition are due to the central control of the spinal interneurons contributing to these spinal neural circuits. Furthermore, several studies have reported that patients with central nervous system disorders, such as strokes or spinal cord injuries, displayed abnormal activation of Ia presynaptic inhibition and/or reciprocal Ia inhibition (Crone *et al.*, 1994; Morita *et al.*, 2001; Okuma *et al.*, 2002; Crone *et al.*, 2003; Kagamihara & Masakado, 2005; Lamy *et al.*, 2009; Bhagchandani & Schindler-Ivens, 2012). In these patients, descending inputs modulating the activity of spinal neural circuits are deficient, indicating that the loss of descending control of spinal interneurons results in an abnormal activation of spinal neural circuits (Field-Fote, 2000). It is suggested that the loss of descending control of supinal neural circuits is involved in the symptoms of upper motor neuron syndromes,

such as hyperreflexia, clonus and spasticity (Crone *et al.*, 2003; Kagamihara & Masakado, 2005), resulting from hyperexcitability of the stretch reflex. Moreover, the degree of Ia presynaptic inhibition or reciprocal Ia inhibition observed in patients has been shown to be related to their functional recovery and walking capacity (Okuma *et al.*, 2002; Bhagchandani & Schindler-Ivens, 2012; Nardone & Trinka, 2015). Therefore, although the extent to which spinal neural circuits are affected depends on the type of diseases and the degree of damage to the motor system above the level of alpha motor neurons, a better understanding of the role of spinal neural circuits in the control of movement is crucial for developing rehabilitative strategies in patients with central nervous system disorders (Field-Fote, 2000; Edgerton *et al.*, 2004).

1.2 Hoffmann reflex

The Hoffmann reflex (H-reflex), an electrical analogous of the monosynaptic stretch reflex, was originally described by Paul Hoffmann (1910). Because, the H-reflex is a valuable tool for non-invasively measuring spinal neural activity, this reflex has been used widely in the research field of clinical neurophysiology and applied physiology (Zehr, 2002; Palmieri *et al.*, 2004; Knikou, 2008). The stretch reflex arc is comprised of muscle spindles, axons of sensory neurons (muscle spindle afferent fibers; group Ia and II afferents), alpha motor neurons, axons of alpha motor neurons, and muscles. The H-reflex arc is almost the same as the stretch reflex arc, and the only difference between the two reflex arcs is that the H-reflex bypasses the muscle spindles (Palmieri *et al.*, 2004). The H-reflex is evoked by electrical stimulation of a mixed peripheral nerve which consists of both sensory and motor axons. Because the diameter of Ia afferent fibers is larger than that of efferent fibers of motor neurons, low-intensity stimulation of

the peripheral nerve initially produces Ia afferent depolarization (Palmieri *et al.*, 2004; Piscione *et al.*, 2012). Action potentials induced by electrical stimulation travel to the spinal cord along Ia afferent fibers. If the terminal of Ia afferents are sufficiently depolarized, the neurotransmitters are released into the synaptic cleft, and EPSPs are evoked in alpha motor neurons. The motor neurons will then fire action potentials if the membrane potential of motor neurons is above a threshold level. The action potentials of motor neurons travel down to the neuromuscular junction along the axons and produce muscle twitches. This twitch response is recorded as an H-reflex in the electromyography (EMG) (Zehr, 2002; Palmieri *et al.*, 2004; Knikou, 2008). Electrical stimulation of a mixed peripheral nerve can also produce motor axon depolarization due to the simultaneous stimulation of efferent nerve fibers. The stimulation directly causes the activation of muscle fibers, which are recorded as motor responses (M-wave) in the EMG (Zehr, 2002; Palmieri *et al.*, 2004; Knikou, 2008). As mentioned above, the diameter of efferent nerve fibers is smaller than that of Ia afferent fibers, thus, the threshold for the M-wave (motor threshold: MT) is higher than that for the H-reflex (Palmieri *et al.*, 2004; Piscione *et al.*, 2012). Therefore, the H-reflex can be observed without an M-wave at low stimulation intensity levels. The amplitude of the H-reflex is increased with increments of stimulation intensity, until reaching the maximum amplitude of the H-reflex (Hmax), and then gradually decreasing with further increments of stimulation intensity. The amplitude of the M-wave is also increased with increments of stimulation intensity, but the maximum value remains stable regardless of further increases in stimulation intensity. The maximum amplitude of the M-wave (Mmax) represents the activation of all motor neurons axons and provides an estimate of the response given by the aggregate of alpha motor neurons (Pierrot-Deseilligny &

Mazevet, 2000). These H-reflex and M-wave recruitment characteristics can be observed by plotting the amplitude of the H-reflex and M-wave at each stimulation intensity, which is referred to as the H-reflex and M-wave recruitment curve. Figure 2 shows the H-reflex and M-wave curve obtained from a SOL muscle.

The ascending part of the H-reflex recruitment curve reflects the increase in the number of recruited alpha motor neurons activated by Ia afferent inputs. Alpha motor neurons are recruited in order of increasing size; recruitment occurs from smallest motor neurons because they are more easily depolarized by Ia afferent inputs according to the size principle (Henneman *et al.*, 1965). Thus, small motor neurons innervating slow motor units are recruited first in the H-reflex (Piscione *et al.*, 2012). By contrast, the M-wave recruitment curve reflects the recruitment order of axons of motor neurons induced by electrical stimulation. As large motor neurons innervating fast motor units have axons with a larger diameter, fast motor units are recruited first in the M-wave (Knikou, 2008). This implies that the H-reflex and M-wave do not represent the response of same motor units. Moreover, the action potentials evoked by electrical stimulation travel to axons of motor neurons not only orthodromically, but also antidromically. These antidromic volleys block the orthodromic volleys generated by Ia afferent depolarization, resulting in a partial cancelation of the H-reflex; the collision of antidromic motor volleys with orthodromic afferent volleys (Palmieri *et al.*, 2004; Knikou, 2008). This is why the H-reflex decreases after reaching maximal values (Fig. 2).

The H-reflex has been utilized as a probe to study spinal neuronal circuits in human

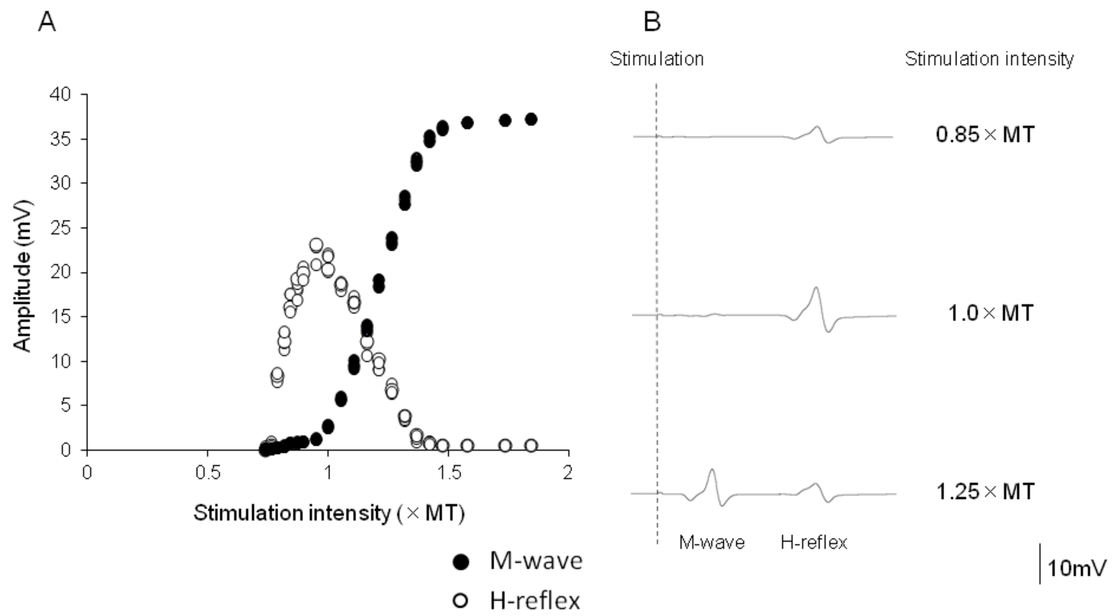

Figure 2. (A) The recruitment curves for a soleus H-reflex (○) and M-wave (●) measured in a healthy subject. Data were obtained during a resting condition. The abscissa shows the stimulation intensity expressed in multiples of motor threshold (×MT). The ordinate shows the amplitude of the H-reflex or M-wave (mV). (B) Typical averaged wave forms of H-reflexes and M-waves (n=5) at each stimulation intensity. The vertical dot line indicates the position of the artifact of electrical stimulation.

subjects (Pierrot-Deseilligny & Mazevet, 2000; Knikou, 2008). One method for investigating spinal neural circuits is to assess the effects of conditioning volleys in peripheral afferents or descending tracts on the H-reflex. In this method, the size of the H-reflex is compared in the presence or absence of the conditioning stimulation. Theoretically, eliciting the test H-reflex in a certain percentage of the maximum M-wave amplitude in each subject allows for the evaluation of almost same proportion of MN pools (Palmieri *et al.*, 2004). However, if the H-reflex elicited from the descending portion of the recruitment curve is used, the effects of conditioning on the H-reflex will be influenced by Ib and recurrent inhibition which are activated by high stimulation intensity (Knikou, 2008). Moreover, the susceptibility of the H-reflex to conditioning inputs has been shown to depend upon the size of the reflex itself (Crone *et al.*, 1990). Therefore, using the same size of test H-reflex (20–30% of Mmax) in the ascending portion of the H-reflex recruitment curve is more appropriate for assessing the effects of conditioning on the H-reflex. The H-reflex amplitude following exposure to conditioning stimuli can be changed by either postsynaptic or presynaptic mechanisms (Pierrot-Deseilligny & Mazevet, 2000; Knikou, 2008). The former is induced by excitatory or inhibitory synaptic inputs into alpha motor neurons, whereas, the latter is induced by presynaptic inhibition of Ia afferents.

The method for studying presynaptic inhibition and reciprocal Ia inhibition in humans was first described by Mizuno *et al.*, (1971), and are summarized as follows; in the lower limb, presynaptic inhibition was determined using long-latency [Conditioning-Test (C-T) intervals of 6–30 ms] suppression of the SOL H-reflexes by conditioning stimuli to the common peroneal nerve (CPN) that innervates the tibialis

anterior (TA) muscle (antagonistic muscle of SOL). This long-latency SOL H-reflex suppression, known as D1 inhibition (Mizuno *et al.*, 1971), is correlated with the presynaptic inhibition of monosynaptic reflexes observed in animal studies in terms of the onset latency and the slow development of H-reflex suppression (Eccles *et al.*, 1962). Reciprocal Ia inhibition was determined by a short-latency (C-T intervals of 2–3 ms) suppression of the SOL H-reflex using a conditioning stimulus to the CPN. H-reflex suppression is believed to reflect reciprocal Ia inhibition (Crone *et al.*, 1987), as the onset latency and response threshold (e.g., strength of the conditioning stimuli) of the H-reflex suppression is comparable with the future of reciprocal Ia inhibition in cats (Eccles *et al.*, 1956). A representative diagram of the neural circuit and the time interval between the conditioning and test stimulation is shown in Figure 3.

1.3 Transcranial magnetic stimulation

TMS was developed by Barker and colleagues in 1985 as a non-invasive method for studying the human brain (Barker *et al.*, 1985; Barker, 1999). They showed that a pulsed magnetic field creates electrical current flow in the brain and can temporarily stimulate a specific area without painful sensations. Since that time, TMS has been widely used as a research tool to study aspects of human brain physiology, including motor function, vision, language and the pathophysiology of brain disorders (Hallett, 2000).

TMS is based on the principle of electromagnetic induction, discovered by Faraday. In this method, a magnetic coil is used to stimulate cortical cells. When a brief high electric current pulse is produced in the electromagnetic coil, which is placed above the scalp, a magnetic field is generated perpendicular to the magnetic coil. The magnetic

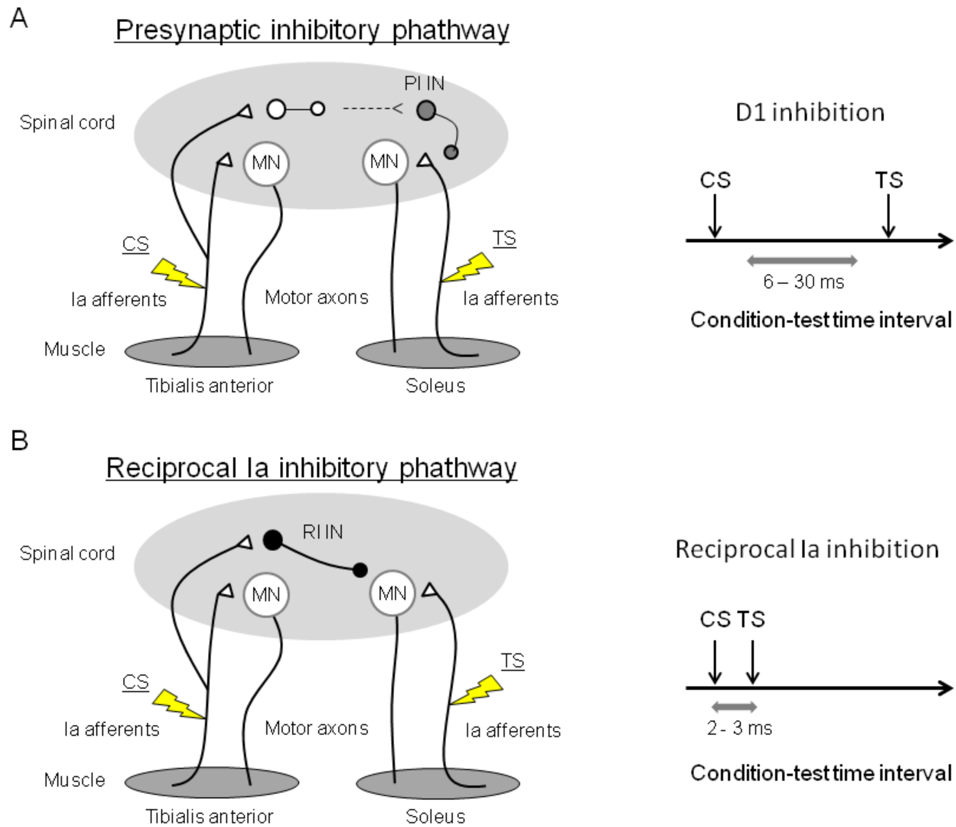

Figure 3. (A) A schematic diagram of the experimental paradigm for Ia presynaptic inhibition and time interval of condition to test stimulation. Conditioning stimulation of the common peroneal nerve (CPN) is applied 6–30 ms before the test stimulation. (B) A schematic diagram of the experimental paradigm for reciprocal Ia inhibition and time interval of condition to test stimulation. Conditioning stimulation of the CPN is applied 2–3 ms before the test stimulation. White circle indicates excitatory interneuron, gray circle indicates presynaptic inhibitory interneuron, and black circle indicates Ia inhibitory interneuron. Lightning bolts indicate electrical stimulation. CS, conditioning stimulation; IN, interneuron; MN, motor neuron; PI, presynaptic inhibition; RI, reciprocal Ia inhibition; TS, test stimulation.

field passes through bone and soft tissue without being affected by the electrical characteristics of a living body, and produces eddy current, which flows in the opposite direction to the electric current of the coil (Barker, 1999). In TMS, cortical cells are stimulated non-invasively by this eddy current, leading to the depolarization of the neuronal membrane (Fig. 4A). Magnetic stimulation is particularly effective for stimulating the human brain because the skull has high levels of electrical resistivity (Barker *et al.*, 1985). The magnetic field produced by TMS is up to 1.5–2.0 Tesla at the face of the magnetic coil, and the induced electric field in the cortex is up to 150 V/m approximately. The most commonly used magnetic coil forms two overlapping loops of wire in a ‘figure of eight’ arrangement (Fig. 5), which produces a more focased and shallower stimulation, within a range of 1 to 2 cm^2, whereas a double-cone coil is used to stimulate deeper cortical areas (Ridding & Rothwell, 2007; Rossi *et al.*, 2009). TMS can stimulate cortical neurons at a depth of 1.5–3.0 cm beneath the scalp, depending on the stimulation intensity (Rossi *et al.*, 2009).

When TMS is applied over the M1, corticospinal neurons are activated and muscle twitch responses are observed. The EMG response induced by TMS, namely the motor evoked potential (MEP), can be recorded in the contralateral limb muscle (Fig. 4B) (Hallett, 2000). The corticospinal pathway provides a direct monosynaptic route from the M1 to the spinal motor neurons (Petersen *et al.*, 2010). TMS can directly activate corticospinal neurons or indirectly activate corticospinal neurons via horizontally oriented interneurons which supply synaptic inputs to the corticospinal neurons (Petersen *et al.*, 2003). The responses evoked by TMS would be influenced by changes in the cortical excitability, and the alpha motor neurons receive multiple EPSPs after the

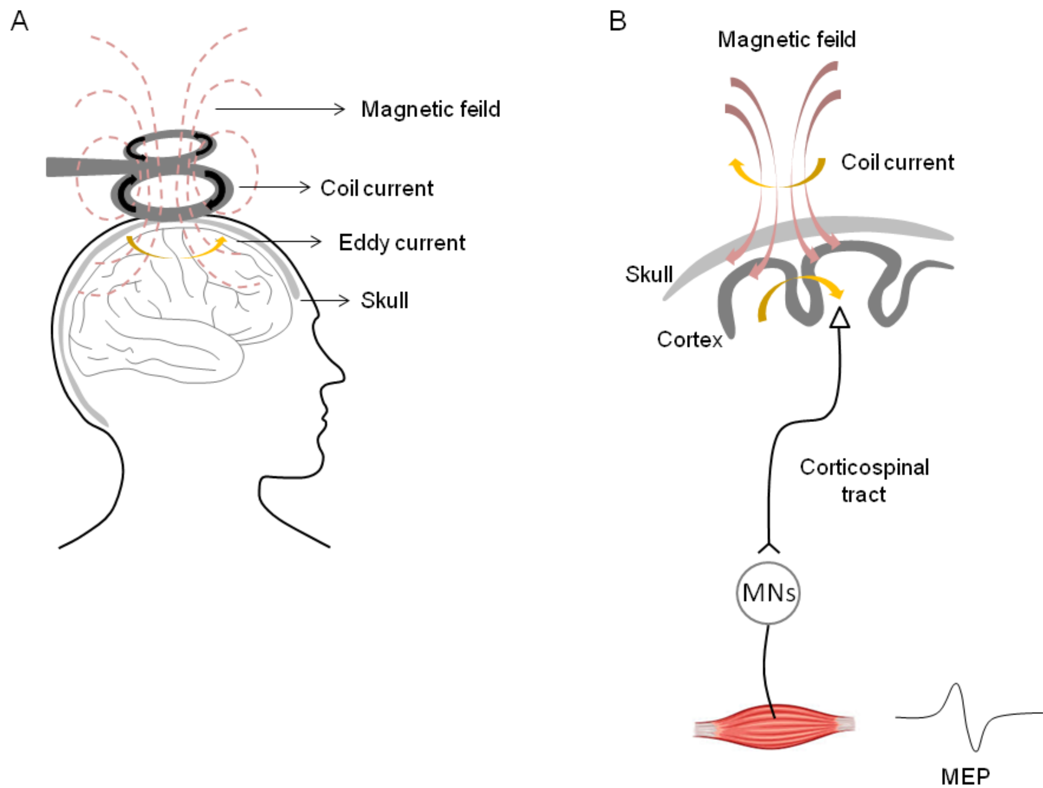

Figure 4. The principles of transcranial magnetic stimulation. In TMS, based on the principle of electromagnetic induction discovered by Faraday, cortical cells are stimulated non-invasively by eddy current (A). When TMS is applied over the primary motor cortex (M1), an EMG response (motor evoked potential) is recorded from the contralateral target muscle (B). MNs, motor neurons; MEP, motor evoked potential.

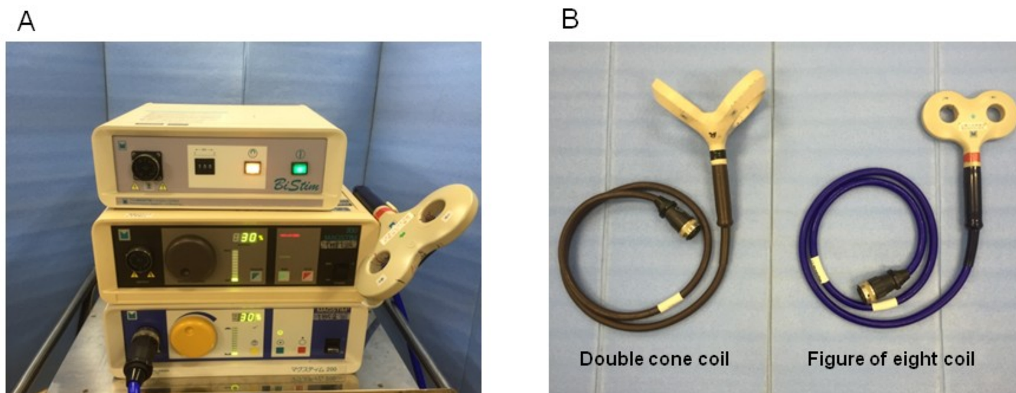

Figure 5. The device used for transcranial magnetic stimulation. (A) A Magstim 200 stimulator. (B) A figure of eight-shaped coil and double cone coil. For magnetic stimulation, a magnetic coil is connected to a Magstim 200 stimulator, and a brief, high current pulse is produced in the coil of wire.

TMS, leading to discharge of motor neurons (Petersen *et al.*, 2010).

Previous studies have shown that TMS can be used for investigating the influence of corticospinal descending inputs to spinal neural circuits by utilizing the H-reflex conditioning-test paradigm (Nielsen *et al.*, 1993b; Meunier & Pierrot-Deseilligny, 1998). In this method, the H-reflex was conditioned by subthreshold TMS at various C-T intervals. It has been reported that conditioning TMS produced a short-latency facilitation of the test H-reflex (Nielsen *et al.*, 1993b; Nielsen & Petersen, 1995a). The timing of short-latency facilitation of the H-reflex corresponds with that of the simultaneous arrival of the both the descending (TMS) and ascending (test H-reflex) volleys at the alpha motor neurons, which is inferred by the conduction time of the stimulation site to S1 spinal level (Herdmann *et al.*, 1991; Meunier *et al.*, 1994). Therefore, this facilitation is considered to reflect monosynaptic excitation from corticospinal neurons with fast-conducting fibers (Nielsen & Petersen, 1995a). Electrophysiological investigation revealed that, the increased membrane potential gradually returns toward the resting membrane potential over a few tens of milliseconds (Landgren *et al.*, 1962) due to the activation of Na^+/K^+ pomps and K^+ leak channels (Koester & Siegelbaum, 2012). Because the subthreshold TMS induces an increase in the membrane potential of motor neurons, the duration of the facilitation of the H-reflex should be expected to follow changes in the membrane potentials. In other words, the facilitation of the H-reflex should last for over a few tens of milliseconds. However, this facilitation was followed by inhibition, which always occurred after a 1–2 ms (short-latency inhibition) (Iles & Pisini, 1992; Nielsen *et al.*, 1993b). Therefore, the rapid termination of facilitation is likely caused by an IPSPs at the motor neurons

(Cowan *et al.*, 1986). Several studies have reported that the alpha motor neurons and Ia inhibitory interneurons that mediate disynaptic reciprocal inhibition are controlled in parallel by the corticospinal tract (Jankowska *et al.*, 1976; Crone *et al.*, 1987; Kasai & Komiyama, 1988). Intracellular recording from alpha motor neurons in baboons demonstrated that motor cortex stimulation produced EPSPs and IPSPs in motor neurons, and that IPSPs always started ~ 1.2 ms later than EPSPs (Landgren *et al.*, 1962). The timing of short-latency inhibition of the H-reflex corresponds with the timing of the late arrival of inhibitory inputs at motor neurons. Therefore, the short-latency inhibitory effect of conditioning TMS on the H-reflex is considered to reflect disynaptic inhibition from Ia inhibitory interneurons (Iles & Pisini, 1992; Nielsen *et al.*, 1993b). Moreover, another facilitation of the H-reflex was also observed approximately 10 ms after the short-latency inhibition. This late facilitation is assumed to represent the activation of different polysynaptic pathways or corticospinal neurons with slow-conducting velocity (long-latency facilitation) (Nielsen & Petersen, 1995b). On the other hand, the effect of corticospinal descending inputs on the presynaptic inhibition of Ia afferents was determined by comparing the amount of D1 inhibition in the presence and absence of TMS (Meunier & Pierrot-Deseilligny, 1998). It has been demonstrated that interneurons mediating Ia presynaptic inhibition are controlled by supraspinal inhibitory and excitatory pathways (Jankowska, 1992), and that stimulation of the corticospinal tract decreases PAD in muscle afferents of lower limbs, generated by the stimulation of group I muscle afferents (Rudomin, 1990). In support of this finding, a previous study has shown that motor cortical stimulation significantly decreases the D1 inhibition of SOL Ia afferents when applied 5–10 ms before CPN stimulation. In these inter-stimulus intervals, cortical conditioning volleys reach the S1

spinal level before the arrival of CPN conditioning volleys because the conduction time to reach the S1 spinal level is approximately the same for the cortical and CPN stimulation (Herdmann *et al.*, 1991; Meunier *et al.*, 1994). Therefore, the reduction of Ia presynaptic inhibition is believed to be the result of activity changes in interneurons interposed in the presynaptic inhibitory pathway. A representative circuit diagram as well as the experimental protocol are shown in Figure 6.

1.4 Summary of the aim of this thesis

The main goal of this thesis is to elucidate the mechanisms underlying spinal cord neuroplasticity induced by motor training, and to provide useful information about how this plasticity may be used for medical treatments. In chapter 2, I investigate the extent to which corticospinal descending inputs delivered to Ia inhibitory interneurons are related to the strength of reciprocal Ia inhibition. In chapter 3, I examine the corticospinal descending control of the Ia presynaptic inhibitory pathway by comparing the effect of motor skill training and non-skilled training on Ia presynaptic inhibition. In chapter 4, I hypothesized that the movement speed of a motor task is one of the important factors for producing changes in spinal neural circuits. To test this hypothesis, I examined whether the movement speed of a motor task alters neuroplasticity in spinal neural circuits. In chapter 5, to reveal the effect of peripheral afferent inputs on neuroplasticity in spinal neural circuits, I investigated whether sensory inputs induced by electrical nerve stimulation could produce changes in spinal neural circuits, and examined the neural mechanisms underlying these changes using TMS conditioning H-reflex techniques. Finally, I summarized the present findings and the conclusions of this thesis in chapter 6. The findings of this study promote a better understanding of

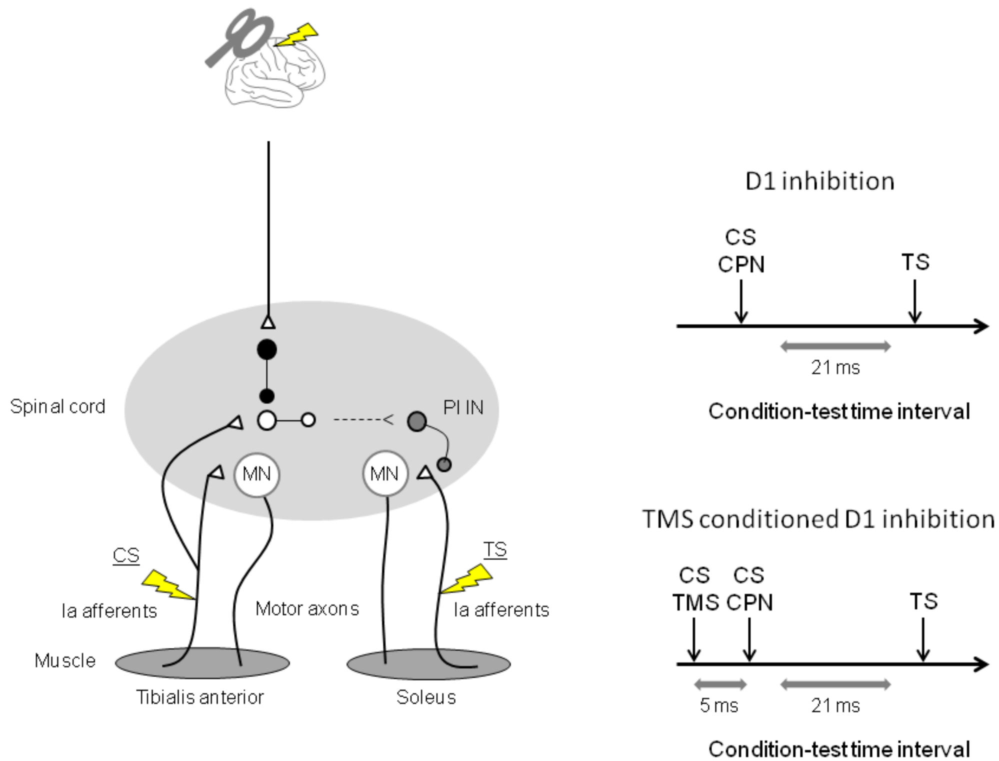

Figure 6. Schematic diagram of experimental paradigm in TMS conditioned Ia presynaptic inhibition (D1 inhibition) and time intervals of condition to test stimulation. The common peroneal nerve (CPN) stimulation is applied 21 ms before the test stimulation, and TMS stimulation is applied 5 ms before the stimulation of the CPN. White circle indicates excitatory interneuron, gray circle indicates presynaptic inhibitory interneuron, and black circle indicates inhibitory interneuron. Thunder indicates electrical stimulation or TMS stimulation. CPN. common peroneal nerve; CS, condition stimulation; IN, interneuron; MN, motor neuron; PI, presynaptic inhibition; TS, test stimulation.

activity-dependent spinal cord neuroplasticity and may lead to the development of effective rehabilitation approaches for improving motor function after trauma or disease.

CHAPTER 2

EFFECTS OF CORTICOSPINAL DESCENDING INPUTS ON THE ACTIVITY OF RECIPROCAL IA INHIBITION

2.1 Introduction

Reciprocal Ia inhibition is a spinal neural mechanism that is considered to play an important role in the control of joint movement (Petersen *et al.*, 1999; Kido *et al.*, 2004; Geertsen *et al.*, 2011). Reciprocal Ia inhibition has been studied extensively in human subjects using the H-reflex technique (Mizuno *et al.*, 1971; Iles, 1986; Crone *et al.*, 1987; Shindo *et al.*, 1995), and several studies have reported that interneurons mediating reciprocal Ia inhibition are controlled by supraspinal motor centers (Kasai & Komiyama, 1988; Kudina *et al.*, 1993; Nielsen *et al.*, 1993b). Crone *et al.*, (1987) reported that the agonist alpha motor neurons and Ia interneurons that project to antagonist alpha motor neurons are controlled in parallel by the brain. Also, in a rat study, Chen *et al.*, (2006) showed that reciprocal Ia inhibition could be operantly conditioned, and that the corticospinal tract was fundamental to the changes in reciprocal Ia inhibition induced by operant conditioning. These results suggested that the corticospinal tract modulates the activity of Ia interneurons that are responsible for reciprocal Ia inhibition. In human subjects, inter-individual variations in the amount of reciprocal Ia inhibition are observed in the resting state (Mizuno *et al.*, 1971; Crone *et al.*, 1987). This variability might reflect the amount of reciprocal Ia inhibition which is regulated by the corticospinal descending inputs to Ia interneurons. However, the relationship between the amount of reciprocal Ia inhibition and the strength of corticospinal inputs on Ia inhibitory interneurons is unknown.

The purpose of this study was to investigate the extent to which the corticospinal inputs delivered to Ia inhibitory interneurons influence the strength of disynaptic reciprocal Ia inhibition. To investigate this issue, I examined the conditioning effect of TMS on the SOL muscle H-reflex at different conditioning-test intervals. It has been reported that conditioning TMS produced short-latency facilitatory and inhibitory effects on the test H-reflex, which may be due to direct or indirect corticospinal mediated connections to the spinal motor neurons (Nielsen *et al.*, 1993b; Nielsen & Petersen, 1995a, b). Hence, I used this method to investigate the influence of corticospinal neurons on the spinal cord. Then, I determined the relationship between the degree of reciprocal Ia inhibition and the strength of corticospinal inputs on Ia inhibitory interneurons. I also carried out a similar experiment in tonic dorsiflexion condition to reveal whether this relationship is modulated by descending inputs related to voluntary motor commands.

2.2 Methods

2.2.1 Subjects

Seventeen healthy subjects, who were aged 21 to 29 years [23.4 ± 1.9 years; mean ± standard deviation (SD)], participated in our study after providing written informed consent. Our study was approved by the Human Ethics Committee of the Graduate School of Integrated Arts and Sciences of Hiroshima University, and all procedures conformed to the Declaration of Helsinki.

2.2.2 Electromyography recording

Subjects were seated in an armchair with the examined leg semi-flexed at the hip (120°)

and the knee (120°) and plantar-flexed at the ankle (110°). The right lower leg was secured with a metal double upright ankle-foot orthosis (Fig. 7).

EMG activity was recorded with bipolar surface electrodes (9-mm diameter Ag/AgCl surface cup electrodes; 20 mm distance between electrodes) placed on the right SOL and TA muscle belly. The electric resistance between the two electrodes was less than 5 kΩ. Raw EMG signals were amplified at 1000 times and band-pass filtered between 5 and 3000 Hz, using an amplifier (model 7S12; NEC San-ei Co., Ltd., Tokyo, Japan). The EMG signals were digitized by an analog/digital (AD) converter with a sampling rate of 10 kHz (PowerLab System Scope version 3.7.6; AD Instruments Pty. Ltd., Dunedin, New Zealand) and stored on a personal computer (PC) for subsequent analyses. The recording period was 200 ms including the pre-stimulus period of 100 ms.

2.2.3 H-reflex

The Sol H-reflex and M-wave were evoked by stimulating the posterior tibial nerve through a monopolar stimulating electrode (1 ms rectangular pulse) using a constant current isolator (SS-102J, Nihon Koden Co., Ltd., Tokyo, Japan) coupled with an electrical stimulator (SEN7203, Nihon Koden Co., Ltd., Tokyo, Japan). A ball cathode electrode was placed at the popliteal fossa, and the anodal electrode was placed on the anterior aspect of the thigh just above the patella. The H-reflex and M-wave response were measured as the peak-to-peak amplitude of the non-rectified reflex. The stimulus intensity was increased gradually from the threshold of the H-reflex to supramaximal value of the M-wave response. After M-wave response was saturated, I recorded five Mmax. As the sensitivity of the H-reflex to facilitatory or inhibitory conditioning inputs

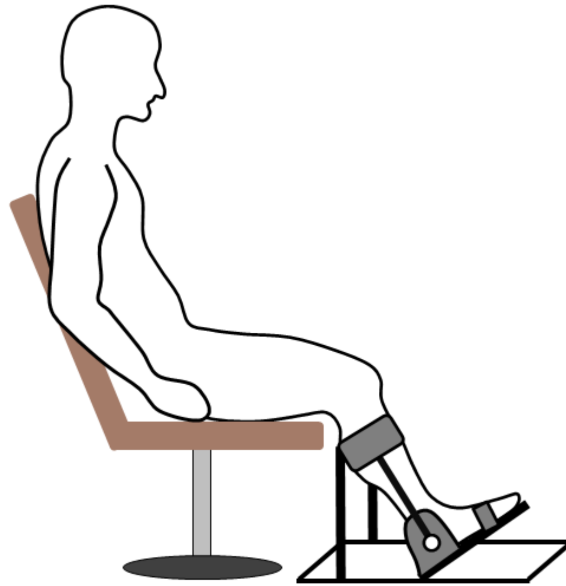

Figure 7. Experimental posture. All measurements were performed in this posture (hip flexed 120 degree, knee flexed 120 degree, and ankle plantar-flexed 110 degree). The right lower leg was secured with an ankle-foot orthosis.

was changed by the test H-reflex size, the size of the control SOL H-reflex was adjusted to 20–30% of Mmax in all conditions (Crone *et al.*, 1990).

2.2.4 Conditioning stimulation

The conditioning stimulus was applied to the CPN (condition 1) or M1 (condition 2). The experimental schema is shown in Figure 8A. The CPN was stimulated with rectangular electric pulse (duration: 1 ms) using a bipolar electrode, which was placed distal to the head of the fibula. The stimulation electrode was positioned carefully so as to avoid activating the peroneus muscles. The strength of the conditioning stimulus was adjusted so that it was slightly higher than the MT of the TA muscle, and the resultant M-waves were monitored throughout the experiment using an online monitor to ensure that they remained constant. This procedure ensured the consistency of the conditioning afferent volley. The motor cortex was stimulated using TMS. The TMS was delivered with a double-cone coil connected to a magnetic stimulator (model 200, Magstim, Whitland, UK). The coil was placed on the scalp to induce a posterior-anterior current flow in the left M1. An optimal stimulus position for evoking MEPs in the right SOL muscle was assessed by moving a coil around the leg motor area. The site at which stimulation with slightly suprathreshold TMS intensity consistently evoked the largest MEP in the right Sol muscle was regarded as the optimal position (approximately 1–2 cm left from Cz). This position was marked with a pen on a swimming cap worn by the subject. Active MT was defined as the minimal stimulus intensity required to induce MEPs of at least 200 μV in the SOL muscle in three of five trials (Rothwell *et al.*, 1999). While measuring the active MT, special care was given to maintain constant EMG activity levels of the SOL muscle (range, 100–150 μV).

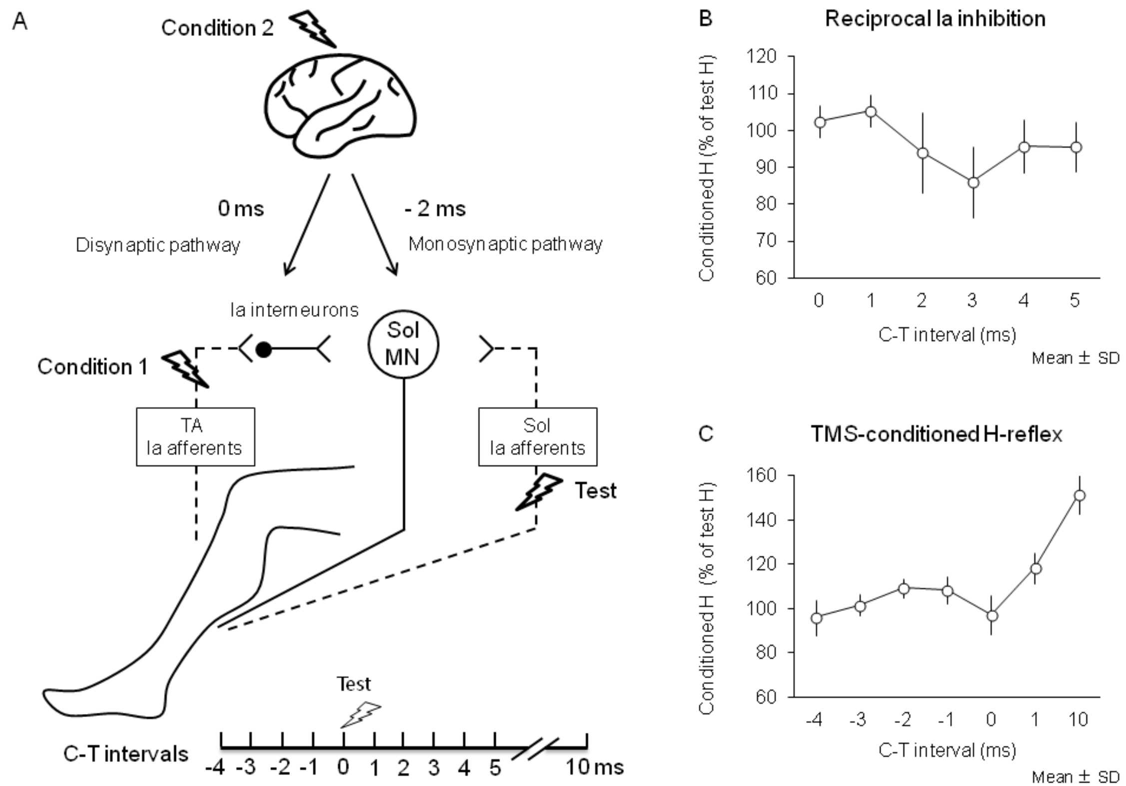

Figure 8. (A) Experimental schema. The soleus (SOL) H-reflex was conditioned by stimulating the common peroneal nerve (CPN) (condition 1) or the motor cortex (condition 2). The H-reflex was evoked at 0 ms by stimulating the posterior tibial nerve (test). Negative conditioning-test intervals (C-T intervals) indicate that the conditioning stimulus was applied after the test stimulus. (B) and (C): Time course of the effects of CPN stimulation (B) or motor cortex stimulation (C) on the SOL H-reflex, which were recorded from one subject. The ordinate shows the amplitude of the conditioned H-reflex expressed as a percentage of the unconditioned H-reflex. The abscissa shows the interval between the conditioning stimulus and test stimulus in milliseconds. Each point represents the mean and standard deviation of seven H-reflexes at each C-T interval. MN, motor neuron; TA, tibialis anterior; TMS, transcranial magnetic stimulation

2.2.5 Study design

Experiment 1

Seventeen healthy subjects participated in experiment 1. Seven subjects were tested twice on different days, in order to ensure the reproducibility of the results. All the experiments were carried out with the subjects in a resting state.

The amount of reciprocal Ia inhibition was determined via conditioning stimulation of the CPN to induce short-latency suppression of the Sol H-reflex. The C-T interval; i.e., the interval between the CPN stimulation and the posterior tibial nerve stimulation, was varied from 0 to 5 ms in 1 ms steps. Figure 8B shows the time course of the effects of CPN stimulation on the amplitude of the Sol H-reflex. In order to estimate the amount of Ia inhibition in each subject, I measured the inhibitory effect at the maximal depression of the H-reflex around 2 ms. The latency of 2 ms indicates a disynaptic linkage (Eccles *et al.*, 1956). The Sol H-reflex was evoked every 3 seconds. The conditioned or unconditioned H-reflexes were applied at random. Seven conditioned and seven unconditioned Sol H-reflexes were recorded at each C-T interval.

To examine the effect of corticospinal inputs on reciprocal Ia inhibitory circuit, the Sol H-reflex was conditioned by subthreshold TMS at different C-T intervals. It is possible that TA muscle response is evoked by the high stimulus intensity, since the MT of MEP in TA muscle was lower than that in Sol muscle (Bawa *et al.*, 2002). Thus, the stimulus intensity was set at 0.95–1.0 × active MT in order to avoid concomitant activation by afferent volleys induced by TA muscle contraction. The Sol H-reflex was conditioned using various C-T intervals (−4, −3, −2, −1, 0, 1, and 10 ms). Negative

C-T intervals indicate that the conditioning stimulus was applied after the test stimulation. Figure 8C shows the time course of the effects of motor cortex stimulation on the amplitude of the Sol H-reflex. The TMS-conditioning effect on the test H-reflex observed around −2 ms is considered to be due to monosynaptic connections from corticospinal neurons (short-latency facilitation) (Nielsen *et al.*, 1993b; Nielsen & Petersen, 1995a, b). This facilitation is followed by inhibition around 0 ms (short-latency inhibition), which is probably mediated by Ia inhibitory interneurons (Kudina *et al.*, 1993; Nielsen *et al.*, 1993b). On the other hand, the TMS-conditioning effect on the test H-reflex observed around 10 ms is assumed to be the activation of different polysynaptic pathways or the slow-conducting pyramidal tract cells (long-latency facilitation) (Nielsen & Petersen, 1995b). Thus, in each subject I measured the change in the conditioned H-reflex amplitude from short-latency facilitation to inhibition in order to estimate the activity of Ia interneurons. The Sol H-reflex was evoked every 6 seconds. The conditioned or unconditioned H-reflexes were applied at random. Seven conditioned and seven unconditioned Sol H-reflexes were recorded at each C-T interval.

Experiment 2

Eleven subjects took part in experiment 2. In this experiment, recordings were performed at rest and during tonic voluntary dorsiflexion at 10% of maximum voluntary contraction (MVC). To ensure a constant muscle contraction level, the integrated EMG (iEMG) value of the TA muscle (as a percentage of the value observed at MVC) was monitored during the 100 ms prior to the stimulation trigger being delivered. Also, feedback auditory signals that depended on the degree of EMG activity were delivered

to the subjects to ensure that a constant EMG level was maintained. Since the size of the H-reflex changed during muscle contraction, I tried to adjust the stimulus intensity to obtain similarly sized control H-reflexes in each condition (20–30% Mmax). Seven conditioned and seven unconditioned Sol H-reflexes at each C-T interval were randomly evoked in the same manner as in experiment 1.

2.2.6 Data analysis

The distributions of all variables were tested for normality using the Shapiro-Wilk test. Test H-reflex size was compared using the paired *t*-test (experiment 1) or one-way repeated-measures analysis of variance (ANOVA) (experiment 2). If significant effects were detected, the Bonferroni post-hoc test was used for multiple comparisons. The relationship between the amount of reciprocal Ia inhibition and the change in the TMS-conditioned H-reflex was analyzed using Pearson's correlation coefficient. iEMG values were compared between the conditions using the paired *t*-test. Also, the changes in reciprocal Ia inhibition and the TMS-conditioned H-reflex during tonic dorsiflexion were analyzed using the paired t-test. P values of < 0.05 were considered significant in all statistical analyses. The data values are presented as the mean ± standard error of mean (SEM) except for some cases in which the mean ± standard deviation or the median value (interquartile range) was used.

2.3 Results

The amplitude of the test H-reflex (% of Mmax) was not statistically different in experiment 1 (condition 1: 23.73 ± 0.95%; condition 2: 24.70 ± 0.98%; $P = 0.069$) and experiment 2 (condition 1: rest 23.48 ± 1.26%, 10% MVC 23.12 ± 1.38%; condition 2:

rest 24.07 ± 1.16%, 10% MVC 24.41 ± 0.92%; $F_{3,30} = 0.233$, $P = 0.872$). The mean TMS stimulus intensity was 45.17 ± 2.96 [% of maximum stimulator output (SO)] in experiment 1 and 46.72 ± 3.15 in experiment 2.

In experimental 1, the median value of reciprocal Ia inhibition was 15.07% (interquartile range: 3.95–25.90) and the median value of change in the amplitude of the TMS-conditioned H-reflex was 16.67% (interquartile range: 10.09–34.08). Inter-individual variations certainly existed in the amount of reciprocal Ia inhibition and the change in the amplitude of the TMS-conditioned H-reflex. Figure 9 depicts the positive relationship observed between the amount of reciprocal Ia inhibition and the change in the amplitude of the TMS-conditioned H-reflex from short-latency facilitation to inhibition. The scatter plot shows the results for total number of the experimental trials (n = 24) in experiment 1 including the data from repeated trials. The correlation coefficient for the relationship was 0.769 ($P < 0.01$). The strength of the reciprocal Ia inhibition tended to increase as the change in the amplitude of the TMS-conditioned H-reflex became greater. Figure 10 shows the effect of tonic dorsiflexion on the amount of reciprocal Ia inhibition and the change in the amplitude of the TMS-conditioned H-reflex in eleven subjects in experiment 2. The background EMG level did not differ significantly between condition 1 and condition 2 (condition 1: 9.78 ± 0.39%; condition 2: 10.02 ± 0.49%; $P = 0.48$). Figure 10A-B represents the mean effects of CPN stimulation and motor cortex stimulation, respectively, on the amplitude of the Sol H-reflex. The amount of reciprocal Ia inhibition did not differ significantly between the resting and tonic dorsiflexion condition (78.99 ± 4.81% vs 70.28 ± 7.23%; $P = 0.13$) (Fig. 10C). In contrast, the amount of change in the amplitude of the TMS-conditioned

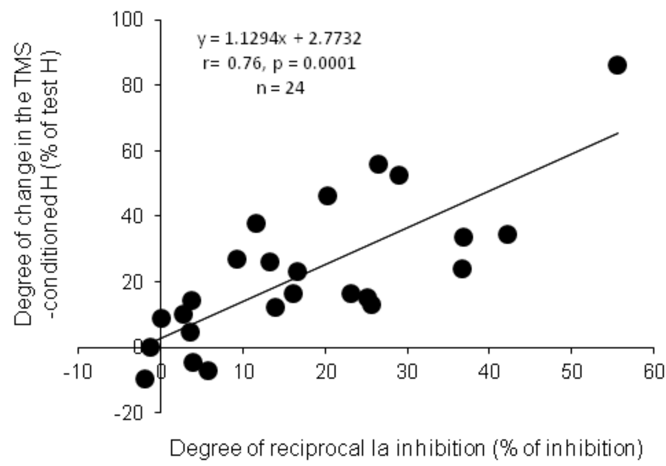

Figure 9. Correlation between the degree of reciprocal Ia inhibition and the change in the amplitude of the TMS-conditioned H-reflex. The total number of experimental trials (n) was 24.

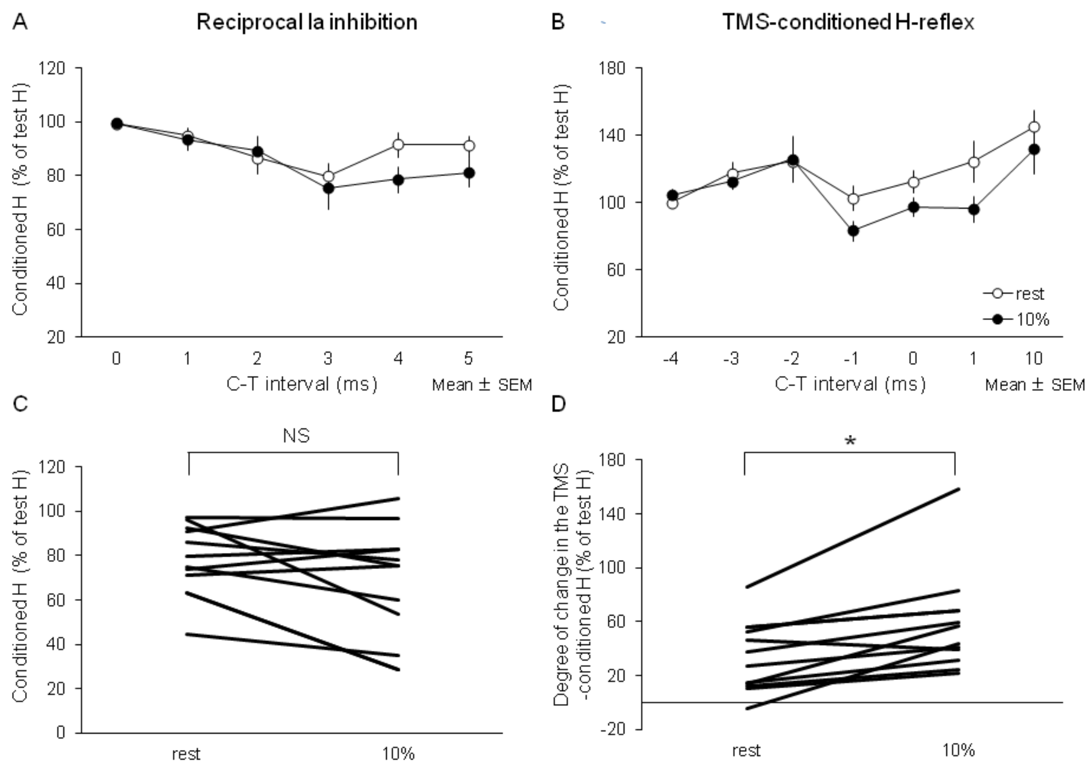

Figure 10. (A) and (B): Time course of the effects of common peroneal nerve stimulation (A) or motor cortex stimulation (B) on the SOL H-reflex in eleven subjects. The abscissa shows the C-T interval in milliseconds. The ordinate shows the normalized amplitude of the conditioned H-reflex. The open circles and closed circles show data obtained at rest and tonic dorsiflexion at 10%MVC conditions, respectively. Each point represents the mean and SEM. (C) and (D): The amount of reciprocal Ia inhibition (C) and the change in the TMS-conditioned H-reflex (D) at rest and during tonic dorsiflexion. The amount of reciprocal Ia inhibition was measured at the interval that produced the largest inhibition (either 2 or 3 ms). The change in the TMS conditioned H-reflex was measured at the intervals that produced the facilitation (around −2 ms) and the inhibition (around 0 ms). Each line represents one subject. Asterisk (*) indicates statistically significant differences (*$P < 0.05$).

H-reflex was increased during dorsiflexion, and the difference between the resting and the tonic dorsiflexion condition was statistically significant (32.12 ± 7.97% vs 57.27 ± 11.55%; $P < 0.01$) (Fig. 10D). The amplitude of the TMS-conditioned H-reflex at 10 ms did not differ significantly between the resting and the tonic dorsiflexion condition (145.23 ± 10.18% vs 131.74 ± 14.54%; $P = 0.15$).

2.4 Discussion

This study showed that the amount of reciprocal Ia inhibition and the change in the amplitude of the TMS-conditioned H-reflex were strongly correlated in the resting state. In addition, the change in the amplitude of the TMS-conditioned H-reflex tended to increase during muscle contraction. These results suggest that Ia inhibitory interneurons are influenced by corticospinal descending inputs, which might explain the inter-individual variation in the amount of reciprocal Ia inhibition.

A number of studies have reported that Ia inhibitory interneurons receive descending inputs from supraspinal centers (Jankowska *et al.*, 1976; Kasai & Komiyama, 1988; Kudina *et al.*, 1993; Nielsen *et al.*, 1993b). Nielsen *et al.,* (1993) reported that the short-latency inhibitory effect of conditioning TMS on the H-reflex was due to Ia inhibitory interneuron-mediated disynaptic reciprocal Ia inhibition. Also, in a previous study, Bawa *et al.,* (2002) showed that TMS-induced corticospinal descending volleys are monosynaptically delivered to the motor neurons innervating the TA and Sol muscles. Thus, I consider that the conditioning effect of TMS on the Sol H-reflex reflects the effects of descending inputs from corticospinal neurons on spinal motor neurons or Ia interneurons.

In this study, I found that the strength of reciprocal Ia inhibition was well correlated with the change in the amplitude of the TMS-conditioned H-reflex. In an earlier study, it was shown that the distribution of synaptic strength within neuron populations is affected by activity-dependent changes (Charpier *et al.*, 1995). Indeed, Crone *et al.,* (1985) reported that the degree of reciprocal Ia inhibition tended to increase with the amount of physical activity. These results imply that activity-dependent neuroplasticity develops at the spinal level (Wolpaw, 2007). Furthermore, it was suggested that reciprocal Ia inhibition can be operantly conditioned and that the excitability of Ia interneurons is controlled by corticospinal descending inputs (Chen *et al.*, 2006b). Because the present study was conducted in a resting state without muscle contraction, and the strength of the stimulus intensity of TMS was relatively same level (i.e., 0.95–1.0 × active MT), it is conceivable that the degree of descending input is affected by the synaptic strength of the corticospinal tract that communicate with Ia interneurons. Thus, observed results may indicate that the variation in the amount of reciprocal Ia inhibition is relevant to the inter-individual differences in the excitability of Ia inhibitory interneurons, which is controlled by the corticospinal descending inputs.

In addition to changes in synaptic plasticity, inter-individual differences in the amount of reciprocal Ia inhibition might be influenced by the motoneuron firing threshold level (Carp & Wolpaw, 1994) and the motoneuron pool background excitability (Funase & Miles, 1999) which affect the amplitude of the H-reflex (Zehr, 2002). Although the amplitude of the test H-reflex was carefully adjusted in the present study (i.e., the constant number of motor neurons recruited within individual subjects), the effects of the intrinsic property of motor neurons (e.g., changes in receptors) cannot

be completely eliminated.

The amount of reciprocal Ia inhibition did not differ significantly between the resting and tonic dorsiflexion condition, which is consistent with the findings of previous studies (Iles, 1986; Crone *et al.*, 1987). Moreover, in the tonic dorsiflexion condition reciprocal Ia inhibition displayed a large degree of variability. These variations might have been explained by contamination of the other spinal inhibitory inputs or saturation of Ia inhibitory interneurons activity, resulting from muscle contraction (Shindo *et al.*, 1995). On the other hand, at a short-latency inhibition phase, I observed a greater change in the amplitude of the TMS-conditioned H-reflex during dorsiflexion. These results indicate that Ia inhibitory interneurons are influenced by the corticospinal descending inputs involved in muscle contraction. Nielsen & Petersen, (1994) investigated the relationship between the corticospinal descending inputs and peripheral afferent inputs and suggested that the corticospinal tract is free from presynaptic control and regulates motor neurons independently of peripheral afferent feedback (Nielsen & Petersen, 1994). Taking these results into account, it is considered that the TMS-conditioned short and long-latency facilitation on the Sol H-reflex are not affected by contaminating inputs derived from muscle contraction and that the activity of Ia inhibitory interneurons is modulated by the corticospinal descending inputs.

2.5 Conclusion

In this study, I confirmed that the strength of reciprocal Ia inhibition is affected by the corticospinal descending inputs delivered to Ia inhibitory interneurons, which may explain the observed inter-individual variation in reciprocal Ia inhibition. These results

indicate that the descending input from corticospinal tracts associated with physical activity is an important factor for producing changes in the reciprocal Ia inhibitory circuit.

CHAPTER 3

THE MECHANISMS OF CHANGES IN THE IA PRESYNAPTIC INHIBITION FOLLOWING SKILLED MOTOR TASK

3.1 Introduction

Neuroplastic changes in the cortical areas induced by motor skill training have been investigated, and results suggest they are related to the acquisition of motor skills (Karni *et al.*, 1995; Pascual-Leone *et al.*, 1995; Muellbacher *et al.*, 2001; Muellbacher *et al.*, 2002; Perez *et al.*, 2004). The activity-dependent neuroplasticity develops not only at the cortical level but also at the spinal level (Wolpaw, 2007). In support of this concept, previous studies showed that motor skill training could induce the reorganization of the spinal cord, which might also account for the improvement of motor performance (Perez *et al.*, 2005a; Mazzocchio *et al.*, 2006; Meunier *et al.*, 2007; Roche *et al.*, 2011a). In these studies, supraspinal descending inputs to the spinal cord associated with the control of limb movements are regarded as an important factor in developing spinal cord neuroplasticity because the changes in spinal neural circuits are only observed following motor skill training.

Spinal reflex responses might interfere with an active voluntary movement due to the unpredictable activation of agonist muscles, and thus spinal reflexes need to be controlled in order to achieve smooth coordinated movement. Several studies have reported that the spinal reflex responses are modulated by motor learning and physical training (Nielsen *et al.*, 1993a; Nadler *et al.*, 2000; Meunier *et al.*, 2007). The modifications have been shown to be accompanied by changes in presynaptic inhibition

(Perez *et al.*, 2005a; Roche *et al.*, 2011a). It has been suggested that descending inputs delivered via the corticospinal tract communicate with interneurons constituting Ia presynaptic inhibitory circuit (Jankowska, 1992). Therefore, the corticospinal descending inputs may play an important role in driving the changes in Ia presynaptic inhibition following the skilled motor task. Although some studies showed that the changes in Ia presynaptic inhibition are induced by motor skill training, how the corticospinal descending inputs influences the activity of Ia presynaptic inhibitory circuit is not well understood. To clarify the mechanisms involved in the modification of Ia presynaptic inhibitory circuit following skilled motor task, the effects of corticospinal descending inputs on the Ia presynaptic inhibitory pathway were examined using TMS conditioning techniques (Meunier & Pierrot-Deseilligny, 1998).

3.2 Methods

3.2.1 Subjects

Sixteen healthy subjects (age, 23.1 ± 2.0 years; mean ± SD) participated in this study after providing written informed consent. Baseline characteristics of participants are shown in Table 1. All experimental procedures were carried out in accordance with the Declaration of Helsinki and were approved by the Human Ethics Committee of the Graduate School of Integrated Arts and Sciences of Hiroshima University.

3.2.2 Electromyography recording

Experimental posutre and EMG recording set up were the almost same as that used in chapter 2. It is summarized here, with several minor modifications noted.

Table 1. Baseline characteristics of subjects (mean ± SD)

	VM group	nVM group
Age	22.37±1.59	23.75 ± 2.25
Sex (Male/Female)	6/2	6/2
SOL Mmax (mV)	16.42 ± 5.53	14.71 ± 4.23
Hmax/Mmax	0.48 ± 0.13	0.48 ± 0.17
active MT (% of SO)	46.5 ± 10.11	48.25 ± 6.18
Stimulus intensity (% of SO)	38.37 ± 7.11	40.87 ± 4.67

Mmax, maximum amplitude of M-wave; Hmax, maximum amplitude of H-reflex; MT, motor threshold; nVM, non-visuomotor; SO, stimulator output; SOL, soleus; VM, visuomotor.

In chapter 3, all experimental measurements were taken while in a resting condition. EMG activity was recorded with bipolar surface electrodes placed on the right SOL and TA muscle belly and the recording period was set to 200 ms including the pre-stimulus period of 50 ms. SOL H-reflex and M-wave were evoked by stimulating the posterior tibial nerve through a monopolar stimulating electrode. An anode was placed above the patella, and a ball cathode was placed at the popliteal fossa. At the beginning of the experiment, the Hmax and Mmax were recorded in all participants. Ten conditioned and ten unconditioned H-reflexes were recorded at each C-T interval, and the conditioned H-reflex amplitude was expressed as a percentage of the unconditioned H-reflex amplitude.

3.2.3 Presynaptic inhibition

The amount of Ia presynaptic inhibition was determined from long latency (C-T intervals of 6–30 ms) suppression of the SOL H-reflexes, by conditioning stimuli to the CPN that innervates the TA muscle. The CPN was stimulated through a bipolar stimulation electrode (1 ms rectangular pulse) placed distal to the head of the fibula. The electrode was carefully positioned to avoid activating the peroneus muscles, and TA M-waves were monitored to ensure constancy of stimulation throughout the experiment. The intensities of the conditioning stimulus was set to just above the MT intensity of the TA muscle (1.1 × MT). The CPN was stimulated with a train of three single pulses at 333Hz. The time interval between CPN stimulation (first shock of a train of three shocks) and test stimulation was kept constant at 21 ms. Conditioned and unconditioned H-reflexes were randomly evoked at 0.33 Hz.

3.2.4 Transcranial magnetic stimulation

A previous study has shown that motor cortical stimulation significantly decreases the D1 inhibition of SOL Ia afferents when applied 5–10 ms before the CPN stimulation (Meunier & Pierrot-Deseilligny, 1998). In these interstimulus intervals, cortical conditioning volleys reach the S1 spinal level before the arrival of CPN conditioning volleys. Therefore, it is assumed that corticospinal descending inputs produce the depressive effect on the interneurons constituting Ia presynaptic inhibitory pathways in the lumber spinal cord (Rudomin *et al.*, 1983). Moreover, it has been demonstrated that a conditioning TMS produces short and long latency facilitation of the H-reflex, due to monosynaptic and polysynaptic connections from corticospinal neurons (Nielsen & Petersen, 1995b). Therefore, it is conceivable that these facilitation indirectly reflect the excitability of the corticospinal tract. In this study, I investigated the effects of corticospinal descending inputs on the Ia presynaptic inhibitory pathway using TMS conditioning techniques.

Magnetic stimulation was delivered to the M1 through a double-cone coil connected to a magnetic stimulator (model 200; Magstim, Whitland, UK). The coil was placed on the scalp to induce a posterior-anterior current flow in the left M1. The coil was attached to the subject’s head and fixed at the optimal position for inducing responses in the right Sol muscle with weak contraction. The site at which stimulation with slightly suprathreshold TMS intensity consistently evoked the largest MEP in the right Sol muscle was regarded as the optimal position. This position was marked with a pen on a swimming cap worn by the subject. The active MT was defined as the lowest stimulus intensity that produced MEPs of at least 200 μV in the Sol in three out of five

trials (Rothwell et al., 1999). The intensity of conditioning TMS was set to 80–90% of the active MT, so that they had minor facilitation effects on the test H-reflex in a resting condition. To examine the effect of corticospinal descending inputs on the Ia presynaptic inhibitory pathway, I compared the amount of D1 inhibition in the presence and absence of TMS. The time interval between TMS and CPN stimulation was set at 5 ms. Moreover, to study the effects of TMS conditioning on the test H-reflex, the amplitude of TMS conditioned H-reflex was evaluated at C-T intervals of 26 ms (21 ms plus 5 ms is 26 ms). For measurement of the excitability of the corticospinal tracts, the intervals between the conditioning stimulation and H-reflex stimulation were set at −3, −2, and −1 ms. The interval that produced the first facilitation effects on the H-reflex (short latency facilitation) was regarded as a suitable C-T interval and was used throughout the experiment. Negative C-T intervals indicated that the conditioned stimulus was applied after the test stimulation. Conditioned and unconditioned H-reflexes were randomly evoked at 0.2 Hz.

3.2.5 Visuomotor task

A custom made program (Labview 2012; National Instruments Co., Tokyo, Japan) was used to set up a visuomotor task. The subjects were allowed free movement of the ankle joint when performing the motor task. For a visuomotor task, subjects repeatedly moved their ankle between target lines according to auditory beep sounds delivered at a frequency of 2.67 Hz (Fig. 11A). In this task, subjects required precise control of joint movement. The movement speed was defined as the time of one cycle of ankle movement (it was set to 750 ms). The target lines representing the point of 10° angle of ankle dorsiflexion and of 10° angle of plantarflexion from the neutral position were

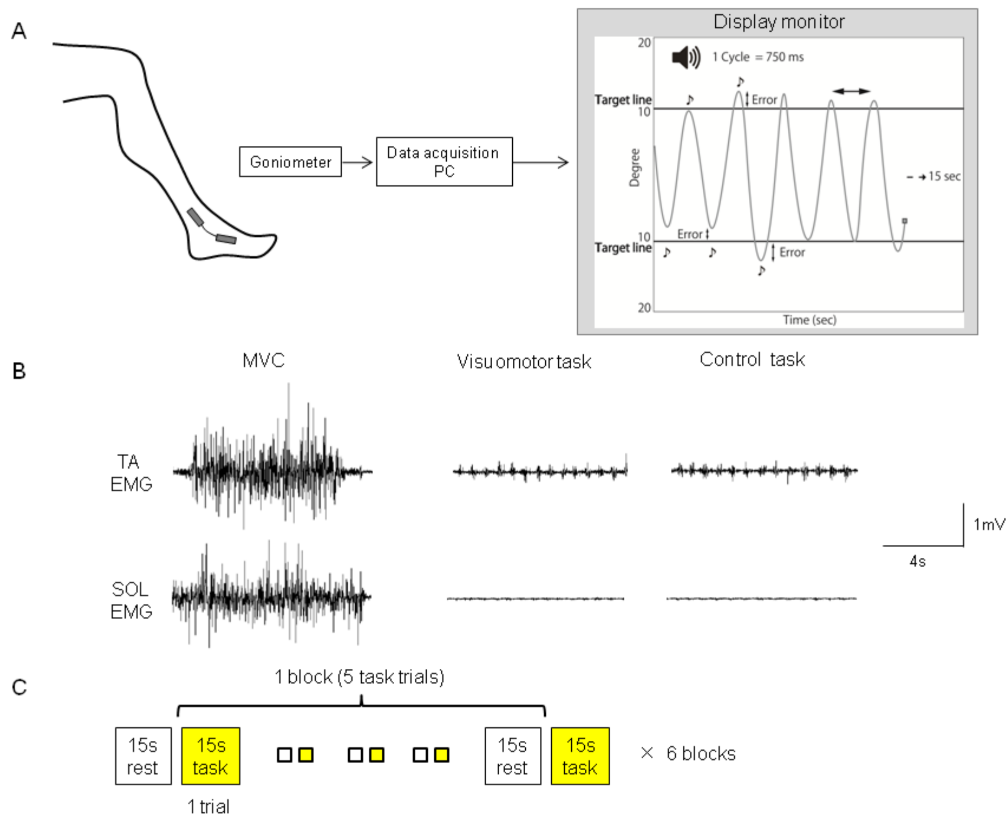

Figure 11. Schematic of the motor task. (A) Experimental set-up. (B) Example of raw electromyography (EMG) activity of tibialis anterior (TA) muscle and soleus (SOL) muscles during isometric maximum voluntary contraction (MVC) and each motor task. (C) Training procedure.

displayed on a monitor (26-inch size), which was set at approximately 1 m in front of the subjects. The subjects were instructed to execute the ankle movement as precisely as possible between the target lines. Ankle angular displacements were measured with a goniometer (SG 100; Biometrics Ltd., Newport, UK) that was mounted on the lateral side of the leg (located at the fifth metatarsal and the fibula) and the goniometer signals were amplified by an amplifier (model 6L01; NEC San-ei Co. Ltd). The signals were recorded on a PC at a sample rate of 100 Hz via an A/D converter (USB6212; National Instruments Co.), and also displayed as a cursor with line trajectory on the display monitor, to control ankle movement. The cursor automatically moved from the left to the right at 15 s (1 trial). Also, during ankle plantarflexion, the cursor moved to the top of the screen, whereas during dorsiflexion, the cursor moved to the bottom of the screen. A single trial example of raw EMG activity of the TA and SOL muscle during isometric MVC and motor task is shown in Figure 11B. MVC was performed before the motor task and measured by pushing against a foot plate or pulling against a non-elastic band which was secured around the foot plate. The subjects performed the motor task for 20 min. The task session consisted of six blocks with five trials (Fig. 11C). In order to minimize muscle fatigue, 15 s resting periods occurred between trials, and each block was separated by 1 min. All subjects were familiarized with the motor task before starting the task session. Motor performance was quantified by subtracting the target degree from the actual degree (10° angle of dorsi- and planter-flexion) at each inflection point (Fig. 11A). The difference was defined as an error and averaged for 16 cycles at each trial (task performance time 12 s). The error data were averaged for each block (mean error) and normalized by the value of the first block in order to confirm the rate of change.

3.2.6 Experimental procedures

Sixteen subjects were randomly allocated to two different groups. Eight subjects performed the visuomotor task (visuomotor group), and the remaining eight subjects performed the control task (non-visuomotor group). The non-visuomotor group subjects performed a control task for 20 min. For the control task, the subjects repeatedly moved their ankle according to auditory beep sounds delivered at a frequency of 2.67 Hz without visual feedback of ankle movement. In this task, the subjects were not required precise control of joint movement. Other task procedures used the same visuomotor task. The amount of D1 inhibition, the amount of TMS conditioned D1 inhibition, the amplitude of TMS conditioned test H-reflex, the amplitude of TMS conditioned H-reflex amplitude at short facilitation phase were measured before and after the task sessions. I also tested the ratio of Hmax vs. Mmax (Hmax/Mmax), which was used as an indicator of motor neuron pool excitability, before and after the task sessions. Hmax and Mmax were evoked every 3 s and calculated from the average of five Hmax and five Mmax values.

3.2.7 Statistical analysis

The baseline characteristics of groups (age, SOL Mmax, Hmax/Mmax, active MT, and stimulation intensity of conditioning TMS) were analyzed using the unpaired *t*-test. The performance data compared among the task sessions used the one-way repeated measure of ANOVA for each group. Test H-reflex size was compared using two-way repeated measures of ANOVA with the factors “time” and “group.” The amount of D1 inhibition, the amount of TMS conditioned D1 inhibition, the difference in the amount of D1 inhibition in the presence and absence of TMS, the amplitude of TMS

conditioned test H-reflex, and the amplitude of TMS conditioned H-reflex at short latency facilitation phase were analyzed by two-way repeated measures of ANOVA with factors "time" and "group." For multiple comparisons, if significant effects were detected, the Bonferroni post hoc test was used. Mauchley's test was used to examine for sphericity. The Greenhouse-Geisser correction was used for non-spherical data. The amount of D1 inhibition was compared with the amount of TMS conditioned D1 inhibition within the group, using the paired *t*-test. The amplitude of TMS conditioned H-reflex at short latency facilitation phase was also compared with the unconditioned H-reflex, using the one-sample paired *t*-test. Moreover, the differences in the Hmax/Mmax and SOL Mmax were compared between before and after the task within the group, using the paired *t*-test. P values of < 0.05 were considered significant in all statistical analyses. Data were analyzed using SPSS version 22 software (IBM SPSS, IBM Japan, Ltd., Tokyo, Japan). The data values are presented as the means ± SEM.

3.3 Results

Baseline characteristics of subjects among groups were well-matched in the Experiment groups (Table 1), and there were no significant differences between all baseline measures (age: t_{14}= 1.41, $P = 0.18$; SOL Mmax: $t_{14} = 0.69$, $P = 0.50$; Hmax/Mmax, $t_{14} = 0.06$, $P = 0.94$; active MT: $t_{14} = 0.41$, $P = 0.68$; stimulation intensity: $t_{14} = 0.83$, $P = 0.42$).

3.3.1 Changes in the task performance

The mean times of one cycle of ankle movement are almost matched the pre-setting times, indicating that the task movement speeds were well controlled throughout the

experiment (visuomotor group = 712.49 ± 19.73, non-visuomotor group = 708.83 ± 23.95). Figure 12 shows an example of ankle joint movement during each motor task and the time course of the mean errors in the motor task. A one-way repeated measures of ANOVA revealed a significant effect of blocks for visuomotor group ($F_{5,35} = 27.8$, $P < 0.01$). In post hoc tests, a significant reduction in the mean errors was observed between the first block and the following blocks ($P < 0.01$). These results indicate that the task performance was certainly improved in the visuomotor task. There were no significant differences in the mean errors across the blocks in the non-visuomotor group ($F_{5,30} = 2.31$, $P = 0.07$).

3.3.2 Effect of corticospinal descending inputs on the Ia presynaptic inhibitory pathway

The mean amplitudes of the test H-reflex (% of Mmax) for all conditions are summarized in Table 2. The values show the average of test H-reflex amplitudes calculated from the mean amplitude of test H-reflex in all subjects. The test H-reflex amplitude was almost the same size throughout the experimental procedures. There were no significant effects of "time" (D1 inhibition: $F_{1,14} = 0.39$, $P = 0.54$; TMS conditioned D1 inhibition: $F_{1,14} = 4.07$, $P = 0.06$; TMS conditioned H-reflex at short latency facilitation phase: $F_{1,14} = 3.85$, $P = 0.07$) and "group" (D1 inhibition: $F_{1,14} = 0.54$, $P = 0.47$; TMS conditioned D1 inhibition: $F_{1,14} = 1.45$, $P = 0.25$; TMS conditioned H-reflex at short latency facilitation phase: $F_{1,14} = 2.03$, $P = 0.18$) on the test H-reflex amplitude. There was also no significant "time" × "group" interaction (D1 inhibition: $F_{1,14} = 0.19$, $P = 0.67$; TMS conditioned D1 inhibition: $F_{1,14} = 0.31$, $P = 0.59$; TMS conditioned H-reflex at short latency facilitation phase: $F_{1,14} = 0.55$, $P = 0.47$).

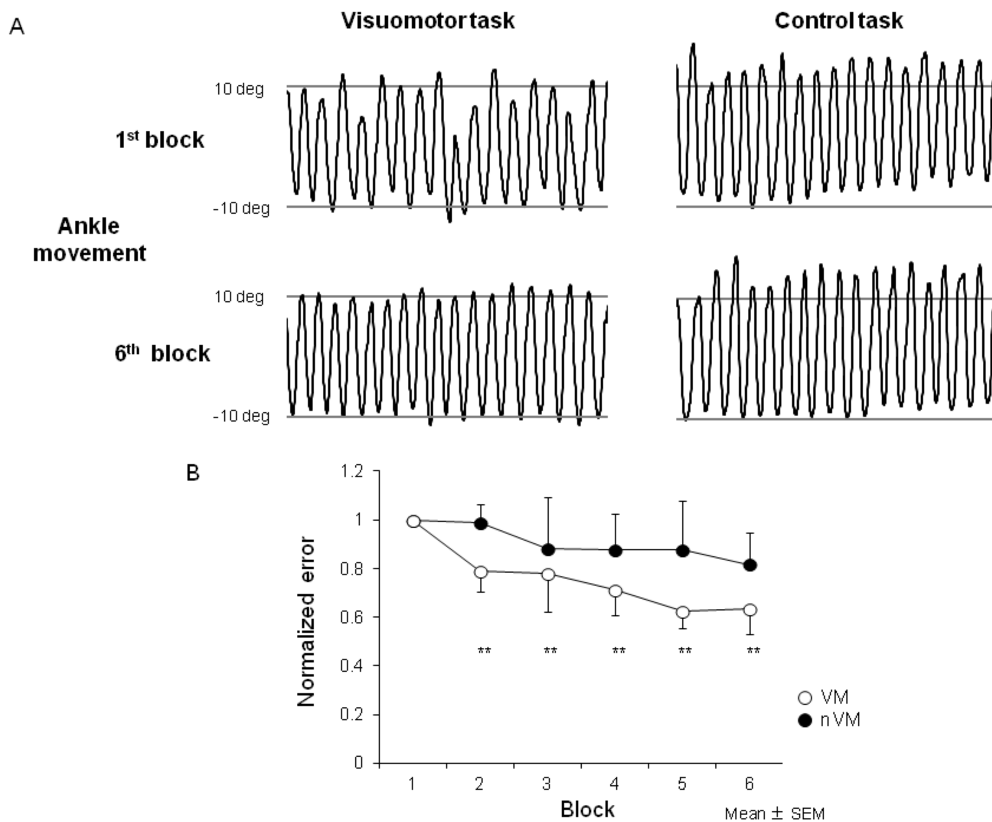

Figure 12. The changes in the motor performance in visuomotor and non-visuomotor groups.

(A) Typical ankle joint movement during each motor task recorded from two representative subjects who performed a visuomotor or a control task. Each line trajectory represents the subject's performance in a first and sixth block. (B) The time course of the changes in the motor performance in the visuomotor and non-visuomotor groups. The ordinate shows mean error values normalized by the value of the first block. The abscissa shows each block. The double asterisks (**) represents a significant difference ($^{**}P < 0.01$) between the first and other blocks in the visuomotor group. Error bar indicates SE. deg, degree; VM, visuomotor; nVM, non-visuomotor.

Table 2. Summary of test H-reflex amplitude (% of Mmax: mean ± SEM)

	D1 inhibition		TMS conditioned D1 inhibition		TMS conditioned H-reflex (short latency facilitation)	
	Pre	Post	Pre	Post	Pre	Post
VM group	25.11 ± 0.94	24.98 ± 0.99	25.53 ± 1.38	24.72 ± 1.01	25.54 ± 0.81	24.12 ± 0.71
nVM group	26.39 ± 1.16	25.68 ± 1.15	27.41 ± 0.48	25.99 ± 0.94	26.42 ± 0.54	25.77 ± 0.81

VM, visuomotor; nVM; non-visuomotor; Mmax, maximum amplitude of M-wave; Pre, before the task sessions; Post, after the task sessions; TMS, transcranial magnetic stimulation.

Figure 13 shows the typical averaged waveforms (n = 10) of the control and conditioned H-reflexes induced by CPN stimulation and TMS stimulation, recorded from one representative subject in each group. Figure 14 A–C show the amount of D1 inhibition, the amount of TMS conditioned D1 inhibition, and TMS conditioned test H-reflex amplitude before and after the visuomotor task or control task, respectively. Because the conditioning stimulation of TMS produced minor facilitation effects on the test H-reflex amplitude (Fig. 14C), the net difference in the amount of D1 inhibition was calculated by subtracting this facilitation effect from the changing amount of D1 inhibition in the presence and absence of TMS [(graph B − graph A) − (100 − graph C)], which is shown in Fig. 14D. A two-way repeated measures of ANOVA for D1 inhibition showed a significant effect of the “time” ($F_{1,14} = 71.43$, $P < 0.01$), but not of “group” ($F_{1,14} = 0.03$, $P = 0.84$). There was significant “time” × “group” interaction ($F_{1,14} = 34.44$, $P < 0.01$). Post hoc analysis indicated that compared to pre, in the visomotor group, the amount of D1 inhibition was significantly increased at post ($P < 0.01$). A two-way repeated measures of ANOVA for TMS conditioned D1 inhibition showed a significant effect of the “time” ($F_{1,14} = 31.19$, $P < 0.01$), but not of “group” ($F_{1,14} = 0.02$, $P = 0.89$). There was significant “time” × “group” interaction ($F_{1,14} = 31.39$, $P < 0.01$). Post hoc analysis indicated that compared to pre, in the visuomotor group, the amount of TMS conditioned D1 inhibition was significantly increased at post ($P < 0.01$). In the visuomotor group, the amount of TMS conditioned D1 inhibition was significantly greater than the amount of D1 inhibition at pre ($t_7 = 4.17$ $P < 0.01$), but not at post ($t_7 = 0.55$, $P = 0.59$). In the non-visuomotor group, the amount of TMS conditioned D1 inhibition was significantly greater than the amount of D1 inhibition at the same period of time (pre: $t_7 = 3.56$, $P < 0.01$; post: $t_7 = 9.16$, $P < 0.01$). However,

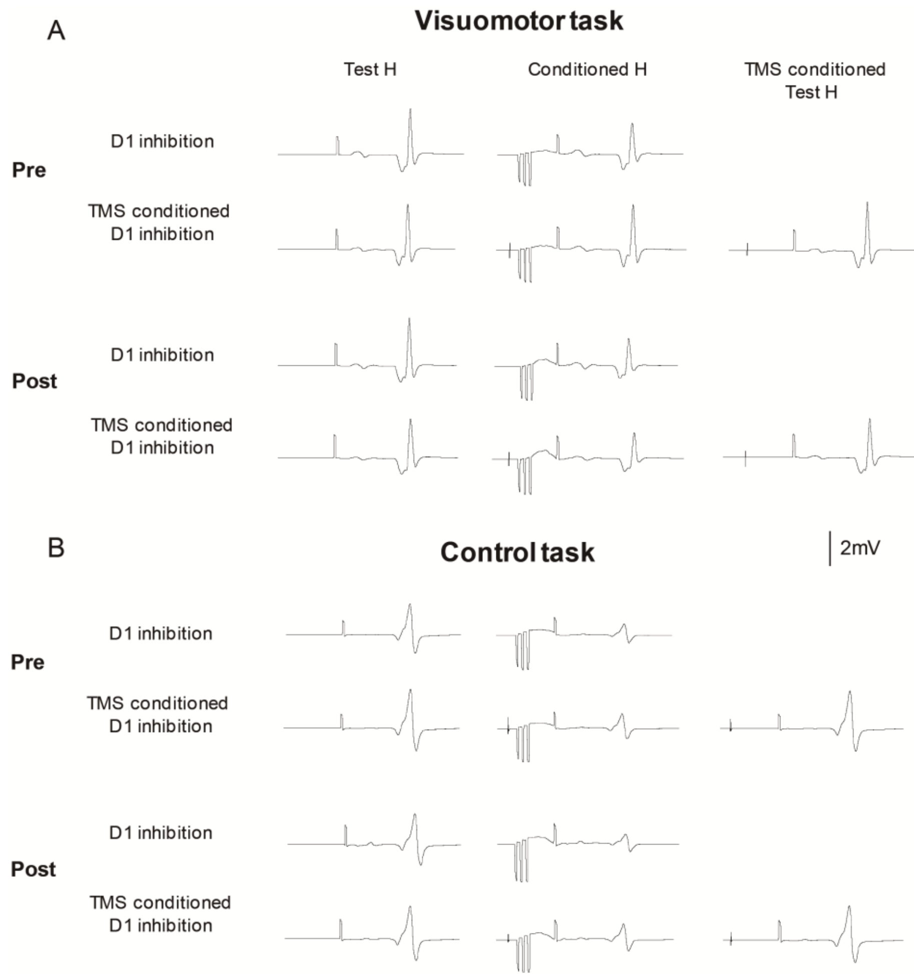

Figure 13. Typical averaged waveforms of H-reflex (n = 10) in each stimulus condition were recorded from two representative subjects who performed a visuomotor task (A) or a control task (B).

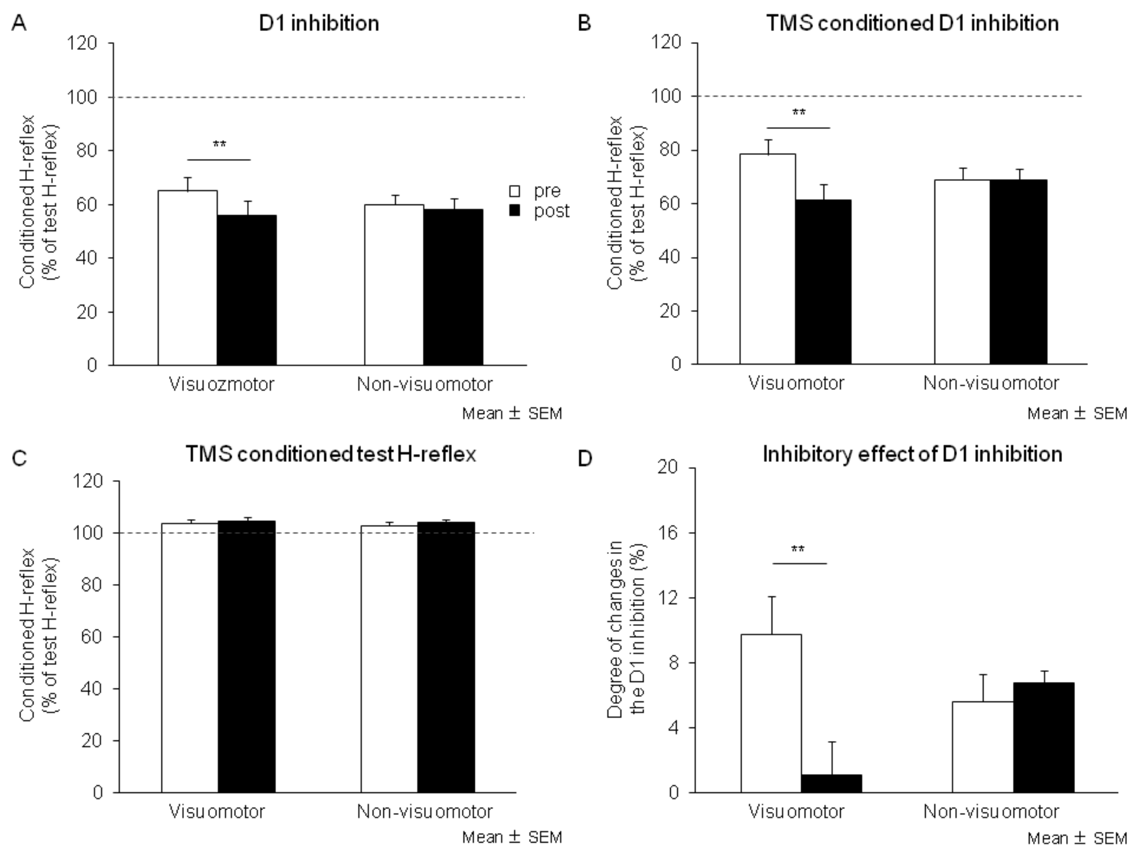

Figure 14. The effect of visuomotor task and control task on the transcranial magnetic stimulation (TMS) conditioned inhibitory effects on the Ia presynaptic inhibitory pathway. The graphs show the mean values of the D1 inhibition (A), TMS conditioned D1 inhibition (B), TMS conditioned test H-reflex amplitude (C), and the net difference in the amount of D1 inhibition (D) in the visuomotor and non-visuomotor group. In (A–C), the ordinate shows the conditioned H-reflex amplitude expressed as a percentage of the test H-reflex amplitude. The dashed line indicates the test H-reflex amplitude (100%). Values below 100% indicate inhibition and values above 100% indicate facilitation. In (D), the ordinate shows the degree of changes in the D1 inhibition which is calculated by subtracting the minor facilitation effect of TMS conditioned test H-reflex amplitude (conditioned H-reflex − test H-reflex) from the changing amount of D1 inhibition in the presence and absence of TMS (difference between graph A and B),

expressed as a percentage of the test H-reflex amplitude. Open and closed bars represent the time at which measurements were taken before (pre) and after (post) the motor task, respectively. The double asterisks (**) represent significant difference (**P < 0.01). Error bar indicates SEM.

there were no significant effects of "time" ($F_{1,14}$ = 0.52, P = 0.48) and "group" ($F_{1,14}$ = 0.19, P = 0.66) on the TMS conditioned test H-reflex amplitude, and there was no significant "time" × "group" interaction ($F_{1,14}$ < 0.01, P = 0.96). Moreover, a two-way repeated measures of ANOVA for the net difference in the amount of D1 inhibition showed a significant effect of "time" ($F_{1,14}$ = 7.31, P = 0.02) but not of "group" ($F_{1,14}$ = 0.22, P = 0.64). There was significant "time" × "group" interaction ($F_{1,14}$ = 10.35, P < 0.01). Post hoc analysis indicated that compared to pre, in the visuomotor task group, the net difference in the amount of D1 inhibition was significantly decreased at post (P < 0.01). The inhibitory effect of D1 inhibition induced by TMS is decreased following the visuomotor task.

Figure 15A shows the typical averaged waveforms (n = 10) of the control and conditioned H-reflexes induced by TMS stimulation with the C-T intervals at short latency facilitation phase, recorded from one representative subject in each motor task. The mean amplitudes of TMS conditioned H-reflex at short latency facilitation phase before and after the motor tasks are shown in Figure 15B. The amplitude of the TMS conditioned H-reflex was significantly larger than that of the unconditioned H-reflex in all conditions (pre: t_7 = 22.21, P < 0.01 in the visuomotor group, t_7 = 23.11, P < 0.01 in the non-visuomotor group; post: t_7 = 17.77, P < 0.01 in the visuomotor group, t_7 = 14.18, P < 0.01 in the non-visuomotor group). There were no significant effects of "time" ($F_{1,14}$ = 1.47, P = 0.25) and "group" ($F_{1,14}$ = 0.59, P = 0.46) on the TMS conditioned H-reflex amplitude, and there was also no significant "time" × "group" interaction ($F_{1,14}$ = 0.03, P = 0.87). The short latency facilitation effects on the H-reflex amplitude did not show any significant difference between pre and post in both groups.

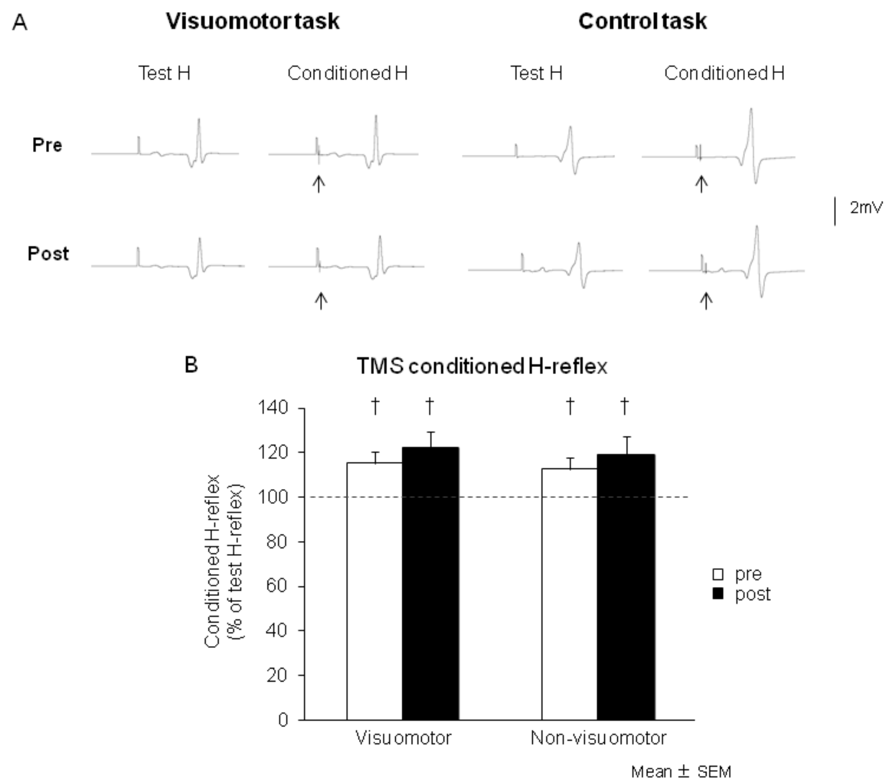

Figure 15. The effect of visuomotor task and control task on the transcranial magnetic stimulation (TMS) conditioned H-reflex at short latency facilitation phase. (A) Typical averaged waveforms of H-reflex (n = 10) in each stimulus condition were recorded from two representative subjects who performed a visuomotor task (left) or a control task (right). The arrows indicate the artifact of TMS stimulation. The conditioning stimulation of TMS was applied after the test H-reflex stimulation. (B) The graphs show the mean values of the TMS conditioned H-reflex amplitude at short latency facilitation phase in the visuomotor and non-visuomotor group. The ordinate shows the conditioned H-reflex amplitude expressed as a percentage of the test H-reflex amplitude. Values below 100% indicate inhibition and values above 100% indicate facilitation. Open and closed bars represent the time at which measurements were taken before (pre) and after (post) the motor task, respectively. The daggers (†) represent significant differences ($^{\dagger}P$

< 0.05) between conditioned H-reflex and baseline test H-reflex, which is shown by the dashed line. Error bar indicates SEM.

Following the motor tasks, the mean SOL Mmax was 16.68 ± 1.99 mV in the visuomotor group and 14.44 ± 1.38 mV in the non-visuomotor motor task group, and the mean Hmax/Mmax was 0.45 ± 0.04 in the visuomotor group and 0.49 ± 0.06 in the non-visuomotor group. There were no significant changes in the SOL Mmax ($t_7 = 0.99$, $P = 0.35$ in the visuomotor group; $t_7 = 0.96$, $P = 0.37$ in the non-visual group), and Hmax/Mmax ($t_7 = 2.10$, $P = 0.07$ in the visuomotor group; $t_7 = 0.29$, $P = 0.78$ in the non-visuaomotor group).

3.4 Discussion

The main findings of this study are that TMS conditioned inhibitory effects on the Ia presynaptic inhibitory pathway are changed following visuomotor tasks. These results may indicate that the changes in Ia presynaptic inhibition are attributed to the activity changes in the inhibitory interneurons that produce inhibitory effects on the Ia presynaptic inhibitory pathway.

3.4.1 Methodological consideration associated with TMS conditioning techniques

In this study, the effects of corticospinal descending inputs on the Ia presynaptic inhibitory pathway were investigated using TMS conditioning techniques. It has been demonstrated that conditioning TMS produces long latency facilitation (observed around 10–20 ms C-T intervals) of the H-reflex (Nielsen *et al.*, 1993b). Because the sensitivity of the H-reflex to the conditioning inputs depends on its size (Crone *et al.*, 1990), the difference in the test H-reflex size is likely to affect the changes in D1 inhibition. Thus, to avoid this test size effect, I adjusted TMS stimulus intensity to evoke minor facilitation on the test H-reflex when TMS was given alone. Moreover, I

observed that TMS conditioned H-reflex amplitude was not changed before and after the motor task, suggesting that test size effects could be negligible. By contrast, it is possible that the decrement of stimulation intensity might be inadequate to produce corticospinal descending volleys to the spinal cord. However, the short latency facilitation effects on the H-reflex amplitude in the adjusted stimulation intensity were observed in this study (Fig. 15). Therefore, it is reasonable to infer that corticospinal descending inputs induced by TMS would reach the spinal cord. Taking these results into account, it is considered that our procedures for measuring the effect of corticospinal descending inputs on the Ia presynaptic inhibitory pathway were appropriate.

3.4.2 Consideration of the muscle fatigue effects

Because muscle fatigue enhances central excitability at the supraspinal levels and changes Ia presynaptic inhibition (Duchateau & Hainaut, 1993), it is conceivable that observed results might be attributed to muscle fatigue. In human studies, muscle responses evoked by supramaximal peripheral nerve stimulation have been used as the index of muscle fatigue following exercise or electrical stimulation (Cupido *et al.*, 1996; Lentz & Nielsen, 2002). In this study, any significant changes in the SOL Mmax were not found before and after the motor task in any task conditions. The lack of changes in the Mmax suggests that muscle fatigue did not take place after the motor tasks. Moreover, the changes in Ia presynaptic inhibition were only observed after visuomotor task, although demanding muscle activity levels were almost the same for the control task (Fig. 11B). Therefore, it is argued that the observed modulations of Ia presynaptic inhibition are not caused by muscle fatigue.

3.4.3 Effects of corticospinal descending inputs on Ia presynaptic inhibition

In the present study, it is observed that Ia presynaptic inhibition was increased following a visuomotor task, but was unchanged by a control task. To determine the influence of descending inputs on the changes in Ia presynaptic inhibition following a visuomotor task, the TMS conditioning effect of Ia presynaptic inhibition were examined. The results showed that the inhibitory effect of Ia presynaptic inhibition induced by TMS was decreased following the visuomotor task, but not following the control task. This disinhibition was not caused by the excitability changes in the corticospinal tract or motoneuron pool; the short latency facilitation effect of TMS stimulation on the SOL H-reflex (Fig. 15) and Hmax/Mmax were not changed by the visuomotor task or the control task. These results suggest that the changes in Ia presynaptic inhibition observed in the present study are attributed to the activity changes in the interneurons that produce inhibitory effects on the Ia presynaptic inhibitory pathway.

Figure 16 shows an illustration of a circuits diagram that represents the possible site of changes induced by a visuomotor task. It has been shown that the corticospinal tract can inhibit the PAD of group Ia afferents by acting on the first order interneurons in the Ia presynaptic inhibitory pathways. This inhibition is accomplished by inhibitory interneurons which receive excitatory inputs from the corticospinal tracts. The reticulospinal tract, on the other hand, would inhibit the PAD of group Ia afferents by acting on the last order interneurons (Rudomin *et al.*, 1983). In skilled motor task, the corticospinal excitability is increased to control joint movements (Fromm & Evarts, 1977; Perez *et al.*, 2004), which might be essential both for producing neuroplastic changes in the spinal cord and for maintaining its changes (Wolpaw, 2007). Thus, the

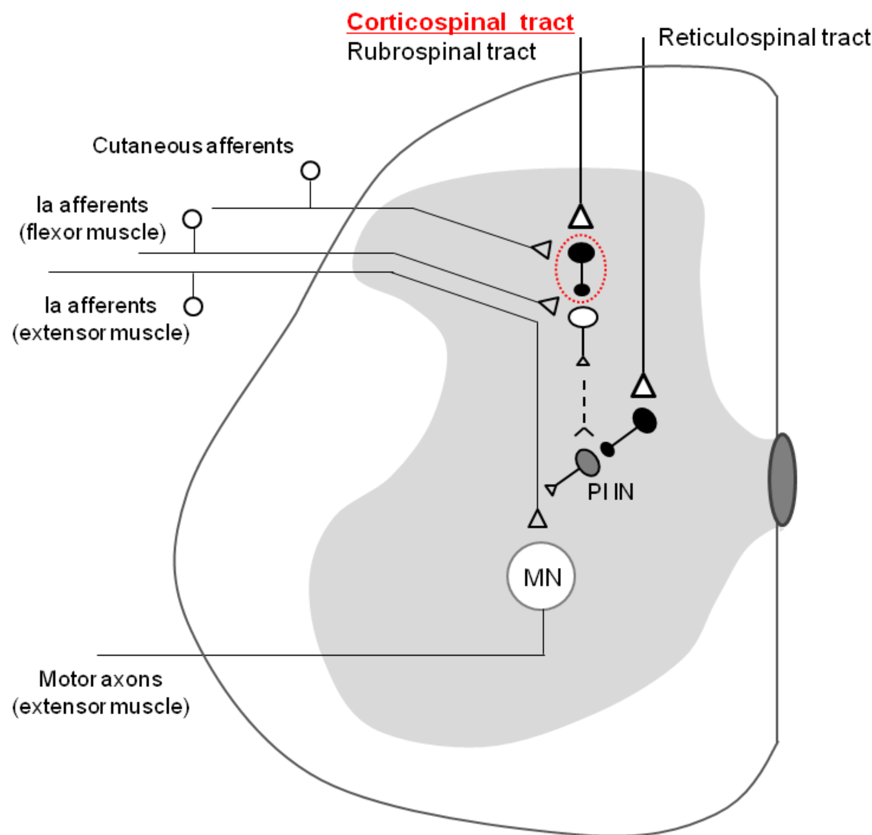

Figure 16. Illustration of a circuit diagram that shows the proposed site of changes induced by a visuomotor task. Interneurons, which produce inhibitory effects on the first order interneurons constituting Ia presynaptic inhibitory pathways, receive excitatory inputs from the corticospinal tract, rubrospinal tract, and the cutaneous afferents. Interneurons, which produce inhibitory effects on the last order interneurons constituting Ia presynaptic pathways, receive excitatory inputs from the reticulospinal tract. White circle indicates excitatory interneuron, gray circle indicates presynaptic inhibitory interneuron, and black circles indicate inhibitory interneurons. Open triangles indicate excitatory synaptic terminals. The red dashed circle indicates the proposed site of changes induced by a visuomotor task. IN, interneuron; MN, motor neuron; PI, presynaptic inhibition.

activity of Ia presynaptic inhibition is presumed to be inhibited during skilled motor task. In fact, however, Ia presynaptic inhibition is increased following a visuomotor task. Although the detailed mechanisms related to the changes in Ia presynaptic inhibition remain unknown, the increased Ia presynaptic inhibition could conceivably be explained by the reduction of inhibitory effects of interneurons activated by the corticospinal tract. However, those interneurons receive inputs from a number of other sources, such as rubrospinal fibers and cutaneous fibers (Rudomin *et al.*, 1983), and might show neuroplastic changes independent of corticospinal inputs.

3.5 Conclusion

The results of this study suggest that the reduction of corticospinal descending inhibitory effects on the Ia presynaptic inhibitory pathway are responsible for the changes in Ia presynaptic inhibition following skilled motor task.

CHAPTER 4
CHANGES IN THE SPINAL NEURAL CIRCUITS ARE DEPENDENT ON THE MOVEMENT SPEED OF THE VISUOMOTOR TASK

4.1 Introduction

Motor skill training has been shown to promote the reorganization of the spinal cord, which is driven by supraspinal descending and peripheral ascending inputs associated with motor tasks. In humans, spinal cord neuroplasticity has been inferred from modifications in the size of the H-reflex that is the electrical analog of the monosynaptic stretch reflex (Thompson & Wolpaw, 2014), and Ia presynaptic inhibition has been suggested to be related to changes in the H-reflex following motor skill training (Perez *et al.*, 2005a; Roche *et al.*, 2011a). Although several studies showed that Ia presynaptic inhibition is modulated by motor skill training, the effects of task movement speed on the changes in Ia presynaptic inhibition have not been clarified. Based on the fact that muscle spindle is sensitive to the velocity of muscle stretch (Poppele & Bowman, 1970; Cronin *et al.*, 2009), it is conceivable that Ia presynaptic inhibition of primary sensory fibers of the muscle spindle (group Ia afferent) is differently modulated, dependent on the task movement speed. Moreover, with increments of task movement speed, agonist/antagonist muscles have to switch their activity as quickly as possible to execute an alternating joint movement. Hence, in this situation it may be necessary to facilitate the spinal reciprocal Ia inhibitory circuit, because this circuit coordinates the contraction and relaxation of opposing sets of muscles (Geertsen *et al.*, 2011). Therefore, it appears reasonable to assume that the changes in these spinal neural circuits are dependent on the task movement speed, and it is hypothesized that Ia presynaptic inhibition and

reciprocal Ia inhibition will be increased following motor skill training performed at a fast movement speed.

The aim of this research is to investigate whether changes in spinal neural circuits are affected by task movement speed. To address this question, the amount of Ia presynaptic inhibition and reciprocal Ia inhibition were examined before and after the visuomotor task that was set to either slow or fast movement speed.

4.2 Methods

4.2.1 Subjects

Twenty-seven healthy subjects (age, 23.1 ± 3.1 years; mean ± SD) participated in this study after providing written informed consent. Baseline characteristics of participants are shown in Table 3. All experimental procedures were carried out in accordance with the Declaration of Helsinki and were approved by the Human Ethics Committee of the Graduate School of Integrated Arts and Sciences of Hiroshima University.

4.2.2 Electromyography recording

Experimental posutre and EMG recording set up were the same as that used in chapter 3.

4.2.3 Ia presynaptic inhibition and reciprocal Ia inhibition

The method for measuring Ia presynaptic inhibition and reciprocal Ia inhibition were the almost same as in chapter 2 & 3. It is summarized here, with several minor modifications noted. The amount of reciprocal Ia inhibition was determined from a

Table 3. Baseline characteristics of subjects (mean ± SD)

	Slow speed group	Fast speed group	Control group
Age	23.77 ± 2.86	23.0 ± 4.24	22.55 ± 2.45
Sex (Male/Female)	6/3	6/3	7/2
SOL Mmax (mV)	13.54 ± 3.31	14.75 ± 6.42	14.05 ± 4.32
Hmax/Mmax	0.53 ± 0.18	0.60 ± 0.19	0.59 ± 0.17

Mmax, maximum amplitude of M-wave; Hmax, maximum amplitude of H-reflex; SOL, soleus

short latency suppression of the SOL H-reflex by a conditioning stimulus to the CPN. The intensity of the conditioning stimulus was set to just above the MT intensity of the TA muscle (1.1 × MT). The CPN stimulus preceded the test stimulus at C-T intervals of 2 or 3 ms and the interval that produced the largest inhibition (either 2 or 3 ms) was used throughout the experiment. Conditioned and unconditioned H-reflexes were randomly evoked at 0.33 Hz.

4.2.4 Visuomotor task

The task program and the set up were the same as that used in chapter 3. Some modification points describe here. For a visuomotor task, subjects repeatedly moved their ankle between target lines according to auditory beep sounds delivered at a frequency of 1 Hz or 2.67 Hz (Fig. 17A). The target lines representing the point of 10° angle of ankle dorsiflexion and of 10° angle of plantarflexion from the neutral position were displayed on a monitor. The subjects were instructed to execute the ankle movement as precisely as possible between the target lines. Ankle angular displacements were measured with a goniometer that was mounted on the lateral side of the leg, and displayed as a cursor with line trajectory on the display monitor, to control ankle movement. The cursor automatically moved from the left to the right at 15 s. For a control task, subjects repeatedly moved their ankle according to auditory beep sounds delivered at a frequency of 2.67 Hz (fast movement speed) without visual feedback of ankle movement. Other task procedures used the same visuomotor task. The movement speed was defined as the time of one cycle of ankle movement; slow movement speed was set to 2000 ms, and fast movement speed was set to 750 ms. A single trial example of raw EMG activity of the TA and SOL muscle and ankle movement during each motor

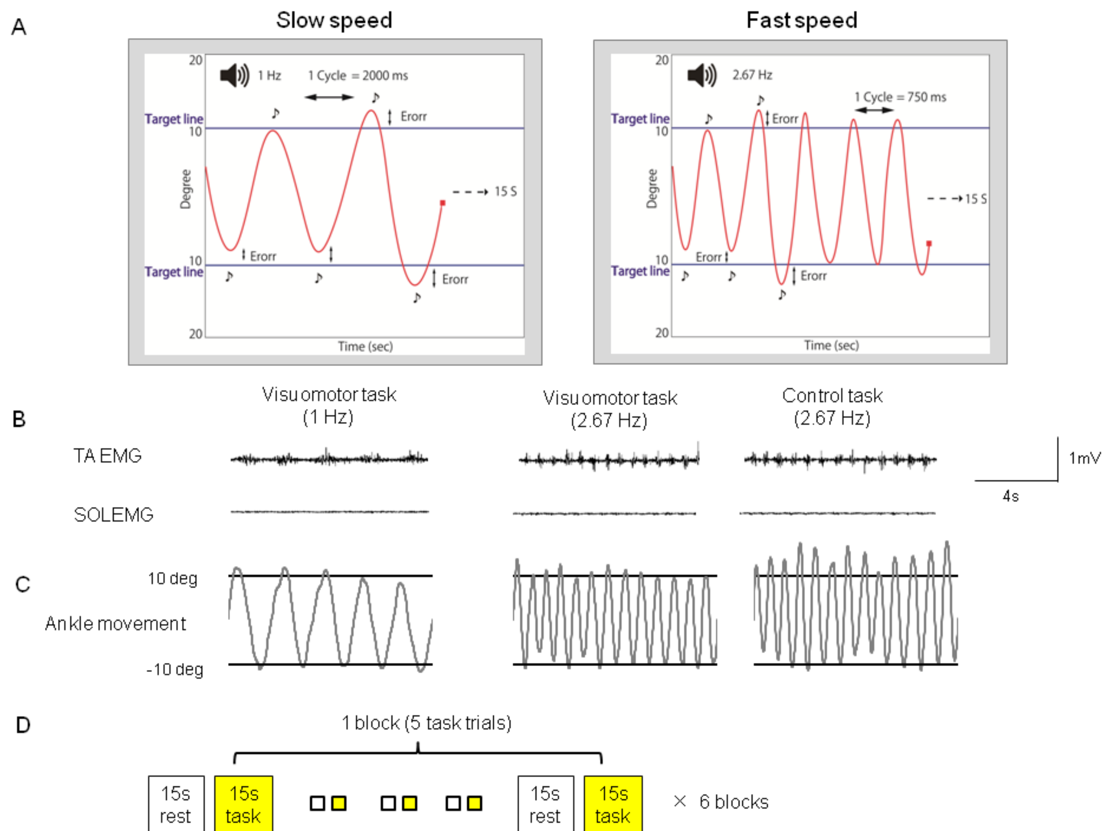

Figure 17. (A) Schematic of the visuomotor task at each rhythm 1 Hz and 2.67 Hz. (B) Example of raw electromyography (EMG) activity of tibialis anterior (TA) muscle and soleus (SOL) muscles during each motor task. (C) Example of the angle joint movement during each motor task, which corresponded to electromyography (EMG) activity of TA and SOL muscles. (D) Training procedure.

task is shown in Figure 17B–C. The subjects performed the motor task for 20 min. The task session consisted of six blocks with five trials (Fig. 17D). In order to minimize muscle fatigue, 15 s resting periods occurred between trials, and each block was separated by 1 min. All subjects were familiarized with the motor task before starting the task session. Motor performance was quantified by subtracting the target degree from the actual degree (10° angle of dorsi- and planter-flexion) at each inflection point (Fig. 17A). The difference was defined as an error and averaged for each trial. As the number of inflection points was different between the slow movement speed condition and fast movement speed condition, the average value was determined from six cycles in the slow and 16 cycles in the fast movement condition at each trial, respectively, which matched the task performance time (12 s). The error data were averaged for each block (mean error) and normalized by the value of the first block in order to confirm the rate of change in each task condition.

4.2.5 Experimental procedure

Twenty-seven subjects were randomly allocated to three different groups: slow speed group (n = 9), fast speed group (n = 9), and control group (n = 9). The subjects who were assigned to slow and fast speed groups performed a visuomotor task, and the control group subjects performed a control task for 20 min. The amount of D1 inhibition and reciprocal Ia inhibition were measured before (pre), 5 min after (post 5), 15 min after (post 15) and 30 min after (post 30) the task sessions. The Hmax/Mmax, which was used as an indicator of motor neuron pool excitability, were also tested before and immediately after the task session. Hmax and Mmax were evoked every 3 s and calculated from the average of five Hmax and five Mmax values.

4.2.6 Statistical analysis

The baseline characteristics of groups (age, SOL Mmax, and Hmax/Mmax) were analyzed using one-way ANOVA. The performance data compared among the task sessions used the one-way repeated measure of ANOVA for each group. Test H-reflex size was compared using two-way repeated measures of ANOVA with the factors "time" and "group." The two-way repeated measures of ANOVA with the factors "time" and "group" were used to evaluate the effects of movement speed of the visuomotor task on the D1 inhibition and reciprocal Ia inhibition. In addition, the amounts of D1 inhibition and reciprocal Ia inhibition were compared using one-way repeated measure of ANOVA in the control group. For multiple comparisons, if significant effects were detected, the Bonferroni post hoc test was used. Mauchley's test was used to examine for sphericity. The Greenhouse-Geisser correction was used for non-spherical data. Moreover, the differences in the Hmax/Mmax and SOL Mmax were compared between before and after the task within the group, using the paired *t*-test. P values of < 0.05 were considered significant in all statistical analyses. Data were analyzed using SPSS version 22 software (IBM SPSS, IBM Japan, Ltd., Tokyo, Japan). The data values are presented as the means ± SEM.

4.3 Results

Baseline characteristics of subjects among groups were well-matched (Table 3), and there were no significant differences between all baseline measures (age: $F_{2,24} = 0.32$, $P = 0.73$; SOL Mmax: $F_{2,24} = 0.13$, $P = 0.87$; Hmax/Mmax: $F_{2,24} = 0.36$, $P = 0.69$).

4.3.1 Changes in the task performance

The mean times of one cycle of ankle movement almost matched the pre-setting times in each group, indicating that the task movement speeds were well controlled throughout the experiment (Slow speed group = 1946.43 ± 5.97 ms; Fast speed group = 742.37 ± 2.36 ms; Control group = 733.77 ± 5.54 ms). Figure 18 shows the time course of the mean errors of motor tasks. A one-way repeated measures of ANOVA revealed a significant effect of blocks for slow and fast speed groups (Slow: $F_{5,40} = 9.08$, $P < 0.01$; Fast: $F_{5,40} = 21.84$, $P < 0.01$). In post hoc tests, a significant reduction in the mean errors was observed between the first block and five ($P = 0.04$) and sixth ($P = 0.01$) blocks in the slow speed group. Similarly, a significant reduction in the mean errors was observed between the first and fourth blocks ($P = 0.01$), between the first and fifth ($P = 0.02$) and sixth ($P < 0.01$) blocks in the fast-speed groups. These results indicated that the task performance was certainly improved in both groups. However, there were no significant differences in the mean errors across the blocks in the control group ($F_{5,40} = 1.49$, $P = 0.21$).

4.3.2 Effects of task movement speed on D1 inhibition and reciprocal Ia inhibition

The mean amplitudes of the test H-reflex (% of Mmax) for all conditions are summarized in Table 4–5. The test H-reflex amplitude was almost the same size throughout experiment procedures. There were no significant effects of “time” (D1 inhibition: $F_{3,72} = 0.95$, $P = 0.41$; reciprocal Ia inhibition: $F_{3,72} = 1.48$, $P = 0.23$) and “group” (D1 inhibition: $F_{2,24} = 0.78$, $P = 0.46$; reciprocal Ia inhibition: $F_{2,24} = 0.35$, $P = 0.71$) on the test H-reflex amplitude. Likewise, there was also no significant “time”

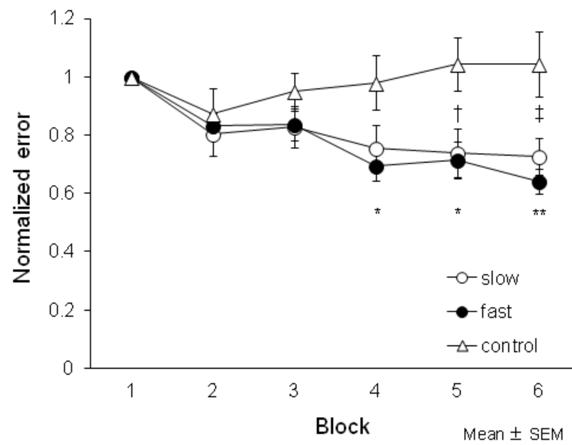

Figure 18. The changes in the motor performance in slow-speed, fast-speed, and control groups

The graph shows the time course of the changes in the motor performance in the slow, fast, and control groups. The ordinate shows mean error values normalized by the value of the first block. The abscissa shows each block. The dagger (†) represents significant difference ($^{\dagger}P < 0.05$) between the first block and fifth block, and the double dagger (‡) represent significant difference ($^{\ddagger}P < 0.01$) between the first block and six block in the slow-speed group. The asterisks (*) represents significant difference ($^{*}P < 0.05$) between the first block and fourth and fifth blocks, and the double asterisks (**) represent significant difference ($^{**}P < 0.01$) between the first block and sixth block in the fast-speed group. Error bar indicates SEM.

Table 4. Summary of test H-reflex amplitude in D1 inhibition (% of Mmax)

	D1 inhibition			
	Pre	Post 5	Post 15	Post 30
Slow speed group	26.79 ± 0.85	25.27 ± 1.04	27.11 ± 1.07	25.28 ± 0.56
Fast speed group	25.76 ± 0.92	25.31 ± 1.12	24.35 ± 1.47	25.19 ± 0.85
Control group	25.99 ± 0.92	25.16 ± 0.91	25.15 ± 0.65	24.54 ± 1.16

Values: mean ± SEM. Mmax, maximum amplitude of M-wave; Pre, before the task sessions; Post 5, 5 min after the task sessions; Post 15, 15 min after the task sessions; Post 30, 30 min after the task sessions.

Table 5. Summary of test H-reflex amplitude in reciprocal Ia inhibition (% of Mmax)

	Reciprocal Ia inhibition			
	Pre	Post 5	Post 15	Post 30
Slow speed group	25.17 ± 0.60	25.69 ± 0.85	25.83 ± 1.01	25.40 ± 0.78
Fast speed group	25.43 ± 0.97	25.59 ± 1.05	23.34 ± 0.8	25.19 ± 0.85
Control group	25.37 ± 0.92	25.97 ± 0.74	24.67 ± 0.68	23.71 ± 1.09

Values: mean ± SEM. Mmax, maximum amplitude of M-wave; Pre, before the task sessions; Post 5, 5 min after the task sessions; Post 15, 15 min after the task sessions; Post 30, 30 min after the task sessions.

×"group" interaction (D1 inhibition: $F_{3,72} = 0.54$, $P = 0.78$; reciprocal Ia inhibition: $F_{3,72} = 1.18$, $P = 0.33$).

4.3.3 Visuomotor task

The effects of the movement speed of a visuomotor task on the D1 inhibition and reciprocal Ia inhibition are shown in Figure 19. The D1 inhibition increased after visuomotor task irrespective of task movement speed. Meanwhile, the reciprocal Ia inhibition was affected by the movement speed of the visuomotor task and only increased after performing the visuomotor task in the fast movement speed condition. A two-way repeated measures of ANOVA for D1 inhibition showed a significant effect of the "time" ($F_{2.06,32.99} = 14.84$, $P < 0.01$), but not of the "group" ($F_{1,16} < 0.01$, $P = 0.97$). There was no significant "time" × "group" interaction ($F_{2.06,32.99} = 1.16$, $P = 0.33$). Post hoc analysis of the "time" factor indicated that compared to pre, the amount of D1 inhibition was significantly increased in post 5 min ($P < 0.01$) and in post 15 min ($P < 0.01$). A two-way repeated measures of ANOVA for reciprocal Ia inhibition showed a significant effect of the "time" ($F_{3,48} = 4.82$, $P < 0.01$), but not of the "group" ($F_{1,16} = 2.01$, $P = 0.18$). Moreover, there were also significant "time" × "group" interactions ($F_{3,48} = 3.49$, $P = 0.02$). Post hoc analysis indicated that, in the fast-speed group, the amount of reciprocal inhibition was significantly increased in post 5 min—there were significant difference between pre and post 5 min ($P = 0.01$), post 5 min and post 15 min ($P < 0.01$), and post 5 min and post 30 min ($P = 0.01$). The amount of reciprocal Ia inhibition was also significantly different between slow and fast speed groups in the post 5 min time period ($P = 0.03$).

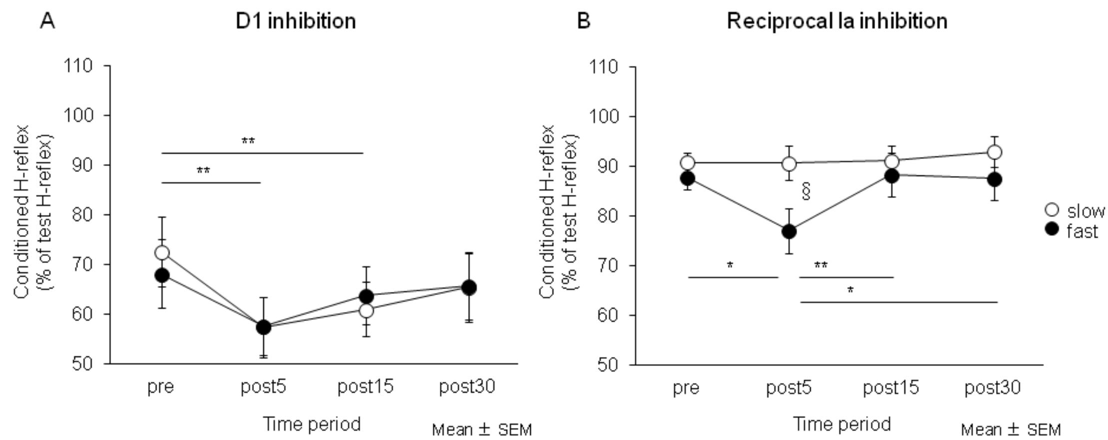

Figure 19. Effects of the movement speed of visuomotor task on the D1 inhibition and reciprocal Ia inhibition. The graphs show the mean values of the D1 inhibition (A) and reciprocal Ia inhibition (B) in the slow- and fast-speed groups. The ordinate indicates the conditioned H-reflex amplitude expressed as a percentage of the test H-reflex amplitude. The abscissa shows the times at which measurements were taken [before (pre), 5 min after (post 5), 15 min after (post 15), and 30 min after (post 30) the visuomotor task]. Open circles represent the slow-speed group and closed circles represent the fast-speed group. Values below 100% indicate inhibition and values above 100% indicate facilitation. The asterisks (*) and the double asterisks (**) represent significant differences (*$P < 0.05$) and (**$P < 0.01$), respectively. The section marked (§) represents a significant difference ($P < 0.05$) between slow- and fast-speed group in the post 5 time period. Error bar indicates SEM.

The mean SOL Mmax after the motor task was 13.86 ± 1.18 mV in the slow-speed group and 14.84 ± 2.09 mV in the fast-speed group. There were no significant differences in the SOL Mmax between pre and post in both groups ($t_8 = 1.22$, $P = 0.26$ in slow-speed group; $t_8 = 0.40$, $P = 0.69$ in fast-speed group). The mean Hmax/Mmax after the motor tasks was 0.51 ± 0.07 in the slow-speed group and 0.58 ± 0.07 in the fast-speed group. There were also no significant differences in the Hmax/Mmax between pre and post in both groups ($t_8 = 1.45$, $P = 0.19$ in the slow-speed group; $t_8 = 1.07$, $P = 0.32$ in the fast-speed group).

4.3.4 Control task

The effects of the control task on the D1 inhibition and reciprocal Ia inhibition are shown in Figure 20. There were no significant differences in the amount of D1 inhibition ($F_{3,24} = 0.64$, $P = 0.57$) and reciprocal Ia inhibition ($F_{3,24} = 1.10$, $P = 0.37$). The mean SOL Mmax after the control task was 14.02 ± 1.33 mV and the mean Hmax/Mmax after the control task was 0.55 ± 0.05. There were also no significant changes in the SOL Mmax ($t_8 = 0.08$, $P = 0.94$), and Hmax/Mmax ($t_8 = 1.66$, $P = 0.14$) following the control task.

4.4 Discussion

The main findings of this study suggest that, (i) the amount of Ia presynaptic inhibition is increased after visuomotor tasks irrespective of task movement speed, (ii) changes in reciprocal Ia inhibition are affected by task movement speed, and are increased in fast movement speed conditions, but unchanged in slow movement speed conditions, (iii) control tasks do not induce any changes in Ia presynaptic inhibition and reciprocal Ia

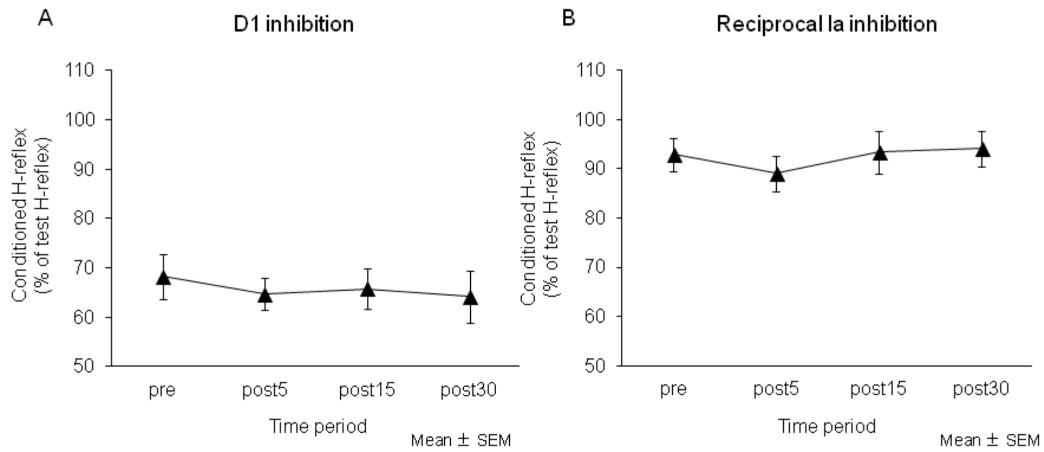

Figure 20. Effects of the control task on the D1 inhibition and reciprocal Ia inhibition. The graphs show the mean values of the D1 inhibition (A) and reciprocal Ia inhibition (B) in the control group. The ordinate shows the conditioned H-reflex amplitude expressed as a percentage of the test H-reflex amplitude. The abscissa shows the time at which measurements were taken [before (pre), 5 min after (post 5), 15 min after (post 15), and 30 min after (post 30) the visuomotor task]. Values below 100% indicate inhibition and values above 100% indicate facilitation. Error bar indicates SEM.

inhibition.

4.4.1 Effects of task movement speed on presynaptic inhibition

The present study observed that Ia presynaptic inhibition was increased following a visuomotor task irrespective of task movement speed, but was unchanged by a control task. Moreover, it was also observed the improvement of the task performance among the trials in the slow and fast movement speed conditions. The increase in the presynaptic inhibition of SOL Ia afferent terminals following a visuomotor task was consistent with the results of a previous investigation which demonstrated that Ia presynaptic inhibition was increased after visuomotor tracking tasks involving alternating ankle movement (Perez *et al.*, 2005a). Ia presynaptic inhibition is a neural mechanisms dedicated to the adjustment of proprioceptive sensory gain during the execution of movement. It has been reported that excessive proprioceptive inputs arising from muscle spindle afferents result in deficits in smooth limb movement and elicit motor oscillation (Fink *et al.*, 2014). Thus, because muscle spindle response increases with increasing the velocity of muscle stretch (Poppele & Bowman, 1970; Bosco & Poppele, 1999), it is hypothesized that Ia presynaptic inhibition will be increased following motor training performed at a fast movement speed, but not in a slow movement state. However, contrary to my hypothesis, the speed-dependent modulation of Ia presynaptic inhibition was not observed in this study. Therefore, the changes in Ia presynaptic inhibition have little to do with the task movement speed. Previous studies have shown that Ia presynaptic inhibition is decreased after performing visoumotor force tracking tasks with thumb and index finger in isometric condition (Roche *et al.*, 2011a) or after isometric strength training on the ankle dorsiflexor muscles (Jessop *et al.*, 2013). The fact that Ia presynaptic inhibition was increased following only the

visuomotor tasks involving dynamic joint movement indicates that descending inputs from corticospinal tract for controlling joint movement might be one of the essential factors to induce the potentiation of Ia presynaptic inhibition. It has been demonstrated that interneurons mediating Ia presynaptic inhibition are controlled by supraspinal centers (Jankowska, 1992), and that the stimulation of the corticospinal tract increases or decreases PAD in muscle afferents (Rudomin, 1990; Meunier & Pierrot-Deseilligny, 1998). Therefore, as mentioned in chapter 3, it is speculated that the changes in the Ia presynaptic inhibition observed herein probably result from modifications of the inhibitory interneurons that produce inhibitory effects on the Ia presynaptic inhibitory pathway, and corticospinal descending inputs play a major role in driving neuroplastic changes in the interneurons.

Several studies have reported that H-reflex amplitude is decreased following skilled motor tasks (Perez *et al.*, 2005a; Mazzocchio *et al.*, 2006; Lungu *et al.*, 2010). However, in this study any changes in the H-reflex amplitude were not observed before and after the visuomotor task. The discrepancy between the changes in the H-reflex observed by this study and the previous studies is probably due to the difference in the motor task used in the study. In the previous studies, the muscle that assessed H-reflex was activated as an agonist during the task. By contrast, in this study, the subjects performed alternating ankle dorsal and plantarflexion movements against gravity so that the ankle dorsiflexion muscles acted as the prime movers for controlling ankle joints. Thus, the antagonist muscle was activated mainly, and the activation of the test muscle was minor (Fig. 17B). Previous studies have reported that homosynaptic depression that reduces synaptic efficacy at the synapse between Ia afferent and motor neurons may be

responsible for the decrease of the H-reflex after the skilled motor task (Mazzocchio *et al.*, 2006; Meunier *et al.*, 2007). Because homosynaptic depression has been suggested to be influenced by the pattern and magnitude of the incoming proprioceptive inputs (Meunier *et al.*, 2007), the difference in the performing task might be attributed to the different results found in the present and previous studies.

4.4.2 Effects of task movement speed on reciprocal Ia inhibition

Training-related changes in reciprocal Ia inhibition have been studied extensively in human subjects, and these studies have shown that there is also task dependency of changes in reciprocal Ia inhibition. For example, the facilitation effect of the reciprocal Ia inhibitory pathway at the onset of ankle dorsiflexion was increased following 4 weeks of explosive isometric dorsiflexion strength training (Geertsen *et al.*, 2008). However, short-term isometric or isotonic strength training on ankle dorsal and planter flexor muscles decreased the reciprocal Ia inhibition (Jessop *et al.*, 2013). Similar results were reported after performing force tracking tasks that required exerting an isometric force between the thumb and index finger (Roche *et al.*, 2011a). Moreover, reciprocal Ia inhibition did not change after visuomotor tracking tasks involving alternating ankle movement (Perez *et al.*, 2005a). In this study, the amount of reciprocal Ia inhibition directed from TA to SOL was only increased when subjects performed a visuomotor task in the fast movement speed condition, but remained unchanged when the subjects performed a visuomotor task in the slow movement speed condition and the control task. These findings suggest that changes in reciprocal Ia inhibition on the ankle muscles have something to do with the task movement speed.

The Ia inhibitory interneurons receive descending inputs from the corticospinal tracts that are likely to influence the Ia inhibitory interneuron excitability (Jankowska *et al.*, 1976; Kasai & Komiyama, 1988; Kubota *et al.*, 2014). Therefore, it could be hypothesized that the increase of reciprocal Ia inhibition observed in this study is due to the modification of the excitability of Ia inhibitory interneurons induced by corticospianl descending inputs. Previous studies showed that the responses of corticomotoneuronal cells vary depending on type of movement (Fromm & Evarts, 1977), and that the activities of these cells are strong during controlled ramp-and-hold movement, compared with their activities during rapid alternating movement (Cheney & Fetz, 1980). However, the results in this study also showed that performing the skilled motor task, itself, could not induce the increment in the strength of reciprocal Ia inhibition. Therefore, the enhancement of the reciprocal Ia inhibition could not be explained solely by the difference in the descending inputs from the corticospinal tract. The activation of Ia interneurons contributes to the hyperpolarization of target motor neurons (Geertsen *et al.*, 2011), suggesting that Ia inhibitory interneurons play an important role in determining the coordination of intralimb flexor-extensor activity (Cowley & Schmidt, 1995). This may be because the reciprocal Ia inhibitory pathway needs to be facilitated in order to achieve alternating rapid movement. In support of this concept, the increase of reciprocal Ia inhibition was only observed following a visuomotor task in the fast movement speed condition. Taking these results into account, it is considered that both the central descending drive for controlling joint movement and task movement speed are important in driving activity changes in reciprocal Ia inhibition.

4.4.3 Time course effects of the visuomotor task on spinal neural circuits

The changes in the Ia presynaptic inhibition and reciprocal Ia inhibition observed in this study were short lasting after the end of a visuomotor task; the increase in Ia presynaptic inhibition lasted up to 15 min and the increase in reciprocal Ia inhibition lasted up to 5 min after the motor task. These temporary modifications of spinal neural circuits almost consist with the previous reports (Perez *et al.*, 2005a; Roche *et al.*, 2011a). Previous studies have shown that structural change in the spinal cord (e.g., axonal sprouting or neurogenesis) occurs gradually over weeks (after 10–12 sessions) (Thompson *et al.*, 2009; Thompson *et al.*, 2013b). Therefore, it is speculated that the observed changes in this study may reflect the early process of activity dependent neuroplasticity in the spinal cord (e.g., changes in the efficacy of synaptic transmission or changes in the supraspinal descending influences), but not the structural changes.

4.5 Conclusion

The results of this study suggest that the supraspinal descending inputs to the spinal cord for controlling joint movement are responsible for changes in Ia presynaptic inhibition, and that task movement speed is one of the critical factors for inducing activity changes in reciprocal Ia inhibition. These results indicate that spinal neural circuits are differentially modulated, dependent on motor tasks, for achieving the task demands. These task-dependent modulations might be related to the precise control of our limb movements.

CHAPTER 5

PATTERNED SENSORY NERVE STIMULATION ENHANCES THE REACTIVITY OF SPINAL IA INHIBITORY INTERNEURONS

5.1 Introduction

Reciprocal Ia inhibition has been established as an important neural mechanism for controlling the coordinated contraction of an agonist muscle and relaxation of an antagonist muscle, and the activity of this spinal circuit was shown to be cyclically modulated in phase-dependent manners (Petersen *et al.*, 1999; Pyndt *et al.*, 2003). The reciprocal Ia inhibition from the ankle flexor to the extensor during walking was suggested to be higher in the swing phase than in the stance phase (Petersen *et al.*, 1999). A previous study using cats reported that flexor-coupled Ia inhibitory interneurons, which convey reciprocal Ia inhibition to the extensor muscle, were more active when their target motor neurons were silent, and suggested that activation of Ia interneurons contributed to the hyperpolarization of target motor neurons (Geertsen *et al.*, 2011). These findings indicated that Ia inhibitory interneurons play a crucial role in switching from the extension phase to flexion phase during locomotion.

Patterned electrical nerve stimulation (PES) resembling sensory feedback from the ankle flexor muscle while walking has been suggested to reinforce the reciprocal Ia inhibitory circuit (Perez *et al.*, 2003; Fujiwara *et al.*, 2011). Since increments in corticospinal excitability induced by non-invasive brain stimulation, such as repetitive TMS or transcranial direct current stimulation, did not increase the strength of reciprocal Ia inhibition (Perez *et al.*, 2005b; Roche *et al.*, 2011b), afferent input from the

ankle flexor muscle is critical for reinforcing this circuit. Although PES has been reported to induce changes in the reciprocal Ia inhibitory circuit, the mechanisms remain largely unknown. Activity-dependent changes in the efficacy of the synaptic transmission (long-term potentiation and long-term depression) are known to develop at the spinal level (Pockett & Figurov, 1993); therefore, it has been hypothesized that changes in the reciprocal Ia inhibitory circuit may be attributed to long-lasting increases in the efficacy of synaptic transmission on Ia afferents to Ia interneurons (i.e., enhancement of transmitter release at the synapse). To address this question, the present study aimed to determine whether changes in the reactivity of supraspinal descending inputs to Ia interneurons could be induced by PES. A previous study reported that motor cortex stimulation produced EPSPs and IPSPs in the spinal motor neurons, and IPSPs always started approximately 1.2 ms later than EPSPs (probably mediated by Ia inhibitory interneuron) (Landgren *et al.*, 1962). In support of this finding, conditioning TMS was shown to produce short-latency facilitation of the test H-reflex (Nielsen *et al.*, 1993b). This facilitation was followed by decrements in the H-reflex (Nielsen *et al.*, 1993b; Kato *et al.*, 2002). Hence, I used the TMS-conditioned H-reflex method to evaluate the influence of supraspinal descending inputs to Ia interneurons.

5.2 Methods

5.2.1 Subjects

Ten (nine males, one female) healthy right-handed individuals (25.1 ± 2.9 years; mean ± SD) participated in our study after providing written informed consent. Nine out of ten participants reported that they preferred to kick a ball with their right foot. Our study was approved by the Human Ethics Committee of the Graduate School of Integrated

Arts and Sciences of Hiroshima University, and the experimental procedures were in accordance with the Declaration of Helsinki.

5.2.2 Electromyography recording

Experimental posutre and EMG recording set up were the same as that used in chapter 2.

5.2.3 Experimental procedures

In order to induce activity changes in the reciprocal Ia inhibitory circuit, patterned electrical stimulation was applied to the CPN every 1 sec for 15 min. An electrical current was delivered using another constant current isolator coupled with an electrical stimulator through a bipolar electrode placed 1–3 cm distal to the head of the fibula. The stimulus pattern comprised a train of five pulses at 100 Hz. The pulse width was 1 ms in duration, and the stimulus intensity was the MT intensity of the TA muscle (Fig. 21). The stimulation electrode was positioned carefully in order to avoid activating the peroneus muscles. Reciprocal Ia inhibition, the TMS-conditioned SOL H-reflex amplitude, and the Hmax/Mmax were recorded before (pre), immediately after (post), and 15 minutes (post15) after PES.

5.2.4 Conditioning stimulation

The method for measuring reciprocal Ia inhibition, Ia presynaptic inhibition, and TMS conditioning effect on the H-reflex were the almost same as in chapter 2 & 3. It is summarized here, with several minor modifications noted. The SOL H-reflex was

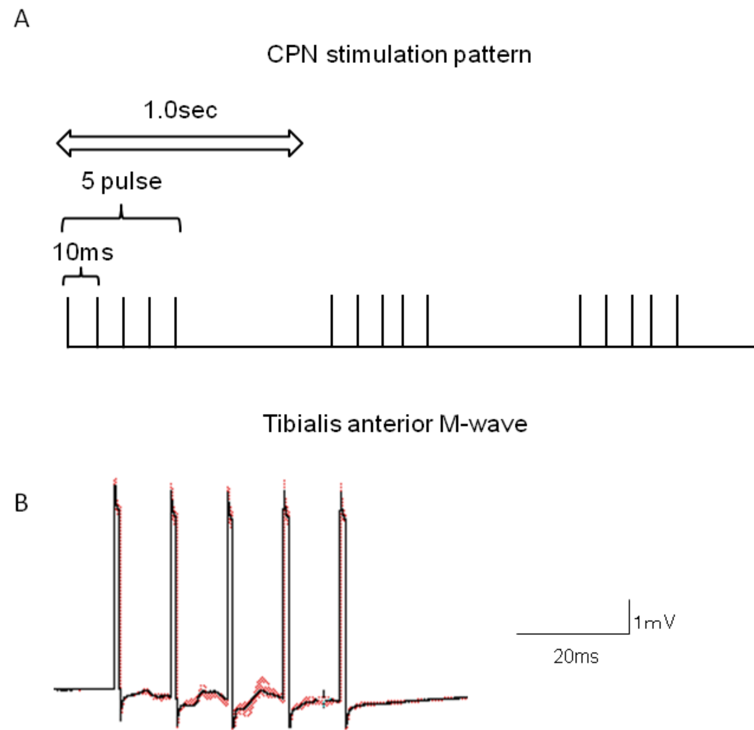

Figure 21. Patterned electrical nerve stimulation (PES). (A) Stimulation pattern of common peroneal nerve (CPN). (B) The typical waveform of tibialis anterior (TA) M-wave. Five representative M-waves are shown in red, with the average M-wave trace in black.

assessed by conditioning stimulation of CPN. The conditioning stimulus strength was set at 1.0 × MT. The amount of reciprocal Ia inhibition was determined via conditioning stimulation of CPN to induce short-latency suppression of the SOL H-reflex. The C-T interval for assessing reciprocal Ia inhibition was varied from 1 to 3 ms in 1-ms steps. The interval that produced the largest inhibition (either 2 or 3 ms) was used throughout the experiment. Also, the amount of Ia presynaptic inhibition was determined via conditioning stimulation of CPN to induce long-latency suppression of the SOL H-reflex. The CPN was stimulated with a train of three single pulses at 333 Hz. The time interval between CPN stimulation (first shock of a train of three shocks) and test stimulation was kept constant at 21 ms. Conditioned and unconditioned H-reflexes were randomly evoked at 0.33 Hz. Ten conditioned and ten unconditioned H-reflexes were recorded at each C-T interval, and the conditioned H-reflex amplitude was expressed as the percentage of the unconditioned H-reflex amplitude.

In order to examine the effects of corticospinal descending inputs on Ia inhibitory interneurons, the test H-reflex was conditioned by TMS. TMS was delivered through a double-cone coil connected to a magnetic stimulator. The optimal stimulus position for eliciting MEPs in the right SOL muscle with a weak contraction was determined as the area in which TMS at a slightly suprathreshold intensity regularly produced the largest MEPs. The active MT was defined as the lowest stimulus intensity that produced MEPs of at least 200 μV in the SOL in three out of five trials (Rothwell et al., 1999). The intensity of TMS was adjusted to 0.95 × active MT in order to avoid muscle responses in the SOL and TA. The SOL H-reflex was conditioned using various C-T intervals (−3, −2, −1, and 0 ms). Negative C-T intervals indicated that the conditioning stimulus was

applied after the test stimulation. The SOL H-reflex was evoked every 6 seconds.

In order to determine whether changes in reciprocal Ia inhibition occurred at Ia inhibitory interneurons, Hmax/Mmax was used as an indicator of motoneuron pool excitability. Hmax/Mmax was recorded in nine subjects. Hmax and Mmax were evoked every 3 seconds and calculated from the average of five Hmax and five Mmax.

5.2.5 Statistical analysis

The test H-reflex size was compared using a one-way repeated-measures ANOVA. In reciprocal Ia inhibition, the conditioned H-reflex was compared to the unconditioned H-reflex using a one-sample paired *t*-test. A one-way repeated-measures ANOVA was used to analyze the effects of PES on reciprocal Ia inhibition, D1 inhibition, and Hmax/Mmax. A two-way repeated-measures ANOVA with factors of “C-T interval” and “Time” was used to evaluate the effects of PES on the TMS-conditioned H-reflex amplitude. If significant effects were detected, the Bonferroni post-hoc test was used for multiple comparisons. Mauchley's test was used to examine for sphericity. The Greenhouse-Geisser correction was used for non-spherical data. P values of < 0.05 were considered significant in all statistical analyses. Data are presented as the mean ± SEM.

5.3 Results

The amplitude of the test H-reflex (% of Mmax) was shown in Table 6. No significant differences were observed in the test H-reflex amplitude between the CPN stimulation ($F_{2,18} = 0.59$, $P = 0.59$) and TMS stimulation ($F_{2,28} = 1.04$, $P = 0.37$). The mean TMS stimulus intensity was 44.54 ± 4.67 (% of the maximum SO).

The degree of reciprocal Ia inhibition and TMS-conditioned H-reflex are shown in Figure 22 A–B. Although the conditioned H-reflex amplitude was weaker than that of the unconditioned H-reflex, a significant difference was only observed between the conditioned H-reflex and unconditioned H-reflex at post ($P < 0.01$) and post15 ($P = 0.02$). Consistent with previous findings, reciprocal Ia inhibition was increased by PES (pre: 92.88 ± 4.52, post: 81.06 ± 3.76, post15: 87.90 ± 2.92 % of test H-reflex, $F_{2,18} = 3.65$, $P = 0.04$). Post-hoc tests revealed a significant difference between pre and post ($P = 0.04$). Furthermore, PES down-regulated the amplitude of the TMS-conditioned H-reflex at the −1 ms interval (pre: 110.85 ± 7.64, post: 99.33 ± 6.32, post15: 110.84 ± 6.82 % of Mmax) in the absence of changes in the other C-T intervals. The two-way repeated measures ANOVA showed a significant main effect of the H-reflex amplitude for the “C-T interval” ($F_{3,81} = 6.29$, $P < 0.01$). In addition, there was no significant “C-T interval” ×“Time” interaction ($F_{6,81} = 0.49$, $P = 0.81$). The Post hoc test revealed that the H-reflex amplitude at −1 ms was significantly different between pre and post ($P = 0.03$) and post and post15 ($P = 0.03$). Figure 23 shows the effects of PES on the D1 inhibition and Hmax/Mmax. No significant differences were observed in the D1 inhibition (pre = 77.15 ± 5.0, post: 77.97 ± 6.72, post15: 67.23 ± 6.36 % of test H-reflex, $F_{2,18} = 1.67$, $P = 0.21$) and Hmax/Mmax (pre: 0.54 ± 0.06, post: 0.51 ± 0.06, post15: 0.51 ± 0.5, $F_{2,16} = 1.35$, $P = 0.28$).

5.4 Discussion

In the present study, it was confirmed that PES increased the amount of reciprocal Ia inhibition, which is consistent with previous findings (Perez *et al.*, 2003; Fujiwara *et al.*, 2011). In addition to increments in reciprocal Ia inhibition, it was found that the

Table 6. Summary of test H-reflex amplitude (% of Mmax: mean ± SEM)

	Pre	Post	Post15
CPN stimulation	25.81 ± 1.14	24.75 ± 1.26	26.10 ± 0.96
TMS stimulation	27.96 ± 1.16	26.75 ± 1.24	26.26 ± 0.83

CPN, common peroneal nerve; Mmax, maximum amplitude of M-wave; Pre, before the task sessions; Post , after the task sessions; Post 15, 15 min after the task sessions; TMS: transcranial magnetic stimulation

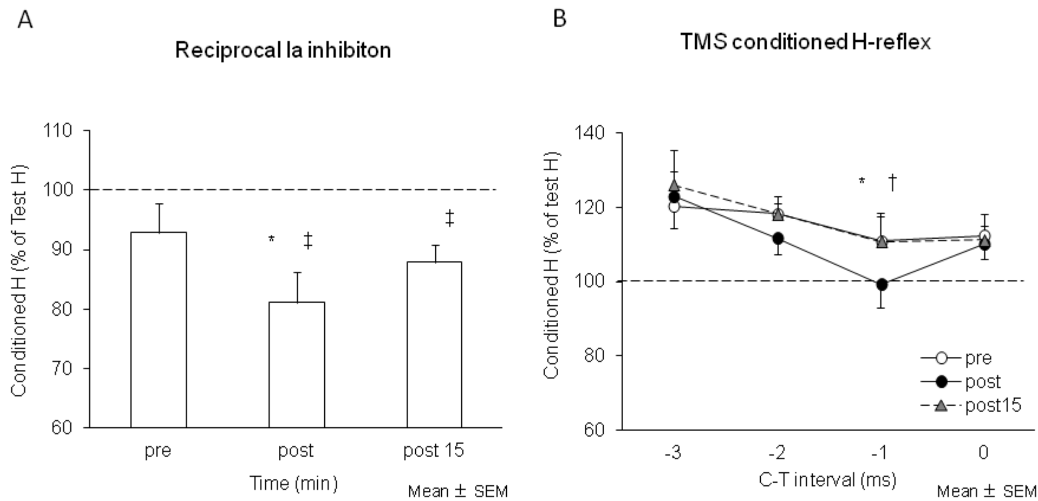

Figure 22. Effects of patterned electrical nerve stimulation (PES) on Ia inhibitory interneurons. The graphs show the effect of PES on reciprocal Ia inhibition (A) and the TMS-conditioned H-reflex (B) from all subjects (n = 10). The ordinate shows the conditioned H-reflex amplitude expressed as a percentage of the test H-reflex amplitude. In A, the abscissa shows the time at which measurements were taken [before (pre), immediately after (post), and 15 minutes (post15) after PES]. In B, the abscissa shows the intervals between the conditioning stimulation and test stimulation in milliseconds (C-T interval). Open circles, closed circles, and triangles indicate pre, post, and post15 conditions, respectively. The asterisks (*) represent significant differences ($^{*}P < 0.05$) between pre and post conditions and the daggers (†) represent significant differences ($^{\dagger}P < 0.05$) between post and post15 conditions. The double daggers (‡) represent significant differences ($^{\ddagger}P < 0.05$) between the conditioned H-reflex and the baseline test H-reflex which is shown by the dashed line. Error bar indicates SEM.

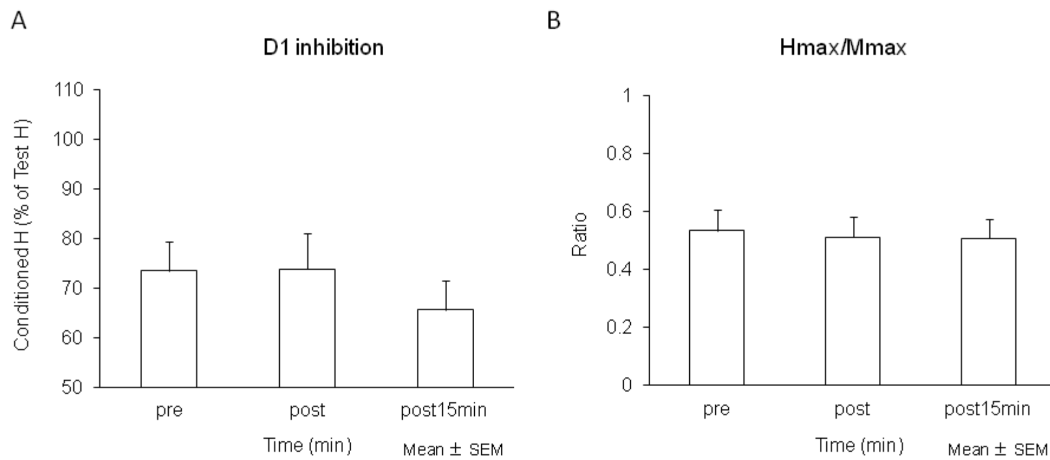

Figure 23. Effects of patterned electrical nerve stimulation (PES) on the D1 inhibition (A) and the ratio of the maximum H-reflex amplitude versus maximum M-wave (Hmax/Mmax) (B). The graph shows group mean values (n = 9). In A, the ordinate shows the conditioned H-reflex amplitude expressed as a percentage of the test H-reflex amplitude. In B, the ordinate shows the values of Hmax/Mmax. The abscissa shows the time at which measurements were taken [before (pre), immediately after (post), and 15 minutes after PES (post15)]. No significant effect of PES was found for D1 inhibition and Hmax/Mmax. Error bar indicates SEM.

amplitude of the TMS-conditioned H-reflex at the −1 ms C-T interval was decreased by PES, and this was not concomitant with changes in D1 inhibition and Hmax/Mmax. Therefore, the results indicate that sensory inputs induced by PES can not cause changes in the Ia presynaptic inhibition. It was hypothesized that the TMS-conditioned H-reflex may not be changed by PES because changes in reciprocal Ia inhibition were attributed to the modulation of synaptic transmission on Ia afferents to Ia interneurons. However, contrary to the hypothesis, the TMS-conditioned H-reflex amplitude was decreased by PES. This result suggested that short-latency facilitation of the H-reflex evoked by TMS reflected monosynaptic excitatory inputs from corticospinal neurons (Nielsen *et al.*, 1993b), and subsequent decrements in the H-reflex amplitude may have reflected inhibitory inputs from Ia inhibitory interneurons (Nielsen *et al.*, 1993b; Kato *et al.*, 2002). Therefore, the results of this study indicate that PES-induced changes in reciprocal Ia inhibition are due to overall changes in the excitability of Ia inhibitory interneurons, but not the changes in either the synaptic modulation of Ia afferents to Ia interneurons or the excitability of postsynaptic motor neurons. Figure 24 shows an illustration of a circuit diagram that represents the proposed site of changes induced by PES.

Several possible mechanisms may have contributed to the changes in reciprocal Ia inhibition and TMS-conditioned H-reflex observed in this study. One possible explanation is that the synaptic efficacy of Ia interneuron terminals may be responsible for these changes. Previous studies reported that tetanic stimulation of the dorsal horn led to long-term potentiation and depression in the ventral horn neurons of the spinal cord in rats (Pockett & Figurov, 1993), and that high-frequency stimulation of afferent

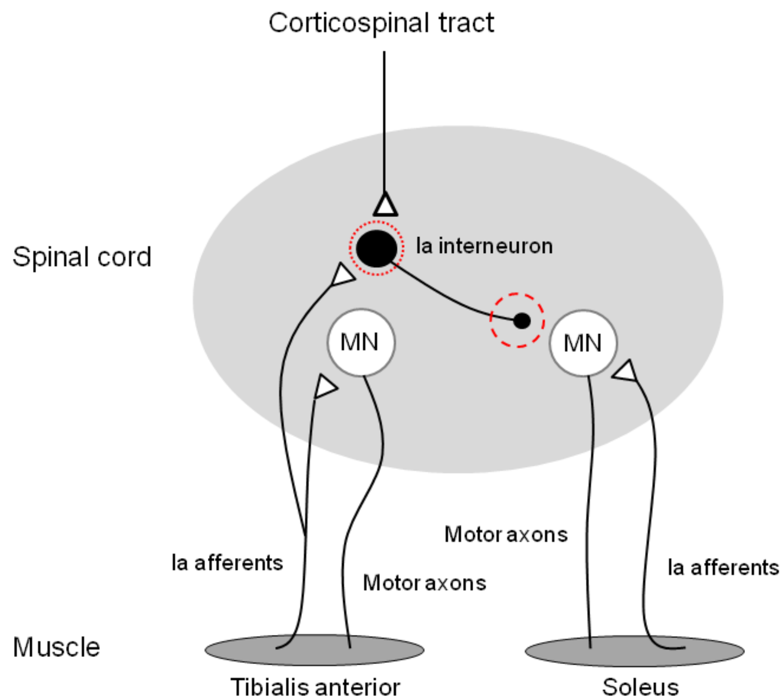

Figure 24. Illustration of a circuit diagram that shows the proposed site of changes induced by patterned electrical nerve stimulation (PES). The Ia inhibitory interneurons, which produce inhibitory postsynaptic potentials (IPSPs) in soleus motor neurons, receive excitatory inputs from the corticospinal tract and the Ia afferents from tibialis anterior muscle. Open triangles indicate excitatory synaptic terminals and close circle indicates inhibitory synaptic terminal. The dashed circle indicates the proposed site of changes induced by PES. The dotted circle indicates another possible site of changes induced by PES. MN; motor neurons.

fibers led to the post-tetanic facilitation of the H-reflex in both animals (Wolpaw *et al.*, 1989) and humans (Kitago *et al.*, 2004). In the present study, I periodically stimulated Ia muscle afferents originating from the ankle flexor muscle at a frequency of 100 Hz every 1 s (1 Hz) for 15 min. The 1 Hz cycle was approximately matched to that of the average gait cycle duration in human self-selected walking speed (Byrne *et al.*, 2007), and 100 Hz was close to the firing rate of Ia afferents of ankle flexor muscles in the early swing phase of locomotion (Geertsen *et al.*, 2011). Therefore, the repetitive activation of Ia inhibitory interneurons evoked by PES that resembled the Ia afferent firing pattern during locomotion may have reinforced the synaptic efficacy of Ia interneuron terminals in targeting motor neurons. Another possible mechanism is the modification of corticospinal excitability. Ia inhibitory interneurons receive descending inputs from the motor cortex through the corticospinal tract (Jankowska *et al.*, 1976), and the excitability of interneurons is controlled by corticospinal descending inputs (Kubota *et al.*, 2014). Fujiwara *et al.*, (2011) showed that the effects of PES on reciprocal Ia inhibition were modulated by supraspinal descending inputs. However, a higher stimulus intensity (approximately 2–3 × MT) than the intensity used in this study appeared to be necessary for increasing TA motor cortex excitability (Khaslavskaia *et al.*, 2002). In support of this finding, the MEPs of the TA muscle could not be changed by PES (Perez *et al.*, 2003). Thus, corticospinal descending inputs to Ia interneurons remained unchanged by PES and did not play a role in the changes observed in reciprocal Ia inhibition. Although the possibility that other mechanisms (e.g., an intrinsic property of Ia interneurons) (Wolpaw, 2007) are also involved cannot be ruled out, it appears reasonable to assume that PES can modulate sensory transmission at the synapse between Ia interneuron terminals and target motor neurons. To the best of our

knowledge, the present study is the first to identify the primary factor involved in changes in the reciprocal Ia inhibitory circuit. These results may lead to a clearer understanding of the spinal cord synaptic plasticity produced by repetitive sensory inputs.

5.5 Conclusion

The results of this study demonstrated that patterned sensory nerve stimulation could modulate the activity of Ia inhibitory interneurons, and these changes may have been caused by synaptic modifications to Ia inhibitory interneuron terminals.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSION

The most important advance in the field of neuroscience over the past half-century is the finding that the central nervous system has the ability to change itself during development and throughout life, which has lead to a breakthrough in our understanding of the neural mechanisms underlying motor control and cognitive processing. In the last few decades, many studies were conducted investigating activity-dependent neuroplasticity, and the results demonstrate that neuroplasticity is an important factor mediating the acquisition of new motor skills (Karni *et al.*, 1995; Pascual-Leone *et al.*, 1995; Nudo *et al.*, 1996; Classen *et al.*, 1998; Kleim *et al.*, 1998; Muellbacher *et al.*, 2001; Remple *et al.*, 2001; Muellbacher *et al.*, 2002; Perez *et al.*, 2004; Perez *et al.*, 2005a; Jackson *et al.*, 2006; Mazzocchio *et al.*, 2006; Meunier *et al.*, 2007; Wolpaw, 2007; Roche *et al.*, 2011a; Thompson & Wolpaw, 2014). Recent studies suggest that neuroplasticity is a major physiological mechanism mediating functional recovery after damage to the central nervous system (Nudo, 2003, 2006; Caemel & Martin, 2011; Ueno *et al.*, 2012; Murata *et al.*, 2015). Therefore, the investigation of neuroplasticity has become an important research topic for clinicians and neuroscientists (Dimyan & Cohen, 2011; Wang & Sun, 2011; Nahum *et al.*, 2013).

In the study of neuroplasticity, many researchers focused on changes in the upper central nervous system in the brain, because the spinal cord was often regarded as a hard-wired organ with no plastic properties. However, recent studies have shown that activity-dependent neuroplasticity occurs not only in the brain but also in the spinal cord

(Wolpaw, 2007, 2010; Thompson & Wolpaw, 2014). Considering that the brain and spinal cord work together during movement execution, it is conceivable that changes in their neural properties will interact with each other. In fact, abnormal descending activity caused by spinal and/or supraspinal lesions has been shown to result in an abnormal activation of spinal neural circuits (Crone *et al.*, 1994; Morita *et al.*, 2001; Okuma *et al.*, 2002; Kagamihara & Masakado, 2005; Lamy *et al.*, 2009; Bhagchandani & Schindler-Ivens, 2012). Therefore, without appropriate recognition of the spinal cord neuroplasticity, neural mechanisms underlying motor control and motor skill acquisition cannot be fully understood (Wolpaw, 2007). In addition, the lack of information about neuroplasticity in the spinal cord may lead to the wrong conclusions about how central nervous system works. For the above reasons, neuroplasticity in the spinal cord needs to be explored; however, to date, little attention has been given to neuroplastic changes in the spinal cord. The main aim of this thesis is to contribute to a better understanding of the neural mechanisms of activity-dependent neuroplasticity in the spinal cord. In particular, this thesis explored the extent to which corticospinal descending inputs influence the activity of spinal neural circuits (Chapter 2–3), and what factors influence the induction of neuroplasticity in the spinal neural circuits (Chapter 4–5).

In chapter 2, I investigated the extent to which corticospinal descending inputs onto Ia inhibitory interneurons influence the degree of reciprocal Ia inhibition, using a TMS conditioning H-reflex technique. The relationship between the amount of reciprocal Ia inhibition and the strength of corticospinal descending inputs to Ia inhibitory interneurons were found to be highly correlated during a resting condition. In addition, the corticospinal descending influence appeared to increase when agonist muscles

contracted. These findings suggest that the activity of Ia inhibitory interneurons is modified by corticospinal descending inputs, which may explain the observed inter-individual variation in reciprocal Ia inhibition.

In chapter 3, I investigated the influence of corticospinal descending inputs on the Ia presynaptic inhibitory pathway by comparing the effect of skilled motor task and non-skilled motor task on TMS conditioning of Ia presynaptic inhibition. The aim of this chapter is to clarify the mechanisms involved in the modification of Ia presynaptic inhibition following skilled motor task. The results showed that Ia presynaptic inhibition was only increased following a skilled motor task, and that the inhibitory effect of Ia presynaptic inhibition induced by TMS was decreased following a skilled motor task, but not following a non-skilled motor task. The increased Ia presynaptic inhibition may be explained by the reduction of inhibitory effects of interneurons activated by the corticospinal tract. These results suggest that modulation of corticospinal descending inhibitory effects on the Ia presynaptic inhibitory pathway may be responsible for the changes in Ia presynaptic inhibition.

Previous studies have shown that spinal neural circuits are modulated by motor skill training. However, the effects of task movement speed on changes in spinal neural circuits have not been clarified. Sensory inputs to the spinal cord vary depending on the task movement speeds (Poppele & Bowman, 1970; Bosco & Poppele, 1999), and are speculated to influence changes in spinal neural circuits. Therefore, in order to find the mechanisms responsible for changes in spinal neural circuits, the effect of task movement speed on changes in spinal neural circuits was examined in chapter 4. The

findings showed that Ia presynaptic inhibition increased following a skilled motor task, irrespective of task movement speed. Reciprocal Ia inhibition was increased by fast movement speed conditioning, but not affected by slow movement speed conditioning. A non-skilled motor task did not induce changes in Ia presynaptic inhibition and reciprocal Ia inhibition. These findings suggest that supraspinal descending inputs controlling joint movement are responsible for changes in Ia presynaptic inhibition and reciprocal Ia inhibition, and that the task movement speed is a critical factor for inducing changes in reciprocal Ia inhibition. It is conceivable that spinal neural circuits are differentially modulated, depending on the motor task, for achieving the demands of the task.

In chapter 5, I investigate whether the sensory inputs induced by electrical stimulation produce changes in spinal neural circuits. Also, although sensory inputs are speculated to be a critical factor in driving changes in spinal neural circuits, the mechanisms underlying these changes are not fully elucidated. Changes in the efficacy of the synaptic transmission develop at the spinal level (Pockett & Figurov, 1993), thus, I hypothesized that changes in spinal neural circuits might result from synaptic modification of excitatory synapses between sensory afferent terminals and interneurons constituting spinal neural circuits. To test this hypothesis, I investigate the neural mechanisms underlying changes in spinal neural circuits induced by sensory nerve stimulation, using a TMS-conditioning H-reflex technique. The sensory nerve stimulation increased reciprocal Ia inhibition and the effect of TMS conditioning on Ia inhibitory interneurons, but did not change Ia presynaptic inhibition. These findings suggest that repetitive sensory inputs can modulate the activity of Ia inhibitory

interneurons. The changes may be caused by synaptic modifications to Ia inhibitory interneuron terminals. The findings of this chapter will help our understanding of spinal cord neuroplasticity produced by sensory inputs, specifically, the location where it occurs.

In summary, the main findings of this thesis are as follows; 1) The activity of Ia inhibitory interneurons is modified by corticospinal descending inputs. 2) Changes in Ia presynaptic inhibition following a skilled motor task are caused by the modulation of corticospinal descending inhibitory effects on the Ia presynaptic inhibitory pathway. 3) Supraspinal descending inputs controlling joint movement are important for producing changes in spinal neural circuits. 4) Task movement speed is a critical factor for inducing changes in reciprocal Ia inhibition, as well as supraspinal descending inputs. 5) Repetitive sensory inputs could modulate the reactivity of Ia inhibitory interneurons. These findings indicate that the changes in spinal neural circuits following motor skill learning result from modulation of interneuron excitability in spinal neural circuits, by corticospinal descending inputs. On the other hand, giving that repetitive sensory nerve stimulation can modulate reciprocal Ia inhibition, it is speculated that reciprocal Ia inhibition is also modulated by non-skilled motor tasks because the activation timing of TA muscle in non-skilled motor task is almost the same as the timing of sensory inputs induced by electrical stimulation. However, a difference was noted, likely due to the effect of the interaction between flexor-coupled Ia interneurons and extensor-coupled Ia interneurons. It has been reported that Ia inhibitory interneurons receive disynaptic IPSPs from antagonist Ia interneurons (mutual inhibition) (Hultborn *et al.*, 1974; Tanaka, 1974). Therefore, during rapid alternating movements, opposing muscles are stretched

in a phasic manner, and then each Ia inhibitory interneuron receives disynaptic IPSPs from each antagonist Ia interneuron. Although the underlying mechanisms are unknown, facilitation elicited by sensory inputs might be offset by these mutual inhibitory effects.

During the learning of new motor skills, a short-lasting changes in spinal neural activity is thought to reflect the change in the supraspinal descending influence on the spinal cord and/or transient changes in the efficacy of synaptic transmission (long-term potentiation and long-term depression) at the spinal level (Bunday & Perez, 2012), while a long-lasting changes in spinal neural activity is thought to reflect structural changes, such as the formation of new synapses (e.g., axonal sprouting, and synaptogenesis) (Carmel & Martin, 2011; Ueno *et al.*, 2012), by the long-term continuation of the descending influence (Wolpaw, 2007). The long-lasting change takes a long time to occur. Therefore, the observed changes in spinal neural circuits may reflect the initial stage of activity dependent neuroplasticity in the spinal cord.

As already mentioned above, rehabilitation methods based on the principles of neuroplasticity are likely to be critical for promoting the recovery of motor function in patients with central nervous system disorders, such as stroke and spinal cord injury. These patients have poor control of limb movements and abnormal activation of Ia presynaptic inhibition and/or reciprocal Ia inhibition (Crone *et al.*, 1994; Morita *et al.*, 2001; Okuma *et al.*, 2002; Crone *et al.*, 2003; Kagamihara & Masakado, 2005). It has been suggested that the modulation of such abnormal spinal function will lead to improvement of impaired motor function (Edgerton *et al.*, 2004; Bhagchandani & Schindler-Ivens, 2012; Thompson *et al.*, 2013b). In this thesis, I described various

factors that influence changes in spinal neural circuits. For example, as repetitive sensory inputs induced by electrical stimulation might change the overall reactivity of Ia inhibitory interneurons, sensory nerve stimulation may be useful for improving the abnormal activation of the reciprocal Ia inhibitory circuit. In addition, when producing changes in the spinal neural circuits by motor task, it is important to consider what type of motor task to use.

The spinal cord processes ensembles of information derived from the supraspinal centers and peripheral sensory receptors and generates motor outputs in a precise and highly coordinated manner in response to changes in the environment. The physiological state of the spinal cord is affected by the nature, intensity and duration of physical activities in later life, and by specific motor training, which influences our motor behavior (Wolpaw, 2007). Therefore, I end this thesis with the conclusion that understanding neuroplasticity in the spinal cord is essential for understanding how the central nervous system controls our body movements and how the system changes with motor skill acquisition.

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Shinji Kubota, Masato Hirano, Takuya Morishita, Kazumasa Uehara, Kozo Funase.

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Shinji Kubota, Masato Hirano, Yoshiki Koizume, Shigeo Tanabe, Kozo Funase.

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