Abstract

Modulation of H reflex in Response to Voluntary Contraction of the Homologous Muscle Group in the Contralateral Limb

By

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Several studies reported that exercising one limb produces gains in motor output in the same muscle of the un-exercised, contralateral limb. This phenomenon is called cross education. There are also data to suggest that muscle and brain activation are different when muscles shorten and lengthen and that the amount of cross education may be also different according to the type of muscle contraction. This thesis is an initial effort in the form of a cross sectional study to shed light on the mechanism of cross education. This project examines the hypothesis that spinal excitability varies in the resting limb according to the type and intensity of muscle contraction in the contralateral limb. The purpose of this study was to compare spinal excitability in the right wrist flexors during and after concentric and eccentric contraction of the left wrist flexors at an intensity of 100% and 60% of the maximum. Ten healthy right-handed subjects (5 females, 5 males, mean age 21 ± 3 years) performed left wrist flexion on a dynamometer using

concentric and eccentric contractions at 20º/s over a 40º range of motion. Statistical analysis showed that spinal excitability decreased ~35% in the left wrist flexors during and for almost 25s after the contraction of the right wrist flexors. Against the hypothesis, there was no main effect of contraction type. During left wrist eccentric contractions at 100% of concentric MVC, right FCR H reflex was 28% less depressed than at 60% concentric MVCs ($p=0.02$, , $F_{(1,9)}=8.1$). During eccentric contractions at 100% and 60% of eccentric MVCs, the weaker contraction produced 24% higher depression of contralateral H reflex ($p=0.101$) H reflex of right wrist flexor throughout the trial was different after100% and 60% concentric MVCs ($p=0.022$). In summary, although longitudinal exercise studies suggested that spinal mechanisms may be involved in cross education, the present data show a long-lasting depression of spinal excitability in the contralateral limb that varies by contraction intensity but not by contraction type.

Modulation of H reflex in Response to Voluntary Contraction of the Homologous Muscle Group in the Contralateral Limb

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Introduction

More than a century ago, researchers first documented positive effects of strength training on the strength of contralateral limb (Scripture, Smith, Brown, 1894).This phenomenon was called cross education or cross training. Cross education is also observed following imagined (Yue & Cole, 1992) and electrical stimulation evoked contractions (Hortobágyi, Scott, Lambert, Hamilton, & Tracy, 1999). Eccentric contractions show 2.5 times more eccentric strength gain in the contralateral untrained quadriceps muscles (77%) in young men than contralateral concentric strength gain(30%) after concentric strength training. Contralateral isometric strength gain was two times greater after eccentric training (39%) than concentric training (22%) (Hortobágyi,Lambert, & Hill, 1997). Thus, cross education is dependent on the type of muscle contraction with biased towards eccentric contractions. Eccentric contractions are also reported having greater strength gain in the contracting muscle itself (46% pre/post change) than concentric contractions (13%) (Hortobágyi et al.,1996). One may speculate that underlying neuromuscular mechanisms might be different between eccentric and concentric contractions. Supporting evidence shows that the spinal excitability is changed according to the type of contraction (Romano & Schieppati, 1987). Active lengthening contractions show significant depression of spinal excitability of more than 8% compared to active shortening contraction (Nordlund, Thorstensson, & Cresswell, 2002). Thus, eccentric contractions have different behavior than concentric.

Although many features of cross education are well known, the mechanisms responsible for cross education have not been clearly identified yet. The most plausible mechanism is transmedian signaling at the level of cortex (Francis et al., 2009; Hortobágyi, Taylor, Petersen, Russell, & Gandevia, 2003; Muellbacher, Facchini, Boroojerdi, & Hallett, 2000), subcortex

(Carroll, Herbert, Munn, Lee, & Gandevia, 2006; Gerloff et al., 1998; Hortobágyi et al., 2003; Lee & Carroll, 2007; Meyer, Roricht, Einsiedel, Kruggel, & Weindl, 1995; Muellbacher et al., 2000) and/ or spinal cord (Hortobágyi et al., 2003) to produce the contralateral strength gain. The present cross sectional study was a first step to investigate how contraction of wrist muscles in one arm affects the excitability of one specific spinal reflex in the resting, contralateral arm and then see by inference if any modulation in this circuit could play a role in the chronically observed cross education. Hortobágyi et al (2003) showed that during forceful isometric contraction of left wrist flexors, the motor neurons controlling the right wrist flexor carpi radialis muscle were inhibited and this inhibition was present for about 35 s after the contraction. They observed more inhibition with more forceful contractions. Although the study was not designed to identify the exact mechanism of trans-median neural effects, the authors suggested presynaptic inhibition of type Ia fibers to be responsible for the observed effect. However, Lagerquist et al (2006) showed that there was no significant change in the excitability of alpha motor neuron controlling the contralateral homologous muscle after a 5 week long isometric strength training program but these investigators examined spinal excitability only at rest and not during contraction. Both of these studies used H reflex which is believed to be a measure of spinal excitability (Zehr, 2002). Since both these studies had different experimental design, different training duration and also different muscle groups, we cannot satisfactorily conclude that spinal pathways are not modulated as a result of contraction in the contralateral limb. Therefore, the present study was designed to investigate the modulation of spinal excitability in the resting right wrist flexors during and after eccentric and concentric contraction of the left wrist flexors

Statement of purpose

The purpose of this study was to compare spinal excitability in the right wrist flexors during and after concentric and eccentric contraction of the left wrist flexors at an intensity of 100% and 60% of the maximum.

Hypothesis

This project examines the hypothesis that spinal excitability varies in the resting limb according to the type and intensity of muscle contraction in the contralateral limb.

Delimitations

This study is designed for college aged healthy, young individuals. Elderly might not have the same effects. Subjects with any type of neuromuscular injury or disorders might have different effects. Any disorders characterized by impaired nerve conduction and/or myopathy might show other results.

Limitations

H Reflex is sensitive to contraction level of the muscle, position of the joint, position of body and posture. It might not be possible to maintain exactly same magnitude of H reflex despite every possible factor is controlled. Accuracy of EMG data might also be a limiting factor. Honesty of subjects is always questionable in research studies involving maximum voluntary contractions. Inability of subject to relax the other arm, shoulder and chest muscles along with other body parts is also potential factor which might modify results.

Review of literature

This section discusses and summarizes previous work related to cross education, mechanisms for cross education and data available on ipsilateral and contralateral effects of exercise on spinal excitability in order to better understand the need of this thesis project.

What is Cross Education?

Cross Education is the improvement in motor performance of a muscle group as a result of training of the homologous muscle group in the contralateral limb. It was first reported by Scripture et al in a report published in *Studies from Yale Psychological Lab* in 1894 (Scripture et al, 1894). Since then, many studies reported the beneficial effect of exercise in one limb on the strength gain in contralateral nonexercising or resting limb (Munn, Herbert, & Gandevia, 2004; Munn, Herbert, Hancock, & Gandevia, 2005). The strength in the resting limb increases by almost 7% of its original state and almost 52% of the strength gain in the contralateral limb according to a meta-analysis (Munn et al., 2004). These results are supported by various studies. Resistance training of elbow flexors increased contralateral elbow flexor strength by 7% (Munn et al., 2005). Less strength loss in the immobilized hand is also observed during post operative recovery (Stromberg, 1986) and also among healthy individuals as a result of contralateral strength training.

Effects of cross education are also observed after electrically evoked contraction (Hortobágyi et al., 1999; Maffiuletti et al., 2006; Toca-Herrera, Gallach, Gomis, & Gonzalez, 2008). Not only that, the contralateral strength gain after electrical muscle stimulation is even greater than the voluntary contraction induced contralateral strength gain(Hortobágyi et al., 1999). Cross Education is also observed after mental rehearsal of the task (Yue & Cole, 1992).

Thus, the interesting observations related with cross educations are that it is muscle specific and also specific to the type of muscle action with eccentric exercise being more effective.

In general two mechanisms (Carroll et al., 2006; Lee & Carroll, 2007) can cause cross training effect: First, facilitation of the motor pathways mediating resting limb may be induced by the excitation of the motor pathways controlling the exercising limb. Second, the motor areas and pathways controlling the exercising limb develop some adaptations which can be accessed by the contralateral side when prompted to produce maximal force. Both of these mechanism can happen simultaneously to carry out the cross training effect to the contralateral side. It is clear so far that cross education requires transmedian signaling in CNS. It is possible because both the sides of the motor pathways are connected by callosal pathways, commissural pathways and interneurones at various levels (cortex, subcortex, spinal cord) throughout the CNS. The communication pathways across the midline can further be divided in two groups: Supraspinal and Spinal. There is plenty of evidence that supraspinal pathways (cortex, subcortex) are involved in modulating the contralateral strength gain.

Not only cerebral cortex?

There is an abundance of evidence that motor cortical excitability is changed during ipsilateral voluntary contraction (Francis et al., 2009; Gerloff et al., 1998; T. Hortobágyi et al., 2003; Muellbacher et al., 2000). Francis et al (2009) showed that during active ankle dorsi flexion there was bilateral brain activity (fMRI) mainly in the areas associated with the motor planning, execution and visuomotor co-ordination. Electrical stimulation induced contraction also showed increased bilateral brain activity in the regions of the bilateral SII (somatosensory area) and insula. (Francis et al., 2009). Bilateral activation of M1, sensory motor area and

cerebellum was found during left wrist movement (Sehm, Perez, Xu, Hidler, & Cohen, 2010). Unilateral wrist ulnar deviation task activated part of ipsilateral temporal lobe. It may be associated with the memory storage during the motor learning (Farthing, Borowsky, Chilibeck, Binsted, & Sarty, 2007). Muellebecher et al (2000) used TMS to find out if the excitability of motor cortical neuron changes during the voluntary forceful activation of ipsilateral hand muscle (Abductor pollicis brevis). They found increased right motor cortical excitability which was reflected by facilitated left MEPs at higher (>120% rMT) and stronger (>50% MVC) of the right abductor pollicis brevis. They also found facilitation of FCR F wave during the contralateral homologous muscle contraction. It means that both upper and lower motor neurons controlling APB are facilitated during forceful contraction of the homologous muscle. The authors suggested it was because of communication between two cortices and subcortices. Since the facilitatory effect of contraction in one hand on the cortically evoked motor potential in the other hand was same in the patients with corpus callosal agenesis (Meyer et al., 1995), it is clear that there are pathways other than transcallosal to allow communication between two sides of the central nervous system. There are evidences that these possible sites could be subcortical and spinal cord. Muellebecher et al (2000) suggests involvement of a subcortical network which might have connections with both the primary motor cortices separately. The facilitated F wave in the same experiment also indicated involvement of spinal pathways. Ironically, Gerloff et al (1998) showed no significant effect of conditioning cortical magnetic stimulation of M1 area on the H reflex evoked in the ipsilateral flexor carpi radialis muscle at rest. This effect was similar to what Ferbert et al (1992) had demonstrated earlier. Ferbert et al (1992) also recorded FCR H reflex during the preactivation of the target muscle after conditioning with a cortical magnetic stimulation. Peak to peak magnitude of H reflex was significantly suppressed when the H reflex

was recorded 30 ms after the TMS. The size of conditioned h reflex was 68±17 as a percentage of unconditioned control value. H reflex is used as a measurement tool for the excitability of alpha motor neuron given the other factors affecting presynaptic inhibition is controlled. The difference noted in H reflex magnitude in active and resting target muscle after ipsilateral cortical stimulation might suggest different mechanisms modulating the interhemisphere excitability along with other results. It has also been suggested that at least a part of this transmedian signaling in order to affect the contralateral excitability is modulated at the spinal level. Gerloff et al (1998) suggested that absence of modulation of H reflex at rest might be due to lack of technical peculiarity of their set up. Thus, they don't rule out the possibility of spinal pathways being involved. Further, they add that the ipsilateral inhibition seen might indicate disfacilitation of an excitatory drive controlling the alpha motor neuron located in the spinal cord. Since the disfacilitation is seen also at the level of brainstem, it is likely that the excitatory drive is generated at the level caudal to the brainstem (Gerloff et al., 1998). A network of spinal interneurons might be responsible for such effect. Unfortunately, spinal interneurons are difficult to study and their physiological effects during rest and during voluntary contraction remain almost unknown.

Modulation of spinal motorneuron excitability in terms of laterality at the same level:

Forceful contraction of one side wrist flexors causes inhibition of the contralateral h reflex in a resting homologous muscle (Hortobágyi et al., 2003). This inhibition statistically significantly increased by 22±18% and 50±17% of the control values, when the strength of contraction was 50% and 75% respectively ($p<0.0001$). Further, the inhibition remained suppressed for approximately 35 seconds before returning to the control value. The author

suggested that alpha motor neuron controlling the target muscle might be inhibited presynaptically as a result of contralateral forceful contraction. This presynaptic inhibition is inhibition of transsynaptic transmission between Ia afferent fibers and motor neurons via a circuit of interneurons. These interneurons also receive inputs from brain, contralateral spinal segments, propriospinal pathways and other unknown factors (Lee & Carroll, 2007; Zehr, 2002). Other studies also show modulation of H reflex during contraction of the contralateral homologous muscle or a remote muscle (Ferbert et al., 1992; Hortobágyi et al., 2003).F wave is facilitated during activation of the contralateral homologous muscle (Muellbacher et al., 2000). H reflex and F wave both target on the spinal motor neuron, but both are not modulated by the same factors. Anyway, there is enough indication that spinal pathways are modulated during contralateral contraction and this modulation may depend on strength of contralateral muscle activation. These observations may explain the contralateral strength gain seen in cross training. However, only one study showing the effects of chronic strength training on the contra lateral motor neurons did not find excitability of the spinal motor neuron modulated even after the significant increase in the MVC of the homologous muscle in the contra lateral limb (Lagerquist, Zehr, & Docherty, 2006). This effect led authors to conclude that there are no spinal pathways involved. This study looked at the effects of isometric training intervention on the spinal motor pathway excitability. This may indicate the possibility that the acute effects of contralateral contraction are different than the chronic effect. To have better understanding of the effects of exercise on spinal excitability in terms of laterality, we need to understand how contralateral movement is affecting the spinal motor pathways of the homologous muscle in the contralateral limb. Studies mentioned above did examine the contralateral modulation during contraction but

only one of them investigated modulation of spinal excitability after contraction which is the main objective of current study.

Concentric and eccentric contractions have different characteristics of contraction. Eccentric contractions are linked with greater force production and therefore, more strength gain in both unilateral (Hortobágyi et al., 1996) and contralateral limb (Hortobágyi et al., 1997). Thus, there is an indication that both the contractions are mediated through different mechanisms- either muscular and/or neural. Further we have already linked contralateral spinal excitability with the amount of force production or the strength of contraction. So we are hoping to see some changes in the contralateral homologous muscle h reflex during and after contraction which are dependent on type of contraction and/or strength of contraction.

Why should we study H reflex?

H reflex is an electrical analogous of the stretch reflex. It's evoked by stimulation of larger diameter afferent Ia fibers which are stimulated first according to the Hennemen's size principle. Ia afferents synapse with alpha motor neuron in the anterior horn of the spinal cord. It has been suggested that ascending H Reflex reliably provides the measurement for the alpha motor neuron excitability(Zehr, 2002).Therefore, almost all the studies examining the excitability of the alpha motor neuron measures the H reflex of the appropriate muscle (Upper Limb-FCR or APB; Lower Limb-Soleus). H Reflex is a reliable measure of the alpha motor neuron excitability given that the level of muscle activity, same position and posture and a proper time interval between two successive stimuli is maintained (Zehr, 2002).Therefore, the present study also relies on the H reflex as a measure of spinal excitability in order to examine the contra lateral spinal segmental effects of the contraction in homologous muscle group.

H reflex during movement:

Ipsilateral homonymous H reflex during contraction:

Although the present study examines the modulation of H reflex in the contra lateral resting limb during unilateral contraction, it is helpful to understand how H reflex and hence spinal excitability is modulated in the same contracting muscle itself. Pinniger et al (Pinniger, Nordlund, Steele, & Cresswell, 2001) found that H reflex in the soleus and medial gastrocnemius was significantly depressed during passive lengthening and facilitated during passive shortening compared to the isometric H reflex magnitude. The depression of reflex during passive lengthening was highest when the H reflex was measured at a latency of less than 60 ms after the onset of movement. This shorter latency suggested that this depression was due to peripheral spinal mechanisms and not the supraspinal influence.

Romano et al (1987) also showed the same results that H reflex depression was higher during passive lengthening than passive shortening. They also found the same results for the active shortening and lengthening of the ankle plantar flexors. The H reflex excitability was significantly depressed during active lengthening contraction and it was elevated during the shortening contraction of ankle plantar flexors (Bikmullina, Rozental, Pleshchinskii, 2005).

To summarize the effects of movement and contraction on the ipsilateral H Reflex, one should point out that Spinal excitability is modulated differently during shortening and lengthening contractions. Lengthening contractions can be associated with decreased spinal motor neuron excitability whereas, shortening contractions seen with increased spinal excitability. However, contra lateral effects may be different.

Contralateral H reflex during voluntary contraction

As mentioned earlier, contralateral h reflex was depressed during and after homologous muscle group contraction (Hortobágyi et al., 2003). This inhibition continued for approximately 30 s after the contraction. They also observed greater depression with the greater strength of contraction. The contraction of ankle ipsilateral ankle dorsi flexors further increased the inhibition of spinal excitability of FCR.

Bikmullina et al (2005) did a slightly different experiment in 2006. They recorded the Soleus H reflex during ipsilateral and contralateral ankle plantar flexion and dorsi flexion. The study design did not include the lengthening contractions of the soleus muscles. Ipsilateral H reflex was facilitated during plantar flexion and depressed dorsi flexion of foot. During plantar flexion of the contralateral foot, H reflex was modulated depending on the strength of contraction. The H reflex was facilitated during 20% MVC ($180.9\pm19.4, p<0.05$), facilitated during 40% MVC(142.1 ± 12.2, $p<0.05$) and inhibited during MVC($80.2\pm7.7\%, p<0.05$) of the contralateral soleus contraction. These results partly conflict with the data by Hortobágyi et al who showed inhibition of FCR even at 25% shortening MVC of the contralateral wrist flexors. However, both the studies show significant inhibition with MVC of contralateral homologous muscle. It is important to note that H reflex in both the extremities may be modulated by different mechanisms and so may be the contralateral modulation of spinal excitability.

It will be interesting to see if contralateral changes in the spinal excitability associated with acute strong voluntary contraction are similar to the chronic strength training. In 2006, Lagerquist (2006) conducted an experiment to see the modulation of H Reflex after the 5 week long strength training of contralateral ankle plantarflexors. They found that there was no

alteration of the H reflex in the untrained side, but there was a significant improvement in the strength as measured by MVIC in the untrained soleus. . This study included only isometric strength training and recording of H reflex associated with isometric contractions only. Since earlier studies (Hortobágyi, Laert, & Hill, 1997) show differences in cross education based on contraction types, it is possible that dynamic contractions have unique effects. The present study is aiming to focus on the effects of type and strength of dynamic voluntary contraction on the contra lateral spinal motor neuron excitability of the homologous muscle.

Summary

The review of previous literature indicates that there is no study documenting the effect of shortening and lengthening contractions at different strength in one limb on the spinal excitability or H reflex magnitude in the homologous muscle in the contralateral limb. Hence, it is the primary aim of the present study to record H reflex during and after contralateral voluntary shortening and lengthening contraction at different strength of contractions.

Methods:

Subjects:

10 healthy right handed subjects with mean age of 21 ± 3 years took part in the study (5 males, 5 females). Mean BMI was 25.7±0.5. Participants were chosen based on their responses to form 1. (Appendix A). N was chosen based on previous literature (Hortobagyi, 2003) that used 7 subjects effectively. We explained the purpose, set up, risks and benefits related to the study to each participant after they arrived. All of them gave their signed informed consent (Appendix C).

Inclusion Criteria:

Right handed young individuals were screened using Edinburgh Inventory (Oldfield et al, 1971). According to this questionnaire, to decide handedness person should be using right hand to write, draw, throw, use scissors, use toothbrush, knife without fork, spoon, broom(upper hand), for striking match and for opening a box. Presence of H reflex recruitment pattern in right side flexor carpi radialis (FCR) muscle was mandatory.

Exclusion Criteria:

The participants should be healthy and with no present or past history of any neuromuscular injury or disorder involving arm. They should not present with any current or past history of any disorder which might affect nerve conduction. They should not be on medications which might alter nerve conduction. They should not have pacemaker implanted because nerve stimulation might interfere and inhibit the pacemaker (Engelhardt, Grosse, Birnbaum, & Volk, 2007). Participants should not show current or past history of fracture of upper limb bones and also, participants should be able to provide informed consent. Despite satisfying every other

inclusion criteria, participants with absence of H reflex in right FCR could not be further involved.

Testing Protocol:

EMG recording:

We used Biopac 1000c system to record EMG from right and left FCR muscle. Analogue to digital board 1401 and Cambridge electronics signal version 3.0 software was used to transfer EMG signals into digital format. Once participant takes his place comfortably, skin over dorsum of both forearms is thoroughly cleaned with alcohol wipes and exfoliating lemon preparation in order to decrease impedance. Participant then performs maximal to submaximal voluntary contraction of FCR, and examiner palpates and finds a spot on FCR muscle belly to secure electrodes. A pair of standard gold cup electrodes with a ground electrode was used to as recording electrodes. Cathode is placed distally and anode proximally on the FCR belly. The place of ground electrodes was generally bony prominence lateral to the cubital fossa.

H reflex screening and recording:

The first step after getting signed informed consent and securing EMG electrodes on right FCR was to find an H reflex in right FCR. We used Digitimer stimulator DSA7 to stimulate right median nerve. A pulse at 400 V with pulse duration of 1 mS was used. The stimulus intensity varied among patients but ranged from 0 to ~25 m A. We used a bipolar metal electrode to stimulate median nerve. It was first made sure that gauze over stimulating electrode was well moistened, then slowly and gently we increased the intensity of current after placing the electrode on a right anterior cubital fossa. Cathode was distal and anode was proximal for the

stimulus electrode too. Median nerve was found just medial to the biceps aponeurosis in the cubital fossa. If not, then we probed for nerve by moving stimulating electrode proximally. In some cases, median nerve was more superficial at medial aspect of mid arm. Once a satisfactory site of median nerve stimulation is found, we strapped the electrode tightly with an arm band. Cutaneous sensation or paraesthesia in the thenar eminence, and palmar aspect of thumb and lateral two and half finger was a reliable indication of median nerve stimulation. Now, stimulus intensity is increased gradually, carefully looking for a long latency curve on the monitor. It is advisable to keep forearm pronated for better H reflex results (Baldissera, Bellani, Cavallari, & Lalli, 2000). As soon as stimulus intensity reaches threshold for larger diameter Ia afferent fibers, we see a long latency deflection on the screen. With the increase of intensity, the magnitude of this curve increases and after certain intensity it reaches maximum amplitude. Further increase in the stimulus intensity depresses the longer latency curve. This long latency curve is known as H reflex. Normal latency for FCR H reflex was noted to be around 15-20 mS in the present study. Before or around this time, another short latency curve appears on the screen which increases and reached maximum level with further increase in the stimulus intensity. This shorter latency curve is known as M wave because it is elicited by direct stimulation of higher threshold motor fibers in the median nerve. The maximum magnitude of H reflex is noted and then H reflex is set on 50% of H max value throughout the experiment. For hard to elicit H reflexes, we tried using very small and gradual increments of stimulus intensity. We also tried flipping the polarity of recording and/or stimulating electrodes. Even after trying these techniques for several times, if we could not elicit H reflex, then participant was excluded from the study.

Experiment Design:

Participant sat on a comfortable chair with back support, both elbows flexed at approximately 90º, arms supported on the table in front and legs supported on the floor. Left forearm was stabilized using foam padding and wooden blocks to minimize the movement.

There were two main experiments: first, contractions at relative force intensities and second, contractions at same absolute force level. Each experiment was further divided into conditions based upon type and strength of contraction. So in total there were eight conditions: Concentric contractions at 100% concentric MVC, Concentric contractions at 60% concentric MVC, Eccentric contractions at 100% eccentric MVC and Eccentric contractions at 60% of eccentric MVC. These four conditions were included in Experiment 1. Experiment 2 included contractions at same absolute force levels. This absolute force level was 100% and 60% of concentric MVC for Maximal and submaximal force levels respectively. Thus, the four conditions were: Concentric contraction at 100% concentric MVC, Concentric contractions at 60% concentric MVC, Eccentric contraction at 100% concentric MVC, Eccentric contractions at 60% concentric MVC. Concentric contractions at 100% and 60% of concentric MVC were required for both the experiments and therefore, practically only six total conditions were included in the experiment. Each condition included five contractions and each contraction was followed by 40 seconds rest period. H reflex was recorded during contraction and at every 5 seconds interval, immediately after contralateral contraction (Figure 1).

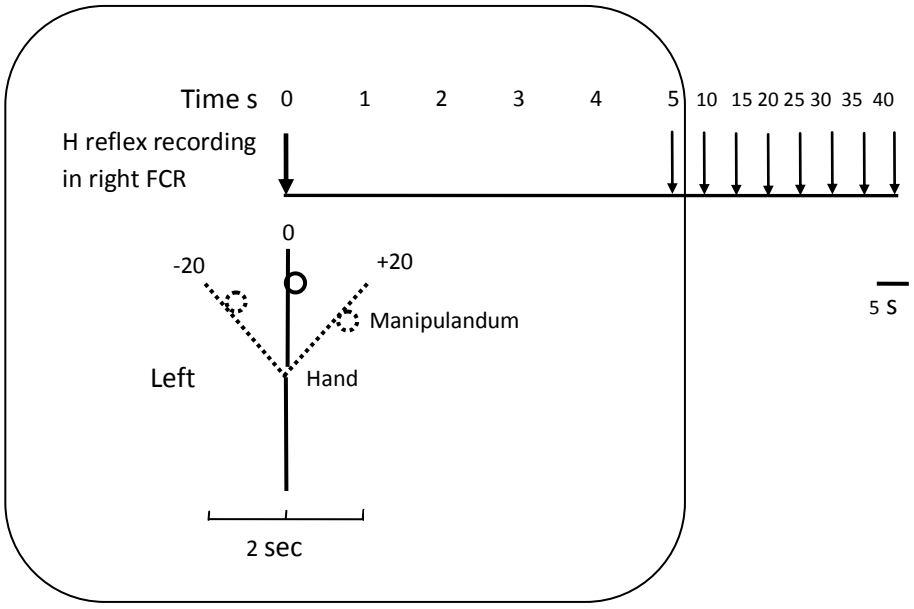

Figure 1: Design of a single trial. Inside the box, left wrist position from -20º to 0º to +20º is shown in the bottom. Left wrist takes total 2 seconds to complete one contraction. Right FCR H reflex is evoked (thick arrow) when left wrist passes 0º position. H reflex is recorded at 5 seconds interval for 40 seconds following contralateral contraction shown by thin arrows. Notice that time scale is magnified inside the box for clarity purposes.

Contraction trials:

Participant sat on a comfortable chair with back support, both elbows flexed at approximately 90º, arms supported on the table in front and legs supported on the floor. Left forearm was stabilized using foam padding and wooden blocks to minimize unwanted trick movement. Participants held left hand in a thumb up and fingers straight position. Left hand pushed against a manipulandum of isokinetic dynamometer (Kin-Com, Chattanooga, TN, USA) at the level of metacarpal heads during contraction. Kin-Com was configured to resist concentric and eccentric movements of left wrist flexors within a 40º range of motion. Neutral position of wrist was 0º, maximum wrist flexion allowed was +20º and maximum wrist extension allowed

was -20º. Speed of all the contractions was 20º/ s. That gives 2 seconds to complete each contraction within complete 40º ROM. Range and direction for concentric contractions were from -20º to +20º and +20º to -20º for eccentric contractions.

Participants were reminded to relax right hand, shoulders, chest, back and leg muscle before each contraction. During a contraction, H reflex in right FCR was evoked as the left wrist passes 0º neutral position. After 1 second of that, when the contraction is over,participant in instructed to relax left hand and other body parts completely. Right FCR H reflex is elicited at every 5 s interval for 40 seconds rest period after each contraction. See figure 1 for graphic presentation of one trial. Optimum verbal encouragement is given during MVCs. Visual feedback by providing a target line on Kin-Com monitor is also included during submaximal contractions.

Data Analysis

Customized computer software was used to analyze EMG Data. Peak to peak amplitude of H reflex will be calculated for each trial using custom software and extracted to MS Excel spreadsheet. H reflex amplitudes were normalized tothe average of last 3 H reflex values within each trial. These normalized values were then compared for type of contraction and force effect across conditions.

Statistical Analysis

Repeated measures ANOVA was used to find significance level of main and interaction effect of type (concentric, eccentric) and intensity (100%, 60%) during trials of experiment 1 and 2. Single tailed student's t test with equal variance assumed was used to find any differences in the background EMG activity.

Results:

The main finding of the present study was that voluntary contractions of left wrist flexors produced almost 40% depression of right FCR H reflex during contraction. H reflex started recovering after the contraction and stabilized at the control value after almost 20-25 seconds. Specifically, the results chapter presents the findings in two main sections: right FCR H reflex 1) during and 2) after left wrist flexors contraction. H reflex amplitude is further reported according to the main and interaction effects of dependent variables, type and intensity of contraction in trials of experiment 1 and 2. In experiment 1, left wrist flexors performed concentric and eccentric contraction at 100% and 60% of the concentric and eccentric 100% MVCs respectively. In experiment 2, left wrist flexors performed concentric and eccentric contractions at 100% and 60% of 100% concentric MVC. Data for all the results discussed here are given in Appendix B in table format.

Right FCR H reflex during left wrist flexors contraction:

Main effect of contraction type:

Figure 2A shows main effect of contraction type during trials of experiment 1 averaged across intensities of contraction. There was no significant difference in right FCR H reflex between concentric and eccentric contraction trials ($p=0.934$, $F_{(1,19)}=0.007$).

Figure 2B shows main effect of contraction type during experiment 2 trials pooled across contraction types. There was no significant main effect of contraction type at $p=0.104$, $F_{(1,19)}=2.911$.

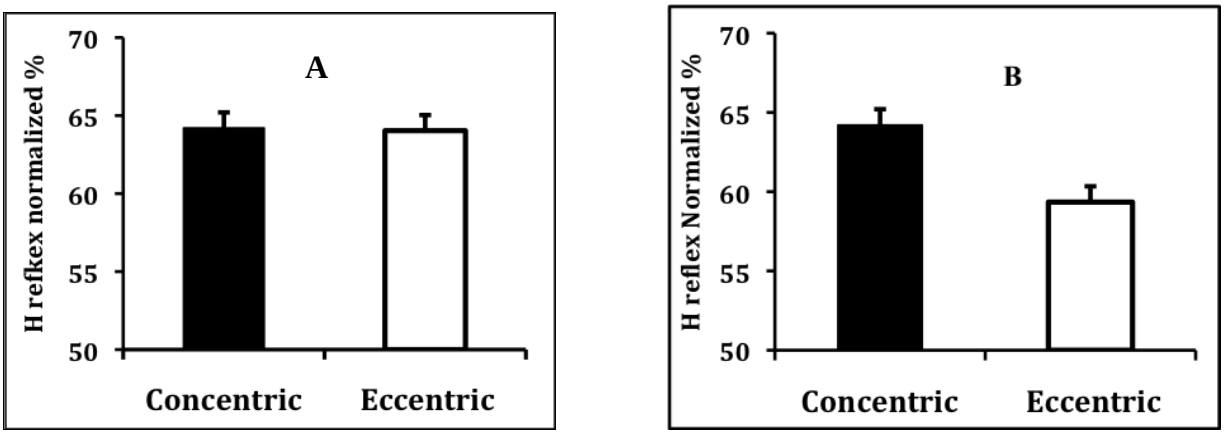

Figure 2: Main effect of contraction type in experiment 1 (A) and 2 (B) trials averaged across contraction intensities. Filled and unfilled bars denote concentric and eccentric contractions respectively. X axis: Type of contraction, Y axis: H reflex amplitudes normalized to control value. Error bars show standard deviations.

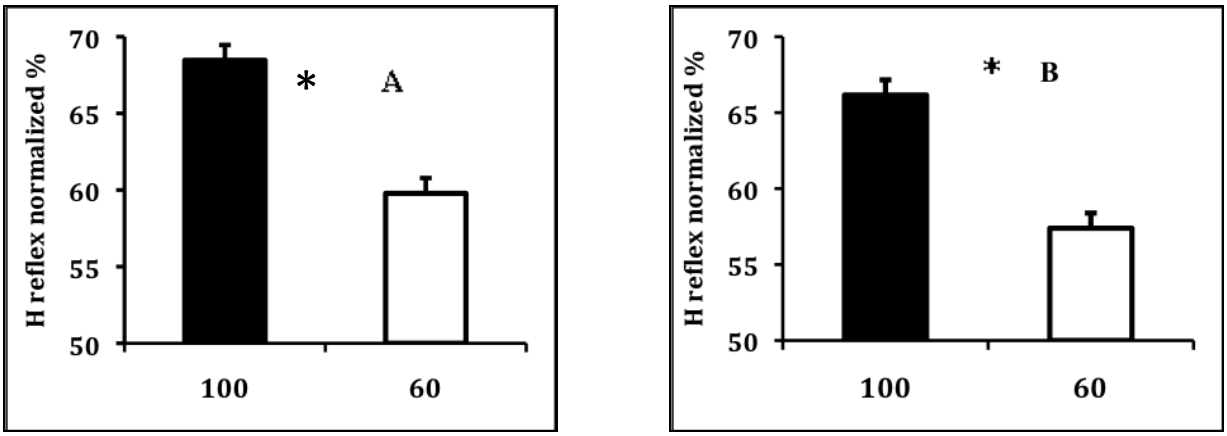

Figure 3: Main effect of contraction intensity in experiment 1 (A) and 2 (B) trials averaged across contraction type. Filled and unfilled bars are 100% and 60% depending on the experiment design respectively. X axis: Intensity of contraction, Y axis: H reflex amplitudes normalized to control value. Error bars show standard deviations. * is significance level at $p<0.05$ level.

Main effect of contraction intensity:

Figure 3A shows main effect of contraction intensity during trials of experiment 1 averaged across intensities of contraction. There was significant difference in right FCR H reflex between 100% and 60% contraction intensities ($p=0.03$, $F_{(1,19)}=5.53$). 100% contraction intensities produced average 68.4±8% of control values and 60% evoked amplitudes similar to 60±21.1% of control value. Thus, stronger the contraction, bigger the H reflex amplitude or weaker the inhibition.

Figure 3B shows main effect of contraction intensity during experiment 2 trials pooled across contraction intensities. Again, there was significant main effect of contraction intensity at $p=0.013$, $F_{(1,19)}=7.5$. Here again, previous pattern of lesser inhibition with stronger contraction was repeated.

Interaction effects:

Figure 4A shows that during concentric contraction, there was almost 18% more depression of H reflex during 60% of contraction intensity than during 100% intensity of contraction. However, this difference was not significant ($p=0.204$, $F_{(1, 9)}=1.9$). During eccentric contractions, contractions at 60% MVC produced approximately 24% higher depression of H reflex compared to contractions at 100% MVC. This difference did not reach the significance level either ($p=0.101$, $F_{(1, 9)}=3.33$).

Figure 4B shows that during eccentric contraction of the left wrist flexors at the intensity same as concentric MVC, right FCR H reflex was 34.9% depressed. This depression increased to 45.9% when the contraction was less strong at the intensity of60% Concentric MVC. This difference in depression was significant ($p=0.02$, $F_{(1, 9)}=8.1$).

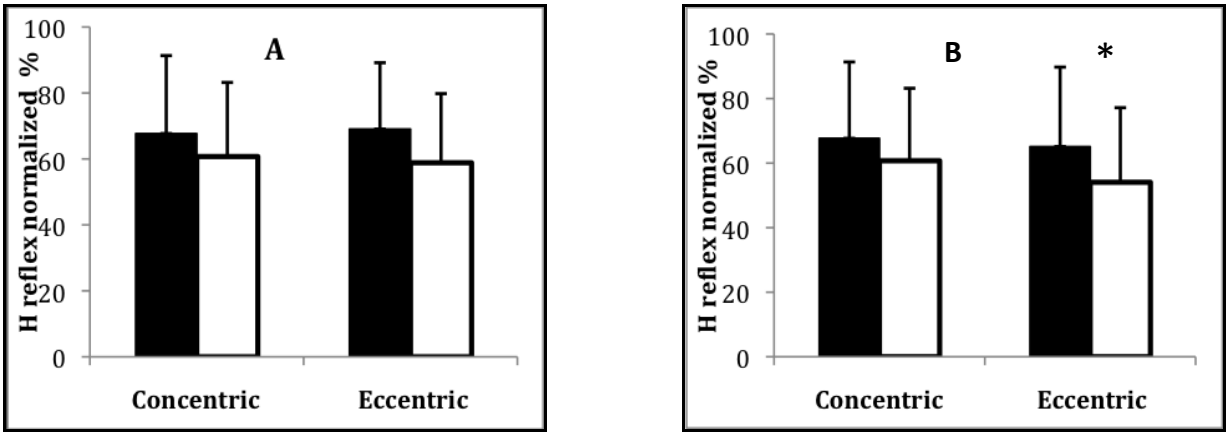

Figure 4: Interaction effect of type and intensity: comparison of normalized H reflex between contractions at 100% and 60% of MVC within concentric and eccentric contractions during experiment 1 (A) and 2(B). Filled and unfilled bars are contractions at 100% and 60% respectively. The error bars denote standard deviation. * is significance level at $p<0.05$ level.

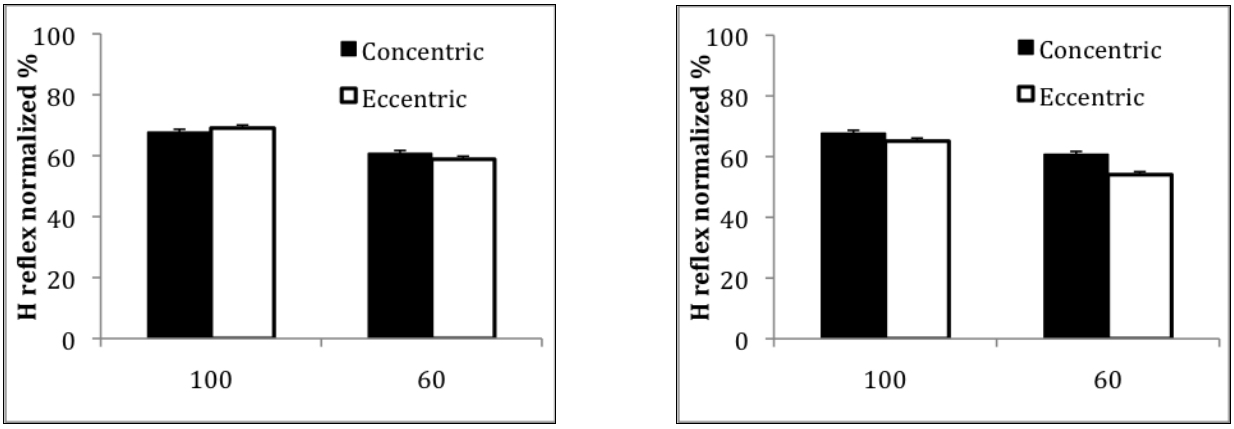

Figure 5: Interaction effect of intensity and type: comparison of normalized H reflex between concentric and eccentric contractions within 100% and 60% MVC contractions intensities during experiment 1 (A) and 2(B). Filled and unfilled bars are concentric and eccentric contractions respectively. The error bars denote standard deviation.

Thus, there was a pattern showing more depression with weaker contralatateral contraction but this pattern was significant only for one condition.

Figure 5A shows that there was no significant main effect of mode of contraction at intensity of 100% MVC ($p=0.868$, , $F_{(1,9)}=0.03$) and 60% MVC ($p=0.479$, , $F_{(1,9)}=0.5$).

Figure 5B shows that when the contralateral contractions were controlled for intensity, there was no main effect of mode of contraction at intensity of 100% concentric MVC ($p=0.523$, $F_{(1, 9)}=0.4$) at a very small observed power of 0.092 and at intensities same as 60% concentric MVC ($p=0.111$, $F_{(1, 9)}=3.1$).

To further understand these results in context with some previous work, two control experiments were designed. The control experiment was performed only in one subject so its results does not support or refute any idea but may help to design future studies.

Control experiments

As noted above, strong maximal contractions showed less inhibition of contralateral spinal excitability which does not match with the previous research (Hortobagyi et al, 2003) and therefore, a small experiment was designed to examine the effect of this protocol using isometric contractions. The results in only one subject (Figure 6) show more inhibition of right FCR H reflex with stronger isometric contraction of the left wrist flexors.

Another control experiment was performed in the same subject to test the excitability of Extensor Carpi Radialis (ECR) during and after left wrist flexors activation using the present study protocol. The subject could not keep the right arm completely relaxed during ECR H reflex recording trials and therefore, data are flawed and cannot be further used.

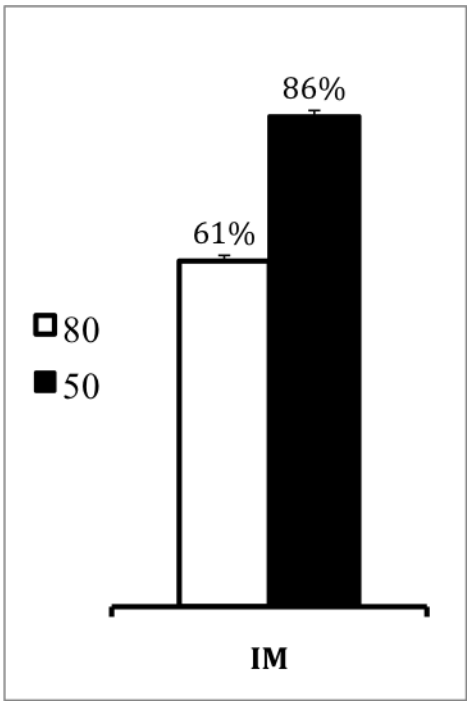

Figure 6:

Effect of isometric contraction on contralateral spinal excitability. Stronger contraction (80% of MVC, filled bar) produced almost 14% inhibition of right FCR H reflex compared to left wrist isometric flexion at 50% of MVC (unfilled bar) which produced almost 39% of inhibition. No statistical tests were performed because only one subject was examined. Error bars show SD.

However, there was no significant difference in background EMG activity during isometric trials for ECR and FCR H reflex recording. Isometric trials showed ~20% facilitation of right ECR H reflex during 100% concentric contraction of the left wrist flexors. Whereas, there was ~40% inhibition of right FCR H reflex during this condition

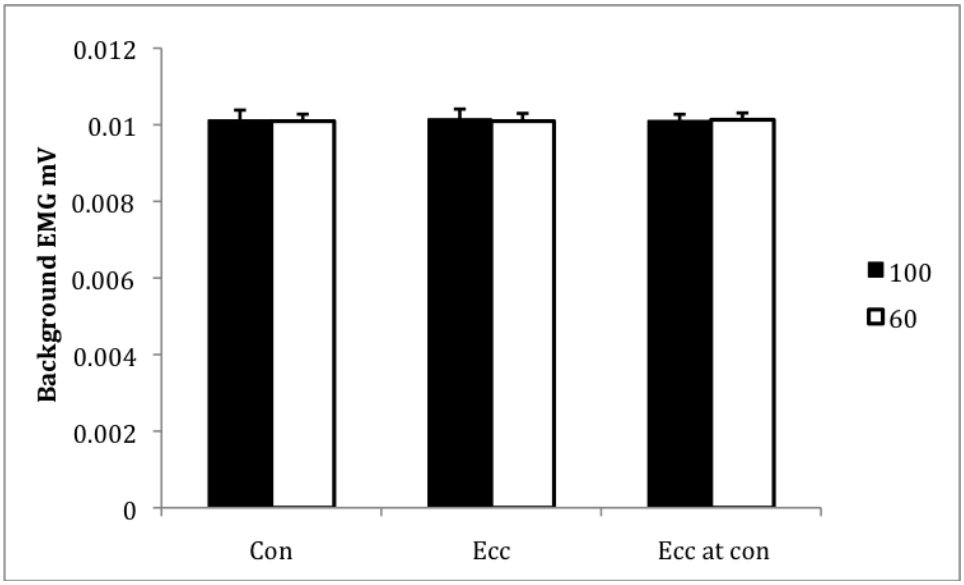

Figure 7: Background EMG activity in right FCR during left wrist contractions by intensity within each condition. X axis: type of contraction, Y axis: Background EMG activity in millivolt. Error bars indicate standard deviations.

Comparison of background EMG activity:

Voluntary activation of right FCR during contractions in left wrist flexors was measured by background EMG activity. There was no significant difference between maximal and submaximal contractions within each condition. There was no significant difference in resting right FCR EMG between 100% and 60% intensities during concentric contractions, ($t(18)=-0.943$, $p=0.12$). No significant difference was noted during eccentric contractions at 100% and 60% intensities ($t(18)=-0.943$, $p=0.185$). Same way, during left eccentric contractions using concentric intensities equal to 100% and 60% MVC, there was no significant difference in right FCR background EMG activity ($t(18)=-0.338$, $p=0.369$).

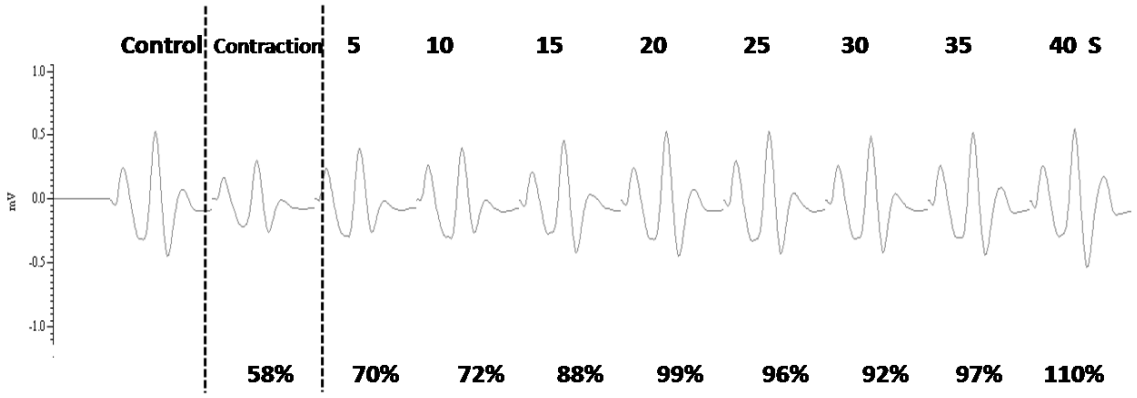

Figure 8: H reflex action potentials in a single trial. H reflexes of subject 4. The individual action potentials show the depression of the H reflex recorded in the resting right FCR during and after an eccentric contraction of the left wrist flexors. H reflex reached control value within 20 seconds in this particular trial.

H reflex recovery following left wrist flexors contraction:

As shown in figure 8, following the depression induced by contralateral wrist flexors contraction, right FCR H reflex recovers over time and stabilizes at control level in approximately 20 seconds.

Effects of contractions at the same relative intensity:

Intensity (2) by time (6) repeated measures ANOVA was used to examine effects of intensity on recovery curve after concentric/ eccentric contractions.

Figure 9 shows that after concentric contractions, interaction between time and intensity was significantly different ($p=0.022$, $F_{(5, 45)} =2.96$) which means that contractions at 100% and 60% of MVC intensity had different H reflex values over time.

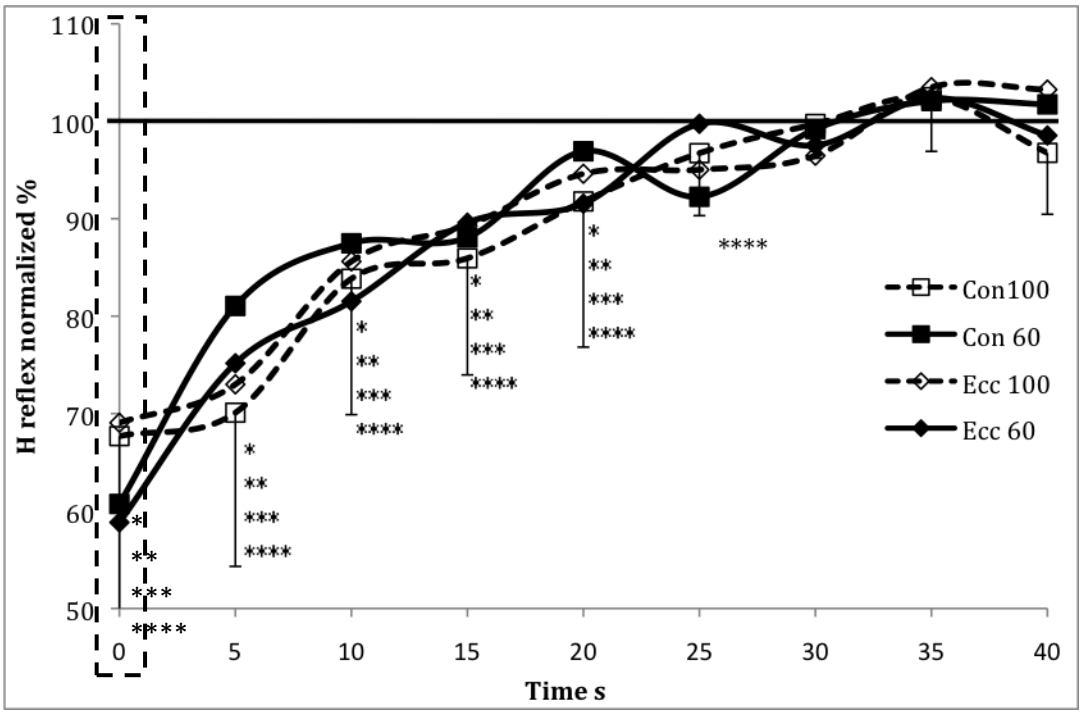

Figure 9: Right FCR H reflex recovery curve after left wrist flexors contraction during concentric contractions at 100% and 60% of concentric MVCs, eccentric contractions at 100 and 60% of eccentric MVCs. Error bars indicate one standard deviation. X-axis shows time in seconds after contraction. 0 second indicates right FCR H reflex recording during left wrist flexors contraction. The box with dotted line indicated contraction. Y-axis shows H reflex values normalized to control level. Error bars are shown only for one condition for the reason of clarity. Significance level at $p<0.05$ are shown by *, **, *** and **** for concentric 100, concentric 60, eccentric 100 and eccentric 60 conditions respectively.

After eccentric contractions, there was no overall effect of intensity on the H reflex recovery pattern ($p=0.548$, $F_{(1, 9)} =0.39$). However, when H reflex values were compared across all the trials for experiment 1 to test the main and interaction effects of variables time, mode and intensity for first 10 seconds (mode(2) by intensity(2) by time(3)), there was significant change in H reflex over time with the change in intensity irrespective to their mode ($p=0.049$, $F_{(2, 18)} =3.576$).

Effects of contractions at the same absolute intensity:

After eccentric contractions at the same intensity as concentric contractions, there was no significant difference in normalized H reflex values between 100% and 60% of contraction intensities ($p=0.132$, $F_{(1, 9)} =2.747$) as examined by intensity by time (two by six) repeated measure ANOVA. Further, there was no effect of intensity on the recovery pattern over time. ($p=0.246$, $F_{(5, 45)} =1.391$).

Based on mode by time (two by six) repeated measure ANOVA, there was no difference in H reflex values based on mode ($p=0.696$, $F_{(1, 9)} =0.16$) at intensities same as concentric 100% MVCs. Also, there was no difference in H reflex recovery pattern based on mode of contraction at intensities same as concentric 100% MVC ($p=0.769$, $F_{(3, 30)} =0.407$). However, at intensities same as 60% concentric MVC, eccentric contraction had significantly more depressed H reflex throughout the trial than concentric contraction ($p=0.027$, $F_{(1, 9)} =7.018$). However, there was no significant interaction between time and mode ($p=0.252$, $F_{(3.3, 29.7)} =1.431$).

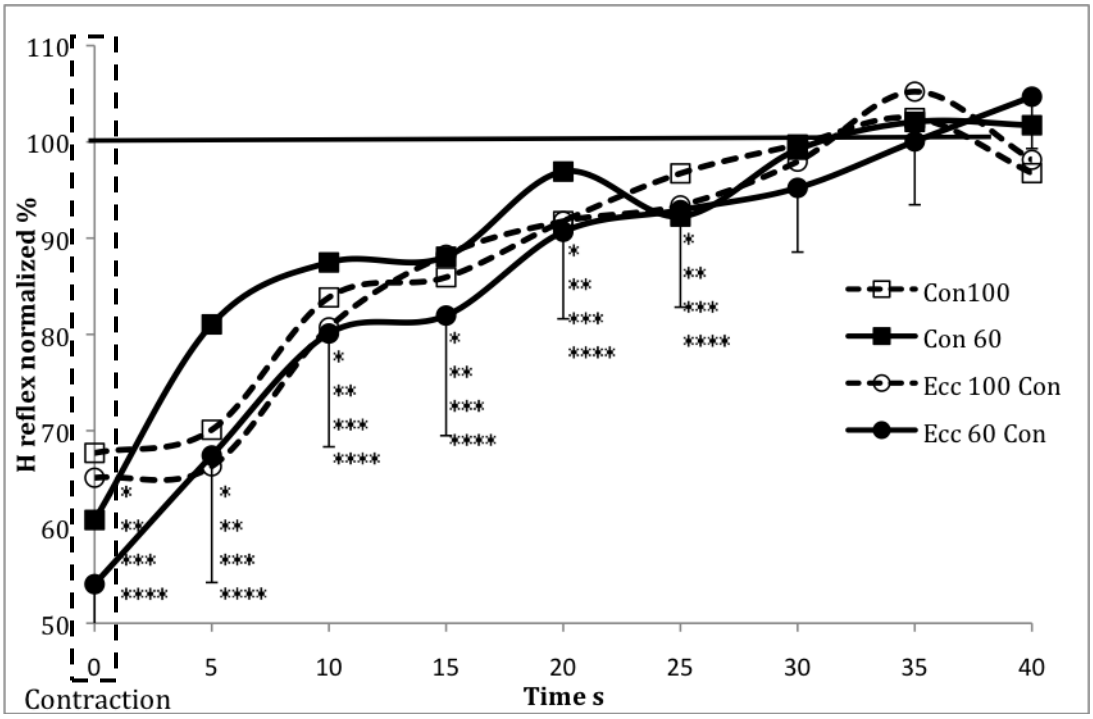

Figure 10: Right FCR H reflex recovery curve after left wrist flexors contraction during concentric contractions at 100% and 60% of concentric MVCs, eccentric contractions at 100 and 60% of concentric MVCs. Error bar indicate one standard deviation. X-axis shows time in seconds after contraction. 0 second indicates right FCR H reflex recording during left wrist flexors contraction. The box with dotted line indicated contraction. Y-axis shows H reflex values normalized to control level. Error bars are shown only for one condition for the reason of clarity. Significance level at $p<0.05$ are shown by *, **, *** and **** for concentric 100, concentric 60, eccentric 100 and eccentric 60 conditions respectively.

Discussion

The purpose of this study was to compare spinal excitability in the right wrist flexors during and after concentric and eccentric contraction of the left wrist flexors at an intensity of 100% and 60% of the maximum. The main finding was that H reflex was depressed during contralateral homologous muscle contraction. Weaker contractions produced more depression than the stronger contractions. After initial depression, the H reflex started to recover and reached control value in ~20s. Concentric and eccentric contractions had no difference in the amount of inhibition and recovery pattern.

Spinal excitability inhibition

Hortobágyi et al (2003) used the exact same protocol as this study to measure contralateral FCR H reflex during and after left wrist isometric flexion. Right FCR H reflex was depressed during contraction and remained inhibited for approximately 35 seconds after contraction. Bikmullina et al (2005) also found that the soleus H reflex was 80% of the control value during contralateral ankle plantar flexion at maximum strength of muscle contraction. H reflex is also depressed in the right FCR while the left wrist flexors are activated to perform wrist flexion with no resistance (Carson, Riek, & Bawa, 1999). Moreover, H reflex is also depressed in response to the contralateral rhythmic arm cycling (Zehr, Collins, Frigon, & Hoogenboom, 2003), pedalling (Cheng, Brooke, Misiaszek, & Staines, 1998), and circumductory arm movements (Delwaide & Pepin, 1991). All of the above mentioned studies described the suppression of H reflex to be presynaptic in nature. FCR H reflex remains unmodulated when contralateral wrist moves a handle bar in small circles in horizontal plane with no resistance (Delwaide & Pepin, 1991; Sabatino et al., 1992). Electrical stimulation at intensity of 0.5x motor

threshold of contralateral median nerve also does not affect FCR H reflex in the ipsilateral hand. Bikmullina et al (2005) also found slight facilitation in the contralateral soleus H reflex when the strength of contraction was 20% and 40% of MVC. In contrast, we found inhibition of FCR H reflex as a result of strong voluntary contraction of contralateral wrist flexors. The data suggests that cortical excitation and/or numbers of motor units fired might be affecting the excitability of spinal motor neuron controlling resting FCR in the other limb.

When wrist moves the Isokinetic manipulandum forcefully, it activates various exteroceptors, proprioceptors and other afferent sensory fibers. It is therefore anticipated that in addition to motor drive, sensory input must have a role to play in the modulation of contralateral H reflex. Suppressed motor neuron activity is found after conditioning with contralateral passive ankle movement (Carson et al., 1999). Evoking a monosynaptic patellar tendon tap also significantly suppresses the contralateral patellar tendon tap response to 10% of control for 25 milliseconds (Kamen & Koceja, 1989). Electrical stimulation of tibial nerve Ia fibers resulted in long latency inhibition of the contralateral homonymous H reflex (Slivko &Teteryatnik, 2005). Least square regression reveals that, for interstimulus intervals longer than 2 seconds, contralateral H reflex will show inhibition for more than 4 seconds. Thus, not only movement, but also artificial production of sensory input similar to voluntary movement is associated with contralateral H reflex depression.

Shortening vs. lengthening

Another finding of the present study is that there was no difference in the H reflex modulation between shortening and lengthening contraction. However, cross education studies show more strength gain after eccentric training. This effect does not seem to be caused by spinal

mechanisms as hypothesized earlier. Unpublished data from our lab shows changes in the ipsilateral motor evoked potentials, intracortical facilitation and inhibition (Hortobágyi et al., June 24-27, 2009). It seems more likely that contralateral strength gain differences in shortening versus lengthening contraction is mediated through supraspinal mechanisms.

Strong vs. weak contractions

Left wrist flexors MVCs exhibited less inhibition of the resting right FCR compared to the submaximal contraction at 60% of MVCs. In contrast, Hortobágyi et al (2003) found more depression with stronger contractions. Right FCR H reflex was almost abolished during left wrist flexor isometric contraction at 75% of MVC. Most feasible explanation is facilitation of H reflex because of higher background EMG activity during higher intensity contractions in our study. However, when background EMG activity was compared across conditions, there was no significant difference. This indicates that isometric and isokinetic movements are controlled by different mechanisms. To be clearer, the same protocol of present study was repeated with one participant using isometric contractions and found same results as Hortobágyi et al (2003).

Most of the studies concluded that the inhibition is due to presynaptic mechanisms because H reflex was depressed even in the presence of some background EMG (Carson et al., 1999). Long latency of this inhibition is also thought to involve presynaptic mechanisms (Slivko &Teteryatnik, 2005; Hortobágyi et al., 2003). It means that sensory input associated with the movement stimulates supraspinal pathways and spinal segmental pathways in a way which depressed alpha motor neuron of the contralateral homonymous muscle either directly or through a complex network of interneurons. Looking at the long latency and continued inhibition, it is most likely that a complex network of spinal and/or supraspinal interneurons is involved.

Spinal segmental mechanism

Reciprocal Inhibition

One possible pathway is facilitation of reciprocal inhibition in the left upper limb as a result of movement in the left wrist flexors. Reciprocal inhibition is depression of agonist by the antagonist muscle activation. Contralateral median nerve stimulation facilitates reciprocal inhibition in the ipsilateral hand exhibited on wrist flexors by wrist extensors as measured by change in FCR H reflex after Radial nerve stimulation (Delwaide & Pepin, 1991; Sabatino et al., 1992; Sabatino et al., 1994).

Delwaide et al (1991) described two possible schemes of the arrangement and connections between interneurons and motor neurons in the spinal cord. Most likely scheme suggests that FCR's MN is connected to an inhibitory interneuron which crosses midline and connects with Ia inhibitory neuron on the other side that synapses with the MN of ECR. Thus, left wrist flexion would cause reduced inhibition of contralateral ECR's MN presynaptically by suppressing Ia inhibitory interneuron. ECR's MN is also attached to another Ia inhibitory interneuron that suppressed FCR's MN. Hence, it causes inhibition of FCR H reflex by facilitation of the reciprocal inhibition. However, there is no data showing the effect of voluntary or passive contraction of contralateral wrist flexors on the spinal excitability of ipsilateral wrist extensors. In a control experiment, we recorded right ECR H reflex during and for 40 seconds after the contraction of the right wrist flexors contraction. The participant could not keep the right wrist flexors relaxed throughout the experiment and therefore, the data are not reliable. However, during isometric flexion of left wrist, there was facilitation of right ECR H reflex which was not violated by differences in background EMG activity.

Supraspinal inhibitory mechanisms

There are two possibilities for modulation of spinal excitability by supraspinal influence.

First is activation of the ipsilateral hemisphere during the movement. Movement on the one side of the body is controlled by contralateral hemisphere. Data from our lab shows that interhemispheric inhibition from right M1 to left M1 was significantly diminished during strong contraction of left wrist flexors (Hortobágyi, Howatson, Rider, Solnik, DeVita, June 24-27, 2009). This finding is in agreement with the previous studies that show that ipsilateral cortical excitability increases as a result of unilateral hand movement (Muellbacher et al., 2000). F-MRI of brain reveals ipsilateral and bilateral activation of certain areas of the brain during ankle dorsiflexion (Francis et al., 2009). In the present study, it does not seem impossible to assume that facilitation of left hemisphere can influence spinal excitability of the resting right FCR.

Second possible mechanism is activation of uncrossed descending corticospinal fibers from the controlling contralateral cerebral cortex. Corticospinal tract originates from primary motor cortex and descends through subcortical structures. Almost 90% of descending fibers crosses midline at the level of medulla which is known as pyramidal decussation. The fibers then descend further through spinal cord as lateral corticospinal tract. However, rest of 10% fibers do not cross midline at pyramidal decussation; they continue further down as anterior corticospinal tract and crosses midline at their specific segment (Kandel ER, Schwartz JH, Jessell TM, 2000) and some of them do not cross midline at all (Nathan, Smith, & Deacon, 1990).Such uncrossed corticospinal fibers might be responsible for carrying cross educatory effects as seen earlier.

It is very much likely that the modulation of H reflex also has some supraspinal contribution through uncrossed corticospinal fibers. Corticospinal volley as measured by

ipsilateral CMEP (evoked by transmastoid stimulation) is inhibited for a long time (~90 sec) after strong voluntary contractions at 75% and 100% MVCs in elbow flexors (Petersen, Taylor, Butler, & Gandevia, 2003). Same result was demonstrated by decreased contralateral CMEP area which was depressed after contraction and recovered to control level within 20 seconds (Hortobágyi et al., 2003). These results are evidence to believe that cortical volley is also modulated like H reflex. It is most likely due to involvement of subcortical pathways.

It is most likely that a combination of multiple supraspinal and/or segmental mechanisms is responsible for the long latency contralateral inhibitory pattern seen in the present study. The present study was not designed to examine the specific mechanisms behind contralateral modulation of homonymous H reflex induced by muscle contraction. This study provided clear evidence of contralateral influence on the spinal excitability. Previous literature strongly suggests role of spinal interneurons (Delwaide & Pepin, 1991; Hortobágyi et al., 2003). We believe that longer latency of inhibition suggests a complex network of spinal interneurons is working with supraspinal control to modulate spinal excitability of homonymous muscle in the contralateral limb. Further basic scientific studies using TMS, CMEP and H reflex methods are needed to explore more about this contralateral control. It will also be useful to examine functional significance of contralateral control. Post contraction inhibition in contracting muscle has been related to the relaxation of motor neuron immediately after contraction and serves as neural principle for techniques like “hold-relax” of proprioceptive neuromuscular facilitation (Moore & Kukulka, 1991). It is of equal importance to examine long term effects and therapeutic importance of contralateral inhibition observed in the present study in normal and neurologic population since this study was limited to normal young population only.

Conclusion

The purpose of this study was to compare spinal excitability in the right wrist flexors during and after concentric and eccentric contraction of the left wrist flexors at an intensity of 100% and 60% of the maximum. The hypothesis was that spinal excitability varies in the resting limb according to the type and intensity of muscle contraction in the contralateral limb. The results show that contraction of the left wrist flexors causes a reduction of spinal excitability, as measured with the H reflex, in the resting right wrist flexors. This depression lasts up to about 25 s after the contraction. In general, these data support the hypothesis. The results show that the depression seemed to be larger at low contraction intensity, supporting the hypothesis. The H reflex depression does not seem to depend on the type of muscle contraction, refuting the specific hypothesis of spinal excitability being contraction-specific. Although directly not investigated, the results suggest a role for the Ia inhibitory interneurons and pre-synaptic inhibition in the contralateral depression of the H reflex during unilateral muscle contraction. The data seem to point to the direction that cross education effects observed in chronic training studies are probably not mediated directly by a change in spinal excitability.

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Appendix A

Contralateral H Reflex Modulation

Telephone/ Personal Interview/ Eligibility Checklist

Name_____Phone #_____Date_____

How old are you? (18 to 40)_____ Weight_____ Height_____

Do you write with your right hand? (Yes)_____ Draw_____ Throw_____ Scissors_____

Toothbrush_____ Knife without fork_____ Spoon_____ Broom (upper hand)_____ Striking

match_____ Opening box_____ (SCORE_____/10)

Did you ever break a bone in your arms and hands? (No)_____

Do you have pain in your arms and hands? (No)_____

Have you ever diagnosed with a neurological disorder in the nerves of your arms? (No)_____

Have you ever been diagnosed with a brain disorder such as Parkinson's disease? (No)_____

Did you ever have a stroke? (No)_____

Are you taking any medications that you know would affect neuronal conduction? (No)_____

Do you have a pacemaker? (No)_____

How many cups of coffee or tea do you drink a day? (1-2)_____

How many glasses of alcoholic beverages do you drink a day? (1-2)_____

Are you willing to sign an informed consent document to enter this study? (Yes) _____

Appendix B: Data table

Figure	Variables		N	mean	SD	P	F	DF
	Main effects							
2A	Type	CON	20	64.2	22.7	0.934	0.007	1,19
		ECC	20	63.9	21.06			
2B	Type	CON	20	64.2	22.7	0.104	2.911	1,19
		ECC	20	59.2	23.9			
3A	Intensity	100	20	68.45	21.8	**0.03**	5.535	1,19
		60	20	59.7	21.1			
3B	Intensity	100	20	66.1	23.5	0.013	7.459	1,19
		60	20	57.4	22.5			
	Interaction effects							
4A	Concentric	100	10	67.8	23.6	0.204	1.876	1,9
		60	10	60.7	22.5			
	Eccentric	100	10	68.9	20	0.101	3.3	1,9
		60	10	58.8	20.8			
4B	Concentric	100	10	67.8	23.6	0.204	1.876	1,9
		60	10	60.7	22.5			
	Eccentric	100	10	64.9	24.6	**0.02**	8.1	1,9
		60	10	54.1	23.2			
5A	100	CON	10	67.8	23.6	0.868	0.029	1,9
		ECC	10	68.9	20			
	60	CON	10	60.7	22.5	0.479	0.54	1,9
		ECC	10	58.8	20.9			
5B	100	CON	10	67.8	23.6	0.523	0.441	1,9
		ECC	10	64.9	24.6			
	60	CON	10	60.7	22.5	0.111	3.125	1,9
		ECC	10	54.1	23.2			
6	Isometric	50	1	86	0.5			
		80	1	61	0.63			
			n	mean	Sd	p	t	df
	Background EMG							
7	Concentric	100	10	0.01	0.0003	0.406	0.240	18
		60	10	0.01	0.0002			
	Eccentric	100	10	0.01	0.0003	0.310	0.504	18
		60	10	0.01	0.0002			
	Eccentric Absolute	100	10	0.01	0.0002	0.369	-0.339	18
		60	10	0.01	0.0002			

			n	mean	sd	p	t	df
Background EMG: Isometric Control Experiment								
FCR	50		1	0.032	0.02	0.23	-0.77	12
	80		1	0.02	0.016			
Background EMG: ECR Control Experiment								
Isometric	ECR		1	0.028	0.012	0.326	0.461	13
	FCR		1	0.02	0.016			
Con 100	ECR		1	0.05	0.012	4.4E-06	7.745	11
	FCR		1	0.01	0.0001			
Ecc 100	ECR		1	0.047	0.007	6.5E-09	13.5	12
	FCR		1	0.01	6.1E-05			

Variables	N		Time								
			0	5	10	15	20	25	30	35	40
Figure 9											
CON	100	10	67.8	70.1	83.8	85.9	91.8	96.7	99.7	102.5	96.8
			23.60	15.8	14.0	12.0	15.0	6.4	3.5	5.6	6.3
	60	10	60.7	81.0	87.5	88.1	96.9	92.2	99.2	102.1	101.7
			22.5	14.3	11.8	11.9	5.7	6.4	5.6	6.4	6.8
ECC	100	10	68.9	71.6	85.9	89.6	93.9	95.9	96.5	103.8	102.0
			20	7.1	9.2	8.1	8.9	8.4	8.0	6.4	5.4
	60	10	58.8	75.2	81.5	89.6	91.5	99.7	97.5	102.4	98.5
			20.8	23.3	11.1	8.4	7.7	7.0	10.3	6.0	7.8
Figure 10											
CON	100	10	67.8	70.1	83.8	85.9	91.8	96.7	99.7	102.5	96.8
			23.6	15.8	14.0	12.0	15.0	6.4	3.5	5.6	6.3
	60	10	60.7	81.0	87.5	88.1	96.9	92.2	99.2	102.1	101.7
			22.5	14.3	11.8	11.9	5.7	6.4	5.6	6.4	6.8
ECC	100	10	64.9	67.0	81.1	88.0	89.8	93.3	97.8	105.0	98.6
			24.6	12.3	11.0	15.4	11.6	10.6	6.3	9.3	6.8
	60	10	54.1	67.4	80.1	82.0	90.7	92.9	95.2	100.0	104.7
			23.2	13.2	11.8	12.5	9.0	10.1	6.7	6.6	5.4
Isometric Control Experiment											
ECR	100	1	115	41.3	66.3	99.8	90.5	78	100.2	86.8	102
FCR	80	1	60.7	38.4	59.3	63.9	77.7	82.7	107.1	101.6	100.7
	50	1	86.2	75.7	98.5	86	93.5	104.9	108	94.4	92.7

Appendix C

Consent Form
Interhemispheric Plasticity in Humans – Version 2

Biomechanics Laboratory
Investigator: Tibor Hortobágyi, Ph.D.
Address: 332A Sports Medicine Building, East Carolina University, Greenville, NC 27858
Telephone: (252) 737 - 4564

I am asked to voluntarily participate in this research project conducted by Tibor Hortobágyi. The **purpose** of this study is to determine how muscle strength is increased in the non-exercised limb after strength training of the muscles in the other limb. The study involves different strength training programs of the right calf muscles, including voluntary, imagined, and electrically evoked muscle contractions. It also involves magnetic stimulation of the brain, electrical stimulation of a leg muscle that is associated with some discomfort, or short-term leg immobilization. My involvement will last for about 8 weeks. I will have to be right-leg dominant determined by ball kicking. There will be about 130 subjects in the study over five years. I understand that my written consent is required before I can participate in this project.

Training procedures. I understand that only the training procedures that are circled will apply to me. A specific training procedure will be randomly assigned to me by chance. For each experiment, the name of each treatment group will be written on a separate piece of paper. The principal investigator will then draw one of these marked papers out of a box. The name of the treatment group written on the paper will be my group assignment.

1. Orientation. There will be two, about 60-minute orientation sessions during which I will be familiarized with the laboratory environment and equipment.

2. If I participate in Experiment 1, I may be randomly assigned to one of the following groups. A. Exercising the right calf muscles with 100% effort. B. Exercising the right calf muscles with 50% effort. C. Exercising the right calf muscles with 10% effort. D. Exercising the right calf muscles with maximal effort imagined muscle actions, or E. Exercising the right calf muscles by having the foot moved by a machine while I relax my leg.

3. If I participate in Experiment 2, I may be randomly assigned to one of the following groups. A. Exercising the right calf muscles with medium intensity voluntary effort. B. Exercising the right calf muscles with muscle contractions produced by therapeutical electrical stimulation. C. Exercising the right calf muscles with medium intensity voluntary effort while my right arm muscles are electrically stimulated, or D. I will not exercise but will report to the laboratory 18 times ("Control group").

4. If I participate in Experiment 3, my left ankle will be put in cast and immobilized for 4 weeks. I will walk around on crutches. I may be randomly assigned to one of the following groups. A. I will not exercise but will report to the laboratory 18 times ("Control group"). B. Exercising the right calf muscles with medium-intensity. C. Exercising the right calf muscles with muscle contractions produced by therapeutical electrical stimulation or D. Exercising the right calf muscles with 100% effort.

Testing procedures. I understand that only the testing procedures that are circled will apply to me. These procedures will be done over two days, totaling 6 hours. I will lie on my stomach on an examination bench.

Voluntary strength. The amount of force I can produce with voluntary effort during ankle extension and ankle flexion will be measured on a computerized strength-measuring device. My foot will be strapped to the measuring arm of the device. As a warm-up, I will do several low-intensity practice trials and my scores will be recorded for six maximal efforts, each lasting 1-2 seconds.

Electrical muscle stimulation. With my foot strapped to the strength-measuring device, water-soaked 2 x 2-inch sponges will be placed on my calf. On the top of these sponges electrodes will be placed and the sponges and the electrodes will be fastened to my leg with Velcro. I will have the opportunity over several practice trials to get familiar with the feeling of my muscle being stimulated. I will feel some discomfort but mostly a "buzzing" sensation. The force my muscles can produce at the highest level of stimulation intensity will be determined. Approximately 6 high-intensity trials will be performed on my muscle. The duration of stimulation will be about 1 to 2 seconds. I will hold the stimulator's safety switch in my hand and I can turn off the stimulation at any time.

Nerve stimulation. The nerve on the back of my knee will be stimulated with a very brief (approximately one hundredth of a second long) electrical pulse. I will receive about 30 pulses at 10 to 15 seconds intervals. These pulses are so short that at low intensities I will not feel anything. At high intensities I will feel my muscle contract.

Magnetic brain stimulation. This technique activates areas of the brain with a magnetic pulse that travels through the skull. A wire coil will be placed near the top of my head. A very brief (one hundred thousand of a second) electrical current is passed through the wire coil and this creates the magnetic pulse that activates or stimulates the brain. When this is done I will hear a click and feel a snapping sensation on the skin under the coil. If the coil is placed over an area of the brain that controls muscles, I will feel a twitch in the muscle, which is often large enough to move the limb. In other cases there I may be feeling a movement or a tingling sensation in my foot. My eyes may blink and my face twitch mildly but I should never feel pain associated with the pulse. The electrical activity in the leg muscle will be recorded with electrodes taped to my skin. My scalp may be marked but these markings will be removed at the end of the session. I will receive about 1000 magnetic pulses with at least 10 seconds between two pulses. I will be told how many stimuli to expect. These experiments last 2 hours. I will be allowed to get up and move around or leave the room.

Exclusion criteria: I may not participate in this project if: I have orthopedic impairments of the lower extremities; I have neurological impairments, including current or past peripheral or central nervous system dysfunction; I am on medications that affect neuronal conduction; I have a pacemaker; I have an implanted medication pump or a metal plate in the skull; I have metal objects in the eye or skull (for example after brain surgery or shrapnel wounds); I am a diabetic, and I consume more than moderate amounts of alcohol or caffeine (more than 4 cups prior to testing). If I am a woman I must use effective means of birth control because the effects of magnetic brain stimulation on embryonic development are unknown and maximal effort contractions are also contraindicated in pregnancy.

Risks: Maximal effort is associated with an increase in heart rate and blood pressure and such changes involve the risk of a heart attack or restriction of blood supply to the heart. Dizziness, overexertion, muscle strain or joint sprain may also occur.

Risks associated with electrical stimulation, such as electrocution or burns, will be avoided by using a so-called isolation unit. This unit isolates me from the main electric line in the wall. Because the duration of the pulse is extremely brief during nerve stimulation, the risks for nerve damage are minimal.

Exposure to magnetic brain stimulation is contraindicated in people who have a pacemaker, an implanted medication pump, a metal plate in the skull, or metal objects inside the eye or skull (for example, shrapnel wounds). Magnetic stimulation may cause slight discomfort lasting less than a second on the scalp near the coil. It may cause some twitching of the face or jaw, which may be unpleasant but not painful.

Magnetic brain stimulation has been used on thousands of individuals in the United States and around the world without any serious problems. The risk of a stroke or other permanent injury is minimal. There are no known long-term risks of magnetic brain stimulation. The principal investigator received training at the National Institutes of Health as well as at the Prince of Wales Medical Research Institute, Sydney, Australia to administer magnetic brain stimulation.

Limb immobilization is inconvenient but pain-free. In extremely rare cases immobilization may cause deep vein thrombosis (DVT). The chance of this occurring in a healthy individual is very small. Please notify the principal investigator at once if symptoms of DVT appear, including swelling of the leg, swelling of the toes, pain inside the leg, or any unusual symptoms while wearing the cast. Individuals with a history of varicose veins (i.e., swollen veins), severe calf muscle injury, leg bone fracture, and smoking are at a greater risk to develop DVT.

I will be fitted with a stump sock and felt pads over bony spots to avoid the bruising of the skin in the cast. I will be asked to report to the laboratory the day after the cast was applied to determine that is comfortable (not too tight or loose). Based on this inspection, the cast will be modified if necessary. I am asked to contact the research staff immediately if the cast causes any discomfort. Immobilization reduces muscle strength but the training protocols will reduce this strength loss.

Benefits: The principal investigator or his associate will explain me the results that came from the specific experiment I participated in after the data will become available (1-2 months after my participation ends). These experiments help us better understand how the two sides of the brain work together and control voluntary movement.

Compensation: If I am in Experiment 1, I will be entitled to $300. If I am in Experiment 2, I will be entitled to $300 (voluntary group), $500 (electrical stimulation group), or $150 (control group). If I am in Experiment 3, I will be entitled to $1,000. The payment will be available to me upon the completion of the study or will be prorated in proportion to the extent of participation according to the following schedule. I will receive about 25%, 50%, 75%, or 100% of the payment for about 25%, 50%, 75%, or 100% completion of the specific experiment.

Withdrawal, Injury, Confidentiality: The nature and purpose of the procedures, the known risks involved, and the possibility of complications has been explained to me, and I understand them. No guarantee of assurance has been given by anyone as to the results that may be obtained. I understand that not all risks and side effects of these treatments are foreseeable.

I understand that participation in these experiments is voluntary and refusal to participate will involve no penalty or loss of benefits to which I am otherwise entitled, and I may discontinue participation at any time without penalty. The principal investigator may terminate my participation in case of I manifest an undesirable response to the training or testing protocol. The principal investigator may also end my participation in the study if I am not abiding by the inclusion criteria in the study. The policy of East Carolina University does not provide for compensation or medical treatment for subjects because of physical or other injury resulting from this research activity. However, every effort will be made to make the facilities of the School of Medicine available for treatment in the event of such physical injury.

I understand that my personal data will be held in strict confidence by the investigators. I understand that if any publications result from this study my name or any identifiable codes will not be used.

Contact person. If I have any questions about the research or possible research-related injury, I may contact Dr. Hortobágyi at home ([252] 355 - 7715) or work ([252] 737- 4564). Also, if questions arise about my rights as a research subject, I may contact the Chair of the University and Medical Center Institutional Review Board ([252] 744 - 2914). I have read the above material and it has been explained to me by Dr. Hortobágyi. I have been encouraged to ask questions about the study and all inquiries have been answered to my satisfaction.

_____
Subject's Name (Print)

_____ _____
Subject's Signature Date

_____
Name of Witness (Print)

_____ _____
Signature of Witness Date

Tibor Hortobágyi
Name of PI

_____ _____
Signature of PI Date

Joseph Armen, DO
Name of Physician

_____ _____
Signature of Physician Date