TASK DEPENDENT EFFECTS OF BARORECEPTOR UNLOADING ON MOTOR CORTICAL AND CORTICOSPINAL PATHWAYS

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Vasiliy E. Buharin

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TASK DEPENDENT EFFECTS OF BARORECEPTOR UNLOADING ON MOTOR CORTICAL AND CORTICOSPINAL PATHWAYS

Approved by:

Dr. T. Richard Nichols,
Committee Chair
School of Applied Physiology
Georgia Institute of Technology

Dr. Boris Prilutsky
School of Applied Physiology
Georgia Institute of Technology

Dr. Minoru Shinohara, Advisor
School of Applied Physiology
Georgia Institute of Technology

Dr. Lewis Wheaton
School of Applied Physiology
Georgia Institute of Technology

Dr. Andrew J. Butler
Department of Physical Therapy
Georgia State University

Date Approved: 21 August 2013

This thesis is for everyone. Read it.

ACKNOWLEDGEMENTS

I want to thank my wife for making me breakfast.

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ABBREVIATIONS

A.U. arbitrary units

AMT active motor threshold

ANOVA analysis of variance

CSP_H cortical silent period elicited by high intensity stimulation

CSP_L cortical silent period elicited by low intensity stimulation

ECG electrocardiogram

ECR extensor carpi radialis

EMG electromyogram

EMGmax electromyogram amplitude during maximal voluntary contraction

EPSP excitatory post-synaptic potential

FCR flexor carpi radialis

FDI first dorsal interosseus

GABA gamma-aminobutyric acid

Glu glutamate

H-reflex Hoffmann reflex

HR heart rate

HR_{LF} fraction of heart rate variability power in the low frequency band

I-waves indirect waves

ICF intracortical facilitation

IPSP inhibitory post-synaptic potential

LBNP lower body negative pressure

M-wave compound muscle action potential

MAP mean arterial blood pressure

MEP motor evoked potential

Mmax maximum M-wave

mmHg millimeters mercury

MSO maximal stimulator output

NMDA N-methyl-D-aspartic acid

NTS nucleus of the solitary tract

PPamp peak-to-peak amplitude

RMS root mean square

RMT resting motor threshold

SICF short-interval intracortical facilitation

RMT resting motor threshold

SICF short-interval intracortical facilitation

SICI short-interval intracortical inhibition

SNA sympathetic nerve activity

TMS transcranial magnetic stimulation

SUMMARY

Corticospinal and intracortical excitability are excitability measures of the central nervous system responsible for motor generation, and are studied for their contribution to fine motor skill execution and learning. Since the need for proper execution of fine motor skills is ever-present and necessary for everyday life, identification of physiological pathways that may disrupt or enhance corticospinal and intracortical excitability is an important research topic. This thesis investigates the effects of baroreceptor unloading on corticospinal and intracortical excitability during various motor tasks. Baroreceptor unloading is a physiological response to common hemodynamic stress (e.g. hypovolemia and orthostasis). The motor tasks investigated are complete muscular relaxation, individual isometric low-force contraction of a muscle, and an isometric co-contraction of a muscle in a joint-stabilizing task. The effects of baroreceptor unloading on corticospinal and intracortical excitability appear to be very task specific. The results are discussed in view of available pharmacological and physiological research, and potential neural pathways for the observed effects are suggested. The overall conclusion is that baroreceptor unloading increases corticospinal excitability and decreases intracortical inhibition in a resting muscle, does not produce any observable effects during individual muscle activity, and decreases corticospinal excitability during joint-stabilizing co-contraction.

CHAPTER I

BACKGROUND

1.1 Introduction

Think back to the time you learned how to write with a pen. Children spend multiple grades improving their handwriting. Penmanship is an example of a fine motor skill, a motor pattern that requires precise coordination of various muscles acting across multiple joints, producing very small and precisely controlled forces [110]. To execute fine motor skills successfully, an individual may need to recruit muscles individually (for movement) or to co-contract multiple muscles (for joint stabilization). The high degree of aptitude for fine motor skills among humans makes tasks that require fine motor skills commonplace. An individuals quality of life, source of income, independence, and, in extreme cases, life itself can all depend on the ability to execute fine motor skills.

Humans talent for executing fine motor skills results from the way the nervous system controls motor tasks. The most direct path for a motor command, originating in the primary motor cortex, to take to reach the spinal motor-neurons is via the corticospinal tract neurons [6, 82]. Corticospinal tack neurons are nerve cells that form monosynaptic connections between the primary motor cortex and the spinal motor-neurons [6, 82]. Muscles commonly involved in fine motor skills, such as muscles that control the hands and wrists, show greater corticospinal innervation than muscles involved in more gross movements, such as the trunk muscles [133]. Excitability of the corticospinal tract (corticospinal excitability) and of the primary motor cortex (intracortical excitability) are, therefore, investigated in studies focusing on the mechanisms of fine motor skill production.

The ability to execute fine motor skills is susceptible to various physiological stressors. To use the example above, compare the appearance of your writing before and immediately after running for 3.14 miles. Under physiological stress muscle force production and coordination [93, 147, 151, 155] are altered, leading to decreased fine motor skill performance [155]. Considering the importance of fine motor skills and the ubiquity of physiological stressors experienced daily, a thorough knowledge of the modification of the mechanisms of fine motor skill would prove useful not only in better characterizing the effects of physiological stress, but also in developing practice regimes for ameliorating such effect.

This thesis establishes the effects of baroreceptor unloading on corticospinal and intracortical excitability during three different motor tasks that would be involved in fine motor skill. Baroreceptor unloading was chosen as the physiological stress of study because of the wealth of knowledge of the mechanisms activated by the baroreceptor reflex and because baroreceptor unloading is a common physiological stress experienced throughout the day [26, 50]. The three motor tasks studied were 1) no muscle activity, 2) individual muscle activity, and 3) co-contraction of muscles in a joint-stabilizing task. Following the relevant scientific background, each Specific Aim (Table 1) is explored separately. Then, the results of all three aims are integrated together, the scientific and functional significance of this work is discussed, and future directions of research are suggested.

1.2 Baroreceptor unloading

Baroreceptors are a group of tonically active stretch-sensitive sensory organs located on arteries whose discharge frequency is directly related to the amount of mechanical distension they experience. Baroreceptors sense blood pressure within arteries indirectly by sensing the amount of arterial distension and provide an input to the baroreceptor reflex – a negative feedback loop for short-term control of arterial blood

pressure mediated via the autonomic nervous system. The main baroreceptor signal comes from the baroreceptors located in the carotid sinus and the aortic arch, key high pressure sites within the circulatory system [10].

The processing of the baroreceptor signal is performed by a group of medullary nuclei, collectively called the medullary cardiovascular center. The medullary cardiovascular center received and integrates input from various parts of the central and peripheral nervous systems, including peripheral baroreceptors and chemoreceptors, the hypothalamus, and the cerebral cortex. The baroreceptors project to and excite a portion of the medulla called the nucleus of the solitary tract (NTS). NTS projects to and inhibits the vasomotor area of the ventrolateral medulla and the cardioacceleratory area of the dorsal medulla. The vasomotor and cardioacceleratory areas projects to sympathetic preganglionic neurons in the spinal cord, which, in turn, excite postganglionic sympathetic nerves, increasing vasoconstriction, heart rate, and heart contractility. Some preganglionic sympathetic neurons innervate the chromaffin cells of the adrenal medulla. Chromaffin cells release epinephrine and norepinephrine into the bloodstream in response to excitation by the preganglionic sympathetic neurons. Inhibition of the dorsal ventrolateral medulla (and subsequent inhibition of sympathetic nerve activity, SNA) by interneurons from the NTS provides the vascular control and a portion of the cardiac control of the baroreceptor reflex. Additional cardiac control of the baroreceptor reflex comes from the cardioinhibitory area, made up of the dorsal motor nucleus of the vagus and nucleus ambiguus, activated by excitatory interneurons from the NTS. The cardioinhibitory area is composed of preganglionic parasympathetic neurons, which project to small parasympathetic ganglia in the atria of the heart. Postganglionic parasympathetic neurons innervate the SA node, the atria, and the ventricles. Activity of the postganglionic parasympathetic fibers slows conduction through the heart [10].

Baroreceptor unloading constitutes any stimulus (e.g. hypovolemia, orthostasis)

that decreases the distension of the baroreceptors. Unloading of the baroreceptors decreases their discharge frequency, thereby decreasing the input to the NTS. Less active NTS, in turn, produces less inhibition of the vasomotor and cardioacceleratory areas and less excitation of the cardioinhibitory area. Removal of inhibition of the vasomotor and cardioacceleratory areas raises SNA, thereby increasing vasoconstriction, heart rate, heart contractility, and plasma epinephrine and norepinephrine. Decrease in excitation of the cardioinhibitory area lowers the parasympathetic input to the heart, diminishing its heart rate lowering effects. Vasoconstriction increases venous return and increased heart rate and heart contractility increase cardiac output, thereby restoring arterial blood pressure back to the pre-stress level.

The lower body negative pressure (LBNP) procedure is a human model of acute central hypovolemia [26, 50]. LBNP of 40 mmHg unloads baroreceptors and results in increased plasma epinephrine and norepinephrine [145, 58], increased discharge rate of muscle sympathetic nerves [143, 145, 31], increased low-frequency content of heart rate variability [85], and increased heart rate with little change in mean arterial blood pressure [143, 145, 31, 137]. During the LBNP procedure the participant lies supine with their lower body inside the LBNP chamber. Baroreceptor unloading is achieved by lowering the pressure inside the chamber. The LBNP procedure was employed in the current studies to unload the baroreceptors.

1.3 Methods for quantifying neuromuscular pathways of fine motor skill

Figure 1 depicts a simplified view of the neuromuscular pathway of a motor command for a fine motor skill. The command originates in the interneurons of the motor cortex. From their, the most direct path to the spinal motor-neurons of the muscle is via the monosynaptic corticospinal neurons [6, 82]. Some corticospinal neurons project to spinal interneurons. The spinal interneurons may modify spinal motor-neuron activity and excitability, influencing the motor command at the spinal level.

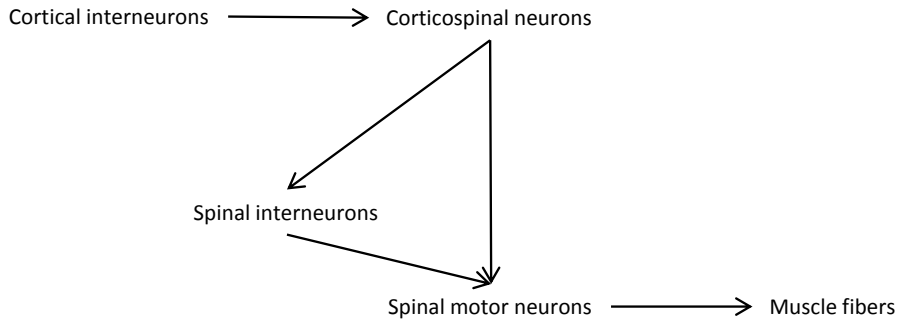

Figure 1: This schematic illustrates the groups of neurons involved in fine motor skill execution. The motor command originates in the interneurons of the motor cortex. From there, the most direct path to the spinal motor-neurons of the muscle is via the monosynaptic corticospinal neurons. Some corticospinal neurons project to spinal interneurons. The spinal interneurons may modify spinal motor-neuron activity and excitability, influencing the motor command at the spinal level. Ultimately, the motor command travels down the spinal motor-neuron to activate the muscle fibers.

Ultimately, the motor command travels down the spinal motor-neuron to activate the muscle fibers. Various techniques, discussed below, exist for measuring the activity and excitability of the different sections of this neuromuscular pathway.

1.3.1 Corticospinal Excitability

Corticospinal excitability is a measure of the excitability of the corticospinal tract neurons. Corticospinal neurons form monosynaptic projections from the motor cortex to spinal interneurons and to spinal motor-neurons of muscle [6, 82]. Muscles commonly involved in fine motor skills, such as muscles that control the hands and wrists, show greater monosynaptic corticospinal innervation than muscles involved in more gross movements, such as the trunk muscles [133]. Monosynaptic corticospinal projections to spinal motor-neurons are greatly involved during execution of fine motor skills [95, 111, 12, 13, 88, 129, 132]. Corticospinal excitability increases during voluntary muscle activity [30], with administration of adrenergic [8, 117, 118, 67] and serotonergic [66, 47] drugs, and during encoding of a novel fine motor skill within the motor cortex [113, 114]. A decrease in corticospinal excitability can be observed with the administration of dopaminergic drugs [78] and following the acquisition of a fine

motor skill [113]. Corticospinal excitability is, therefore, studied as one of the mechanisms of voluntary fine motor skill production [88, 129, 132] and of neural plasticity of the primary motor cortex [113, 114].

Various tools exist for studying corticospinal excitability. Transcranial magnetic stimulation (TMS) is a novel technique that is able to assess corticospinal excitability noninvasively and painlessly, using principles of magnetic induction. TMS induces an electrical field in the brain. At threshold TMS intensity, the induced electrical field activates the corticospinal neurons proximally to the axon hillock [130, 36]. The precise mode of activation is not clear – threshold TMS may active the corticospinal neurons trans-synaptically, by activating the axons of the low-threshold excitatory interneurons that synapse onto corticospinal neurons [33, 32, 131, 130], or at the soma of the corticospinal neurons themselves [38, 39, 101]. Pharmacological studies reveal that the TMS threshold is mainly dependent on Na^+ and K^+ channel kinetics [156]. It is likely that at threshold intensity, the electrical field set up by TMS creates depolarizing currents in the axons of the low-threshold excitatory interneurons, generating action potentials which cause excitatory neurotransmitter release onto the corticospinal neurons; or it results in enough depolarizing currents at the soma of the corticospinal neurons to generate an action potential. Either way, however, responses evoked by TMS are influenced by cortical pathways.

As TMS intensity increases, higher threshold cortical excitatory interneurons are recruited (Fig. 2) [36]. The latency of the descending spinal volleys generated by TMS is used to infer the populations of excitatory interneurons activated by TMS. For a resting hand muscle, with the TMS coil held with the handle pointing posteriorly at an angle of approximately 45 degrees to the sagittal plane yielding an E-field perpendicular to the central sulcus, at threshold intensity, TMS generates the first indirect wave (I-1 wave) [11, 36]. As TMS intensity increases later indirect waves (e.g. I-2 and I-3 waves) are generated [36]. At sufficiently high TMS intensity direct

excitation of the corticospinal tract is possible [36]. Due to the cortical nature of corticospinal neuron activation by TMS, reversing the direction of the induced current within the brain increases the motor threshold [11]. With this "reversed orientation" TMS, the first descending volleys elicited are I-3 waves [150].

TMS delivered over a muscle's motor representation area at threshold intensity and higher produces a motor evoked potential (MEP) in the corresponding muscle. The size of the MEP is often used to quantify corticospinal excitability [115]. However, the MEP is not a straight forward measure of the monosynaptic corticospinal neuron excitability. If TMS activates corticospinal neurons at the soma, an increase in MEP size for a given stimulation could result from an increase in the number of discharges of a single corticospinal axons or an increase in the total number of corticospinal neurons activated. If TMS activates corticospinal neurons trans-synaptically, an increase in MEP size may additionally result from an increase in recruitment of excitatory interneurons leading to activation of more corticospinal neurons. Since the MEP reflects descending activity in multiple corticospinal neurons, synchronicity of the descending activity will affect MEP size. Changes in synchronicity of the descending activity can be inferred from differences in the response of MEP area and MEP peak-to-peak amplitude. Disynaptic contribution to MEP size cannot be excluded [115]. An addition of a chemical synapse to otherwise direct descending projections to the motor-neurons may increase the conduction time by as much as 2 ms [97], which is not enough to discern in an MEP with a duration of 20 ms. Therefore, parts of the MEP may reflect corticospinal activity influencing motor-neurons via spinal interneurons. Other variables that may affect MEP size include, spinal interneuron activity, spinal motor-neuron excitability, and muscle fiber excitability. Consideration of these variables is important in properly interpreting MEP data.

Somatosensory activity, which is present during voluntary contraction, can augment corticospinal excitability. In a resting muscle, electrical stimulation over somatosensory afferents of a muscle was shown to be capable of increasing and decreasing MEP size in that muscle, depending on the interval between the peripheral stimulation and TMS [34]. This suggests that somatosensory activity provides an input to both inhibitory and excitatory motor pathways within the central nervous system. Of course, the short, precisely timed pulses of somatosensory input caused by electrical stimulation are physiologically different from the tonic increase in somatosensory activity during voluntary contraction. During small isometric contraction, corticospinal excitability increases and MEP variability decreases [30]. This increase in corticospinal excitability likely reflects the net effect of somatosensory input on various motor pathways, as well as the contributions of central motor command and the activation of descending motor pathways, and underscores the need for investigating corticospinal excitability during different tasks for a thorough understanding of the motor system.

1.3.2 Intracortical Excitability

Intracortical excitability is a measure of the excitability of the excitatory and inhibitory interneurons within the motor cortex. The cortical interneurons can contribute to motor command by modifying corticospinal excitability [80]. Some intracortical excitability measures are associated with activity via the NMDA receptor, a molecular coincidence detector underlying cortical neural plasticity [89, 136]. Intracortical excitability is, thus, studied for its contribution to neural plasticity and motor command.

By delivering two stimuli in rapid succession of each other, paired-pulse TMS can be used to infer the excitability of distinct populations of intracortical excitatory and inhibitory interneurons. Depending on the interstimulus interval and the order of

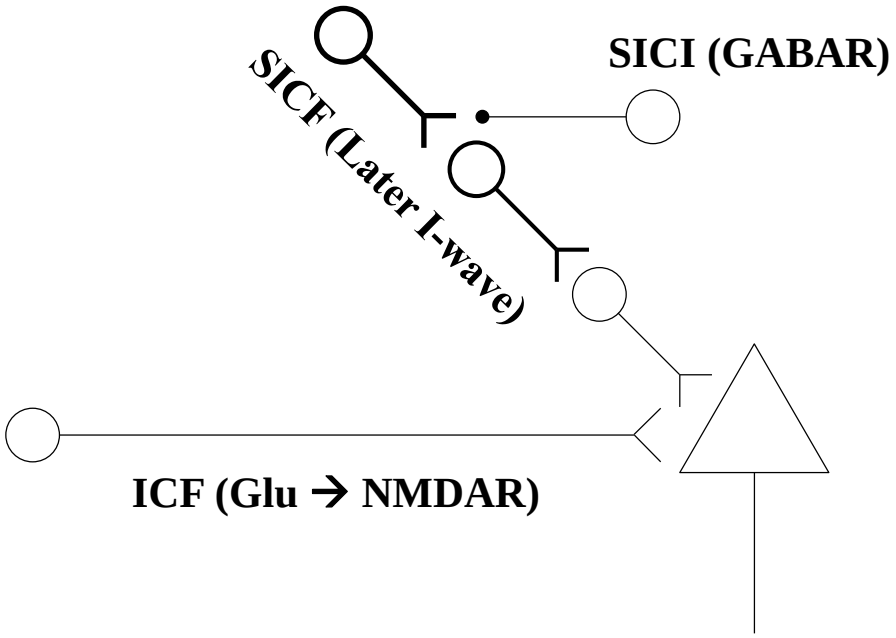

Figure 2: Schematic of populations of intracortical neurons and the paired-pulse protocols used to assess their excitability. Excitatory interneurons are denoted with forks at their axon terminals; inhibitory interneurons are denoted with solid circles. SICF, short-interval intracortical facilitation; SICI, short-interval intracortical inhibition; ICF, intracortical facilitation; GABAR, GABA receptor pathway; NMDAR, NMDA receptor pathway; Glu, glutamate.

the two stimulation pulses, a subthreshold conditioning TMS pulse can decrease or increase the amplitude of the suprathreshold test pulse MEP [80, 56]. In order to ensure that the paired-pulse stimulation is assessing cortical effects, the subthreshold conditioning pulse is often set below the MEP threshold for the active muscle. Such low stimulation is unlikely to produce any spinal volleys, supporting the notion that any measured effects are localized supraspinally [37].

The excitability of a portion of the excitatory glutamatergic interneurons that generate later I-waves can be investigated with short-interval intracortical facilitation (SICF, Fig. 2) [56]. In this paired-pulse stimulation design, the subthreshold conditioning pulse is delivered 1.5 ms after the suprathreshold test pulse. The 1.5 ms interstimulus interval corresponds to the delay between I-waves [36]. In the SICF procedure, the suprathreshold test stimulation activates the later I-wave generating

interneurons. Approximately 1.5 ms later these interneurons produce excitatory post-synaptic potentials (EPSP) at their target neurons (I-1 wave generating 0neurons). At this moment, the subthreshold conditioning stimulation is able to activate the I-1 wave generating interneurons that were brought closer to threshold by the test stimulation [56]. Since, by itself, the test stimulation also activates the lower threshold I-1 wave generating interneurons, comparison of the MEP in response to paired stimulation versus test stimulation can reveal the excitability of later I-wave generating interneurons. Study of SICF during voluntary contraction may provide additional perspective on the SICF pathway, as low-force voluntary contraction activates the excitatory interneurons that synapse onto the corticospinal tract neurons and decreases the threshold for SICF [109].

When the conditioning pulse precedes the test pulse by 1-5 ms, the test pulse MEP is decreased in amplitude [80]. This stimulation design is termed short-interval intracortical inhibition (SICI). SICI has two peaks of maximal inhibition around 1 and 2.5 ms [44, 127]. The decrease in MEP amplitude during SICI is primarily caused by synaptic inhibition [127] and allows for the assessment of the excitability of a population of inhibitory interneurons (Fig. 2) (though neuronal refractoriness may contribute at the 1 ms interstimulus interval [44]). The subthreshold conditioning stimulation activates low-threshold interneurons. 1-5 ms after stimulation net inhibition of the motor cortex is observed. This is most likely caused by inhibitory interneurons, which produce inhibitory post-synaptic potentials (IPSP) on their target neurons, including later I-wave generating interneurons [37]. When the suprathreshold test stimulation is administered, it encounters hyperpolarized later I-wave generating interneurons. Since they are hyperpolarized, fewer later I-wave generating interneurons are recruited by the test stimulation, which leads to lower corticospinal tract activation and a smaller MEP. The short interstimulus interval used in SICI suggests involvement of the fast-acting ionic gamma-aminobutyric acid

(GABA) A receptor. The amount of inhibition produced during SICI can be increased with the administration of $GABA_A$ agonists [159]. Therefore, SICI is often used as an indirect measure of intracortical $GABA_A$-ergic activity [98].

Though robust, the SICI measurement can sometimes be contaminated by facilitation [98]. In a resting condition, if the interstimulus interval used to assess SICI corresponds to an SICF peak, inhibition will be reduced [116]. This can be avoided by choosing an interstimulus interval corresponding to an SICF trough, such as 2 ms [116]. SICI decreases during voluntary muscle contraction [126, 109]. Part of this decrease in SICI during contraction has been attributed to contamination from SICF, primarily due to a decrease in SICF threshold with contraction [109]. Decreasing the intensity of the subthreshold conditioning stimulus was shown to allow SICI assessment free from SICF interference during voluntary contraction [109].

The decrease in SICI during voluntary contraction [126, 109] may also be due to somatosensory input. Electrical stimulation over motor axons [141, 34] and electrical stimulation of digits [125, 124, 92] was shown to decrease SICI. However, the decrease in SICI is abolished if the interval between digit stimulation and TMS is altered [77]. Hence, the potential effect of somatosensory afferent input on SICI is not robust.

When the conditioning pulse precedes the test pulse by 10-25 ms, the test pulse MEP is increased in amplitude [80]. Termed intracortical facilitation (ICF), this stimulation design allows for the assessment of the excitability of a portion of excitatory interneurons that are physiologically distinct from the population of interneurons assessed with SICF (Fig. 2). Similar to SICI, the subthreshold conditioning stimulation activates low-threshold interneurons. 10-25 ms after the conditioning stimulation the excitatory glutamatergic interneurons enact their effects on their target neurons, generating EPSP. If a suprathreshold test stimulation is delivered while the cortical neurons are brought closer to threshold by the EPSP, the resulting MEP will be greater than when the test stimulation is given alone. The ICF measure disappears during

voluntary muscle activity [109], making it impossible to assess the excitability of the underlying interneuron pathways for a contracting muscle. ICF is decreased with administration of N-Methyl-D-aspartic acid (NMDA) receptor antagonists [134, 158], suggesting that ICF can be used as an indirect measure of excitatory glutamatergic intracortical excitability mediated via the NMDA receptor. The NMDA receptor has been shown to function as a molecular coincidence detector [89, 136], making the NMDA receptor pathway one potential molecular pathway underlying cortical neural plasticity. Hence, assessment of ICF is of particular interest to the study of neural plasticity and motor learning. However, the ICF response is also decreased with $GABA_A$-ergic activity [159]. Therefore, assessment of both SICI and ICF, and their interpretation in concert, is necessary to properly infer effects on NMDA-ergic activity.

When TMS is delivered during voluntary muscle activity, the MEP is followed by a momentary absence of contractile activity [90]. Past 50 ms, this absence of activity, termed the cortical silent period, is attributed to lasting effects of intracortical inhibitory pathways activated by TMS [150]. During voluntary muscle activity, intracortical inhibition is suppressed [126, 109]. TMS delivered during voluntary muscle contraction activates intracortical inhibitory interneurons. The inhibitory interneurons produce IPSP in the excitatory cortical interneurons that were generating muscle activity. The IPSP hyperpolarize the excitatory cortical interneurons, temporarily blocking the descending cortical drive to the muscle. Pharmacological evidence suggests that the duration of the cortical silent period elicited by low intensity stimulation is indicative of excitability of the $GABA_A$-ergic pathway [74], while the duration of the cortical silent period elicited by high intensity stimulation is indicative of $GABA_B$-ergic pathway excitability [74]. Though the cortical silent period and SICI are both used to assess the inhibitory GABA-ergic pathway within the motor cortex, the two TMS protocols assess slightly different, potentially overlapping populations

of interneurons. The intensity of stimulation used in cortical silent period assessment is suprathreshold, allowing for the recruitment of higher threshold inhibitory interneurons. The cortical silent period assesses inhibition over interneurons recruited during voluntary motor activity, while SICI represents inhibition of later I-waves, i.e. interneurons recruited by TMS. At present, it is not known if TMS recruitment of cortical interneurons follows voluntary interneuron recruitment.

Methodological considerations exist for assessing the cortical silent period. The duration of the cortical silent period can depend on the contraction intensity and steadiness of contraction before and after TMS [90]. Variability of the cortical silent period can be diminished by increasing the contraction intensity and by requiring the subjects to sustain a steady contraction past the stimulation [90]. However, a greater contraction intensity would recruit a greater number of intracortical excitatory interneurons that generate muscle activity and may induce fatigue. Therefore, care must be taken when designing a procedure to assess the cortical silent period.

1.3.3 Spinal motor-neuron excitability

Spinal motor-neurons are neurons that originate in the central nervous system and synapse onto muscle. This thesis will focus on somatic motor-neurons. These are motor-neurons that innervate skeletal muscle. Alpha motor-neurons innervate extrafusal muscle fibers – the main force generating muscle fibers; gamma motor-neurons innervate intrafusal muscle fibers, which adjust the sensitivity of muscle spindles – one of the somatosensory organs in the muscle [25].

All descending motor signal, including TMS-generated activity that results in an MEP, has to travel via the spinal motor-neurons to reach the muscle. Consequently, motor-neuron excitability can influence MEP size. Motor-neuron pool excitability can be estimated using the Hoffmann reflex (H-reflex) [112]. One of the inputs to alpha motor-neurons is from somatosensory afferents of the muscle. By electrically

stimulating the somatosensory afferent axons and observing the size of the evoked motor activity the H-reflex can estimate alpha motor-neuron pool excitability. Since the stimulation is given over somatosensory axons, the size of the H-reflex depends primarily on excitability of the alpha motor-neurons (though presynaptic inhibition of the somatosensory afferents can also contribute).

The H-reflex technique only provides a partial estimate of alpha motor-neuron pool excitability. Anatomically, the somatosensory afferent axons and the motor-neuron axons lie in the same nerve bundle. Electrical stimulation over the nerve bundle meant to activate somatosensory afferents can also activate the motor-neurons. This is especially true at higher intensities of stimulation [112]. The antidromic activity generated in the motor-neurons by electrical stimulation will interfere with and diminish the H-reflex [112]. Furthermore, the motor-neurons activated in the H-reflex may not be the ones activated by TMS [115]. As such, H-reflex results should be interpreted appropriately.

1.3.4 Spinal interneuron excitability

Spinal interneurons are neurons in the spinal chord that allow for local processing of the motor signal. The Ia inhibitory interneurons, for example, inhibit the alpha motor-neurons of a muscle. Ia inhibitory interneurons receive input from group I somatosensory afferents of various muscles [99, 148, 103] and from descending corticospinal neurons [43]. Inhibition of alpha motor-neurons by the Ia inhibitory interneuron due to somatosensory activity from a muscle is called disynaptic inhibition. Changes in the activity and excitability of spinal interneurons can modify spinal motor neuron excitability and affect MEP size.

For true antagonist pairs, such as ankle plantar- and dorsiflexors, somatosensory afferents from one muscle inhibit alpha motor-neurons of the antagonist [99]. Thus, during activation of the soleus muscle, disynaptic inhibition insures minimal opposing

activity from the tibialis anterior muscle, and vice-versa [99]. This is beneficial when the muscle is activated as a primary mover [103]. During a co-contraction task, such as for joint stabilization, the excitability of the Ia inhibitory interneuron is greatly reduced [99, 103]. Though not true antagonist pairs [148], flexor (FCR) and extensor carpi radialis (ECR) muscles of the forearm exhibit similar disynaptic inhibitory behavior [99]: somatosensory afferents from the ECR inhibit FCR alpha motor-neurons via the Ia inhibitory interneurons, and the excitability of the Ia inhibitory interneurons is reduced during co-contraction of the two muscles in a joint-stabilizing task.

In the forearm, it is possible to quantify disynaptic inhibition from the extensor to the flexor, and from it infer the excitability of the Ia inhibitory interneuron pool, using the conditioned H-reflex technique. Electrical stimulation of the ECR afferents would result in excitation of the Ia inhibitory interneurons and subsequent inhibition of the alpha motor-neurons of the FCR muscle. By timing the conditioning electrical stimulation of the ECR afferents with the H-reflex of the FCR muscle, and comparing the evoked motor activity with the unconditioned FCR H-reflex, it is possible to assign a value to the amount of disynaptic inhibition present at the spinal level.

1.3.5 Muscle fiber excitability

Muscle fibers are similar to neurons in that their excitability is determined by a membrane potential set up by ion channels. To check whether muscle fiber excitability remains constant during the course of an experiment, electrical stimulation can be used. By stimulating the alpha motor-neuron axons a compound muscle action potential (M-wave) will be elicited. Since the stimulation is delivered over the motor-neuron axons, the M-wave represents excitability at and distal to the motor end-plate [112].

1.4 Joint-stabilizing co-contraction

The previous section gave an overview of the motor pathways involved in fine motor skill generation, methods of assessing their excitability, and the influence that individual voluntary muscle contraction may have over these pathways. However, in addition to individual muscle contraction, fine motor skills may require co-contraction of multiple muscles to stabilize a joint. As already alluded to in the spinal interneuron subsection, neural control of joint-stabilizing co-contraction is different from individual muscle contraction [103], and differences have been observed at the corticospinal neuron level, as well [1].

At comparable activation levels, corticospinal excitability of a muscle is greater during individual muscle recruitment than during co-contraction of muscles in a joint-stabilizing task [1]. This reduction due to co-contraction has been attributed to decreased excitability of the monosynaptic corticospinal neurons [1] for two reasons: 1) a decrease in the short-latency facilitation of the H-reflex by TMS [102], and 2) a decrease in the short-latency TMS induced peak of the post-stimulus time histogram of single motor unit discharges [1]. Due to their temporal dynamics, both, the short-latency facilitation of the H-reflex and the short-latency peak of the motor unit post-stimulus time histogram assess the monosynaptic projections from the motor cortex to the spinal motor-neuron pool [103]. When the H-reflex is adjusted to produce a comparable amplitude between single muscle and co-contraction tasks, the amount of TMS induced H-reflex facilitation is not affected by spinal motor-neuron excitability. Maintenance of comparable muscle contraction levels between the two tasks ensures that the observed effects are not influenced by the number of recruited spinal motor-neurons [103]. Finally, transcranial electrical stimulation, at threshold levels, activates the axons of corticospinal neurons [36] and is therefore unaffected by changes in the excitability of the soma of the corticospinal neurons [130]. The monosynaptic corticospinal explanation for the reduction in MEP size during co-contraction

is further supported by the absence of significant effects of co-contraction on the short-latency facilitation of the H-reflex [102] and on short-latency peak of the post-stimulus time histogram of single motor unit discharges in response to transcranial electrical stimulation [1].

The reduction in corticospinal excitability during joint-stabilizing co-contraction is not due to greater inhibition from the GABA-ergic interneurons assessed with SICI. Comparison of SICI during ECR contraction and ECR-FCR co-contraction did not reveal any differences [1]. Put another way, the SICI pathway is not influenced by joint-stabilizing co-contraction.

Other potential causes of the reduced corticospinal excitability during joint-stabilizing co-contraction are 1) cortical reciprocal inhibition and 2) recruitment of co-contraction specific corticospinal tract neurons (Fig. 3). Anatomical studies of the cat motor cortex reveal reciprocal pathways between cortical representations of the wrist flexors and extensors [17]. Functional cortical reciprocal inhibition was recently demonstrated in human electrophysiological studies [7, 62]. Electrical stimulation of the somatosensory afferents of the FCR muscle inhibited TMS evoked MEP in the ECR muscle [7, 62]. The cortical nature of such inhibition is supported by the latency of the inhibition, the dependence of the inhibition on TMS coil orientation, and the lack of inhibition of MEP evoked by transcranial electrical stimulation [7, 36, 150]. Activation of such cortical reciprocal inhibitory pathway during joint-stabilizing co-contraction can decrease corticospinal excitability during co-contraction.

Joint-stabilizing co-contraction may involve co-contraction specific corticospinal neurons that are distinct from those recruited during individual muscle activity. In the monkey motor cortex, multiple representations of a single muscle have been found, suggesting the existence of multiple subgroups of descending neurons, possibly used for functionally distinct tasks [71]. Similarly, an imaging study in humans comparing cortical activation during ankle plantar/dorsiflexion with co-contraction shows

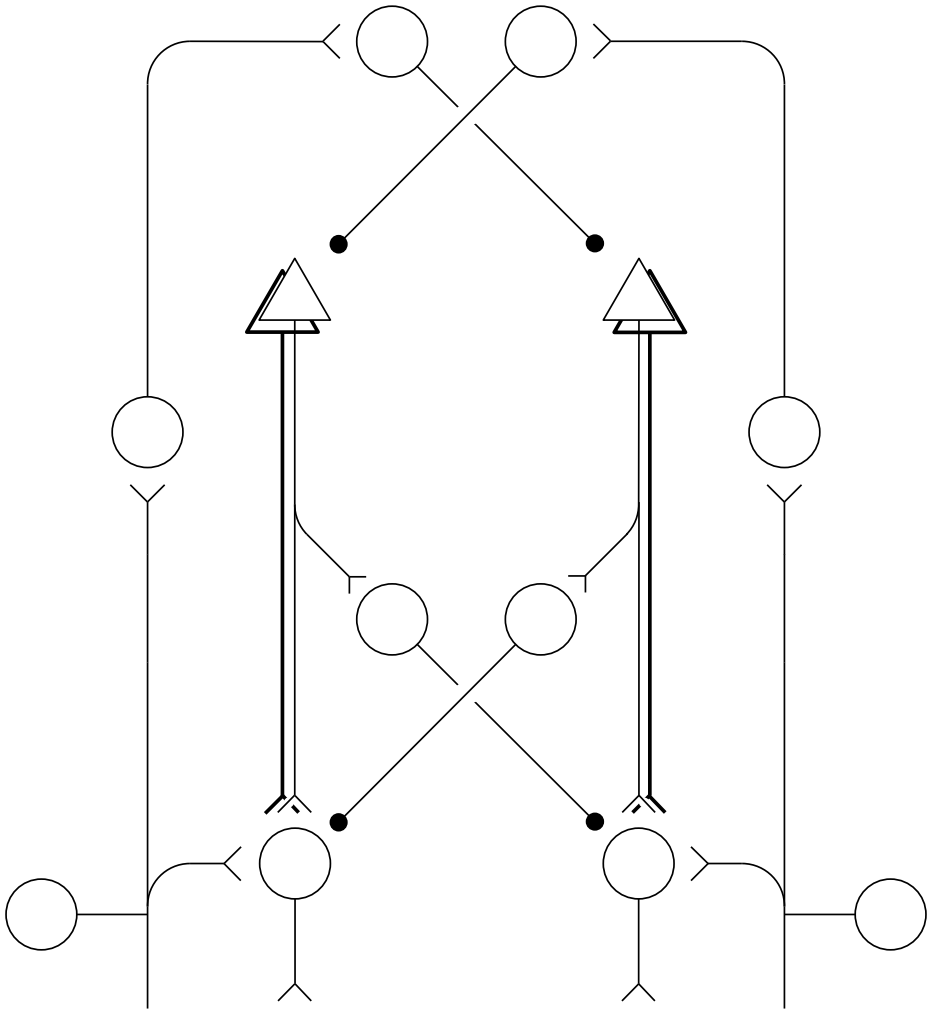

Figure 3: Schematic model of corticospinal neurons (denoted with triangular soma) in a co-contraction task. The low threshold corticospinal neurons in the foreground project to spinal motor-neurons and to Ia inhibitory interneurons. The higher threshold co-contraction specific corticospinal neurons project to the spinal motor-neurons, only. They are in the background, denoted with a thicker contour. Spinal Ia inhibitory interneurons and cortical reciprocal interneurons are denoted with solid circles at their axon terminals.

a shift in the location of cortical peak activation [69]. Groups of cortical neurons exist that are active during co-contraction only, but not during flexion/extension [65]. In the spinal chord, groups of monosynaptic corticospinal neurons with excitatory projections to wrist flexors and inhibitory projections to the extensors show a decrease in activity during co-contraction, as compared to simple flexion/extension [43]. Other descending tract neurons have been identified that are vigorously active during wrist co-contraction, and project to the wrist flexors without presenting any inhibition to the extensors [43]. It is, therefore, possible that different groups of corticospinal neurons are recruited for individual muscle contraction and for joint-stabilizing co-contraction tasks. In this case, the decrease in corticospinal excitability during co-contraction may simply reflect neurophysiological differences between the corticospinal neurons specific to flexion/extension and to co-contraction.

1.5 Potential for influence of baroreceptor unloading over motor pathways of fine motor skill

Baroreceptor unloading may influence the motor pathway via central or peripheral pathways. Baroreceptor unloading dis-inhibits the dorsal ventrolateral medulla, which projects to the locus coeruleus [41, 64]. Mild hypovolemia increases catecholamine release from the locus coeruleus [138, 70], which robustly innervates the neocortex [45, 35]. The locus coeruleus also innervates the dorsal raphe nucleus from which serotonergic neurons spread to higher brain structures. Serotonin release is increased with adrenergic stimulation of the raphe nucleus [23]. Hence, baroreceptor unloading may result in increased function of neuromodulatory monoamines (i.e. norepinephrine, dopamine, and serotonin) within the motor cortex. Drug studies of monoaminergic agents demonstrate profound influence of norepinephrine, dopamine, and serotonin over corticospinal and intracortical excitability [156]. In general, norepinephrine and serotonin agonists increase corticospinal excitability and decrease intracortical inhibition [152, 8, 117, 118, 66, 67, 47], while dopamine agonists have the opposite effect

[157, 160, 78].

Similarly, activation of the locus coeruleus and raphe nucleus by baroreceptor unloading may affect the descending motor pathway at the spinal level [94]. Both, the locus coeruleus and raphe nuclei project to the spinal cord [24, 105, 153, 81] and secrete norepinephrine and serotonin, respectively. The locus coeruleus and raphe nuclei innervate spinal motor-neurons [49, 61, 120, 2], various spinal interneurons [52, 91, 21, 68], descending neurons [52, 53], and motor-sensory neurons [52, 53, 68]. In general, both norepinephrine and serotonin, acting at the spinal cord level, can increase excitability of spinal interneurons and motor-neurons [40, 107, 144, 140, 154, 28, 63, 29], can increase responses evoked by group I motor afferents [68], and can have varying effects on responses evoked by group II motor afferents [104, 123, 52, 68]. Thus activation of locus coeruleus and raphe nuclei by baroreceptor unloading can influence the neural motor pathways at various levels of the central nervous system.

The primary dopaminergic input to the spinal cord comes from the hypothalamus [60, 119, 139]. Dopaminergic neurons innervate spinal motor-neurons [54], various spinal interneurons [54, 55] and motor sensory afferents [4, 20, 22]. Dopamine appears to increase spinal motor-neurons excitability [54] and decrease afferent monosynaptic transmission [20, 22]. The hypothalamus provides one of the inputs to the medullary cardiovascular center [10]. In a purely peripheral unloading of baroreceptors the hypothalamus is unlikely to change in activity. Therefore, dopaminergic influence of motor pathways at the spinal level in response to baroreceptor unloading is not as likely as that of norepinephrine and serotonin.

Baroreceptor unloading also disinhibits sympathetic nerve activity [143]. Sympathetic nerves innervate muscle spindles [3]. Though controversy exists over whether increased sympathetic nerve activity influences muscle spindle discharges [87], a possible increase in somatosensory afferent input is suggested from an increased stretch

Table 1: Table of the motor tasks performed in each Specific Aim

	Specific Aim 1	Specific Aim 2	Specific Aim 3
Target muscle	At rest	At rest/Active	Active/Active
Secondary muscle	At rest	At rest/At rest	At rest/Active

reflex [59, 72] in the presence of an unaltered H-reflex [72] with physiologically heightened sympathetic nerve activity. Electrical stimulation of motor axons and digits has opposing effects on SICI and MEP [125, 141, 124, 92, 34, 77], depending on the timing of the somatosensory input. Thus, baroreceptor unloading may alter the neural motor system by influencing the peripheral motor pathways. Collectively, baroreceptor unloading may influence neuromuscular pathway centrally and peripherally.

1.6 Specific aims

This thesis establishes the effects of baroreceptor unloading on corticospinal and intracortical excitability during three different motor tasks that would be involved in fine motor skill. Baroreceptor unloading was chosen as the physiological stress of study because of the wealth of knowledge of the mechanisms activated by the baroreceptor reflex and because baroreceptor unloading is a common physiological stress experienced throughout the day [26, 50]. The three motor tasks studied were 1) no muscle activity, 2) individual muscle activity, and 3) co-activation of muscles in a joint-stabilizing task. Each Specific Aim (Table 1) is explored separately. Then, the results of all three aims are integrated together, the functional implications of this work are discussed, and future directions of research are suggested.

1.6.1 Specific Aim 1

Purpose: Observe the net effect of baroreceptor unloading on corticospinal excitability for a resting muscle. **Hypothesis:** Unloading of the baroreceptors with 40 mmHg

LBNP will increase corticospinal excitability, as measured by the size of MEP in response to TMS.

Rationale: The first Specific Aim was meant as exploratory. Since influence of physiological stress over the motor system is a novel line of research, a simple investigation into the effects during rest was undertaken. Hemodynamic stress is a common physiological stress that results in baroreceptor unloading; baroreceptor unloading dis-inhibits the dorsal ventrolateral medulla, which projects to the locus coeruleus [41, 64]. Mild hypovolemia increases catecholamine release from the locus coeruleus [138, 70], which robustly innervates the neocortex [45, 35]. The locus coeruleus also innervates the dorsal raphe nucleus from which serotonergic neurons spread to higher brain structures. Serotonin release is increased with adrenergic stimulation of the raphe nucleus [23]. Hence, baroreceptor unloading may result in increased function of neuromodulatory monoamines (i.e. norepinephrine, dopamine, and serotonin) within the motor cortex, which have been shown to affect corticospinal excitability in opposing fashion [156]. While norepinephrine and serotonin agonists increase corticospinal excitability [8, 117, 118, 66, 67, 47], dopamine agonists have the opposite effect [78].

Similarly, activation of the locus coeruleus and raphe nucleus by baroreceptor unloading may affect the descending motor pathway at the spinal level [94]. Both, the locus coeruleus and raphe nuclei project to the spinal cord [24, 105, 153, 81] and innervate spinal motor-neurons [49, 61, 120, 2], various spinal interneurons [52, 91, 21, 68], descending neurons [52, 53], and motor-sensory neurons [52, 53, 68]. In general, both norepinephrine and serotonin, acting at the spinal cord level, can increase excitability of spinal interneurons and motor-neurons [40, 107, 144, 140, 154, 28, 63, 29], can increase responses evoked by group I motor afferents [68], and can have varying effects on responses evoked by group II motor afferents [104, 123, 52, 68].

Finally, baroreceptor unloading heightens sympathetic nerve activity (SNA) [143],

which may alter somatosensory input peripherally. Peripheral effects on the neuromuscular system are suggested by an increased stretch reflex [59, 72] in the presence of an unaltered H-reflex [72] with physiologically heightened SNA. Depending on the timing, somatosensory input has the potential to increase or suppress corticospinal excitability [34]. Collectively, baroreceptor unloading may alter corticospinal excitability by influencing the neuromuscular pathways centrally and peripherally; the directionality of the net effect is not clear. Due to the high increase in norepinephrine in blood plasma with the application of LBNP [145, 58], corticospinal excitability was hypothesized to increase with LBNP.

1.6.2 Specific Aim 2

Purpose: Examine the effects of baroreceptor unloading on intracortical excitability for a muscle at rest and during voluntary contraction. **Hypothesis:** Unloading of the baroreceptors with 40 mmHg LBNP will increase intracortical facilitatory pathways or decrease intracortical inhibitory pathways

Rationale: Results of Specific Aim 1 demonstrated an increase in corticospinal excitability. The increase in corticospinal excitability was observed only at higher TMS intensity. TMS of higher intensity recruits interneurons that generate later I-waves (i.e. I-2 and I-3 waves) in the motor cortex [36], suggesting that greater contribution of later I-waves leads to increased corticospinal excitability with baroreceptor unloading. Contribution of later I-waves to corticospinal excitability may be increased by 1) increasing the activity of the intracortical excitatory glutamatergic pathway responsible for later I-wave generation, assessed with SICF [56] or 2) decreasing the activity of the intracortical inhibitory $GABA_A$-ergic [159] pathway that inhibits later I-waves [37], assessed with SICI [80].

Baroreceptor unloading can affect interneuron excitability at varying levels of the motor system. Baroreceptor unloading dis-inhibits the dorsal ventrolateral medulla,

which projects to the locus coeruleus [41, 64]. Mild hypovolemia increases catecholamine release from the locus coeruleus [138, 70], which robustly innervates the neocortex [45, 35]. The locus coeruleus also innervates the dorsal raphe nucleus from which serotonergic neurons spread to higher brain structures. Serotonin release is increased with adrenergic stimulation of the raphe nucleus [23]. Hence, baroreceptor unloading may result in increased function of neuromodulatory monoamines (i.e. norepinephrine, serotonin, and dopamine) within the motor cortex. Norepinephrine and serotonin agonists decrease SICI [117, 118, 67, 152, 66, 47] while dopamine agonists increase SICI and decrease SICF [157, 160, 78].

Intracortical excitability may also be affected by somatosensory afferent input. Electrical stimulation of motor axons and digits was shown to decrease SICI [125, 141, 124, 92, 34, 77], depending on the timing of the somatosensory input. Baroreceptor unloading can alter somatosensory input centrally and peripherally. Both, the locus coeruleus and raphe nuclei project to the spinal cord [24, 105, 153, 81] and modulate activity of motor-sensory neurons [52, 53, 68]. Activation of the locus coeruleus and raphe nuclei by baroreceptor unloading may lead to increased norepinephrine and serotonin activity at the spinal cord level, which was shown to increase responses evoked by group I motor afferents [68], and have varying effects on responses evoked by group II motor afferents [104, 123, 52, 68]. Finally, baroreceptor unloading heightens SNA [143], which may alter somatosensory input peripherally. Peripheral effects on the neuromuscular system are suggested by an increased stretch reflex [59, 72] in the presence of an unaltered H-reflex [72] with physiologically heightened SNA. Collectively, baroreceptor unloading may alter intracortical excitability by influencing the motor system centrally and peripherally. It was predicted that baroreceptor unloading would change at least one of the TMS measures of intracortical excitatory and inhibitory pathways in the direction for increasing intracortical excitability. Intracortical excitability was studied at rest and during voluntary muscle contraction

because muscle contraction alters intracortical excitability [126, 109] and, as such, may interfere with the effects of LBNP.

1.6.3 Specific Aim 3

Purpose: Examine the effects of baroreceptor unloading on corticospinal excitability for a muscle contracting in a joint-stabilizing co-contraction and individually. **Hypothesis:** Unloading of the baroreceptors with 40 mmHg LBNP will not affect corticospinal excitability of a muscle contracting individually and will increase corticospinal excitability of a muscle co-contracted in a joint-stabilizing task, as measured by the size of the motor potentials evoked by transcranial magnetic stimulation.

Rationale: Specific Aims 1 and 2 demonstrated that, for a resting muscle, baroreceptor unloading increases corticospinal excitability [15], and this increase is, at least partially, explained by decreased GABA-ergic inhibition (as assessed with SICI) at the motor cortex. No effect of baroreceptor unloading on intracortical excitability was observed during voluntary muscle contraction. Voluntary contraction also decreases GABA-ergic inhibition [126, 109]. The lack of effect of baroreceptor unloading on intracortical excitability during voluntary muscle contraction implies that either the disinhibiting effects of baroreceptor unloading were present, however, eclipsed by the disinhibiting effects of contraction or that the effects of baroreceptor unloading on the neuromuscular system were shut off during voluntary contraction. Observing some motor effect of baroreceptor unloading during voluntary contraction would rule out the latter and suggest the former possibility.

From Specific Aim 2 it is known that baroreceptor unloading, assessed with the lower body negative pressure (LBNP) procedure [26, 50], can modulate cortical pathways, in general, and disinhibits the motor cortex, in particular (by suppressing the GABA-ergic pathway). It is, then, possible that the LBNP procedure exerts influence over other cortical inhibitory pathways, as well. Joint-stabilizing co-contraction

is a motor task during which corticospinal excitability decreases [1]. This decrease in corticospinal excitability appears to be cortical in nature and is not mediated via the GABA-ergic pathway [1]. Joint-stabilizing co-contraction, therefore, presents an opportunity to further test if LBNP exerts influence over the neuromuscular system during voluntary contraction.

The purpose of this study was to observe the changes in corticospinal excitability for two tasks – joint-stabilizing co-contraction and individual muscle contraction – during baroreceptor unloading by means of the LBNP technique. Individual muscle contraction was investigated in addition to co-contraction for better interpretation of the data in the context of previous research. In Specific Aim 2, LBNP was shown to exert disinhibitory effects at the cortical level. The decrease in corticospinal excitability during joint-stabilizing co-contraction suggests inhibitory cortical activity [1]. An increase in corticospinal excitability with 40 mmHg LBNP was predicted during joint stabilizing co-contraction because this would imply cortical disinhibition. No effect of LBNP on corticospinal excitability during individual muscle contraction was predicted because of the lack of cortical inhibition during this task [126, 109] and because intracortical excitability was not altered by LBNP in Specific Aim 2.

CHAPTER II

AIM 1: CORTICOSPINAL EXCITABILITY AT REST

2.1 Introduction

Corticospinal excitability, as measured by transcranial magnetic stimulation (TMS), serves as a representation of the summed excitabilities of the central and peripheral nervous systems, and the muscle fibers. Corticospinal excitability is associated not only with the voluntary activation of the motor cortex [88, 129, 132], but with the plasticity of the primary motor cortex [113, 114]. Accordingly, identification of physiological pathways and interventions that enhance corticospinal excitability in humans is an important research topic.

Hemodynamic stress is a common physiological stress that results in baroreceptor unloading. Baroreceptor unloading can influence the motor system centrally and peripherally. Baroreceptor unloading dis-inhibits the dorsal ventrolateral medulla, which projects to the locus coeruleus [41, 64]. Mild hypovolemia increases catecholamine release from the locus coeruleus [138, 70], which robustly innervates the neocortex [45, 35]. The locus coeruleus also innervates the dorsal raphe nucleus from which serotonergic neurons spread to higher brain structures. Serotonin release is increased with adrenergic stimulation of the raphe nucleus [23]. Hence, baroreceptor unloading may result in increased function of neuromodulatory monoamines (i.e. norepinephrine, dopamine, and serotonin) within the motor cortex, which have been shown to affect corticospinal excitability in opposing fashion [156]. While norepinephrine and serotonin agonists increase corticospinal excitability [8, 117, 118, 66, 67, 47], dopamine agonists have the opposite effect [78]. Similarly, activation of the locus coeruleus and raphe nucleus by baroreceptor unloading may affect the descending motor pathway

at the spinal level [94]. Both, the locus coeruleus and raphe nuclei project to the spinal cord [24, 105, 153, 81] and innervate spinal motor-neurons [49, 61, 120, 2], various spinal interneurons [52, 91, 21, 68] and descending neurons [52, 53], and motor-sensory neurons [52, 53, 68]. In general, both norepinephrine and serotonin, acting at the spinal cord level, can increase excitability of spinal interneurons and motor-neurons [40, 107, 144, 140, 154, 28, 63, 29], can increase responses evoked by group I motor afferents [68], and can have varying effects on responses evoked by group II motor afferents [104, 123, 52, 68]. Finally, baroreceptor unloading heightens sympathetic nerve activity (SNA) [143], which may alter somatosensory input peripherally. Peripheral effects on the neuromuscular system are suggested by an increased stretch reflex [59, 72] in the presence of an unaltered H-reflex [72] with physiologically heightened SNA. Depending on the timing, somatosensory input has the potential to increase or suppress corticospinal excitability [34]. Collectively, baroreceptor unloading may alter corticospinal excitability by influencing the neuromuscular pathways centrally and peripherally; the directionality of the net effect is not clear.

The purpose of this study was to observe the net effect of baroreceptor unloading on corticospinal excitability for a resting muscle, quantified as the size of the MEP evoked by TMS. To accomplish this purpose, the size of MEP was compared between physiological conditions with and without the application of lower body negative pressure (LBNP), a human model of acute central hypovolemia [26], in healthy human adults. Due to the high increase in norepinephrine concentration in blood plasma with the application of LBNP [145, 58], corticospinal excitability was hypothesized to increase with LBNP.

Table 2: Baseline characteristics of participants in the Specific Aim 1 study, separated by group. Measurements were taken prior to Trial 1. % MSO, % maximum stimulator output. No significant differences were observed.

	Experimental group	Control group
N	12	9
Age, years	20.9 ± 1.70	20.1 ± 1.45
Handedness index	0.81 ± 0.14	0.82 ± 0.16
Heart rate, beats/min	60.9 ± 8.2	61.0 ± 7.1
Mean arterial blood pressure, mmHg	89.0 ± 7.6	87.9 ± 10.0
Resting motor threshold, % MSO	45.3 ± 7.01	45.9 ± 7.39

2.2 Methods

2.2.1 Subjects

Twenty-one healthy adults (20.6 ± 1.6 years of age, 9 women) were randomly assigned to either the Experimental ($n = 12$) or Control ($n = 9$) group. This subject number was originally based on an *a priori* sample size calculation performed with GPower version 3.1.5 (Universität Kiel, Germany). For a between-within study design with two groups, two measurements of each dependent variable, an alpha error probability of 0.05, power of 0.95, and a small effect size of 0.2, the *a priori* sample size was twenty subjects. All subjects were right-handed, as confirmed with the Edinburgh handedness inventory (Table 2) [106]. Volunteers were free of any signs of chronic altered sympathetic nerve activity: no history of diabetes, cardiovascular problems, brain or nerve disorder, obesity, hypertension, or hypotension [83]. They did not perform extensive hand grip activity, exhibit skilled use of hands report arthritis of the hands, or take any medication that may affect motor control and/or brain and nerve function. In addition, subjects were not allowed to participate if they had a family history of seizure or epilepsy, skin allergies, were pregnant, were prone to severe headaches, or had metal in their head other than dental fillings [73]. To minimize the variability in the basal physiologic level and responsiveness of SNA across subjects,

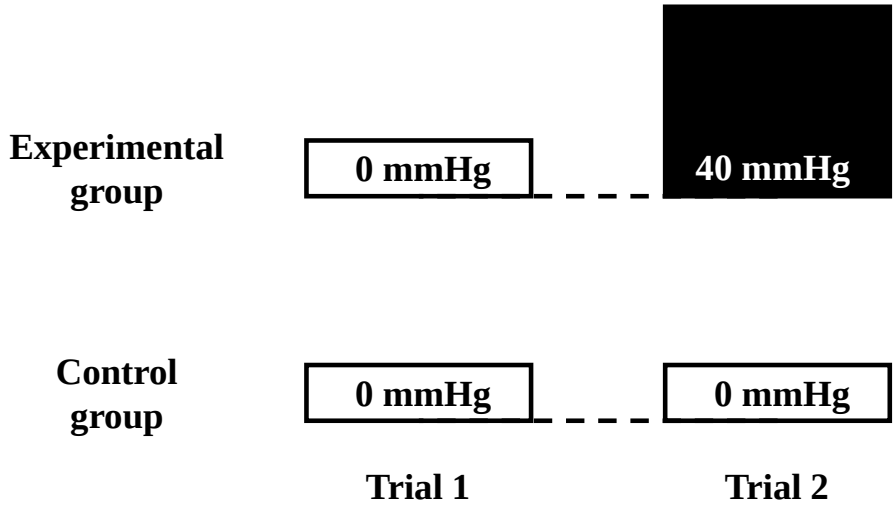

Figure 4: Schematic of protocol used in the Specific Aim 1 study. Both groups of subjects went through two trials of data collection. Lower body negative pressure (LBNP) was applied only in Trial 2 of the Experimental group.

all experiments were conducted at 8 am; participants abstained from food and drink, with the exception of water, for 10 hours prior to the experiment [5]. Women were tested during their follicular phase to avoid potential confounding effects of estrogen and progesterone [96]. All subjects gave written informed consent. Local Institutional Review Boards approved the study.

2.2.2 Experimental approach

Figure 4 shows a schematic of the protocol the subjects went through. MEP of the resting first dorsal interosseus (FDI) muscle was measured in both groups. In the Experimental group, MEP was recorded during 0 mmHg LBNP (basal condition) in Trial 1 and during 40 mmHg LBNP (unloaded baroreceptors) in Trial 2. The two trials were separated by 1 hour. The experimental protocol followed an ordered design to avoid interference of any possible carryover effects of the LBNP procedure. To account for possible effects of order, the Control group went through the same study procedure as the Experimental group above, except MEP was measured during 0 mmHg LBNP (basal condition) in both Trials 1 and 2. Effects of baroreceptor

unloading on corticospinal excitability were assessed as the difference in MEP size between Trial 1 and Trial 2, compared between the two groups.

2.2.3 Baroreceptor unloading

Baroreceptor unloading was accomplished using the LBNP procedure. The participants lay supine with their lower body inside the LBNP chamber (1.2 m $\times$ 0.6 m $\times$ 0.5 m). Subjects wore a neoprene belt about their hips at the level of the iliac crest. An airtight flexible nylon cover was fit over the opening of the LBNP chamber to form a seal between the chamber and the belt. A bicycle saddle in the chamber prevented subject displacement during the application of LBNP. A commercial vacuum (Dayton Industrial, Dayton, OH, USA) attached to the chamber was used to lower the pressure inside the chamber. The LBNP was adjusted with a valve between the vacuum and the chamber. This setup has been used repeatedly in LBNP studies by Seals and colleagues [135, 145, 31]. During Trial 2 in the Experimental group, the pressure in the chamber was gradually reduced to -40 mmHg relative to ambient pressure in 20 s. LBNP of 40 mmHg is known to significantly and substantially increase plasma norepinephrine concentration [145, 58] and heart rate with little changes in blood pressure [137]. The vacuum remained on during the recording of MEPs across trials including when the valve was closed for 0 mmHg LBNP (Trial 1 in the Experimental group and Trials 1 and 2 in the Control group). Blood pressure at the brachial artery in the left arm and heart rate (averaged over 5-7 s) at the fingertip were monitored noninvasively (Cardiocap/5, GE Healthcare, Giles, UK) and recorded at each TMS intensity, 30 s into the block. Measurement of blood pressure took approximately 30 s.

2.2.4 Corticospinal excitability

Corticospinal excitability was assessed noninvasively with single-pulse TMS (Magstim 200^2, by way of BiStim module, Magstim Co, Wales, UK) of the left primary motor

cortex. The head was oriented in neutral position on a pillow; the arms lay at the subjects sides with the right hand resting on the bed in pronation. The subjects were instructed not to move their arms or hands during measurement. The orientation of the right hand was monitored visually throughout the experiment.

The experiment was conducted in an electrically shielded room. Surface electromyogram (EMG) was recorded using 2 Ag-AgCl electrodes (E224A, IVM, Healdsburg, CA, USA) placed on the skin overlying the right FDI in a belly-tendon montage. One electrode was placed over the belly of the FDI muscle and the other was attached to the skin over the base of the proximal phalanx of the index finger, after abrasion of the skin. A wet circumferential strap electrode (F-E10SG1, Grass Technologies, West Warwick, RI, USA) was placed around the right wrist for a reference. The EMG was differentially pre-amplified 300 times (Y03-000, MotionLabs, NY, USA) and further amplified with a custom-built amplifier for a total gain of 1,000.

A figure-of-eight coil (Magstim second generation double 70 mm remote coil, Magstim Co, Wales, UK) was held over the left primary motor cortex at the optimum position (i.e. hotspot) for eliciting an MEP in the resting FDI muscle of the right hand. The coil was held with the handle pointing posteriorly at an angle of approximately 45 degrees to the sagittal plane yielding an E-field perpendicular to the central sulcus [11]. A TMS coil navigation system (NDI TMS Manager, Northern Digital Inc, Waterloo, Ontario, Canada) was used to maintain the coil position in 3-dimensional space relative to the head. The data were sampled at 5,000 samples/s with an analog-to-digital converter (Power 1401, Cambridge Electronic Design Ltd, Cambridge, UK) and data acquisition software (Signal 4.0, Cambridge Electronic Design Ltd, Cambridge, UK) for online monitoring, storage, and offline analysis. Visual feedback of the pre-stimulus EMG was provided to ensure relaxation of the FDI.

The resting motor threshold (RMT) was determined as the smallest TMS intensity needed to elicit an MEP with peak-to-peak amplitude (PPamp) greater than 50 μV

in 5 out of 10 consecutive stimulations [16, 30]. RMT was measured in percentage of maximal stimulator output for each subject at basal SNA, with the vacuum turned off. Previous studies show that the effects of norepinephrine, serotonin, or dopamine agonists on corticospinal excitability may depend on TMS intensity [8, 117, 118, 66, 67, 47, 78]. Therefore, MEPs were collected in response to six intensities of TMS, i.e., 90%, 100%, 110%, 120%, 130%, and 140% of RMT in each subject. Measurements were made in blocks, with 12 MEP responses per block (i.e. per TMS intensity), and the order of the TMS intensity blocks was randomized. In Trial 1, MEPs were collected at ambient pressure (0 mmHg LBNP) in both groups. One hour later, MEPs were collected again at ambient pressure in the Control group and at 40 mmHg LBNP in the Experimental group in Trial 2.

2.2.5 M-wave (supplemental experiment)

After completing the TMS data analysis, a supplemental experiment was performed in a separate group of three subjects to explore the effect of LBNP on the compound muscle action potential (M-wave) of the FDI. These subjects were tested on two days, separated by at least one week, in the same experimental setup as for the TMS experiment. On the Experimental day, the M-wave was measured at 0 mmHg (Trial 1) followed by 40 mmHg (Trial 2). On the Control day, the M-wave was recorded at 0 mmHg in both Trials. The two trials were separated by 1 h, and the testing days were randomized. Electrical stimulation was delivered to the ulnar nerve transcutaneously via two stimulating electrodes at the wrist using a constant current stimulator (Digitimer DS7AH, Digitimer Limited, Herdfordshire, UK). Three levels of submaximal stimulation intensities were set to elicit target M-wave PPamps within the range of 1-3 mV in Trial 1. The target amplitudes were chosen to approximately cover the amplitude range of the MEP observed during the TMS at higher intensities. Supramaximal M-wave was also collected at 150% of stimulation intensity for the

maximal M-wave. Twenty-five stimulations were delivered at each intensity, separated by 10 s. The supramaximal M-wave was collected first, followed by the submaximal M-waves, in random order.

2.2.6 Data reduction

The first 2 MEP responses in each block were discarded to control for any possible startle responses. All remaining recordings were inspected, and, recordings that showed obvious pre-stimulus EMG activity 100 ms preceding the TMS were discarded by visual inspection. The MEP PPamp, the area bound by the MEP and the 0 mV axis (MEP area), and the root mean square (RMS) amplitude of the pre-stimulus EMG were calculated for each response (Fig. 5). Figure 5 shows EMG recordings from Trials 1 and 2 of two representative subjects, one from the Experimental group (top row) and one from the Control group (bottom row). The EMG in the period between 20 and 50 ms following application of TMS was used to measure MEP PPamp and MEP area. In addition to PPamp, MEP area was analyzed to account for potential changes in MEP that may not be reflected in PPamp. The RMS amplitude of the pre-stimulus EMG was calculated from data 100 ms preceding the application of TMS. Values in each response were averaged together within each intensity block.

As supplemental assessment for exploring the potential alteration in the association between MEP and TMS intensity, the linear phase of MEP stimulus-response curve was approximated with the standardized slope of the regression line through the middle four intensities (100-130% RMT). The standardized regression slope was calculated by dividing the slope of a regression line by the ratio of the SDs of each variable, using SPSS software (SPSS Statistics, IBM, Armonk, NY, USA). The standardized regression slope was used because it accounts for differences in the distribution of variables.

M-wave data were analyzed similar to MEP data. The first 2 responses at each

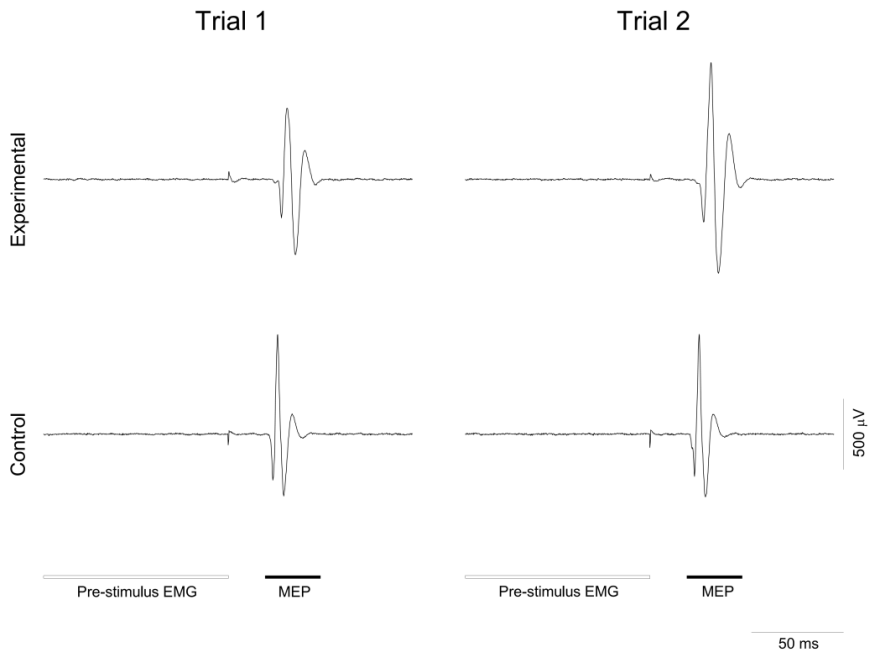

Figure 5: Representative recordings of pre-stimulus electromyogram (EMG) and motor evoked potential (MEP) recorded from the right hand first dorsal interosseus muscle in the Experimental and Control groups. The recordings in response to 130% resting motor threshold stimulation were taken from a subject in the Experimental group during Trial 1 (0 mmHg LBNP) and Trial 2 (40 mmHg LBNP) on the top row and from a subject in the Control group during Trial 1 (0 mmHg LBNP) and Trial 2 (0 mmHg LBNP) on the bottom row. Pre-stimulus EMG 100 ms preceding the transcranial magnetic stimulation (TMS) is indicated (open underscore). Peak-to-peak amplitude and area of MEP were measured in 20-50 ms post stimulus artifact (dark underscore).

intensity of stimulation were discarded to control for possible startle effects. Recordings that showed obvious pre-stimulus EMG activity 100 ms preceding the stimulation were discarded. For the remaining responses, the M-wave PPamp between 3 and 25 ms following stimulation were averaged at each intensity. The relative change in M-wave PPamp from Trial 1 to Trial 2 was calculated by subtracting Trial 1 value from Trial 2, and dividing by Trial 1 value.

2.2.7 Statistical analysis

Subject characteristics include the age, handedness index, baseline heart rate, baseline mean blood pressure, and RMT. The baseline heart rate, baseline mean blood pressure and heart rate were measured with the vacuum turned off before Trial 1. These variables were compared between the two groups with a Students independent samples t-test. The main dependent variables for corticospinal excitability were MEP PPamp and MEP area. The independent variables were Group (Control and Experimental), Trial (1 and 2), and Intensity (90%-140% RMT). To test the effects of baroreceptor unloading on corticospinal excitability, a three-factor (Group, Trial, Intensity) analysis of variance (ANOVA) with repeated measures for Trial and Intensity was used. A significant three-factor interaction with significant differences between Trials 1 and 2 in the Experimental group would indicate effects of LBNP on MEP PPamp and MEP area. Standardized slope of the regression line was analyzed with a two-factor (Group, Trial) ANOVA with repeated measures for Trial. RMS amplitude of the pre-stimulus EMG, heart rate, and mean arterial blood pressure were analyzed with a three-factor (Group, Trial, Intensity) ANOVA with repeated measures to test for differences in pre-stimulus muscle activation and measures of SNA. Inclusion of the Control group in the ANOVAs ensured that any differences seen between Trials 1 and 2 in the Experimental group were not due to order effect. An alpha level of 0.05 was used for all significance testing and $P > 0.05$, textitP < 0.05, and textitP < 0.01

were noted where appropriate. In all repeated measures ANOVA models, Mauchly Test of Sphericity for the interaction was found to be significant. All P-values were adjusted using the Huynh-Feldt Epsilon correction factor. Significant interactions were tested with the Bonferroni post hoc test. Statistical analyses were performed using Statistica 9.0 (StatSoft Inc., Tulsa, OK, USA). Unless stated otherwise, the data are presented as mean ± SD in the text and tables and as mean ± standard error of mean in the figures.

2.3 Results

Basic subject characteristics including age, handedness index, baseline heart rate, baseline mean blood pressure, and RMT were not significantly different between the groups ($P > 0.05$, Table 2). Pre-stimulus EMG did not vary with Intensity, Trial, or Group significantly ($P > 0.05$). Pre-stimulus EMG values were 3.60 ± 0.17 μV and 3.62 ± 0.12 μV in Trials 1 and 2 in the Control group, and were 3.78 ± 1.29 μV and 3.77 ± 1.26 μV in Trials 1 and 2 in the Experimental group, respectively.

2.3.1 Cardiovascular response

In the Control group, heart rate was 60.0 ± 7.1 and 61.8 ± 6.9 beats/min in Trials 1 and 2, respectively. In the Experimental group, heart rate increased from 60.0 ± 7.0 beats/min in Trial 1 to 70.4 ± 9.2 beats/min in Trial 2. After detecting the significant effect of Trial ($P < 0.01$) and Group × Trial interaction ($P < 0.01$), post-hoc testing revealed that heart rate increased significantly from Trial 1 to Trial 2 only in the Experimental group ($P < 0.01$). Mean arterial blood pressure was 86.8 ± 8.7 and 89.0 ± 8.8 mmHg in Trials 1 and 2, respectively, in the Control group. Mean arterial blood pressure was 88.4 ± 6.3 and 89.6 ± 8.6 mmHg in Trials 1 and 2, respectively, in the Experimental group. Although there was a significant effect of trial ($P < 0.05$), the change in mean arterial pressure from Trial 1 to Trial 2 was as small as 1.6 mmHg when collapsed across groups. Mean arterial blood pressure was not influenced by

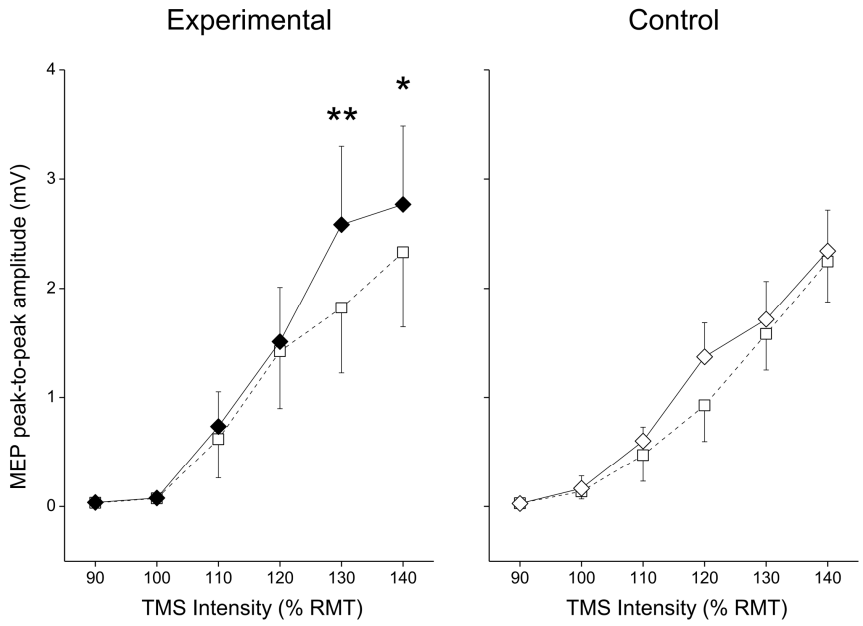

Figure 6: MEP peak-to-peak amplitude in two trials as a function of TMS intensity in the Experimental and Control groups. Square and diamond symbols indicate measurements taken during Trial 1 and Trial 2, respectively. LBNP of 40 mmHg was applied during Trial 2 (filled diamond) in the Experimental group on the left. MEP was enhanced with LBNP in the Experimental group. * $P < 0.05$, ** $P < 0.01$ between trials at the corresponding intensity, revealed by post hoc test of the Group $\times$ Trial $\times$ Intensity interaction. MEP, motor evoked potential; RMT, resting motor threshold.

any interaction.

2.3.2 Corticospinal excitability

The results of two indices of MEP size (PPamp and MEP area) were comparable, and hence only the PPamp results are presented (Fig. 6). The MEP PPamp was influenced by stimulus Intensity ($P < 0.01$), Trial ($P < 0.01$), their interaction ($P < 0.01$), and an interaction of Group, Trial, and stimulus Intensity ($P < 0.01$). Post-hoc testing showed the MEP PPamp in Trial 2 was greater compared with Trial 1 only at 130% RMT (by 42%, $P < 0.01$) and 140% RMT (by 19%, $P < 0.05$) in the Experimental group (Fig. 6, left), but not in the Control group (Fig. 6, right). The standardized

slope of the linear regression of the MEP PPamp was not significantly influenced by Group or Trial, but had a significant Group × Trial interaction ($P < 0.05$). The slope was 0.673 ± 0.147 and 0.619 ± 0.154 in Trials 1 and 2, respectively, of the Control group, and 0.610 ± 0.184 and 0.727 ± 0.144, in Trials 1 and 2, respectively, of the Experimental group. Although the post-hoc test did not detect statistical significance, the slope showed a trend for a 19% increase of MEP PPamp from Trial 1 to Trial 2 in the Experimental group, on average.

2.3.3 M-wave (supplemental experiment)

The elicited M-wave PPamp at each intensity were 1.4 ± 0.3, 2.0 ± 0.2, 2.9 ± 0.6, and 18.5 ± 6.3 mV when averaged across trials, days, and subjects. The changes in M-wave PPamp from Trial 1 to Trial 2 were variable with no clear trend of consistent increase or decrease on the Experimental day. The relative changes in M-wave PPamp from Trial 1 to Trial 2 in each subject were -19.9 ± 14.6% vs. -9.0 ± 31.7%, 9.5 ± 6.7% vs. -3.4 ± 9.2%, and -11.3 ± 5.3% vs. 13.3 ± 11.1% for Control vs. Experimental days, respectively, when averaged across intensities. When further averaged across subjects, the relative change in M-wave PPamp between Trials 1 and 2 was -7.2 ± 15.6% on the Control day and 0.3 ± 20.7% on the Experimental day.

2.4 Discussion

The main findings are the significant increases in the MEP PPamp during the application of LBNP compared with ambient pressure at 130% and 140% RMT TMS intensity in the Experimental group.

2.4.1 Cardiovascular response

Baroreceptor unloading with LBNP in the Experimental group was supported by cardiovascular measurements. In response to LBNP, heart rate increased significantly, while there was little change in mean arterial blood pressure. The mild increase

in heart rate with little change in blood pressure has been observed using similar LBNP procedures (see [50] for review). The LBNP procedure is a commonly used intervention that is regarded as a human model for acute central hypovolemia [26], reducing central venous pressure. The drop in central venous pressure is sensed by baroreceptors, resulting in the heightening of SNA via the baroreflex response [143]. Heightened SNA increases heart rate, cardiac contractility, and peripheral resistance of the cardiovascular system to maintain mean arterial blood pressure [145]. The increased heart rate with little change in mean arterial blood pressure observed in Trial 2 of the Experimental group suggests that the current LBNP protocol was effective at unloading the baroreceptors and heightening SNA [26].

2.4.2 Corticospinal excitability

The influence of baroreceptor unloading on MEP size was examined between trials with and without LBNP in the Experimental and Control groups, respectively. The absence of pre-stimulus EMG activity and lack of difference in the level of pre-stimulus EMG between groups and trials indicated that subjects maintained a resting state across measurements. Significantly greater values in both indices of MEP at 130% and 140% RMT in Trial 2 (with LBNP) compared with Trial 1 (no LBNP) in the Experimental group indicated increased corticospinal excitability with LBNP regardless of the index of MEP size. This indication was strengthened by the significant Group $\times$ Trial interaction for the standardized slope of the MEP size. In the supplemental experiment employing the same LBNP protocol as was used for recording MEP, no clear trend of consistent change was observed in M-wave amplitude due to 40 mmHg LBNP. Hence, the mechanisms responsible for the increased MEP amplitude with LBNP appear to mostly likely be proximal to the neuromuscular junction.

2.4.3 Potential mechanisms

As explained in the Introduction of this chapter, baroreceptor unloading may influence corticospinal excitability via somatosensory afferent input and via central activity of monoamines (norepinephrine, serotonin, dopamine). Neuromuscular studies employing conditioning of TMS MEP by electrical stimulation over motor axons [34] show that somatosensory afferent input can increase or decrease MEP size, depending on the delay. Centrally mediated increase in somatosensory afferent input may arise from norepinephrine and serotonin activity at the spinal level. Both, norepinephrine and serotonin can increase responses evoked by group I motor afferents [68], while having varying effects on responses evoked by group II motor afferents [104, 123, 52, 68]. Peripherally, baroreceptor unloading may increase somatosensory afferent input by increasing SNA [143]. While no influence of SNA on muscle spindle discharge is observed in humans [87], an increased stretch reflex [59, 72] in the presence of an unaltered H-reflex [72] due to physiological heightening of SNA suggests an SNA-induced increase in gain of the somatosensory afferent input. The impact of the potential increase in somatosensory afferent input, or in its gain, in the resting FDI is questionable considering that the corticospinal excitability is not increased with LBNP through TMS intensity of 120% RMT.

The influences of monoamines on corticospinal excitability may be inferred by comparing the current physiological results with previous studies using pharmacological agents for norepinephrine, serotonin, and dopamine in healthy humans. Administration of yohimbine and reboxetine, both norepinephrine facilitating agents, increases corticospinal excitability at and above 130% and 150% RMT TMS intensity, respectively [117, 118]. Administration of the serotonin agonists sertraline and paroxetine, both selective serotonin reuptake inhibitors, also increases corticospinal excitability, but immediately above the resting motor threshold [66, 47]. On the other hand, the dopamine agonist cabergoline decreases corticospinal excitability and does so at TMS

intensities 120% RMT and higher [78]. Methylphenidate and d-amphetamine, both indirect agonists of the dopamine and norepinephrine systems, also increase corticospinal excitability. While methylphenidate starts doing so around 135% RMT [67], d-amphetamine, which has a much greater influence (10 fold at threshold levels) on dopamine function [79] has no significant effect on MEP size at intensities of stimulation as high as 150% RMT [8], potentially reflecting the net result of opposing influences from the two monoamines. In all, the observed increase in corticospinal excitability with LBNP is likely indicative of noradrenergic function, and potentially serotonergic and somatosensory afferent functions, overriding dopaminergic function with baroreceptor unloading.

2.4.4 Implication

Facilitation of MEP at higher TMS intensities provides possibilities that higher TMS intensity is needed to either directly reach (e.g. depth-wise) or activate the populations of neurons most responsive to upregulated noradrenergic function. At the motor cortex, increased monoamine concentration may modulate the responsiveness of corticospinal tract neurons or interneurons, such as the inhibitory gamma-Aminobutyric acid interneurons and facilitatory N-Methyl-D-aspartic acid interneurons. TMS activates the corticospinal tract neurons either at the soma [38, 39, 101] or transsynaptically, by depolarizing the axons of interneurons that synapse onto the tract neurons [33, 32, 131, 130]. As TMS intensity increases, higher threshold interneurons are recruited [36]. The indifferent MEP response to 90-110% RMT TMS intensity with the manipulation of the baroreflex (Fig. 6) indicates that the excitability of the corticospinal tract neurons remains unaffected. Increased synaptic strength or increased excitability of higher-threshold interneurons are, then, the cortical mechanisms potentially underlying the enhanced MEP at higher TMS intensities due to acute mild central hypovolemia induced by LBNP. Both, increased synaptic strength

and increased excitability of the higher-threshold interneurons would recruit more corticospinal tract neurons, leading to a larger MEP.

In conclusion, corticospinal excitability was enhanced with baroreceptor unloading by means of lower body negative pressure [15].

CHAPTER III

AIM 2: INTRACORTICAL EXCITABILITY AT REST AND DURING INDIVIDUAL MUSCLE CONTRACTION

3.1 Introduction

Intracortical excitability contributes to motor command and neural plasticity. In Specific Aim 1 it was shown that corticospinal excitability increases with baroreceptor unloading [15]. Baroreceptor unloading accompanies increased motor cortex activation in an imaging study [75]. The increase in corticospinal excitability with baroreceptor unloading observed in Specific Aim 1 was specific to higher TMS intensity [15]. TMS of higher intensity recruits interneurons that generate later I-waves (i.e. I-2 and I-3 waves) in the motor cortex [36], suggesting that greater contribution of later I-waves leads to increased corticospinal excitability with baroreceptor unloading. Contribution of later I-waves to corticospinal excitability may be increased by 1) increasing the activity of the intracortical excitatory glutamatergic pathway responsible for later I-wave generation, assessed with short-interval intracortical facilitation (SICF) [56] or 2) decreasing the activity of the intracortical inhibitory $GABA_A$-ergic [159] pathway that inhibits later I-waves [37], assessed with short-interval intracortical inhibition (SICI) [80]. The effects of baroreceptor unloading on intracortical excitatory and inhibitory pathways in the motor cortex are unknown.

Baroreceptor unloading can affect interneuron excitability at varying levels of the motor system. The lower body negative pressure (LBNP) procedure, a human model of acute central hypovolemia, can be used to study baroreceptor unloading [26]. Baroreceptor unloading dis-inhibits the dorsal ventrolateral medulla, which projects to the locus coeruleus [41, 64]. Mild hypovolemia increases catecholamine release from

the locus coeruleus [138, 70], which robustly innervates the neocortex [45, 35]. The locus coeruleus also innervates the dorsal raphe nucleus from which serotonergic neurons spread to higher brain structures. Serotonin release is increased with adrenergic stimulation of the raphe nucleus [23]. Hence, baroreceptor unloading may result in increased function of neuromodulatory monoamines (i.e. norepinephrine, serotonin, and dopamine) within the motor cortex. Norepinephrine and serotonin agonists decrease SICI [117, 118, 67, 152, 66, 47] while dopamine agonists increase SICI and decrease SICF [157, 160, 78].

Intracortical excitability may also be affected by somatosensory afferent input. Electrical stimulation of motor axons and digits was shown to decrease SICI [125, 141, 124, 92, 34, 77], depending on the timing of the somatosensory input. Baroreceptor unloading can alter somatosensory input centrally and peripherally. Both, the locus coeruleus and raphe nuclei project to the spinal cord [24, 105, 153, 81] and modulate activity of motor-sensory neurons [52, 53, 68]. Activation of the locus coeruleus and raphe nuclei by baroreceptor unloading may lead to increased norepinephrine and serotonin activity at the spinal cord level, which was shown to increase responses evoked by group I motor afferents [68], and have varying effects on responses evoked by group II motor afferents [104, 123, 52, 68]. Finally, baroreceptor unloading heightens SNA [143], which may alter somatosensory input peripherally. Peripheral effects on the neuromuscular system are suggested by an increased stretch reflex [59, 72] in the presence of an unaltered H-reflex [72] with physiologically heightened SNA. Collectively, baroreceptor unloading may alter intracortical excitability by influencing the motor system centrally and peripherally.

Based on the results in Specific Aim 1, it was predicted that baroreceptor unloading would change at least one of the TMS measures of intracortical excitatory and inhibitory pathways in the direction for increasing intracortical excitability. Intracortical excitability was studied at rest and during voluntary muscle contraction

because muscle contraction alters intracortical excitability [126, 109] and, as such, may interfere with the effects of LBNP.

3.2 Methods

3.2.1 Subjects

Sixteen healthy adults (19.8 ± 1.5 years of age, 6 women) participated in the study on two days separated by approximately four weeks. This subject number was originally based on an *a priori* sample size calculation performed with GPower version 3.1.5 (Universität Kiel, Germany). For a within-group study design with two measurements of each dependent variable, an alpha error probability of 0.05, power of 0.95, and a small effect size of 0.2, the *a priori* sample size was nineteen subjects. Since fewer subjects were recruiter, it should be noted that the obtained sample size is sufficient for statistical testing with power of 0.90, not 0.95. Women were tested during their follicular phase to avoid potential confounding effects of estrogen and progesterone [96]. All subjects were right-handed, as confirmed with the Edinburgh handedness inventory (laterality quotient: 0.82 ± 0.155) [106]. Volunteers were free of any signs of chronic altered SNA: no history of diabetes, cardiovascular problems, brain or nerve disorder, obesity, hypertension, or hypotension [83]. They did not perform extensive hand grip activity, exhibit skilled use of hands, report arthritis of the hands, or take any medication that may affect motor control or brain and nerve function. In addition, subjects were excluded if they had a family history of seizure or epilepsy, skin allergies, were pregnant, were prone to severe headaches, or had metal in their head, excluding dental fillings [128]. To minimize the variability in the basal physiological level and responsiveness of SNA across subjects, all experiments were conducted at 8 am; participants abstained from food and drink, with the exception of water, for 10 hours prior to the experiment [5]. All subjects gave written informed consent. The study conforms to the Code of Ethics of the World Medical Association [122], and

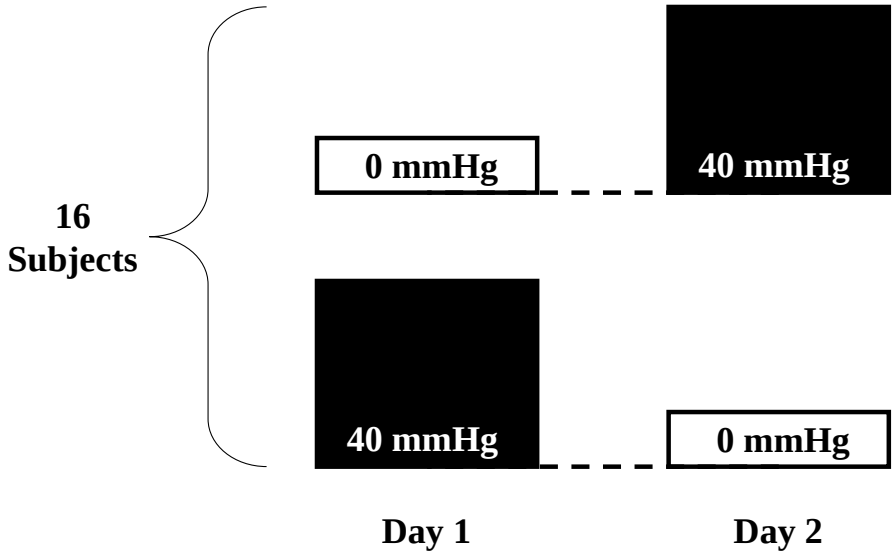

Figure 7: Schematic of protocol used in the Specific Aim 2 study. In this cross-over study design, a total of 16 subjects were tested on two separate days. Intracortical excitability was assessed on both days, at basal condition on the Control day and at 40 mmHg LBNP on the LBNP day. The order of the days was assigned at random.

was approved by the Georgia Institute of Technology and Emory Institutional Review Boards.

3.2.2 Experimental approach

Figure 7 depicts the cross-over study design of Specific Aim 2. Intracortical excitability was assessed with TMS, delivered over the motor representation area of the first dorsal interosseus (FDI) muscle. Motor evoked potentials (MEP) were recorded from the FDI muscle in the right hand with unloaded baroreceptors on the LBNP day and with non-manipulated baroreflex on the Control day. The duration of the experiment session and the tolerability of the experiment procedure by the subjects dictated assessment of intracortical excitability on two separate days. Since intracortical excitability may change with the time of day [84], the experiment protocol of each subject was temporally comparable between the two days. On a given day, intracortical excitability was assessed at rest (Resting stage), followed by FDI contraction (Active stage) with a 10-min intermission (Intermission stage) in between.

The LBNP vacuum was turned off during the Intermission stage. Effects of LBNP on intracortical excitability were interpreted from the differences of corresponding measures between the Control day and the LBNP day. The order of the two days was randomized across subjects.

3.2.3 Baroreceptor unloading

The LBNP procedure in our previous study [15] was employed to unload the baroreceptors. The participants lay supine with their lower body inside the LBNP chamber (1.2 m $\times$ 0.6 m $\times$ 0.5 m). Subjects wore a neoprene belt about their hips at the level of the iliac crest. An air tight flexible nylon cover was fit over the opening of the LBNP chamber to form a seal between the chamber and the belt. A bicycle saddle in the chamber prevented subject displacement during the application of LBNP. A commercial vacuum (Model 3Z708B, Dayton Industrial, Dayton, OH, USA) attached to the chamber was used to lower the pressure inside the chamber. The LBNP was adjusted with a valve between the vacuum and the chamber. This setup has been used repeatedly in LBNP studies by Seals and colleagues [135, 145, 31] and in our previous study [15]. On the LBNP day, baroreceptors were unloaded by gradually reducing the pressure in the chamber to -40 mmHg relative to ambient pressure in 20 s. MEP testing commenced 30 s after application of LBNP. LBNP of 40 mmHg is known to physiologically unload the baroreceptors and heighten SNA in general, increasing epinephrine and norepinephrine concentration in plasma [58] and increasing heart rate with little changes in blood pressure [15]. On the Control day, the vacuum remained on and the pressure was set to 0 mmHg during the recording of MEPs. Blood pressure at the brachial artery in the left arm and heart rate at the fingertip were monitored noninvasively (Cardiocap/5, GE Healthcare, Giles, UK), and mean arterial blood pressure was recorded in each TMS block. The ECG (Cardiocap/5, GE Healthcare, Giles, UK) was sampled at 10,000 samples/s with an analogue-to-digital

converter (Power 1401, Cambridge Electronic Design Ltd, Cambridge, UK) and data acquisition software (Spike2 v7.0, Cambridge Electronic Design Ltd, Cambridge, UK) for offline analysis of heart rate variability. ECG was not obtained in one subject due to technical issues. These cardiovascular data were recorded during TMS, as well as, before delivery of TMS (Baseline stage) and during the 10-min intermission (Intermission stage).

3.2.4 Intracortical excitability

Intracortical excitability was assessed noninvasively with single- and paired-pulse TMS (BiStim, Magstim Co, Wales, UK) of the left primary motor cortex. The head was oriented in neutral position on a pillow. The arms of each subject lay at his/her sides with the right hand in pronation, in a wooden brace when producing isometric contractions, or resting on the bed when not. The subjects were instructed not to move their arms or hands during measurement. The orientation of the arms and hands was monitored visually throughout the experiment. Subjects did not have vision of their hands because it was interrupted by the LBNP tube.

The experiment was conducted in an electrically shielded room. Surface EMG was recorded using Ag-AgCl electrodes (E224A, IVM, Healdsburg, CA, USA) placed on the skin overlying the right FDI in a belly-tendon montage. One electrode was placed over the belly of the muscle and the other was attached to the skin over the distal tendon, after abrasion of the skin. A wet circumferential strap electrode (F-E10SG1, Grass Technologies, West Warwick, RI, USA) was placed around the right wrist for a reference. The EMG was differentially amplified 300 times and bandpass filtered between 15 and 2,000 Hz (Y03-000, MotionLabs, NY, USA). The EMG data were sampled at 5,000 samples/s with an analog-to-digital converter (Power 1401, Cambridge Electronic Design Ltd, Cambridge, UK) and data acquisition software (Signal 5.0, Cambridge Electronic Design Ltd, Cambridge, UK) for online monitoring,

storage, and offline analysis.

Contraction intensity was determined based on the EMG amplitude of FDI during maximal voluntary isometric contraction. A rectified running average EMG with an averaging window of 0.175 s was used to provide visual feedback to subjects and to calculate the maximal EMG (EMGmax) of the FDI. With their right hand secured in the hand brace, subjects increased their EMG to maximum in a ramp fashion over 3 s, and maintained it at maximum for 2 s before relaxing. Verbal instruction and encouragement were provided while the right hand of subjects was visually monitored.

TMS intensity was adjusted based on MEP in FDI. A figure-of-eight coil (Magstim second generation double 70 mm remote coil, Magstim Co, Wales, UK) was held over the left primary motor cortex at the optimum position (i.e. hotspot) for eliciting an MEP in the resting FDI of the right hand. The coil was held with the handle pointing posteriorly at an angle of approximately 45 degrees to the sagittal plane yielding an E-field perpendicular to the central sulcus [11]. A TMS coil navigation system (NDI TMS Manager, Northern Digital Inc., Waterloo, Ontario, Canada) was used to maintain the coil position in 3-dimensional space relative to the head. A continuous running visual feedback of the EMG was provided to the subject to ensure relaxation or appropriate activation of the FDI when necessary. The pre-stimulus EMG was monitored by the experimenter.

The resting motor threshold (RMT) was determined as the smallest TMS intensity needed to elicit an MEP with peak-to-peak amplitude (PPamp) greater than 50 μV in 5 out of 10 consecutive stimulations in the relaxed FDI [16, 30]. Active motor threshold (AMT) was assessed during the isometric contraction of FDI at 10% EMGmax. Due to difficulty in differentiating between MEP and background contraction EMG in single stimulation traces, the AMT was defined as the largest TMS intensity that produced an EMG response less than 50 μV above background EMG activity in the triggered average of ten consecutive rectified stimulation traces [109]. RMT and

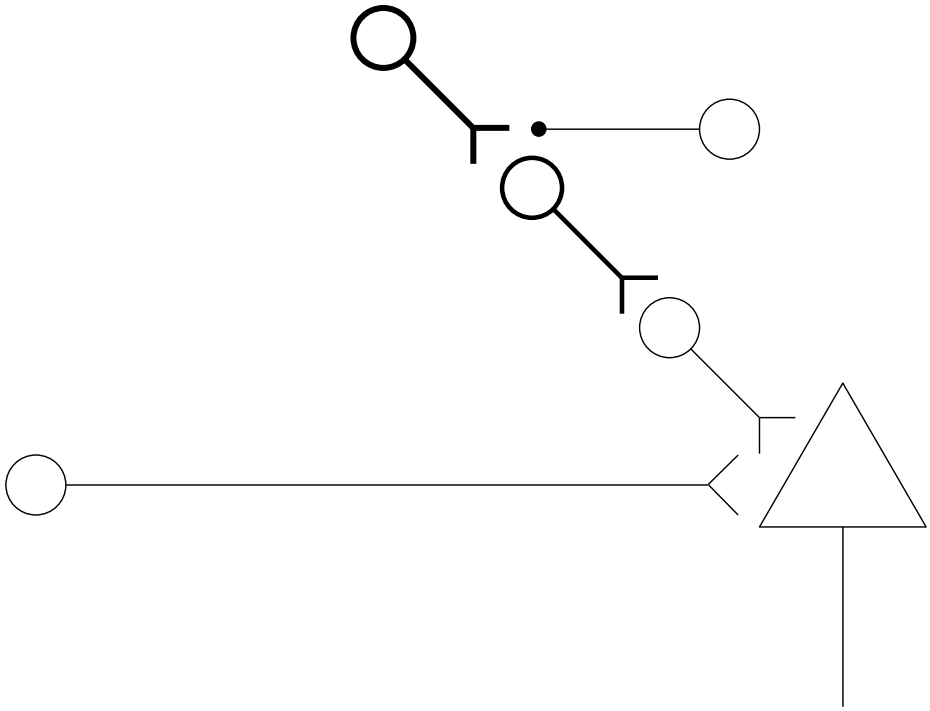

Figure 8: Schematic of populations of intracortical neurons assessed in Specific Aim 2. Excitatory interneurons are denoted with forks at their axon terminals; inhibitory interneurons are denoted with solid circles.

AMT were measured in percentage of maximal stimulator output.

During the Resting stage, activity of the intracortical later I-wave, $GABA_A$-ergic, and NMDA-ergic pathways (Fig. 8) in the resting FDI were assessed with the paired-pulse TMS paradigms for SICF [56], SICI [159], and ICF [158, 134], respectively (Fig. 9). Test stimulus for the paired-pulse stimulation in the Resting stage was determined as the TMS intensity that produced an average MEP with PPamp of 1 mV for ten consecutive stimulation pulses in the relaxed FDI [109]. SICF was measured by delivering the 0.9 $\times$ AMT pulse 1.5 ms after the suprathreshold test stimulus pulse [109]. For SICI and ICF, a subthreshold 0.9 $\times$ AMT pulse was followed by the suprathreshold test stimulus pulse with the interval of 2 and 10 ms, respectively [80, 109]. An interstimulus interval of 2 ms was used for SICI assessment to avoid contamination of SICI measurement by SICF peaks [116]. MEPs in response to test stimulation were collected first, followed by SICF, SICI, and ICF in random order.

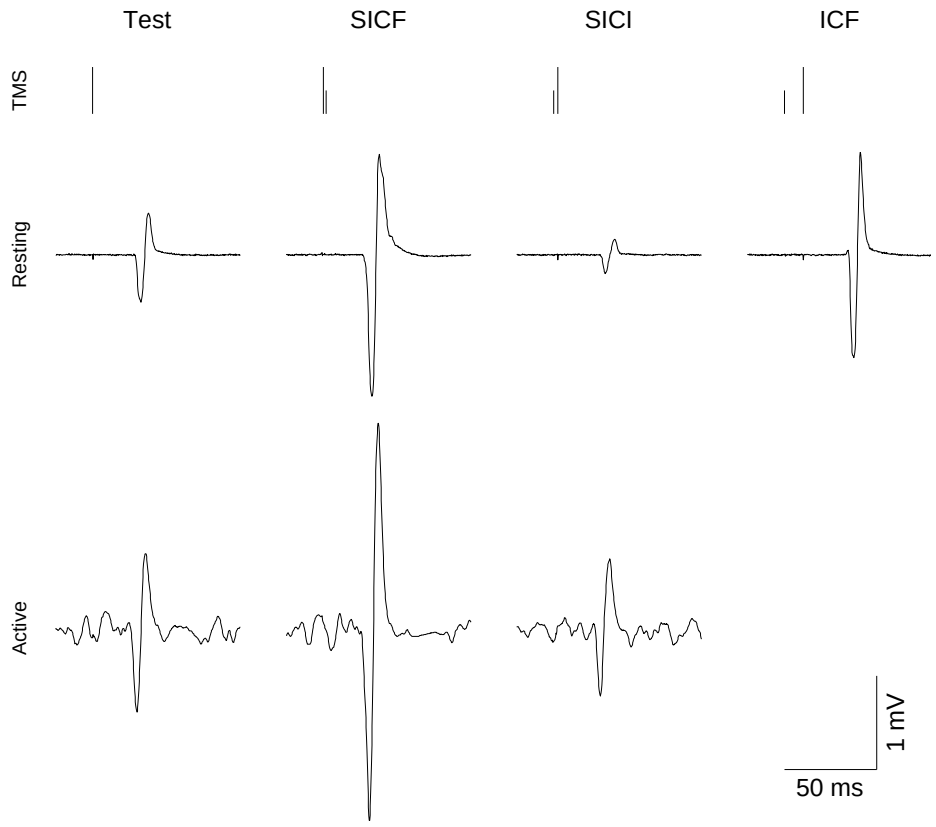

Figure 9: Representative recordings of motor evoked potentials (MEP) in response to transcranial magnetic stimulation (TMS) recorded from the first dorsal interosseus muscle in one subject on the Control day. The traces represent different TMS protocols (row 1), a single MEP in the Resting stage (row 2), and an average of 10 MEPs in the Active stage (row 3) without lower body negative pressure. Test, single-pulse test stimulation; SICF, short-interval intracortical facilitation; SICI, short-interval intracortical inhibition; ICF, intracortical facilitation. The vertical scale is for rows 2 and 3.

TMS was delivered every 6 s. Seventeen MEPs were collected for each TMS paradigm in the Resting stage. Due to technical issues, SICF was not measured in one subject.

During the Active stage, the primary variables of interest were activity of the intracortical $GABA_A$-ergic and $GABA_B$-ergic pathways, assessed with cortical silent period in the contracting FDI. Cortical silent period was measured during isometric contraction at 50% EMGmax to minimize variability of the cortical silent period due to contraction strategy of subjects [90] and to delineate potential effects of LBNP on intracortical $GABA_A$ and $GABA_B$-ergic pathways [74]. Subjects were instructed

to ramp up to the 50% EMGmax contraction, maintain the contraction through the ensuing stimulation, and to relax only when told to do so by the experimenter, at least 1 s after TMS [90]. Subjects who failed to follow this instruction were removed from analysis of 50% EMGmax data (2 subjects). Cortical silent period was measured in response to TMS at RMT (CSP_L) and TMS at 2 × RMT (CSP_H), separately, because the response to low and high intensity TMS is indicative of $GABA_A$-ergic and $GABA_B$-ergic activity, respectively [74]. For those subjects whose 2 × RMT was greater than 100% maximal stimulator output ($n = 4$), TMS at 100% maximal stimulator output was used to assess CSP_H. TMS was delivered every 15 s. CSP_L and CSP_H were collected in this order, and twelve responses were collected for each.

Additionally, activity of the later I-wave and $GABA_A$-ergic pathways of the active FDI were investigated with the paired-pulse protocols for SICF and SICI during isometric contraction at 10% EMGmax (Fig. 9). Note that activity of the intracortical NMDA-ergic pathway cannot be measured during muscle contraction due to the disappearance of ICF [109]. The test stimulus for paired-pulse stimulation in the Active stage was determined as the TMS intensity that produced a peak amplitude between 0.5 mV and 1 mV in the average of ten consecutive rectified stimulation traces during 10% EMGmax contraction in FDI [109]. SICF was measured by delivering the 0.9 × AMT pulse 1.5 ms after the suprathreshold test pulse [109]. For SICI, a subthreshold 0.7 × AMT pulse was followed by suprathreshold test stimulus pulse at 2 ms. Previously, it was suggested that the decrease in SICI with voluntary muscle contraction [126, 109] may also be due to contamination of the response by SICF [109]. The 2 ms interval was chosen to avoid SICF contamination [116]. The conditioning stimulus for SICI was set at 0.7 × AMT, rather than 0.9 × AMT used in the Resting stage, to prevent interference from SICF during voluntary contraction [109]. Response to 0.9 × AMT single-pulse stimulation was also measured to check whether the conditioning stimulation produced an MEP. MEPs in response to test stimulation were collected

first, followed by SICF, SICI, and single-pulse TMS at 0.9 × AMT, in random order, during 10% EMGmax contraction of FDI. TMS was delivered every 6 s, and the subject maintained the contraction level for the duration of each TMS paradigm block. Twelve MEP responses were collected for each TMS paradigm.

3.2.5 Data collection sequence

The following data recording order was chosen to prevent potential residual effects of muscle contraction on subsequent recordings of intracortical excitability at rest. On a given day, the cardiovascular measurements in the Baseline stage were assessed once the subject was situated in the experiment arrangement. After identifying EMGmax, RMT, and AMT, determination of test stimulus and data collection for the Resting stage were performed with the LBNP set to the appropriate value for the day. At the end of a 10-min Intermission stage following the Resting stage, cardiovascular measurements were recorded. After identifying the test stimulus for the Active stage with the LBNP set to the appropriate value for the day, data for Active stage were collected with the contraction at 10% EMGmax followed by the contraction at 50% EMGmax.

3.2.6 Data reduction

The first 2 recordings in each TMS paradigm were discarded to control for possible startle responses. In the Resting stage, EMG recordings that showed pre-stimulus EMG activity above baseline 100 ms preceding TMS were discarded. The root mean square (RMS) amplitude of the pre-stimulus EMG, the MEP PPamp, and the MEP area bound by the MEP and the 0 mV axis (MEP area) were calculated for each response. The RMS amplitude of the pre-stimulus EMG was calculated from data 100 ms preceding the application of TMS. The EMG in the period between 20 and 50 ms following application of TMS was used to measure MEP PPamp and MEP area. MEP area was analyzed to account for potential changes in MEP that may not be

reflected in PPamp. MEP PPamp and area of each response were averaged together within each paradigm block and expressed relative to the corresponding values for test stimulation.

During the Active stage, collected recordings were inspected for comparable muscle activation. At each contraction level, those recordings whose RMS amplitude of the pre-stimulus EMG fell 2 standard deviations outside of the mean were discarded. The recordings collected during subthreshold 0.9 $\times$ AMT TMS were averaged together. To observe whether sub-threshold TMS produced an MEP during 10% EMGmax contraction, the PPamp of the pre-stimulus EMG and the PPamp between 20 and 50 ms post TMS were calculated. PPamp was calculated 1) to take into account possible fluctuations in the pre-stimulus EMG and 2) to not underestimate the MEP response. All other recordings were rectified and averaged together within each paradigm block. The automated cumulative sum method was used to measure the cortical silent period [76]. The start and end of the CSP_L and CSP_H were defined as the times, following the MEP, from when the average rectified EMG fell below and increased back up to the pre-stimulus EMG level, respectively. Each cortical silent period was expressed in ms, and, in addition, normalized to the peak amplitude and area of the accompanying MEP to take the variability in TMS response into consideration [108]. The normalized value for CSP_H was not available in one subject due to a technical issue in the EMG gain for measuring MEP size during CSP_H. The MEP peak amplitude and MEP area of paired-pulse stimulations were calculated and expressed relative to the corresponding values for test stimulation.

As measures of baroreflex-dependent SNA, heart rate and heart rate variability were assessed from the ECG recordings taken during Baseline, Resting, Intermission, and Active stages. All ECG recordings used in calculation of heart rate variability were greater than two minutes in duration. From the ECG recording, all R-wave peaks were identified, marked, and visually inspected to rule out artifacts. Then the

power spectrum of the R-to-R interval was calculated. Low frequency (0.05-0.15 Hz) power was expressed relative to the power in total frequencies (0.05-0.50 Hz) and was used as a measure of SNA [19].

3.2.7 Statistical analysis

In this within-subject study design, the main independent variable was Day (Control vs. LBNP). First, to test whether the conditioning stimulation produced the expected facilitation or inhibition of the test stimulation on the Control day, the paired-pulse MEP was compared to the test MEP on the Control day for all paired-pulse TMS protocols using a paired samples t-test. The main dependent variables for intracortical excitability were the appropriate ratios of MEP PPamp, MEP area, and MEP peak amplitude of SICI, ICF and SICF to the corresponding values for test stimulation, and the absolute and normalized durations of CSP_L and CSP_H. EMGmax, RMT, AMT, test stimulation intensity for Resting and Active stages, pre-stimulus EMG from the Resting stage, and the main dependent variables were individually tested for difference between the two days using a two-tailed paired samples t-test. To test whether the sub-threshold stimulation produced motor activity, PPamp of the unrectified pre-stimulus EMG (i.e. 100 ms period preceding TMS) was tested against the PPamp within the MEP period of the unrectified sub-threshold 0.9 $\times$ AMT TMS response during 10% EMGmax contraction, using a two-tailed paired samples t-test. Effects of LBNP on heart rate, mean arterial blood pressure, and HR_{LF} were each tested with a two-factor (day, stage) ANOVA with repeated measures, where factor stage had four levels: Baseline, Resting, Intermission, and Active. Significant main effects and interaction of all ANOVA were further tested with the Bonferroni post hoc. To test the effects of experiment day order, the data were reorganized such that the independent variable Day reflected either day 1 or day 2 of the experiment, and the same statistical analyses were repeated. An alpha level of 0.05 was used for all

Table 3: Neuromuscular characteristics in the first dorsal interosseus muscle used for the TMS protocol in Specific Aim 2. The left two columns compare between LBNP and Control days. The right two columns compare the same variables between days 1 and 2. Lower body negative pressure was applied on the LBNP day. EMGmax, electromyogram amplitude during maximal voluntary contraction; RMT, resting motor threshold; MSO, maximal stimulator output; AMT, active motor threshold; TS_R, test stimulation intensity during the Resting stage; PPamp, peak-to-peak amplitude; TS_A, test stimulation intensity during the Active stage. No significant difference between day types (LBNP vs. Control) or between days 1 and 2.

	LBNP	Control		Day 1	Day 2
EMGmax, mV	0.95 ± 0.32	0.95 ± 0.29		1.00 ± 0.32	0.90 ± 0.28
RMT, % MSO	44.3 ± 7.1	44.7 ± 7.15		44.3 ± 7.56	44.7 ± 7.20
AMT, % MSO	32.7 ± 8.09	32.4 ± 5.75		33.1 ± 7.93	32.0 ± 5.92
TS_R PPamp, mV	1.0 ± 0.36	1.2 ± 0.37		1.1 ± 0.48	1.2 ± 0.33
TS_R area, mV·ms	3.4 ± 1.31	4.7 ± 2.00		3.9 ± 1.87	4.2 ± 1.77
TS_R, % MSO	55.6 ± 12.52	53.9 ± 10.04		54.8 ± 12.80	54.7 ± 9.76
TS_R, % RMT	122.5 ± 11.23	118.7 ± 9.44		120.8 ± 11.83	120.4 ± 9.09
TS_A peak, mV	0.9 ± 0.26	0.8 ± 0.31		0.9 ± 0.32	0.8 ± 0.25
TS_A area, mV·ms	7.2 ± 2.30	7.2 ± 2.69		7.2 ± 2.53	7.2 ± 2.48
TS_A, % MSO	39.8 ± 8.64	38.5 ± 7.93		39.4 ± 8.64	38.6 ± 7.81

significance testing. If the MauchlyS sphericity test was violated, the Huyn-Feldt adjusted P-value was used. $P < 0.05$ and $P < 0.01$ were noted where appropriate. Statistical analyses were performed using Statistica 9.0 (StatSoft Inc., Tulsa, OK, USA). Unless stated otherwise, the data are presented as mean ± SD in the text and tables and as mean ± standard error of mean in the figures.

3.3 Results

3.3.1 Subject characteristics

Basic subject characteristics, including EMGmax, RMT, AMT, and test stimulation MEP and intensity for Resting and Active stages were not different between day types (LBNP vs. Control) or between days 1 and 2 (Table 3). On average, MEP PPamp during the single-pulse stimulation was 1.10 ± 0.38 mV in the resting muscle and MEP peak amplitude was 0.88 ± 0.29 mV in the contracting muscle, respectively,

when collapsed across days.

3.3.2 Cardiovascular response

With the application of 40 mmHg LBNP on the LBNP day, both heart rate and HR_{LF} were increased while mean arterial blood pressure was not significantly different compared with Control day (Fig. 10). There were main effects of Day ($P < 0.01$), Stage ($P < 0.01$), and their interaction ($P < 0.01$) on heart rate. Similarly, main effects of Day ($P < 0.05$), Stage ($P < 0.05$), and their interaction ($P < 0.01$) were detected for HR_{LF}. Heart rate was greater on the LBNP day when 40 mmHg LBNP was applied in Resting (by 11 bpm, $P < 0.01$) and Active (by 12 bpm, $P < 0.01$) stages, respectively, compared with the Baseline and Intermission stages of the same day. HR_{LF} was also greater on the LBNP day by 48% in Resting stage ($P < 0.01$) and by 40% in Active stage ($P < 0.01$) compared with the Baseline and Intermission stages of the same day. There was no significant difference in heart rate or HR_{LF} between days during Baseline or Intermission stage. There was no significant effect of day, but there was a main effect of Stage ($P < 0.05$) on mean arterial blood pressure. Mean arterial blood pressure was slightly greater at the end of data collection (Active stage, 88.6 ± 7.3 mmHg when collapsed across days) compared with the beginning of data collection (Baseline stage, 86.1 ± 6.8 mmHg, $P < 0.05$). No effects of day order were observed for heart rate, HR_{LF}, or mean arterial blood pressure (Table 4).

3.3.3 Intracortical excitability, at rest

In the Resting stage, RMS amplitude of the pre-stimulus EMG did not vary between days in either muscle (Table 5). The stimulation intensity needed to elicit 1 mV PPamp MEP in the resting FDI did not vary significantly between days (Table 3), and was 120.6 ± 10.4% of RMT when averaged across days. MEP PPamp and MEP area during SICF, SICI, and ICF paired-pulse protocols were expressed relative to the MEP PPamp and MEP area during the single-pulse test stimulation, respectively

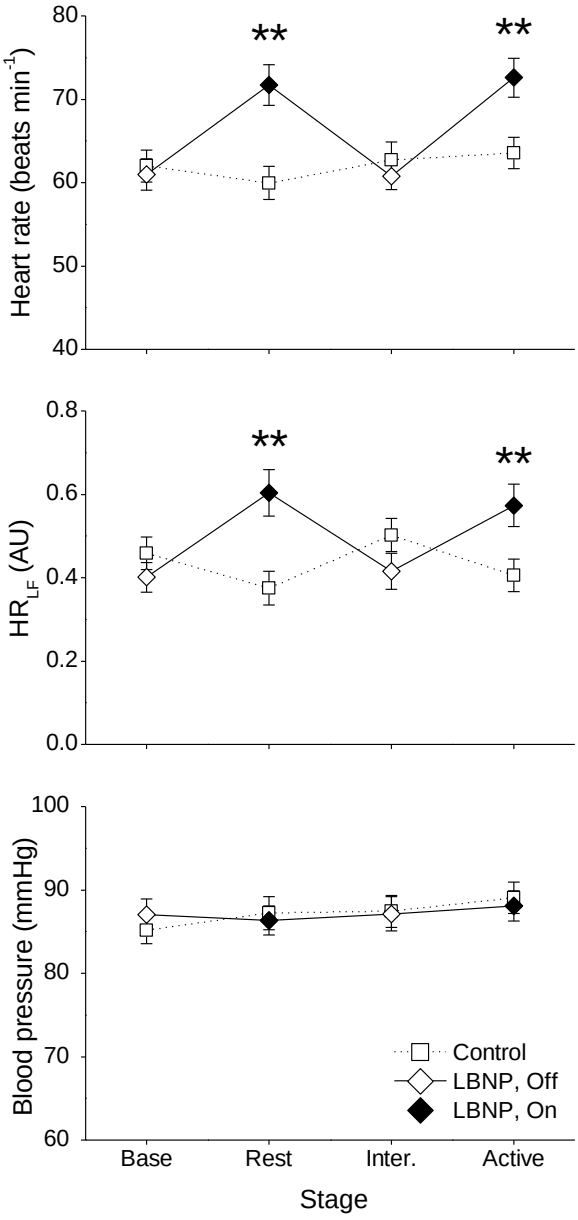

Figure 10: Cardiovascular measurements during different stages of the Specific Aim 2 experiment on the Control and LBNP days. Filled symbols indicate when sympathetic nerve activity was heightened (Resting and Active stages of the LBNP day, only). AU, arbitrary units of heart rate variability power in the low frequency (0.05-0.15 Hz) expressed relative to the power in total frequencies (0.05-0.50 Hz); Base, Baseline stage; Rest, Resting stage; Inter., Intermission stage. $^{**}P < 0.01$ between stages during LBNP day, as tested with Bonferroni post hoc test of significant Day $\times$ Stage interaction.

Table 4: Cardiovascular measures at each stage on Days 1 and 2 in the Specific Aim 2 study. HR, heart rate; bpm, beats per minute; HR_{LF}, low-frequency (0.05 – 0.15 Hz) power of heart rate variability relative to the total power of the R-to-R interval spectrum; MAP, mean arterial blood pressure. No significant effect of Day order or significant interaction was detected for the comparison between Day 1 and Day 2.

		Baseline	Resting	Intermission	Active
HR	Day 1	62.1 ± 7.98	68.9 ± 10.51	61.5 ± 6.07	70.5 ± 9.07
(bpm)	Day 2	60.9 ± 7.26	62.8 ± 10.06	61.9 ± 8.99	65.6 ± 9.69
HR_{LF}	Day 1	0.41 ± 0.149	0.53 ± 0.244	0.42 ± 0.174	0.53 ± 0.229
(A.U.)	Day 2	0.45 ± 0.150	0.44 ± 0.193	0.49 ± 0.156	0.44 ± 0.154
MAP	Day 1	87.1 ± 7.14	87.2 ± 7.23	87.8 ± 8.10	88.3 ± 7.48
(mmHg)	Day 2	85.1 ± 6.56	86.4 ± 7.64	86.8 ± 7.78	88.8 ± 7.40

Table 5: Root mean square amplitude of EMG preceding TMS application in Specific Aim 2. Left two columns compare between LBNP and Control days. The right two columns compare the same variables between days 1 and 2. EMG, electromyogram; TMS, transcranial magnetic stimulation; EMGmax, amplitude of electromyogram during maximal voluntary contraction. No significant differences observed between days.

	LBNP	Control		Day 1	Day 2
Resting stage, μV	4.17 ± 0.61	3.80 ± 0.29		4.16 ± 0.56	3.81 ± 0.39
10% EMGmax, μV	123 ± 44.1	152 ± 71.4		117 ± 40.8	157 ± 70.6
50% EMGmax, μV	474 ± 129.5	473 ± 207.4		441 ± 99.6	506 ± 218.2

(Fig. 11). No significant effects of day order were observed for any of the intracortical excitability measures (Table 6).

The SICF protocol resulted in facilitation on the Control day. On the Control day, the conditioned MEP PPamp and MEP area were 52.6% and 46.6% greater than the test MEP PPamp and MEP area, respectively ($P < 0.01$). There was no significant difference in SICF ratios between LBNP and Control days (Fig. 11).

The conditioned MEP PPamp and MEP area were 56.1% and 48.6% lower than the test MEP PPamp and MEP area, respectively, on the Control day during the

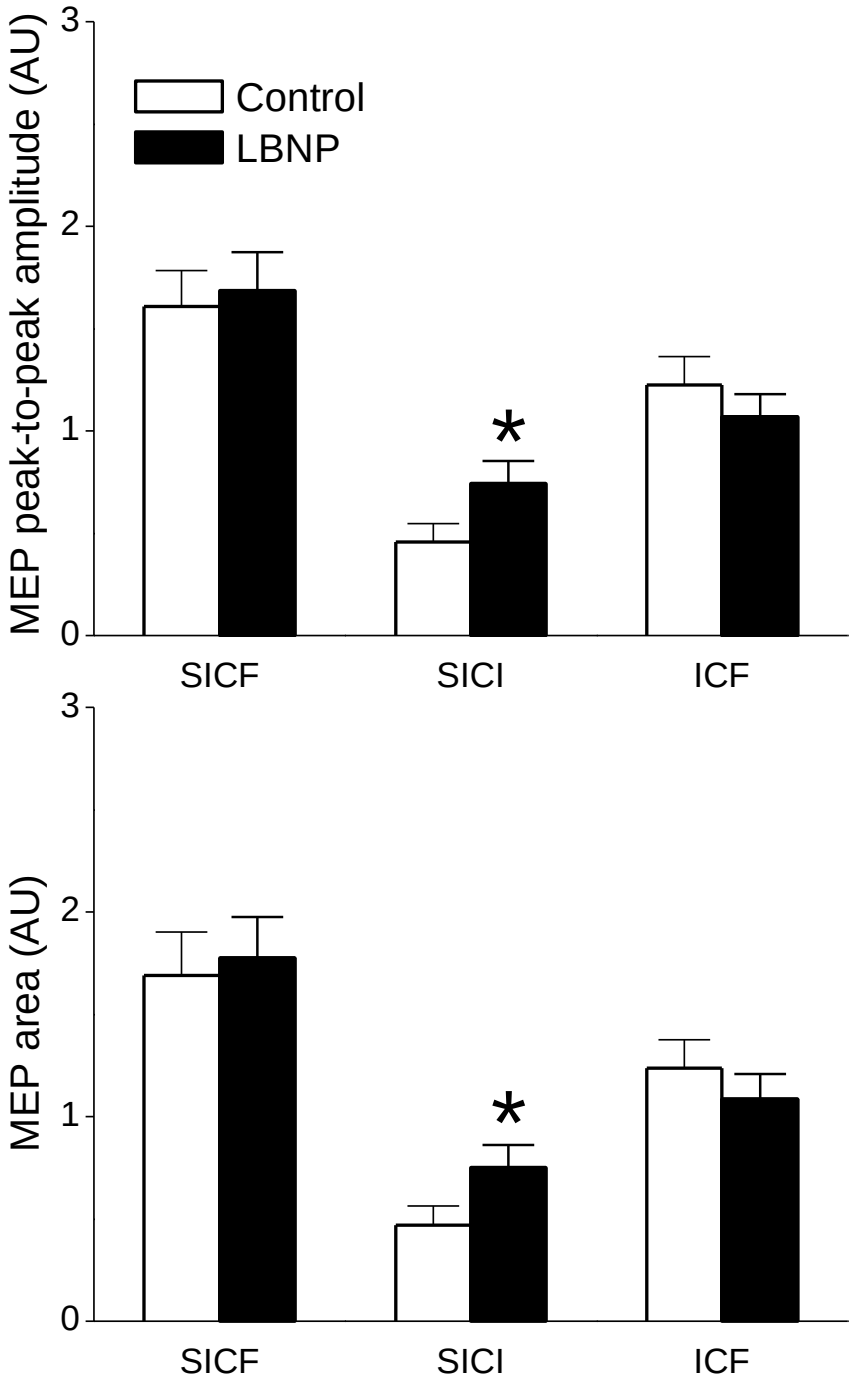

Figure 11: Intracortical excitability in the first dorsal interosseus muscle during the Resting stage on the Control and LBNP days of the Specific Aim 2 study. The y-axis shows the MEP peak-to-peak amplitude (top panel) and MEP area (bottom panel), in response to paired-pulse stimulation, normalized to the corresponding value during single-pulse test stimulation. Unloading of baroreceptors was performed on the LBNP day. * $P < 0.05$ compared with Control in the respective measure.

Table 6: Measures of intracortical excitability in resting first dorsal interosseus muscle on Days 1 and 2 of the Specific Aim 2 study. Data are relative to the values for single-pulse test stimulation. SICF, short-interval intracortical facilitation (n = 15); SICI, short-interval intracortical inhibition (n = 16); ICF, intracortical facilitation (n = 16); MEP, motor evoked potential; PPamp, peak-to-peak amplitude; No statistical differences observed between Day 1 and Day 2.

		SICF	SICI	ICF
MEP PPamp	Day 1	1.64 ± 0.70	0.62 ± 0.41	1.07 ± 0.49
	Day 2	1.65 ± 0.71	0.58 ± 0.44	1.23 ± 0.50
MEP Area	Day 1	1.70 ± 0.74	0.63 ± 0.41	1.07 ± 0.52
	Day 2	1.76 ± 0.87	0.59 ± 0.45	1.25 ± 0.53

SICI protocol ($P < 0.01$). Inhibition was significantly reduced with LBNP, resulting in 63% greater MEP PPamp ($P < 0.05$) and 60% greater MEP area ($P < 0.05$) ratios on the LBNP day compared with the Control day (Fig. 11).

During the ICF protocol only 11 out of the 16 subjects showed facilitation on the Control day. Across all subjects, the conditioned MEP was not significantly greater than the test MEP on the Control day. Subjects presented average ratios of 15% and 16% of MEP PPamp and MEP area, respectively, when collapsed across days. No significant effect of LBNP was observed on these ICF measures (Fig. 11).

3.3.4 Intracortical excitability, during muscle activity

In assessing intracortical excitability in the active FDI muscle, the RMS amplitude of pre-stimulus EMG of the FDI did not differ significantly between LBNP and Control days during 10% EMGmax or during 50% EMGmax (Table 5). There was no significant difference in the duration of the cortical silent period for either the CSP_L or CSP_H when measured in ms, or when normalized to the corresponding MEP peak or area, between days (Table 7). On average, CSP_L and CSP_H were 55.9 ± 26.6 ms and 242.9 ± 52.8 ms, respectively, when collapsed across days. In 4 subjects, the TMS intensity for CSP_H was less than 2 × RMT due to their high RMT relative to the

Table 7: MEP and cortical silent period in the first dorsal interosseus muscle during 50% EMGmax voluntary muscle contraction tested with high- and low- intensity stimulations in the Specific Aim 2 study. First two columns compare between LBNP and Control days. Second two columns compare the same variables between Days 1 and 2. Peak, peak amplitude of the average rectified MEP; Area, area of the average rectified MEP; CSP_L, cortical silent period in response to low intensity TMS at resting motor threshold; CSP_H, cortical silent period in response to high intensity TMS at 2 $\times$ resting motor threshold. No significant differences observed between days.

	LBNP	Control		Day 1	Day 2
Low stimulation					
Peak, mV	5.77 ± 2.45	4.74 ± 2.13		5.61 ± 2.33	4.90 ± 2.32
Area, mV·ms	40.48 ± 13.75	34.80 ± 13.10		41.15 ± 14.08	34.14 ± 12.38
CSP_L, ms	53.5 ± 20.26	58.4 ± 32.36		61.2 ± 23.39	50.7 ± 29.40
CSP_L $Peak^{-1}$	10.9 ± 5.83	17.4 ± 18.58		12.4 ± 5.71	15.9 ± 19.05
CSP_L $Area^{-1}$	1.4 ± 0.66	1.9 ± 1.20		1.6 ± 0.62	1.8 ± 1.26
High stimulation					
Peak, mV	6.10 ± 3.35	5.70 ± 2.87		5.92 ± 3.44	5.87 ± 2.78
Area, mV·ms	50.97 ± 24.89	45.95 ± 22.46		50.77 ± 25.84	46.15 ± 21.42
CSP_H, ms	249.1 ± 57.38	236.6 ± 49.03		253.1 ± 50.68	232.6 ± 54.65
CSP_H $Peak^{-1}$	64.8 ± 53.99	73.8 ± 92.29		68.9 ± 54.28	69.6 ± 92.35
CSP_H $Area^{-1}$	6.8 ± 4.76	7.7 ± 7.59		7.0 ± 4.68	7.5 ± 7.66

capacity of the TMS equipment. Removal of these subjects from the analysis did not influence the statistical results.

During voluntary muscle activity, the conditioned MEP PPamp and MEP area were 112% and 83% greater than the test MEP PPamp and MEP area, respectively ($P < 0.01$) in the SICF protocol on the Control day. There was no significant difference in SICF ratios between LBNP and Control days (Fig. 12).

The SICI protocol did not result in any significant differences between the conditioned and test MEP on the Control day, during voluntary muscle activity. No significant effects of day were observed for MEP ratios (Fig. 12). MEP PPamp and MEP area ratios during the SICI protocol were 1.16 ± 1.06 AU and 1.12 ± 0.78 AU, respectively.

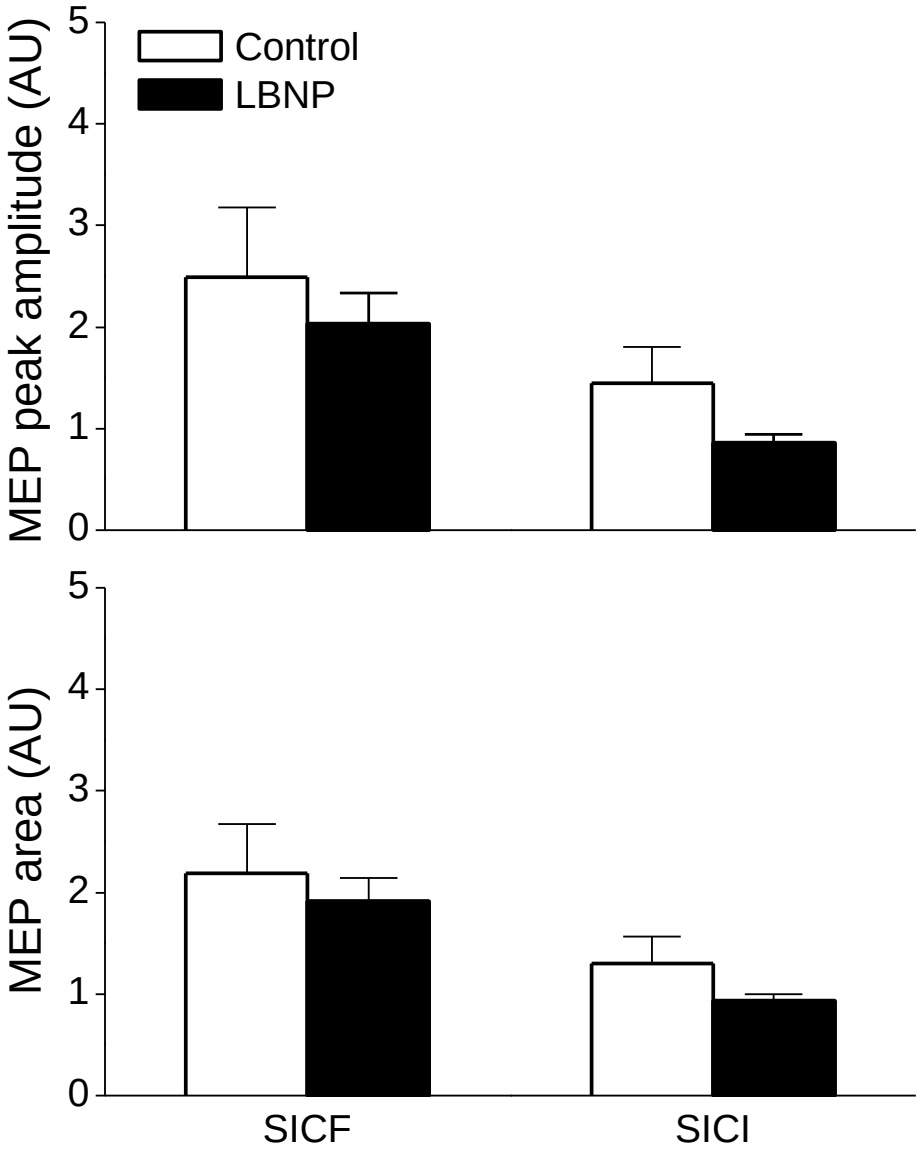

Figure 12: Intracortical excitability in the 10% EMGmax active first dorsal interosseus muscle on the Control and LBNP days of the Specific Aim 2 study. The y-axis shows the MEP peak-to-peak amplitude (top panel) and MEP area (bottom panel), in response to paired-pulse stimulation, normalized to the corresponding value during single-pulse test stimulation. Unloading of baroreceptors was performed on the LBNP day. No significant differences were observed between days.

To confirm that sub-threshold conditioning TMS at 0.9 × AMT did not produce measurable motor activity, the PPamp of the potential response to the sub-threshold TMS was compared with the PPamp of unrectified EMG during the background contraction. The PPamp after the sub-threshold TMS (304 ± 143 μV) was not significantly different from the PPamp before the sub-threshold TMS (338 ± 206 μV) during 10% EMGmax contraction.

3.4 Discussion

The major finding is that SICI in the Resting stage was decreased on the LBNP day when LBNP of 40 mmHg was applied compared with the Control day. No significant differences between days were found in other measures in the Resting stage (SICF and ICF) or any measures (cortical silent period, SICI, and SICF) in the Active stage.

3.4.1 Cardiovascular response

LBNP of 40 mmHg unloads the baroreceptors, thereby heightening SNA for various organs, as evidenced by increases in norepinephrine concentration in the plasma [145, 58], muscle sympathetic nerve discharges [143, 145, 31], heart rate [143, 145, 31, 15], and low-frequency content of heart rate variability [85]. In the present study, heart rate and HR_{LF} were significantly greater on the LBNP day during application of LBNP of 40 mmHg, while mean arterial blood pressure was maintained. The 11-12 bpm increase in heart rate with minimal change in blood pressure due to LBNP of 40 mmHg is comparable to previous studies [143, 145, 31, 15]. These findings support that the current LBNP protocol was effective for unloading the baroreceptors and heightening SNA [26].

3.4.2 Intracortical excitability

SICI in the Resting stage decreased on the LBNP day when LBNP of 40 mmHg was applied. SICI represents the inhibition of later I-waves (i.e. I-2 and I-3 waves)

[37] and probes the activity of the intracortical inhibitory pathway that modulates the excitability of corticospinal neurons through $GABA_A$ receptors [159]. Several technical considerations ensured the proper measurement of intracortical excitability. Care was taken to ensure that only resting data were analyzed since SICI is influenced by muscle activation [126, 109]. Lack of difference in pre-stimulus EMG activity between days further supports the maintenance of resting status across days. LBNP of 40 mmHg increases corticospinal excitability in the resting muscle when stimulated only at 130% or higher RMT [15]. Since the employed intensity of test stimulation was below 130% RMT and was not different between days, it is likely that the current measurements were made within the range for consistent input-output properties of corticospinal neurons. Since the order of LBNP and Control days was assigned randomly, it is unlikely that the tested interneurons were systematically different between days. Since the conditioning stimulation was chosen below the AMT and no discernible MEP was produced during the Active stage, the employed conditioning stimulation likely did not produce descending volleys. Additionally, the amount of SICI in the Resting stage on the Control day was comparable to previous reports [126, 109]. Hence, the decreased SICI in the Resting stage on the LBNP day indicates a decrease in SICI due to baroreceptor unloading, supporting our prediction. The decrease in SICI suggests that baroreceptor unloading results in decreased activity of the intracortical inhibitory $GABA_A$-ergic pathway [159], leading to less inhibition of later I-waves [37].

In contrast to the resting muscle, there was no significant effect of day on the measures of intracortical inhibitory pathways in the Active stage. SICI was used to assess $GABA_A$-ergic activity, and CSP_L and CSP_H assessed $GABA_A$-ergic and $GABA_B$-ergic activities, respectively [74]. The absence of significant effect of days on these measures suggests that baroreceptor unloading did not influence $GABA_A$-ergic

or $GABA_B$-ergic activity during muscle contraction. Note that inhibition was not observed during SICI in the contracting muscle, in contrast to the approximately 38% inhibition in a previous report [109]. It is possible that peculiarities of the experimental setup, such as lack of vision of the hand that influences somatosensory intracortical inhibition [18] may have influenced these measures of intracortical inhibition in the contracting muscle.

SICF in the current protocol tested the activity of a portion of the intracortical pathway implicated in generating later I-waves [56]. The absence of significant effect of day on SICF during resting and active muscle states suggests that baroreceptor unloading did not influence the intracortical excitatory later I-wave generating pathways.

A limitation in interpreting the effects of LBNP on ICF mechanisms is that the ICF protocol did not result in significant facilitation of the test MEP on the Control day. Though not anticipated during the study design, the 69% facilitatory response rate among participants on the Control day is comparable to literature [149]. Currently, with the lack of significant effect of LBNP on ICF it is impossible to state whether LBNP does not influence the excitatory NMDA-ergic pathway probed with the ICF protocol [158, 134], or whether the NMDA-ergic pathway simply was not assessed.

3.4.3 Potential mechanisms

While the identification of the actual mechanisms for the current observations is beyond the scope of this study, it would be worthwhile to explore the potential mechanisms for the significant effect of LBNP (i.e. decrease in SICI). Based on the literature, the decreased SICI due to LBNP may involve the potential influences of baroreflex processes on 1) somatosensory afferent input and/or 2) cortical monoamines (i.e. norepinephrine, serotonin, dopamine). As presented in the Introduction, baroreceptor unloading may influence somatosensory afferent activity centrally, via release of

norepinephrine and serotonin from descending projections of the locus coeruleus and raphe nuclei [104, 123, 52, 68], or peripherally, via an increase in SNA [143]. Electrical stimulation over motor axons [141, 34] and electrical stimulation of digits [125, 124, 92] can decrease SICI. However, the decrease in SICI is abolished if the interval between digit stimulation and TMS is altered [77]. Hence, the potential effect of somatosensory afferent input on SICI is not robust. Furthermore, there is controversy over whether physiologically heightened SNA can modulate somatosensory afferent input. While no influence of SNA on muscle spindle discharge is observed in humans [87], an increased stretch reflex [59, 72] in the presence of an unaltered H-reflex [72] due to physiological heightening of SNA suggests an SNA-induced increase in gain of the somatosensory afferent input. However, the impact of this possible increase in somatosensory afferent input, or its gain, in the resting FDI is questionable considering that the corticospinal excitability is not increased with LBNP through TMS intensity of 120% RMT in the resting FDI [15].

The potential influences of baroreflex processes on cortical monoamines affecting SICI are inferred from the following evidence. As explained in the Introduction to this chapter, baroreceptor unloading may result in increased noradrenergic, serotonergic, and dopaminergic function within the motor cortex. The influences of these neuromodulatory monoamines on SICI are variable. Administration of pharmacological agents that facilitate noradrenergic or serotonergic functions, including noradrenergic agonists [57, 67, 48] and serotonergic agonists [152] can decrease SICI and, similar to the application of LBNP [15], increase corticospinal excitability [8, 117, 66, 118, 67, 47]. The decreased SICI with LBNP contrasts with the increased SICI due to the administration of dopamine agonists [157, 160, 78], which were also shown to decrease corticospinal excitability [78]. Hence, baroreceptor unloading may inhibit intracortical inhibitory $GABA_A$-ergic activity possibly due to facilitation of noradrenergic and potentially serotonergic functions overriding the dopaminergic

functions on $GABA_A$-ergic activity.

3.4.4 Implication

Collectively, baroreceptor unloading decreases the intracortical $GABA_A$-ergic pathway in the resting hand muscle, probably by altering intracortical neuromodulatory activity and perhaps somatosensory afferent input. The current findings may be further integrated into exploring the potential mechanisms for the previous observation of increased corticospinal excitability with baroreceptor unloading only at higher TMS intensities (130% RMT and greater) in Specific Aim 1 [15]. Such higher TMS intensity is necessary to produce descending volleys with 2 ms delay and longer (later I-waves) [36]. As judged from the decrease in SICI with LBNP, baroreceptor unloading may decrease the activity of intracortical inhibitory $GABA_A$-ergic pathway that inhibits later I-waves 2 ms after excitation [37], and thus allow the excitatory later I-wave generating interneurons to recruit a larger number of corticospinal neurons and generate greater descending corticospinal activity.

The disinhibiting effects of LBNP were not seen during muscle contraction. Voluntary muscle contraction decreases SICI [126, 109]. In this study, SICI was decreased to the point of not being observed. The lack of effect of LBNP on intracortical excitability during voluntary muscle contraction implies either that the disinhibiting effects of LBNP were present, however, eclipsed by the disinhibiting effects of contraction or that the effects of LBNP on the neuromuscular system were shut off during voluntary contraction. Observing some motor effect of LBNP during voluntary contraction would rule out the latter and suggest the former possibility. Since, from the resting state data, LBNP appears to influence the GABA-ergic inhibitory pathway at the cortical level, it may influence other cortical inhibitory pathways, as well. Joint-stabilizing co-contraction is a motor task during which corticospinal excitability is decreased [1]. This decrease in excitability does not depend on the GABA-ergic

activity [1]. Co-contraction, therefore, provides an opportunity to further test the effects of LBNP during contraction.

In conclusion, baroreceptor unloading significantly decreased SICI, and did not affect SICF or ICF, in a resting hand muscle. In an active muscle, baroreceptor unloading had no effect on cortical silent period, SICF, or SICI. These findings suggest that baroreceptor unloading diminishes intracortical inhibition, at least in the resting muscle.

CHAPTER IV

AIM 3: CORTICOSPINAL EXCITABILITY DURING INDIVIDUAL MUSCLE CONTRACTION AND DURING JOINT-STABILIZING CO-CONTRACTION

4.1 Introduction

Specific Aims 1 and 2 demonstrated that, for a resting muscle, baroreceptor unloading increases corticospinal excitability [15], and this increase is, at least partially, explained by decreased GABA-ergic inhibition (as assessed with short-interval intracortical inhibition, SICI) at the motor cortex. No effect of baroreceptor unloading on intracortical excitability was observed during voluntary muscle contraction. Voluntary contraction also decreases GABA-ergic inhibition [126, 109]. The lack of effect of baroreceptor unloading on intracortical excitability during voluntary muscle contraction implies that either the disinhibiting effects of baroreceptor unloading were present, however, eclipsed by the disinhibiting effects of contraction or that the effects of baroreceptor unloading on the neuromuscular system were shut off during voluntary contraction. Observing some motor effect of baroreceptor unloading during voluntary contraction would rule out the latter and suggest the former possibility.

From Specific Aim 2 it is known that baroreceptor unloading, assessed with the lower body negative pressure (LBNP) procedure [26, 50], can modulate cortical pathways, in general, and disinhibits the motor cortex, in particular (by suppressing the GABA-ergic pathway). It is, then, possible that the LBNP procedure exerts influence over other cortical inhibitory pathways, as well. Joint-stabilizing co-contraction is a motor task during which corticospinal excitability decreases [1]. This decrease in corticospinal excitability appears to be cortical in nature and is not mediated via

the GABA-ergic pathway [1]. Joint-stabilizing co-contraction, therefore, presents an opportunity to further test if LBNP exerts influence over the neuromuscular system during voluntary contraction.

The purpose of this study was to observe the changes in corticospinal excitability for two tasks – joint-stabilizing co-contraction and individual muscle contraction – during baroreceptor unloading by means of the LBNP technique. Individual muscle contraction was investigated in addition to co-contraction for better interpretation of the data in the context of previous research. In Specific Aim 2, LBNP was shown to exert disinhibitory effects at the cortical level. The decrease in corticospinal excitability during joint-stabilizing co-contraction suggests inhibitory cortical activity [1]. An increase in corticospinal excitability with 40 mmHg LBNP was predicted during joint stabilizing co-contraction because this would imply cortical disinhibition. No effect of LBNP on corticospinal excitability during individual muscle contraction was predicted because of the lack of cortical inhibition during this task [126, 109] and because intracortical excitability was not altered by LBNP in Specific Aim 2.

4.2 Methods

4.2.1 Subjects

Twenty two healthy young adults (22.2 ± 4.4 years of age, 7 women) participated in the study. This subject number was originally based on an *a priori* sample size calculation performed with GPower version 3.1.5 (Universität Kiel, Germany). For a between-within study design with two groups, two measurements of each dependent variable, an alpha error probability of 0.05, power of 0.95, and a small effect size of 0.2, the *a priori* sample size was twenty subjects. All subjects were right-handed, as confirmed with the Edinburgh handedness inventory (Table 1) [106]. Participants did not present any signs of altered autonomic nervous activity (e.g.: diabetes, cardiovascular problems, brain or nerve disorder, obesity, hypertension, or hypotension [83]).

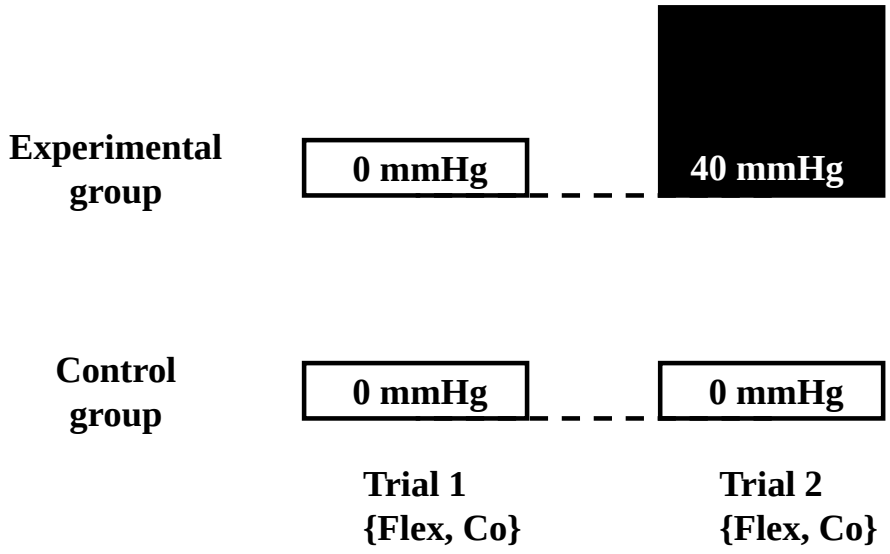

Figure 13: Schematic of protocol used in the Specific Aim 3 study. Both groups of subjects went through two trials of data collection. During each Trial corticospinal excitability was assessed for two tasks: individual muscle contraction and joint-stabilizing co-contraction. Lower body negative pressure (LBNP) was applied only in Trial 2 of the Test group.

They did not exhibit skilled use of hands or perform extensive hand grip activity. Subjects did not take any medication that may affect motor control or brain and nerve function. In addition, subjects were not allowed to participate if they had a family history of seizure or epilepsy, skin allergies, were pregnant, were prone to severe headaches, or had metal in their head, besides dental fillings [128]. To minimize the variability in the basal physiologic level and responsiveness of SNA to the LBNP procedure across subjects, all experiments were conducted at 8 am; participants abstained from food and drink, with the exception of water, for 10 hours prior to the experiment [5] and from all forms of exercise for 12 hours prior to the experiment. To avoid potential confounding effects of estrogen and progesterone on SNA, women were tested during their follicular phase [96]. All subjects gave written informed consent. The local Institutional Review Board approved the study.

4.2.2 Experimental approach

Figure 13 depicts the protocol used in the Specific Aim 3 study. Subjects were randomly assigned to either the Test (n = 12) or Control (n = 10) group. In both groups, subjects performed two motor tasks: isometric wrist flexion and isometric co-contraction of the wrist flexor and extensor muscles in the right forearm. The muscle of interest was the flexor carpi radialis muscle (FCR). Motor evoked potentials (MEP) were measured in both groups in response to transcranial magnetic stimulation (TMS) over the motor hotspot during the motor tasks. Supramaximal compound muscle action potential (Mmax) during rest and disynaptic inhibition during co-contraction were also measured. In the Test group, measurements were made without LBNP intervention (basal condition) in Trial 1 and with LBNP of 40 mmHg (condition with unloaded baroreceptors) in Trial 2. An ordered protocol was followed to avoid interference of possible residual effects of the LBNP procedure. To account for potential effects of order, a Control group, which was tested at basal condition in both Trials 1 and 2, was included in the study design. Effects of LBNP on corticospinal excitability during Flexion and Co-contraction were assessed from the MEP area during Trial 1 and Trial 2 for each task, compared between the two groups.

4.2.3 Motor task

The experiment was conducted in an electrically shielded room. The head was oriented in neutral position on a pillow; the right shoulder was abducted approximately 30 degrees and the forearm was secured midway between pronation and supination in a brace with a dowel inside the palm. The subjects were instructed to either flex their wrist against the dowel (Flexion) or co-contract their wrist flexor and extensor muscles (Co-contraction), depending on the task being assessed. Prior to data collection the subjects were instructed that the Co-contraction task was meant to stabilized the wrist in neutral position and increase the wrist joint stiffness and were

given approximately 10 minutes to become comfortable with executing the task. The orientation of the right hand was monitored throughout the experiment to ensure consistency. Bipolar surface electromyogram (EMG) was recorded from the right FCR and extensor carpi radialis (ECR) muscles using pairs of Ag-AgCl electrodes (E224A, IVM, Healdsburg, CA, USA) placed on the skin over the muscle bellies, in line with the long axis of the muscle. On each muscle the electrodes were separated by 2 cm. A sticky disposable electrode (Telectrode/T716, Bio Protech, Wonju si, Gangwon-do, Korea) was placed on the medial epicondyle of the right arm to serve as the reference. The EMG was differentially pre-amplified 300 times and bandpass filtered 15 – 2,000 Hz (Y03-000, MotionLabs, NY, USA).

Contraction intensity was determined based on the EMG amplitude of FCR during maximal voluntary isometric contraction that was performed in the following manner. With their wrist clamped between two boards with C-clamps midway between flexion and extension, subjects increased their EMG amplitude to maximum in a ramp fashion over 3 s, and maintained it at maximum for 2 s before relaxing. Subjects were told to use only their FCR, and to relax all other muscles. Verbal instruction and encouragement were provided while the right hand of subjects was visually monitored. A rectified running average EMG with an averaging window of 0.175 s was used to provide visual feedback to subjects and to calculate the maximal EMG (EMGmax) of the FCR. We decided to use the average window of 0.175 s because, during the pilot study, we found the use of this window reduces the distracting high-frequency signals for judging the level of muscle activity while it does not lose the responsiveness of detecting the changes in muscle activity.

Subjects were provided visual feedback of FCR and ECR activity and instructed to match their FCR activity level to a target set no greater than 10% EMGmax. The target level did not change through out the experiment. Flexion and Co-contraction tasks were performed using the same visual feedback of EMG (Fig. 14). Subjects were

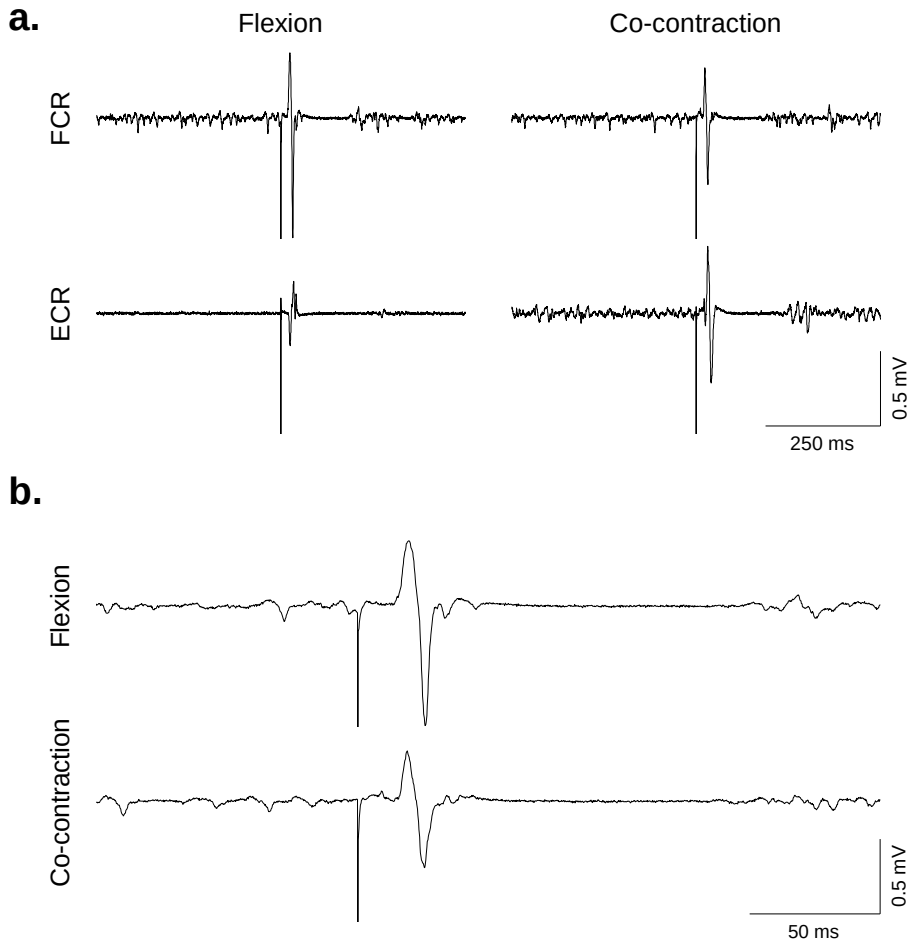

Figure 14: **a.** The traces are individual 0.8 s recordings of interference electromyogram recorded from a single subject during Trial 1 in the Specific Aim 3 study in response to transcranial magnetic stimulation delivered 0.4 s into the presented recordings. The top row shows recordings from the flexor carpi radialis (FCR), the bottom row – - extensor carpi radialis (ECR). Left column was recorded during Flexion, while the right column was recorded during Co-contraction. **b.** Traces from the FCR in a., zoomed in on the time axis to demonstrate the waveform.

repeatedly asked of their fatigue perception and breaks were given in between TMS blocks as necessary. The instruction to the subjects during Flexion was to flex their hand against the dowel. During Co-contraction, subjects were instructed to stabilize or harden the wrist joint, and not radially deviate the wrist or squeeze the fingers. Instructions with regard to a specific target level of ECR activity were not provided because of the difficulty in adjusting the amount of ECR activity to reach the same level across subjects. Instead, subjects were instructed to maintain the achieved level of ECR activity throughout the Co-contraction task. One subject in the Test group was unable to complete the experiment during Co-contraction of Trial 2.

4.2.4 Baroreceptor unloading

The LBNP technique was used to unload the baroreceptors, similar to our previous studies [15] and Specific Aim 2. The lower body of the subjects was sealed inside an airtight LBNP chamber, at the level of the iliac crest. A bicycle seat inside the chamber insured the subject remained in a stable position during application of 40 mmHg LBNP. The pressure inside the chamber was controlled with the aid of a valve and a commercial vacuum (Dayton Industrial, Dayton, OH, USA). In Trial 2 of the Test group, the pressure in the chamber was gradually reduced to -40 mmHg relative to ambient pressure and maintained at this value during data collection. LBNP of 40 mmHg is known to unload the baroreceptors and increase SNA, as evidenced by increased muscle sympathetic nerve discharges [143, 31, 145], increased epinephrine and norepinephrine concentration in plasma [58], and increased heart rate with little changes in blood pressure [15, 137] and Specific Aim 2. During Trial 1 for the Test group and Trials 1 and 2 for the Control group (i.e. all trials besides Trial 2 in the Test group), data collection was performed with the pressure set to ambient (0 mmHg LBNP) and the vacuum turned on. Blood pressure at the brachial artery of the left arm and heart rate (averaged over 5-7 s) from the electrocardiogram (ECG) were

monitored noninvasively (Cardiocap/5, GE Healthcare, Giles, UK) and recorded in between TMS blocks. Measurement of blood pressure took approximately 30 s. The ECG was sampled at 100 samples/s with an analog-to-digital converter (Power 1401, Cambridge Electronic Design Ltd, Cambridge, UK) and data acquisition software (Spike 2 v.7, Cambridge Electronic Design Ltd, Cambridge, UK) for offline analysis of heart rate variability.

4.2.5 Corticospinal excitability

Corticospinal excitability was assessed noninvasively with single-pulse TMS (Magstim 200^2, by way of BiStim module, Magstim Co, Wales, UK) of the left primary motor cortex. A figure-of-eight coil (Magstim second generation double 70 mm remote coil, Magstim Co, Wales, UK) was held over the left primary motor cortex at the optimum position (i.e. hotspot) for eliciting an MEP in the resting FCR muscle of the right forearm. The coil was held with the handle pointing posteriorly at an angle of approximately 45 degrees to the sagittal plane yielding an E-field perpendicular to the central sulcus [11]. A TMS coil navigation system (NDI TMS Manager, Northern Digital Inc, Waterloo, Ontario, Canada) was used to maintain the coil position in 3-dimensional space relative to the head. The EMG data were sampled at 5000 samples/s with an analog-to-digital converter (Power 1401, Cambridge Electronic Design Ltd, Cambridge, UK) and data acquisition software (Signal v.5, Cambridge Electronic Design Ltd, Cambridge, UK) for online monitoring, storage, and offline analysis.

The resting motor threshold (RMT) for the FCR muscle was determined as the smallest TMS intensity needed to elicit an MEP with peak-to-peak amplitude greater than 50 μV in 5 out of 10 consecutive stimulations [30, 16] in the resting FCR. RMT was measured in percentage of maximal stimulator output for each subject, with the LBNP vacuum turned off. MEPs were collected at TMS intensity of 110% of RMT

and greater, at 10% RMT increments. TMS was delivered every 6 s. Measurements were made in blocks, with 12 MEP responses per block (i.e. per TMS intensity), and the order of the TMS intensity blocks was randomized. Breaks were given in between blocks as needed to prevent fatigue. MEPs were collected during Flexion and Co-contraction tasks. In Trial 1, MEPs from both tasks were collected at ambient pressure (0 mmHg LBNP) in both groups. In Trial 2, MEPs were collected again at ambient pressure in the Control group and at 40 mmHg LBNP in the Test group. Assessment of corticospinal excitability was not possible in one subject from the Test group and one subject from the Control group due to equipment issues.

4.2.6 H-reflex and Mmax

Effect of LBNP on Mmax and on the disynaptic inhibition of FCR [99, 148] were investigated using transcutaneous bipolar electrical stimulation of the median nerve and radial nerve. The electrical stimulation was delivered via pairs of spherical stimulating electrodes, separated by 2 cm, connected to a constant current stimulator (S88-SIU5-CCU1, Grass Products, Natus Neurology Inc., Warwick, RI, USA). Potential increase in SNA due to pain of the electrical stimulation would present a confounding variable to increase in SNA with LBNP [143, 31, 145]. Mmax and disynaptic inhibition were assessed only in those subjects who did not perceive the electrical stimulation as painful. Mmax of the FCR muscle was assessed with a 1 ms square-wave electrical stimulation delivered to the median nerve at supramaximal intensity: 150% of intensity that elicited maximum compound muscle action potential. Twelve Mmax responses were measured from the resting FCR, while electrical stimulation was delivered every 10 s. Mmax was obtained from nine and eight subjects from the Test and Control groups, respectively.

Disynaptic inhibition was assessed during Co-contraction as the reduction of the H-reflex in FCR due to conditioning stimulation of the radial nerve (Fig. 15) [99].

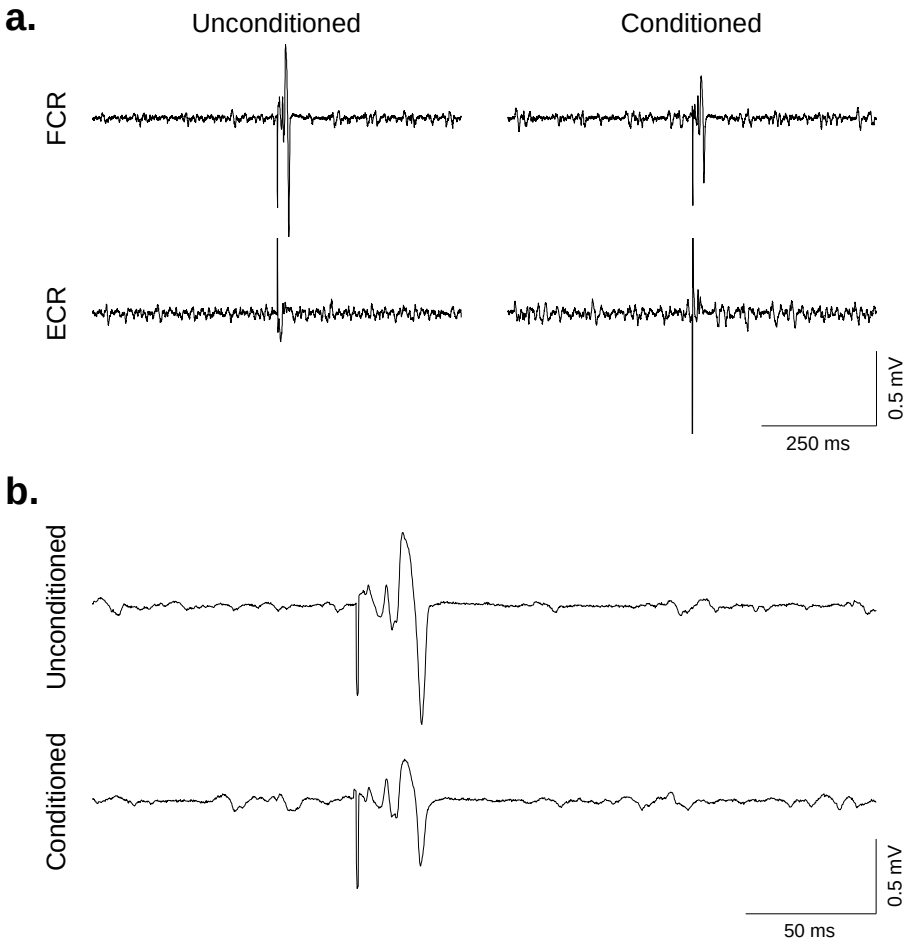

Figure 15: **a.** The traces are individual 0.8 s recordings of interference electromyogram recorded from a single subject during Trial 1 in the Specific Aim 3 study in response to electrical stimulation delivered 0.4 s into the presented recordings. The top row shows recordings from the flexor carpi radialis (FCR), the bottom row – - extensor carpi radialis (ECR). Left column shows the H-reflex elicited by median nerve stimulation. The right column shows the H-reflex conditioned by stimulation of the radial nerve. **b.** Traces from the FCR in a., zoomed in on the time axis to demonstrate the waveform.

The H-reflex in FCR was produced by the electrical stimulation of median nerve at an intensity on the ascending limb of the recruitment curve for the H-reflex. Between trials, intensity of stimulation was adjusted as necessary to maintain an H-reflex of comparable size. The radial nerve was conditionally stimulated at motor threshold, as judged by the appearance of a muscle compound action potential in the EMG of resting ECR. Both nerves were stimulated with a 1 ms square-wave pulse. First, the FCR H-reflex was observed in the resting FCR muscle. Then, the reduction of the resting FCR H-reflex by electrical stimulation of the radial nerve preceding the median nerve stimulation by 0 and 1 ms was measured. The interval that gave the greatest reduction in resting FCR H-reflex was subsequently used to assess disynaptic inhibition during Co-contraction. Responses to twelve unconditioned and twelve conditioned stimulations delivered every 6 s were recorded from the FCR. H-reflexes were successfully measured in eight and six subjects from the Test and Control groups, respectively.

4.2.7 Data collection sequence

During Trial 1, Mmax was measured first, followed by corticospinal excitability during Flexion, corticospinal excitability during Co-contraction, and disynaptic reciprocal inhibition during Co-contraction in random order. Trial 2 followed the same order as Trial 1.

4.2.8 Data reduction

The first 2 MEP responses in each block were discarded to control for possible startle responses. Mmax recordings that showed obvious pre-stimulus EMG activity in FCR and Flexion task recordings that showed obvious EMG activity in ECR 400 ms preceding the TMS were discarded by visual inspection. All remaining EMG recordings were rectified and averaged within stimulation blocks. The mean pre-stimulus EMG amplitude in FCR and ECR were calculated for all recordings; the pre-stimulus EMG

amplitude in FCR was expressed relative to EMGmax in FCR. The MEP was defined as the EMG response between 12 and 50 ms post TMS. Peak amplitude of the average rectified MEP (MEP peak) and the area bound by the average rectified MEP and the 0 mV axis (MEP area) were calculated for each TMS response. The area measurement in response to electrical stimulation was performed similarly. For assessing disynaptic inhibition, the ratio of the H-reflex area during conditioned stimulation to the area during unconditioned stimulation was calculated (H-reflex ratio).

The automated cumulative sum method was used to measure the cortical silent period [76] in the FCR produced by TMS during the motor task. The start and end of the cortical silent period were defined as the times, following the MEP, from when the average rectified EMG fell below and increased back up to the pre-stimulus EMG level, respectively.

To examine autonomic responses to LBNP, heart rate variability was assessed from the ECG recordings taken during measure of corticospinal excitability during the motor task. All ECG recordings used in calculation of heart rate variability were greater than three minutes in duration. From the ECG recording, all R-wave peaks were identified, marked, and visually inspected to rule out artifacts. Then the power spectrum of the R-to-R interval was calculated. Low frequency (0.05 – 0.15 Hz) power was expressed relative to the power in total frequencies (0.05 – 0.50 Hz, HR_{LF}) and was used as a measure of SNA [19].

4.2.9 Statistical analysis

Subject characteristics include the age, handedness index, baseline heart rate, baseline mean arterial blood pressure (MAP), and RMT that was measured with the vacuum turned off before Trial 1. These variables were compared between the two groups with a Students independent samples t-test. Effects of LBNP on Mmax, unconditioned H-reflex, and H-reflex ratio were assessed with a two-factor (Group $\times$ Trial) analysis

of variance (ANOVA) with repeated measures for Trial. Effects of LBNP on heart rate, HR_{LF}, MAP, pre-stimulus EMG amplitude, MEP peak, MEP area, and cortical silent period were assessed using a three-factor (Group × Trial × Task) ANOVA with repeated measures for Trial and Task. Effects of LBNP were judged from within-Group effects of Trial. Inclusion of the Control group in the ANOVAs ensured that any differences seen between trials 1 and 2 in the Experimental group were not due to order effect. When a 3-factor interaction was found, to clarify if Task affected the variables without the influence of LBNP, the variables in Trial 1 were assessed with a two-factor (Group × Task) ANOVA with repeated measures for Task. An alpha level of 0.05 was used for all significance testing, and $P < 0.05$ and $P < 0.01$ were noted where appropriate. Tukey post-hoc test was used to test significant interactions when appropriate. Statistical analyses were performed using Statistica 9.0 (StatSoft Inc., Tulsa, OK, USA). Unless stated otherwise, the data are presented as mean ± SD in the text and tables and as mean ± standard error of mean in the figures.

4.3 Results

4.3.1 Subject characteristics

Basic subject characteristics, including age, L.Q. value, EMGmax, heart rate, mean arterial blood pressure, and RMT were not different between groups (Test vs. Control, Table 8)

4.3.2 Cardiovascular response

For heart rate, there were a main effect of Trial ($P < 0.01$), Task ($P < 0.01$), an interaction of Group and Trial ($P < 0.01$), and an interaction of Group, Trial, and Task ($P < 0.05$). As a within-Group effect, heart rate in Trial 2 was greater compared with Trial 1 by 15.9 bpm ($P < 0.01$) in the Test group, but not in the Control group (Fig. 16). During Trial 1 (i.e. without effects of LBNP) heart rate was found to be affected by the Task ($P < 0.01$). In Trial 1, heart rate during Co-contraction (61.2

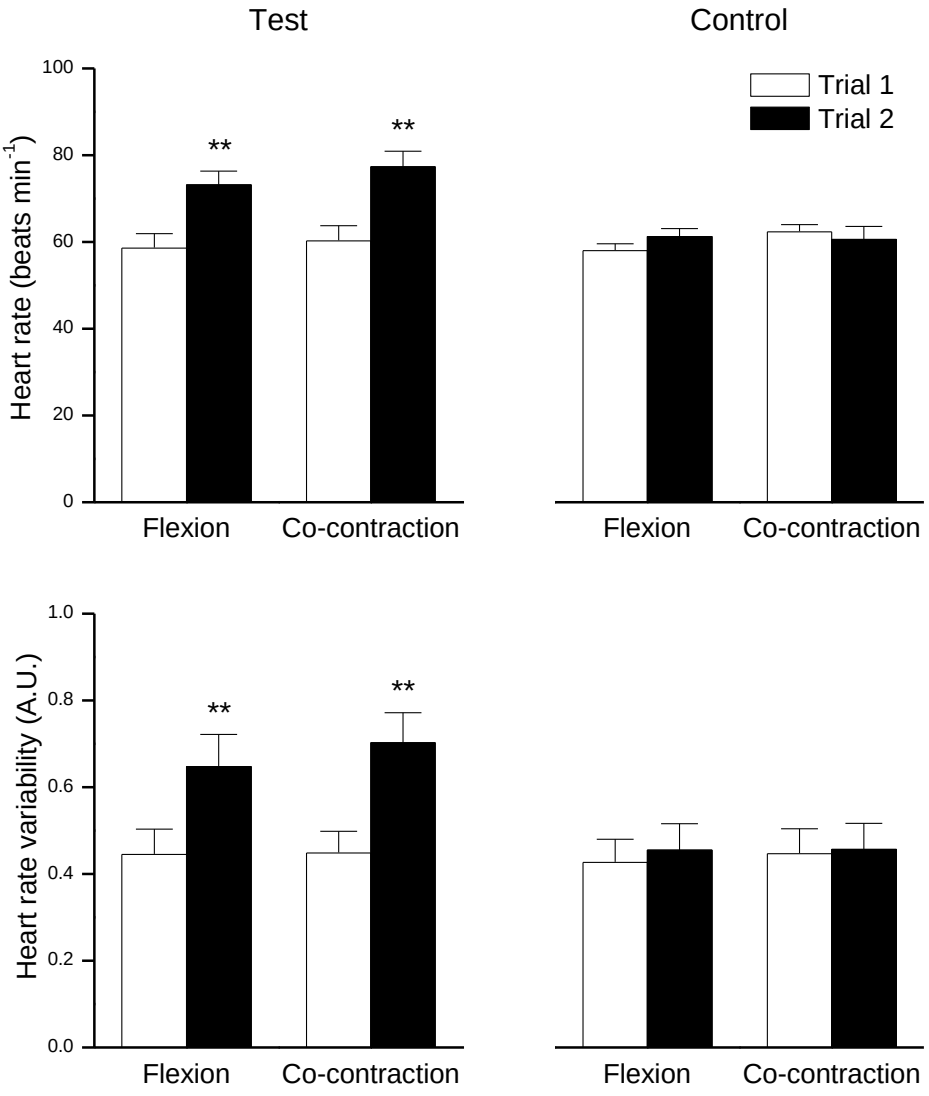

Figure 16: Cardiovascular measurements during different stages of the Specific Aim 3 experiment in the Test and Control groups. Heart rate (top) and heart rate variability (bottom, 0.05 – 0.15 Hz power fraction of heart rate variability relative to the total power of the R-to-R interval spectrum); A.U., arbitrary units of heart rate variability power fraction. $^{**}P < 0.01$ post hoc analysis of significant Group $\times$ Trial interaction.

Table 8: Baseline characteristics of each group in Specific Aim 3 study. L.Q., laterality quotient for right handedness; EMGmax, electromyogram amplitude during maximal voluntary contraction; bpm, beats per minute; MAP, mean arterial blood pressure; RMT, resting motor threshold; MSO, maximal stimulator output. No significant difference between groups

	Test group	Control group
N	12	10
Age, years	22.67 ± 5.45	21.70 ± 2.98
L.Q. value	0.76 ± 0.22	0.68 ± 0.19
EMGmax, mV	0.31 ± 0.28	0.39 ± 0.27
Heart rate, bpm	57.3 ± 10.35	61 ± 5.46
MAP, mmHg	85.4 ± 9.75	83.5 ± 7.82
RMT, % MSO	51.00 ± 10.39	50.10 ± 7.84

± 8.79 bpm) was greater than during Flexion (58.3 ± 8.28 bpm) by 2.9 bpm, on average, across groups.

For HR_{LF}, there were a main effect of Trial ($P < 0.01$) and an interaction of Group and Trial ($P < 0.01$). HR_{LF} increased from Trials 1 to 2 in the Test group (47.4%, $P < 0.01$, Fig. 16), but not in the Control group.

Mean arterial blood pressure was maintained around 84.1 ± 8.5 mmHg, on average, throughout the experiment. There was no significant effect or interaction of Group, Trial, or Task on mean arterial blood pressure.

4.3.3 Background contraction

Subjects were asked to produce a steady contraction with their FCR less than 10% of EMGmax during the motor tasks. With a main effect of Group ($P < 0.05$), pre-stimulus EMG amplitude in FCR resulted in 6.9 ± 2.8% EMGmax in the Test group and 5.3 ± 2.2% EMGmax in the Control group. There were no significant effects or interactions of Task or Trial on pre-stimulus EMG amplitude in FCR.

For ECR, with a main effect of Task ($P < 0.01$), pre-stimulus EMG amplitude of ECR increased approximately 8 fold from 3.2 ± 0.6 μV during Flexion to 24.0 ± 19.2

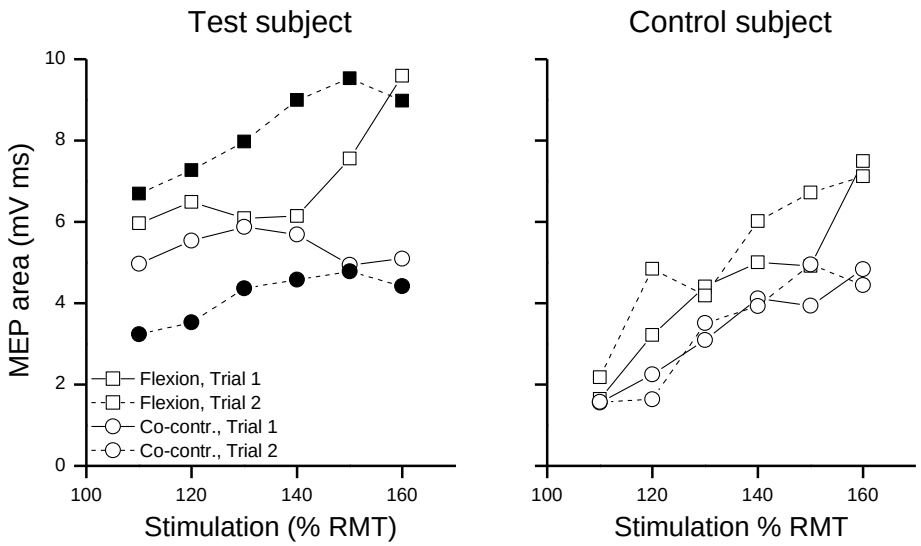

Figure 17: FCR MEP response of two representative subjects in the Specific Aim 3 study. The subject on the left is from the Test group and the subject on the right is from the Control group. Solid lines indicate response during Trial 1; broken lines indicate response during Trial 2; square symbols denote response during wrist flexion; circles denote response during wrist co-contraction; filled symbols indicate when sympathetic nerve activity was heightened (Trial 2 of Test group, only). MEP, motor evoked potential; RMT, resting motor threshold.

μV during Co-contraction. There were no significant effects or interactions of Group or Trial on pre-stimulus EMG amplitude of ECR.

4.3.4 Corticospinal excitability

Figure 17 shows the MEP area response of FCR in two representative subjects during Flexion and Co-contraction in trials 1 and 2. One subject is from the Test group (left panel) and another one is from the Control group (right panel). In Trial 2, the subject from the Test group, i.e. with the application of LBNP, appeared to show increased MEP amplitude during Flexion and, conversely, decreased MEP amplitude during Co-contraction, compared with Trial 1.

These observations in individual subjects were statistically tested in the grouped data. There were a main effect of Task ($P < 0.01$) and Group $\times$ Task ($P < 0.01$) and Trial $\times$ Task ($P < 0.05$) interactions for MEP peak; and a main effect of Task (P

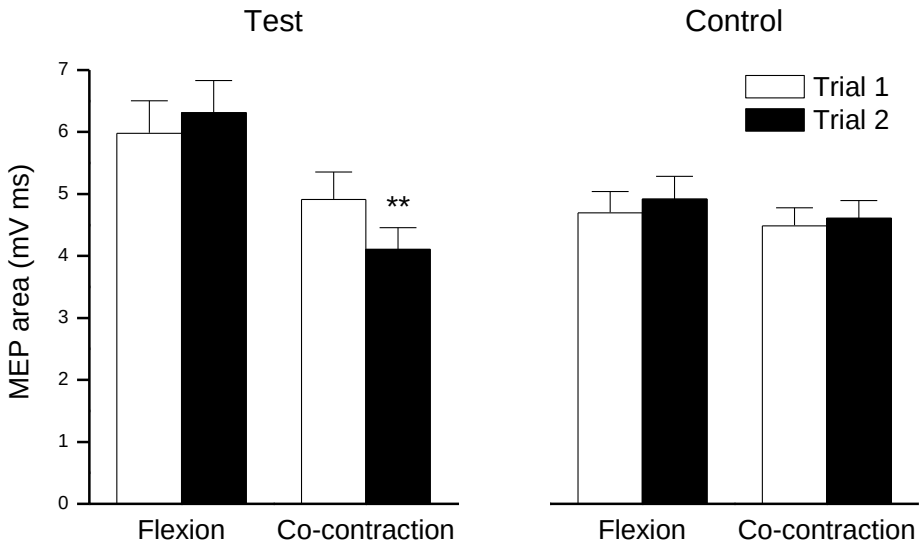

Figure 18: Corticospinal excitability in the Specific Aim 3 study. Response of Test group and Control group subjects during Flexion and Co-contraction tasks in Trials 1 and 2. MEP, motor evoked potential. $^{**}P < 0.01$ between trials for the specified Task in the Group.

< 0.01) and Group $\times$ Task ($P < 0.01$), Group $\times$ Trial ($P < 0.05$), Trial $\times$ Task ($P < 0.01$), and Group $\times$ Trial $\times$ Task ($P < 0.01$) interactions for MEP area. Post hoc analysis did not reveal any significant within-Group effects of Trial for MEP peak. Post hoc analysis of the Group $\times$ Trial $\times$ Task interaction of MEP area revealed that, in the Test group, MEP area in Trial 2 during Co-contraction was decreased by 22% compared with Trial 1 ($P < 0.01$, Fig. 18). During Flexion in the Test group, there was no significant difference between Trial 1 and Trial 2. In the Control group, MEP area was not significantly different across tasks and trials. When the effect of Task on MEP area without the influence of LBNP was further examined by testing Trial 1 data only, both groups presented lower MEP area during Co-contraction (4.90 ± 2.83 mV·ms) by 8.6% compared with Flexion (5.36 ± 3.25 mV·ms) in Trial 1, with the presence of a main effect of Task in a 2-factor ANOVA ($P < 0.05$).

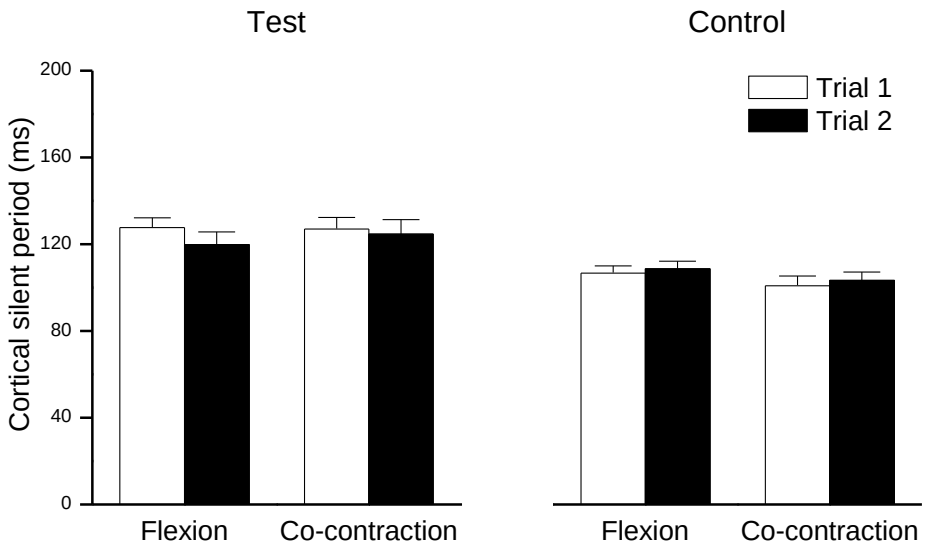

Figure 19: Cortical silent period in the Specific Aim 3 study, measured, in ms, from the flexor carpi radialis.

4.3.5 Cortical silent period

There was a main effect of Group ($P < 0.01$) and a Group × Trial interaction ($P < 0.05$) on cortical silent period. On average, cortical silent period in the Test group (125 ± 42 ms) was 20 ms longer compared with the Control group (105 ± 27 ms). In each group, cortical silent period was not significantly different across tasks and trials as post hoc analysis of the Group × Trial interaction did not reveal any significant within-Group effects (Fig. 19).

4.3.6 H-reflex and Mmax

To examine the amount of disynaptic inhibition in the FCR, H-reflex ratio between unconditioned and conditioned stimulation was obtained with the peripheral stimulation intensity that was expected to yield the comparable unconditioned H-reflex in the FCR across trials. On average, the unconditioned H-reflex was 3.81 ± 2.73 mV·ms and was not significantly different between trials or groups. With a main effect of Group ($P < 0.05$), H-reflex ratio in Test group (0.89 ± 0.18 A.U.) was greater than in the Control group (0.64 ± 0.25 A.U.) across trials (Table 9). There was no

Table 9: Peripheral effects of lower body negative pressure, measured from the flexor carpi radialis in the Specific Aim 3 study. Lower body negative pressure of 40 mmHg was applied during Trial 2 in the Test group, only. A.U. arbitrary units; Mmax, compound muscle action potential in response to supramaximal electrical stimulation. A main effect of Group was detected for the H-reflex ratio.

		Test group	Control group
H-reflex ratio (A.U.)	Trial 1	0.86 ± 0.11	0.61 ± 0.23
	Trial 2	0.92 ± 0.23	0.67 ± 0.29
Mmax (mV·ms)	Trial 1	25.5 ± 16.5	25.1 ± 13.1
	Trial 2	26.1 ± 15.9	24.5 ± 13.0

significant main effect of Trial or interaction on the H-reflex ratio.

To examine the excitability at and distal to motor endplate, Mmax in the FCR was obtained with supramaximal peripheral stimulation. There was no significant effect or interaction of Group or Trial on Mmax (Table 9).

4.4 Discussion

The major findings of this study are the decrease in MEP area during Co-contraction but not during Flexion in Trial 2 in the Test group, in which LBNP of 40 mmHg was applied. In the Control group, there were no differences in MEP area across tasks and trials.

4.4.1 Cardiovascular response

LBNP of 40 mmHg is known to unload the baroreceptors and heighten SNA as evidenced by increases in muscle sympathetic nerve discharges [143, 31, 145], concentration of epinephrine and norepinephrine in the plasma [145, 58], heart rate [143, 15, 31, 145], and HR_{LF} [85]. In the current study, LBNP of 40 mmHg in Trial 2 of the Test group increased heart rate and HR_{LF}, while maintaining mean arterial blood pressure. In the Control group, there were no significant differences in these cardiovascular measures. These cardiovascular responses in the Test group are

comparable to previous studies employing LBNP [143, 15, 31, 145, 85], and support unloading of baroreceptors with the application of LBNP.

4.4.2 Corticospinal excitability

Within each group, subjects maintained comparable contraction intensity across trials and tasks as shown by the indifferent pre-stimulus EMG amplitude. A slight difference in pre-stimulus EMG amplitude between groups (less than 2% EMGmax) is not related to baroreceptor unloading because there was no significant interaction of Group and Trial. No significant difference in resting Mmax across groups and tasks is consistent with the absence of effect of LBNP on Mmax at rest observed in Specific Aim 1 [15], indicating that the efferent pathways at and distal to the motor-end plate are not influenced by baroreceptor unloading.

No influence of baroreceptor unloading on corticospinal excitability during agonist contraction is suggested by the absence of significant effect of Trial on MEP peak and MEP area during Flexion in the current study. This finding in FCR is in line with the absence of effects of LBNP on measures of intracortical excitability during index finger abduction with the first dorsal interosseus muscle, previously observed in Specific Aim 2. The current findings thus extend the absence of effect of baroreceptor unloading on intracortical excitability during agonist contraction from an intrinsic hand muscle to absence of effect on corticospinal excitability during agonist contraction of a more proximal forearm muscle.

A reduction in corticospinal excitability due to co-contraction is suggested by smaller MEP area during Co-contraction compared with Flexion during Trial 1 (i.e. without LBNP) in the current study. This finding is consistent with a similar study during wrist co-contraction in which ECR corticospinal excitability was shown to decrease during co-contraction [1], and thus supports that the current subjects completed the tasks appropriately. Comparable pre-stimulus EMG amplitude between

Flexion and Co-contraction suggests comparable activation of spinal motor-neurons between the tasks and supports the previous suggestion that the decline in corticospinal excitability with co-contraction is likely not caused by potential alterations at the spinal motor-neuron level, but by a reduction in the excitability of the monosynaptic corticospinal neurons at the motor cortex [1].

MEP area proved more responsive than MEP peak amplitude to LBNP during Co-contraction as the latter measure did not show any significant effects of LBNP during Co-contraction. This discrepancy between the two measures of MEP size can be statistical or physiological in nature. The MEP is measured from the EMG signal, an interference signal composed of the temporal summation of the action potentials generated along the muscle fibers underlying the recording electrodes [42]. Since the MEP peak amplitude measure is dependent on the precise alignment of action potentials, it is more susceptible to variability of the EMG generating components (e.g. proximity of the muscle fibers stimulated by TMS to the recording electrodes). The variability of the MEP peak response during Co-contraction may have been too great to observe any significant effects of LBNP. On the other hand, the MEP peak amplitude may have remained unaltered in the presence of a decrease in MEP area if there was a physiological change in the onset of muscle fiber action potentials (due to changes in conduction velocity or arrival delay of descending activity). A reduction in corticospinal excitability accompanied by a decrease in the arrival delay of later MEP generating neural activity, for example, would maintain the MEP peak amplitude and shorten the MEP duration, thereby decreasing MEP area.

A reduction in corticospinal excitability due to LBNP during Co-contraction is suggested by the decrease in MEP area of FCR during Co-contraction in Trial 2 (i.e. with LBNP) compared with Trial 1 (i.e. without LBNP) in Test group in the current study. This finding is contrary to our hypothesis of increased corticospinal excitability due to baroreceptor unloading. Maintenance of comparable pre-stimulation EMG

amplitude in FCR between Flexion and Co-contraction suggests comparable voluntary activation of spinal motor-neurons between the two tasks and supports that the decline in corticospinal excitability during co-contraction is likely not caused by a potential alteration in the excitability of the spinal motor-neurons [1, 103]. Since there appears to be no influence of baroreceptor unloading on the major neural pathways within FCR during Flexion, the decrease in MEP area of FCR during Co-contraction is most likely induced by the effect of baroreceptor unloading on the neural pathways that are additionally recruited or modulated with the wrist co-contraction. The major additional neural pathways due to co-contraction include 1) disynaptic inhibition in the spinal cord [99, 148], 2) cortical reciprocal inhibition [7, 62], and 3) the corticospinal neurons recruited specifically during co-contraction [43]. These pathways are summarized in Figure 20.

As a first potential pathway, disynaptic inhibition refers to the inhibition of spinal motor-neurons by Ia inhibitory interneurons [99, 148, 103]. For the FCR muscle, the Ia inhibitory interneurons (i.e. the ones that inhibit the FCR motor-neurons) can be recruited by group I afferents of the FCR and ECR [148] and can be modulated by corticospinal neurons of the ECR [43]. An increase in disynaptic inhibition with baroreceptor unloading could potentially decrease the excitability of the FCR spinal motor-neurons, and result in a smaller MEP during wrist co-contraction. However, this possible influence of baroreceptor unloading on disynaptic inhibition in the spinal cord is not supported by the invariable H-reflex ratio observed between trials in the present study.

A second potential pathway is cortical reciprocal inhibition that refers to inhibitory action of somatosensory afferents of ECR muscle on corticospinal outputs to the FCR muscle via cortical interneurons [7, 62, 17]. An increase in cortical reciprocal inhibition could result from a) increased activity of the somatosensory afferents

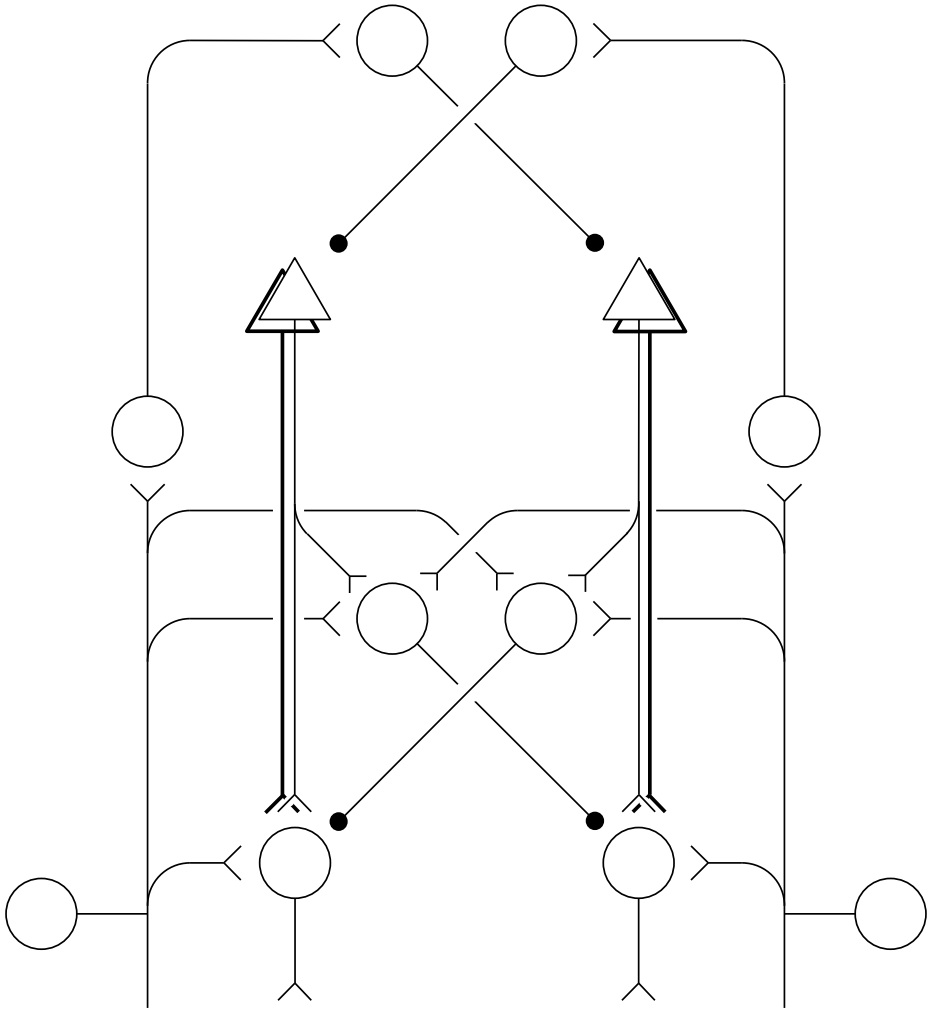

Figure 20: Schematic model of co-contraction specific neuromuscular pathways potentially affected by baroreceptor unloading. The model depicts disynaptic inhibition, cortical reciprocal inhibition, and co-contraction specific corticospinal neurons. Corticospinal neurons are denoted with triangular soma. The low threshold corticospinal neurons in the foreground project to spinal motor-neurons and to Ia inhibitory interneurons. The higher threshold co-contraction specific corticospinal neurons project to the spinal motor-neurons, only. They are in the background, denoted with a thicker contour. Spinal Ia inhibitory interneurons and cortical reciprocal interneurons are denoted with solid circles at their axon terminals.

or b) increased excitability of the reciprocal inhibitory interneurons in the cortex. Increased activity of the somatosensory afferents with baroreceptor unloading was not supported by the invariable H-reflex ratio between trials during Co-contraction. Additionally, the invariable MEP area during Flexion between trials does not support increased activity of the somatosensory afferents with baroreceptor unloading. In contrast, there are no available data or report that may help suggest whether baroreceptor unloading increases the excitability of the reciprocal inhibitory interneurons in the cortex.

A third potential pathway suggested involves corticospinal neurons that are recruited specifically during co-contraction [43]. According to studies on the primate neuromuscular system, there exist at least two functionally distinct corticospinal neurons that are preferentially recruited based on the contraction task [43, 65]. The corticospinal neurons that synapse onto both spinal motor-neurons of a muscle and the coupled Ia inhibitory interneurons are suggested to be recruited during simple agonist contraction, while the ones that only synapse onto spinal motor-neurons of a muscle are suggested to be recruited during co-contraction [43, 65]. In humans, recruitment of distinct corticospinal neurons during co-contraction is supported by a shift in the peak activation area in the motor cortex between tasks of agonist contraction and antagonist co-contraction in a brain imaging study [69]. The possibility of whether or not baroreceptor unloading exerts a net inhibitory effect on the corticospinal neurons specific to co-contraction cannot be inferred or refuted from the current data.

Taken together, the LBNP-induced reduction in corticospinal excitability during Co-contraction may involve greater cortical reciprocal inhibition or inhibition of the corticospinal neurons specific to co-contraction due to baroreceptor unloading. Further research is warranted to delineate these mechanisms.

4.4.3 Cortical silent period

Cortical silent period was not significantly affected by trials during either Flexion or Co-contraction in either group, in contrast to MEP. The duration of the cortical silent period can be affected by the contraction intensity and steadiness of contraction before and after TMS [90]. Given feedback of their EMG amplitude, all subjects maintained a steady contraction through stimulation, and pre-stimulus EMG activity did not show significant differences between trials or tasks, suggesting comparable contraction steadiness and intensity between tasks and trials. Therefore, the results suggest that baroreceptor unloading does not affect the cortical silent period during contraction of wrist flexors or co-contraction. These findings corroborate a finding in the first dorsal interosseus muscle during index finger abduction (Specific Aim 2) and extend the knowledge in that an absence of effect of baroreceptor unloading on the cortical silent period does not depend on contraction tasks with regard to the involvement of co-contraction.

4.4.4 Implication

The current findings demonstrate that the effects of LBNP on MEP size show a task dependency, while effects of LBNP on cortical silent period do not. This contrasting effect of LBNP implies different influences of baroreceptor unloading on distinct intracortical mechanisms for MEP size and cortical silent period duration. As stated above, the reduction in MEP size with LBNP during Co-contraction may involve greater cortical reciprocal inhibition or inhibition of corticospinal neurons specific to co-contraction. The cortical silent period, on the other hand, indicates intracortical GABA-ergic activity [74]. While GABA-ergic activity would influence MEP size, GABA-ergic activity is not affected by co-contraction [1]. Nor is GABA-ergic activity affected by LBNP during muscle contraction (Specific Aim 2). Therefore, the task dependency of the effects of LBNP on MEP area and the apparent absence of such

task dependency for cortical silent period imply that the decrease in MEP size during Co-contraction with baroreceptor unloading is not due to GABA-ergic mechanisms.

The task dependent effects of baroreceptor unloading may have been caused by the novelty and difficulty of the Co-contraction task. It can be argued that subjects had less experience with isometrically contracting both the wrist flexors and extensors while keeping their palm open and around a dowel than with isometrically flexing the hand into the dowel. Certainly, nearly all subjects reported having more difficulty with the Co-contraction task and used the 10 minute practice window prior to data collection for practice of the Co-contraction task more than the Flexion task. Corticospinal excitability increases during encoding of novel fine motor skills [113, 114]. If, during the course of data collection, the subjects were inadvertently encoding a the Co-contraction task, their corticospinal excitability may have been increased. This increase in corticospinal excitability may then have allowed for the observation of a decrease in corticospinal excitability with LBNP. Such possibility does not argue against effects of LBNP over the motor system during voluntary contraction, since both the Control and Test group would presumably experience motor encoding during the course of the study. However, the effects of LBNP may not be evident once the Co-contraction task is equally learned as Flexion and corticospinal excitability is no longer increased due to encoding [113]. This possibility can be tested with a training paradigm.

In conclusion, baroreceptor unloading exerts influence over the neuromuscular system during voluntary contraction and this influence appears task dependent. Baroreceptor unloading by means of 40 mmHg LBNP did not influence corticospinal excitability during wrist flexion but diminished corticospinal excitability during wrist joint-stabilizing co-contraction.

CHAPTER V

INTEGRATION

5.1 Neuromuscular pathways

Figure 21 shows a simplified schematic of the corticospinal pathway and associated neuromuscular pathways investigated in this thesis for influence of baroreceptor unloading. Motor generating mechanisms at and distal to the motor endplate do not appear to be influenced by baroreceptor unloading, as demonstrated by the lack of significant effect of LBNP on maximum compound muscle action potential presented in Specific Aims 1 and 3.

Spinal motor-neuron excitability was not tested directly in any of the Specific Aims. However, none of the results collected from any of the aims support an influence of baroreceptor unloading over spinal motor-neuron excitability. In Specific Aim 1, MEP was increased at higher intensities of stimulation. This suggests that at least the low threshold motor-neurons are not influenced by LBNP. The lack of effect of LBNP on MEP during wrist flexion and the reduction of corticospinal excitability during wrist co-contraction, but not during wrist flexion with LBNP, at comparable levels of motor pool recruitment observed in Specific Aim 3 argues against an effect of baroreceptor unloading on a partially active motor-neuron pool. Baroreceptor unloading increases peripheral sympathetic nerve activity [143]. Lack of effect of heightened sympathetic nerve activity on the H-reflex in a previous study [72] further supports a lack of effect of baroreceptor unloading on spinal motor-neuron excitability.

Effects of baroreceptor unloading on the Ia inhibitory interneuron excitability were tested with the conditioned H-reflex technique during joint-stabilizing co-contraction. 40 mmHg LBNP had no effect on the disynaptic inhibitory pathway, suggesting no

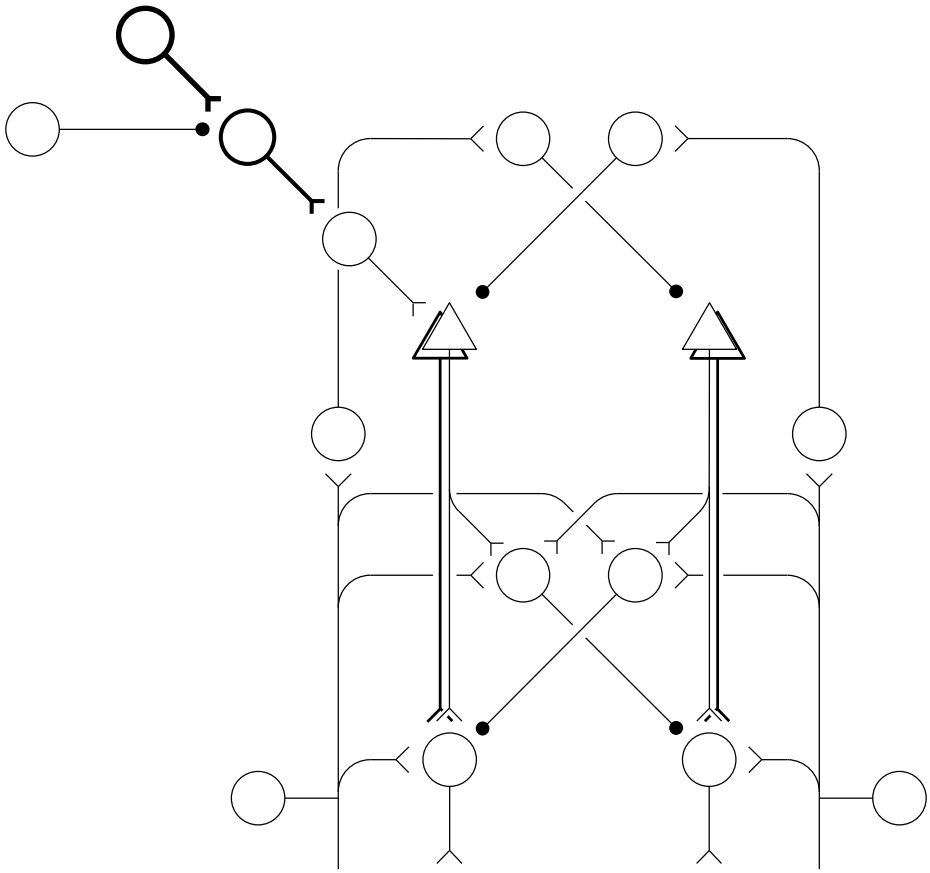

Figure 21: A summary schematic model. The model depicts GABA-ergic intracortical inhibition, disynaptic inhibition, cortical reciprocal inhibition, and co-contraction specific corticospinal neurons. Corticospinal neurons are denoted with triangular soma. The low threshold corticospinal neurons in the foreground project to spinal motor-neurons and to Ia inhibitory interneurons. The higher threshold later I-wave generating interneurons and co-contraction specific corticospinal neurons are denoted with a thicker contour. The higher threshold co-contraction specific corticospinal neurons are in the background and project to the spinal motor-neurons, only. GABA-ergic inhibitory interneurons, spinal Ia inhibitory interneurons, and cortical reciprocal interneurons are denoted with solid circles at their axon terminals.

influence over the Ia inhibitory interneuron excitability. However, excitability of the Ia inhibitory interneurons is greatly suppressed during co-contraction [99]. It is possible that the lack of effect is due to the suppression of the excitability of the Ia inhibitory interneurons by co-contraction. This possibility can be tested with the disynaptic inhibition technique performed at rest.

The main finding of Specific Aim 1 was an increase in corticospinal excitability of a resting muscle with baroreceptor unloading at TMS intensity of 130% and greater [15]. The lack of effect at lower intensities of stimulation suggests that at rest, the low-threshold corticospinal neurons were not affected by LBNP. Since higher intensity TMS activates higher threshold later I-wave generating interneurons [36], thereby recruiting more corticospinal neurons, the results of Specific Aim 1 can suggest that LBNP affects 1) higher threshold spinal motor-neurons, 2) higher threshold corticospinal neurons and 3) higher threshold later I-wave generating interneurons. As already discussed, though the effects of LBNP on spinal motor-neurons have not been investigated, the findings of the three aims do not support an influence. Nonetheless, an effect of LBNP over higher-threshold spinal motor-neurons cannot be excluded.

LBNP may have increased corticospinal excitability in Specific Aim 1 by exerting influence over the higher-threshold corticospinal neurons. By bringing the membrane potential of corticospinal neurons closer to threshold, LBNP may have caused higher-threshold corticospinal neurons to activate at a lower TMS intensity, increasing the MEP. However, the lack of effect at lower TMS intensities suggests that corticospinal neurons' membrane potential is not affected by LBNP, at least for low-threshold corticospinal neurons. LBNP may have also exerted influence over the higher-threshold corticospinal neurons by decreasing presynaptic inhibition at the corticospinal-motor-neuronal synapse, thereby increasing the number of spinal motor-neurons recruited.

Aside from preferential control of higher-threshold corticospinal neurons, this possibility requires presynaptic inhibition of corticospinal neurons at the spinal level. Previous findings do not support presynaptic inhibition of corticospinal fibers [100]. The intensity-dependent increase in corticospinal excitability with baroreceptor unloading requires an influence over the higher-threshold corticospinal neurons, specifically.

Baroreceptor unloading may have increased corticospinal excitability at higher intensities of TMS indirectly by influencing the higher-threshold interneurons that activate corticospinal neurons. TMS of higher intensity recruits interneurons that generate later I-waves (i.e. I-2 and I-3 waves) in the motor cortex [36], suggesting that greater contribution of later I-waves leads to increased corticospinal excitability with baroreceptor unloading. Contribution of later I-waves to corticospinal excitability may be increased by 1) increasing the activity of the intracortical excitatory glutamatergic pathway responsible for later I-wave generation, assessed with SICF [56] or 2) decreasing the activity of the intracortical inhibitory $GABA_A$-ergic [159] pathway that inhibits later I-waves [37], assessed with SICI [80].

This final possibility was investigated in Specific Aim 2. In the resting muscle, LBNP did not influence SICF and decreased SICI. The lack of influence of LBNP over SICF suggests that baroreceptor unloading does not alter the excitatory later I-wave generating interneurons. The decrease in SICI was interpreted as a decrease in $GABA_A$-ergic inhibition of the motor cortex with baroreceptor unloading. This implies that baroreceptor unloading releases the excitatory higher-threshold later I-wave generating interneurons from $GABA_A$-ergic inhibition. Disinhibited later I-wave generating interneurons would activate a larger number of corticospinal neurons, leading to a greater MEP. However, since the later I-wave generating interneurons have a higher TMS threshold [36], the increase in MEP would only be evident at higher intensities of TMS, just as was the case in Specific Aim 1. LBNP may have decreased activity of the $GABA_A$-ergic pathway by hyperpolarizing the GABA-ergic neurons or

by decreasing the synaptic strength (e.g. via blockade of the post synaptic GABA receptors of the excitatory interneurons) between the GABA-ergic and excitatory interneurons.

No effect of baroreceptor unloading on intracortical excitability was observed during voluntary muscle contraction. The lack of effect on SICF is inline with the lack of baroreceptor unloading on later I-wave generating interneurons, observed in the resting muscle. The lack of effect of LBNP on SICI and the cortical silent period, both measures of the GABA-ergic pathway, was different from the decrease in SICI observed during rest. Since voluntary contraction also decreases GABA-ergic inhibition [126, 109], the lack of effect of baroreceptor unloading on intracortical excitability during voluntary muscle contraction likely implies that the $GABA_A$ suppressing effects of baroreceptor unloading are present during voluntary contraction, but they are difficult to measure using the TMS technique. This notion is further supported by the observation of an influence of LBNP over the neural command during a voluntary motor task in Specific Aim 3.

In Specific Aim 3 baroreceptor unloading did not affect corticospinal excitability during individual muscle contraction. This is inline with the lack of effect of baroreceptor unloading on measures of GABA-ergic inhibition during voluntary contraction in Specific Aim 2 and further supports cortical disinhibition as the underlying mechanism of the increase in corticospinal excitability in Specific Aim 1. Since voluntary contraction suppresses GABA-ergic inhibition [126, 109], it can act as a control for the GABA-ergic effects of LBNP. With the GABA-ergic effects removed by voluntary contraction, any changes in corticospinal excitability with baroreceptor unloading would indicate an influence of LBNP over pathways other than the inhibitory GABA-pathway. The lack of effect of baroreceptor unloading over corticospinal excitability during voluntary contraction does not support the mechanisms previously listed to

explain the increase in resting corticospinal excitability, other than the cortical interneuron explanation.

Co-contraction of the wrist FCR and ECR decreased FCR corticospinal excitability relative to activation of the FCR muscle by itself. This result is similar to the decrease in ECR corticospinal excitability during co-contraction of FCR and ECR [1] and suggests that the effects of co-contraction are not muscle (extensor vs. flexor) specific.

Corticospinal excitability during joint-stabilizing co-contraction decreased with baroreceptor unloading in Specific Aim 3. Since a similar effect was not seen during individual muscle contraction at comparable intensity, the result cannot be explained by pathways recruited during individual muscle contraction. Additional pathways recruited during co-contraction include spinal disynaptic inhibition [99], cortical reciprocal inhibition [7, 62, 17], and co-contraction specific corticospinal neurons [43]. As explained above, an effect of LBNP over the disynaptic inhibition is not supported by the obtained data. The decrease in corticospinal excitability can be explained by increased cortical reciprocal inhibition. An increase in the activity of the somatosensory afferents or an increase in the activity of the cortical reciprocal inhibitory neurons with LBNP would result in greater cortical reciprocal inhibition (Fig. 21). The lack of change in disynaptic inhibition suggests that the potential effects of LBNP on the cortical reciprocal inhibitory pathway are enacted post-synaptically at or proximal to the peripheral somatosensory afferents.

Finally, the decrease in corticospinal excitability with baroreceptor unloading during joint-stabilizing co-contraction can also be explained by an effect of LBNP exclusively on the co-contraction specific corticospinal neurons. At present, it is not known if the co-contraction specific corticospinal neurons are influenced differently by LBNP. This can be tested by comparing the effects of LBNP on FCR corticospinal excitability during joint-stabilizing co-contraction with the sum of the effects on FCR

corticospinal excitability during FCR and during ECR individual contraction. If the results during co-contraction can be explained by the sum of the results during individual contractions, a preferential influence of LBNP over the co-contraction specific corticospinal neurons would not be supported.

5.2 Potential pathways

The main effects of baroreceptor unloading observed in this thesis – an increase in corticospinal excitability and decrease in intracortical $GABA_A$-ergic inhibition during rest, lack of effect during individual muscle contraction, and decrease in corticospinal excitability during joint-stabilizing co-contraction – can be explained by the potential influence of baroreceptor unloading over central and peripheral motor pathways. Baroreceptor unloading dis-inhibits the dorsal ventrolateral medulla, which projects to the locus coeruleus, the main site of norepinephrine synthesis in the central nervous system [41, 64]. Mild hypovolemia increases catecholamine release from the locus coeruleus [138, 70], which robustly innervates the neocortex [45, 35]. The locus coeruleus also innervates the dorsal raphe nucleus from which serotonergic neurons spread to higher brain structures. Serotonin release is increased with adrenergic stimulation of the raphe nucleus [23]. Hence, baroreceptor unloading may result in increased function of neuromodulatory monoamines (i.e. norepinephrine, dopamine, and serotonin) within the motor cortex. Drug studies of monoaminergic agents demonstrate profound influence of norepinephrine, dopamine, and serotonin over corticospinal and intracortical excitability [156]. In general, norepinephrine and serotonin agonists increase corticospinal excitability and decrease intracortical inhibition [152, 8, 117, 118, 66, 67, 47], while dopamine agonists have the opposite effect [157, 160, 78].

Similarly, activation of the locus coeruleus and raphe nucleus by baroreceptor unloading may affect the descending motor pathway at the spinal level [94]. Both,

the locus coeruleus and raphe nuclei project to the spinal cord [24, 105, 153, 81] and innervate spinal motor-neurons [49, 61, 120, 2], various spinal interneurons [52, 91, 21, 68], descending neurons [52, 53], and motor-sensory neurons [52, 53, 68]. In general, both norepinephrine and serotonin, acting at the spinal cord level, can increase excitability of spinal interneurons and motor-neurons [40, 107, 144, 140, 154, 28, 63, 29], can increase responses evoked by group I motor afferents [68], and can have varying effects on responses evoked by group II motor afferents [104, 123, 52, 68]. Thus activation of locus coeruleus and raphe nuclei by baroreceptor unloading can influence the neural motor pathways at various levels of the central nervous system.

Baroreceptor unloading also disinhibits sympathetic nerve activity [143]. Sympathetic nerves innervate muscle spindles [3] and may influence the neuromuscular system peripherally. Though controversy exists over whether increased sympathetic nerve activity influences muscle spindle discharges [87], a possible increase in somatosensory afferent input is suggested from an increased stretch reflex [59, 72] in the presence of an unaltered H-reflex [72] with physiologically heightened sympathetic nerve activity.

Electrical stimulation of motor axons and digits has opposing effects on SICI and MEP [125, 141, 124, 92, 34, 77], depending on the timing of the somatosensory input. Thus, the effects of peripheral somatosensory afferent activity on corticospinal and intracortical excitability are not robust. Furthermore, as discussed in the Neuromuscular pathways subsection, effects of LBNP at the peripheral somatosensory afferents, as well as spinal motor-neurons and spinal interneurons, are not supported by the collected data. The results of this thesis study are, thus, best explained by facilitation of norepinephrine and serotonin activity overriding dopamine activity in the motor cortex and potentially at the the central motor afferents of the spinal cord. The question of location of effects can be answered with further drug studies, deafferentation studies, and motor imagery studies. For example, a lack of increase in

corticospinal excitability with baroreceptor unloading during peripheral nerve block would suggest a peripheral somatosensory mechanism for the effects presented here.

5.3 Significance

An increase in corticospinal excitability of a resting muscle at higher intensities of stimulation with baroreceptor unloading suggests that the response to a small suprathreshold cortical motor signal would generate greater motor activity than otherwise. The decrease in intracortical $GABA_A$-ergic activity points to less inhibition of cortical motor signal during the resting state. The decrease in corticospinal excitability during joint-stabilizing co-contraction suggests the need for greater cortical input for modulation of motor activity during co-contraction under baroreceptor unloading. These findings hold immediate significance, in the field of motor control and otherwise, to individuals that may experience baroreceptor unloading (e.g. due to blood loss, sudden change to an upright posture, flying a jet fighter plane in combat, and returning to earth from microgravity [50]).

A decrease in SICI of resting muscle has been observed to precede muscle activation and movement initiation [121, 46]. Reduction of SICI and increase of corticospinal excitability during situations of baroreceptor unloading may lead to quicker movement initiation and to unintended movement. This can potentially allow individuals to produce greater muscle force and more rapid movement with less intracortical excitatory input at the cost of earlier onset of movement execution than intended.

Termination of motor activity was shown to be an active act of motor control that relies on increased GABA-ergic inhibition prior to decrease in corticospinal excitability and termination of voluntary activity [14, 146]. Though no effect of LBNP was observed for intracortical and corticospinal excitability during individual muscle contraction, the decreased $GABA_A$-ergic activity and increased corticospinal excitability in the resting condition may influence termination of motor activity, preventing the

increase in GABA-ergic inhibition and prolonging the termination response time.

The concept of surround inhibition (e.g. high pass filtering) has recently been applied to the motor system. In the study of motor control, surround inhibition refers to active inhibition of unwanted movement (using any and all inhibitory pathways available) during generation of wanted movement [51]. $GABA_A$-ergic inhibition may contribute to surround inhibition. During phasic activation of the FDI muscle, increased $GABA_A$-ergic inhibition of the movement-unrelated abductor pollicis brevis muscle was observed [142]. GABA-ergic inhibition may play a role in suppressing unwanted motor activity during movement execution. Decreased intracortical $GABA_A$-ergic inhibition may impair surround inhibition and decrease movement accuracy.

In the no-go task, subjects are instructed to volitionally inhibit a prepared action. Intracortical $GABA_A$-ergic inhibition contributes to performance on the no-go task [27] decreasing corticospinal excitability [86, 27]. Decreased intracortical inhibition and increased corticospinal excitability due to baroreceptor unloading may impair volitional inhibition of prepared action in "finger on the trigger" situations, leading to execution of the prepared movement at an inappropriate time (and hopefully nothing worse).

The findings that baroreceptor unloading increases corticospinal excitability and decreases intracortical excitability in a resting muscle, and decreases corticospinal excitability during joint-stabilizing co-contraction hold functional significance, as well. As already described, reduction of SICI and increase of corticospinal excitability during rest in situations of baroreceptor unloading may lead to unintended contraction, resulting in increased antagonist co-contraction. Furthermore, once a co-contraction is achieved, either intentionally or not, the decreased corticospinal excitability during co-contraction would require greater intracortical input to modulate the joint stiffness. This may be beneficial to maintaining a steady co-contraction (e.g. stabilizing a

load in space) without perturbation, but detrimental to situations that require modulation of co-contraction (e.g. stability against perturbation, manipulation of objects in space). Fine motor skill execution requires precise control of muscle activation and activity during varying motor tasks. The effects of baroreceptor unloading presented in this thesis alter motor control and would potentially decrease fine motor skill execution.

The seeming trend towards co-contraction with baroreceptor unloading may share common mechanisms with (and may potentially explain) the freeze response of the colloquial fight-flight-freeze response to a perceived harmful event. Similar to baroreceptor unloading, the integrated acute stress response activates the medullary cardiovascular center and (potentially similarly) the locus coeruleus and increases sympathetic nerve activity. However, unlike baroreceptor unloading, the integrated acute stress response originates entirely in the central nervous system, in the sensory centers of the cortex, and depends on the activity of the amygdalae and hypothalamic nuclei [9]. An investigation of the effects of additional central pathways activated during the integrated acute stress response would be the next logical step in pursuing this question further.

Individuals whose livelihood depends on fine motor skill execution, such doctors and musicians, spend days, months and years practicing their fine motor skills. And yet for a learned piece, compared to the rehearsal condition, pianists show greater EMG magnitude of proximal muscles (biceps brachii and upper trapezius) and greater co-contraction of antagonistic muscles in the forearm (extensor digitorum communis and flexor digitorum superficialis) during competition condition [155]. This change in motor control during a situation perceived as stressful can negatively affect motor performance. As stated above, a situation perceived as stressful activates a lot of the same central regions as baroreceptor unloading. The findings in this thesis, then, may present the mechanisms potentially responsible for the changes in motor

control and may translate into novel practice suggestions to ameliorate said effects of perceived stressful situations. For example, since during baroreceptor unloading co-contraction appears unavoidable and corticospinal excitability during co-contraction is diminished, it could prove more beneficial to practice modulating the levels of co-contraction rather than trying to avoid co-contraction.

5.4 Future directions

This thesis established the existence of acute effects of baroreceptor unloading on motor-cortical and corticospinal excitability during rest and voluntary motor activity. In doing so, it has opened up new and exciting avenues of research. The most immediate is the further identification of effects of baroreceptor unloading on cortical reciprocal inhibition and on co-contraction specific corticospinal neurons discussed in Specific Aim 3. As stated throughout the thesis, the effects of baroreceptor unloading may be of central or peripheral origin. Future drug and deafferentation studies can be designed to narrow down the location of action. This thesis focused on neuromuscular mechanisms of motor generation. Equally interesting on its own, a study of baroreceptor unloading on somatosensory pathways would compliment the findings of this thesis. In this thesis, a physiological stress that unloads the baroreceptors was chosen because of the (relatively) clearly delineated pathways it affects. Further research into other physiological, as well as psychological stressors would expand our understanding of the human motor-sensory system in various daily situations. As mentioned in the introduction, corticospinal and intracortical excitability have been implicated in neural plasticity and motor learning. Having observed effects of baroreceptor unloading on corticospinal and intracortical excitability, studies of baroreceptor unloading on neural plasticity and motor learning are now justified. Finally, this thesis was concerned with acute effects of baroreceptor mediated physiological stress. Though perhaps more difficult to study, researching the effects of chronic physiological stress

on fine motor skill pathways may benefit the rehabilitation practices for patients with chronic conditions.

CHAPTER VI

CONCLUSION

In conclusion, baroreceptor unloading increased corticospinal excitability and decreased intracortical inhibition in a resting muscle, did not produce any effects during individual muscle activity, and decreased corticospinal excitability during joint-stabilizing co-contraction.

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VITA

Vasya was here