Functional Implications of H-reflex Modulation and Modification in Human Soleus, Medial Gastrocnemius, and Lateral Gastrocnemius Muscles

Yukiko Makihara

A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the School of Medicine (Curriculum in Human Movement Science)

Chapel Hill
2011

Approved by:

Richard L. Segal

J. Troy Blackburn

Michal D. Lewek

Vicki S. Mercer

Jonathan R. Wolpaw

ABSTRACT

YUKIKO MAKIHARA: Functional Implications of H-reflex Modulation and Modification
in Human Soleus, Medial Gastrocnemius, and Lateral Gastrocnemius Muscles
(Under the direction of Richard L. Segal)

Sensorimotor integration of the central nervous system (CNS) plays an important role in motor control. In order to evaluate this highly complicated phenomenon, the H-reflex has been used as a window to assessing neural activity in the spinal cord. This dissertation investigated principles governing functional association among the H-reflexes in three calf muscles. First, the H-reflexes of the soleus, and medial and lateral gastrocnemii (MG and LG) were examined during walking, and between standing and walking. The H-reflexes of all three muscles showed similar phase-dependent and task-dependent modulations, and thus, the H-reflexes of three muscles are synergistically modulated to facilitate ongoing motor task. Second, long-term modifications of the H-reflexes in the three muscles, induced by the soleus H-reflex operant down-conditioning, were examined. Operant conditioning induced acute adaptation in the soleus and MG H-reflexes, however, long-term change occurred only in the soleus H-reflex. It appeared that compensatory plasticity may occur to prevent long-term change in the MG H-reflex, and in turn, to preserve the existing repertoire of motor skills. Third, effects of long-term change in the soleus H-reflex induced by operant conditioning were investigated during locomotion. Although decrease of the soleus H-reflex was retained across conditioning sessions, the soleus locomotor H-reflex did not change. Other EMG activity as well as joint kinematics were maintained the same after conditioning, and thus, the normal locomotion was preserved. Minimum adjustment of reflex gain could be the mechanism responsible for maintaining the appropriate reflex during walking. Based on findings from the three studies, the present dissertation demonstrated that the CNS controls the H-reflexes of the three synergist muscles to be synergistic and/or possibly compensatory, which are logical directions to facilitate or preserve the motor skills in the current behavioral repertoire. Furthermore, especially in the existing repertoire, normal locomotion is maintained due to minimum adjustment by the CNS. Thus, the CNS is shown to be capable of accommodating a new skill while preserving the current motor skills. Preserving the existing repertoire may not occur in patients with abnormal movement pattern, and thus, similar investigations with patients should occur in the future.

ACKNOWLEDGEMENTS

I would like to take this opportunity to thank everyone who has helped me reaching this career. I would like to express my gratitude to all of the members of my dissertation committee, Drs. Rick Segal, Troy Blackburn, Mike Lewek, Vicki Mercer, and Jonathan Wolpaw, for their valuable insight, expertise, and time that helped me accomplishing this project. I could not have asked for better mentors to support me throughout my time as a doctoral student. Specifically, I can't thank my advisor Dr. Rick Segal enough for his understanding and support. He has been incredibly caring for not only my academic work but my life. Additionally, I need to thank Dr. Aiko Thompson for her invaluable support for me to achieve the dissertation project as well as to write manuscripts for publication.

I would also like to thank my friends and family for the love and support throughout my many years as a student. Thank you for being with me, encouraging me, and loving me unconditionally. Without the help from each of you, I would not have been able to complete this long journey. Thank you especially to my husband, Josh Shaffer. He has always believed in me and has been so incredibly patient and understanding. I would not be where I am today without his support. I will forever be grateful.

TABLE OF CONTENTS

IV. THIRD MANUSCRIPT

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1. Control of a movement

Movement plays a crucial role in human life allowing us to purposefully achieve specific goals. Impairments in movement due to injury or disease can cause disabilities in almost all aspects of quality of life by limiting one's ability to participate in normal social activity. Thus, principles governing human movements are one of the main interests in behavioral science and clinical fields.

In natural circumstances (as opposed to experimental situations in animals such as decerebration or deafferentation), the human motor system and sensory system interact with each other in complex ways through activity of the central nervous system (CNS). The CNS comprises two major different levels for motor control; the cerebral cortex and the spinal cord, which interact directly or indirectly through the brain stem (Ghez, 1991). The interaction of the cortex and spinal cord is also under continuous influence from two subcortical systems; the basal ganglia and the cerebellum. Through the sensorimotor integration of the CNS in motor control, so-called "integrative action" of the CNS (Sherrington, 1947), humans are able to produce appropriate movements and function in the environment. It is assumed that information (i.e., input) available in the environment is accepted by the sensory system, integrated by the function of the CNS, and converted into output as observable response in the motor system (Schmidt and Lee, 2005). Thus, sensorimotor integration of the CNS is information processing that occurs in the interval between the presentation of the stimulus and the beginning of the response (i.e., reaction-time). There are at least three processing stages in the sensorimotor integration of the CNS, which are organized hierarchically and in parallel. First, sensory input from receptors in many different sites such as muscles, joints, tendons, skin, eyes, and ears are processed in parallel to sense and identify the stimulus. This stage is called *stimulus-identification stage*. After the stimulus has been properly identified, the information is processed in two sequential stages. The second stage following the stimulus identification is called *response-selection stage* in which a response to be made is decided. The response could be either one of a number of actions in the existing repertoire, or no action at all (i.e., the stimulus is ignored). Finally, after the response has been selected, the organization of the selected response is programmed. This stage is called *response-programming stage*, which is the final stage of the information processing that lead to execution of the appropriate motor response.

As shown in the concept of information processing, one way to model motor control is that humans heavily rely on sensory information to regulate movements. This notion is represented in closed-loop control system theory (Schmidt and Lee, 2005). Closed-loop systems are important particularly during a movement that lasts for a period of time. Once the action is initiated, feedback signals from the sensory receptors occurred as a consequence of the current movement are sent back to the information processing centers. In the information processing centers, the feedback signals are received and processed in which the difference between the actual and desired states (i.e., error) is computed. Then, the response is programmed so as to reduce the error. Thus, decisions about future action are continuously made throughout the movement in a closed loop to provide ongoing control. In contrast to the closed-loop system is an open-loop system, in which the motor response is programmed in advance without considering any sensory information (i.e., feed-forward system). For example, movement can be initiated in response to internally driven needs (i.e., voluntary movement); however, control of ongoing movement is dependent on sensory input and can be modified by feedback.

One feature of studying sensorimotor integration of human CNS in motor control is that, in most cases, it is not directly measurable, probably because CNS activity underlying control of movement is a highly complicated and distributed phenomenon. Thus, experiments studying motor control need to be designed to have a specific window to measure CNS activation. For example, imaging techniques such as functional MRI or positron emission tomography have been used to identify changes in brain activity during a sequence of simple motor skill learning (Jenkins et al., 1994; Penhune and Doyon, 2002; Floyer-Lea and Matthews, 2005; Lehericy et al., 2005). Transcranial magnetic stimulation (TMS) has been used to investigate corticospinal tract (CST) activity (Perez et al., 2007; Thompson et al., 2011). Spinal cord reflexes have been studied as a window into evaluating activity in the spinal cord (Schieppati, 1987; Brooke et al., 1997; Wolpaw, 2010).

The H-reflex, sometimes referred to as the "electrical analogue" of the stretch reflex, has been extensively examined in both healthy and neurologically impaired humans as a window to neural processing in the spinal cord (Schieppati, 1987; Brooke et al., 1997; Pierrot-Deseilligny and Mazevet, 2000; Zehr, 2002; Misiaszek, 2003). Thus, in the general introduction of the present dissertation, fundamental knowledge about the H-reflex will be reviewed in the next section. The H-reflex is largely monosynaptic (Magladery et al., 1951), and electrically induced spinal reflex. While the pathway of the H-reflex is wholly spinal, it is also influenced by descending inputs from the brain (Wolpaw, 2010). Since the H-reflex participates in other motor tasks such as locomotion as a part of the stretch reflex (Yang et al., 1991), examining the H-reflex provides insights about how the Ia afferent pathway is modulated within a task or modified over a course of motor learning, aging, and development. Current knowledge regarding modulation and modification of the H-reflex will be reviewed in the sections 3 and 4 following the section 2 below.

2. The H-reflex

Low intensity percutaneous electrical stimulation on a mixed (i.e., containing both afferent and efferent axons) peripheral nerve evokes the Hoffman reflex (or the H-reflex) in humans. The pathway of the H-reflex was demonstrated to be monosynaptic consisting of primary afferent fibers, its synapse on the motoneuron, and the motoneuron (Magladery et al., 1951). This pathway is considered to be the electrical analogue of the stretch reflex, but it bypasses fusimotor drive and muscle spindle discharge. In addition, there are more differences such as the afferent fibers contributing to the reflex, activity pattern of activated afferents, and patterns of afferent input dispersion between the stretch reflex and the H-reflex (Burke, 1983).

The H-reflex can be seen in most muscles in which the nerve is accessible for surface stimulation. Indeed, the H-reflex has been investigated in more than 20 muscles in the human body (Misiaszek, 2003). Moreover, in a research setting, the strength of the electrical stimulation can easily be adjusted, which allows an experimenter to control the H-reflex systematically. Due to the relative ease of handling, the H-reflex has become an attractive clinical/research tool (Misiaszek, 2003) and has been used widely as a window to study neural plasticity of the spinal cord (Zehr, 2002).

2.1 Evoking the H-reflex

Recording of the H-reflex as EMG activity is possible in the following scenario (Zehr, 2002). If low intensity percutaneous stimulation is sufficient enough (i.e., above the threshold) for activation of Ia afferents and neurotransmitter release at the Ia afferent and alpha-motoneuron synapse, it causes postsynaptic depolarization of alpha-motoneurons. If excitatory postsynaptic potentials (EPSPs) exceed the threshold, then the motoneurons fire action potentials resulting in neurotransmitter release at the neuromuscular junction. This causes depolarization of the muscle fibers, which is recorded in EMG activity as an H-reflex, and results in muscle contraction. A simple schema for monosynaptic part of the H-reflex pathway is shown in Figure 1.1.

Stimulation of a mixed peripheral nerve may evoke both a response through sensory (afferent)-motor (efferent) arc (i.e., H-reflex) and a direct motor response (i.e., M-wave) depending on stimulus intensity. When intensity of percutaneous stimulation increases from a low level, the axons of Ia afferents are recruited before the axons of alpha motoneurons because axon diameter of the Ia afferent is larger than that of motoneuron (Kukulka, 1992). Thus, when the stimulation strength changes from low to high, the H-reflex is recorded without the M-wave, and then with the M-wave. In addition, as the stimulation strength increases, additional Ia afferents and motoneurons are recruited resulting in a larger H-reflex and M-wave up to the intensity when the H-reflex starts to decrease (i.e., maximum H-reflex, review in the next section). Because of this corresponding relationship, the M-wave size is often used as an indication of stimulation constancy for the H-reflex.

2.2 Recruitment curve

When the stimulus intensity incrementally increases from a low level to a high level, recruitment curves of the H-reflex and the M-wave are concurrently recorded. The H-reflex size increases linearly with the stimulation intensity until it reaches the maximimum H-reflex (Hmax). During this ascending limb of the H-reflex recruitment curve, the motoneurons are recruited into the H-reflex activation from the smallest to the largest in accordance with the size principle due to differences in input resistance (Henneman et al., 1965). On the other hand, the order of the recruitment for the M-wave is from the largest motoneurons to the smallest motoneurons because electrical stimulation first activates axons with larger diameters (Pierrot-Deseilligny and Mazevet, 2000). Since impulses on the motoneuron axons that are elicited by the electrical stimulation propagate in both directions (i.e., to the spinal cord: antidromic and to the muscle: orthodromic), the reflex activation of relatively large motoneurons collide with antidromic motor volley on the same motor axons. This collision causes the typical shape of the H-reflex recruitment curve in which the size of the H-reflex falls to almost silent after it passes the Hmax (Schieppati, 1987). Indeed, in the human soleus, activation of slow-twitch fibers (i.e., innervated by smaller motoneurons) is mainly responsible for the H-reflex (Buchthal and Schmalbruch, 1970).

2.3 Factors that affect the H-reflex

When H-reflex size is compared across sessions to look into specific changes in the reflex pathway, any external factors that affect the H-reflex size must be maintained the same between sessions. There are several factors that have been suggested to have substantial influence on H-reflex size.

Background excitability

Generally, H-reflex size is linearly associated with level of voluntary contraction because the number of motoneurons that are recruited in the reflex activity increases as the excitability of the motoneuron pool raises toward threshold (Burke et al., 1989). Thus, when comparing H-reflex size between different tasks or different time line, maintaining the background activity of the target muscle at the same relative level [e.g., percentage of maximum voluntary contraction (MVC)] when the reflex is evoked is important. The motoneuron pool excitability (i.e., background excitability) can be monitored through surface EMG of the muscle of interest. Contraction of the muscle is also useful for reducing variability in the latency and size of the H-reflex (Burke et al., 1989; Funase and Miles, 1999), and thus it is recommended that the H-reflex be tested when the target muscle is activated at a certain relative level (Zehr, 2002). There are some studies reporting non-linear part of the H-reflex size vs. background excitability association in humans especially in a high background EMG level (Edamura et al., 1991; Loscher et al., 1996). When the background EMG activity exceeds approximately 50% of the MVC, increment of the H-reflex becomes smaller in some subjects. However, most data are well fitted by a straight line.

Stimulus efficacy

When comparing H-reflex size between tasks or in a series of sessions, the Ia afferent volley that is received by alpha motoneurons must be consistent so that any changes occurring in the H-reflex pathway (i.e., presynaptic and postsynaptic facilitation and inhibition) can be accurately recorded. During H-reflex measurement using surface electrodes, the size of the M-wave is used as an indication of the stimulus efficacy. By monitoring and keeping the M-wave size at the same level, the stimulus efficacy can be maintained consistent, and changes in the H-reflex pathway such as size modulation can be measured.

One typical methodological error arises from movement of the stimulating electrodes on the skin, particularly when movement or change of a posture is involved in a test condition. Changes in distance between the nerve and the stimulus electrodes easily alter the activation of the Ia afferent, causing changes in the H-reflex size that are independent of changes in the reflex pathway (Brooke et al., 1997). This error can be avoided by using a consistent posture and/or adjusting the stimulus intensity during the experiment so as to maintain the same M-wave size.

Presynaptic inhibition

The H-reflex was originally proposed as a monosynaptic reflex (Magladery et al., 1951). Due to the direct synaptic connection between the Ia afferent and alpha motoneuron, the H-reflex has been thought to reflect directly the excitability of the motoneuron pool. Zehr pointed out that this earlier notion leading to misinterpretation of the H-reflex still continues to the present day (Zehr, 2002). It is clear that the synaptic connection between the Ia afferent and the alpha motoneuron is under continuous modification. Thus, the H-reflex size is not a direct measure of the excitability of the motoneuron pool. Presynaptic inhibition (PSI) at the synapse between the Ia afferent and the alpha motoneuron has been discussed extensively as a primary source for modification of the H-reflex size (Capaday and Stein, 1987a, 1989; Stein, 1995; Brooke et al., 1997).

Presynaptic inhibition is mediated by inhibitory spinal interneurons acting on the Ia afferent terminals (Eccles et al., 1962). PSI is a mechanism that affects neurotransmitter release at the Ia terminals, which results in reduction of the motoneuron depolarization. Postsynaptic properties such as the membrane potential of the motoneuron are not directly affected by PSI. Thus, the H-reflex size and the excitability of the motoneuron are separately regulated due to the presence of PSI (Cook and Cangiano, 1972). That is, the H-reflex size can be altered by the PSI with consistent background activity, or conversely, changes in background activity with constant PSI can also alter H-reflex size.

The activity of inhibitory interneurons that mediate PSI are influenced by many factors including peripheral feedback from the muscle spindle and Golgi tendon organ both in a target muscle and other muscles, cutaneous receptors, joint receptors, vestibular receptors, and neck receptors, and descending supraspinal inputs (Schieppati, 1987; Stein, 1995; Brooke et al., 1997). Zehr suggested that those factors can be controlled by maintaining the subject's posture, intension, and contraction of other muscles (Zehr, 2002).

Inputs from other afferent pathways

Another piece of evidence indicating that the H-reflex is not a pure monosynaptic reflex was presented by Burke et al (1984). Considering the fact that the electrical stimulation to elicit the H-reflex is applied over the skin covering a "mixed" nerve, afferent volleys would not be exclusively composed of homonymous Ia activity, but rather contaminated by other afferent signals. Burke et al. (1984) estimated the rising times of the composite EPSPs evoked by subthreshold electrical stimulation in single motor units of the soleus motoneurons in humans. They found that much of the EPSPs were summed in raising the motoneuron membrane potential to threshold, and that motoneuron discharge occurred a few milliseconds after the onset of the EPSPs. A few milliseconds would be ample time for oligosynaptic inputs such as group Ib activity to reach the motoneuron pool. In addition, they showed, using surface EMG recordings, that the rising phase of the H-reflex was long enough for the oligosynaptic inputs to contaminate the H-reflex waveform. Thus, they suggested that the H-reflex waveform, especially the later portion of the signal, is likely to be affected by the oligosynaptic inputs. However, they also suggested that the H-reflex, when compared to the stretch reflex, contains significant excitation that is contributed by the monosynaptic pathway due to its shorter duration of the rising phase.

3. Modulation of the H-reflex

Although the H-reflex can be evoked in many muscles, the soleus is the most commonly used muscle in human lower extremity studies (Brooke et al., 1997; Capaday, 1997; Zehr, 2002; Misiaszek, 2003). Therefore, this section will review current findings regarding modulation of the soleus H-reflex pathway.

3.1 Task-dependent modulation

It was shown by Akazawa et al. that size of the soleus stretch reflex in mesencephalic cats is strongly modulated depending on the ongoing-task (i.e., standing or walking, Akazawa et al., 1982). Task-dependent modulation of the human soleus H-reflex was shown for standing vs. walking (Capaday and Stein, 1986; Kido et al., 2004a) and walking vs. running (Capaday and Stein, 1987b; Edamura et al., 1991). In those studies, the H-reflex sizes evoked at the same background level were compared between two different tasks. The results demonstrated that the reflex is largest during standing, smaller during walking, and even smaller during running. In addition, Edamura et al. (1991) reported that the form of locomotion (i.e., walking or running), not the speed or level of EMG activity, is the most important determinant for the task-dependent modulation of the soleus H-reflex. It is suggested that lowering the reflex gain would reduce saturation of the stretch reflex pathways (or motoneuron pool), which would be appropriate for the muscle to be prepared for an unexpected stretch, to contribute to locomotor activity, and to reduce possible instability (i.e., tremor) (Capaday and Stein, 1987b; Edamura et al., 1991).

The task-dependent modulation of the soleus H-reflex is also reported in other movements. Dyhre-Poulsen et al. (1991) investigated the size of the human soleus H-reflex during landing (from a downward jump) and hopping. The H-reflex size was low at landing (i.e., touchdown the ground) while the reflex was high at the touchdown during hopping. Those results clearly indicate that the soleus stretch reflex pathway is modulated in an appropriate way to support execution of the intended task.

3.2 Phase-dependent modulation

Akazawa et al. also suggested phase-dependency of the stretch reflex in mesencephalic cats based on the gait cycle (Akazawa et al., 1982). Later, the phase-dependent modulation within a gait cycle was demonstrated in various studies using human soleus stretch reflex (Yang et al., 1991) or, more intensively, the H-reflex (Capaday and Stein, 1986; Yang and Whelan, 1993; Kido et al., 2004a; Krauss and Misiaszek, 2007). It was commonly shown that, in healthy human subjects, the H-reflex of the soleus during walking reaches its peak during the late stance phase, falls rapidly to a low level at the beginning of the swing phase, and stays low throughout the swing phase. Those results suggest that the stretch reflex pathway of the soleus is prepared so that walking is carried out effectively even in the face of unexpected perturbations. That is, the stretch reflex activity of the soleus during the stance phase could support propulsion of the body while suppression of such reflex activity in the swing phase ensures that the muscle would not oppose ankle dorsiflexion, even if a sudden stretch is applied on the muscle.

3.3 Context-dependent modulation

The H-reflex size of the soleus during walking is also modulated based on context of the task. Krauss and Misiaszek (2007) investigated the soleus H-reflex size in healthy humans when postural threat (perturbation in anterior-posterior direction) was applied during walking. Their results showed that the reflex size at heel contact significantly increased when compared to normal walking. Similarly, Schneider and Capaday (2003) reported that, during backward walking, the soleus H-reflex size in untrained subjects increased significantly in mid-swing phase. Since the increase of the reflex size disappeared immediately when the subject was allowed to hold handrails in both studies, those results suggested that the increase of the H-reflex would be related to task uncertainties, such as the balance perturbation and foot contact with the ground. They also suggested that the increase of the H-reflex size at the heel contact (forward walking) or mid-swing (backward walking) may be important to control ankle joint angle and stiffness at ground contact.

On the other hand, Llewellyn et al. (1990) compared the human soleus H-reflex size between treadmill walking and walking on a narrow beam (3.5 cm wide, 34 cm from the floor) at the same background EMG levels. They found that, during the beam walking, the

H-reflex size was considerably suppressed and that the EMG pattern was dominated by co-contraction of the soleus and tibialis anterior instead of typical reciprocal pattern. In this context, the H-reflex itself would be a source of instability, and thus, the authors suggested that this suppression of the H-reflex size is probably related to technique to walk when postural stability is threatened.

3.4 Mechanisms underlying the H-reflex modulation

As reviewed above, the H-reflex size is generally correlated with background activation level. It is also well known that the soleus H-reflex modulation and EMG activity have similar patterns during a gait cycle (i.e., high in the stance phase and almost silent in the swing phase). However, the H-reflex modulation is apparently not a simple consequence of the background excitability of the agonist or antagonist activity. In the study by Yang and Whelen (1993), healthy human subjects were trained to activate the soleus voluntarily during swing phase or trained to walk without activating the TA. In both cases, the H-reflex was modulated based on the phase of the gait cycle just as seen in normal walking. Capaday and Stein (1989) showed that, in decerebrate cats, postsynaptic inhibition of the motoneurons cannot separately control the reflex size and the background activity. As mentioned above, the H-reflex size is modulated independently of the background EMG activity. Thus, by exclusion, presynaptic inhibition is discussed most extensively as a neural mechanism that modulates the H-reflex pathway (Capaday and Stein, 1987b, a; Stein and Capaday, 1988; Edamura et al., 1991; Yang and Whelan, 1993; Stein, 1995). Nevertheless, the exact site of those modulations needs to be further examined.

4. Modification of the H-reflex

The CNS, thought to be hard-wired and inflexible, is now recognized to have capability for change (Wolpaw, 2010). This ability of the CNS to change is defined as neural plasticity. According to Ludlow et al. (2008), "neural plasticity is the ability of the central nervous system (CNS) to change and adapt in response to environmental cues, experience, behavior, injury or disease". Furthermore, they added that neural plasticity results from changes in function of neural pathways such as neural connectivity that occur through changes in synaptic strength and some other mechanisms. Thus, changes in function of neural pathways constitute the basis for neural plasticity. Neural plasticity occurs in response to environmental influences, experience, practice, learning, development, aging, change in use, injury or disease, and produces changes in behavior. Neural plasticity occurs continuously throughout life to induce movement adaptations, some of which are achieved quickly while others take a long time. For example, coordinated steps by a dancer, accurate fingering technique in a pianist, walking capability gradually acquired during development, and abnormal movement pattern after stroke or spinal cord injury are all products of neural plasticity, producing positive or negative consequences. As will be reviewed below, modifications of the H-reflex caused by neural plasticity may represent adaptation of the nervous system to meet particular requirements for each task, and thus, neural plasticity in the H-reflex pathway may contribute a specific motor skill.

4.1 Cross-sectional studies

It is well known that trained ballet dancers have smaller soleus H-reflexes than other aerobically trained individuals and control subjects (Nielsen et al., 1993). The modification of the soleus H-reflex size induced by long-term exercise training may represent specific adaptations to meet particular requirements of the training. That is, frequent use of cocontraction between antagonistic ankle muscles in ballet dancers may lead to suppression of the soleus H-reflex (Nielsen et al., 1993). On the other hand, smaller soleus H-reflex size is also reported in volleyball players and sprinters when compared to non-trained subjects (Casabona et al., 1990). Since the H-reflex is mainly constituted by activation of smaller motoneurons (i.e., slow twitch fibers, Buchthal and Schmalbruch, 1970), the H-reflex activity may not be involved in explosive muscle contractions used in volleyball and sprinting (Casabona et al., 1990). Thus, application of the H-reflex for assessing adaptation of large motoneurons that would be induced by explosive contraction training is limited (Ross et al., 2001). Furthermore, the number of those cross-sectional studies in humans is still limited and there is always possibility of contamination of intrinsic genetic factors (Casabona et al., 1990; Zehr, 2002).

4.2 Longitudinal studies

After four weeks of hopping training, inhibition of the H-reflex during hopping compared to standing that was evident before the training disappeared (Voigt et al., 1998). After 20 days of bed rest, the soleus maximum H-reflex/maximum M-wave ratio (Hmax/Mmax ratio) decreased from 63.3 % to 26.7 % (Yamanaka et al., 1999). As a very short-term change, the H-reflex size can voluntarily be decreased in a day of balance training (Trimble and Koceja, 1994). Furthermore, the soleus Hmax/Mmax ratio became smaller after 30-minute training of cocontraction of the ankle dorsi- and plantarflexors (Perez et al., 2007). Those observed modifications seem to reflect neural plasticity in the H-reflex pathway, which underlies functional adaptation during motor learning.

The effects of aging ("detraining" effects) on the soleus H-reflex size in healthy humans have also been examined in a few studies by comparing "young" (i.e., 19-31 years old) and "old" (i.e., 60-84 years old) subject groups. Generally, the Hmax/Mmax ratio in the old group was significantly smaller than that in the young group (Sabbahi and Sedgwick, 1982; Koceja et al., 1995). Kido et al. (2004a) investigated H-reflex decreases during the aging process by testing subjects ranging in age from 22 to 82 years, instead of comparing two representatives groups (i.e., "young" or "old"), and found that the H-reflex size decreased gradually with age. In addition, Koceja et al. (1995) reported that the Hmax/Mmax ratios of the young individuals were smaller when the subjects were standing than when lying prone (i.e., posture-dependent H-reflex modulation) whereas this modulation was not measured in the old individuals. Similarly, Kido et al. (2004a) demonstrated that, although the H-reflex size was always larger during standing than during walking in young subjects, this task-dependent H-reflex modulation was less pronounced in the old subjects. Interestingly, the rhythmic H-reflex modulation during a gait cycle (i.e., phase-dependent modulation) was preserved in the old individuals despite the gradual decrease of the Hmax/Mmax ratio with aging (Kido et al., 2004a). An increase in the presynaptic inhibition acting on Ia terminals with aging has been shown and proposed as a possible explanation for the decrease of the H-reflex size with aging (Morita et al., 1995).

Neural plasticity in the human soleus H-reflex also occurs during development. Hodapp et al. (2007) reported that the soleus H-reflex size during mid-stance phase in healthy children (15-16 years old) was significantly depressed when compared to children with diplegic cerebral palsy (CP) in the same age group. The mid-stance H-reflex sizes were not different between healthy children and children with CP in 5-11 aged groups, suggesting that age-dependent H-reflex depression during walking that occurs with maturation of the CNS is most likely to involve plasticity in the supraspinal structures and corticospinal tract, which are affected in children with CP.

4.3 Operant conditioning of the H-reflex

As reviewed above, among several approaches, the H-reflex has been used widely as a convenient tool to study neural plasticity. Nevertheless, the H-reflex is only a window to evaluate changes in the spinal cord activity associated with changes in motor behavior. Operant conditioning, proposed by Wolpaw and his colleagues, utilizes this H-reflex as a target task (i.e., increase or decrease the H-reflex size, Wolpaw, 1987). Because the H-reflex pathway is influenced by descending inputs from the brain, this pathway can be operantly conditioned. That is, animals or human subjects are trained to be able to produce descending influence that is appropriate to the target task to earn a reward. According to a general definition of a skill as a behavior that is adaptive and acquired through practice (Compact Oxford English Dictionary, 1993), this change in the reflex size is a simple motor skill. Hence, operant conditioning paradigms have been used as a model for studying long-term neural plasticity during motor skill learning (Wolpaw and Tennissen, 2001; Wolpaw, 2010).

A series of operant conditioning studies showed that the size of the spinal stretch reflex (SSR) or the H-reflex can be operantly conditioned in monkeys, rats, mice, and humans (Wolpaw et al., 1983; Wolpaw, 1987; Evatt et al., 1989; Chen and Wolpaw, 1995; Carp et al., 2006a; Thompson et al., 2009). In this laboratory model, animals or subjects were conditioned to make the reflex smaller or larger so as to increase the reward contingency. Additional studies revealed many possible neural mechanisms underlying this reflex change. Down-conditioning causes changes in motoneuron properties [firing threshold and axonal conduction velocity (Carp and Wolpaw, 1994, 1995; Carp et al., 2001)] as well as changes in synaptic terminals on the motoneuron and spinal interneurons [GABAergic terminals and interneurons (Wang et al., 2006, 2009)]. Up-conditioning has been less understood compared to down-conditioning, however; it is suggested that up-conditioning is associated with plasticity in spinal interneurons (Carp and Wolpaw, 1995; Wolpaw and Chen, 2001), and that down-conditioning and up-conditioning are not mirror images (Wolpaw, 2007, 2010). In addition, animal data were used to demonstrate that the sensorimotor cortex (hindlimb area) and cerebellum are essential for successful conditioning (Chen and Wolpaw, 2005; Chen et al., 2006a; Wolpaw and Chen, 2006), and that, of the major descending and ascending tracts, only the corticospinal tract (CST) conveys this training-inducing descending influence from the brain to the spinal cord (Chen et al., 2002; Chen and Wolpaw, 2002; Chen et al., 2006a). Furthermore, detailed analysis revealed two distinct phases of the reflex change over the course of the operant conditioning, each of which is suggested to reflect plasticity at two sites, the brain (i.e., supraspinal) and the spinal cord (Wolpaw and O'Keefe, 1984; Thompson et al., 2009). Those results suggest that interaction of the supraspinal plasticity and the spinal cord plasticity account for the overall reflex change.

5. Interaction of soleus H-reflex and locomotion

Ia afferent pathways of the soleus have been shown to have important roles in locomotion. In addition, since the part of the Ia afferent pathway is the target pathway to be conditioned during the H-reflex operant conditioning, the effects of soleus H-reflex operant conditioning on locomotion have been studied in rats.

5.1 Contributions of the Ia afferent pathway to locomotion

Yang et al. (1991) used a pneumatic device to apply small and rapid dorsiflexion to the ankle joint to stretch the soleus of human subjects. The size of the consequent soleus stretch reflex was correlated with velocity of the disturbance. The authors estimated that 30-60% of the soleus activation was contributed by reflex activity induced by velocity-sensitive (i.e., Ia afferents) inputs particularly during early stance phase. Bennett et al. (1996) demonstrated using decerebrated and spinal cats that ankle extensor force was substantially contributed by the stretch reflex of the triceps surae (25% during tonic contraction and 50% during locomotion) by comparing the extensor force before and after deafferentation. Furthermore, Stein et al. (2000) showed in decerebrate cats that the ankle extensor force measured when the triceps surae muscles were stretched in a way mimicking the normal pattern was 35% more than when the muscles were isometrically held. Thus, in normal walking, the muscle spindle afferent pathways contribute to generation of the triceps surae EMG activity and force production during stance phase of locomotion.

5.2 Effects of soleus H-reflex operant conditioning on locomotion in rats

In the study by Chen et al. (2005), normal rats performed two treadmill sessions before and after the soleus H-reflex operant conditioning and locomotion changes were assessed by soleus H-reflex sizes (locomotor H-reflex), soleus EMG bursts (locomotor EMG), and the duration, length, and symmetry of a step cycle. The soleus locomotor H-reflex and locomotor EMG at the second gait assessment was larger in the up-conditioned rats while smaller in the down-conditioned rats when compared to the first gait assessment, and the change of locomotor EMG and locomotor H-reflex had strong positive correlation. Since other fundamental gait parameters such as length, duration, and symmetry of the step cycle did not change after operant conditioning, the authors suggested that operant conditioning may have induced compensatory changes, probably in the activity of other muscles, so that a locomotion pattern was still produced properly after the conditioning.

Another locomotion study was conducted by Chen et al. (2006b) to determine the effects of soleus operant up-conditioning on locomotion using rats with spinal cord injury. Rats prepared with midthoracic spinal cord injury were either exposed or not exposed to right soleus H-reflex up-conditioning. Two treadmill sessions were performed before and after the conditioning period. Before the conditioning, the treadmill locomotion showed clear asymmetry in the onset times of the right and left soleus EMG activity. After the conditioning, this asymmetry was corrected in the conditioned group whereas it persisted in the control group. The soleus locomotor H-reflex and locomotor EMG activity during the second treadmill session in the conditioned group were significantly bigger than those recorded in the first session and had significant positive correlation while there was no significant increase in the control group. Together with the finding from the previous study, it seems likely that operant conditioning of the soleus H-reflex in rats would induce compensatory plasticity to preserve the locomotion pattern when locomotion is performed properly (i.e. normal rats) whereas it would trigger different plastic changes to correct locomotion when a gait abnormality, such as asymmetry, already exists (i.e. spinal cord injury rats). Thus, they suggested that the protocol of operant conditioning might be a useful approach for people with spinal cord injury to improve their walking function.

6. Synergism among the soleus, MG, and LG

As reviewed above, acute modulation and long-term modification of the soleus H-reflex have been extensively studied. Surprisingly, previous studies exclusively focused on the soleus H-reflex, and to date, there have been very limited numbers of studies investigating modification and modulation of the H-reflex in its synergists, medial and lateral gastrocnemii (MG and LG, respectively). Those three muscles are innervated by the tibial nerve (i.e., synergists), and therefore, changes in the soleus H-reflex pathway would most likely affect MG and LG H-reflex pathways. Traditionally, the soleus, MG, and LG muscles were categorized as a group of synergist muscles for ankle plantar flexion (i.e., the triceps surae); however, many studies report that they should not be treated the same. Thus, we cannot simply assume that the MG and LG H-reflexes are modified and modulated in the same way as the soleus H-reflex.

Anatomically, the soleus is a single-joint plantar flexor whereas the gastrocnemii muscles are two-joint muscles crossing both the ankle and knee joints. Accordingly, their muscle length and consequent motor output are affected differently by each joint motion (Kawakami et al., 1998). Furthermore, in cats, the LG has been shown to consist of anatomically defined subvolumes (i.e., compartments) (English and Letbetter, 1982), and each compartment has a different activation pattern during locomotion (English, 1984). The human LG is also compartmentalized by its architecture (Segal et al., 1991) and EMG activity (Wolf et al., 1993) whereas the human MG is not likely to be compartmentalized

(Wolf and Kim, 1997). Differences in histochemical properties regarding muscle fibers types have also been reported (Johnson et al., 1973; Gollnick et al., 1974; Edgerton et al., 1975); while the human soleus has a higher percentage of type I fibers (slow-twitch fiber, 70-90%) compared to type II fibers (fast-twitch fiber, 10-30%), the gastrocnemii muscles contain a similar portion of both fiber types (50%). The fiber type differences are associated with their resistance to fatigue (Ochs et al., 1977), contractile properties (Vandervoort and McComas, 1983), and characteristics of EMG activity during functional tasks such as standing and walking (Joseph and Nightingale, 1952; Campbell et al., 1973; Duysens et al., 1991).

As for EMG activity, the soleus is often categorized as a tonic muscle and the gastrocnemii as phasic muscles because the soleus has a main role in tonic activity such as postural control (Joseph and Nightingale, 1952; Fitzpatrick et al., 1992) whereas the gastrocnemii are silent until phasic activity such as walking starts to provide impetus to a motion (Campbell et al., 1973). Furthermore, forward propulsion of the trunk during walking is primarily given by the soleus whereas the MG mainly contributes to vertical body support (McGowan et al., 2008). Differences in activation of the soleus, MG, and LG during plantarflexion task have also been reported. Segal and Song (2005) demonstrated, using functional MRI, that human MG and LG activated differentially during 1-min unilateral heel-raise exercise, with the MG being activated significantly more than the LG. Moreover, their results showed that, in the soleus, MG, and LG, activation of a proximal part of the muscle was significantly more than that of a distal part in all three muscles. During concentric ankle plantarflexion exercise in a sitting position, the activation of the LG was reported to be more than that of the soleus and MG (Giordano and Segal, 2006). In this study, only MG and LG, not the soleus, showed differential activation between the proximal and distal part of the muscle (proximal was larger than distal). Interestingly, despite the difference in medial-lateral spatial location between the MG and LG, force directions generated by those muscles were downward with subject-specific medial and lateral deviations, and were not significantly different (Giordano et al., 2009). However, the MG and LG in cats were reported to have substantial off-sagittal torques (i.e., toe-out and eversion torques) in addition to the classical sagittal plane torque (i.e., plantarflexion) (Lawrence et al., 1993).

Levy (1963) measured the stretch reflexes as well as the H-reflexes of the human soleus and MG in a prone position and found that the amplitudes of both stretch and H-reflexes were always greater in the soleus than in the MG. He linked the amplitude differences to an observation of previous animal studies, which demonstrated that density of the muscle spindles is greater in the soleus than in the MG in cats (Hagbarth and Wohlfart, 1952; Swett and Eldred, 1960). In addition, the motoneurons of the soleus have a larger total EPSP, which was exerted by Ia afferent volleys in a wide variety of muscles, than that of the MG and LG in cats (Eccles et al., 1957; Scott and Mendell, 1976) and in monkeys (Carp, 1993).

7. Development of dissertation project

Based on available anatomical, histological, and electrophysiological evidence, it is conceivable that the MG and LG H-reflex modulation and modification would be independent from those in the soleus H-reflex. That is, the H-reflexes of those three muscles would not necessarily be modulated and modified in the same direction (i.e., synergistic association). In order to establish solid basis of our understanding for modulation and modification of the H-reflexes occurring in the three synergist muscles, the first project of this dissertation investigated the H-reflex size modulation of the soleus, MG, and LG during walking and between standing and walking (Chapter 2), and the H-reflex modification of the three muscles induced by operant conditioning of the soleus H-reflex (Chapter 3).

The second project of the dissertation was developed to fill another gap in the literature: effects of the soleus H-reflex operant conditioning on locomotion in humans. There is no systematic human study to look into interaction between the soleus H-reflex operant conditioning and locomotion, which might lead to development of a new rehabilitative strategy to restore locomotor function in patients with neurological damage. Thus, as an attempt to provide insights about the functional implication of the operant conditioning, effects of the soleus H-reflex operant down-conditioning on locomotion in healthy humans was investigated (Chapter 4). The specific aims and hypotheses are listed below:

Chapter 2

Specific Aim

> To delineate characteristics of MG and LG H-reflex modulation during walking and between standing and walking, and to compare it to the well-known soleus H-reflex modulation in healthy humans. Experiments for this aim involved examining the soleus, MG, and LG H-reflexes during standing and walking.

Hypothesis

> The H-reflexes of the three muscles would be modulated similarly to facilitate the execution of ongoing motor tasks.

Chapter 3

Specific Aim

To determine whether and to what extent the H-reflexes of the MG and LG are concurrently modified after successful operant down-conditioning of the soleus H-reflex in healthy humans. Experiments for this aim involved operant down-conditioning of the soleus H-reflex with periodic concurrent monitoring of the MG and LG H-reflexes.

Hypothesis

The MG and LG H-reflexes would decrease in response to successful down-conditioning of the soleus H-reflex. However, the decrease of the H-reflex would be less in the MG and LG than in the soleus.

Chapter 4

Specific Aim

To investigate interaction of soleus H-reflex operant down-conditioning with locomotion in healthy humans. Experiments for this aim involved locomotion assessment using EMG activity and kinematic analyses before and after the soleus H-reflex down-conditioning.

Hypothesis

Operant down-conditioning would induce primary changes in the locomotor EMG and locomotor H-reflex of the soleus, but compensatory changes in other muscles would also occur so that the joint kinematics as well as other locomotor parameters would not change.

The conceptual model for developing those dissertation projects is presented in Figure 1.2.

8. General methodology

Operant conditioning protocols for human soleus H-reflex

In manuscripts 2 and 3 for this dissertation, operant down-conditioning technique for human soleus H-reflex was used as method to induce neural plasticity. Protocols for the H-reflex conditioning in human soleus have been established and reported thoroughly by Thompson et al. (2009). Instead of describing details of the protocols in each of the manuscripts, complete methodology for the human soleus operant down-conditioning is given below. A brief description of the conditioning method is also provided in manuscripts 2 and 3.

Session schedule

There are 6 baseline sessions and 30 down-conditioning sessions spread over 12 weeks (i.e., 3 sessions per week). Each session takes less than 90 minutes and is performed at the same time of the day to control for diurnal variations in H-reflex size (Wolpaw and Seegal, 1982; Chen and Wolpaw, 1994; Carp et al., 2006b; Lagerquist et al., 2006; Thompson et al., 2009). Each subject participates in one or two preliminary sessions to determine MVC value and appropriate M-wave size and background EMG activity range of the soleus which are used as targets for the rest of the sessions. Electrical stimulation that is set at just above the M-wave threshold is applied, and the M-wave size at this stimulus intensity is chosen as the target M-wave. The target M-wave stimulus level should elicit the

H-reflex on the ascending part of the H-reflex recruitment curve (see below: Session protocol). The target background activity range is selected to be a similar range of the activity during natural standing (i.e., usually 10-15% of MVC). The subject is asked to maintain the background activity in the target range with an aid of visual feedback (see below: Visual feedback).

Session protocol

EMG recording electrodes are placed on the skin overlying soleus and tibialis anterior (TA), and the stimulating electrodes on the skin overlying the tibial nerve (see below for details: Electrical stimulation and EMG recording). Then all sessions begin with an H-reflex/M-wave recruitment curve measurement while the subject stands naturally and keeps the background activity of the soleus within the target range. To obtain the recruitment curve, stimulus intensity is increased in steps of 1.25–2.5 mA from the soleus H-reflex threshold to just above the level that is required to elicit the Mmax (Zehr and Stein, 1999; Kido et al., 2004a; Thompson et al., 2009). Four sets of EMG responses recorded at each intensity are averaged.

Following the recruitment curve measurement, protocols are separated between baseline and conditioning sessions. In either protocol, the subject stands with the soleus activity within the target range, and the stimulus level is set so as to elicit the target soleus M-wave size (i.e., just above the M-wave threshold). Small adjustments in stimulus strength are occasionally needed to maintain the same soleus M-wave size.

During baseline sessions, 225 control H-reflexes are elicited after the recruitment curve measurement. The 225 trials are partitioned into three blocks of 75 trials. The H-reflexes are simply recorded and the subject does not receive any encouragement or feedback regarding the reflex size.

During the down-conditioning sessions, the recruitment curve measurement is followed by a block of 20 control H-reflexes. This control block is performed in the same way as the baseline sessions described above. Then, 225 conditioned H-reflexes (i.e., three blocks of 75 trials) are elicited, in which the subject is provided immediate visual feedback on the soleus H-reflex size at each trial (see below: Visual feedback) and asked to decrease the reflex size.

Electrical stimulation and EMG recording

Surface electrodes are placed in the popliteal fossa to stimulate the tibial nerve using a stimulator. For the EMG activity recording, a pair of surface electrodes is placed longitudinally on the skin over the soleus below the gastrocnemii (i.e., just above the insertion to the Achilles tendon) with an interelectrode distance of 3 cm. Locations for the nerve stimulating electrodes are determined to minimize the H-reflex threshold and maximize the Hmax and the Mmax sizes of the soleus. To evaluate antagonist activity during the conditioning, another pair of EMG recording electrodes is placed on the skin over the center of the TA. Locations for all electrodes are mapped using permanent skin marks such as scars and moles at the preliminary session to minimize session-to-session variability. For the subsequent sessions, the electrodes are placed based on those landmarks.

The EMG and nerve stimulus signals are recorded for a period of 200 ms in response to each electrical stimulus pulse including a pre-stimulus period of 50 ms. In addition, the soleus EMG signals are rectified and averaged every 100 ms. The result is immediately shown on the computer screen as a bar graph for visual feedback (see below: Visual feedback). The visual feedback is provided to help the subject maintain the soleus background EMG activity within the specified range. If the soleus background EMG is kept in the target range for 2 s and if 5 s is passed since the last stimulus (i.e., the minimum interstimulus period is 5 s), a square stimulus pulse with 1 ms of duration is delivered to elicit the H-reflex and M-wave.

Visual feedback

Visual feedback is provided on the computer screen and contains two bar graphs (Figure 1.3): one for the background EMG activity of the soleus (left) and the other for the soleus H-reflex size (right).

The background graph has a shaded area that shows the target background range specifically defined for each subject. The bar, which is updated every 100 ms, represents current level of rectified soleus EMG activity. The bar turns green when the top is in the shaded area. If the bar stays green for at least 2 s (and if 5 s is passed since the last stimulus), stimulation is delivered. The subject is allowed to move between trials, and asked to make the bar green for more than 2 s only when he/she is ready to receive the stimulation. Thus, the inter-stimulus interval is controlled by the subject and varies within and across sessions. The visual feedback screen for the background activity is exactly the same for both the control and conditioning trials.

The feedback graph regarding the H-reflex size is different between the control and conditioning trials. During the control trials, the graph shows only a green vertical bar 200 ms after the each stimulus, which represents a mean rectified EMG activity in H-reflex interval of the soleus (typically 30-45 ms after the stimulus). During the conditioning trials, the feedback graph includes a shaded area that represents a reward criterion (see below: The reward criterion). The vertical bar is updated 200 ms after each stimulus, and is green if the top of the bar is below the reward criterion (i.e., successful trial) and otherwise red (i.e., failure trial). Thus, the bar is used by the subject to determine whether the trial is a success. The reflex size feedback graph during the conditioning trials also constantly shows a thick horizontal line that represents the average H-reflex size of the soleus during the six baseline sessions for each subject. This baseline average line informs the subject how much the reflex size is "improved" (i.e., decreased) during the conditioning sessions when compared to the baseline sessions. In addition, during the conditioning trials, ongoing success rate updated at each stimulus is provided at the bottom of the screen.

The reward criterion

The reward criterion during the conditioning trials is based on the performance of the immediately preceding block of trials. Thus, for the first block of 75 conditioning trials, the reward criterion is based on the average of the block of 20 control trials. For the second and third blocks of 75 conditioning trials, the reward criterion values are based on the preceding conditioning block. The reward criterion is set at 60% from the lowest end of the soleus reflex size distribution. Therefore, 60% of the trials would be successful (i.e., less than the criterion) if the distribution of the soleus H-reflex size for the current block is similar to that of the previous block. For each block during the conditioning sessions, the subject earns an extra monetary reward (up to ten dollars) if the success rate exceeds 50% or the average reflex size is lower than the average of the baseline sessions.

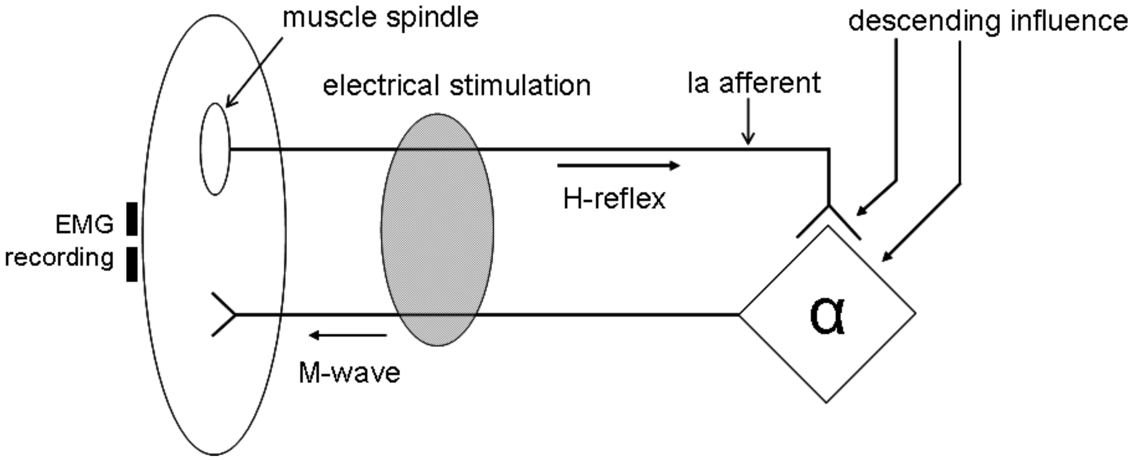

Figure 1.1: Spinal pathway of the H-reflex
The spinal pathway of the H-reflex is mainly monosynaptic, consisting of Ia afferent, alpha motoneuron, and a synapse between them. Electrical stimulation is percutaneously applied on a “mixed nerve”, which elicits afferent volley to evoke an H-reflex and a direct motor response (M-wave).

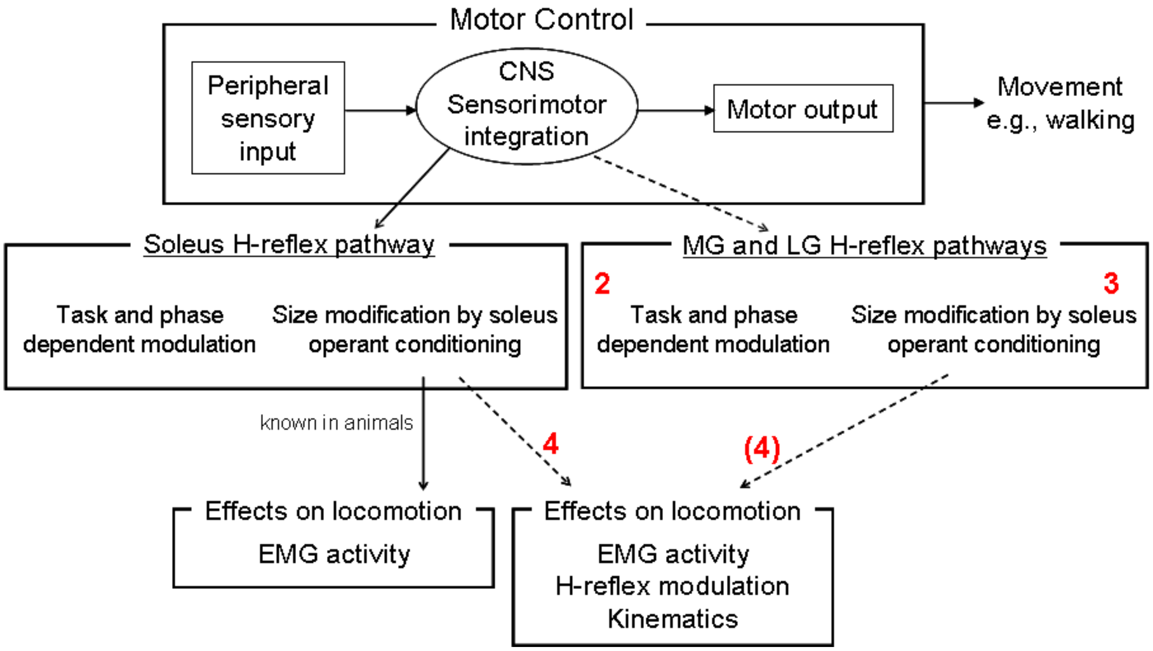

Figure 1.2: Conceptual model that led to development of the dissertation project
Solid arrows indicate findings already reported in the literature, and dotted arrows indicate lack of knowledge to date. Numbers in red indicate chapter numbers in the dissertation.
Solid arrows: Control of a movement such as walking is done by complex integration between sensory and motor systems through activity of the central nervous system (CNS). Sensorimotor integration of the CNS induces H-reflex size modulation of the soleus during walking. In addition, neural plasticity in the CNS occurring during operant conditioning modifies the soleus H-reflex. Modification of the soleus H-reflex by operant conditioning has been shown to have effects on locomotion in animals.
Dotted arrows: Previous studies have exclusively investigated the soleus H-reflex, and H-reflexes in other muscles such as its synergists, medial and lateral gastrocnemii (MG and LG) have not been well studied. Thus, whether or not the three muscles are modulated and modified in the same direction is unknown. The first project of this dissertation studied modulation of the MG and LG H-reflexes during locomotion (Chapter 2) and modification of the MG and LG H-reflexes induced by soleus operant conditioning (Chapter 3). As the second project, effects of modified H-reflex in the soleus (and possibly in the MG, and LG, if the soleus conditioning affects those synergists) on locomotion were analyzed (Chapter 4) in terms of EMG activity, H-reflex modulation, and kinematics.

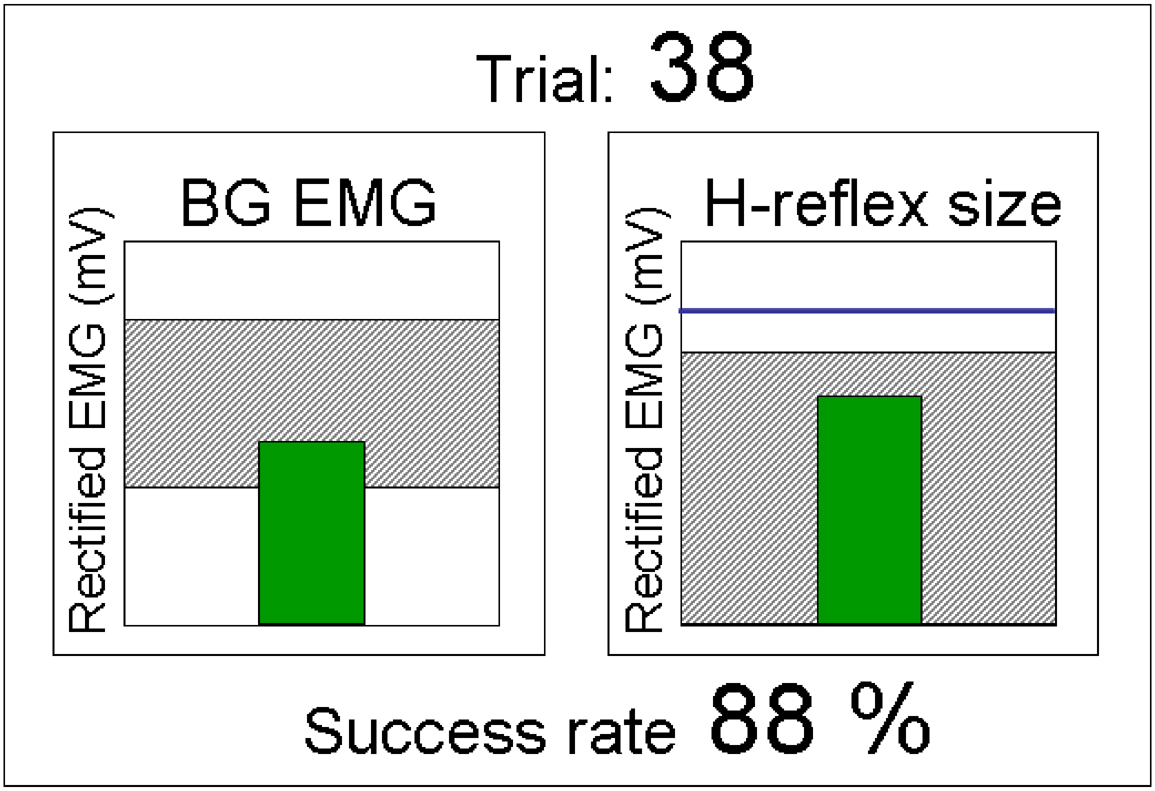

Figure 1.3: Visual feedback screen

There are two graphs on the visual feedback screen: feedback on soleus background EMG activity (left) and on soleus H-reflex size (right).

Left: This graph is the same between baseline and conditioning sessions. The target background EMG activity, which is set at each subject's natural standing level, is shown as shaded area. If the background EMG activity remains in the target range for 2 s, the H-reflex is elicited.

Right: This graph is presented only during the conditioning trials. The H-reflex size bar, representing mean rectified soleus EMG in the H-reflex interval (typically 30-45 ms after stimulus), appears 200 ms after the stimulus pulse. The horizontal thick line shows the subject's average H-reflex size during the baseline sessions. The shaded area indicates the range of the H-reflex sizes that satisfies the reward criterion. The criterion is based on the H-reflex sizes of the immediately preceding block: the criterion for the first block of 75 trials is based on the 20 control trials, the second block is based on the first bock, and third block is based on the second block. The bar turns green if its height is within the shaded area and the trial is counted as successful. If the height of the bar exceeds the shaded area, it turns red and the trial is counted as a failure. The ongoing success rate is shown at the bottom of the screen and updated after each trial.

References

Akazawa K, Aldridge JW, Steeves JD, Stein RB (1982) Modulation of stretch reflexes during locomotion in the mesencephalic cat. J Physiol 329:553-567.

Bennett DJ, De Serres SJ, Stein RB (1996) Gain of the triceps surae stretch reflex in decerebrate and spinal cats during postural and locomotor activities. J Physiol 496 (Pt 3):837-850.

Brooke JD, Cheng J, Collins DF, McIlroy WE, Misiaszek JE, Staines WR (1997) Sensori-sensory afferent conditioning with leg movement: gain control in spinal reflex and ascending paths. Prog Neurobiol 51:393-421.

Buchthal F, Schmalbruch H (1970) Contraction times of twitches evoked by H-reflexes. Acta Physiol Scand 80:378-382.

Burke D (1983) Critical examination of the case for or against fusimotor involvement in disorders of muscle tone. In: Desmedt JE (ed) Motor control mechanisms in health and disease Raven Press, New York, pp 133-150.

Burke D, Gandevia SC, McKeon B (1984) Monosynaptic and oligosynaptic contributions to human ankle jerk and H-reflex. J Neurophysiol 52:435-448.

Burke D, Adams RW, Skuse NF (1989) The effects of voluntary contraction on the H reflex of human limb muscles. Brain 112 (Pt 2):417-433.

Campbell KM, Biggs NL, Blanton PL, Lehr RP (1973) Electromyographic investigation of the relative activity among four components of the triceps surae. Am J Phys Med 52:30-41.

Capaday C (1997) Neurophysiological methods for studies of the motor system in freely moving human subjects. J Neurosci Methods 74:201-218.

Capaday C, Stein RB (1986) Amplitude modulation of the soleus H-reflex in the human during walking and standing. J Neurosci 6:1308-1313.

Capaday C, Stein RB (1987a) Difference in the amplitude of the human soleus H reflex during walking and running. J Physiol 392:513-522.

Capaday C, Stein RB (1987b) A method for simulating the reflex output of a motoneuron pool. J Neurosci Methods 21:91-104.

Capaday C, Stein RB (1989) The effects of postsynaptic inhibition on the monosynaptic reflex of the cat at different levels of motoneuron pool activity. Exp Brain Res 77:577-584.

Carp JS (1993) Monosynaptic EPSPs in primate lumbar motoneurons. J Neurophysiol 70:1585-1592.

Carp JS, Wolpaw JR (1994) Motoneuron plasticity underlying operantly conditioned decrease in primate H-reflex. J Neurophysiol 72:431-442.

Carp JS, Wolpaw JR (1995) Motoneuron properties after operantly conditioned increase in primate H-reflex. J Neurophysiol 73:1365-1373.

Carp JS, Chen XY, Sheikh H, Wolpaw JR (2001) Operant conditioning of rat H-reflex affects motoneuron axonal conduction velocity. Exp Brain Res 136:269-273.

Carp JS, Tennissen AM, Chen XY, Wolpaw JR (2006a) Diurnal H-reflex variation in mice. Exp Brain Res 168:517-528.

Carp JS, Tennissen AM, Chen XY, Wolpaw JR (2006b) H-reflex operant conditioning in mice. J Neurophysiol 96:1718-1727.

Casabona A, Polizzi MC, Perciavalle V (1990) Differences in H-reflex between athletes trained for explosive contractions and non-trained subjects. Eur J Appl Physiol Occup Physiol 61:26-32.

Chen XY, Wolpaw JR (1994) Circadian rhythm in rat H-reflex. Brain Res 648:167-170.

Chen XY, Wolpaw JR (1995) Operant conditioning of H-reflex in freely moving rats. J Neurophysiol 73:411-415.

Chen XY, Wolpaw JR (2002) Probable corticospinal tract control of spinal cord plasticity in the rat. J Neurophysiol 87:645-652.

Chen XY, Wolpaw JR (2005) Ablation of cerebellar nuclei prevents H-reflex down-conditioning in rats. Learn Mem 12:248-254.

Chen XY, Carp JS, Chen L, Wolpaw JR (2002) Corticospinal tract transection prevents operantly conditioned H-reflex increase in rats. Exp Brain Res 144:88-94.

Chen XY, Carp JS, Chen L, Wolpaw JR (2006a) Sensorimotor cortex ablation prevents H-reflex up-conditioning and causes a paradoxical response to down-conditioning in rats. J Neurophysiol 96:119-127.

Chen Y, Chen XY, Jakeman LB, Schalk G, Stokes BT, Wolpaw JR (2005) The interaction of a new motor skill and an old one: H-reflex conditioning and locomotion in rats. J Neurosci 25:6898-6906.

Chen Y, Chen XY, Jakeman LB, Chen L, Stokes BT, Wolpaw JR (2006b) Operant conditioning of H-reflex can correct a locomotor abnormality after spinal cord injury in rats. J Neurosci 26:12537-12543.

Cook WA, Jr., Cangiano A (1972) Presynaptic and postsynaptic inhibition of spinal motoneurons. J Neurophysiol 35:389-403.

Duysens J, Tax AA, van der Doelen B, Trippel M, Dietz V (1991) Selective activation of human soleus or gastrocnemius in reflex responses during walking and running. Exp Brain Res 87:193-204.

Dyhre-Poulsen P, Simonsen EB, Voigt M (1991) Dynamic control of muscle stiffness and H reflex modulation during hopping and jumping in man. J Physiol 437:287-304.

Eccles JC, Eccles RM, Lundberg A (1957) The convergence of monosynaptic excitatory afferents on to many different species of alpha motoneurones. J Physiol 137:22-50.

Eccles JC, Schmidt RF, Willis WD (1962) Presynaptic inhibition of the spinal monosynaptic reflex pathway. J Physiol 161:282-297.

Edamura M, Yang JF, Stein RB (1991) Factors that determine the magnitude and time course of human H-reflexes in locomotion. J Neurosci 11:420-427.

Edgerton VR, Smith JL, Simpson DR (1975) Muscle fibre type populations of human leg muscles. Histochem J 7:259-266.

English AW (1984) An electromyographic analysis of compartments in cat lateral gastrocnemius muscle during unrestrained locomotion. J Neurophysiol 52:114-125.

English AW, Letbetter WD (1982) A histochemical analysis of identified compartments of cat lateral gastrocnemius muscle. Anat Rec 204:123-130.

Evatt ML, Wolf SL, Segal RL (1989) Modification of human spinal stretch reflexes: preliminary studies. Neurosci Lett 105:350-355.

Fitzpatrick RC, Gorman RB, Burke D, Gandevia SC (1992) Postural proprioceptive reflexes in standing human subjects: bandwidth of response and transmission characteristics. J Physiol 458:69-83.

Floyer-Lea A, Matthews PM (2005) Distinguishable brain activation networks for short- and long-term motor skill learning. J Neurophysiol 94:512-518.

Funase K, Miles TS (1999) Observations on the variability of the H reflex in human soleus. Muscle Nerve 22:341-346.

Ghez C (1991) The control of movement. In: Kandel E (ed) Principles in Neroscience Third edition. Elsevier, New York, pp 533-547.

Giordano SB, Segal RL (2006) Leg muscles differ in spatial activation patterns with differing levels of voluntary plantarflexion activity in humans. Cells Tissues Organs 184:42-51.

Giordano SB, Segal RL, Abelew TA (2009) Differences in end-point force trajectories elicited by electrical stimulation of individual human calf muscles. J Appl Biomech 25:330-339.

Gollnick PD, Sjodin B, Karlsson J, Jansson E, Saltin B (1974) Human soleus muscle: a comparison of fiber composition and enzyme activities with other leg muscles. Pflugers Arch 348:247-255.

Hagbarth KE, Wohlfart G (1952) The number of muscle-spindles in certain muscles in cat in relation to the composition of the muscle nerves. Acta Anat (Basel) 15:85-104.

Henneman E, Somjen G, Carpenter DO (1965) Functional Significance of Cell Size in Spinal Motoneurons. J Neurophysiol 28:560-580.

Hodapp M, Klisch C, Mall V, Vry J, Berger W, Faist M (2007) Modulation of soleus H-reflexes during gait in children with cerebral palsy. J Neurophysiol 98:3263-3268.

Jenkins IH, Brooks DJ, Nixon PD, Frackowiak RS, Passingham RE (1994) Motor sequence learning: a study with positron emission tomography. J Neurosci 14:3775-3790.

Johnson MA, Polgar J, Weightman D, Appleton D (1973) Data on the distribution of fibre types in thirty-six human muscles. An autopsy study. J Neurol Sci 18:111-129.

Joseph J, Nightingale A (1952) [Electromyography of muscles of posture: leg muscles in males.]. J Physiol 117:484-491.

Kawakami Y, Ichinose Y, Fukunaga T (1998) Architectural and functional features of human triceps surae muscles during contraction. J Appl Physiol 85:398-404.

Kido A, Tanaka N, Stein RB (2004) Spinal excitation and inhibition decrease as humans age. Can J Physiol Pharmacol 82:238-248.

Koceja DM, Markus CA, Trimble MH (1995) Postural modulation of the soleus H reflex in young and old subjects. Electroencephalogr Clin Neurophysiol 97:387-393.

Krauss EM, Misiaszek JE (2007) Phase-specific modulation of the soleus H-reflex as a function of threat to stability during walking. Exp Brain Res 181:665-672.

Kukulka CG (1992) Principles of neuromuscular excitation. In: Gersh MR (ed) Electrotherapy in rehabilitation (contemporary perspectives in rehabilitation) Davis, Philadelphia, pp 3-25.

Lagerquist O, Zehr EP, Baldwin ER, Klakowicz PM, Collins DF (2006) Diurnal changes in the amplitude of the Hoffmann reflex in the human soleus but not in the flexor carpi radialis muscle. Exp Brain Res 170:1-6.

Lawrence JH, 3rd, Nichols TR, English AW (1993) Cat hindlimb muscles exert substantial torques outside the sagittal plane. J Neurophysiol 69:282-285.

Lehericy S, Benali H, Van de Moortele PF, Pelegrini-Issac M, Waechter T, Ugurbil K, Doyon J (2005) Distinct basal ganglia territories are engaged in early and advanced motor sequence learning. Proc Natl Acad Sci U S A 102:12566-12571.

Levy R (1963) The relative importance of the gastrocnemius and soleus muscles in the ankle jerk of man. J Neurol Neurosurg Psychiatry 26:148-150.

Llewellyn M, Yang JF, Prochazka A (1990) Human H-reflexes are smaller in difficult beam walking than in normal treadmill walking. Exp Brain Res 83:22-28.

Loscher WN, Cresswell AG, Thorstensson A (1996) Excitatory drive to the alpha-motoneuron pool during a fatiguing submaximal contraction in man. J Physiol 491 (Pt 1):271-280.

Ludlow CL, Hoit J, Kent R, Ramig LO, Shrivastav R, Strand E, Yorkston K, Sapienza CM (2008) Translating principles of neural plasticity into research on speech motor control recovery and rehabilitation. J Speech Lang Hear Res 51:S240-258.

Magladery JW, Porter WE, Park AM, Teasdall RD (1951) Electrophysiological studies of nerve and reflex activity in normal man. IV. The two-neurone reflex and identification of certain action potentials from spinal roots and cord. Bull Johns Hopkins Hosp 88:499-519.

McGowan CP, Neptune RR, Kram R (2008) Independent effects of weight and mass on plantar flexor activity during walking: implications for their contributions to body support and forward propulsion. J Appl Physiol 105:486-494.

Misiaszek JE (2003) The H-reflex as a tool in neurophysiology: its limitations and uses in understanding nervous system function. Muscle Nerve 28:144-160.

Morita H, Shindo M, Yanagawa S, Yoshida T, Momoi H, Yanagisawa N (1995) Progressive decrease in heteronymous monosynaptic Ia facilitation with human ageing. Exp Brain Res 104:167-170.

Nielsen J, Crone C, Hultborn H (1993) H-reflexes are smaller in dancers from The Royal Danish Ballet than in well-trained athletes. Eur J Appl Physiol Occup Physiol 66:116-121.

Ochs RM, Smith JL, Edgerton VR (1977) Fatigue characteristics of human gastrocnemius and soleus muscles. Electromyogr Clin Neurophysiol 17:297-306.

Penhune VB, Doyon J (2002) Dynamic cortical and subcortical networks in learning and delayed recall of timed motor sequences. J Neurosci 22:1397-1406.

Perez MA, Lundbye-Jensen J, Nielsen JB (2007) Task-specific depression of the soleus H-reflex after cocontraction training of antagonistic ankle muscles. J Neurophysiol 98:3677-3687.

Pierrot-Deseilligny E, Mazevet D (2000) The monosynaptic reflex: a tool to investigate motor control in humans. Interest and limits. Neurophysiol Clin 30:67-80.

Ross A, Leveritt M, Riek S (2001) Neural influences on sprint running: training adaptations and acute responses. Sports Med 31:409-425.

Sabbahi MA, Sedgwick EM (1982) Age-related changes in monosynaptic reflex excitability. J Gerontol 37:24-32.

Schieppati M (1987) The Hoffmann reflex: a means of assessing spinal reflex excitability and its descending control in man. Prog Neurobiol 28:345-376.

Schmidt RA, Lee TD (2005) Motor control and learning: A behavioral emphasis. Fourth edition. Human Kinetics, Champaign.

Schneider C, Capaday C (2003) Progressive adaptation of the soleus H-reflex with daily training at walking backward. J Neurophysiol 89:648-656.

Scott JG, Mendell LM (1976) Individual EPSPs produced by single triceps surae Ia afferent fibers in homonymous and heteronymous motoneurons. J Neurophysiol 39:679-692.

Segal RL, Song AW (2005) Nonuniform activity of human calf muscles during an exercise task. Arch Phys Med Rehabil 86:2013-2017.

Segal RL, Wolf SL, DeCamp MJ, Chopp MT, English AW (1991) Anatomical partitioning of three multiarticular human muscles. Acta Anat (Basel) 142:261-266.

Sherrington C (1947) The integrative action of the nervous system. Second edition. Yale University Press, New Haven.

Stein RB (1995) Presynaptic inhibition in humans. Prog Neurobiol 47:533-544.

Stein RB, Capaday C (1988) The modulation of human reflexes during functional motor tasks. Trends Neurosci 11:328-332.

Stein RB, Misiaszek JE, Pearson KG (2000) Functional role of muscle reflexes for force generation in the decerebrate walking cat. J Physiol 525 Pt 3:781-791.

Swett JE, Eldred E (1960) Distribution and numbers of stretch rceptors in medial gastrocnemius and soleus muscles of the cat. Anat Rec 137:453-460.

Thompson AK, Chen XY, Wolpaw JR (2009) Acquisition of a simple motor skill: task-dependent adaptation plus long-term change in the human soleus H-reflex. J Neurosci 29:5784-5792.

Thompson AK, Lapallo B, Duffield M, Abel BM, Pomerantz F (2011) Repetitive common peroneal nerve stimulation increases ankle dorsiflexor motor evoked potentials in incomplete spinal cord lesions. Exp Brain Res.

Trimble MH, Koceja DM (1994) Modulation of the triceps surae H-reflex with training. Int J Neurosci 76:293-303.

Vandervoort AA, McComas AJ (1983) A comparison of the contractile properties of the human gastrocnemius and soleus muscles. Eur J Appl Physiol Occup Physiol 51:435-440.

Voigt M, Chelli F, Frigo C (1998) Changes in the excitability of soleus muscle short latency stretch reflexes during human hopping after 4 weeks of hopping training. Eur J Appl Physiol Occup Physiol 78:522-532.

Wang Y, Pillai S, Wolpaw JR, Chen XY (2006) Motor learning changes GABAergic terminals on spinal motoneurons in normal rats. Eur J Neurosci 23:141-150.

Wang Y, Pillai S, Wolpaw JR, Chen XY (2009) H-reflex down-conditioning greatly increases the number of identifiable GABAergic interneurons in rat ventral horn. Neurosci Lett 452:124-129.

Wolf SL, Kim JH (1997) Morphological analysis of the human tibialis anterior and medial gastrocnemius muscles. Acta Anat (Basel) 158:287-295.

Wolf SL, Segal RL, English AW (1993) Task-oriented EMG activity recorded from partitions in human lateral gastrocnemius muscle. J Electromyogr Kinesiol 3:87-94.

Wolpaw JR (1987) Operant conditioning of primate spinal reflexes: the H-reflex. J Neurophysiol 57:443-459.

Wolpaw JR (2007) Spinal cord plasticity in acquisition and maintenance of motor skills. Acta Physiol (Oxf) 189:155-169.

Wolpaw JR (2010) What can the spinal cord teach us about learning and memory? Neuroscientist 16:532-549.

Wolpaw JR, Seegal RF (1982) Diurnal rhythm in the spinal stretch reflex. Brain Res 244:365-369.

Wolpaw JR, O'Keefe JA (1984) Adaptive plasticity in the primate spinal stretch reflex: evidence for a two-phase process. J Neurosci 4:2718-2724.

Wolpaw JR, Tennissen AM (2001) Activity-dependent spinal cord plasticity in health and disease. Annu Rev Neurosci 24:807-843.

Wolpaw JR, Chen XY (2001) Operant conditioning of rat H-reflex: effects on mean latency and duration. Exp Brain Res 136:274-279.

Wolpaw JR, Chen XY (2006) The cerebellum in maintenance of a motor skill: a hierarchy of brain and spinal cord plasticity underlies H-reflex conditioning. Learn Mem 13:208-215.

Wolpaw JR, Braitman DJ, Seegal RF (1983) Adaptive plasticity in primate spinal stretch reflex: initial development. J Neurophysiol 50:1296-1311.

Yamanaka K, Yamamoto S, Nakazawa K, Yano H, Suzuki Y, Fukunaga T (1999) The effects of long-term bed rest on H-reflex and motor evoked potential in the human soleus muscle during standing. Neurosci Lett 266:101-104.

Yang JF, Whelan PJ (1993) Neural mechanisms that contribute to cyclical modulation of the soleus H-reflex in walking in humans. Exp Brain Res 95:547-556.

Yang JF, Stein RB, James KB (1991) Contribution of peripheral afferents to the activation of the soleus muscle during walking in humans. Exp Brain Res 87:679-687.

Zehr EP, Stein RB (1999) Interaction of the Jendrassik maneuver with segmental presynaptic inhibition. Exp Brain Res 124:474-480.

Zehr PE (2002) Considerations for use of the Hoffmann reflex in exercise studies. Eur J Appl Physiol 86:455-468.

CHAPTER II

FIRST MANUSCRIPT

H-reflex modulation in the human medial and lateral gastrocnemii during standing and walking

(Submitted to Muscle and Nerve)

ABSTRACT

Introduction: The soleus H-reflex is dynamically modulated during walking. However, modulation of the gastrocnemii H-reflexes has not been studied systematically.

Methods: The medial and lateral gastrocnemii (MG and LG) and soleus H-reflexes were measured during standing and walking in humans.

Results: Maximum H-reflex amplitude was significantly smaller in MG (mean 1.1 mV) and LG (1.1 mV) than in soleus (3.3 mV). Despite these size differences, the reflex amplitudes of the three muscles were positively correlated. The MG and LG H-reflexes were phase- and task-dependently modulated in ways similar to the soleus H-reflex.

Discussion: Although there are anatomical and physiological differences between the soleus and gastrocnemii muscles, the reflexes of the three muscles are similarly modulated during walking and between standing and walking. The present findings support the hypothesis that these reflexes are synergistically modulated during walking to facilitate the ongoing movement.

INTRODUCTION

The soleus H-reflex is dynamically modulated during motor tasks in humans. The soleus H-reflex amplitude at a given background electromyography (EMG) level decreases from standing to walking, and from walking to running (i.e., task-dependent modulation) (Capaday and Stein, 1986, 1987a; Edamura et al., 1991; Kido et al., 2004a). Furthermore, the soleus H-reflex is modulated during walking depending on the phase of the gait cycle (i.e., phase-dependent modulation) (Capaday and Stein, 1986; Yang and Whelan, 1993; Kido et al., 2004a). In contrast to the large number of studies investigating modulation of the soleus H-reflex, the number of studies on H-reflexes of its synergists, medial and lateral gastrocnemii (MG and LG, respectively) has been limited to specific tasks such as hopping (Moritani et al., 1990; Dyhre-Poulsen et al., 1991), landing (Dyhre-Poulsen et al., 1991; McDonagh and Duncan, 2002), and lengthening and shortening contraction (Pinniger et al., 2001). In general, the H-reflexes of the soleus and the gastrocnemii were modulated during a motor task in similar ways, although Pinniger et al. (2001) found that the ratio of maximum H-reflex to maximum M-wave was larger in the soleus than in the MG. On the other hand, Moritani et al. (1990) reported that the amplitude of the MG H-reflex during hopping elicited shortly after foot contact increased as force and speed of the motor task increased whereas the soleus H-reflex showed an opposite pattern. However, this study had only one subject and the H-reflex size was not evaluated in relation to the background EMG level; thus, it is not clear how differently the H-reflex is modulated during hopping between the soleus and the MG. The MG and LG, together with the soleus, are traditionally considered as one functional unit of ankle plantarflexor muscles (i.e., the triceps surae) (Murray et al., 1976).

However, to date, questions of whether and to what extent the MG and LG H-reflexes are modulated between and during standing and walking have not been studied.

There are several clear differences between the gastrocnemii and soleus. Anatomically, the gastrocnemii muscles are two-joint muscles operating at both the ankle and knee joints, whereas the soleus is a single-joint plantarflexor muscle. Thus, ankle and/or knee joint motion influences their lengths and consequent motor outputs differently (Kawakami et al., 1998). Histochemical properties (i.e., muscle fiber type) are also different between the soleus and gastrocnemii (Johnson et al., 1973; Gollnick et al., 1974; Edgerton et al., 1975). Their fiber type differences affect their resistance to fatigue (Ochs et al., 1977), contractile properties (Vandervoort and McComas, 1983), and EMG activity patterns during standing and walking (Joseph and Nightingale, 1952; Campbell et al., 1973). The gastrocnemii are often categorized as phasic muscles because they are mainly activated during phasic activity (e.g., walking) to provide impetus to a motion (Campbell et al., 1973), whereas the soleus is categorized as a tonic muscle because it has a main role in tonic actions such as postural control (Joseph and Nightingale, 1952; Fitzpatrick et al., 1992). Duysens et al. (1991) reported that the soleus was constantly activated during standing while the MG was almost silent, causing the EMG activity of the soleus to be always larger than that of the MG. During walking, the MG was nearly as active as the soleus but its activation started later in the stance phase.

As for the differences in spinal reflexes, Levy (1963) measured stretch reflexes and H-reflexes of the human soleus and MG in a prone position and found that the amplitudes of both reflexes were always greater in the soleus than in the MG. He linked the amplitude differences to an observation in previous animal studies, which demonstrated that muscle spindle density is greater in the soleus than in the MG in cats (Hagbarth and Wohlfart, 1952; Swett and Eldred, 1960). Furthermore, in cats, the soleus motoneurons were reported to have larger total excitatory post-synaptic potentials (EPSPs) produced by Ia afferent volleys from a variety of muscles than its synergists MG and LG (Eccles et al., 1957; Scott and Mendell, 1976). The larger total EPSP in the soleus than in the MG and LG was also reported in a non-human primate study (Carp, 1993). Moreover, in decerebrated cats, Nichols (1989) showed that organizations of the heterogenic reflexes (either excitatory or inhibitory) among the three muscles were different between quiescent condition (i.e., resting) and activated condition, and thus, suggested that the soleus and gastrocnemii have different roles in coordinating posture and movement.

In sum, based on the available anatomical, histological, and electrophysiological evidence, it is conceivable that gastrocnemii and soleus spinal reflexes are differently modulated during motor tasks. However, to our knowledge, there has been no study systematically investigating task- and/or phase-dependent modulation of the MG or LG H-reflexes. Thus, in the present study, we examined the MG and LG H-reflexes during standing and walking to delineate the characteristics of MG and LG H-reflex modulation, and to compare it to the well-known soleus H-reflex modulation.

MATERIALS AND METHODS

General Procedure

Twenty-four subjects with no known neurological disorders (13 men and 11 women aged 21-54 yrs) participated in the study. The subjects understood the purposes and procedures of the experiments and signed the consent form before participation. All protocols were approved by the Institutional Review Boards for Human Research of the University of North Carolina at Chapel Hill (Chapel Hill, NC) and Helen Hayes Hospital (West Haverstraw, NY).

In all experiments, EMG activity was recorded from the soleus, MG, LG, and tibialis anterior (TA) muscles with self-adhesive surface Ag-AgCl electrodes (Vermed, Bellows Falls, VT). After placing the EMG electrodes on the skin over the muscles, maximum voluntary contraction (MVC) was measured for soleus, MG, and LG during standing. Then, the H-reflexes and the M-waves of the three muscles were elicited by stimulating the tibial nerve while the subject was standing upright or walking on a treadmill.

Electrical Stimulation and EMG Recording

Self-adhesive surface Ag-AgCl electrodes (2.2 × 2.2 cm for the cathode and 2.2 × 3.5 cm for the anode; Vermed, Bellows Falls, VT) were placed on the skin over the popliteal fossa to stimulate the tibial nerve using a Grass S48 stimulator (with CCU1 constant current unit and SIU5 stimulus isolation unit; Astro-Med, West Warwick, RI). For EMG activity recording, a pair of self-adhesive surface Ag-AgCl electrodes (2.2 × 3.5 cm, Vermed, Bellows Falls, VT) was placed longitudinally on the skin over the soleus just below the gastrocnemii with an interelectrode distance of 3 cm. EMG recording electrodes were also placed over the center of the muscle bellies of the MG, LG, and TA. Pairs of electrodes for the soleus, MG, and LG muscles were placed at least 7 cm apart between the muscles to minimize the cross-talk. Locations for the nerve stimulating electrodes were determined to minimize the H-reflex threshold and maximize the maximum H-reflex (Hmax) and M-wave (Mmax) amplitudes of the soleus. Thus, the stimulus location was optimized to the soleus H-reflex. However, in a pilot experiment, stimulation at the optimum location for the soleus H-reflex also maximized the Hmax and Mmax of the MG and LG. Therefore, the same stimulus location was used for eliciting the soleus, MG, and LG H-reflexes simultaneously, and presumably, this location was optimum for all three muscles.

EMG signals were amplified, band-pass filtered (10-1000 Hz), and recorded with a custom-made system and Axoscope (Molecular Devices Inc., Sunnyvale, CA) at 5000 Hz (for standing data) or 2000 Hz (for walking data). During standing, the EMG and nerve stimulus signals were recorded for a period of 200 ms in response to each test stimulus pulse including a prestimulus period of 50 ms. In addition, the soleus EMG signals were rectified, averaged every 100 ms, and shown on the computer screen as a bar graph for visual feedback. If the soleus background EMG was kept in the specified range (typically 10-15% MVC) for 2 s and if 5 s had passed since the last stimulus (i.e., the minimum interstimulus period was 5 s), a square stimulus pulse with 1 ms of duration was delivered to elicit the H-reflex and M-wave. During the measurement of the standing H-reflexes with various background activity levels, the visual feedback was used to help the subject grade the background EMG activity from 5 to 100% MVC levels. In order to simplify the task, no feedback of the MG and LG background EMG activity was provided. During walking, the EMG activity, heel contact, and nerve stimulation signals were continuously recorded while the H-reflexes were elicited at pseudo-random intervals (i.e., interstimulus interval of 2.5-4.5 s). This was to ensure that the H-reflexes were obtained at various phases throughout the entire gait cycle. No more than one stimulus was delivered per gait cycle, and there was at least one gait cycle without stimulation between stimulated cycles (Yang and Stein, 1990; Kido et al., 2004a, b).

MVC measurements of the soleus, MG, and LG

The MVC values of the soleus, MG, and LG were determined as the maximum rectified EMG level. The subject stood on both feet with slightly raised heels from the ground, and often shifted body weight onto the tested leg as the level of contraction increased. Also, if necessary, vertical resistance was applied by either pressing down the subject's shoulders or asking the subject to push up against the hand-rail that was placed in front of the subject for balancing. The subject was asked to exert maximum effort to activate the plantarflexors for 5 s. Three MVC measurements were made with short breaks in between. Average EMG activity of the middle 3 s was calculated for each muscle and the highest value of the three trials was taken as the muscle's MVC.

H-reflex measurements of the soleus, MG, and LG

For all 24 subjects, the H-reflex and M-wave recruitment curves were obtained from the soleus, MG, and LG simultaneously while the subject stood and maintained a pre-defined level (usually 10-15% of the MVC) of rectified soleus EMG activity (see *Electrical stimulation and EMG recording*). Stimulus intensity was increased in steps of 1.25–2.5 mA from the soleus H-reflex threshold to just above the level that was required to elicit the Mmax in all three muscles (Zehr and Stein, 1999; Kido et al., 2004a; Thompson et al., 2009). Generally, ten different stimulus intensities were used to obtain the recruitment curve, and four EMG responses were recorded and averaged at each stimulus intensity.

For 19 subjects, the H-reflexes of the soleus, MG, and LG were elicited 225 times (3 blocks of 75 trials) to examine the relation among the three muscles' H-reflexes. The trial occurred during standing while the soleus EMG activity was maintained within a pre-defined range (i.e. 10-15% MVC) (see *EMG recording and electrical stimulation*). Stimulus level was set at just above the soleus M-wave threshold. Small adjustments were occasionally needed to maintain the same soleus M-wave amplitude throughout the 225 trials.

For 11 subjects, the H-reflexes of the soleus, MG, and LG were measured during standing with various levels of background activity and during walking to assess task- and phase-dependent modulation of the H-reflex. During standing, the subject was instructed to match the soleus background activity at target levels (4 to 5 different levels within 5-100% MVC range) while approximately 10 consecutive stimuli were applied at each level (see *EMG recording and electrical stimulation*). Thus, roughly 50 H-reflexes were recorded from the soleus, MG, and LG. Small adjustments of stimulus current were occasionally made to maintain the same soleus M-wave amplitude.

After completing the H-reflex measurement during standing, the subject walked on a non-inclined treadmill at his/her comfortable speed (average 0.9 m/s) while the tibial nerve was pseudo-randomly stimulated to elicit the H-reflexes (see Electrical stimulation and EMG recording). This self-selected speed was slower than the typical normal walking speed (i.e., 1.1-1.2 m/s, Duysens et al., 1991) probably because loud noise from the treadmill motor made the subject feel they walked at a faster speed than the actual speed. Foot switches (Bortec Biomedical, Calgary, Canada) were inserted between the subject's shoe and the heel to detect heel contact during walking. Several different stimulus intensities were used to obtain the H-reflexes with the same M-wave size across different phases of the gait cycle (Capaday and Stein, 1986; Llewellyn et al., 1990; Edamura et al., 1991) (see *Data analysis*).

Data analysis

All analyses were done with a custom-written MATLAB program (Mathworks, Natick, MA). To measure the background activity level during standing, rectified EMG activity in the 50-ms prestimulus period was averaged. For the background activity during walking, the EMG data measured during unstimulated steps were averaged and used as the control EMG activity (Yang and Stein, 1990; Kido et al., 2004a, b). Thus, the background activity of the stimulated step at a certain time in the gait cycle was calculated from the averaged EMG activity during the unstimulated steps at a corresponding time.

The H-reflex and the M-wave amplitudes were measured as the peak-to-peak values in each reflex window (i.e., typically 30-45 ms post-stimulus for the H-reflex and 5-23 ms post-stimulus for the M-wave). For all three muscles, the Hmax, Mmax, and Hmax/Mmax ratio were obtained from the recruitment curve measurement. In order to evaluate the MG and LG H-reflex amplitudes during standing in relation to the soleus H-reflex amplitudes, the MG or LG H-reflexes were plotted against the soleus H-reflexes. Then, using linear regression analysis, their correlations were measured as the coefficient of determination (R^2).

To investigate the task- and phase-dependent modulation of the H-reflex, trials with consistent M-wave size were selected for data analysis so as to compare between tasks (i.e. standing and walking) or across various phases of the gait cycle at equal stimulus intensities (Capaday and Stein, 1986; Llewellyn et al., 1990; Edamura et al., 1991). Thus, some of the responses with too large or too small M-waves were eliminated from further analyses. The standing data with various background EMG levels were sorted based on the background activity, and the H-reflex amplitude and background activity were averaged every 4-5 trials to yield approximately ten data points for each muscle. For the walking data, the gait cycle was determined using heel contact signals. Then, the entire gait cycle was divided into 12 equal bins, and each muscle's H-reflex amplitudes and background activity were averaged for each bin (Kido et al., 2004a). Approximately 10 responses were averaged in each bin. To compare the H-reflex amplitudes between the two tasks, the standing and walking H-reflexes were plotted as a function of the background EMG activity for each muscle (Capaday and Stein, 1986, 1987a). To evaluate H-reflex modulation during walking, the modulation index (i.e., 100 × (maximum H-reflex – minimum H-reflex) / maximum H-reflex) (Zehr and Kido, 2001; Kido et al., 2004a) was calculated over the gait cycle. For statistical comparison between muscles and tasks, the background EMG activity and the H-reflex amplitude were normalized using the MVC value and the Mmax amplitude, respectively, and averaged across the subjects. All group average data were presented as mean ± standard error (SE). Statistical differences among the muscles were examined using paired t-test, if not indicated otherwise. The α level was set at 0.05. To correct the significance level for multiple comparisons, Sidak correction ($1-(1-\alpha)^{1/n}$) was used to determine the p-value threshold. That is, for comparing among the three triceps muscles (i.e., 3 potential comparisons), we used $1-(1-0.05)^{1/3} = 0.017$ as the threshold for significance.

RESULTS

Hmax and Mmax during standing

Typical examples of H-reflexes and M-waves during standing in the soleus, MG, and LG are shown in Figure 2.1. The H-reflexes and the M-waves of the three muscles were simultaneously recorded in response to a test stimulus, while the background EMG activity levels of the soleus, MG, and LG remained within a narrow range [mean ± 1 standard deviation (SD) range for soleus: 10.9 ± 1.6 – 16.8 ± 2.8, MG: 4.5 ± 1.0 – 9.5 ± 1.6, LG: 4.4 ±

1.0 – 7.6 ± 1.7 (%MVC, mean ± SE)] throughout the measurement. The average Hmax/Mmax ratios (± SE) from 24 subjects for the soleus, MG, and LG were 0.45 (± 0.05), 0.20 (± 0.03), and 0.20 (± 0.03), respectively. The average Hmax/Mmax ratio of the soleus was significantly larger than that of the MG and LG ($p<0.01$, paired t-test, for both MG and LG). There were no significant differences in the average Mmax among the three muscles (soleus: 8.12 ± 0.58 mV, MG: 6.84 ± 0.56 mV, and LG: 6.47 ± 0.57 mV, paired t-test). The average Hmax of the soleus was significantly larger than that of the MG and LG ($p<0.01$, paired t-test, for both MG and LG, soleus: 3.33 ± 0.36 mV, MG: 1.14 ± 0.12 mV, and LG: 1.13 ± 0.14 mV).

Each trace in Figure 2.1a, c, and e is an average of 225 trials at a constant stimulus level during standing. The stimulus intensity was set at just above the soleus M-wave threshold, indicated by arrows on Figure 2.1b, d, and f. Thus, the reflexes of all three muscles were elicited in the ascending part of the recruitment curve (i.e., before the Hmax level. These panels show that the absolute H-reflex amplitude of the soleus was much larger than that of the MG and LG at a given stimulus level. In addition, the latencies of both the H-reflex and the M-wave were slightly shorter for the MG and LG than for the soleus (for the soleus, MG, and LG, M-wave latencies were 10ms, 6ms, and 9ms, and H-reflex latencies were 32ms, 31ms, and 30ms, respectively), due to the more proximal locations of the MG and LG electrodes compared to the soleus electrodes.

MG and LG H-reflexes in relation to the soleus H-reflex

The correlations among the H-reflex amplitudes of the soleus, MG, and LG were examined during standing with consistent background activity (see above) and M-wave amplitude [mean ± 1 standard deviation (SD) range for soleus: 4.1 ± 0.8 – 5.6 ± 1.0, MG: 12.5 ± 2.9 – 18.0 ± 4.0, LG: 17.5 ± 6.2 – 20.2 ± 7.0 (%Mmax, mean ± SE)]. Sample correlation plots from one subject are shown in Figure 2.2a-c. In Figure 2.2a and b, all data points were below the unity slope lines (i.e. dotted lines), suggesting that the amplitudes of the MG and LG H-reflexes were smaller than that of the soleus. Although there were amplitude differences between the soleus and both gastrocnemii, the H-reflex amplitudes in the three muscles were strongly correlated with each other. Significant linear correlations were present in all pairs in all subjects ($p<0.01$), and the average R^2 values (± SE) among all subjects for the soleus-MG, soleus-LG, and MG-LG pair were 0.47 ± 0.06, 0.57 ± 0.05, 0.48 ± 0.06, respectively. There were no statistical differences in R^2 values among the pairs.

Task-dependent modulation of the H-reflex

Figure 2.3 shows the H-reflexes during standing and walking in one subject as a function of the background activity. The group average M-wave amplitudes (± SE) were matched between the tasks: standing vs. walking, 0.60 ± 0.08 mV vs. 0.67 ± 0.08 mV for the soleus ($p=0.14$, paired t-test), 0.68 ± 0.10 mV vs. 0.66 ± 0.09 mV for the MG ($p=0.38$), and 0.86 ± 0.23 mV vs. 0.77 ± 0.20 mV for the LG ($p=0.14$). In Figure 2.3, the linear regression lines are fitted to aid the H-reflex amplitude comparison between the tasks. R^2 values of the fitted lines for the soleus, MG, and LG are 0.74, 0.74, and 0.83, respectively, for standing, and 0.63, 0.55, and 0.82 for walking. In group data, slopes of the regression lines for the soleus H-reflex were 36.0 ± 7.7 (standing) vs. 39.3 ± 5.8 (walking), for the MG were 17.0 ± 3.5 vs. 14.8 ± 1.9, and for the LG were 21.5 ± 4.5 vs. 27.6 ± 4.2. None of the slopes in the three muscles showed significant differences between standing and walking ($p=0.68$, 0.53, and 0.22, for the soleus, MG, and LG, respectively, paired t-test). Intercepts of the regression lines for the soleus H-reflex were 2.64 ± 0.37 (standing) vs. 0.14 ± 0.13 (walking), for the MG were 0.93 ± 0.14 vs. 0.15 ± 0.04, and for the LG were 1.44 ± 0.2 vs. 0.12 ± 0.06, and differences between the tasks were significant in all three muscles ($p<0.01$, paired t-test). As seen in Figure 2.3, the soleus H-reflex amplitude was greater during standing than during walking, similar to the finding of Capaday and Stein (1986). This task-dependent modulation of the reflex amplitude was also seen in the MG and LG.

Since there were relatively few data points below 20% of the MVC during standing and above 60% MVC during walking, the data from background EMG levels between 20% and 60% MVC were used for the rest of the analysis (Kido et al., 2004a). For the statistical comparison between the tasks, the background EMG activity was normalized using the MVC value, and the H-reflex amplitude was normalized using the Mmax value. The average H-reflex amplitudes (%Mmax), when the background EMG level was 20-60% MVC, were calculated for each subject. The mean values among all subjects are shown in Figure 2.4. The H-reflex amplitude was significantly larger during standing than during walking in all three muscles ($p<0.01$ in the soleus and MG, $p<0.05$ in LG, paired t-test). The relative size difference between the tasks (i.e. walking amplitude/standing amplitude ratio) was calculated for each muscle and for each subject. The group average ratios (± SE) for the soleus, MG, and LG were 0.64 ± 0.08, 0.49 ± 0.06, and 0.71 ± 0.10, respectively, which did not differ significantly from each other ($p=0.17$ for soleus vs. MG, $p=0.61$ for soleus vs. LG, and $p=0.07$ for MG vs. LG).

Phase-dependent modulation of the H-reflex

Figure 2.5 shows H-reflex modulation during walking as a function of the gait cycle phases as well as the locomotor EMG activity in the soleus, MG, and LG in one subject. The locomotor EMG activity for each muscle was obtained by averaging 73 unstimulated steps. The M-wave amplitudes were consistent throughout the gait cycle as indicated by dotted lines. The average M-wave amplitudes showed no significant changes throughout the 12 bins ($p=0.12$ for soleus, $p=0.86$ for MG, and $p=0.10$ for LG, one-way repeated measures ANOVA). Similar to the previous studies (Capaday and Stein, 1986; Llewellyn et al., 1990; Edamura et al., 1991; Kido et al., 2004a), the soleus H-reflex amplitude was low at heel-contact, gradually increased to its maximum value toward late stance phase, and rapidly decreased at the beginning of the swing phase. This strong phase-dependent modulation was also seen in the MG and LG. Indeed, average modulation indices (± SE) of the three muscles among all subjects were high (98.3 ± 0.3 for the soleus, 96.1 ± 0.9 for the MG, and 96.1 ± 0.7 for the LG), indicating that the H-reflexes in each muscle are highly modulated during walking based on gait cycle phase. The modulation indices for the MG and LG were statistically smaller than that for the soleus ($p<0.01$ for both MG and LG, paired t-test), probably due to smaller H-reflex amplitudes in the MG and LG. Patterns of the EMG activity and H-reflex modulation were similar during locomotion in each muscle (i.e., high in the late stance phase and silent during the swing phase).

DISCUSSION

The purpose of this study was to examine the H-reflexes of the MG and LG in humans, in comparison to the well-known soleus H-reflex. The H-reflexes of the soleus, MG, and LG were measured during standing and walking to examine the phase- and task-dependency of the reflex amplitudes. Although the Hmax/Mmax ratios were significantly different between the soleus and the gastrocnemii, the H-reflex amplitudes in response to single test stimuli were strongly correlated among the three muscles during standing. The MG and LG H-reflex amplitudes were modulated depending on the task (i.e., standing vs. walking) and on the phase of the gait cycle. The pattern of the modulation was similar to that seen with the soleus H-reflex.

The Hmax/Mmax ratios of the MG and LG were smaller than that of the soleus

The Hmax amplitudes calculated from the recruitment curves of the three muscles were significantly different: the soleus Hmax was larger than the MG or LG Hmax. While there were no statistical differences in the Mmax of all three muscles, the Hmax/Mmax ratio of the soleus was significantly larger than that of the MG or LG. Those results are consistent with previous animal data (Messina and Cotrufo, 1976). Several possible explanations can be given regarding what make the Hmax/Mmax ratios different between the soleus and the gastrocnemii. First, the soleus and the gastrocnemii have different compositions of muscle fiber types. Type I ("slow") muscle fibers are predominant in the soleus (70-90%) whereas type I and II ("fast") muscle fibers are equally common (50% for both types) in the gastrocnemii (Johnson et al., 1973; Gollnick et al., 1974; Edgerton et al., 1975). Thus, motoneurons that innervate the soleus and the gastrocnemii have different properties such as axon diameter and cell size, which will greatly influence the recruitment of H-reflex and M-wave at different stimulus levels in each muscle (Henneman and Mendell, 1981). During the recruitment curve measurement, the motoneurons are recruited into the H-reflex activation by the Ia afferent volley from the smallest to largest in an orderly manner in accordance with the size principle (Henneman et al., 1965) while the order of the recruitment is reversed for the M-wave (i.e., from the largest motoneurons to the smallest motoneurons) as electrical stimulation first activates axons with larger diameters (Pierrot-Deseilligny and Mazevet, 2000). Thus, the reflex activation of relatively large motoneurons would be occluded by the antidromic motor volley on the same motor axons, contributing to the falling part of the H-reflex recruitment curve (Schieppati, 1987). Indeed, in the human soleus, activation of slow-twitch fibers (i.e., innervated by smaller motoneurons with smaller axon diameters) is mainly responsible for the H-reflex (Buchthal and Schmalbruch, 1970). The fact that the soleus motoneuron pool contains more small motoneurons to be recruited into the H-reflex than the MG or LG pools would, at least partially, explain the smaller Hmax amplitudes of the MG and LG when compared to that of the soleus. Second, it is known that the size of Ia EPSP is larger in soleus motoneurons than in MG and LG motoneurons in cats (Eccles et al., 1957; Mendell and Henneman, 1971; Scott and Mendell, 1976) and non-human primates (Hongo et al., 1984; Carp, 1993). This would partially explain why the H-reflex amplitudes of the MG and LG were smaller than that of the soleus (see Figure 2.1). In addition, in cats, the number of muscle spindles is greater in the soleus than in the MG (Hagbarth and Wohlfart, 1952). If the same uneven distribution of the spindles exists in human, the number of Ia afferents is presumably smaller in the gastrocnemii than in the soleus, possibly leading to less homonymous Ia excitation of the motoneuron at a given stimulus level in the gastrocnemii when compared to the soleus. However, the counts of the muscle spindles in cats are not consistent between studies (Hagbarth and Wohlfart, 1952; Swett and Eldred, 1960), and human data are unavailable. Other factors that affect the H-reflex amplitude, such as presynaptic inhibition and postsynaptic inhibition (Capaday and Stein, 1987b, 1989; Stein, 1995), may differently influence H-reflex recruitment (i.e., Hmax and Hmax/Mmax ratio) between the soleus and gastrocnemii (see Discussion by Schieppati, 1987), although, to our knowledge, there has been no study reporting the different influence of pre- or postsynaptic inhibition among the three muscles.

The amplitudes of the MG and LG H-reflexes co-vary with soleus H- reflex amplitude

In the present study, the intensity of the electrical stimulation for eliciting the H-reflex in all three muscles was set at just above the soleus M-wave threshold during standing (Figure 2.2). Thus, the reflexes of all three muscles were elicited in the ascending part of the recruitment curve (i.e., before the Hmax level, see arrows in Figure 2.1b, d, and f). H-reflex and M-wave sizes of the three muscles did not either positively or negatively correlate with each other ($R^2 < 0.03$), further confirming that the H-reflexes of the three muscles were elicited in the ascending part of the recruitment curve, and therefore, possibility that antidromic motor volley of the MG and LG motor axons would decrease the size of the MG and LG H-reflexes can be avoided. Despite the smaller amplitudes of the MG and LG H-reflexes compared to the soleus, the H-reflex amplitudes of the three muscles were strongly positively correlated with each other. This suggests that, although there are several mechanical and physiological differences between the soleus and the gastrocnemii (Joseph and Nightingale, 1952; Campbell et al., 1973; Ochs et al., 1977; Vandervoort and McComas, 1983; Duysens et al., 1991), the excitability of the H-reflex pathway is modulated in similar ways among the three muscles.

The MG and LG H-reflexes are task- and phase-dependently modulated similarly to the soleus H-reflex

The excitability of the H-reflex pathway is similarly modulated between the soleus and gastrocnemii not only during standing (as discussed above) but also during walking. Although the H-reflex amplitudes of the MG and LG were smaller than that of the soleus during standing and walking, the standing reflexes were larger than the walking reflexes in all three muscles (Figure 2.4). The extent of this task-dependent modulation (indicated by the walking reflex/standing reflex ratio) was not significantly different among the three muscles, which suggests that the reflexes were similarly modulated between standing and walking among the three muscles. In addition, the modulation indices during walking showed high values for all three muscles (>96%), indicating that the reflex amplitudes of all three muscles are similarly modulated with gait cycle phase.

The fact that the MG and LG H-reflexes are task- and phase-dependently modulated in ways similar to the soleus H-reflex supports the hypothesis that the soleus, MG, and LG H-reflex pathways function as one synergistic unit. That is, similar modulations of the H-reflex pathways of soleus, MG, and LG contribute to successful walking. As suggested by Capaday et al. (1987a) and Edamura et al. (1991), lowering the reflex gain from standing to walking (i.e., task-dependent modulation) would ensure that the stretch reflex pathways (or net motor outputs) are not saturated during walking so that those muscles can contribute to locomotor activity. Also, during walking, activation of the soleus, MG, and LG motoneurons by Ia excitatory inputs (i.e., stretch reflex activity) in the stance phase would contribute to upward and forward propulsion of the body (Capaday and Stein, 1986), while suppression of such reflex activity in the swing phase would prevent it from opposing ankle dorsiflexion (i.e, phase-dependent modulation) (Capaday and Stein, 1986).

In sum, the H-reflex amplitudes of the MG and LG are smaller than that of the soleus. Despite these amplitude differences and the functional differences previously reported, the soleus, MG, and LG H-reflexes are similarly modulated between tasks (i.e., standing vs. walking) and within a task (i.e., across the gait cycle). These results suggest that the reflex pathways of the three muscles function synergistically to support successful execution of motor tasks such as walking.

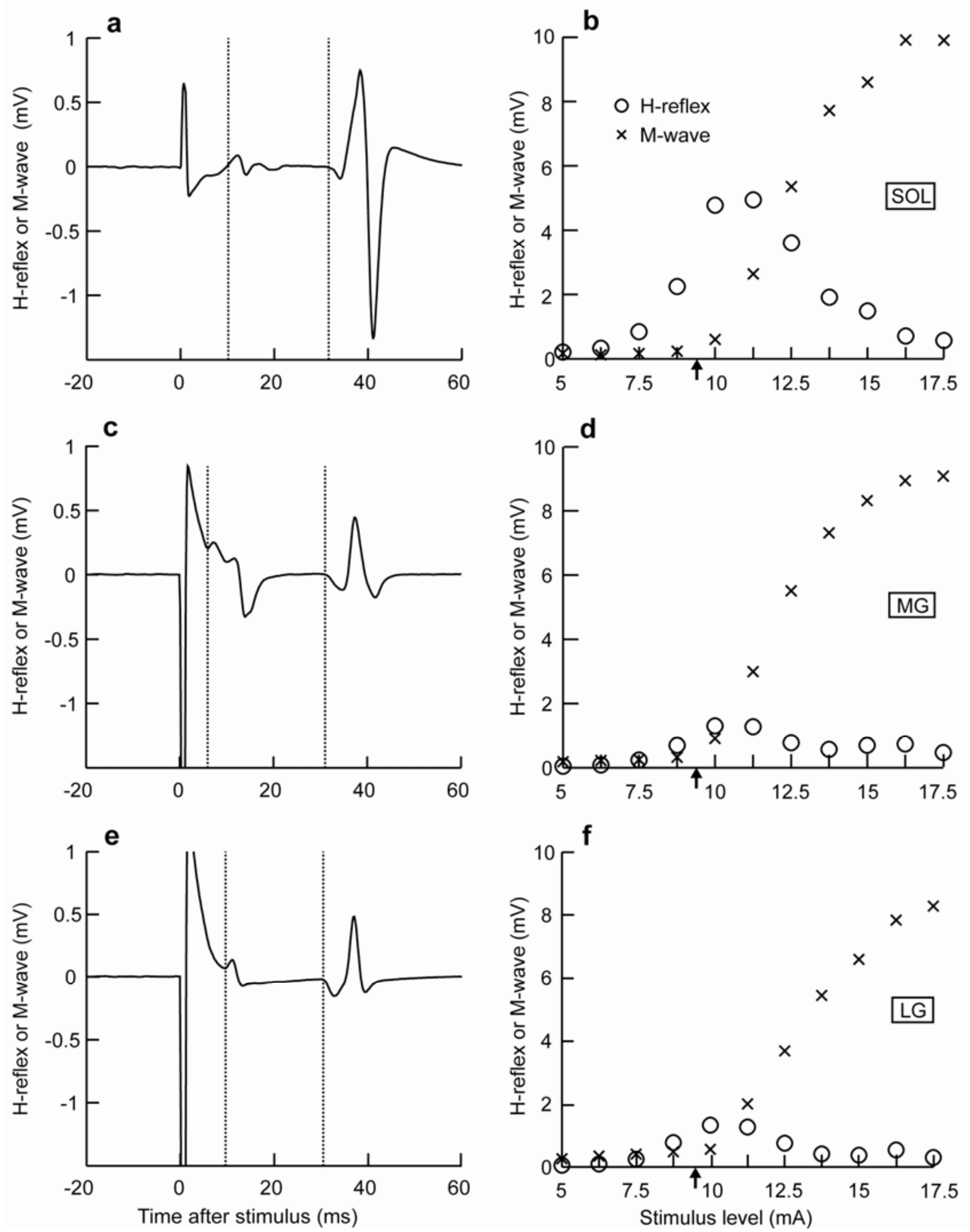

Figure 2.1: Standing H-reflexes and recruitment curves in the soleus, MG, and LG Examples of typical standing H-reflexes (a, c, and e) and recruitment curves (b, d, and f) elicited in the soleus (SOL), MG, and LG from one subject. The H-reflexes and the M-waves of the three muscles were simultaneously recorded in response to a test stimulus, and the background activity levels of the soleus, MG, and LG were maintained throughout the measurement. Each data point of the recruitment curve was an average of four reflexes at the same stimulus intensity. The vertical dashed lines indicate the onsets of the M-wave and H-

reflex. The Hmax/Mmax ratios calculated from the recruitment curves (b, d, and f) were 0.50, 0.14, and 0.16 for the soleus (Mmax 9.9 mV, Hmax 4.9 mV), MG (Mmax 9.1 mV, Hmax 1.3 mV), and LG (Mmax 8.3 mV, Hmax 1.3 mV), respectively. Each trace in a, c, and e is an average of 225 trials at the same stimulus intensity, which was set at just above the soleus M-wave threshold (indicated by the arrows on b, d, and f). The absolute H-reflex amplitude of the soleus was much larger than that of the MG and LG at this stimulus level. Also the latencies of both the H-reflex and the M-wave were slightly shorter for the MG and LG than for the soleus (for the soleus, MG, and LG, M-wave latencies were 10ms, 6ms, and 9ms, and H-reflex latencies were 32ms, 31ms, and 30ms, respectively).

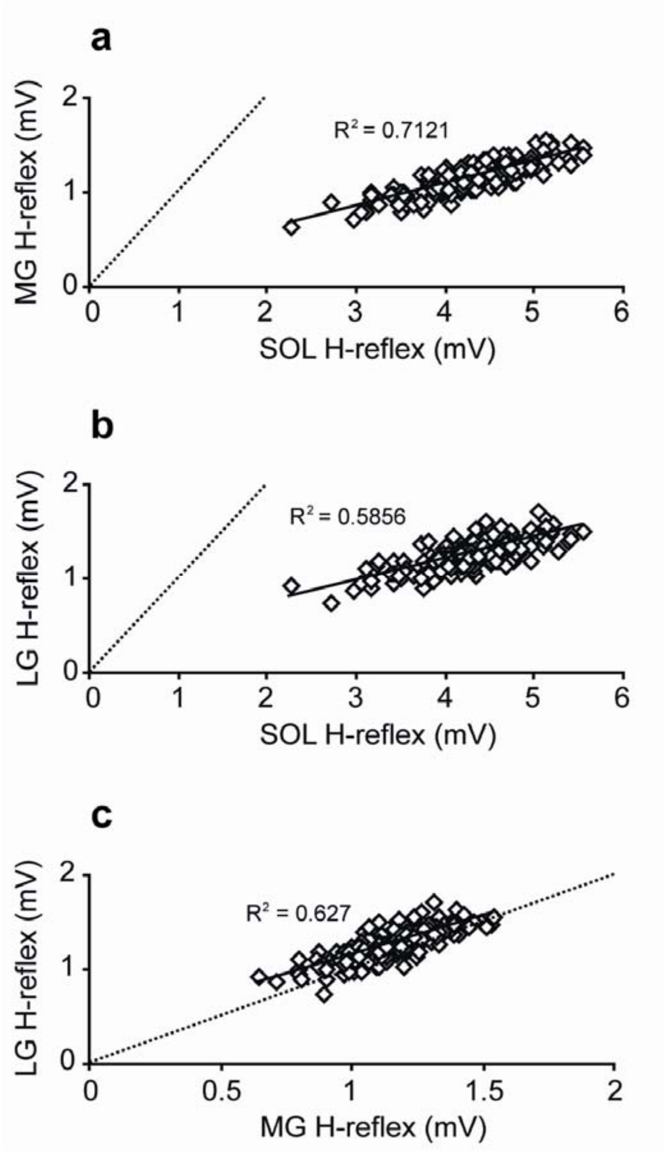

Figure 2.2: Correlation of the peak-to-peak H-reflex amplitudes
Correlation of the peak-to-peak H-reflex amplitudes among the soleus (SOL), MG, and LG measured during standing in a single subject (a: SOL-MG, b: SOL-LG, and c: MG-LG). The dotted lines are unity lines, on which amplitudes of two given muscles are the same size. All the data points in a and b were below the unity lines. Although the absolute amplitudes of the MG and LG are much smaller than the soleus, they are strongly correlated in a linear fashion. R^2 values are shown in each panel.

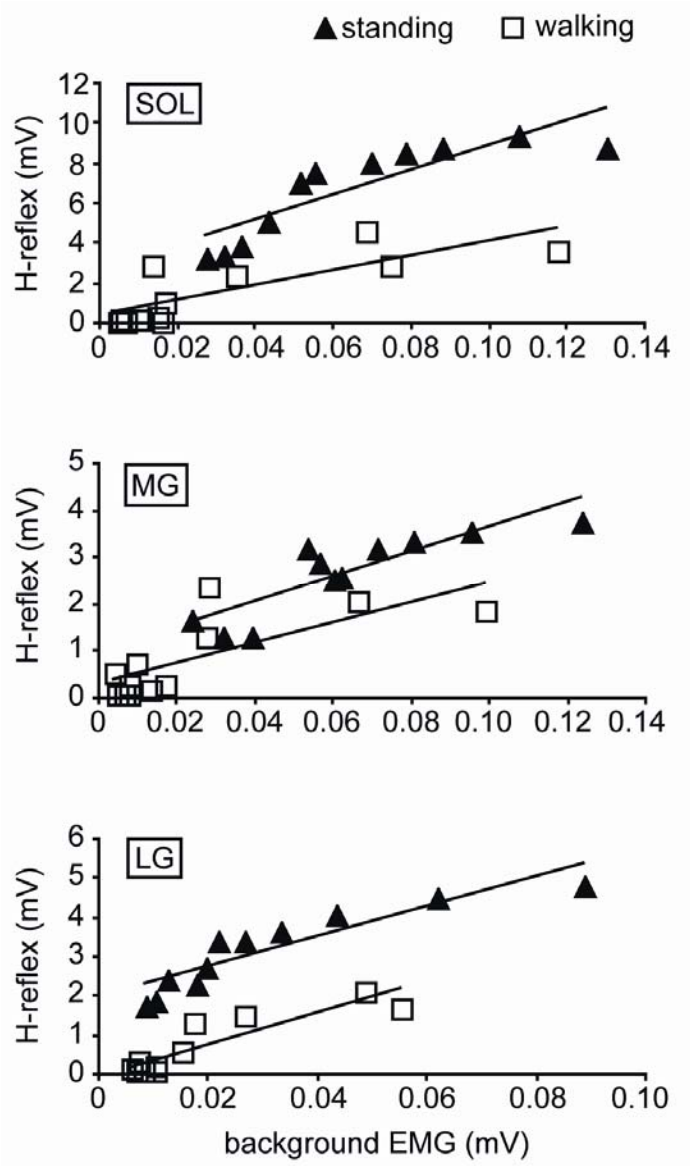

Figure 2.3: Sample task-dependent modulation of the H-reflexes
The H-reflex amplitudes during walking and standing plotted against the background EMG activity for each muscle in one subject. The M-wave amplitudes were well-matched between the two tasks. The H-reflex amplitudes at a given EMG level were larger during standing than during walking in all three muscles.

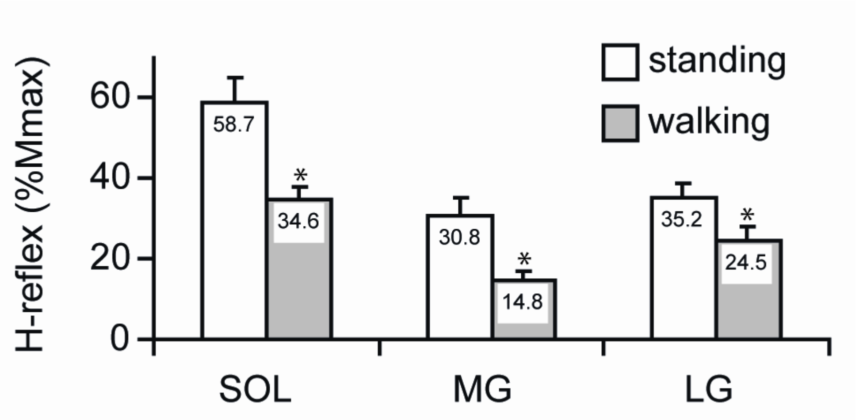

Figure 2.4: Task-dependent modulation of the H-reflexes
The mean H-reflex amplitudes (normalized using the Mmax amplitude) in the background activity range of 20-60% MVC averaged among all subjects (n=11) for each task. *: The H-reflex amplitudes during walking were significantly smaller than those during standing in all three muscles [$p<0.01$ in the soleus (SOL) and MG, $p<0.05$ in LG, paired t-test]. The relative size differences between the tasks were not statistically different among the three muscles.

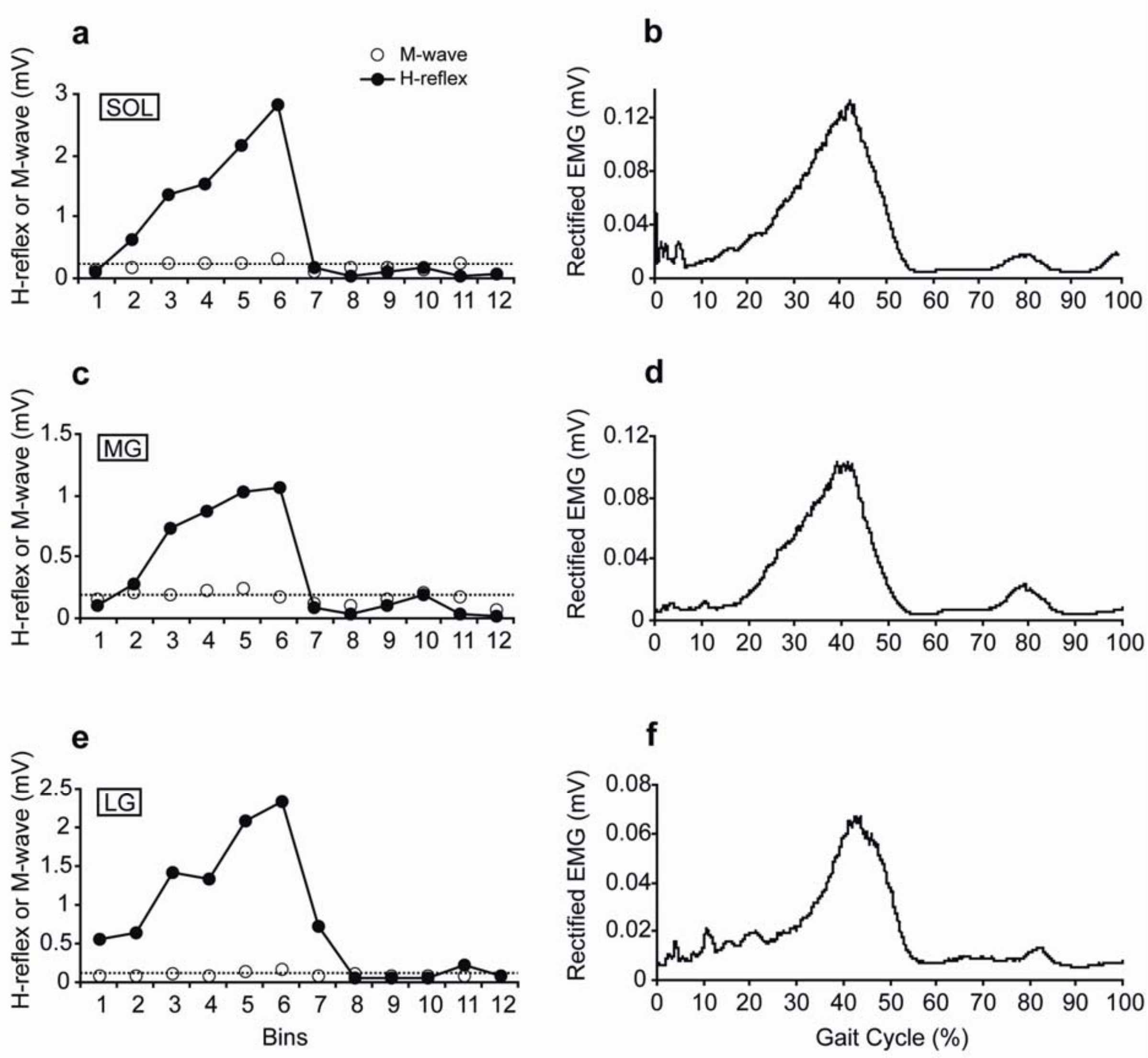

Figure 2.5: Phase-dependent modulation of the H-reflexes and EMG activity during walking The H-reflex and M-wave amplitudes during walking as a function of a gait cycle (a, c, and e) as well as EMG activity during walking (b, d, and f) in one subject. a, c, e) The gait cycle (from heel contact to the next heel contact) was divided into 12 equal bins and the reflexes elicited in the same bin were averaged together. The M-wave amplitudes (dotted lines) were kept consistent throughout the gait cycle for each muscle. The H-reflex amplitudes were highly modulated between phases in all muscles. The modulation indices for this subject were 98.9, 97.6, and 97.4 for the soleus, MG, and LG, respectively. b, d, f) The locomotor EMG activity for each muscle was obtained by averaging 73 unstimulated steps. The locomotor EMG activity and the amplitude modulation of the H-reflex were similar in each muscle.

References

Buchthal F, Schmalbruch H (1970) Contraction times of twitches evoked by H-reflexes. Acta Physiol Scand 80:378-382.

Campbell KM, Biggs NL, Blanton PL, Lehr RP (1973) Electromyographic investigation of the relative activity among four components of the triceps surae. Am J Phys Med 52:30-41.

Capaday C, Stein RB (1986) Amplitude modulation of the soleus H-reflex in the human during walking and standing. J Neurosci 6:1308-1313.

Capaday C, Stein RB (1987a) Difference in the amplitude of the human soleus H reflex during walking and running. J Physiol 392:513-522.

Capaday C, Stein RB (1987b) A method for simulating the reflex output of a motoneuron pool. J Neurosci Methods 21:91-104.

Capaday C, Stein RB (1989) The effects of postsynaptic inhibition on the monosynaptic reflex of the cat at different levels of motoneuron pool activity. Exp Brain Res 77:577-584.

Carp JS (1993) Monosynaptic EPSPs in primate lumbar motoneurons. J Neurophysiol 70:1585-1592.

Duclay J, Robbe A, Pousson M, Martin A (2009) Effect of angular velocity on soleus and medial gastrocnemius H-reflex during maximal concentric and eccentric muscle contraction. J Electromyogr Kinesiol 19: 948-956.

Duysens J, Tax AA, van der Doelen B, Trippel M, Dietz V (1991) Selective activation of human soleus or gastrocnemius in reflex responses during walking and running. Exp Brain Res 87:193-204.

Dyhre-Poulsen P, Simonsen EB, Voigt M (1991) Dynamic control of muscle stiffness and H reflex modulation during hopping and jumping in man. J Physiol 437:287-304.

Eccles JC, Eccles RM, Lundberg A (1957) The convergence of monosynaptic excitatory afferents on to many different species of alpha motoneurones. J Physiol 137:22-50.

Edamura M, Yang JF, Stein RB (1991) Factors that determine the magnitude and time course of human H-reflexes in locomotion. J Neurosci 11:420-427.

Edgerton VR, Smith JL, Simpson DR (1975) Muscle fibre type populations of human leg muscles. Histochem J 7:259-266.

Fitzpatrick RC, Gorman RB, Burke D, Gandevia SC (1992) Postural proprioceptive reflexes in standing human subjects: bandwidth of response and transmission characteristics. J Physiol 458:69-83.

Gollnick PD, Sjodin B, Karlsson J, Jansson E, Saltin B (1974) Human soleus muscle: a comparison of fiber composition and enzyme activities with other leg muscles. Pflugers Arch 348:247-255.

Hagbarth KE, Wohlfart G (1952) The number of muscle-spindles in certain muscles in cat in relation to the composition of the muscle nerves. Acta Anat (Basel) 15:85-104.

Henneman E, Mendell LM (1981) Funtional Organization of Motoneuron Pool and Inputs. In: Handbook of Physiology (Brooks VB, ed), pp 423-507. Baltimore: Williams and Wilkins.

Henneman E, Somjen G, Carpenter DO (1965) Functional Significance of Cell Size in Spinal Motoneurons. J Neurophysiol 28:560-580.

Hongo T, Lundberg A, Phillips CG, Thompson RF (1984) The pattern of monosynaptic Ia-connections to hindlimb motor nuclei in the baboon: a comparison with the cat. Proc R Soc Lond B Biol Sci 221:261-289.

Johnson MA, Polgar J, Weightman D, Appleton D (1973) Data on the distribution of fibre types in thirty-six human muscles. An autopsy study. J Neurol Sci 18:111-129.

Joseph J, Nightingale A (1952) [Electromyography of muscles of posture: leg muscles in males.]. J Physiol 117:484-491.

Kawakami Y, Ichinose Y, Fukunaga T (1998) Architectural and functional features of human triceps surae muscles during contraction. J Appl Physiol 85:398-404.

Kido A, Tanaka N, Stein RB (2004a) Spinal excitation and inhibition decrease as humans age. Can J Physiol Pharmacol 82:238-248.

Kido A, Tanaka N, Stein RB (2004b) Spinal reciprocal inhibition in human locomotion. J Appl Physiol 96:1969-1977.

Levy R (1963) The relative importance of the gastrocnemius and soleus muscles in the ankle jerk of man. J Neurol Neurosurg Psychiatry 26:148-150.

Llewellyn M, Yang JF, Prochazka A (1990) Human H-reflexes are smaller in difficult beam walking than in normal treadmill walking. Exp Brain Res 83:22-28.

McDonagh MJ, Duncan A (2002) Interaction of pre-programmed control and natural stretch reflexes in human landing movements. J Physiol 544:985-994.

Mendell LM, Henneman E (1971) Terminals of single Ia fibers: location, density, and distribution within a pool of 300 homonymous motoneurons. J Neurophysiol 34:171-187.

Messina C, Cotrufo R (1976) Different excitability of type 1 and type 2 alpha-motoneurons. The recruitment curve of H- and M-responses in slow and fast muscles of rabbits. J Neurol Sci 28:57-63.

Moritani T, Oddsson L, Thorstensson A (1990) Differences in modulation of the gastrocnemius and soleus H-reflexes during hopping in man. Acta Physiol Scand 138:575-576.

Murray MP, Guten GN, Baldwin JM, Gardner GM (1976) A comparison of plantar flexion torque with and without the triceps surae. Acta Orthop Scand 47:122-124.

Nichols TR (1989) The organization of heterogenic reflexes among muscles crossing the ankle joint in the decerebrate cat. J Physiol 410:463-477.

Ochs RM, Smith JL, Edgerton VR (1977) Fatigue characteristics of human gastrocnemius and soleus muscles. Electromyogr Clin Neurophysiol 17:297-306.

Pierrot-Deseilligny E, Mazevet D (2000) The monosynaptic reflex: a tool to investigate motor control in humans. Interest and limits. Neurophysiol Clin 30:67-80.

Pinniger GJ, Nordlund M, Steele JR, Cresswell AG (2001) H-reflex modulation during passive lengthening and shortening of the human triceps surae. J Physiol 534:913-923.

Schieppati M (1987) The Hoffmann reflex: a means of assessing spinal reflex excitability and its descending control in man. Prog Neurobiol 28:345-376.

Scott JG, Mendell LM (1976) Individual EPSPs produced by single triceps surae Ia afferent fibers in homonymous and heteronymous motoneurons. J Neurophysiol 39:679-692.

Stein RB (1995) Presynaptic inhibition in humans. Prog Neurobiol 47:533-544.

Swett JE, Eldred E (1960) Distribution and numbers of stretch rceptors in medial gastrocnemius and soleus muscles of the cat. Anat Rec 137:453-460.

Thompson AK, Chen XY, Wolpaw JR (2009) Acquisition of a simple motor skill: task-dependent adaptation plus long-term change in the human soleus H-reflex. J Neurosci 29:5784-5792.

Vandervoort AA, McComas AJ (1983) A comparison of the contractile properties of the human gastrocnemius and soleus muscles. Eur J Appl Physiol Occup Physiol 51:435-440.

Yang JF, Stein RB (1990) Phase-dependent reflex reversal in human leg muscles during walking. J Neurophysiol 63:1109-1117.

Yang JF, Whelan PJ (1993) Neural mechanisms that contribute to cyclical modulation of the soleus H-reflex in walking in humans. Exp Brain Res 95:547-556.

Zehr EP, Stein RB (1999) Interaction of the Jendrassik maneuver with segmental presynaptic inhibition. Exp Brain Res 124:474-480.

Zehr EP, Kido A (2001) Neural control of rhythmic, cyclical human arm movement: task dependency, nerve specificity and phase modulation of cutaneous reflexes. J Physiol 537:1033-1045.

CHAPTER III

SECOND MANUSCRIPT

Operant down-conditioning of soleus H-reflex in humans: effects on gastrocnemius H-reflexes

ABSTRACT

Long-term modification of the medial and lateral gastrocnemii (MG and LG) H-reflexes induced by successful operant down-conditioning of the soleus H-reflex was investigated in humans. Previously, final conditioned soleus H-reflex has been shown to be a sum of rapid within-session task-dependent adaptation (difference between control and conditioned H-reflex within a session) and gradual across-session long-term change (time-course change in control reflex). The conditioning protocol is suggested to induce task-dependent adaptation originating in supraspinal plasticity, which in turn, produces long-term changes reflecting spinal plasticity. There were six baseline and 30 down-conditioning sessions. The MG and LG H-reflexes were concurrently recorded with the soleus H-reflex every six sessions. Each conditioning session had 20 control trials and 225 conditioning trials. After the conditioning, the soleus H-reflex showed significant decrease in conditioned H-reflex, control H-reflex, and within-session decrease whereas, for synergists, significant within-session change occurred only in the MG H-reflex, and neither the MG nor the LG control H-reflexes decreased. Thus, the within-session decrease was differentially induced among the three muscles. Additionally, since the MG H-reflex had a continuous within-session decrease throughout the conditioning, compensatory plasticity may have occurred to prevent long-term change in the MG H-reflex pathway. A compensatory plasticity would reflect a strategy of the brain and spinal cord to impose the least impact of the conditioning on the existing wide repertoire of motor skills. The present study suggests that operant conditioning has differential effects on the target muscle and the synergist muscles.

INTRODUCTION

The H-reflex, sometimes referred to as the "electrical analogue" of the stretch reflex, has been extensively studied in both healthy and neurologically impaired humans (Schieppati, 1987; Brooke et al., 1997; Pierrot-Deseilligny and Mazevet, 2000; Zehr, 2002; Misiaszek, 2003). Our recent study showed that, in humans, H-reflex size of the soleus is highly correlated with H-reflex sizes of its synergists medial gastrocnemius (MG) and lateral gastrocnemius (LG) during standing (Makihara et al., 2010). Furthermore, the H-reflexes of the three muscles are similarly modulated during walking (i.e., phase-dependent modulation) and between walking and standing (i.e., task-dependent modulation), supporting the hypothesis that the reflexes of the three muscles are synergistically modulated to facilitate the execution of ongoing motor tasks.

The H-reflex is frequently used as a tool to investigate adaptive plasticity of the spinal cord (Zehr, 2002; Misiaszek, 2003; Knikou, 2008). Over the last three decades, it has been shown that the H-reflex or the spinal stretch reflex can be operantly conditioned in different species including monkeys, rats, mice, and humans (Wolpaw et al., 1983; Wolpaw, 1987; Evatt et al., 1989; Chen and Wolpaw, 1995; Carp et al., 2006b; Thompson et al., 2009). Recently, Thompson et al. (2009) showed in humans that the soleus H-reflex change induced by operant conditioning was the sum of rapid within-session (i.e., task-dependent) adaptation and gradual across-session (i.e., long-term) change. The protocol was designed to turn the rapid component on and off while leaving the slow across-session component unaffected, enabling the measurement of the two distinguishable phenomena that differ in time of onset, rate of development, and flexibility. It was suggested that the conditioning protocol induces task-dependent adaptation originating from the brain, and in turn, produces long-term change in the spinal cord.

While the supraspinal and spinal mechanisms underlying the H-reflex changes induced by operant conditioning have been intensively studied (Wolpaw and O'Keefe, 1984; Carp and Wolpaw, 1994, 1995; Carp et al., 2001; Chen et al., 2002; Chen and Wolpaw, 2002, 2005; Chen et al., 2006; Wang et al., 2006; Wolpaw and Chen, 2006; Thompson et al., 2009; Wang et al., 2009), the impact of conditioning on muscles that are not directly conditioned has been much less investigated (Wolpaw et al., 1983). In monkeys, Wolpaw et al. (1983) reported that effects of operant conditioning of the biceps brachii stretch reflex were relatively specific to the target muscle (i.e., biceps brachii), and the reflexes in its synergists (i.e., brachialis and brachioradialis) were changed to a lesser extent. Similar results have been reported for the biceps brachii stretch reflex in humans (Wolf and Segal, 1996). However, in the lower extremities, despite a large number of studies done in the past 20 years (Chen and Wolpaw, 1995, 2005; Carp et al., 2006b; Chen et al., 2006; Thompson et al., 2009; Chen et al., 2010; Wolpaw, 2010), effects of the soleus H-reflex conditioning on its synergists, the MG and LG H-reflexes have not been studied. Thus, the purpose of the present study was to investigate whether and to what extent the H-reflexes of the MG and LG are modified by successful operant down-conditioning of the soleus H-reflex. Specifically, the present study focused on evaluating the extent of task-dependent adaptation and long-term change of the MG and LG H-reflexes in relation to the soleus H-reflex.

MATERIALS AND METHODS

Study Overview

The H-reflexes of the MG and LG were concurrently measured with the soleus H-reflex at every six sessions over the course of operant conditioning. The study protocol was approved by the Institutional Review Board of Helen Hayes Hospital and all subjects gave written consent prior to participation. Eight subjects (two women and six men, aged 21-54 years) with no known history of neurological disease or injury participated in the study. All subjects were physically active; four subjects (21, 27, 29, and 31 years old) had a regular exercise routine such as going to a gym or playing basketball at least 1-2 times per week. Although the other three subjects (31, 33, and 43 years old) did not exercise regularly, their jobs and life styles were not sedentary but rather including physical activity. The last subject who was the oldest in the subject group (i.e., 54 years old) was retired, but doing volunteer work at a senior home 3-4 times per week.

In the preliminary session prior to operant conditioning, the maximum voluntary contraction (MVC) values for the soleus, MG, and LG were measured as the maximum rectified EMG levels during isometric contraction in standing. Then, for each subject, target soleus background EMG window and target M-wave size for H-reflex trials were determined during natural standing. The target background window was set around the subject's natural standing level (i.e., usually 10-15% MVC). Tibial nerve stimulation just above the soleus M-wave threshold was selected, and the M-wave size at this stimulus level was used as the target M-wave size. For all the H-reflex trials throughout the study, the soleus M-wave size and the soleus background EMG levels were kept constant (Thompson et al., 2009).

Operant conditioning of the soleus H-reflex

The operant conditioning protocol for the human soleus H-reflex has been described elsewhere (Thompson et al., 2009) and is briefly summarized here. The protocol comprised six baseline sessions and 30 down-conditioning sessions spread over 12 weeks (i.e., 3 sessions per week). Each subject's sessions always occurred at the same time of day to control for diurnal variations in H-reflex size (Wolpaw and Seegal, 1982; Chen and Wolpaw, 1994; Carp et al., 2006a; Lagerquist et al., 2006).

Each session began with an H-reflex/M-wave recruitment curve measurement during standing while the subject maintained the soleus background EMG activity in the target window. Then, in each of the baseline sessions, 225 control H-reflexes were elicited, which was divided into three blocks of 75 trials. In each conditioning session, 20 control H-reflexes were elicited as in the baseline sessions and then 225 conditioned H-reflexes were elicited. The difference between the control and conditioning trials was that, in the control trials, the H-reflex was simply elicited without any feedback or encouragement to change H-reflex size. On the other hand, in the conditioning trials, the subject was asked to decrease the soleus H-reflex and was given visual feedback regarding the reflex size immediately after each stimulus. The visual feedback showed whether the resulting H-reflex was smaller than a criterion value (i.e., successful or not). The reward criterion was based on the performance of the immediately preceding block of trials (i.e., for the first block of 75 conditioning trials, the reward criterion was based on the average of the block of 20 control trials, the criterion for the second block was based on the first block, and the criterion for the third block was based on the second block). The reward criterion was set at 60% from the lowest end of the soleus reflex size distribution. Therefore, if the distribution of the soleus H-reflex size in the current block was the same as that in the preceding block, 60% of the trials would have been successful. The visual feedback screen also showed a thick horizontal line representing the average soleus H-reflex size in the baseline sessions for each subject, and the ongoing successful rate was also printed to screen.

During the H-reflex measurements, the subject was asked to maintain a natural standing posture and not to contract (i.e., tense up) any muscle in the body. Between each trial, the subject was allowed to move within a limited area. Although some subjects preferred to stand still throughout a block, others tended to move, such as taking steps in a same place or shaking legs. During the conditioning sessions, the subject was encouraged to maximize success rate, to focus on every trial, and to decrease the H-reflex size as much as possible within a session. The thick horizontal line representing the baseline average was useful for the subject to tell how much he/she improved during the conditioning. To maximize the subject's motivation, attention, and conscious involvement in improving success rate, verbal encouragement was given between the conditioning blocks. The subject was instructed to explore different techniques for decreasing the soleus H-reflex size and to identify the most effective strategy.

The MG and LG H reflexes were measured at every six sessions concurrently with the soleus H-reflex. Note that there was no feedback regarding the background EMG activity and H-reflex size of the MG or LG.

Electrical Stimulation and EMG Recording

In order to elicit the H-reflex and M-wave, the tibial nerve was stimulated in the popliteal fossa using a Grass S48 stimulator (with CCU1 constant current unit and SIU5 stimulus isolation unit; Astro-Med, West Warwick, RI) with self-adhesive surface Ag-AgCl electrodes (2.2 × 2.2 cm for the cathode and 2.2 × 3.5 cm for the anode; Vermed, Bellows Falls, VT). The stimulating electrode pair was placed so as to minimize the H-reflex threshold, maximize the maximum H-reflex (Hmax) and the maximum M-wave (Mmax) sizes of the soleus, and to avoid stimulation of other nerves. Thus, the stimulus location was optimized to the soleus H-reflex. However, in a pilot experiment, stimulation at the optimum location for the soleus H-reflex also maximized the Hmax and Mmax for the MG and LG. Therefore, as in a previous study (Duclay et al., 2009), the same stimulus location was used for eliciting the soleus, MG, and LG H-reflexes simultaneously, and presumably, this location was optimum for all three muscles. Soleus EMG activity was recorded using a pair of self-adhesive surface Ag-AgCl electrodes (2.2 × 3.5 cm, Vermed, Bellows Falls, VT) placed longitudinally just below the gastrocnemii with the centers of electrodes ≈3 cm apart. To evaluate antagonist activity during the conditioning, another pair of EMG recording electrodes was placed on the skin over the belly of tibialis anterior (TA). To measure the MG and LG H-reflexes, in every six sessions, pairs of EMG recording electrodes were placed on the skin over the center of MG and LG muscle bellies (see *Operant conditioning of the soleus H-reflex*). EMG electrodes for the soleus, MG, and LG muscles were placed at least 7cm apart to minimize the cross-talk. To avoid session-to-session variability in electrode placement, locations of all electrodes were mapped in relation to permanent skin marks such as scars and moles in the preliminary session and the electrodes were placed based on this mapping in all subsequent sessions.

EMG signals were amplified, band-pass filtered (10-1000 Hz), sampled at 5,000 Hz, and stored. The soleus EMG activity was rectified and averaged every 100 ms, and the result was immediately provided as visual feedback to help the subject maintain the soleus background EMG activity within the specified window (see Thompson et al., 2009 for details). If the soleus background EMG was kept in the target window (typically 10-15%MVC) for 2 s and if 5 s had passed since the last stimulus, a 1 ms square stimulus pulse was delivered to elicit the H-reflex and M-wave. The EMG and nerve stimulus signals were recorded for a period of 200 ms in response to each test stimulus pulse including a prestimulus period of 50 ms.

Data analysis

H-reflex and M-wave sizes of the soleus, MG, and LG were defined as the mean rectified size in each reflex window (i.e., for the soleus, typically 30-45 ms post stimulus for the H-reflex and 5-23 ms post stimulus for the M-wave) minus average background EMG. We calculated the average H-reflex sizes for the entire 225 H-reflex trials (i.e., three 75-trial blocks together) and the first 20 within-session control trials (i.e., the first 20 trials of a baseline session, which were obtained from the first 75 H-reflex block, or 20 control H-reflex trials of a conditioning session) for each session. Changes in the soleus control and conditioned H-reflex sizes across sessions were quantified as a percentage of the average for the six baseline sessions. That is, the control H-reflex size at each of the conditioning sessions was normalized using the average of the first 20 trials of six baseline sessions, and the conditioned H-reflex size was normalized using average of the entire 225 trials of six baseline sessions (i.e., 100% means no change during the conditioning). For the H-reflexes of the MG and LG, although there was no feedback on the MG and LG H-reflex sizes or encouragement to decrease them, the MG and LG H-reflexes during the soleus conditioning trials are referred to as conditioned reflexes of the MG and LG, as they were measured during the exposure to the conditioning paradigm. The MG and LG H-reflexes during the soleus control trials are referred to as control reflexes of the MG and LG. The control reflexes or conditioned reflexes of the MG and LG during the conditioning sessions were normalized by the average of the first 20 trials or the entire 225 trials in the baseline session 6, respectively. We also evaluated the extent of H-reflex size changes within a session by calculating the difference between the averaged control H-reflex size and the conditioned H-reflex size.

To determine for each subject whether down-conditioning was successful, the soleus H-reflex sizes during the six baseline sessions (# 1-6) were compared to the conditioned reflex sizes in the last six conditioning sessions (# 25-30) using unpaired t-test (Thompson et al., 2009). Although the analysis was performed to compare six data points between the baseline and conditioning sessions for each subject, they were not paired (i.e., data from baseline session #1 was not matched to data from conditioning session # 25), and thus, unpaired t-test, not paired, was used. Only data from successfully conditioned subjects were used for further analyses of the MG and LG H-reflexes. Then, using group data, the H-reflexes of the MG and LG were compared between the baseline session and the last conditioning session by a paired t-test. In addition, the relation between the soleus and MG (or LG) H-reflexes was evaluated by plotting the MG (or LG) H-reflex as a function of the soleus H-reflex for each session in each subject. Linear regression analysis was used to obtain the coefficient of determination (R^2).

RESULTS

Seven out of eight subjects showed significant decrease of the soleus H-reflex size after 30 down-conditioning sessions (i.e., successfully conditioned, see *Data analysis*). Conditioning for one subject who failed (31 years old) was terminated at conditioning session #10 because this subject did not follow instructions from the investigator. Since the purpose of this study was to examine the effects of successful soleus H-reflex down-conditioning on the gastrocnemii H-reflexes, only the data from successfully conditioned subjects were used for further analysis.

Stability of EMG recording and conditioning stimulation

In order to verify that the quality of EMG recording and the strength of stimulus were consistent throughout the study, the Mmax (and Hmax) size, background EMG level, and M-wave size were compared across sessions and between control reflexes and conditioned reflexes. Neither the Mmax nor the Hmax changed significantly throughout the entire study period in all three muscles (for soleus, Mmax: p=0.69 and Hmax: p=0.88, for MG, Mmax: p=0.45 and Hmax: p=0.46, and for LG, Mmax: p=0.61 and Hmax: p=0.40, one-way repeated-measures ANOVA). The background EMG activity level remained within a narrow window for each muscle [mean ± 1 standard deviation (SD) range for soleus: 10.9 ± 1.6 – 16.8 ± 2.8, MG: 4.5 ± 1.0 – 9.5 ± 1.6, LG: 4.4 ± 1.0 – 7.6 ± 1.7 (%MVC, mean ± standard error)] throughout the course of the study (p=0.23, 0.75, and 0.91 for soleus, MG, and LG, respectively, one-way repeated-measures ANOVA). M-wave size was maintained in a narrow window for each muscle [mean ± 1 SD range for soleus: 4.1 ± 0.8 – 5.6 ± 1.0, MG: 12.5 ± 2.9 – 18.0 ± 4.0, LG: 17.5 ± 6.2 – 20.2 ± 7.0 (%Mmax, mean ± standard error)] throughout the course of the study (p=0.22, 0.70, and 0.61 for soleus, MG, and LG, respectively, one-way repeated-measures ANOVA). When the background EMG activity level and M-wave size were compared between the initial 20 trials and the entire 225 trials, there were no significant differences for any of the three muscles (p=0.09, 0.42, and 0.95, for the soleus, MG, and LG background EMG level, respectively: p=0.84, 0.67, and 0.92, for the soleus, MG, and LG M-wave size, respectively, paired t-test) although soleus background activity had a trend towards significance. Furthermore, the background activity of the antagonist muscle (i.e., TA) was stable throughout the study (p=0.11, one-way repeated-measures ANOVA).

Change in the soleus H-reflex

Figure 3.1A shows sample sweeps of averaged H-reflex of the soleus (i.e., average of entire 225 trials) in the sixth baseline session and the last conditioning session from a representative subject. The soleus H-reflex in the last conditioning was clearly smaller than that in the baseline. For the group of seven subjects, the soleus conditioned H-reflex in the last conditioning session was 76.1 ± 3.9 % [mean ± standard error (SE)] of the baseline value, comparable to the final H-reflex sizes in the previous animal and human studies (Wolpaw, 1987; Chen and Wolpaw, 1995; Carp et al., 2006b; Thompson et al., 2009). This final conditioned H-reflex size was significantly smaller than that of the baseline (p<0.01, paired t-test, see Figure 3.3).

Figure 3.1B shows typical examples of the averaged soleus control H-reflexes in the sixth baseline session and the last conditioning session in one subject. The soleus control H-reflex was decreased after conditioning. In the last conditioning session, the group mean soleus control reflex was significantly smaller than that in the baseline, 88.8 ± 4.3 % of the baseline value ($p<0.05$, paired t-test, Figure 3.3).

During the baseline sessions, the soleus H-reflex size in the first 20 control reflexes and all 225 control reflexes were not different ($p=0.08$, paired t-test). This result indicates that any differences between the 20 control reflexes and the 225 conditioned reflexes during the conditioning sessions were due to the exposure to the operant conditioning paradigm. In order to evaluate the extent of this within-session change, the control reflex size was subtracted from the conditioned reflex size.

Figure 3.2A is an example of within-session change in the soleus H-reflex size between the control H-reflex and conditioned H-reflex in the last conditioning session from a representative subject. The conditioned H-reflex was clearly smaller than the control H-reflex. As a group, the mean within-session decrease was 12.7 ± 2.9 % after down-conditioning, which was significantly different from the baseline value ($p<0.01$, paired t-test, Figure 3.3).

Changes in the MG H-reflex

The MG H-reflexes were concurrently recorded with the soleus H-reflexes during 20 control trials and 225 conditioning trials. Figure 3.1C shows sample sweeps of the averaged 225 MG H-reflexes in the baseline session and the last conditioning session in one subject. The MG conditioned H-reflex size was smaller in the last conditioning session than in the baseline session. Group data showed that the mean MG conditioned H-reflexes in the last conditioning session was 85.5 ± 5.1 % of the baseline value, significantly smaller than that in the baseline ($p<0.05$, paired t-test, see Figure 3.3).

Figure 3.1D shows typical examples of the averaged control H-reflexes in the baseline and last conditioning sessions in one subject. Unlike the soleus control reflex, the MG control reflex did not change after conditioning. As a group, the mean MG control reflex in the last conditioning session was 98.9 ± 6.8 % of the baseline value ($p=0.88$, paired t-test, Figure 3.3).

There was no significant difference between the 20 control reflexes and all 225 control reflexes in the baseline session for the MG ($p=0.25$, paired t-test). Figure 3.2B shows typical examples of the control and conditioned H-reflexes of the MG in the last conditioning session in a single subject. The conditioned H-reflex was clearly smaller than the control H-reflex. As a group, this within-session decrease of the MG H-reflex was 13.4 ± 5.1 % in the last conditioning session, significantly different from the baseline session ($p<0.05$, paired t-test, Figure 3.3).

Changes in the LG H-reflex

As in the MG, the LG H-reflexes were concurrently recorded with the soleus H-reflexes during 20 control trials and 225 conditioning trials. Figure 3.1E shows examples of the averaged 225 H-reflexes of the LG in the baseline session and the last conditioning session from one subject. Unlike the soleus and MG conditioned H-reflex, the LG conditioned H-reflexes did not change after conditioning. In the last conditioning session, the group mean was 93.1 ± 12 % of the baseline value, not different from the baseline ($p=0.59$, paired t-test, Figure 3.3).

Figure 3.1F shows typical examples of the LG control H-reflexes in the baseline and last conditioning sessions from one subject. Unlike the soleus control reflex, the LG control reflex did not change after conditioning. The group mean of the LG control reflex in the last conditioning session was 99.1 ± 11.7 % of the baseline value, not different from the baseline (p=0.94, paired t-test, Figure 3.3).

During the baseline session, there was no difference between the first 20 control H-reflexes and all 225 control H-reflexes in the LG H-reflex size (p=0.97, paired t-test). Figure 3.2C shows typical examples of the averaged control and conditioned H-reflexes of the LG in the last conditioning session in a single subject. Unlike the soleus and MG, there was no apparent within-session change in the LG H-reflex. The group mean of within-session change was -6 ± 4.2% in the last conditioning session, not significantly different from the baseline session (p=0.20, paired t-test, Figure 3.3).

Relation between the soleus and gastrocnemius H-reflexes

In order to investigate whether the soleus H-reflex operant conditioning has any effects on the correlations of H-reflex sizes among the three muscles, coefficient of determination (i.e., R^2 value) was calculated for the relation between the soleus and MG 225 H-reflexes, and the soleus and LG 225 H-reflexes in the baseline and last conditioning sessions. Results showed that both pairs (i.e., soleus and MG, and soleus and LG) showed strong linear correlations in the baseline session (R^2 =0.71 ± 0.06 and 0.73 ± 0.07, mean ± SE, for soleus-MG and soleus-LG, respectively). The R^2 values stayed high in the last conditioning session (R^2 =0.71 ± 0.05 and 0.62 ± 0.08, for soleus-MG and soleus-LG, respectively) and did not show significant changes from the baseline (p=0.99 for soleus-MG, p=0.24 for soleus-LG, paired t-test). Those results showed that the positive linear correlations between soleus and MG H-reflexes, and soleus and LG H-reflexes were well maintained. There were no significant differences in the R^2 values between the two pairs (i.e., soleus-MG and soleus-LG) either in the baseline (p=0.78, paired t-test) or the last conditioning session (p=0.23, paired t-test).

Figure 3.4 shows size distributions of the 225 soleus H-reflexes (in relation to the MG H-reflexes) during the baseline and last conditioning sessions in two representative subjects. Ranges of mean ± 2 SD are indicated by shaded areas for the baseline (gray) and the last conditioning session (blue). In the subject in Figure 3.4A, the soleus H-reflexes (x-axis) exhibited more low values in the last conditioning session, which made its distribution (i.e., mean ± 2SD margin) extend towards the lower end from 0.98-1.35 mV (baseline session, mean rectified EMG) to 0.74-1.25 mV (last conditioning session). In another example (Figure 3.4B), the distribution for the soleus H-reflex became narrower from 0.32-0.63 mV (baseline session) to 0.17-0.39 mV (last conditioning session) by eliminating large responses while producing some smaller responses.

In response to the soleus H-reflex down-conditioning, size distribution of the MG H-reflexes changed from the baseline session to the last conditioning session (see y-axes in Figure 3.4), in similar ways to that of the soleus H-reflexes. In the example shown in Figure 3.4A, size distribution of the 225 MG H-reflexes became wider from 0.33-0.50 mV (baseline) to 0.22-0.45 mV (last conditioning), by expanding the range towards the lower end. In another example of 3.4B, MG H-reflex distribution became narrower from 0.10-0.28 mV (baseline) to 0.07-0.21 mV (last conditioning), by eliminating larger responses. These patterns of changes in the MG conditioned H-reflexes were associated with soleus conditioned H-reflex change summarized above, suggesting that the soleus and MG conditioned H-reflexes changed concurrently in the same direction. Three out of seven subjects presented this corresponding pattern of changes between the soleus and MG H-reflexes by expanding the mean ± 2SD ranges to the lower end (i.e., 3.4A pattern), and another three subjects showed the narrowed mean ± 2SD ranges of the two muscles at the last conditioning session (i.e., 3.4B pattern). Thus, there were different ways available for the subjects to decrease the mean reflex size. One subject showed a smaller mean ± 2SD range for the soleus H-reflex and a larger range for the MG H-reflex at the last conditioning session than at the baseline session.

Size distribution of the 225 LG H-reflexes tended to change from the baseline session to the last conditioning session in similar ways as in the soleus and MG H-reflexes (see Figure 3.4). However, the final LG conditioned H-reflex size was 93% of the baseline (see Figure 3.3) and changes in size distribution were much less pronounced than those in the soleus or MG (data are not shown).

Within-session change and control H- reflex in the soleus and gastrocnemii

In order to examine how within-session change of the MG or LG H-reflex correlates with that of the soleus H-reflex across sessions, the amount of within-session changes in the MG or LG H-reflex at every six conditioning sessions (i.e., down-conditioning sessions 6, 12, 18, 24, and 30) were plotted as a function of the soleus within-session change (Figure 3.5A and 3.5B). Each data point represents the average of all subjects with standard errors in two muscles at each session. As Figure 3.5A and 3.5B show, within-session changes occurred rapidly in the three muscles (i.e., down-conditioning session 6). In addition, within-session changes of the two muscles are significantly correlated ($R^2 = 0.82$, $p<0.01$ for soleus-MG plot, $R^2 = 0.87$, $p<0.01$ for soleus-LG plot) in a positive linear fashion across sessions and most data points are in the area where changes in the two muscles are negative. Thus, within-session decreases concurrently existed among the three muscles in most sessions in most subjects. However, although all data points in the soleus-MG plot (Figure 3.5A) are close to the unity slope line (i.e., dotted line) indicating that the amount of within-session changes in the soleus and MG are similar, data points in the soleus-LG plot (i.e., Figure 3.5B) are above the unity slope line suggesting that the extent of LG H-reflex within-session change is less than that of the soleus or MG. The smaller within-session decrease of the LG H-reflex, compared to the soleus or MG H-reflex, would probably lead to non-significant result of the LG H-reflex within-session change between baseline and the last conditioning sessions shown earlier by using the paired t-test (see Figure 3.3). Furthermore, in both Figure 3.5A and 3.5B, within-session decreases in the three muscles across sessions fluctuated independent of the time line. That is, throughout the five sessions (i.e., down-conditioning sessions 6, 12, 18, 24, and 30), the amount of within-session change did not necessarily increase as the session proceeded.

Association of the control H-reflex between the soleus and the MG or LG across sessions was examined by plotting the amount of control reflexes in the MG or LG as a function of the soleus control reflex at every six conditioning sessions (i.e., down-conditioning sessions 6, 12, 18, 24, and 30) (Figure 3.5C and 3.5D). Each data point represents the average of all subjects with standard errors in two muscles at each session. In both 3.5C and 3.5D, linear correlations of the control H-reflexes between the two muscles are not significant ($R^2 = 0.35$, $p=0.29$ for soleus-MG plot, $R^2 = 0.03$, $p=0.77$ for soleus-LG plot) suggesting that across-session changes in the control H-reflexes of the soleus and MG or LG are not in a linear relationship. However, particularly in the soleus and MG association, gradual decrease of the soleus control reflex was accompanied by decrease of the MG control reflex up to the down-conditioning session 24. Between the session 24 and 30, however, the synergistic association between the two muscles (i.e., change in the same direction) was reversed. That is, despite a slight decrease of the soleus control reflex from session 24 to 30, the MG control reflex increased. Therefore, the MG control H-reflex at the last conditioning session was not different from that in the baseline session (Figure 3.3). For the soleus and LG association, the synergistic changes from session 6 to session 24 were less clear compared to the soleus-MG association (e.g., at session 12, the LG control reflex went up while the soleus control reflex went down). However, between session 24 and 30, the reverse of the synergistic association occurred in the soleus and LG control reflexes, similar to the soleus and MG association. The control H-reflexes of the MG and LG in each subject at down-conditioning 24 and 30 are plotted in Figure 3.6A and B. Five subjects out of seven showed increased MG control reflex from session 24 to 30, suggesting that the reverse of the synergistic association was not induced by an outlier but rather it might indicate a trend (i.e., MG control reflex returns to the baseline value between the session 24 and 30) among those subjects. For the LG control H-reflex, three subjects showed increases from session 24 to 30.

DISCUSSION

The present study explored for the first time the effects of soleus H-reflex down-conditioning on H-reflexes in synergist muscles. Similar to the previous findings in the upper limb (Wolpaw et al., 1983; Wolf and Segal, 1996), the changes in the conditioned H-reflex of synergists (i.e., MG and LG) were less than that of the target muscle (i.e., soleus, Figure 3.3). Beyond this finding, in the present study, we were able to further differentiate the conditioning effects into the two distinct components of H-reflex changes: rapid task-dependent adaptation (i.e., within-session change) and long-term change (i.e., across-session change in the control H-reflex). We found that the soleus down-conditioning induces task-dependent adaptation in the MG and LG (although less) across sessions but not long-term change in either of these synergists at the last conditioning session. Thus, the present results suggest that operant conditioning does not simply have less effect on synergists but rather has differential effects between the target muscle and synergist muscles with regard to task-dependent adaptation and long-term change.

Task-dependent adaptation in the MG and LG H-reflexes

In the present study, within the conditioning sessions, the MG H-reflex size decreased from the 20 control trials to 225 conditioning trials, closely following the changes in the soleus H-reflex size (see Figure 3.3 and 3.5A). For the soleus, in the baseline session, there seemed to be a natural trend in which five out of seven subjects had larger reflexes in the 225 control trials than in the first 20 control trials (p=0.08). Thus, within-session decreases in the soleus and MG H-reflexes during the conditioning sessions reflect the subject's adaptation to the task: to decrease the soleus H-reflex (Thompson et al., 2009). Therefore, operant conditioning concurrently induced task-dependent adaptation in the soleus and MG H-reflexes. This was somewhat expected as the soleus and MG H-reflex sizes are highly correlated during standing (Makihara et al., 2010) (see also pre-conditioning data in Figure 3.4A). For the soleus, the background activity tended to be lower during the 225 conditioning trials than during the first 20 control trials in five subjects (p=0.09), however, the variation in the MG or soleus H-reflex size was not associated with the variation in the background EMG activity ($R^2 < 0.03$) or M-wave size ($R^2 < 0.06$). Thus, task-dependent changes in the H-reflex sizes were most likely presynaptic in origin (Stein and Capaday, 1988; Stein, 1995; Brooke et al., 1997). Previously, Thompson et al. (2009) suggested that cortical and corticospinal tract (CST) activity produce task-dependent changes of H-reflex size. Thus, the observed within-session decreases in the soleus and MG H-reflexes probably reflect such supraspinal plasticity that acts through the mechanisms of presynaptic inhibition (Meunier and Pierrot-Deseilligny, 1998; Baudry et al., 2010).

For the LG H-reflex, within-session decrease was not significant at the last conditioning session (Figure 3.3), different from those in the soleus or MG. In general, the extent of within-session decrease in the LG H-reflex was less than that in the MG H-reflex (see Figure 3.5A and 3.5B). As shown in Figure 3.5B, the LG H-reflex did not decrease as much as the soleus H-reflex. This smaller within-session decrease in the LG H-reflex, compared to that in the soleus or MG H-reflex, was unexpected, as there were strong positive correlations among the H-reflex sizes of those three muscles during standing (Makihara et al., 2010). These results indicate that the soleus H-reflex conditioning does not necessarily induce task-dependent adaptation of the H-reflex equally among its synergists, even though their H-reflex sizes are tightly correlated outside (or even during) the operant conditioning paradigm. As in the soleus and MG, the LG background EMG levels and M-wave sizes were not associated with the LG H-reflex sizes ($R^2 < 0.05$ for background EMG vs. H-reflex, $R^2 < 0.03$ for M-wave vs. H-reflex), and thus, the origin of such rapid (i.e., within-session) differential control of the LG H-reflex from the soleus and MG H-reflexes is likely presynaptic (Stein and Capaday, 1988; Stein, 1995; Brooke et al., 1997). Differential autogenic (homonymous) and heterogenic (heteronymous) excitation and inhibition among the three muscles were reported in decerebrated cats (Nichols, 1989) and presynaptic inhibition has been demonstrated to be selective enough to induce different effects between collaterals from the same muscle afferent (Rudomin et al., 1998). However, actual mechanisms that differentiate task-dependent adaptation between the soleus (and MG) and LG H-reflexes are unknown.

Long-term change in the MG and LG H-reflexes

In the previous study of soleus H-reflex operant conditioning, Thompson et al. (2009) suggested that repeatedly induced task-dependent adaptation (i.e., within-session change) of the H-reflex, likely a product of supraspinal plasticity, gradually induces plasticity in the spinal cord and changes the control H-reflex. In the present study, to our surprise, after successful down-conditioning of the soleus H-reflex, there was no change in the MG control H-reflex in spite of the persistent presence of within-session decrease in the MG H-reflex (see Figure 3.5A). Since the amount of within-session H-reflex decrease was essentially the same between the soleus and MG, the absence of control H-reflex change (i.e., long-term change) in the MG at the last conditioning session cannot be explained by the difference in the extent of task-dependent adaptation.

Questions arise: why repeatedly induced task-dependent adaptation did not lead to long-term change in the MG H-reflex pathway, and, how that happened. As mentioned above, task-dependent adaptation of the soleus H-reflex reflects supraspinal plasticity (i.e., most likely changes in CST activity), and when such supraspinal plasticity persists, it changes the spinal cord in long-term (Wolpaw and O'Keefe, 1984; Wolpaw et al., 1994; Chen et al., 2001; Thompson et al., 2009). Since the task-dependent within-session decrease in the MG H-reflex was just as much as that in the soleus H-reflex, it can be assumed that supraspinal plasticity in the H-reflex pathways induced by the operant conditioning paradigm was no different between the soleus and MG. The control reflex of the two muscles decreased synergistically up to session 24, and the association was reversed at session 30. Thus, presumably, there would have been a mechanism occurred between the session 24 and 30 that prevented the supraspinal (i.e., CST activity) plasticity from further inducing long-term changes in the MG H-reflex pathways. It could be compensatory plasticity of the spinal cord (Wolpaw 2010), which maintained the MG control H-reflex size while the within-session task-dependent decrease of the MG H-reflex was repeatedly induced. There have been several studies showing compensatory plasticity of the spinal cord, especially among the spinal pathways of synergists (Decima and Morales, 1983; Whelan et al., 1995; Whelan and Pearson, 1997; Pearson et al., 1999; Bouyer et al., 2001). For the LG H-reflex, the extent of within-session decrease was less than that in the soleus and MG H-reflexes, and thus, it is unclear whether a lack of long-term change in the LG H-reflex at the last conditioning session has any functional meaning. However, as shown in Figure 3.5D, the reverse of the synergistic association between the soleus and LG control reflexes occurred between session 24 and 30, just as in the soleus and MG association. Thus, there might be compensatory plasticity in the LG H-reflex pathway to maintain the LG control reflex at the last conditioning session.

When the soleus H-reflex operant conditioning induces long-term changes in the soleus H-reflex pathway in healthy humans, minimizing the impact of conditioning on other pathways would be an urgent issue. Thus, it might be the brain and the spinal cord's best interest to preserve the pathways that were not directly conditioned. The absence of long-term change in the MG (and possibly the LG) H-reflex pathway at the last conditioning session might reflect the brain and spinal cord's strategy to accommodate a new skill (i.e., to decrease the soleus H-reflex) with minimum impact on synergists' pathway so as to impose the least impact on the existing wide repertoire of motor skills (Chen et al., 2005; Wolpaw, 2010).

Functional implications

Modification of the H-reflex size, as studied in operant conditioning, is not merely an experimental phenomenon (Wolpaw and Tennissen, 2001). Many studies reported changes in the soleus H-reflex size that accompanied acquiring of new motor skills (Casabona et al., 1990; Nielsen et al., 1993; Trimble and Koceja, 1994; Voigt et al., 1998; Perez et al., 2007). Those modifications of the H-reflex size induced by exercise training may represent specific adaptations to meet particular requirements of the training. For example, a small soleus H-reflex in professional ballet dancers (Nielsen et al., 1993) would be a consequence of frequent cocontraction in the agonist and antagonist muscles, which would facilitate precision of dance skills (Wolpaw, 2010). Thus, changes of the soleus H-reflex are thought to contribute to specific skills across different populations. As Figure 3.4 shows, a pattern of the H-reflex change would vary between individuals probably based on the learning strategy (Thompson et al., 2009) as well as the nature and intensity of daily physical activity. We did not find any apparent association between the pattern of the reflex decrease (i.e., extend or narrow the distribution) and other parameters such as the original reflex size or the Hmax/Mmax ratio.

When long-term change of the soleus H-reflex is induced by operant conditioning, the current behavioral repertoire would be affected. In order to avoid disruptive influence on the current motor behavior while accommodating the change in the soleus H-reflex, minimizing the impact of conditioning on synergists (i.e., MG and/or LG) would be the most economical strategy because of their functional similarities. Thus, compensatory plasticity may be necessary to maintain the MG control reflex, and as a result, to protect the other behaviors. The compensatory plasticity may be constantly present throughout the conditioning period as the soleus control reflex decreases to automatically calibrate the MG control reflex. Indeed, Chen et al., (2005) reported in rats that the normal locomotion pattern is preserved despite the significant decrease or increase of the soleus H-reflex induced by operant conditioning, probably due to compensatory changes in activity of other muscles. Although the animal protocol involves a much higher dosage and volume of conditioning than the human protocol (i.e., for rats, 2500-10000 trials per day distributed throughout the 24 hours for 30-70 days, Chen and Wolpaw, 1995), final H-reflex size is generally similar between species [for down-conditioning, monkeys 68% (of control value, Wolpaw, 1987), rats 67% (Chen and Wolpaw, 1995), mice 71% (Carp et al., 2006b), and humans 69% (Thompson et al., 2009) and 76% (present study)]. Thus, effects of soleus H-reflex operant conditioning on functional behaviors such as locomotion will need to be investigated to further obtain insights about compensatory plasticity in humans.

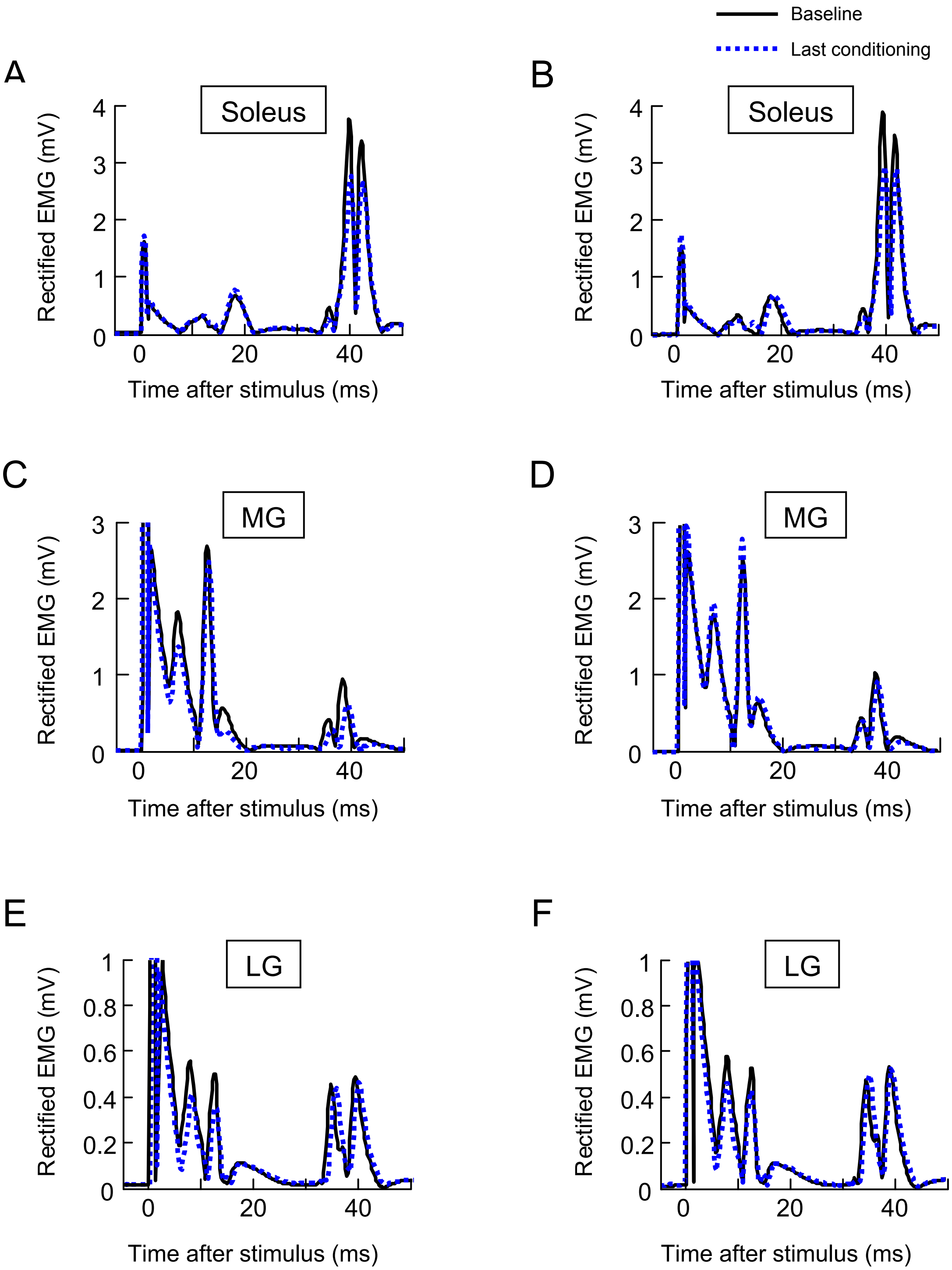

Figure 3.1: Conditioned H-reflexes (left) and control H-reflexes (right)
Typical examples of averaged conditioned H-reflexes and control H-reflexes in the soleus (A and B), MG (C and D), and LG (E and F) in the sixth baseline session (solid) and the last conditioning (dotted) from a single subject. For each sweep, 225 responses were averaged together. The conditioned H-reflex size decreased after the soleus H-reflex down-

conditioning in the soleus and MG, but not in the LG. The control H-reflex decreased after conditioning only in the soleus. Background EMG and M-wave size did not change between the baseline and last conditioning sessions.

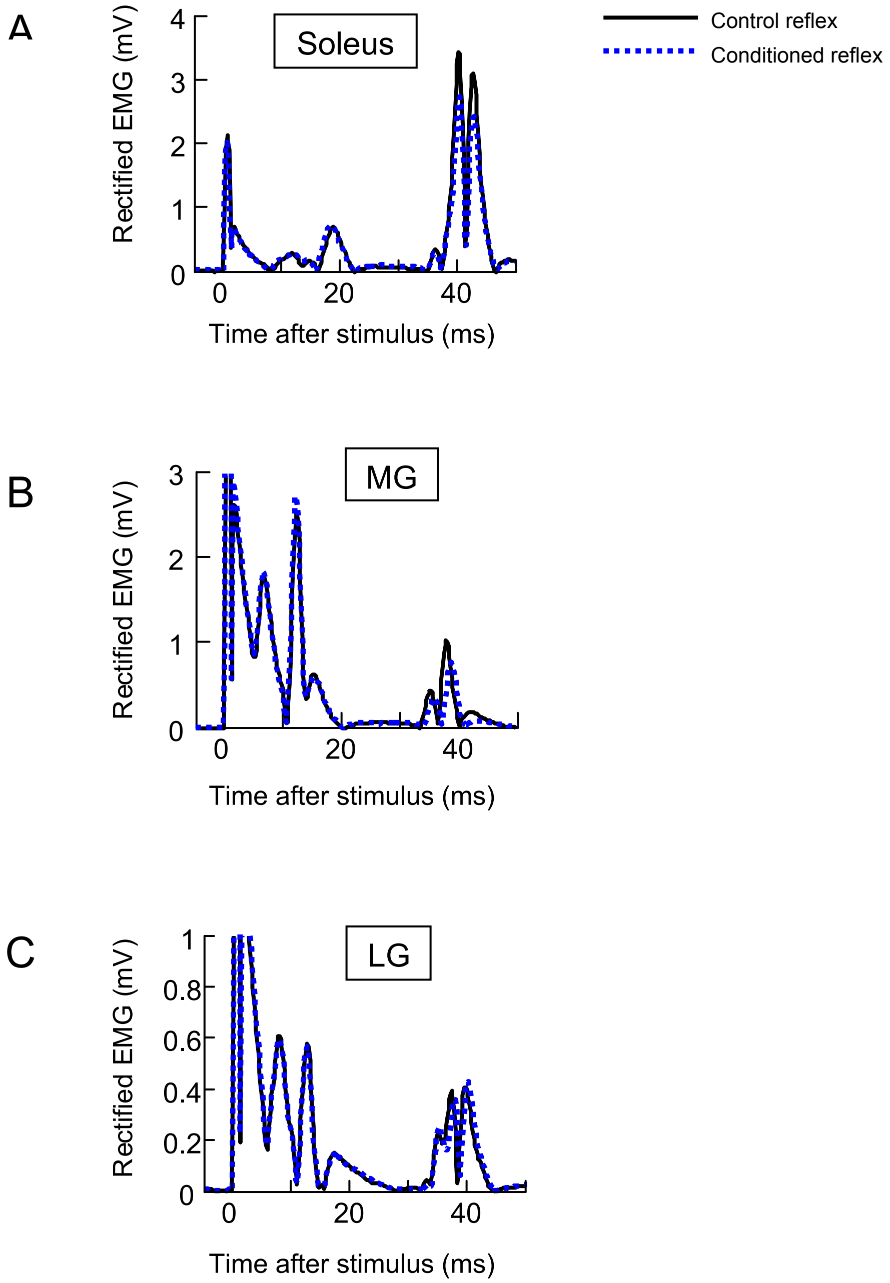

Figure 3.2: Within-session change in the last conditioning session
Typical examples of control H-reflexes (solid) and conditioned H-reflexes (dotted) of the soleus (A), MG (B), and LG (C) in the last conditioning session. The soleus and MG showed within-session decrease in H-reflex size from the control H-reflex, but the LG did not.

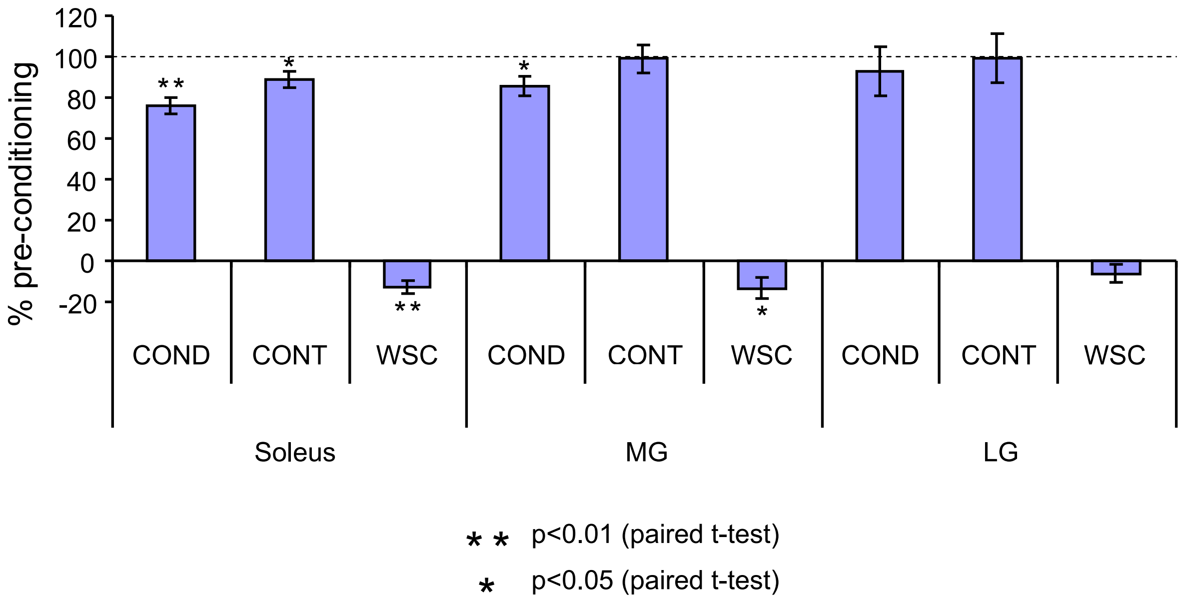

Figure 3.3: Group average of the conditioned H-reflexes, control H-reflexes, and within-session changes

Bars in the graph represent values in the last conditioning session of each category, which are normalized by baseline values. For the conditioning and control H-reflexes, 100% means that the reflex did not change from the baseline. For within-session change, 0% means that there was no difference between the control reflex and conditioned reflex in the last conditioning session (i.e., within-session change was 0% in the baseline). The soleus shows significant changes from the baseline to the last conditioning session with regard to the conditioned H-reflex, control reflex, and within-session change. The conditioned H-reflex and within-session change of the MG changed significantly, however, the control reflex stayed at the baseline level. For the LG, none of the three measurements changed significantly in the last conditioning session. COND: conditioned H-reflexes, CONT: control H-reflexes, WSC: within-session change

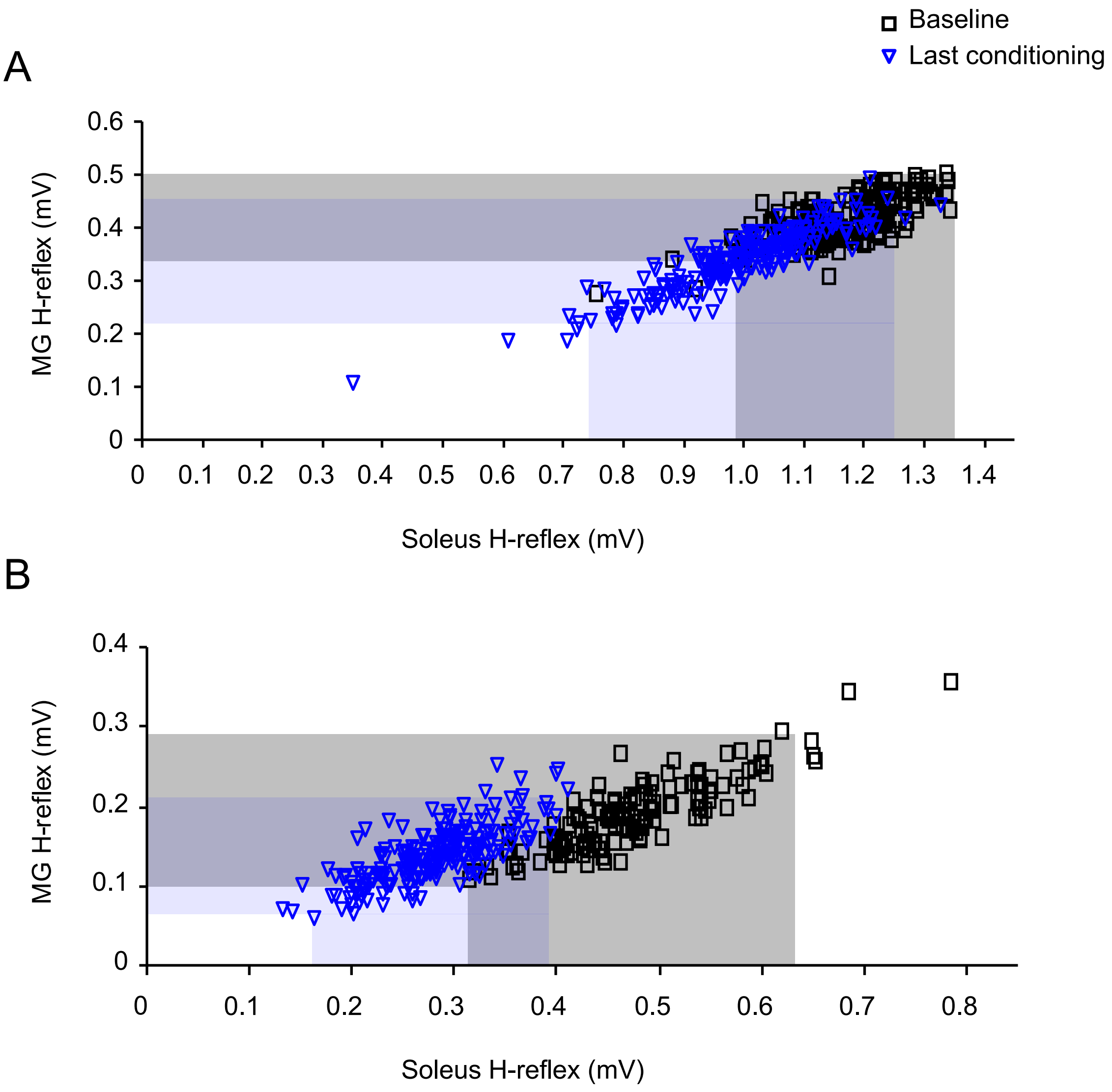

Figure 3.4: Correlation between the soleus and MG conditioned H-reflexes
Two examples of correlation between the soleus and MG conditioned H-reflexes in the baseline and the last conditioning sessions. The size distribution (i.e., mean ± 2 SD) became wider in the last conditioning session in 3.4A whereas it became narrower in 3.4B. Three subjects showed 3.4A pattern, and another three subjects showed 3.4B pattern.

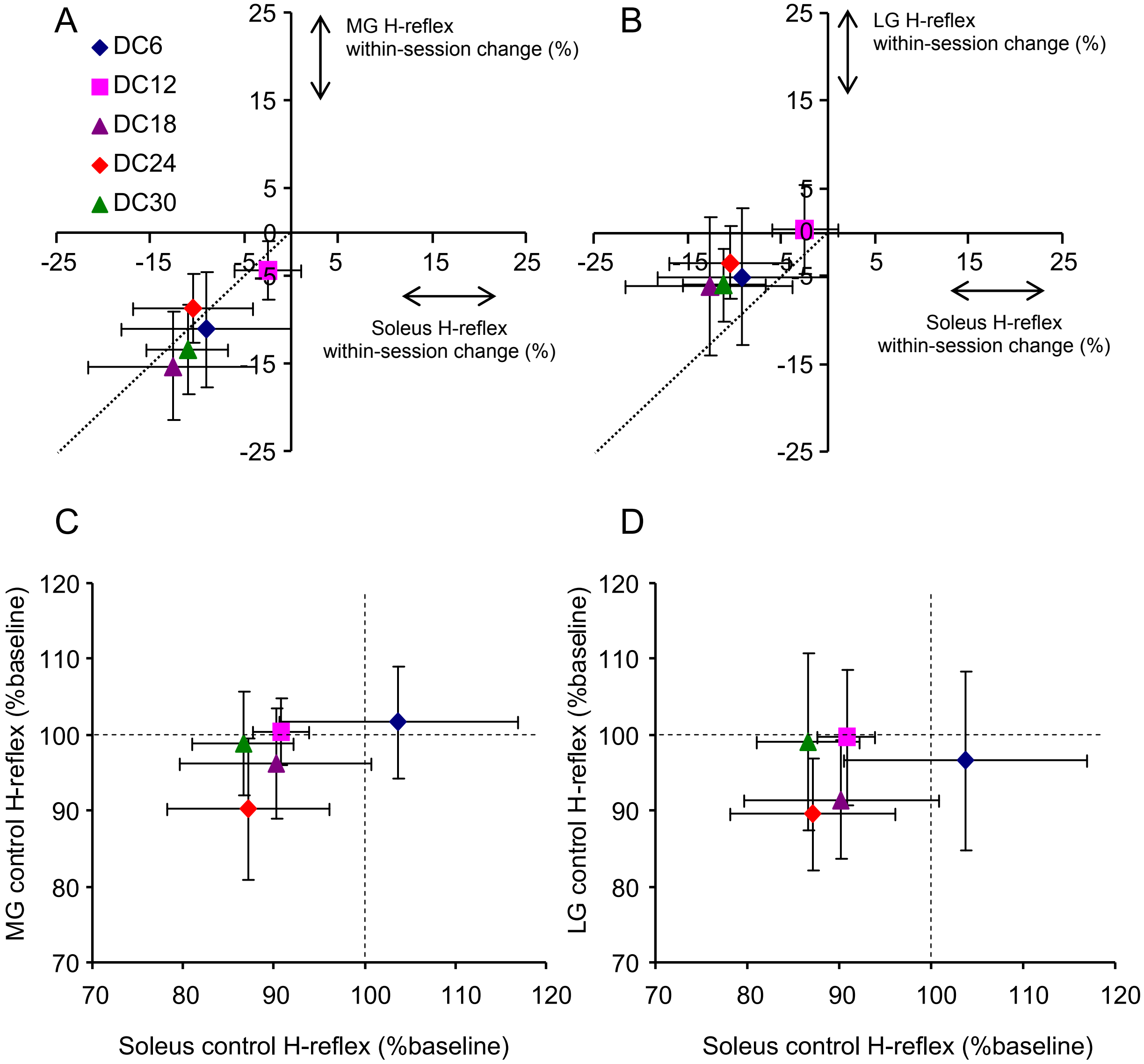

Figure 3.5: Correlation plots of with-session changes and control reflexes
Correlation plots of within-session changes between the soleus and MG H-reflexes (A), and soleus and LG H-reflexes (B), and of control H-reflexes between the soleus and MG (C), and soleus and LG (D) at conditioning sessions 6, 12, 18, 24, and 30. Each data point represents the average of all subjects with standard errors in two muscles at each session. Dotted lines on the A and B are unity slope lines. On C and D, between the down-conditioning session 24 and 30 (i.e., orange diamond and green triangle, respectively), the MG or LG control H-reflex increased while the soleus H-reflex slightly decreased. DC: down-conditioning.

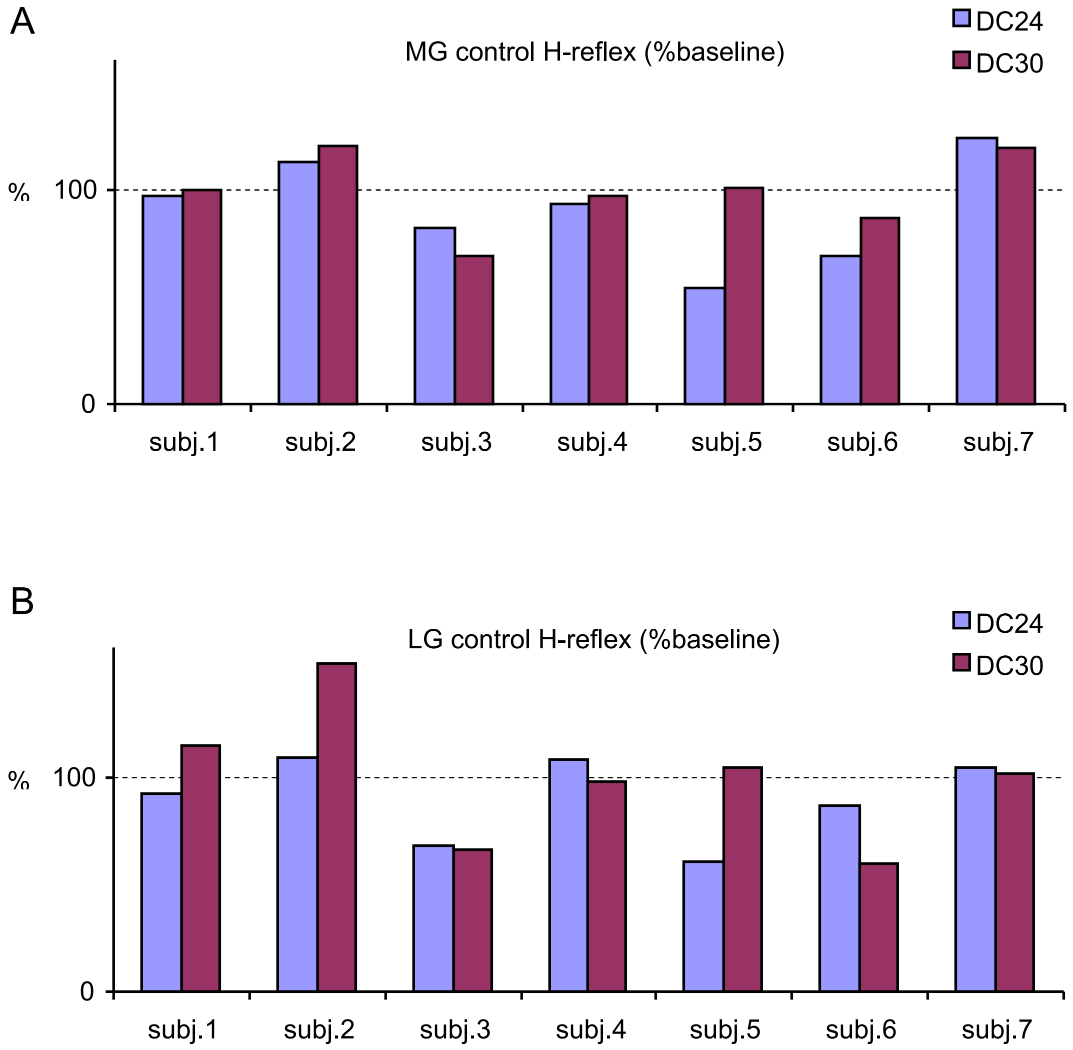

Figure 3.6: Individual data for control H-reflexes of the MG and LG
The control H-reflexes of the MG (A) and LG (B) in each subject at down-conditioning (DC) 24 and 30 represented as a percentage of the baseline value are shown. The average of the MG control reflex was 90% at DC24 and 99% at DC30. Five subjects out of seven showed increased MG control reflex from DC24 to DC30. For the LG control H-reflex, the average was 90% at DC24 and 99% at DC30. Three subjects showed increases from DC24 to DC30.

References

Baudry S, Maerz AH, Enoka RM (2010) Presynaptic modulation of Ia afferents in young and old adults when performing force and position control. J Neurophysiol 103:623-631.

Bouyer LJ, Whelan PJ, Pearson KG, Rossignol S (2001) Adaptive locomotor plasticity in chronic spinal cats after ankle extensors neurectomy. J Neurosci 21:3531-3541.

Brooke JD, Cheng J, Collins DF, McIlroy WE, Misiaszek JE, Staines WR (1997) Sensori-sensory afferent conditioning with leg movement: gain control in spinal reflex and ascending paths. Prog Neurobiol 51:393-421.

Carp JS, Wolpaw JR (1994) Motoneuron plasticity underlying operantly conditioned decrease in primate H-reflex. J Neurophysiol 72:431-442.

Carp JS, Wolpaw JR (1995) Motoneuron properties after operantly conditioned increase in primate H-reflex. J Neurophysiol 73:1365-1373.

Carp JS, Chen XY, Sheikh H, Wolpaw JR (2001) Operant conditioning of rat H-reflex affects motoneuron axonal conduction velocity. Exp Brain Res 136:269-273.

Carp JS, Tennissen AM, Chen XY, Wolpaw JR (2006a) Diurnal H-reflex variation in mice. Exp Brain Res 168:517-528.

Carp JS, Tennissen AM, Chen XY, Wolpaw JR (2006b) H-reflex operant conditioning in mice. J Neurophysiol 96:1718-1727.

Casabona A, Polizzi MC, Perciavalle V (1990) Differences in H-reflex between athletes trained for explosive contractions and non-trained subjects. Eur J Appl Physiol Occup Physiol 61:26-32.

Chen XY, Wolpaw JR (1994) Circadian rhythm in rat H-reflex. Brain Res 648:167-170.

Chen XY, Wolpaw JR (1995) Operant conditioning of H-reflex in freely moving rats. J Neurophysiol 73:411-415.

Chen XY, Wolpaw JR (2002) Probable corticospinal tract control of spinal cord plasticity in the rat. J Neurophysiol 87:645-652.

Chen XY, Wolpaw JR (2005) Ablation of cerebellar nuclei prevents H-reflex down-conditioning in rats. Learn Mem 12:248-254.

Chen XY, Chen L, Wolpaw JR (2001) Time course of H-reflex conditioning in the rat. Neurosci Lett 302:85-88.

Chen XY, Carp JS, Chen L, Wolpaw JR (2002) Corticospinal tract transection prevents operantly conditioned H-reflex increase in rats. Exp Brain Res 144:88-94.

Chen XY, Carp JS, Chen L, Wolpaw JR (2006) Sensorimotor cortex ablation prevents H-reflex up-conditioning and causes a paradoxical response to down-conditioning in rats. J Neurophysiol 96:119-127.

Chen XY, Chen Y, Wang Y, Thompson A, Carp JS, Segal RL, Wolpaw JR (2010) Reflex conditioning: a new strategy for improving motor function after spinal cord injury. Ann N Y Acad Sci 1198 Suppl 1:E12-21.

Chen Y, Chen XY, Jakeman LB, Schalk G, Stokes BT, Wolpaw JR (2005) The interaction of a new motor skill and an old one: H-reflex conditioning and locomotion in rats. J Neurosci 25:6898-6906.

Decima EE, Morales FR (1983) Long-lasting facilitation of a monosynaptic pathway as a result of "partial" axotomy of its presynaptic elements. Exp Neurol 79:532-551.

Duclay J, Robbe A, Pousson M, Martin A (2009) Effect of angular velocity on soleus and medial gastrocnemius H-reflex during maximal concentric and eccentric muscle contraction. J Electromyogr Kinesiol 19: 948-956.

Evatt ML, Wolf SL, Segal RL (1989) Modification of human spinal stretch reflexes: preliminary studies. Neurosci Lett 105:350-355.

Knikou M (2008) The H-reflex as a probe: pathways and pitfalls. J Neurosci Methods 171:1-12.

Lagerquist O, Zehr EP, Baldwin ER, Klakowicz PM, Collins DF (2006) Diurnal changes in the amplitude of the Hoffmann reflex in the human soleus but not in the flexor carpi radialis muscle. Exp Brain Res 170:1-6.

Makihara Y, Thompson AK, Abel BM, Segal RL (2010) Operant down-conditioning of soleus H-reflex in humans: effects on synergist muscles. Program No 8215 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2010. Online.

Meunier S, Pierrot-Deseilligny E (1998) Cortical control of presynaptic inhibition of Ia afferents in humans. Exp Brain Res 119:415-426.

Misiaszek JE (2003) The H-reflex as a tool in neurophysiology: its limitations and uses in understanding nervous system function. Muscle Nerve 28:144-160.

Nichols TR (1989) The organization of heterogenic reflexes among muscles crossing the ankle joint in the decerebrate cat. J Physiol 410:463-477.

Nielsen J, Crone C, Hultborn H (1993) H-reflexes are smaller in dancers from The Royal Danish Ballet than in well-trained athletes. Eur J Appl Physiol Occup Physiol 66:116-121.

Pearson KG, Fouad K, Misiaszek JE (1999) Adaptive changes in motor activity associated with functional recovery following muscle denervation in walking cats. J Neurophysiol 82:370-381.

Perez MA, Lundbye-Jensen J, Nielsen JB (2007) Task-specific depression of the soleus H-reflex after cocontraction training of antagonistic ankle muscles. J Neurophysiol 98:3677-3687.

Pierrot-Deseilligny E, Mazevet D (2000) The monosynaptic reflex: a tool to investigate motor control in humans. Interest and limits. Neurophysiol Clin 30:67-80.

Rudomin P, Jimenez I, J. Q (1998) Selectivity of the presynaptic control of synapic effectiveness of group I afferents in the mammalian spinal cord. In: Rudomin P, Romo R, Mendell LM (eds) Presynaptic inhibition and neural control Oxford University Press, New York, pp282-302.

Schieppati M (1987) The Hoffmann reflex: a means of assessing spinal reflex excitability and its descending control in man. Prog Neurobiol 28:345-376.

Stein RB (1995) Presynaptic inhibition in humans. Prog Neurobiol 47:533-544.

Stein RB, Capaday C (1988) The modulation of human reflexes during functional motor tasks. Trends Neurosci 11:328-332.

Thompson AK, Chen XY, Wolpaw JR (2009) Acquisition of a simple motor skill: task-dependent adaptation plus long-term change in the human soleus H-reflex. J Neurosci 29:5784-5792.

Trimble MH, Koceja DM (1994) Modulation of the triceps surae H-reflex with training. Int J Neurosci 76:293-303.

Voigt M, Chelli F, Frigo C (1998) Changes in the excitability of soleus muscle short latency stretch reflexes during human hopping after 4 weeks of hopping training. Eur J Appl Physiol Occup Physiol 78:522-532.

Wang Y, Pillai S, Wolpaw JR, Chen XY (2006) Motor learning changes GABAergic terminals on spinal motoneurons in normal rats. Eur J Neurosci 23:141-150.

Wang Y, Pillai S, Wolpaw JR, Chen XY (2009) H-reflex down-conditioning greatly increases the number of identifiable GABAergic interneurons in rat ventral horn. Neurosci Lett 452:124-129.

Whelan PJ, Pearson KG (1997) Plasticity in reflex pathways controlling stepping in the cat. J Neurophysiol 78:1643-1650.

Whelan PJ, Hiebert GW, Pearson KG (1995) Plasticity of the extensor group I pathway controlling the stance to swing transition in the cat. J Neurophysiol 74:2782-2787.

Wolf SL, Segal RL (1996) Reducing human biceps brachii spinal stretch reflex magnitude. J Neurophysiol 75:1637-1646.

Wolpaw JR (1987) Operant conditioning of primate spinal reflexes: the H-reflex. J Neurophysiol 57:443-459.

Wolpaw JR (2010) What can the spinal cord teach us about learning and memory? Neuroscientist 16:532-549.

Wolpaw JR, Seegal RF (1982) Diurnal rhythm in the spinal stretch reflex. Brain Res 244:365-369.

Wolpaw JR, O'Keefe JA (1984) Adaptive plasticity in the primate spinal stretch reflex: evidence for a two-phase process. J Neurosci 4:2718-2724.

Wolpaw JR, Tennissen AM (2001) Activity-dependent spinal cord plasticity in health and disease. Annu Rev Neurosci 24:807-843.

Wolpaw JR, Chen XY (2006) The cerebellum in maintenance of a motor skill: a hierarchy of brain and spinal cord plasticity underlies H-reflex conditioning. Learn Mem 13:208-215.

Wolpaw JR, Seegal RF, O'Keefe JA (1983) Adaptive plasticity in primate spinal stretch reflex: behavior of synergist and antagonist muscles. J Neurophysiol 50:1312-1319.

Wolpaw JR, Maniccia DM, Elia T (1994) Operant conditioning of primate H-reflex: phases of development. Neurosci Lett 170:203-207.

Zehr PE (2002) Considerations for use of the Hoffmann reflex in exercise studies. Eur J Appl Physiol 86:455-468.

CHAPTER IV

THIRD MANUSCRIPT

Locomotor EMG activity is preserved after operant conditioning of the soleus H-reflex in healthy human subjects

ABSTRACT

Long-term change in the soleus H-reflex induced by operant conditioning has been shown to persist outside of the conditioning paradigm. Thus, it is conceivable that such long-term change in the spinal cord pathway would influence walking, in which the activity of the changed pathway has significant roles. Effects of soleus H-reflex operant down-conditioning on locomotion were investigated in healthy human subjects. For successfully conditioned subjects, locomotion analyses were performed pre- and post-conditioning and locomotor H-reflexes, locomotor EMG, and joint kinematics were compared between the two locomotion sessions. During conditioning, the soleus control reflex showed a significant decrease, however, this decrease was not reflected in the locomotor H-reflex. None of the locomotor variables at post-conditioning changed from those at pre-conditioning, and thus, the normal locomotion pattern was preserved. It is suggested that, in the process of H-reflex modulation between standing and walking, decreased H-reflexes induced during standing would be adjusted during walking so that the appropriate reflex gain for walking could be maintained. Locomotor reflex activity was preserved, as well as locomotor EMG activity and kinematics. Those results were different from the previous rat study showing that the normal locomotion pattern was preserved despite the decrease of locomotor H-reflexes. For humans who were conditioned only an hour per day, behavioral repertoire outside the conditioning paradigm is much larger than that for rats who were conditioned most of the day. Therefore, preserving the existing wide variety of skills (e.g., normal walking) with minimum adjustments might be essential in humans.

INTRODUCTION

Operant conditioning of the soleus H-reflex changes the activity of the H-reflex pathways in monkeys, rats, mice, and humans (Wolpaw, 1987; Chen and Wolpaw, 1995; Carp et al., 2006a; Thompson et al., 2009). A large part of this H-reflex change continues and gradually develops over a long-term period of conditioning. This long-term change of the H-reflex is suggested to reflect spinal cord plasticity (Wolpaw and O'Keefe, 1984; Wolpaw et al., 1994; Chen et al., 2001; Thompson et al., 2009). Since the change of the reflex is retained across sessions, spinal cord plasticity persists outside of the conditioning paradigm. Thus, it is conceivable that such long-term change in the spinal cord pathway would influence movements in which the activity of the changed pathway has significant roles. Ia afferent pathways, which would be changed by successful soleus H-reflex conditioning, contribute to generation of the triceps surae EMG activity and force production during stance phase of locomotion (Yang et al., 1991; Bennett et al., 1996; Stein et al., 2000). Also, the activity of Ia afferent pathways is phase-dependently modulated during walking (i.e., high in the stance phase and low in the swing phase) to facilitate proper and effective execution of the task (Capaday and Stein, 1986; Llewellyn et al., 1990; Edamura et al., 1991) even in the face of unexpected perturbation (Schmidt and Lee, 2005). Thus, when the soleus H-reflex is changed due to changes in efficacy of Ia (and possibly II) excitation of motoneurons by operant conditioning (i.e., spinal cord plasticity, Carp and Wolpaw, 1994; Wolpaw, 1997, for review), movement function, such as locomotion, might be changed.

In rats, Chen et al. (2005) found that the soleus H-reflex and EMG burst during locomotion became larger after successful up-conditioning and smaller after successful down-conditioning. In their study, duration, length, and right/left symmetry of the step cycle were not affected, despite the changes in the locomotor H-reflex and EMG activity. Recent studies by Chen et al. showed that the EMG burst and H-reflex size of the vastus lateralis during locomotion changed after the soleus H-reflex operant conditioning (Chen et al., 2009a; Chen et al., 2009b). These findings suggest that as the soleus H-reflex changes in response to operant conditioning, compensatory changes occur in the activity of other leg muscles, in order to preserve the normal locomotion pattern (Wolpaw, 2010).

In the present study, we investigated, in healthy human subjects, effects of soleus H-reflex operant conditioning on locomotion. Specifically, we examined H-reflex modulation, EMG activity, and kinematics of the legs during locomotion before and after successful soleus H-reflex down-conditioning.

MATERIALS AND METHODS

General procedure

Eight healthy subjects (two women and six men, age 21-54 yrs old) with no known neurological disorders participated in the study. Only data from successfully conditioned subjects (n=7) were used for locomotion analysis. Each subject walked on a treadmill at self-selected speed while EMG activity, H-reflexes, and joint angle data were measured. Locomotion data were compared before and after the operant conditioning. All subjects gave informed consent before the participation. All protocols were approved by the Institutional Review Board for Human Research at Helen Hayes Hospital, West Haverstraw, NY.

Preliminary session and Operant conditioning sessions

Detailed methodology for the operant conditioning has been reported previously (Thompson et al., 2009), and thus briefly summarized here. There were six baseline sessions and 30 down-conditioning sessions, which occurred 3 times per week. Each subject's sessions always occurred at the same time of day to control for diurnal variations in H-reflex size (Wolpaw and Seegal, 1982; Chen and Wolpaw, 1994; Carp et al., 2006b; Lagerquist et al., 2006; Thompson et al., 2009).

In the initial preliminary session prior to the first baseline session, the maximum voluntary contraction (MVC) value was determined as maximum rectified EMG level in the soleus, MG, and LG. Next, target soleus background EMG window and M-wave size were determined for each subject. The target background activity was chosen while the subject maintained natural standing (i.e., usually 10-15% MVC). The target M-wave size was the reflex amplitude elicited by stimulation set at just above the M-wave threshold. For all the H-reflex trials throughout the study, the soleus M-wave size and the soleus background EMG levels were kept constant

Each session began with an H-reflex/M-wave recruitment curve measurement during standing while the subject maintained the soleus background EMG activity in the target window. The soleus EMG activity was rectified and averaged every 100 ms, and the result was immediately provided as visual feedback to help the subject maintain the soleus background EMG activity within the specified window (see Thompson et al., 2009 for details). In each baseline session, 225 control H-reflexes separated into three blocks of 75 trials were elicited. In each down-conditioning session, 20 control H-reflexes were measured as in baseline sessions, and then, 225 conditioned H-reflexes divided into three blocks of 75 trials were elicited. During the control trials, the H-reflex amplitudes were simply recorded and the subject did not receive any encouragement or feedback regarding the reflex amplitude. During the conditioning trials, the subject was asked to decrease the soleus reflex size and provided immediate visual feedback of the soleus H-reflex size at each trial. The visual feedback indicated whether the H-reflex size was smaller than a pre-defined reward criterion (i.e., successful or not). The MG and LG H-reflexes were concurrently measured with the soleus H-reflex at every six sessions, but there was no feedback about the background EMG activity and the H-reflex size of the MG and LG. The M-wave sizes of the MG and LG were kept the same throughout all measurements.

Locomotion sessions

There were two locomotion sessions, one before and one after the conditioning period. The post-conditioning locomotion session occurred on the next day or two days after the last conditioning session. At the beginning of each locomotion session, the H-reflex and M-wave recruitment curves were simultaneously obtained from the conditioned side soleus, MG, and LG. Then, the maximum voluntary contraction (MVC) values for each of the 12 muscles were determined. Typically, two MVC measurements were made with short breaks in between. The subject was asked to perform the maximum isometric contraction of each tested muscle for 5 s, and average rectified EMG in the middle 3 s was used as the MVC value for each muscle.

In order to detect heel-contact signal, foot switches (Bortec Biomedical, Calgary, Canada) were inserted between the subject's shoe and the heel. Then, the subject walked on a treadmill at his/her comfortable speed (average 0.9 m/s) that was kept the same between the two locomotion sessions. This self-selected speed was slower than the typical normal walking speed (i.e., 1.1-1.2 m/s, Duysens et al., 1991) probably because loud noise from the treadmill motor made the subject feel they walked at a faster speed than the actual speed. The EMG activity of the bilateral soleus, tibialis anterior (TA), MG, LG, vastus lateralis (VL), and biceps femoris (BF), and heel-contact signal were continuously recorded during the first walking trial. Next, the tibial nerve was pseudo-randomly stimulated for the measurement of locomotor H-reflexes of the soleus, MG, and LG on the conditioned side (see Electrical stimulation and EMG recording). In order to compare the H-reflexes elicited at the same stimulus efficacy throughout the gait cycle, various stimulus intensities were used (Capaday and Stein, 1986; Llewellyn et al., 1990; Edamura et al., 1991). By combining all data and eliminating responses with too large or too small M-wave from further analysis, H-reflexes with constant size M-wave (i.e., target M-wave size) across different phases of the gait cycle were obtained. This target M-wave size was kept consistent between the two locomotion sessions for each subject.

After finishing the measurement of locomotor EMG activity and locomotor H-reflexes, active infrared markers were placed on five landmarks (acromion, greater trochanter, lateral epicondyle of the femur, lateral malleolus, and tips of the toes) and the subject returned to the treadmill to walk at the same speed. Two-dimensional (2D) trajectories of the markers in the sagittal plane were captured using a custom-built infrared motion capture system at a sampling frequency of 66 Hz. The 2D joint angle data on the sagittal plane (i.e., flexion-extension for the hip and knee, and dorsiflexion and plantarflexion for the ankle) were calculated as an angle between adjacent segments.

EMG recordings and electrical stimulation

For H-reflex recording, self-adhesive surface Ag-AgCl electrodes (2.2 × 2.2 cm for the cathode and 2.2 × 3.5 cm for the anode; Vermed, Bellows Falls, VT) were placed unilaterally (i.e., conditioned side) in the popliteal fossa to stimulate the tibial nerve using a Grass S48 stimulator (with CCU1 constant current unit and SIU5 stimulus isolation unit; Astro-Med, West Warwick, RI). For reflex EMG activity recording, a pair of self-adhesive surface Ag-AgCl electrodes (2.2 × 3.5 cm, Vermed, Bellows Falls, VT) was placed longitudinally on the skin over the soleus just below the gastrocnemii with an interelectrode distance of 3 cm. Locations for the nerve stimulating electrodes were determined to minimize the H-reflex threshold and maximize the maximum H-reflex (Hmax) and the M-wave (Mmax) amplitudes of the soleus. Pairs of EMG recording electrodes were also placed over the center of the muscle belly of the TA. During operant conditioning, pairs of EMG electrodes were placed on the skin over medial and lateral gastrocnemii (MG and LG) at every six sessions. Centers of electrodes for the triceps surae muscles were placed at least 7 cm apart between the muscles to minimize the cross-talk. For locomotor EMG recording, pairs of EMG recording electrodes were placed bilaterally on the soleus, TA, MG, LG, VL, and BF. To avoid session-to-session variability, locations for all electrodes were mapped using permanent skin marks such as scars and moles at the first locomotion session. For the second locomotion session, the electrodes were placed based on those landmarks.

EMG signals were amplified, band-pass filtered (10-1000 Hz), and recorded with a custom-made system and Axoscope (Molecular Devices Inc., Sunnyvale, CA) at 5000 Hz (for standing data) or 2000 Hz (for walking data). During walking, EMG and nerve stimulation signals were continuously recorded while the H-reflexes of the soleus, MG, and LG were elicited by 1 ms square pulse with pseudo-random intervals (i.e., interstimulus interval of 2.5-4.5 s). This was to ensure that the H-reflexes were obtained at various phases throughout the entire gait cycle but no more than one stimulus per gait cycle was delivered and there was at least one gait cycle without stimulation between the stimulated cycles (Yang and Stein, 1990; Kido et al., 2004b, a).

Data analysis

All analyses were done with a custom-written MATLAB program (Mathworks, Natick, MA). For conditioning data, changes in the control and conditioned H-reflex sizes across sessions were quantified as a percentage of the average for the baseline (see Thompson et al., 2009 for details). The H-reflex and the M-wave amplitudes during walking were measured as peak-to-peak values in each reflex window (i.e., typically 30-45 ms post stimulus for the soleus H-reflex and 5-23 ms post stimulus for the soleus M-wave). The H-reflex sizes of the soleus, MG, and LG during locomotion were normalized using the Mmax size of each muscle and plotted in the function of the gait cycle. A gait cycle was determined by heel contact signals (from one heel contact to the next heel contact of the same side). Then, the entire gait cycle for each muscle was divided into 12 equal bins, and H-reflex amplitudes recorded in the same bin (i.e., bin 1-12, typically 10 responses in each bin) were averaged. The group average of H-reflex size in each bin was compared between pre- and post-conditioning using the paired t-test ($\alpha = 0.05$). To correct the significance level for multiple comparisons, Sidak correction ($1-(1-\alpha)^{1/n}$) was used to determine the p-value threshold. That is, for comparing between pre- and post-conditioning for 12 bins (i.e., 12 potential comparisons), we used $1-(1-0.05)^{1/3} = 0.004$ as the threshold for significance. In addition, the extent of H-reflex modulation during walking was evaluated by the modulation index [i.e., (maximum H-reflex - minimum H-reflex) / maximum H-reflex ×100; calculated over the gait cycle for each muscle (Zehr and Kido, 2001; Kido et al., 2004b)].

The EMG activity of the 12 muscles during locomotion (without the stimulation) were rectified and normalized using the MVC value of each muscle. An average locomotor EMG sweep for each muscle throughout the gait cycle was calculated from many steps (more than 200 steps). The entire gait cycle for each muscle was divided into 12 equal bins, and the EMG activity in the same bin was averaged. The group average EMG activity in each bin was compared between the two locomotion sessions by a paired t-test ($\alpha = 0.004$ with previous Sidak correction). For analyses of total EMG activity in a gait cycle, area under the EMG activity curve was calculated from the binned EMG sweep for each muscle and for each subject, and compared between the two locomotion sessions using a paired t-test ($\alpha = 0.05$).

For kinematic analysis, angle displacements in several phases of a gait cycle for each joint were calculated. Sample sweeps of ankle, knee, and hip joint angle in the function of the gait cycle (normalized as 100%) are presented in Figure 4.1. Vertical bars on the sweeps indicate beginning and end of phases in which each joint displacement was calculated. For the ankle joint, the displacements were calculated between first maximum dorsiflexion (DF1) and first maximum plantarflexion (PF1), PF1 and second maximum DF (DF2), DF2 and second maximum PF (PF2), and PF2 and third maximum DF (DF3). For the knee joint, the displacements were calculated between first maximum extension (ext1) and first maximum flexion (flex1), flex1 and second maximum extension (ext2), ext2 and second maximum flexion (flex2), and flex2 and third maximum extension (ext3). For the hip joint, the displacement was calculated between first maximum extension (ext1) and first maximum flexion (flex1). Then, the group average of displacements in the same phase were compared between pre- and post-conditioning using the paired t-test [$\alpha = 0.05$ for the hip, and $\alpha = 0.013$ for 4 potential comparisons (i.e., ankle and knee) with Sidak correction]. Gait cycle time (from heel contact of one side to the next heel contact of the same side) and step time (from heel contact of one side to heel contact of the other side, expressed in %gait cycle) were also calculated and compared between pre- and post-conditioning using the paired t-test ($\alpha = 0.05$).

RESULTS

The soleus H-reflex operant down-conditioning was successful in seven subjects (identified individually by unpaired t-tests between average soleus H-reflex size of the six baseline sessions and average conditioned H-reflex size of the last six conditioning sessions). All data for locomotion analyses are from those successfully conditioned subjects.

Changes of the soleus, MG, and LG H-reflexes after soleus H-reflex down-conditioning

At the last conditioning session, the soleus showed significant decreases both in the conditioned [76.1 ± 3.9 % mean ± standard error (SE), $p<0.01$, paired t-test] and control (88.8 ± 4.3 %, $p<0.05$, paired t-test) H-reflexes when compared to baseline. Furthermore, change of the reflex size within a session (i.e., conditioned H-reflex size minus control reflex size) decreased significantly (-12.7 ± 2.9 %, $p<0.01$, paired t-test). For the MG, the conditioned H-reflex (85.5 ± 5.1 %, $p<0.05$, paired t-test) and within-session change (-13.4 ± 5.1 %, $p<0.05$, paired t-test) decreased significantly while the control reflex did not change

(98.9 ± 6.8%). For the LG, none of those three categories (93.1 ± 12 %, 99.1 ± 11.7 %, and -6.0 ± 4.2 %) showed significant changes.

Locomotor H-reflex

In order to verify that the quality of EMG recording and strength of conditioning stimulation were consistent, the Mmax (and Hmax) size and M-wave size were compared between two locomotion sessions. The Mmax size was not significantly different between the two sessions in all three muscles (p=0.24, 0.46, and 0.32, for soleus, MG, and LG, respectively, paired t-test). Also, the Hmax size did not differ between the two locomotion sessions in all three muscles (p=0.72, 0.92, and 0.56, for soleus, MG, and LG, respectively, paired t-test). The size of the M-wave during the locomotor H-reflex measurement was maintained the same for each subject between the two locomotion sessions (p=0.65, 0.54, and 0.65, for soleus, MG, and LG, respectively, paired t-test).

In Figure 4.2, the average H-reflexes of the soleus, MG, and LG (%Mmax) in each bin were plotted (mean ± SE). The stimulation level was set at just above the M-wave threshold, and thus, all H-reflexes were elicited in the ascending limb of the recruitment curve. The size of the H-reflex in the MG or LG during walking was smaller than that of the soleus, however, the H-reflexes of the three muscles showed a very similar pattern: increased gradually towards the late stance phase, dropped at the beginning of the swing phase, and stayed low throughout the swing phase. The modulation indices of the three muscles were high (>0.97) and did not change significantly between the two locomotion sessions (p=0.50, 0.61, and 0.07, for soleus, MG, and LG, respectively, paired t-test). When comparing the H-reflex in each bin between pre- and post-conditioning, no significant differences were found for any of the 12 bins for the three muscles (paired t-test with Sidak correction). Thus, the size of the locomotor H-reflexes as well as phase-dependent H-reflex modulation in the soleus, MG, and LG did not change after the operant conditioning.

Locomotor EMG

The EMG activity was recorded in the 6 leg muscles bilaterally (i.e., 12 in total) during walking at the subject's comfortable speed (typically at 0.9 m/s). All data were normalized using the MVC value for each muscle at each session and shown in Figure 4.3 (mean ± SE). The MVC values were not statistically different between the two sessions in all 12 muscles (paired t-test). The EMG patterns were very similar between pre- and post-conditioning sessions. There were no significant differences in EMG activity between pre- and post-conditioning for any of the bins for the 12 muscles investigated (paired t-test with Sidak correction). The area under the binned EMG activity curve, where the muscle was activated, was calculated from bins 1-7 for the soleus, MG, and LG, from bins 8-12 and 1-2 for the TA, and from bins 11-12 and 1-3 for the VL and BF. Comparison of those areas between the pre- and post-conditioning locomotion sessions for each muscle did not show significant differences in any of the 12 muscles (paired t-test).

Joint displacement and gait parameters

Joint angle displacements in each phase (indicated by vertical bars on the sweeps in Figure 4.1) were calculated (mean ± SE) and compared between two locomotion sessions. For the ankle joint, the displacements were calculated in four phases, and there were no significant differences between pre- and post-conditioning in any of the phases (paired t-test with Sidak correction, Figure 4.4A). For the knee joint, the gait cycle was divided into four phases in which the angle displacements were calculated. No significant differences were found between the two sessions in any of the phases (paired t-test with Sidak correction, Figure 4.4B). For the hip joint, the displacements were compared between pre- and post-conditioning in one phase, which was not significantly different (paired t-test, Figure 4.4C).

The gait cycle time at pre-conditioning (1.17 ± 0.04 s, mean ± SE) was not significantly different from that at post-conditioning (1.19 ± 0.04, p=0.22, paired t-test). That also indicates that a stride length did not differ between the two sessions because the walking speed was the same. The step times for both side (i.e., from conditioned side to contralateral side, and from contralateral side to conditioned side) were calculated and expressed as a percentage of the gait cycle. For both pre- and post-conditioning, the step time for one side occupied 50% of the gait cycle, which indicated that step symmetry was maintained between the two sessions.

DISCUSSION

The present study investigated effects of the soleus H-reflex down-conditioning on locomotion in healthy human subjects. The final conditioned H-reflex size in the soleus was 76 % of the baseline value in the present group of subjects. In these subjects, changes in the MG and LG H-reflexes were also measured after operant conditioning; the H-reflex size in the last conditioning session was 86% of the baseline in the MG and 93% of the baseline in the LG. However, none of these changes were reflected in the locomotor H-reflex or EMG activity.

In the present group of subjects, the final control H-reflex was also decreased to 89 % of the baseline in the soleus after successful down-conditioning. Because the control reflex probably represents the long-term plasticity in the reflex pathway, we expected the locomotor H-reflex to also be changed after conditioning. However, the conditioning-induced smaller H-reflex during standing did not transfer to a smaller H-reflex during walking. For individual data, there were no associations between the final soleus control H-reflex size and soleus locomotor H-reflexes during the stance phase (early stance phase: average in bin 1-2, middle stance: average in bin 3-4, and late stance phase: average in bin 5-6) at post-conditioning (p=0.15, 0.29, and 0.88, for soleus control reflex vs. locomotor H-reflex in early, middle, and late stance phase, respectively, Figure 4.5). A possible explanation for this might be found in task-dependent modulation of the H-reflex (i.e., decrease of H-reflex size from standing to walking, Capaday and Stein, 1986; Stein and Capaday, 1988). This rapid modulation in reflex size between the tasks is strongly influenced by the activity of the corticospinal tract (Hodapp et al., 2007) and occurs through the mechanisms of presynaptic inhibition (Stein and Capaday, 1988; Stein, 1995). Probably, in the process of reflex gain modulation between standing and walking, the decreased H-reflex that occurred in standing was adjusted for walking so that the appropriate reflex gain for walking could be maintained after conditioning. Since the Ia afferent pathways have important roles in locomotion (Yang et al., 1991; Bennett et al., 1996; Stein et al., 2000), preserving the reflex activity during locomotion would be functionally the most relevant. Locomotor reflex activity was preserved, as well as locomotor EMG activity and kinematics (see Figure 4.2, 4.3, and 4.4). This is different from animal studies.

In rats, Chen et al. (2005) showed that, after successful conditioning, the locomotor H-reflex and EMG activity were changed in the direction of conditioning (i.e., increase or decrease) to a comparable extent to the conditioned H-reflex change, while the duration and symmetry of the step cycle were unchanged. In their study, the average final conditioned H-reflex size was about 50% of the baseline value, different from 73 % in the present subject group. Thus, one might suspect that the difference in the conditioning effect on locomotion could be due to the difference in the amount of conditioned H-reflex decrease between rats and humans. However, in the present study, even the subject who showed the smallest conditioned H-reflex after conditioning (53% of the baseline, similar to the rats in Chen et al., 2005) showed no change in the locomotor H-reflex and EMG activity (Figure 4.6). Thus, it is unlikely that a lack of change in locomotion in the present subject group is due to the limited decrease in the final conditioned H-reflex, compared to rats.

In response to the conditioning-induced changes in the H-reflex pathway, the rats maintained their normal locomotion pattern by compensatory changes in the activity of other leg muscles (Chen et al., 2009a; Chen et al., 2009b), whereas the human subjects maintained their locomotion by preserving the activity of primary afferents (i.e., by not transferring the changed reflex activity from standing to walking). In humans, this is probably the most effective way to maintain normal locomotion, while accommodating a new skill of a smaller H-reflex (i.e., operant down-conditioning effects) in standing. Unlike laboratory animals that were continuously exposed to operant conditioning [e.g., 2500-8000 trials per day (distributed throughout the 24 hours), for 50 days], humans were only exposed to a discrete conditioning schedule (i.e., 1 hour per session, three times a week, 30 conditioning sessions). Thus, for humans, the behavioral repertoire outside the conditioning paradigm is much larger than that for rats, and therefore, preserving the existing wide variety of skills (e.g., normal walking) with the minimum adjustments at the acquisition of a new motor skill might be essential. Finally, it should also be mentioned that many factors such as anatomical differences and locomotion pattern (i.e., quadruped vs. biped) (Courtine et al., 2007) could contribute to different effects of conditioning on locomotion between rats and humans. In particular, biped locomotion in humans is much more demanding in maintaining the balanced upright posture than quadruped locomotion in rats. Thus, minimizing any changes during normal locomotion, rather than compensating for a change (e.g., smaller soleus locomotor H-reflex) by inducing additional changes, may be more critical for humans than for rats.

The present study showed that the successful down-conditioning of the soleus H-reflex does not change locomotion in healthy human subjects. Does this imply that there is no therapeutic effect of conditioning? In rats with incomplete spinal cord injury, Chen et al. (2006) found that asymmetric locomotion was improved after successful conditioning. In humans, preliminary results suggest that operant down-conditioning of the H-reflex improves spastic gait in people with incomplete spinal cord injury (Pomerantz et al., 2010). Thus, effects of conditioning on locomotion might be different in people after injury or disease, in which locomotion is already abnormal.

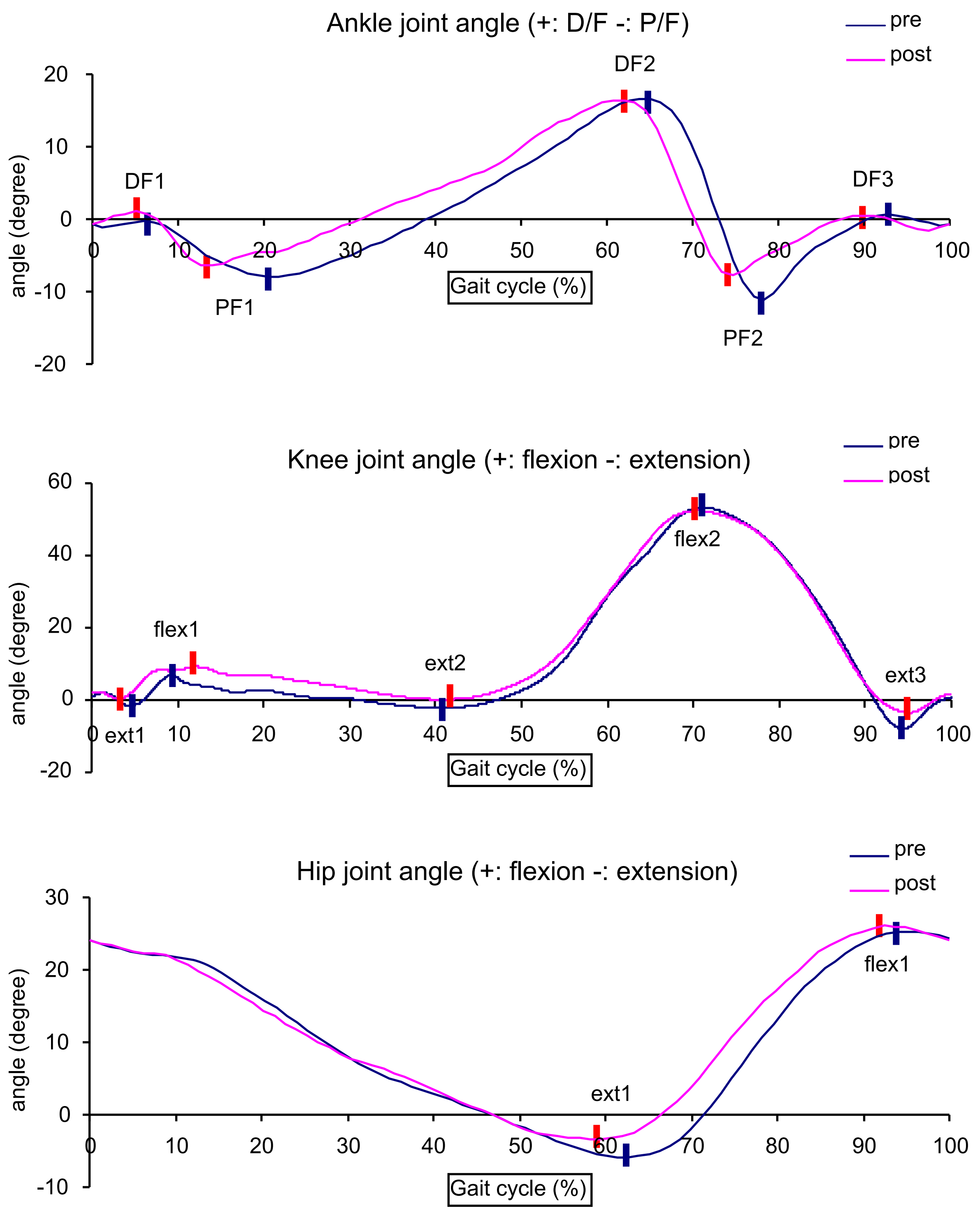

Figure 4.1: Sample sweeps of joint angles
Sample sweeps of joint angles throughout the gait cycle. Angle displacement in each phase (DF1-PF1, PF1-DF2, DF2-PF2, and PF2-DF3 for the ankle, ext1-flex1, flex1-ext2, ext2-

flex2, and flex2-ext3 for the knee, and ext1-flex1 for the hip) was calculated. DF: dorsiflexion, PF: plantarflexion, ext: extension, flex: flexion, pre: pre-conditioning, post: post-conditioning

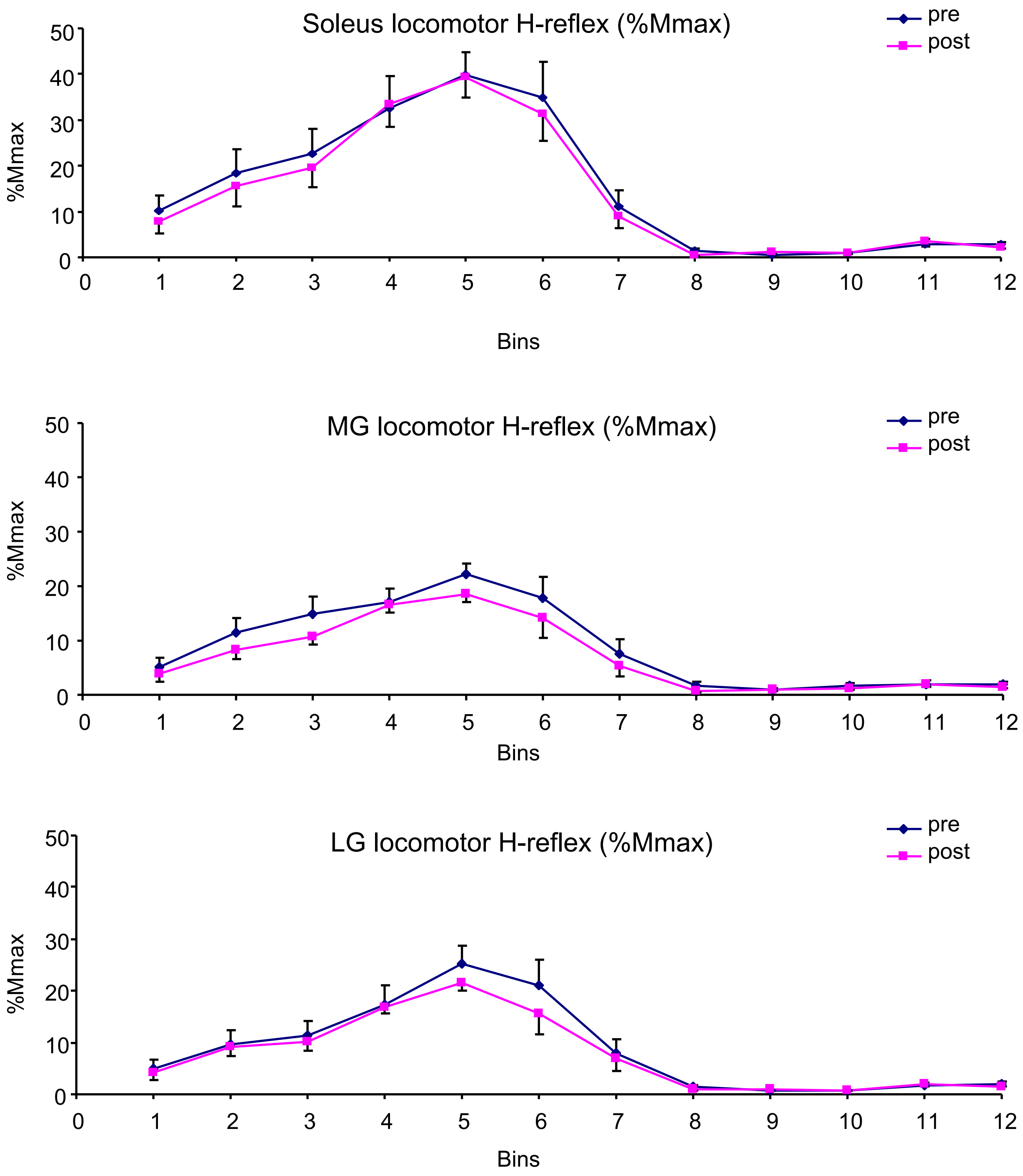

Figure 4.2: Locomotor H-reflexes
Locomotor H-reflexes of the soleus, MG, and LG averaged from all subjects. Data are expressed in a percentage of the Mmax size (mean ± SE). The gait cycle was divided into 12 equal bins and average reflex size in a bin was calculated. There were no significant differences between pre- and post-conditioning in all 12 bins in all three muscles.

Figure 4.3: Locomotor EMG

Locomotor EMG of the 12 muscles averaged from all subjects (left column: conditioned side, right column: contralateral side). Data are normalized by MVC values (mean ± SE). As in the locomotor H-reflexes, the locomotor EMG was also divided into 12 bins and compared between pre- and post-conditioning. There were no significant differences between pre- and post-conditioning in all 12 bins in all muscles. In addition, the area under the binned EMG activity curve, where the muscle was activated, was calculated for each muscle. Those areas between the pre- and post-conditioning locomotion sessions were not significantly different in all 12 muscles.

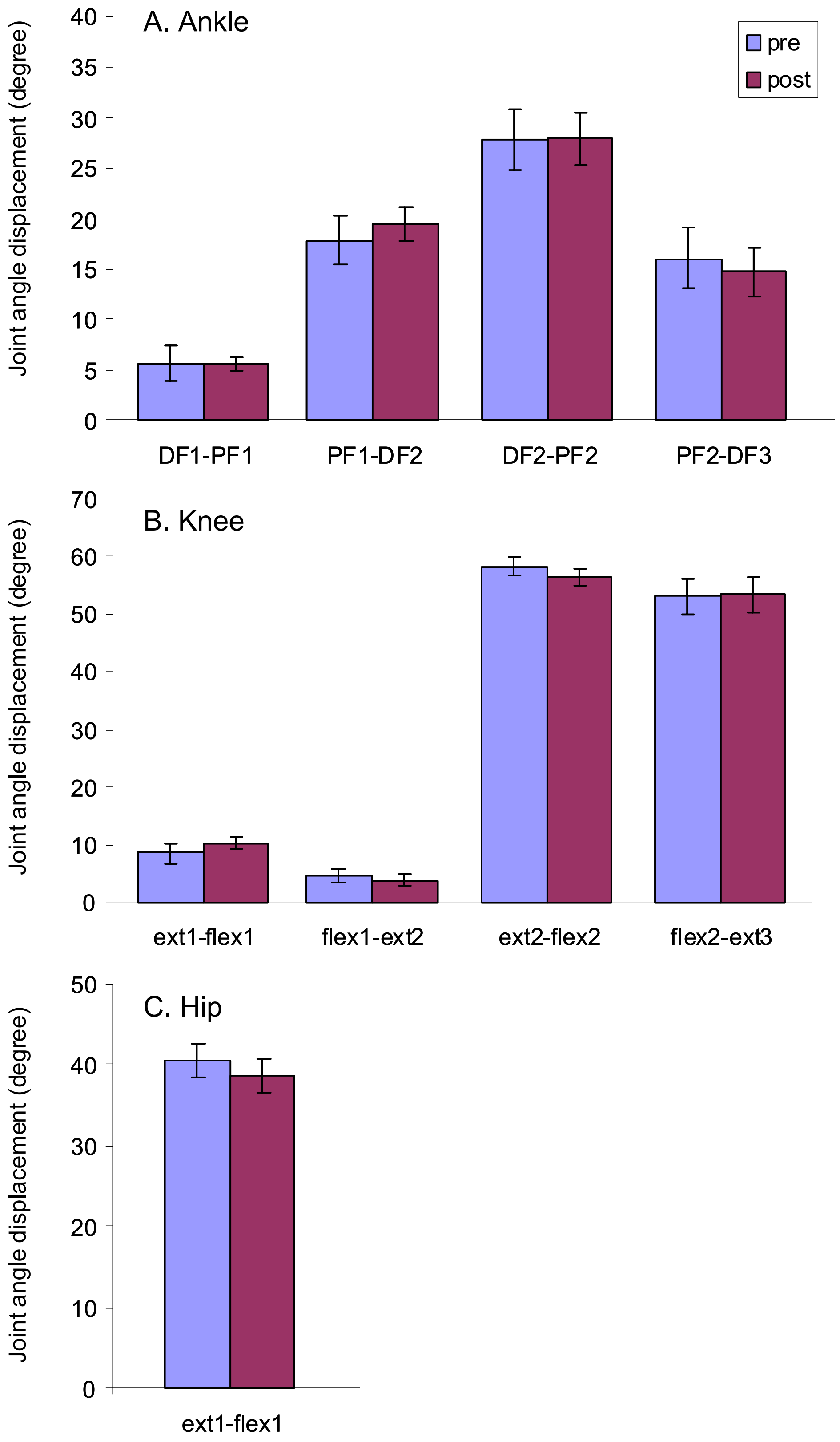

Figure 4.4: Joint displacement of the ankle, knee, and hip

Group average of joint angle displacements in the ankle (A), knee (B) and hip (C) in each phase at pre-conditioning and post-conditioning locomotion sessions (mean ± SE). There were no significant differences between pre- and post-conditioning in all phases in all three joints. DF: dorsiflexion, PF: plantarflexion, ext: extension, flex: flexion

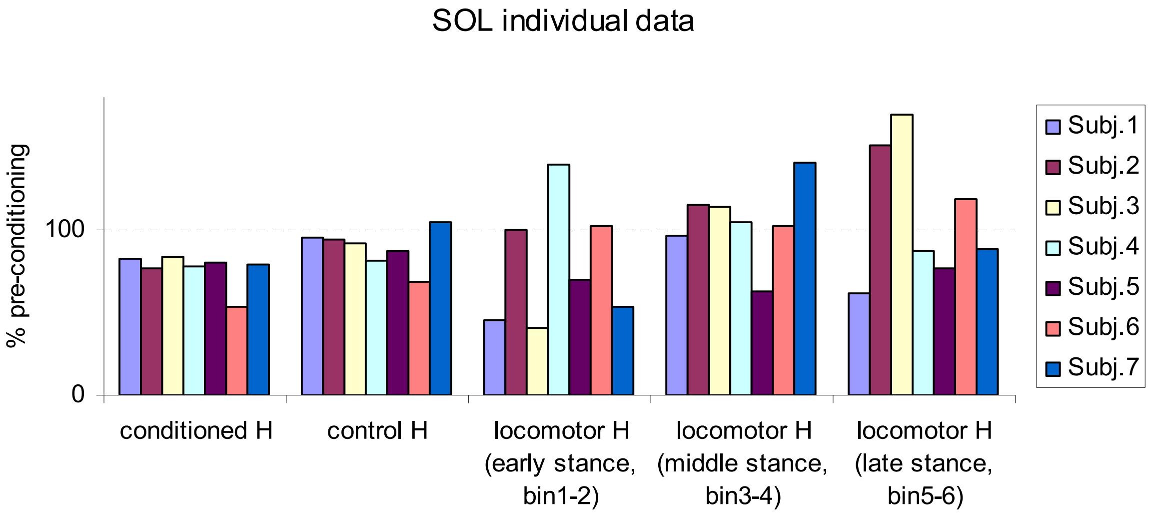

Figure 4.5: Individual conditioned, control, and locomotor H-reflexes
Individual differences in the soleus conditioned and control H-reflexes after operant conditioning, and soleus locomotor H-reflex during stance phase (early stance phase: average in bin 1-2, middle stance phase: average in bin 3-4, late stance phase: average in bin 5-6). Each value was expressed in percentages of pre-conditioning values. There was no apparent association between decreases of the conditioned or control H-reflex and changes of locomotor H-reflexes.

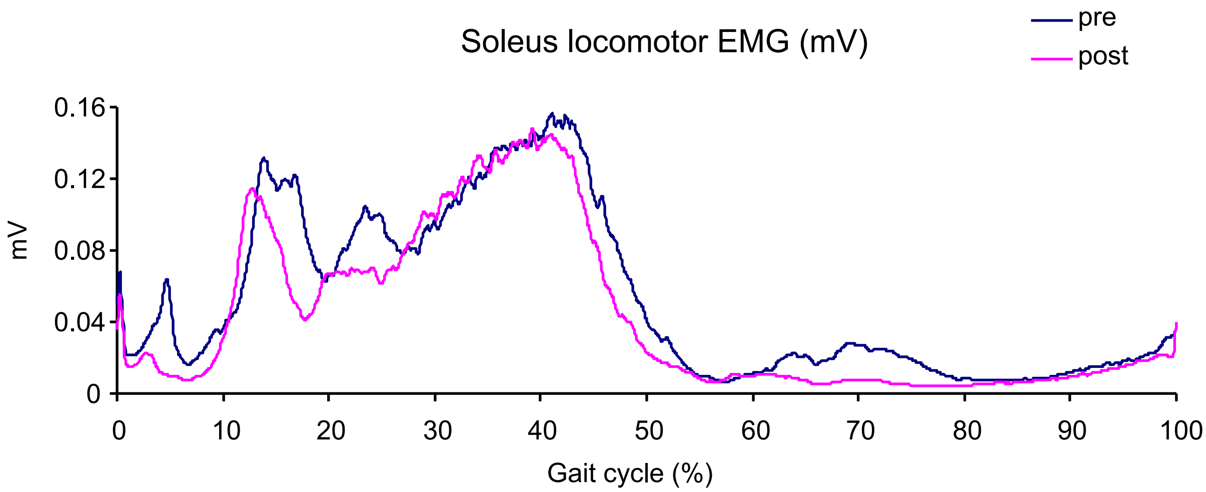

Figure 4.6: Sample soleus locomotor EMG sweeps
Sample EMG sweeps at pre- and post-conditioning from the subject who showed the largest decrease of the conditioned soleus H-reflex.

References

Bennett DJ, De Serres SJ, Stein RB (1996) Gain of the triceps surae stretch reflex in decerebrate and spinal cats during postural and locomotor activities. J Physiol 496 (Pt 3):837-850.

Capaday C, Stein RB (1986) Amplitude modulation of the soleus H-reflex in the human during walking and standing. J Neurosci 6:1308-1313.

Carp JS, Wolpaw JR (1994) Motoneuron plasticity underlying operantly conditioned decrease in primate H-reflex. J Neurophysiol 72:431-442.

Carp JS, Tennissen AM, Chen XY, Wolpaw JR (2006a) H-reflex operant conditioning in mice. J Neurophysiol 96:1718-1727.

Carp JS, Tennissen AM, Chen XY, Wolpaw JR (2006b) Diurnal H-reflex variation in mice. Exp Brain Res 168:517-528.

Chen L, Chen XY, Chen Y, Liu RL, Wang Y, Wolpaw JR (2009a) Conditioning of soleus H-reflex is accompanied by an opposite change in vastus lateralis H-reflex in freely moving rats. Program No 791 Neuroscience Meeting Planner. Chicago, IL: Society for Neuroscience, 2009. Online.

Chen XY, Wolpaw JR (1994) Circadian rhythm in rat H-reflex. Brain Res 648:167-170.

Chen XY, Wolpaw JR (1995) Operant conditioning of H-reflex in freely moving rats. J Neurophysiol 73:411-415.

Chen XY, Chen L, Wolpaw JR (2001) Time course of H-reflex conditioning in the rat. Neurosci Lett 302:85-88.

Chen Y, Chen XY, Jakeman LB, Schalk G, Stokes BT, Wolpaw JR (2005) The interaction of a new motor skill and an old one: H-reflex conditioning and locomotion in rats. J Neurosci 25:6898-6906.

Chen Y, Chen XY, Jakeman LB, Chen L, Stokes BT, Wolpaw JR (2006) Operant conditioning of H-reflex can correct a locomotor abnormality after spinal cord injury in rats. J Neurosci 26:12537-12543.

Chen Y, Chen L, Liu RL, Wang Y, Wolpaw JR, Chen XY (2009b) Soleus H-reflex conditioning may change locomotor EMG and kinematics. Program No 792 Neuroscience Meeting Planner. Chicago, IL: Society for Neuroscience, 2009. Online.

Courtine G, Bunge MB, Fawcett JW, Grossman RG, Kaas JH, Lemon R, Maier I, Martin J, Nudo RJ, Ramon-Cueto A, Rouiller EM, Schnell L, Wannier T, Schwab ME, Edgerton VR (2007) Can experiments in nonhuman primates expedite the translation of treatments for spinal cord injury in humans? Nat Med 13:561-566.

Duysens J, Tax AA, van der Doelen B, Trippel M, Dietz V (1991) Selective activation of human soleus or gastrocnemius in reflex responses during walking and running. Exp Brain Res 87:193-204.

Edamura M, Yang JF, Stein RB (1991) Factors that determine the magnitude and time course of human H-reflexes in locomotion. J Neurosci 11:420-427.

Hodapp M, Klisch C, Mall V, Vry J, Berger W, Faist M (2007) Modulation of soleus H-reflexes during gait in children with cerebral palsy. J Neurophysiol 98:3263-3268.

Kido A, Tanaka N, Stein RB (2004a) Spinal reciprocal inhibition in human locomotion. J Appl Physiol 96:1969-1977.

Kido A, Tanaka N, Stein RB (2004b) Spinal excitation and inhibition decrease as humans age. Can J Physiol Pharmacol 82:238-248.

Lagerquist O, Zehr EP, Baldwin ER, Klakowicz PM, Collins DF (2006) Diurnal changes in the amplitude of the Hoffmann reflex in the human soleus but not in the flexor carpi radialis muscle. Exp Brain Res 170:1-6.

Llewellyn M, Yang JF, Prochazka A (1990) Human H-reflexes are smaller in difficult beam walking than in normal treadmill walking. Exp Brain Res 83:22-28.

Pomerantz F, Wolpaw JR, Lichtman SW, Abel BM, DeFrancesco E, Thompson AK (2010) Operant down conditioning of the soleus H-reflex in people with spasticity due to chronic incomplete spinal cord injury. Program No 8214 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2010. Online.

Schmidt RA, Lee TD (2005) Motor control and learning: A behavioral emphasis. Fourth edition. Human Kinetics, Champaign.

Stein RB (1995) Presynaptic inhibition in humans. Prog Neurobiol 47:533-544.

Stein RB, Capaday C (1988) The modulation of human reflexes during functional motor tasks. Trends Neurosci 11:328-332.

Stein RB, Misiaszek JE, Pearson KG (2000) Functional role of muscle reflexes for force generation in the decerebrate walking cat. J Physiol 525 Pt 3:781-791.

Thompson AK, Chen XY, Wolpaw JR (2009) Acquisition of a simple motor skill: task-dependent adaptation plus long-term change in the human soleus H-reflex. J Neurosci 29:5784-5792.

Wolpaw JR (1987) Operant conditioning of primate spinal reflexes: the H-reflex. J Neurophysiol 57:443-459.

Wolpaw JR (1997) The complex structure of a simple memory. Trends Neurosci 20:588-594.

Wolpaw JR (2010) What can the spinal cord teach us about learning and memory? Neuroscientist 16:532-549.

Wolpaw JR, Seegal RF (1982) Diurnal rhythm in the spinal stretch reflex. Brain Res 244:365-369.

Wolpaw JR, O'Keefe JA (1984) Adaptive plasticity in the primate spinal stretch reflex: evidence for a two-phase process. J Neurosci 4:2718-2724.

Wolpaw JR, Maniccia DM, Elia T (1994) Operant conditioning of primate H-reflex: phases of development. Neurosci Lett 170:203-207.

Yang JF, Stein RB (1990) Phase-dependent reflex reversal in human leg muscles during walking. J Neurophysiol 63:1109-1117.

Yang JF, Stein RB, James KB (1991) Contribution of peripheral afferents to the activation of the soleus muscle during walking in humans. Exp Brain Res 87:679-687.

Zehr EP, Kido A (2001) Neural control of rhythmic, cyclical human arm movement: task dependency, nerve specificity and phase modulation of cutaneous reflexes. J Physiol 537:1033-1045.

CHAPTER V

SYNTHESIS

1. Summary of findings

In the first project of this dissertation, the H-reflexes of the MG and LG were examined with regard to acute modulation during walking and between standing and walking (Chapter 2), and gradual modification induced by operant down-conditioning of the soleus H-reflex (Chapter 3). The modulation and modification of the MG and LG H-reflexes were compared with those in the soleus H-reflex to investigate the functional association of the H-reflexes among the three muscles. In the second project, effects of successful soleus H-reflex operant conditioning on locomotion were examined in healthy humans (Chapter 4). Major findings of the three chapters are summarized in the following sections.

Synergistic modulation of the H-reflexes

In Chapter 2, the H-reflexes of the soleus, MG, and LG showed task-dependency and phase-dependency in size modulation, and the modulation patterns during walking (i.e., phase-dependent modulation) and between standing and walking (i.e., task-dependent modulation) were very similar among the three muscles (Table 5.1). Those findings support the hypothesis that the three muscles function as one synergistic unit to facilitate ongoing movements. The functionality of the soleus H-reflex modulation was suggested to support successful walking (Capaday and Stein, 1986, 1987; Edamura et al., 1991), and thus, walking would be further secured by synergistic modulation of the H-reflexes in the three synergist muscles.

The task-dependent modulation of the soleus H-reflex from standing to walking was shown to be acquired with maturation of the CNS (Hodapp et al., 2007). Thus, rapid modulation based on the ongoing task is a learned motor skill through neural plasticity during development. Based on the finding that the MG and LG H-reflexes are modulated similarly to the soleus H-reflex, it appears that neural plasticity during development occurred concurrently in the MG and LG H-reflex pathways to establish the synergistic modulation of the three muscles.

It should be mentioned that the synergistic modulation of the soleus, MG, and LG H-reflexes is not necessarily present in other movements such as running. Nichols (1989) demonstrated that heterogenic inputs from the soleus stretch reflex increase the MG and LG stretch reflexes whereas heterogenic inputs from the MG and LG stretch reflexes decrease the soleus stretch reflex when the muscles are highly activated. Inhibition of the soleus stretch reflex by the MG and LG heterogenic inputs increased as the MG and LG force increased. In this case, absence of mutual synergism among the three muscles, with suppression of the monoarticular muscle (i.e., soleus) and facilitation of the biarticular muscles (i.e., MG and LG), is suggested to promote interjoint coordination between the ankle and knee joints. Thus, during a motor task that requires increased contribution of the gastrocnemii compared to walking, such as fast running (Duysens et al., 1991), the H-reflex modulations among the three muscles would show different associations from those during walking.

Compensatory modification of the H-reflexes

Findings from Chapter 3 supported the hypothesis that operant conditioning has less effect on synergists than on the target muscle. In addition, rapid within-session task-dependent adaptation (i.e., differences between conditioned and control H-reflexes) and gradual across-session long-term change (i.e., changes in control H-reflexes) were shown to be differentially induced among the three muscles by operant conditioning. Task-dependent adaptation, likely originated in plasticity of the brain, occurred in the soleus and MG H-reflexes to a similar extent throughout the conditioning period. However, long-term changes, which would reflect spinal cord plasticity, occurred only in the soleus H-reflex, not in both the MG and LG H-reflexes at the end of conditioning (Table 5.1). It has previously been shown during human soleus H-reflex operant conditioning that, when task-dependent adaptation (i.e., supraspinal plasticity) is repeatedly induced, long-term changes (i.e., spinal cord plasticity) are hierarchically induced (Thompson et al., 2009). Thus, the finding of the MG H-reflex showing task-dependent adaptation but no long-term change suggests that there may be compensatory plasticity in the MG H-reflex pathway to prevent long-term change of the MG H-reflex. Since long-term changes in the soleus H-reflex persisted outside the conditioning paradigm, this lack of synergism (i.e., compensation) may reflect CNS's strategy to preserve other motor skills in which the soleus H-reflex pathways are involved. Therefore, compensatory plasticity may be necessary to preserve the current repertoire of motor skills. For the LG H-reflex, the extent of within-session change was less than that in the MG and soleus. However, synergistic association between the soleus and LG control reflexes were reversed just as that in soleus and MG control reflexes, resulting in no change in the LG control reflex at the end of conditioning. Thus, there may be compensatory plasticity occurred in the LG H-reflex pathway. Nevertheless, neural mechanisms underlying the differential control of the LG H-reflex from the soleus and MG H-reflexes with regard to task-dependent adaptation need to be explored. Finally, differences in H-reflex changes appeared among the three muscles during the conditioning would indicate potential evidence of some independence among those muscles.

The H-reflexes of the soleus and MG showed rapid within-session decreases. This acute adaptation of synergists in the same direction is opposite from findings reported by Akima et al. (2002). They reported that fatigue of the vastus lateralis induced by 30 minutes of electromyostimulation increased the activity of its synergists, vastus medialis and rectus femoris during knee extension exercise. This compensatory reaction of synergists to achieve the intended task was available immediately after the electromyostimulation suggesting that the CNS is capable of sending commands for acute compensation by activity of synergists. One difference of the present study and Akima's study with regard to acute adaptation in synergist muscles would be the task in which the activity of muscles is measured. That is, if changes in activity of one muscle directly affect the performance as the knee extension exercise in Akima's study, synergists are rapidly recruited to provide compensatory activity so that the intended task is still executed properly. On the other hand, during natural standing as in this operant conditioning paradigm, the H-reflex activity is not functionally relevant because background activity is sufficient to maintain upright posture. Thus, synergists could change in the same direction. However, once the decrease of the soleus H-reflex persists outside the conditioning (i.e., control reflex change), compensation by synergists may be necessary to maintain the normal motor skill.

Preservation of normal locomotion

Long-term changes in the soleus H-reflex shown in Chapter 3, possibly reflecting spinal cord plasticity in the soleus H-reflex pathway, persist across sessions. Since Ia afferent pathways are also functionally involved in locomotion, the soleus H-reflex during locomotion (i.e., locomotor H-reflex) was hypothesized to change to a similar extent as changes in the control reflex after conditioning. However, contrary to the hypothesis, operant conditioning did not change the soleus locomotor H-reflex. Adjustment of reflex gain in the process of task-dependent modulation between standing and walking is suggested to be responsible for maintaining the appropriate reflex gain during locomotion despite persistent decrease of the H-reflex in standing. As the primary change (i.e., soleus control reflex change) did not transfer to locomotion, no changes occurred in locomotor EMG activity of other muscles or in joint kinematics (Table 5.1). Adjusting the reflex gain during walking is probably the most effective way to maintain normal locomotion, while accommodating operant down-conditioning effects in standing (i.e., a smaller control H-reflex).

As proposed in Chapter 1 (General Introduction), sensorimotor integration of the CNS plays an important role in motor control. The present dissertation could promote our understanding for principles governing human motor control by the CNS, specifically with regard to association among the three calf muscles. In sum, the dissertation demonstrated that the CNS controls the H-reflexes of the three synergistic muscles to be synergistic or compensatory, which are logical directions to facilitate or preserve the motor skills in the current behavioral repertoire. Synergism or compensation is available when there are multiple muscles with a similar function (i.e., synergists), and the CNS appears to apply this advantage in motor control. Furthermore, especially in the existing repertoire of motor skills, normal locomotion is preserved after operant conditioning. This would emphasize importance of normality in human walking and indicate that even a small change (e.g., decrease of the soleus locomotor H-reflex) could cause critical influence on human walking.

Therefore, it would be the CNS's role to eliminate the change by minimum adjustment and to provide solid control to maintain normal locomotion.

2. Strengths and weaknesses

Strengths

In the past, studies investigating modulation and modification in the H-reflex pathway almost exclusively examined the soleus. The soleus and two of its synergists, the MG and LG have been treated the same, and therefore, it would probably be a natural tendency to assume that same or similar modulation and modification take place in those three muscles. However, the present dissertation did demonstrate that those three muscles do not always behave in the same direction to the same extent. Capability of the CNS to control the H-reflexes of the three muscles similarly or differentially based on the context is applied in motor control, which would facilitate or maintain the existing motor skills. Synergistic or compensatory association of the H-reflexes in synergist muscles is new knowledge and would establish a more solid basis of our understanding regarding how the CNS concurrently controls synergist muscles.

In addition, this dissertation involved measurements during functional tasks such as walking. Walking is one of the most fundamental motor skills in humans, and also it is destructively affected by injury or disease. By adding pre- and post-conditioning locomotion analyses, this dissertation not only provided new knowledge regarding motor control in synergist muscles but introduced the possibility of further developing the operant conditioning paradigm as a new therapeutic method. Clinical implications are discussed in the next section.

Weaknesses

In Chapters 3 and 4 in which effects of successful soleus H-reflex operant conditioning on synergists' H-reflexes or on locomotion were reported, developing a "successfully" conditioned group was critically important. Considering the latest study of human soleus H-reflex operant conditioning (Thompson et al., 2009), the subject group in this dissertation was appropriate as the sample size and the final reflex size were very similar between the two studies. However, the dissertation involved measurements of the synergists' H-reflexes, for which the effects of conditioning were expected to be more subtle and variable than for the target muscle. Thus, although variability in the effects of conditioning on synergists was an inherent feature, having more subjects would have improved the quality of statistical analyses. This weakness is also applied to measurements of the locomotor variables.

Another weakness would be the number of sessions in which the MG and LG H-reflexes were recorded. Since a brief session length (i.e., about 60 minutes) is a key for successful conditioning in humans (Thompson et al., 2009), we conducted additional measurement of the MG and LG H-reflexes only at every six sessions. Thus, data for final statistical analyses were taken from one session, which might not have represented the true value. In addition, the compensation of MG (and possibly LG) H-reflexes seemed to occur at the last conditioning session. In order to verify that the compensation at session 30 represents a trend, not a random change, adding more sessions would have been ideal. There is an experimental conflict between increasing the amount of MG and LG data and minimizing the session length. An acceptable compromise to both is necessary in the future.

For the locomotion analyses in Chapter 4, the absence of change in the soleus locomotor H-reflex was suggested to be due to an adjustment of reflex gain in the process of task-dependent modulation. In order to strengthen this suggestion, task-dependent modulation of the soleus H-reflex between standing and walking should have been measured in a similar range of background activation before and after the operant conditioning. In that way, whether or not the reflex gain was adjusted by task-dependent modulation (i.e., changes in ratio of walking H-reflex vs. standing H-reflex) after the conditioning could be clearly shown. Furthermore, for Chapter 4, we hypothesized that the soleus locomotor H-reflex would change after successful conditioning based on a previous animal study (Chen et al., 2005). However, that was not the case for humans, which emphasizes that inherent differences between species always need to be considered when applying animal data to humans. In particular for the conditioning paradigm, although the final H-reflex size is generally similar between species, the animal protocol includes much more dosage and volume of conditioning than the human protocol. The most critical difference is the conditioning nature, where humans are conditioned only for an hour, three times per week. Thus, humans spend considerable time doing other behaviors resulting in having a much wider variety of motor skills than animals during the conditioning period.

For locomotion analysis, locomotor H-reflexes, locomotor EMG activity, and joint kinematics were measured in separate walking trials. We chose not to measure all variables in a same trial to reduce the amount of accessories such as electrodes, cables, and infrared markers attached to the subject, in order to have the subject walk as normal as possible. However, this adjustment in protocol made comparison among the locomotor variables less than ideal. Future studies will have to overcome this weakness.

As for the EMG activity data during walking, the maximum voluntary contraction (MVC) values were used for normalization. Although widely used, inaccuracy of the MVC normalization was suggested (De Luca, 1997). Thus, use of a dynamometer to provide secure stabilization during the MVC measurement would be preferable, if the equipment is accessible. If a peripheral nerve innervating the muscle of interest is available over the skin (e.g., the tibial nerve), using the maximum M-wave value for normalization is also desirable. Finally, biomechanical differences between treadmill walking and overground walking have been reported in healthy humans (Lee and Hidler, 2008). Thus, particularly for patient populations in the future, fundamental differences between the two forms of locomotion need to be taken into consideration, and preferably, functional assessment to look into effects of operant conditioning on locomotion should be performed over ground.

3. Future direction

In Chapter 4, successful down-conditioning of the soleus H-reflex in healthy humans was shown to have no effect on locomotion. However, there is a study showing the effects of operant up-conditioning on locomotion using rats with spinal cord injury (SCI) (Chen et al., 2006). Before the conditioning, treadmill locomotion showed clear asymmetry in the onset times of the right and left soleus EMG. After the conditioning, this asymmetry was corrected in the conditioned group whereas it persisted in the control group. Furthermore, in humans, preliminary results suggest that operant down-conditioning of the H-reflex improves walking in people with incomplete spinal cord injury (Pomerantz et al., 2010). Thus, it seems effects of operant conditioning on locomotion may appear differently in patients with neurological damage, whose walking is already abnormal.

Yang et al. (1991) reported that soleus H-reflex modulation during walking is significantly less in patients with spastic paretic resulting from neurological damage such as spinal cord injury or traumatic brain injury. The most common pattern was 1) exaggerated H-reflex during early stance phase, where the reflex activity would counteract forward movement of the body, and 2) less depression of the H-reflex during the swing phase than normally occurs (Chapter 2). In this case, decrease of the soleus H-reflex could be beneficial for regaining normal reflex modulation. Therefore, unlike healthy humans (i.e., Chapter 4), changes in the H-reflexes induced by operant down-conditioning would more likely be transferred to the locomotor H-reflexes because those are functionally relevant changes. Indeed, preliminary data showing improvement of reflex modulation after soleus down-conditioning were reported (Thompson et al., 2010). Furthermore, improvement of the H-reflex modulation appeared to be associated with functional improvement (Thompson et al., 2010). Thus, operant down-conditioning may have the possibility of restoring or improving locomotor function in patients with spasticity who have exaggerated H-reflexes with impaired modulation during walking. Detailed measurement and analysis of soleus H-reflex modulation during walking for each patient will be necessary to decide eligibility for operant conditioning. If, in the future, all system (i.e., software) and equipment for operant conditioning of the soleus H-reflex can be converted into a small portable unit, it might be possible to perform the conditioning as a home-based therapy. The largest hurdles will be a development of user friendly software and hardware as well as education of patients. Particularly, this kind of unit must come with a feedback screen that can be effectively used by a patient to maintain background EMG activity. Most importantly, the software and hardware must include an automated way for M-wave size to be controlled throughout baseline and conditioning. Furthermore, performing operant conditioning during locomotion (i.e., treadmill walking) would likely maximize transfer of the conditioning effects to locomotion (Barnett et al., 1973), which would facilitate functional benefit of operant conditioning in patients.

Another common locomotor abnormality in spastic patients with incomplete spinal cord injury was found in locomotor EMG activity pattern of the soleus. Unlike the normal soleus EMG activity during the stance phase of walking, which shows a burst-like curve with the peak activation in the late stance phase, the soleus in patients is activated throughout the stance phase without apparent spikes (Leroux et al., 1999; Pepin et al., 2003). It is reported that the normal burst-like activation pattern of the ankle plantarflexors is the most energetically efficient method of powering walking (Kuo, 2002). Thus, increasing the soleus activity during the late stance phase to restore the burst would be beneficial to improve walking in patients. Chen et al. (2006) showed that, in rats with incomplete spinal cord injury, operant up-conditioning of the soleus H-reflex enhanced the soleus EMG activity during locomotion. Hence, one might expect that up-conditioning would have potential for increasing the peak activity of the soleus in the late stance phase. However, Sinkjaer et al. (2000) reported that, in human soleus stance EMG activity, contribution of the Ia excitation, which is targeted in the H-reflex conditioning, is confined to the early stance, and the peak activation in the late stance phase is mainly contributed by group Ib afferents. Therefore, based on the current knowledge, it seems down-conditioning of the soleus H-reflex is likely the appropriate direction for patients with spasticity and exaggerated reflexes to improve their locomotion function. On the other hand, for patients who have problem in propulsion during walking due to lack of the burst-like activation of the soleus, operant conditioning of

Ib afferents might be beneficial. Along the same line, if, in the future, we could expand the functional implications of the operant conditioning paradigm to other spinal reflexes and other muscles, it would be possible to customize conditioning protocols based on each patient's specific problem.

Table 5.1: Summary of the major findings in three chapters
“×” indicates a variable in each column was measured in each corresponding muscle/joint. “no” indicates a variable in each column was not measured in each corresponding muscle/joint. MG: medial gastrocnemius, LG: lateral gastrocnemius, TA: tibialis anterior, VL: vastus lateralis, BF: biceps femoris, TDA: task-dependent adaptation, LTC: long-term change, N/A: not available

Chapter 2

	Task-dependent modulation	Phase-dependent modulation
Soleus	×	×
MG	×	×
LG	×	×

Synergistic modulation
- facilitate the skill

Chapter 3

	Conditioned H-reflex	TDA	LTC
Soleus	×	×	×
MG	×	×	no
LG	no	no	no

Compensatory modification
- preserve the skill

Chapter 4

	Changes in locomotor H-reflex	Changes in locomotor EMG
Soleus	no	no
MG	no	no
LG	no	no
TA	N/A	no
VL	N/A	no
BF	N/A	no
Contralateral muscles	N/A	no

	Changes in joint kinematics
Ankle/knee/hip	no

Maintain normal walking

References

Akima H, Foley JM, Prior BM, Dudley GA, Meyer RA (2002) Vastus lateralis fatigue alters recruitment of musculus quadriceps femoris in humans. J Appl Physiol 92:679-684.

Barnett ML, Ross D, Schmidt RA, Todd B (1973) Motor skills learning and the specificity of training principle. Res Q 44:440-447.

Capaday C, Stein RB (1986) Amplitude modulation of the soleus H-reflex in the human during walking and standing. J Neurosci 6:1308-1313.

Capaday C, Stein RB (1987) Difference in the amplitude of the human soleus H reflex during walking and running. J Physiol 392:513-522.

Chen Y, Chen XY, Jakeman LB, Schalk G, Stokes BT, Wolpaw JR (2005) The interaction of a new motor skill and an old one: H-reflex conditioning and locomotion in rats. J Neurosci 25:6898-6906.

Chen Y, Chen XY, Jakeman LB, Chen L, Stokes BT, Wolpaw JR (2006) Operant conditioning of H-reflex can correct a locomotor abnormality after spinal cord injury in rats. J Neurosci 26:12537-12543.

De Luca CJ (1997) The use of surface electromyography in biomechanics. J Appl Biomech 13:135-163.

Duysens J, Tax AA, van der Doelen B, Trippel M, Dietz V (1991) Selective activation of human soleus or gastrocnemius in reflex responses during walking and running. Exp Brain Res 87:193-204.

Edamura M, Yang JF, Stein RB (1991) Factors that determine the magnitude and time course of human H-reflexes in locomotion. J Neurosci 11:420-427.

Hodapp M, Klisch C, Mall V, Vry J, Berger W, Faist M (2007) Modulation of soleus H-reflexes during gait in children with cerebral palsy. J Neurophysiol 98:3263-3268.

Kuo AD (2002) Energetics of actively powered locomotion using the simplest walking model. J Biomech Eng 124:113-120.

Lee SJ, Hidler J (2008) Biomechanics of overground vs. treadmill walking in healthy individuals. J Appl Physiol 104:747-755.

Leroux A, Fung J, Barbeau H (1999) Adaptation of the walking pattern to uphill walking in normal and spinal-cord injured subjects. Exp Brain Res 126:359-368.

Nichols TR (1989) The organization of heterogenic reflexes among muscles crossing the ankle joint in the decerebrate cat. J Physiol 410:463-477.

Pepin A, Norman KE, Barbeau H (2003) Treadmill walking in incomplete spinal-cord-injured subjects: 1. Adaptation to changes in speed. Spinal Cord 41:257-270.

Pomerantz F, Wolpaw JR, Lichtman SW, Abel BM, DeFrancesco E, Thompson AK (2010) Operant down conditioning of the soleus H-reflex in people with spasticity due to chronic incomplete spinal cord injury. Program No 8214 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2010. Online.

Sinkjaer T, Andersen JB, Ladouceur M, Christensen LO, Nielsen JB (2000) Major role for sensory feedback in soleus EMG activity in the stance phase of walking in man. J Physiol 523 Pt 3:817-827.

Thompson AK, Chen XY, Wolpaw JR (2009) Acquisition of a simple motor skill: task-dependent adaptation plus long-term change in the human soleus H-reflex. J Neurosci 29:5784-5792.

Thompson AK, Abel BM, Wolpaw JR (2010) Soleus H-reflex modulation during locomotion in people with chronic incomplete spinal cord injury. Program No 8211 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2010. Online.

Yang JF, Fung J, Edamura M, Blunt R, Stein RB, Barbeau H (1991) H-reflex modulation during walking in spastic paretic subjects. Can J Neurol Sci 18:443-452.