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Use-dependent plasticity of the human central nervous system: the influence of motor learning and whole body heat stress

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USE-DEPENDENT PLASTICITY OF THE HUMAN CENTRAL NERVOUS SYSTEM: THE INFLUENCE OF MOTOR LEARNING AND WHOLE BODY HEAT STRESS

by

Andrew Edwards Littmann

An Abstract

Of a thesis submitted in partial fulfillment
of the requirements for the Doctor of
Philosophy degree in Physical Rehabilitation Science
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Thesis Supervisor: Professor Richard K. Shields

ABSTRACT

The human central nervous system (CNS) is capable of significant architectural and physiological reorganization in response to environmental stimuli. Novel sensorimotor experiences stimulate neuronal networks to modify their intrinsic excitability and spatial connectivity within and between CNS structures. Early learning-induced adaptations in the primary motor cortex are thought to serve as a priming stimulus for long term CNS reorganization underlying long-lasting changes in motor skill. Recent animal and human studies suggest that whole body exercise and core temperature elevation as systemic stressors also recruit activity-dependent processes that prime the motor cortex, cerebellum, and hippocampus to process sensorimotor stimuli from the environment, enhancing overall CNS learning and performance. A primary goal of rehabilitation specialists is to evaluate and design activity-based intervention strategies that induce or enhance beneficial neuroplastic processes across the lifespan. As such, an investgation of the influence of physical, non-pharmacological interventions on cortical excitability, motor learning, and cognitive function provide the central theme of this dissertation.

The first study investigated the effects of a visually-guided motor learning task on motor cortex excitability at rest and during voluntary activation measured via transcranical magnetic stimulation (TMS). Motor learning significantly increased resting cortical excitability that was not accompanied by changes in excitability as a function of voluntary muscle activation. The cortical silent period, a measure of inhibition, increased after learning and was associated with the magnitude of learning at low activation. These findings suggest that separate excitatory and inhibitory mechanisms may influence motor output as a function of learning success.

The following studies investigated the influence of systemic whole-body thermal stress on motor cortex excitability, motor learning and cognitive performance. We

established the reliability of a novel TMS cortical mapping procedure to study neurophysiological responses after whole-body heat stress. Heat stress significantly potentiated motor cortex excitability, though acute motor learning and cognitive test performance did not differ between subjects receiving heat stress and control subjects. Future research is needed to delineate the potential of whole body heat stress as a therapeutic modality to influence central nervous system plasticity and performance.

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CERTIFICATE OF APPROVAL

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PH.D. THESIS

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This is to certify that the Ph.D. thesis of

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To my family

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CHAPTER I

INTRODUCTION

Overview

The human central nervous system (CNS) is capable of significant architectural and physiological reorganization in response to environmental stimuli. Novel sensorimotor experiences stimulate neuronal networks to modify their intrinsic excitability and spatial connectivity within and between CNS structures (52, 169). The primary motor cortex (M1) in particular demonstrates significant modulation of neuronal excitability as new skills are learned or practiced, adapting in a manner highly specific to the activity (36, 38, 146, 147). Early learning-induced modulations of M1 excitability are thought to influence long-term CNS reorganization that underlies changes in motor skill. Recent animal and human studies suggest that whole body exercise and core temperature elevation as systemic stressors also recruit activity-dependent neuroplastic processes that prime the motor cortex, cerebellum, and hippocampus to process sensorimotor stimuli from the environment, enhancing overall CNS motor learning and cognitive performance [for reviews see (41, 56, 111, 179)]. In the following series of studies, neuroplasticity is understood as the inherent capacity of the CNS to adapt to changing environmental demands. That capacity may be influenced not only by internal factors such as an individual's age or genetic predisposition for neural reorganization, but also by external influences including physical activity or task specific learning. A primary goal of rehabilitation specialists is to evaluate and design activity-based intervention strategies that induce or enhance beneficial neuroplastic processes across the lifespan in health and disease. As such, the central theme of this dissertation encompasses an investigation of the influence of physical, non-pharmacological interventions on plasticity of cortical excitability, motor learning, and cognitive performance.

Background

Assessment of Cortical Excitability

Much of our knowledge of mechanisms underlying M1 plasticity derives from studies measuring modulation of neuronal excitability through the use of transcranial magnetic stimulation (TMS) (77). TMS provides a safe, non-invasive method to determine how activity-based therapeutic interventions influence acute or chronic neuroplastic changes and, importantly, how such adaptations translate to improved functional performance and quality of life (117). Magnetic brain stimulation uses a high-intensity electric current flowing through a wire coil held over the scalp to create a perpendicularly directed magnetic field of typically 1.5-2.0 tesla (186). The field induces electric currents in the cortex running parallel to the surface that depolarize cortical neurons (163). Depending on stimulus intensity, the field is able to activate cortical neurons to a depth of 1.5-3.0 cm, penetrating the skin and bone overlying the cortex with little to no attenuation (167). TMS currents spread from the point of surface stimulation through the superficial cortical layers, exciting excitatory and inhibitory interneurons that converge on the corticospinal neuron somata in layer V (49). The descending volley of corticospinal impulses produces a muscular response, known as the motor evoked potential (MEP), which can be recorded from skeletal muscle by surface electrodes. The magnitude of MEP wave amplitude or area represents the sum of intrinsic neuron excitability plus the extrinsic excitatory and inhibitory interneuron connections converging on corticospinal neurons (45). Thus, larger MEP amplitude signifies a greater state of corticospinal excitability.

By manipulating stimulation intensity or timing, distinct properties of cortical excitability specific to various sensorimotor experiences are revealed. Threshold represents the excitability and density of motor neurons at the focal point of the electrical field and the interposed cortical axons (178). Motor threshold is the minimum intensity

of stimulation needed to evoke a small motor response of a pre-determined size (commonly 50μV) with a 50% probability from a target muscle (162, 164). Single pulse TMS induces repetitive firing of pyramidal tract neurons leading to multiple excitatory postsynaptic potentials (EPSPs) in spinal motoneurons which are necessary to bring the spinal motoneurons to firing threshold.(45). Blockade of voltage-gated Na^+ channels by administration of phenytoin increases motor threshold stimulation 10-20% without parallel changes in other measures of cortical inhibition (30). From these findings, it is hypothesized that attenuation of membrane excitability blocks the repetitive pyramidal cell firing that-induces EPSPs. One primary limitation of threshold to describe excitability is that low stimulation intensity characterizes only the most excitable elements of the corticospinal pathway at a single point (27). The locus of greatest excitability may vary with therapeutic interventions (22, 118, 206). As a result, measurement across multiple locations has greater potential to characterize spatial changes in excitability across a large distribution of neural somata as a response to interventions.

Representations of muscles (or movements) in the motor cortex comprise a population of neurons distributed over the cortical surface, following a general somatotopic organization. Across any muscle distribution, several locations may produce MEPs, with one site typically showing the greatest amplitude (frequently described as the motor "hotspot"). In its simplest form, the hotspot represents the point of greatest corticospinal neuron density in the somatic representation of the target muscle The hotspot is frequently used as the primary site of stimulation in TMS studies; however, while somatotopic organization holds for general subregions of the extremities and head, the precise topography for individual muscle (or movement) representations are better viewed as a neural network with broadly distributed functions involving large populations of neurons (170). The wide spatial distribution and overlap of cortical topography suggests that single-point TMS activation may not fully characterize cortical

modulation induced by activity, as selective activation of individual muscles in the upper extremity is potentially difficult to perform (12, 53, 171). Motor training may induce expansion of cortical representations (35, 102, 148), necessitating widespread assessment. Repeated mapping provides a global index of change across the distribution with high sensitivity to large or directionally specific expansions (36, 37). Expansion of the representation is frequently quantified by the number of locations over the cortex producing MEPs (22). Physiological or architectural shifts in map area may also be represented by movement of the amplitude weighted average of all active MEP locations in the distribution, or center of gravity (CoG) (200). This method assumes added importance when mapping over a fixed number of points, when the absolute area of the map is not directly measured.

MEP intensity curves measure the input-output relationship between stimulus strength and MEP amplitude. Curves generated at a single site reveal the excitability of neurons with progressively higher activation thresholds compared to single intensity mapping. Instead of assessing purely localized excitability of one area in the cortex, intensity curves reflect neuronal excitability spread from the center of activation. Plotting such data yields a characteristic sigmoid curve, with MEP amplitude increasing as a function of a rising stimulus intensity (47). Motor training has been shown to increase the MEP intensity curve slope with dynamic tracking using the wrist (122), and leg (153), and with an isometric force matching task using the wrist (151). Ridding and Rothwell observed during acute (ischemic) and chronic (amputation) modulation of upper extremity cortical maps that increases in recruitment curve slope qualitatively mirrored increases in motor map area of the biceps brachii and forearm flexors(157). The authors suggested that recruitment curves can be also used to detect changes in motor map size. Thus, intensity curves complement mapping to reveal changes in map excitability. This occurs by recruiting neurons not activated at lower intensities used during mapping to reflect excitability across neurons of differing thresholds.

Variation of Cortical Excitability using TMS

The use of TMS as an investigative tool has greatly increased over the last 20 years (97). As the scope of investigation increases, it is important to understand the reliability of the measure to provide confidence that observed changes are due to physiological responses in the subject and not simply an artifact of natural variation in the measures itself. In practice, MEP amplitudes are often highly variable within and between subjects (104). Physiological variability of MEP within and between sessions can also differ widely depending on the measurement variable, target muscle, stimulating coil positioning, and subject alertness (27, 104, 206). Each study in this dissertation examines excitability across the representation of the first dorsal interosseus muscle in the hand. Due to the complexity of factors that may influence cortical excitability, a testing procedure that was short, reliable and valid was explored in Chapter 3.

While there is no clear definition of what constitutes an acceptable level of reliability, intraclass correlation coefficients ranging from 0.7-0.8 are typically considered to indicate high reliability (6). Kamen and colleagues reported moderate to good reliability (ICC = 0.6-0.81) of MEP amplitude in the first dorsal interosseus muscle in the hand using a round coil between sessions separated by 24 hour (97). Between-session reliability was similarly high in MEP intensity curve slope (ICC = 0.82) and lateral coordinate (corresponding to frontal plane shift) of the CoG (ICC = 0.85) when tested with a figure of eight coil over a two week period, though anterior-posterior (saggital plane) coordinate reliability was low (ICC = 0.38)(97, 127). In normal subjects, cortical map shape often mirrors the direction of induced current flow, being elongated along the axis of the coil (204). As a result, there is a larger area over which the CoG may fall in the anterior-posterior direction, increasing the room for variation of this measure across sessions. Scalar CoG displacement is generally reproducible within 2-3 mm (133). Variation in measurements depends in part on consistent positioning of the stimulating coil. Coil positioning errors result in a different population of neurons

activated by TMS pulses. Stereotactic positioning systems allow investigators to retrieve a stimulation site to within 2.5 mm and retain the coil position with low spatial divergence during stimulation. All of the studies in this dissertation utilize a 3-D stereotactic positioning camera system to track coil position variability. One previous study documented localization accuracy of 1.6 mm and 2.5 mm within-session and between session, respectively (173). In our hands, we discovered that we can be accurate to within 1.0 to 1.3 mm for coil placement between testing sessions.

The influence of physical stress on cortical excitability and motor learning

Physical rehabilitation interventions are intended to place a physiological load, or stress, on tissues to create an adaptive response, whether through mechanical loading or stimulating metabolic function. While stress is often perceived as detrimental, appropriate doses of stress administered at safe biological levels are necessary to trigger adaptations of various tissues, for example, stimulating muscular hypertrophy through tissue overload (110). Sensorimotor stimuli during learning or practice of motor skills stress CNS metabolism increasing local blood flow and availability of serotonin, dopamine, and norepinephrine in the motor and sensory cortices contributing to activity-specific changes in resting motor cortex excitability and functional connections with surrounding cortical cells (25, 26, 73). Using intracortical microstimulation mapping in non-human primates, Nudo and colleagues elegantly demonstrated expansion of M1 topography contributing to hand and digit manipulation induced by acquisition of complex reaching and grip tasks (140). In humans, cortical somatotopic representations enlarge after practice of complex skills such as piano playing (146) or complicated finger tapping sequences(102). Potentiation of MEP amplitude (35, 95, 136) and slope of the MEP intensity curve (95, 151) accompany gains in performance of fine motor tasks using the hand. The nature and dose of physiological loading or stress necessary to trigger

adaptations appears to be central to neuroplastic adaptation. Cortical map representations decrease in size during immobilization, but can be quickly reversed by resuming voluntary muscle contraction (120); however, significant MEP potentiation is most associated with learning tasks involving a high degree of cognitive processing (24, 95, 153). In contrast, short-duration strength training and passive movements elicit only minor or no change in MEP amplitude (95, 122, 146).

Acute increases in excitability with motor learning precede a consolidation period of several hours during which the motor memory becomes resistant to change (14, 102, 135, 175). The early learning-induced MEP facilitation in M1 has been proposed as a key factor contributing to long-term reorganization of the CNS (136, 169). Early changes in voluntary activation EMG patterns appear as a result of practice, including shifts in timing of muscle recruitment (176), and coordination of spatial organization of muscle synergies (100) as task competence increases. However, early M1 modulations are typically measured during resting conditions. Execution of skilled motor tasks requires the coordinated participation of multiple structures in the motor hierarchy, including motor cortex, basal ganglia, cerebellum and spinal cord. Consequently, modulation of cortical excitability under conditions of differing motor output is not well understood. It is reasonable to assume that the action of other brain centers may influence the nature by which cortical excitability changes observed at rest are manifested during voluntary activation. Weak voluntary contraction increases MEP size dramatically (183); however, during strong contractions, MEPs in the biceps brachii (189) and FDI (128) decrease progressively as contractions approach maximum. The somewhat paradoxical decrease in cortical excitability parameters as contraction increases may be explained in part by altered excitability of the motoneuron pool (128). Determining whether cortical motor output during volitional activity differentially changes across levels of activation in response to learning warrants further investigation. Pearce and Kidgell reported an 11% increase in MEP amplitude in the FDI at 10% MVC activation following an isometric

learning task (149, 150) however they did not examine MEPs at various levels of activation. Excitability of evoked potentials at differing levels of muscle activation after learning has not been extensively explored.

Inhibitory inputs on cortical cells also play an important role shaping output from the motor cortex. TMS delivered during a voluntary contraction stimulates inhibitory interneurons connected to corticospinal neurons. Consequently, the MEP is followed by a period of electrical silence in the EMG signal, termed the cortical silent period (SP), the duration of which is believed to estimate the net influence of inhibitory cortical networks on motor output (64, 78). While the specific physiological mechanisms underpinning the SP remain controversial, resumption of EMG activity is thought to depend on recovery of motor cortical excitability from gamma-aminobutyric acid-B ($GABA\text{-}_B$) inhibition after the TMS pulse (29, 64, 184). The SP may last several hundred milliseconds, with the initial portion representing reduced motoneuron excitability, and later portions (after about 100 ms) reflecting intracortical inhibition (29).

The silent period modulates according to movement complexity and synergies used in the task, although consistent patterns of SP modulation across different tasks are not clear. Complex grip tasks (pincer grip or power gripping) requiring activation of synergistic muscles elicit shorter silent periods than simple isometric abduction of the FDI, presumably because synergist muscles uninvolved in the abduction task activated cortical inhibitory circuits(187). Systematically increasing TMS intensity at 10% MVC, and thus recruiting a larger proportion of the corticospinal neurons pool, increased FDI MEP recruitment curve slopes to a greater extent with precision grip than simple abduction but SP recruitment slope was not altered (109). Increasing task difficulty during a simple isometric FDI abduction task potentiates MEP amplitude but not SP duration at a constant level of voluntary activation (149). However, repetitive extension and relaxation of the wrist at 0.4 Hz simultaneously potentiated MEP amplitude and significantly reduced SP duration (82), although SP measurements were recorded at 4%

of MVC. Moreover, Gallasch and colleagues investigated modulation of inhibition in the FDI with a ballistic target tracking task. The authors reported that intracortical inhibition (ICI) measured at rest was decreased together with MEP potentiation (66).Taken together, the data suggest a repetitive motor task of high complexity should decrease cortical inhibition. An investigation of the influence of variable descending motor drive on MEP and silent period modulation after learning forms the basis of Chapter 2.

Whole body stress

The link between movement-specific motor skill practice and increased cortical excitability with motor learning in the upper extremity is well established; however, recent studies support that whole-body systemic activity may also enhance motor cortex plasticity (34) and motor performance (8). Metabolic and muscular stress from aerobic exercise enhances synaptic transmission in several brain regions including the hippocampus, cerebellum, and the primary motor cortex, suggesting systemic stress may have a general priming effect on plastic change throughout the CNS (195, 197). Cirillo and colleagues used a paired associative stimulation (PAS) TMS technique to compare long-term potentiation-like corticospinal plasticity in the M1 representation of the abductor pollicis brevis muscle of the hand in physically active and sedentary individuals (34). When appropriately timed, PAS induces a lasting increase in corticospinal excitability which is interpreted as a marker of plasticity within the primary motor cortex (48). PAS induced significantly greater increases in MEP amplitude and recruitment curve slopes in the trained individuals. Despite the greater adaptive capacity observed with regular physical activity, the effect of systemic whole body stress on fine motor performance and motor learning has not been widely investigated. Elderly individuals who underwent 8 weeks of aerobic training showed significant gains in accuracy on a sinusoidal finger tracking task despite no intervening practice during the training period (8). Interestingly, accuracy was not associated with an aerobic training effect, leading the

authors to suggest that exercise may directly affect central nervous system components responsible for motor performance, although electrophysiological measurements were not performed.

Despite the limited information related to whole body stress and motor learning, exercise participation has consistently emerged as a key indicator of improved cognitive function (18, 56, 58, 190, 197). Voluntary running enhances spatial learning in mice that is accompanied by neurogenesis and hippocampal long-term potentiation (a neural analog of long-term memory formation)(195) and facilitates acquisition of hippocampus-dependent learning (197). Protective neurotrophins are implicated as one mechanism underlying cognitive improvements. Brain-derived neurotrophic factor (BDNF), insulin-like growth factor (IGF-1), and nerve growth factor (NGF) facilitate production of new neurons in the hippocampus and promote synaptic plasticity in the hippocampus and cerebral cortex (198, 199). BDNF and its mRNA are particularly increased in the brain after a regimen of daily physical exercise in rats and positively correlated with faster learning and better retention in mice over an exercise period of 1 week duration(198).

In humans, the beneficial effect of systemic exercise stress on cognitive plasticity is most evident in older adults. Long-term aerobic exercise is associated with improvements in attention, processing speed, and executive function (180). Acute bouts of aerobic exercise improve response speed and accuracy, problem solving and inhibition (190). Colcombe and Kramer suggested the brain regions related to executive function might be the most physiologically pliable and thus most sensitive to aerobic exercise training (39). Not surprisingly, executive function domains show the largest benefit from improved fitness (39, 112).

Cognitive test performance during or after acute bouts of exercise is sensitive to stress dosage. During prolonged exercise at a moderate intensity the physiological effects of exercise duration are well known. Numerous studies have shown a progressive increase in metabolic load with increased sweating, elevated heart rate, or a change in

plasma levels of epinephrine or norepinephrine (19). Several studies suggest an inverted U-shaped facilitation of cognitive function with tests of reaction time (20, 31). Though exercise type, duration, intensity, and cognitive tasks differ widely among studies, executive function gains appear strongest with moderate intensity whole body stress that lasts from 10-60 minutes (18, 39). Subject performance on the Stroop Test, an executive function test of cognitive interference, also showed a quadratic inverted-U facilitation, with the greatest gains in executive performance occurring at workloads from 40-70% maximal intensity for resistance exercise (28). Acute Stroop Test performance gains after bouts of moderate cycling (≈50% of maximal O_2 uptake) for >10 minutes were accompanied by increased frontal cortex blood flow and oxygenation (208) and acute elevation of serum BDNF (60). Taken together, these findings suggest whole body stress, via exercise, represents a powerful systemic stimulus to enhance motor and cognitive plasticity. However, many patients with physical constraints may not have a capacity to exercise (e.g. severe osteoarthritis, limited neural control) at a level sufficient to induce these systemic protective chaperones. Thus, we explore the extent to which an alternative systemic stressor may enhance cortical motor and cognitive plasticity in Chapter 4.

Systemic Adaptation through Whole Body Heat Stress

Aerobic exercise induces sweating in response to active hyperthermia up to 1° C core body temperature or more (76, 168). Similar to active exercise, passive exposure to high ambient temperatures stimulates the sympathetic nervous system (166), increasing heart rate (185) and serum catecholamines concentrations (114, 115). This raises the question of whether systematic elevation of core temperature alone, in appropriate doses, can enhance CNS physiology and functional performance in humans.

Several of the cardiovascular responses during passive whole body heat stress resemble those observed after whole body exercise (92). Cutaneous vascular

conductance increases markedly (165), leading to a decrease in peripheral vascular resistance, shunting blood away from the core to facilitate heat exchange. As a result, heart rate increases significantly to maintain a stable cardiac output (105). Within our lab we demonstrated that 30 minutes of passive heat stress at 73° C (10-15% relative humidity) increased core body temperature between 0.71 to 1.20 °C, increased heart rate up to 60% of age-predicted maximum, increased norepinephrine by 58%, increased prolactin 285%, and increased Hsp72 by 49%, but minimally influence systolic blood pressure (90, 92). Increased core body temperature (88) stimulates marked changes in neurotransmitter concentrations in the central nervous system, in particular, serotonin. Serotonin (5-hydroxytryptamine or 5-HT) is a monoamine neurotransmitter which influences regulation of mood, appetite, sleep, and muscle contraction. Serotonin has a stimulatory effect on the hypothalamus, disinhibiting dopaminergic action that inhibits prolactin (PRL) secretion from the anterior hypothalamus (61). Change in prolactin levels have been indirectly linked to change in the dopaminergic-serotonergic pathways (61, 63) allowing PRL plasma concentrations to provide an indirect noninvasive marker of serotonergic activity in the CNS. Plasma concentrations of PRL have been shown to increase significantly in response to increased core body temperature in our lab and others (5, 90, 92, 138). Acute modulation by serotonin or noradrenergic reuptake inhibitors are known to enhance cortical excitability of the motor system (131, 195). Single dose administration of fluoxetine led to acute enlargement of the cortical motor map of the abductor pollicis muscle of the hand after stroke (154). In healthy subjects, single-dose sertraline induced an increase in recruitment curve slope evoked by TMS (the relative gain of CNS excitement) (93). A single dose of paroxetine has been shown to improve motor performance for sensorimotor dexterity tasks such as the 9-hole Peg Test in healthy subjects (123, 124) and post-stroke patients (145). An acute enhancement of central noradrenergic activity by reboxetine, a norepinephrine reuptake inhibitor, increased TMS-induced MEP amplitudes and intra-cortical facilitationin the hand (84)

and elbow(84, 155, 156) and enhanced visual motor task skill in the elbow (155). The link between serotonergic or noradrenergic transmitters and enhanced cortical excitability is not entirely clear as chronic administration of serotonin agonists or norepinephrine agonists has not demonstrated consistent effects on motor cortical activation (2, 69, 116). At the same time, serotonin and norepinephrine neurotransmission are thought to influence exercise-induced regulation of brain-derived neurotrophic factor (BDNF), a protective neurotrophin that promotes neuronal survival and is implicated in learning and cognitive function (94). Recent evidence supports that increasing core body temperature enhances the development of protective neurotrophins that promote neurogenesis and synaptic plasticity (72). Thus, while the modulatory effects of these transmitters are complex, upregulation of dopaminergic and serotonergic availability, as supported by our prolactin studies (90, 92), and increased noradrenergic concentrations observed during our heat stress test (90, 92) supports a transmitter link that may directly or indirectly influence cortical excitability and learning.

Summary

Rehabilitation specialists prescribe activity-based interventions designed to place physiological stress on tissues to stimulate beneficial adaptations. The central theme of this dissertation is to understand the effects of various types of stress on motor cortical excitability, motor learning, and cognitive function. The fact that over 70% of the population does not exercise at a level to prevent the development of chronic disease is troubling. However, after a CNS insult, severe pain from arthritis, or prolonged inactivity, individuals may have an even more difficult time engaging in a whole body physical stress program. Accordingly, in this dissertation we take the first steps to explore whether whole body heat stress may represent a more palatable intervention as a stepping stone to a healthier and more active lifestyle. Importantly, to our knowledge, no

previous study has evaluated the effect of systemic heat stress on cortical motor excitability, motor learning, and cognitive function in humans.

Purpose of the study

The first project (Chapter 2) of this series was developed to test if a quantifiable dose of motor learning translated to enhanced cortical excitability. Enhanced cortical excitability at rest after motor learning is well documented; however, the relationship between cortical excitability changes at rest and during various levels of volitional background motor output is elusive. Moreover, few studies have attempted to relate the degree of learning to the magnitude of change in cortical excitability variables. The goal of the first study was to compare cortical excitability at rest and with progressive levels of descending cortical drive before and after a fine motor learning task using the first dorsal interosseus (FDI). The primary outcome variables were magnitude of motor learning (quantified by reduction in task error), MEP amplitude during resting and voluntary activation, and cortical silent period.

The second project (Chapter 3) represents a methodological study related to TMS. This study was prompted by the desire to streamline the TMS collection phase as the duration of these studies may impact the variability of outcome measures (104). Specifically, we developed a grid-based mapping technique and tested the reliability of the MEP amplitude across the map, stability of the map center of gravity, and test-retest variation of the stimulus-response characteristics. Our goal was to use this map to ascertain the change in excitability after a whole body stress (Chapter 4).

The primary aims of the final project (Chapter 4) were to determine the effects of 30 minutes of passive heat stress as a novel intervention of whole body stress on 1) the global cortical excitability of the M1 representation of the FDI, 2) motor performance on a precision tracking task using the FDI, and 3) acute cognitive test performance.

Specific aims and hypotheses

Specific Aim 1 (Chapter 2): To investigate the acute influence of a visually-guided motor learning task on cortical excitability of the first dorsal interosseus muscle representation at rest and at differing levels of background muscle activation.

Hypothesis 1a: Motor learning, defined as the reduction in error during a precision tracking task, will lead to a significant increase of the mean MEP amplitude of the resting MEP intensity curve when compared to pre-training. This finding supports that motor learning enhances cortical excitability during this novel dynamic motor learning task using the FDI.

Hypothesis 1b: The mean MEP amplitude, superimposed on various backgrounds of volitionally active muscle, will be increased as a function of volitional background force after learning a dynamic motor learning task. The silent period (SP) will be significantly reduced after the learning task as a function of volitional background force after learning. This finding supports that excitatory and inhibitory cortical inputs shape cortical modulations involved in motor learning.

Hypothesis 1c: There will be an association between those who showed the greatest learning and enhancement of the mean MEP of the intensity curve and cortical silent period. This will show that motor learning is scaled to measured changes in cortical electrophysiological measures of excitability and inhibition.

Specific Aim 2 (Chapter 3): To determine the reproducibility of a primary motor cortex mapping and recruitment procedure evoked by single pulse TMS of the first dorsal interosseus muscle in healthy subjects.

Hypothesis 2a: The mean MEP amplitude of a 15-point map will show high reliability with intra-class correlation coefficients (ICC) > 0.8 between 2 mapping sessions separated by 30 minutes. Mean peak-to-peak amplitude will be closely associated across activation sites (>0.8) before and after mapping sessions. The mean

MEP amplitude of the recruitment curve will show no systematic change between the two testing sessions.

Hypothesis 2b: The absolute movement of the individual X- and Y-coordinates will not exceed 4 mm between two mapping sessions separated by 30 minutes. The x-coordinate will show greater reliability than the y-coordinate because of the elongation of the map in the direction of induced current flow, where larger map area provides a greater area over which the y-coordinate may fall.

Specific Aim 3 (Chapter 4): To determine if passive whole-body heat stress affects motor cortex excitability, motor learning performance, and cognitive function.

Hypothesis 3a: Passive heat stress will increase cortical excitability as evident by an increase in MEP amplitude of the FDI motor map. The mean MEP peak-to-peak amplitude will not differ significantly after no heat stress. Passive heat stress will lead to a significant increase of the mean amplitude of the resting MEP intensity curve when compared to no heat stress.

Hypothesis 3b: Passive heat stress will cause individuals to perform a dynamic movement task with less absolute and variable error compared to control subjects not receiving heat stress, This finding will support that heat stress improves acute motor learning.

Hypothesis 3c: Passive heat stress will improve performance on two tests of cognitive function. Components of the tests measuring executive function will show comparatively greater improvement after heat stress. This finding will support that improvements in cognitive performance observed with moderate doses of exercise may also be induced by systemic thermal stress.

CHAPTER II

CORTICAL EXCITABILITY DURING REST AND ACTIVITY ARE MODULATED AFTER A FINE MOTOR LEARNING TASK USING THE FIRST DORSAL INTEROSSEUS MUSCLE

Introduction

Acquisition of motor skills induces activity-specific architectural and physiological adaptations in the primary motor cortex (M1) (102, 169). In humans, rapid gains in motor performance are accompanied by localized increase of motor evoked potential (MEP) amplitude or increased gain in stimulus-response behavior in M1 evoked by transcranial magnetic stimulation (TMS)(35, 54, 102, 122, 146-148, 153). MEP facilitation recorded from the cortical representation of the hand may be evident as soon as 90 seconds of fine motor task practice (35, 68, 95). Such modulations in M1 excitability have been observed within minutes after task practice and may persist up to one hour after longer interventions (135, 209). The acute MEP enhancement precedes a consolidation period of several hours during which performance gains become increasingly stable (14, 102, 135, 175). Significant MEP potentiation is most associated with learning tasks involving a high degree of cognitive processing (24, 95, 153). The early learning-induced MEP changes are believed to serve as a key stimulus of long-term reorganization of the CNS (136, 169). However, the extent to which change in cortical excitability are reflected in motor output are not well understood. A deeper understanding of the relationship between early cortical changes and motor performance will provide important insights regarding the role M1 plays in motor skill acquisition.

MEP potentiation after motor learning is well documented in quiescent muscles in the hand (35, 68, 95, 122, 148, 180). At the same time, the influence of motor learning on cortical excitability variables during muscle activation, particularly at various levels of descending cortical drive, has been less widely investigated. Complex hand dexterity

tasks potentiate MEP amplitude in the first dorsal interosseus (FDI) to a greater extent than simple index finger abduction under equal background muscle activation (5% MVC) (62). Pearce and Kidgell (150) reported 11.8% greater MEP amplitude in the FDI during a visually-guided static abduction task at voluntary activation equal to 10% MVC when performed under "difficult" visuomotor conditions in which visual feedback sensitivity was increased. Thus, tasks with greater visuomotor demands, involving more cognitive processing, appear to induce increased corticospinal excitability with muscle activation. Recent evidence suggests that visually-guided motor learning modulates cortical excitability differently during volitional muscle activation than at rest. A 16-minute tracking task using the elbow led to significant increase in the resting recruitment curve slope of the biceps brachii. MEP amplitude was increased during tonic contraction 5% of maximal integrated EMG amplitude after 2 weeks of training though no significant modulation of MEP was present immediately following task acquisition (95); however, no comparisons were made at differing levels of activation or in relation to learning success. Regional cerebral blood flow (46), fMRI signal (42), and MEP amplitude (152) in M1 increase as a function of volitional force output. As such, motor learning may differentially influence the net neural drive observed at various levels of volitional muscle activation.

Inhibitory inputs also modulate to shape motor output after learning. TMS delivered during a voluntary contraction stimulates inhibitory interneurons connected to corticospinal neurons producing a period of electrical silence in the EMG signal, known as the cortical silent period (SP). The duration of the SP is used to estimate the net influence of inhibitory cortical networks on motor output (64, 78). Repetitive practice of wrist extension and relaxation at 0.4 Hz simultaneously potentiated MEP amplitude and significantly reduced SP duration during tonic wrist extension at 4% MVC compared to baseline measures (82) Gallasch and colleagues measured the influence of a ballistic target tracking paradigm using FDI abduction on modulation of MEP amplitude and

intracortical inhibition (ICI). The authors reported that ICI decreased together with MEP potentiation (66) after significant motor learning. Taken together, the data suggest a repetitive motor learning task with high attentional demand should decrease cortical inhibition.

To our knowledge, no prior studies have tested cortical excitability during various levels of descending volitional motor drive in response to learning a motor skill. We contend that acute motor learning will show enhancement of resting MEP amplitude as a result of increased descending volitional drive, and that cortical inhibition should decrease after motor learning. Moreover, we expect there will be an association between motor learning and selected measures of change in cortical excitability. Accordingly, the purpose of this study is to investigate whether a visually-guided motor learning task modulates select measures of M1 excitability at rest and during voluntary drive of the FDI.

Methods

Subjects

Ten healthy right-handed subjects [(mean ± SD) age = 25.0 ± 5.7 years] participated in the motor learning component of this study. Equal number of males and females were included. A subset (n=4) repeated the resting cortical excitability measurements without the intervening motor task at a separate session at least 7 days after the first session to serve as controls. A separate cohort of 6 healthy subjects (1 female; aged 26.7 ± 7.8 years) underwent resting TMS measurements serving as controls. Subjects with a history of neurological or cardiovascular disorders, history of seizures, implanted electrodes or pacemakers, any non-dental metal in the head, or long term specialized use of the hands such as playing a musical instrument (159) were excluded from participation. Subjects completed a safety screening prior to participation to screen for potential contraindications to TMS (103). After receiving a description of the

protocol, subjects provided written informed consent in accordance with the University of Iowa Institutional Review Board.

Experimental apparatus

We used a custom designed apparatus to measure motor learning of a visually-guided tracking task during which subjects used concentric abduction and eccentric adduction of the index finger to track a sinusoidal target trace. During the tracking task, subjects sat facing a computer screen with the left hand stabilized palm down on a custom metal frame (Figure 2.1A). The index finger was attached to a pulley housing a potentiometer by a cuff and weighted lanyard which resisted abduction at 5% of maximum voluntary isometric contraction force (MVC). Due to the continuous FDI activation during the tracking task (alternating concentric and eccentric contractions), 5% MVC resistance was chosen to minimize static fatigue over the task that might influence MEP measures (158). Custom computer software (126, 177) generated a progressive sine wave trace across the screen that served as the tracking displacement target for the task. Subjects controlled the vertical position of a cursor using abduction (downward cursor displacement) and adduction (upward displacement) of the index finger, attempting to overlay the target trace with the cursor trace (Figure 2.1B). This displacement constituted approximately 15 degrees range of motion at the metacarpal phalangeal joint (MCP). Both traces were simultaneously generated in real time, such that subjects did not have prior visual feedback of the target position. An individual training block comprised 5 sinusoidal movement cycles at 0.4 Hz, with one minute rest given between blocks to prevent fatigue. A numerical error score (absolute error) was displayed on the computer screen after each trial, providing the subject with knowledge of results.

Transcranial magnetic stimulation

Motor evoked potentials were elicited by transcranial magnetic stimulation of the first dorsal interosseus somatic representation of M1 immediately prior to and following

the tracking task. Subjects were seated semi-reclined in a custom-designed chair with headrest. The left shoulder was flexed and abducted to approximately 30°, with the hand resting palm down on a table. The hand was positioned with the index finger in neutral abduction, supported in a custom-molded modeling clay splint to ensure consistent muscle length during TMS measurements. Stimulation of the right M1 was delivered by a Magstim 200^2 stimulator (Magstim Company Ltd., Whitland, Dyfed, UK) equipped with a 70 mm diameter figure-of-eight coil. This stimulator/coil combination produces a monophasic magnetic pulse with a 100μs rise time (1000μs duration). The coil was positioned tangentially to the skull surface and held at a 45° angle to the sagittal plane with the handle oriented posterolaterally creating a posterior-to-anterior current flow over the cortical surface (16, 203). The coil was moved in 0.5-1 cm increments systematically across the scalp surface until the site eliciting the largest average MEP in response to a moderately suprathreshold intensity (50-60% maximal stimulator output) was located (the motor "hotspot"). A Polaris infrared 3-D positional tracking camera recorded optimal coil position over the hotspot (Northern Digital, Inc., Waterloo, Ontario, Canada) which was referenced to a 3-D head marker affixed to the subject's forehead and digitized to four anatomical landmarks (ear tragi, tip of nose, and skull vertex). Subsequent coil position during stimulation was maintained within a maximum error tolerance of 2 mm of tangential translation and 2° of planar deviation (pitch/roll) and coil rotation (yaw).

The resting motor threshold (RMT) was determined by stimulating over the hotspot with a suprathreshold magnetic pulse. Intensity was decreased in 5% increments of maximal stimulator output until the stimulus became sub-threshold (162). RMT was defined as the minimum intensity sufficient to elicit MEPs with amplitude ≥ 50 μV for at least 4 of 8 consecutive pulses. Stimulus intensities utilized during the experimental procedures were normalized as a percentage of each subject's RMT.

Maximal voluntary contraction EMG was recorded prior to the recruitment procedures to provide a baseline for active EMG and MEP normalization. After 2-3 warm-up contractions, subjects performed three 5-second abduction MVCs with the index finger. Subjects were given visual feedback of the exerted torque on a computer screen, and were verbally encouraged. Subjects rested approximately one minute between MVCs. The MVC producing the greatest peak torque was used as the normalizing factor for all torque and EMG measurements.

Electromyographic recordings

Motor evoked potentials were recorded from the first dorsal interosseus (FDI) muscle of the left hand with bipolar Ag-AgCl electrodes (8mm diameter with 20 mm inter-electrode distance). A common ground electrode was affixed to the anterior tibia of the ipsilateral leg. EMG signals were preamplified on-site by a factor of 35 before being differentially amplified. The differential amplifier had an input impedance of 15MΩ at 100Hz, a frequency response of 15-1000 Hz, a common mode rejection ratio of 87 dB at 60 Hz and gain of 500-10K times. EMG data were amplified (1-5k), band pass filtered (20-4000Hz), digitally sampled at 2 KHz, and stored on computer for offline analysis. 100 ms of pre-stimulation activity and 150 ms of post stimulation activity were recorded. MEP amplitude was quantified as the peak-to-peak amplitude taken from a time window 20-50ms after the magnetic stimulus pulse onset. Volitional EMG amplitude was quantified as the mean root-mean-square amplitude of the rectified, filtered signal of a 500 ms window centered at the peak isometric finger abduction torque. EMG data were normalized as the percentage of MVC. During offline analysis, the cortical silent period duration was measured as the time between the onset of the TMS pulse and the recurrence of continuous voluntary EMG activity after the TMS. Examiners were blinded to conditions during all analysis.

Experimental Protocol

Figure 2.1A illustrates the timeline of experimental procedures. Subjects first performed 5 isometric abduction contractions of the index finger, each lasting 7 to 8-seconds at torques equal to 20, 40, 60, 80, and 100% of MVC. When the contraction torque stabilized, a single TMS pulse was superimposed on each contraction to obtain the MEP and cortical silent period (SP). Subjects rested 1 minute between contractions. Next, 10 resting MEPs were evoked at the motor hotspot at each of 13 stimulus intensities ranging from 80-200% RMT in 10% increments (130 pulses total). Pulses were delivered at a frequency of ≤ 0.1 Hz with the order of intensities pseudorandomized.

Subjects then performed 10 blocks of the tracking task with each block separated by 1 minute. MEP intensity curves at rest and MEPs during voluntary activation were collected immediately preceding and following the tracking task. After completion of the tracking task, the voluntary and resting recruitment measures were repeated.

Data analysis

To quantify learning performance for the tracking task, we measured absolute and variable error for each block. Absolute error was calculated as the mean absolute value of the difference in displacement between subject finger trace and the target trace every 100 ms of the task. Variable error was calculated as the standard deviation of absolute error within a block every 100 ms and averaged for each phase (abduction or adduction) of the task. Taken together, the error scores represent the positional deviation from the target and consistency of tracking performance within a block, respectively. Error scores were normalized as a percentage of error in Block 1 for each subject and averaged across blocks to determine an overall mean error score. The degree of motor learning was quantified as the reduction in tracking error from Block 1 to Block 10.

All analog EMG signals were digitized and analyzed using Datapac 2K2 ver. 3.18 (Run Technologies Inc, Laguna Hills, CA). We calculated MEP amplitude for each

stimulus intensity of the resting recruitment procedure as the peak to peak amplitude of the digitized waveform and averaged by subject according to TMS intensity or level of voluntary background muscle activation. Due to the duration of the recruitment procedure, we examined whether recruitment data could be reliably represented by a subset of curve intensities as a means to minimize subject fatigue and discomfort. We found the 92% of the variation in the full recruitment curve could be explained using only intensities of 80, 100, 120, 140, and 160% RMT. Later control data were collected using this shortened protocol. MEP amplitude during voluntary recruitment was normalized as a percent of mean active EMG of a 500 ms window prior to the TMS pulse. SP duration was calculated as the time between the TMS pulse onset and the resumption of continuous voluntary EMG activity. We used visual inspection to determine the resumption of EMG activity. Prior validation studies showed that the visual approach to quantify the SP duration resulted in lower between-visit variation (43) when compared to an automated mathematical approach (44).

Repeated measures analysis of variance (ANOVA) was performed to assess for systematic differences in absolute and variable error, cortical excitability (MEP), and voluntary activation across various levels both within and between subject groups (learning vs control). After testing for significant interaction, main effects and simple effects analyses were carried out. Post-hoc comparisons of the association between the magnitude of learning and MEP amplitudes were made using Pearson correlation. Results of all analyses were considered significant at $P \leq 0.05$. All statistical analyses were performed using SPSS 19 for Windows software package.

Results

Tracking Task Learning

Absolute error decreased by 44.7% and 28.9% from Block 1 to Block 10 for the abduction and adduction cycles, respectively (Figure 2.2A), while variable error

decreased by 28.2 and 25.8% for abduction and adduction cycles, respectively (Figure 2.2 B). No significant Cycle x Block interactions were found indicating that learning error did not differ significantly between the concentric phase (abduction) and eccentric phase (adduction) for either absolute or variable error. There was a significant main effect of Block for absolute error ($F_{(9,198)} = 4.951$, $P<0.001$) and variable error ($F_{(9,199)} = 7.479$, $P<0.001$). Pair wise comparison showed absolute and variable error during Blocks 6-10 were significantly less than Trial 1 ($P<.05$) though error was not significantly changed after Block 6.

The Influence of Learning on Cortical Excitability at Rest

Representative MEPs collected from a single subject before and after the training task are shown in Figure 2.3A. The primary biphasic wave shape was maintained across stimulation intensities and between testing sessions. The tracking task induced a significant increase in mean MEP amplitude. Mean MEP amplitude averaged across all stimulus intensities of the intensity curve rose from 150.3% MVC measured pre-training to 193.4% MVC measured after training. There was a significant Condition (pre vs. post) x Intensity interaction ($F_{(12,59)} = 1.99$, $P=0.032$). Post-hoc comparison showed that MEP amplitudes at 120-130%RMT, and 150-200% RMT were significantly increased after training ($P<0.05$) (Figure 2.3B). Resting MEP amplitudes after the tracking task were compared with resting MEP measures obtained under control conditions. There was a main effect of Group (learning vs. control) ($F_{(1,79)}= 4.677$, $P=.034$) indicating that mean MEP amplitude was significantly greater in the learning group. Pairwise comparison revealed that MEP amplitude was greater in the learning group at stimulus intensity of 100% RMT (Figure 2.3C).

The influence of learning on voluntary activation

Representative torque-time traces from a single subject during the voluntary recruitment procedure show the superimposed twitch torque evoked by the TMS,

followed by reduction in torque corresponding to the cortical silent period (SP) (Figure 2.4A). A detailed view of a 350 ms window following the TMS pulse shows the volitional EMG signal, evoked MEP, and silent period for each respective background activation level (Figure 2.4B). The dotted vertical line marks the timing of the TMS pulse. The small vertical lines on the EMG trace indicate the resumption of active EMG which defined the end of the SP. Figure 2.5A shows the mean MEP amplitude at each target torque during the recruitment procedure. EMG amplitude increased as a function of target torque. A two-way repeated measures ANOVA revealed no significant interaction ($F_{(4,99)} = 0.139$, $P = 0.967$) in EMG amplitude as a function of target torque or significant main effect of condition (pre vs. post-training) ($F_{(1,99)} = 0.715$, $P = 0.42$). The target force generated by subjects at each test increment did not differ between pre and post training Conditions ($F_{(1,99)} = 0.018$, $P = 0.895$) (Figure 2.5A). As such, net voluntary EMG drive to the FDI did not differ after training at any tested level of background activation.

A single TMS pulse was superimposed on the isometric FDI contraction to ascertain the peak-to-peak amplitude of the MEP during voluntary contraction at each target force increment. There was a significant Condition x Force interaction for MEP amplitude ($F_{(4,98)} = 3.335$, $P = .020$). Post-hoc analysis showed MEP amplitude was significantly less at 20% MVC, decreasing 22% after the tracking task, though no differences in MEP amplitude between groups were measured at target torques greater than 20% MVC (Figure 2.5B).

Effect of training on cortical silent period

In follow-up analysis, we measured the duration of the cortical silent period of the isometrically contracted FDI muscle at each level of background activation to determine if cortical inhibition changed as a function of activation level. There was a significant main effect of Condition ($F(1,88) = 7.03$, $P = .029$), indicating mean SP duration

increased significantly after training (220.1 ± 41.2 ms) compared to the pre-training condition (205.2 ± 38.6 ms) (Figure 2.5C). There was no interaction by intensity indicating silent period duration did not differ according to level of background muscle activation.

Association of learning and corticospinal excitability

Correlational analysis was performed to test for association between excitability measures and the magnitude of learning of the task including absolute error and variable error (Table 2.1). There was no significant correlation between the MEP amplitude ratio and the magnitude of absolute error (r =0.213, P = 0.55) or variable error (r = 0.263, P = 0.46); however, there was a significant positive correlation between the duration of the silent period and the magnitude of learning (r = 0.67, $P < 0.05$), indicating that subjects who decreased error the greatest amount showed greater duration of SP, and thus greater inhibition. Further analysis of silent period across background activation levels showed that SP at 20% MVC was significantly correlated to reduction in absolute error (0.83, P = 0.006) and variable error (0.804, P = 0.009)(Figure 2.6). No significant correlations were found between error reduction and silent period duration at 40, 60, 80, or 100% MVC.

Discussion

In this study we investigated the effect of visually-guided motor learning on cortical excitability recruitment of the first dorsal interosseus representation at rest and during volitional activation. The key findings were: (1) Subjects demonstrated a significant reduction in tracking task error that reached a stable lower limit by Trial 6. Improvement in task accuracy did not differ between the concentric and eccentric phases of tracking; (2) task learning induced significant MEP potentiation in the resting MEP intensity curve at stimulus intensities above 110% RMT, (3) mean duration of the cortical silent period was significantly longer after motor training, and showed a significant positive correlation with the magnitude of error reduction at background activation of

20%MVC, (4) peak-to-peak MEP amplitude during background activation of 20% MVC was significantly decreased after training, and (5) mean FDI volitional EMG amplitude did not differ after task learning with background activation 20, 40, 60, 80, or 100% MVC.

Influence of task acquisition on resting cortical excitability

Subjects rapidly improved performance the motor task, showing significant reduction in tracking displacement errors (absolute error) and consistency of tracking (variable error) after 10 training blocks. Both error measures were significantly reduced by Trial 6 and remained stable through the remainder of the task, supporting that subjects had reached an asymptotic level of performance during skill acquisition. The 10 training blocks (50 total sinusoidal movement repetitions) were sufficient to increase resting MEP amplitude by 43.1% at stimulus intensities greater than 110% RMT. The significant MEP facilitation is consistent with prior studies reporting MEP enhancement following complex sensorimotor tasks of peg manipulation (68) or finger opposition (23) comprising as little as three 30 s trials. Durations of facilitation of MEPs after fine motor tasks range from 15 minutes (68) up to 60 minutes (146). Because the duration of facilitation in this study was not known, we collected pilot data for the present study showing that reduction in task error was maintained after a 30-minute rest period during which no competing motor activity of the hand was performed. Thus, the behavioral component hypothesized to underlie changes in excitability was still present beyond the completion of testing.

Positive correlations between motor performance improvement and the magnitude of MEP facilitation have been reported in the upper extremity (135, 137, 209); however, these tasks differed from the present study in that significant MEP increases were induced by ballistic contractions using concentric biceps brachii flexion (209) or isometric pinch force (135). In the latter study, the association between learning and

MEP enhancement was observed only with ballistic contractions and not with graded ramp contractions using visual feedback of the EMG signal, similar to the visual position feedback of the present study. We did not find a relationship between the magnitude of task error reduction and change in cortical excitability at rest. One explanation may be that subjects reached similar levels of competence with the task such that the spread of error data was small compared to the level of individual variation between subjects in MEP modulation. Smyth et al. reported a relationship between the degree of motor learning performance on a visual tracking task and MEP amplitude in the extensor carpi radialis (180). The authors reported the degree of MEP increase was greater 24 hours following the learning task when given a 50% feedback schedule compared to a 100% feedback schedule. However, the relationship was present only after the time period attributed to motor consolidation. Though the nature of the learning tasks differed from the present study, the findings of both studies suggest that initial MEP potentiation itself was not indicative of the degree of learning. Research suggests that consolidation is an important part of the process of motor skill learning (14). This period may be central to the development of a stable cognitive representation of the task. The current protocol used a constant practice structure, in which subjects performed the same task over a block of 10 repetitions. Compared to variable practice structure, where practice includes different practice conditions or tasks, constant practice produces greater performance improvement immediately following task acquisition, but less skill retention after the consolidation period (98, 99). Thus, initial gains in MEP amplitude may likely be influenced by additional components of the task (feedback type and schedule) to create a stable motor representation reflective of the degree of learning.

Influence of training on volitional drive

The human and non-human primate motor cortex undergoes representational modulation in response to motor skill acquisition and practice, including expansion of the

representation (101, 141, 146, 148), changes in kinematic movements evoked by TMS (21, 35), and facilitation of MEPs in trained muscle (23, 136, 146, 209). A novel component of this study was to investigate how changes in resting corticospinal excitability influence motor output during precision learning. Despite significant increases in resting M1 excitability, volitional EMG did not differ after the motor learning task across levels of muscle force. Acute changes in neural drive are observed in strength training (55), although many of the gains in force are due to greater spatial organization of neural drive in muscle synergies or decreased activity of antagonists. We chose the FDI to obtain reliable MEPs in a single joint muscle to minimize the influence of spatial muscle synergies.

The lengthening of the SP measured across levels of background activation was unexpected. The SP is thought to reflect the neural mechanisms by which output from M1 is attenuated by GABAergic inhibitory interneuron transmission (130). The latter part of the SP is attributed to intracortical inhibition and thus a lack of corticospinal excitation to the motor neurons (64). As such, this finding supports increased inhibition as a result of the tracking task. This finding is at odds with prior studies. In the absence of training, SP duration has been reported to decrease with increasing tonic contraction (79, 204) or show no change (81, 183, 207). Upper extremity training has previously induced shortening of the SP after precision learning (65) that could not be attributed to fluctuations in background EMG or stimulus intensity, given that muscle force and TMS intensity were controlled (144). Our lab, as well as others, has showed a significant increase in silent period duration with muscle fatigue (91, 184) . Taylor and colleagues (184) measured an increase in SP of more than 50 ms during a sustained MVC (120s) though SP recovered within 120s. Iguchi and Shields showed a complete recovery of SP duration at 1 and 10 minutes after repeated bouts of MVCs for 10 minutes(91). Single MVCs were generated in the current protocol, lasted approximately 7 seconds, and a full one minute rest was given between contractions with subjects reporting no subjective

fatigue nor showing any decrease in MEP amplitude after training (15, 17) or change in MEP latency. Though the cause of the SP increase in the present study is not known, it adds further evidence supporting the dissociation of excitatory and inhibitory intracortical processes underlying excitability in M1 with motor learning.

One intriguing finding was the decrease in MEP amplitude at the lowest level of voluntary activation (20% MVC), combined with the significant association of learning success and the SP duration at 20%. Motor training induces highly specific motor changes depending on the motor task, whether strength training (95) or precision skill training (3, 11). This study differed from prior reports in that the tracking task entailed cyclical concentric and eccentric finger movements with a resistance of approximately 5% MVC. When performed in a dynamic task, EMG activity is comparatively greater during concentric than isometric muscle activation(125). Neural commands associated with eccentric contractions alter the recruitment order, discharge rate, and thresholds of motor units within a muscle and also influence relative activity of motor units among synergists(172). (83). Motor evoked potentials evoked in muscle after eccentric contractions in the biceps brachii and brachioradialis are less than following concentric or isometric contractions of identical load (1). Eccentric contractions may thus involve differing excitability according to motoneuron size and recruitment during the training task. Moreover, during eccentric contractions, force output is of muscle is enhanced because of muscle intrinsic properties and activation of stretch receptors. These effects may need to be countered by inhibitory mechanisms. Taken together, the current findings argue that changes in excitability measured during descending drive were specific to low level muscle activation similar to the specific force demands of the training task.

Conclusions

The present study demonstrated that significant reduction of task error during the skill acquisition phase of learning induced a significant increase in corticospinal excitability recruitment at rest. Facilitation of resting MEP recruitment was observed in parallel with increased inhibition at low force muscle activation. The magnitude of cortical inhibition during low level activation was correlated to the magnitude of motor learning. Our findings support that early facilitation of M1 excitability may be an important component of motor learning and that excitability changes are specific to the demands of the task. Future studies will be beneficial to determine the cortical excitability variables most associated with initial motor learning and the time course over which excitability is manifested in voluntary motor output.

Table 2.1

MEASURES	VarErr	AbsErrSlope	MEP Ratio	SP %Change	SP 20%MVC
AbsErr	0.794 **	-0.801**	0.213	0.671*	0.830**
VarErr	--	--	0.263	0.859 **	0.804**
AbsErr Slope	--	--	-0.316	-0.532	-0.713*
MEP Ratio	--	--	--	0.586	0.236

Note: *AbsErr*: Reduction in absolute error Block 1-10 as % of initial error; *VarErr:* reduction in variable error Block 1-10 as % of initial error; *AbsErr Slope*: rate of absolute error reduction Block 1-6; *MEP Ratio*: ratio of post:pre mean motor map amplitudes (%MVC). * = $P < 0.05$, ** = $P < 0.01$.

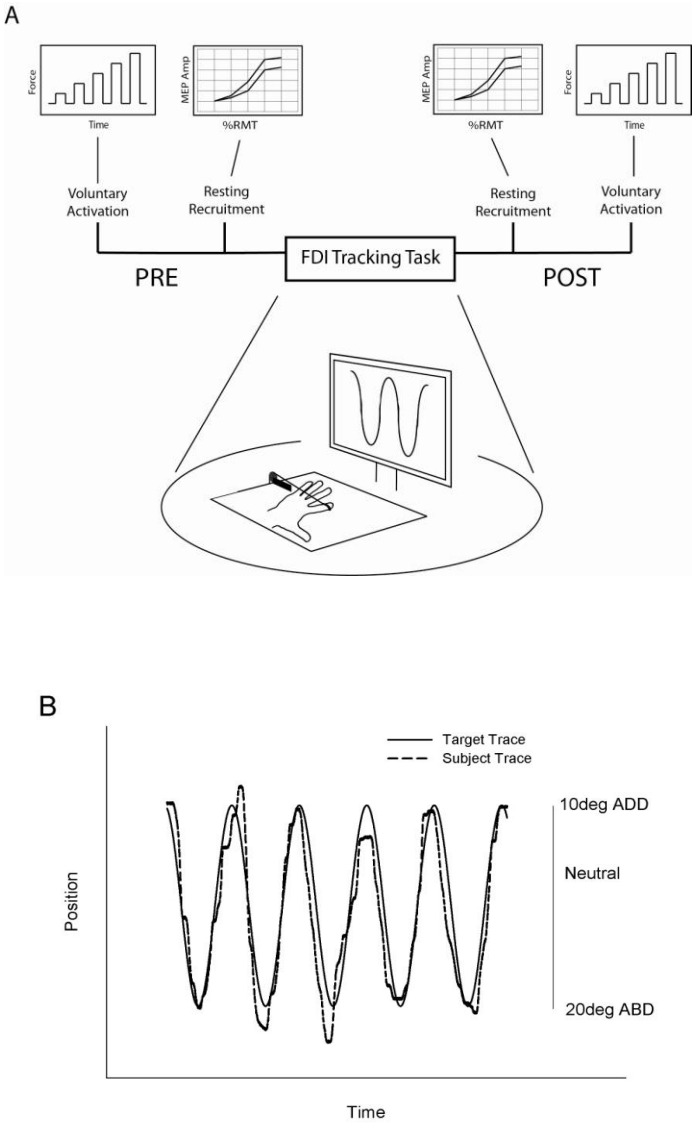

Figure 2.1 Experimental protocol. (A) Schematic timeline of data collection showing illustration of tracking task set up. (B) Detailed representative example recorded from a single subject showing a single training block of the sinusoidal tracking task. The subject trace (dashed line) is superimposed on the target trace (solid line). The target trace was generated from left to right progressively across the screen during a single trial. Subjects attempted to match the cursor position to the instantaneous position of the target trace.

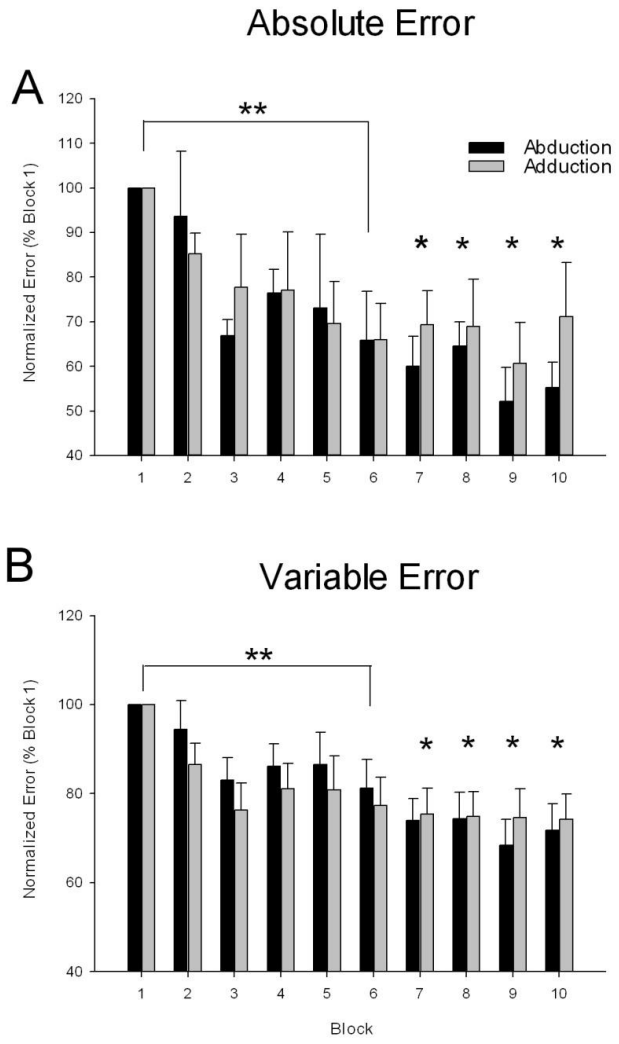

Figure 2.2 Mean error scores (expressed as a percentage of Block 1 error). (A) Absolute error and (B) variable error across the 10 blocks of the FDI tracking task. Error scores shown correspond to the flexion (abduction; black bars) and extension (adduction, gray bars) cycles of the sinusoidal tracking task. Error bars are standard errors. **= indicates significant difference from Block 1 to Block 6. *= indicates no significant difference from Block 7 to Block 10.

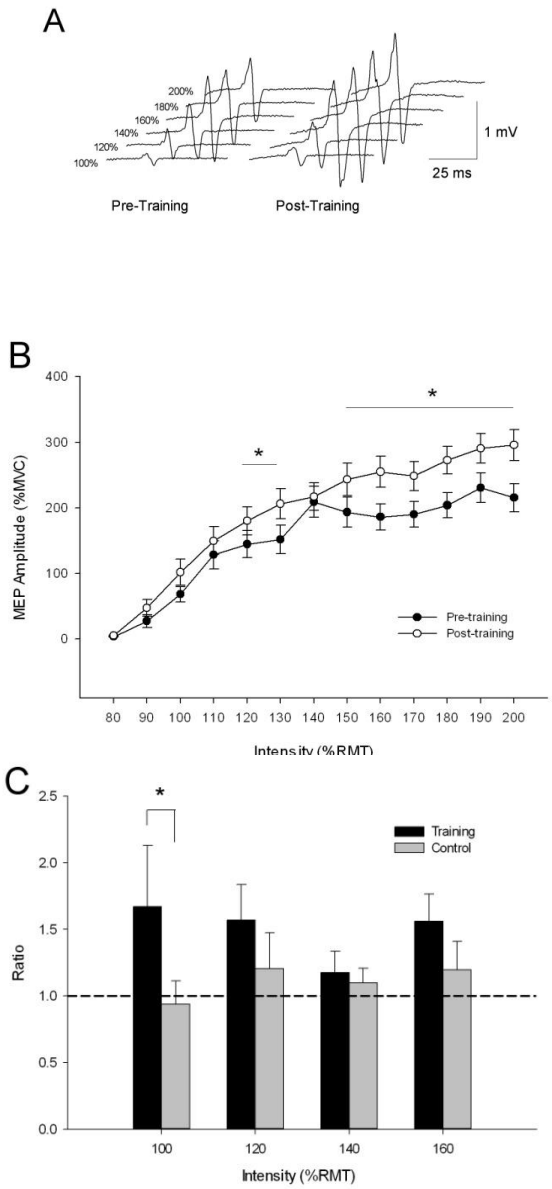

Figure 2.3 Motor evoked potential behavior measured before and after the tracking task. (A) Representative potentiation of MEPs in the post-training condition acquired from a single subject during the resting recruitment curve procedure. Percentages indicate TMS intensity as %RMT. (B) Mean MEP amplitudes (±SE) for the Pre-Training (filled circles) and Post-Training (open circles) conditions by intensity (%RMT). (C) Mean MEP amplitude comparison between training and control groups during MEP recruitment procedure. *$P <$ 0.05.

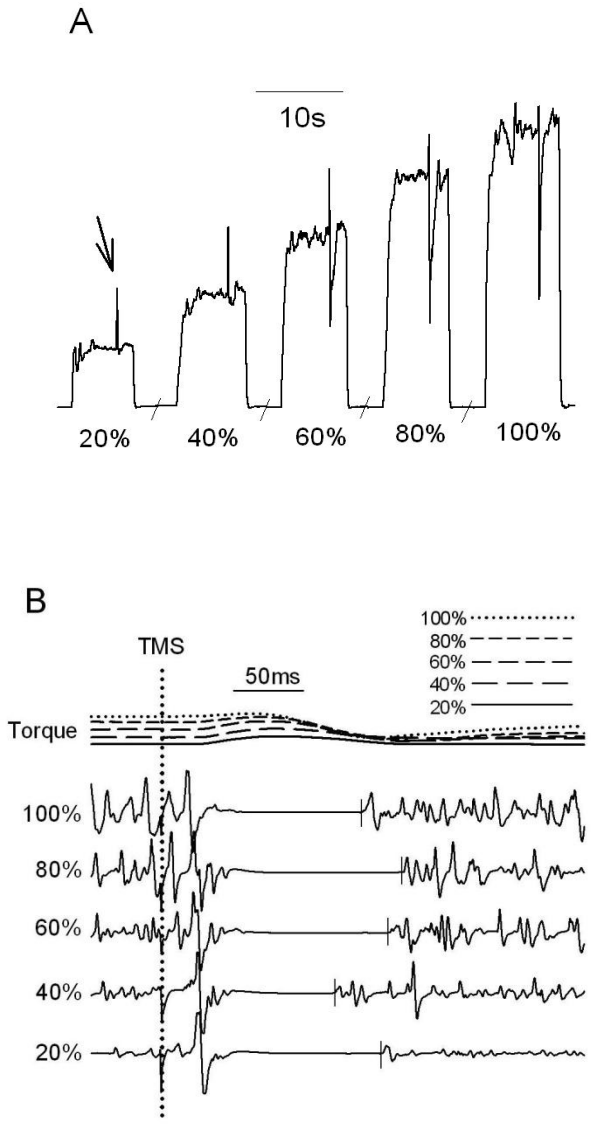

Figure 2.4 Representative EMG and Torque Data during voluntary activation (A) Torque-time traces recorded from a single subject during the voluntary recruitment procedure. Subjects rested for 1 minute between contractions (rest periods indicated by diagonal slash are not shown to scale for time). The arrow indicates the superimposed torque evoked by the TMS pulse shown for the first trace only. (B) Detailed view of torque and EMG recordings showing MEP, silent period (SP) and torque traces evoked by TMS for each target torque value (%MVC). The dotted vertical line indicates the timing of TMS delivery. The short vertical lines on the EMG signal indicate the termination of the SP.

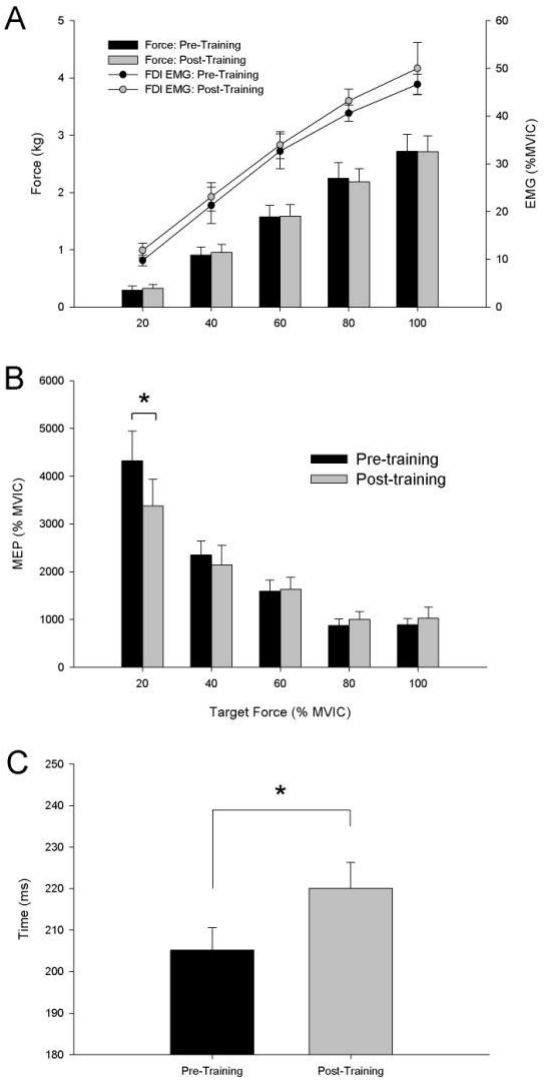

Figure 2.5 TMS excitability measured during background muscle activation. (A)Volitional EMG amplitude (expressed as a percentage of maximum voluntary isometric contraction EMG [%MVC], right ordinal axis) of the first dorsal interosseus muscle as a function of FDI torque. Vertical bars represent the mean force production (%MVC; left ordinal axis) obtained during a 2-second window preceding the TMS pulse at each target force category during generation of voluntary FDI recruitment curves. No significant difference was found between Pre- and Post-Training conditions for EMG amplitude and FDI force production. Error bars are standard errors. (B) Motor evoked potential (MEP) peak-to-peak amplitude (expressed as a percentage of mean MVC EMG) obtained during voluntary recruitment curves. (C) Mean duration of the cortical silent period recorded during the Pre- and Post-Training conditions of the voluntary recruitment task across each target torque. *indicates significant difference between Pre-Training and Post-Training conditions ($P < 0.05$).

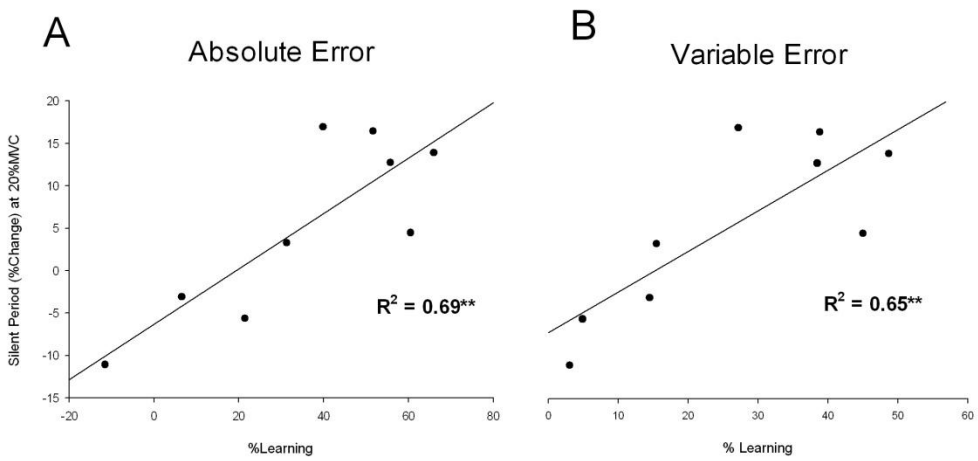

Figure 2.6 Association of motor learning and silent period duration. The abscissa shows the magnitude of learning as defined by the reduction in absolute (A) and variable error (B) after 10 training blocks. **indicates $P < 0.01$.

CHAPTER III

VARIABILITY OF MOTOR CORTICAL EXCITABILITY USING A NEWLY DEVELOPED MAPPING PROCEDURE

Introduction

Transcranial magnetic stimulation (TMS) offers a safe, non-invasive technique (77, 161) to quantify modulations in excitability or topography of specific muscle or movement representations in the primary motor cortex (M1) that accompany changes in motor behavior (38, 52, 169). With novel motor or sensory inputs, cortical representations may undergo significant task-dependent facilitation of motor evoked potential (MEP) amplitude (95), potentiation of TMS input-output characteristics (95, 122), or movement of the amplitude-weighted locus of MEP activity, or center of gravity (CoG) (118). Typically, somatic cortical representations are spatially distributed and show considerable overlap with adjacent muscles such that selective stimulation of individual muscles in the upper extremity is difficult (12, 53, 171, 204). As such, single-point measurement of cortical excitability, most commonly measured at the site of greatest MEP amplitude (hotspot), may be insufficient to adequately characterize excitability behavior across the distribution, most notably directionally-specific expansions or modulations. Comprehensive mapping of M1 offers utility to investigate spatial variation in excitability or reorganization of the distribution in response to therapeutic interventions such as skill learning, exercise, or manipulation of sensory input.

M1 mapping has been shown to reliably quantify cortical modulations in response to motor skill learning in healthy individuals (146, 147) and those with central nervous system lesions (118, 119, 151). However, extensive systematic mapping procedures may be time consuming, lasting potentially as long as 45-60 minutes (106), such that acute cortical adaptations induced by interventions may be inadequately characterized if

mapping durations outlast the effect. Moreover, fluctuations in subject comfort or alertness may increase MEP variability during long duration testing (210). We sought to design a short duration mapping procedure to measure cortical excitability changes observed during the novel whole-body heat stress intervention in Chapter 4 for which the duration of modulations in cortical excitability was unknown. Due to the systemic nature of the intervention, excitability changes were likely to be widespread. Therefore, a reliable, comprehensive mapping of MEP excitability was necessary to maximize the potential to capture modulations in excitability, while minimizing potential for subject fatigue or fluctuations in alertness. Moreover, we sought to investigate whether certain regions of the cortical map might show greater reliability, and as such, show potential as a marker of global excitability changes measured during interventions. For those reasons, we devised a short-duration cortical mapping and recruitment curve procedure based on a fixed grid of 15 points for mapping the first dorsal interosseus (FDI) muscle in the hand.

Previous studies report moderate to good reliability of MEP amplitude at the FDI motor hotspot using a round coil (ICC = 0.6-0.81) between sessions separated by 24 hours (97). Similar between-session reliability was observed in MEP intensity curve slope (ICC = 0.82) and in the mediolateral coordinate of the CoG (ICC = 0.85) when tested with a figure of eight coil over a two week period, though anterior-posterior coordinate reliability was low (ICC = 0.38)(97, 127). Reproducibility of the CoG in hand muscle representations is generally within 1-3 mm (127, 133, 206). A recent study used a similar 15-point (3 x 5 cm) grid to derive cortical maps and CoG modulation in the in the upper extremity representation in subjects with hand amputation; as such, the reliability of the method for muscles in the hand was not established (65). As with any new methodology, determining reliability is crucial to establish the clinical utility of the measures. Hence, the purpose of this study was to determine between-session reliability of MEP amplitude, MEP intensity curve, and map CoG of the FDI cortical representation. We expect high reproducibility with this method to assess cortical excitability.

Methods

Subjects

Six healthy right-handed volunteers (1 female), aged 19-39 (mean 26.8 ± 8.0) years participated in the study. Exclusion criteria were history of neurological or cardiovascular disorders, history of seizures, implanted electrodes or pacemakers, any non-dental metal in the head, or long-term specialized use of the hands such as playing a musical instrument (159). Each subject completed a safety inventory prior to participation to screen for potential contraindications to TMS (103) and provided written informed consent in accordance with the University of Iowa Institutional Review Board. Subjects refrained from exhaustive exercise and consumption of alcohol and caffeine in the 24 hours preceding the experimental session.

Data Collection

Motor evoked potentials were recorded from the first dorsal interosseus (FDI) muscle of the left hand with bipolar Ag-AgCl electrodes (8mm diameter with 20mm inter-electrode distance). The skin overlying the muscle was abraded with sandpaper and cleaned with alcohol to minimize skin impedance. The common ground electrode was affixed to the anterior tibia of the ipsilateral leg. EMG data were amplified (1-5k), filtered (20-4000Hz), digitally sampled at 2 KHz, and stored on computer for offline analysis. 100 ms of pre-stimulation activity and 150 ms of post stimulation activity were recorded. MEP amplitude was measured as the peak-to-peak amplitude taken from a time window 20-50ms after the magnetic stimulus pulse onset. Trials in which active contraction contaminated the MEP were omitted. EMG data were digitized and analyzed using Datapac 2K2 ver. 3.18 (Run Technologies Co., CA). For analysis, MEPs were normalized to the mean maximal voluntary isometric contraction (MVC) amplitude. Voluntary EMG was rectified and band pass filtered (10-200 Hz). EMG amplitude was

calculated as the mean RMS amplitude of the rectified, filtered signal taken from a 1000 ms window centered at the MVC force peak.

Stimulation of the right motor cortex was delivered by a Magstim 200^2 stimulator (Magstim Company Ltd., Whitland, Dyfed, UK) equipped with a 70 mm diameter figure-of-eight coil. The coil was positioned tangentially to the skull surface, held at a 45° angle to the sagittal plane with the handle oriented posterolaterally creating a posterior-to-anterior current flow over the cortical surface (16, 203). Starting approximately 2 cm anterior and 4 cm lateral to the skull vertex, the coil was moved in 0.5-1 cm increments systematically across the anterolateral scalp surface until the site eliciting the largest average MEP in response to a moderately suprathreshold intensity (50-60% maximal stimulator output) was located (motor "hotspot"). The optimal coil position for eliciting MEPs (hotspot) in the resting FDI was recorded and monitored by a Polaris infrared 3-D stereotactic positional tracking camera (Northern Digital, Inc., Waterloo, Ontario, Canada). Coil position was monitored with respect to a 3-D head reference marker affixed to the subject's forehead and digitized to four anatomical landmarks (ear tragic, tip of nose, and skull vertex).

Justification of Mapping Grid Design

The map geometry was chosen to measure cortical motor excitability over the FDI representation with emphasis along the approximate longitudinal axis of the central sulcus (Figure 1), while minimizing focal stimulation over the primary sensory cortex located approximately 3 cm posterior to the hand motor hotspot (108) or the lateral premotor cortex, located approximately 2.5-3 cm anterior to the FDI motor hotspot (70). Comprehensive mapping often includes recording all active sites producing MEPs over a somatic representation (22, 33, 40, 119) though may involve hundreds of stimulation pulses. Fixed area mapping grids have been used estimate changes in cortical representation range in size from 3 x 3 cm (96), 5 x 5 cm (127) to 7 x 7 cm (35). Wilson

and colleagues found the majority of activity in abductor pollicis brevis muscle occurred within a 5 x 7 grid up to 2-3 cm from the hotspot (204). We chose 5 x 7 cm grid dimensions (15 points) with maximum distance of 3 cm from the hotspot as an acceptable representative sampling of active MEP sites, while still maintaining a clinically feasible duration of 12-15 minutes to collect 75 MEPs.

Cortical Mapping

FDI motor maps were created by applying 5 consecutive stimuli at each point of a 15-point grid centered at the FDI hotspot for each subject (Figure 3.1). Stimuli were delivered at 120% RMT with stimulation frequency ≤ 0.1 Hz, starting with the hotspot. The order of stimulation over the remaining grid points was randomized, with the position and orientation of the coil at each point monitored by the tracking camera. Stimulation was limited to 5 pulses at 15 points to achieve session durations feasible for clinical use yet allow characterization of potential brief excitation modulations. Pilot data of cortical mapping showed no significant differences in mean MEP amplitude of individual grid points whether derived from 5 or 10 stimuli, thus 5 stimuli were used to minimize time necessary for the procedure. Moreover, recruitment curves were not significantly did not differ when using 20% intensity increments versus 10% increments to derive the curve.

Stimulus-response measurements were measured via five stimuli delivered per intensity at 80, 100, 120, 140, and 160% RMT. Stimuli were delivered in pseudorandom order at a frequency ≤ 0.1 Hz.

Center of Gravity

The CoG of the motor map was derived from the mapping grid. The motor map CoG X-coordinate was calculated by $\mathbf{X = \Sigma (x*z)/ \Sigma z}$ and the Y-coordinate by $\mathbf{Y = \Sigma(y*z)/ \Sigma z.}$ *X* and *Y* are expressed in centimeters in relation to the motor hotspot, while *Z* represents the mean MEP peak-to-peak amplitude at each grid point. The Euclidian

equation was applied to determine the mean scalar distance the CoG shifted between sessions.

Experimental Procedures

Subjects were seated comfortably in a semi-reclined position with the head supported on a headrest. The left shoulder was flexed and abducted to approximately 30°, with the hand resting palm down on a table. The hand was positioned with the index finger in neutral abduction, supported in a modeling clay splint custom-molded to each subject's hand. Following identification of the FDI hotspot, we determined the resting motor threshold (RMT) starting at suprathreshold stimulus intensity, then decreasing the intensity in 5% increments of maximal stimulator output until the stimulus intensity became sub-threshold (162). RMT was defined as the minimum stimulator intensity eliciting MEP amplitude of 50μV in at least 4 of 8 consecutive trials. The mapping and recruitment curve procedures were performed consecutively and then repeated using stimulus intensities identical to the first session after a 30-minute rest interval. Between sessions, the investigator marked the location of the camera head reference marker and EMG electrode and removed them from the subject. Electrophysiological reliability when removing the marker and electrode was of methodological interest for future studies necessitating removal during therapeutic interventions. Subjects sat quietly in a separate chair during the rest interval. Prior to the second mapping session, the electrode and marker were reapplied. The position of the head reference marker was re-digitized to the four anatomical landmarks on the subject's head to ensure accurate replacement. The total re-digitizing error across 4 digitizing points after reapplication of the head reference marker was 1.14 ± 0.6 mm. Pilot data measuring re-digitizing error without removal of the reference marker from the head was 1.18 ± 0.8 mm.

Data Analysis

The relative reliability of the TMS-related measurements was analyzed using the intraclass correlation coefficient (ICC) based on a one-way repeated measures analysis of variance (ANOVA). Grid map test-retest reliability and stimulus-response recruitment were analyzed using paired t-tests and Pearson correlation coefficients to assess consistency and average agreement between sessions. Stability of the CoG between sessions was testing using the ICC. For all tests, the alpha level was set to 0.05.

Results

Motor Cortex Mapping

Figure 3.2 shows the change in mean MEP amplitude at each map grid point between-sessions expressed as a ratio of the repeated measure to baseline measure. The MEP ratio of individual grid points ranged from 0.81-2.75). Motor map mean MEP amplitude of the 15 combined grid points did not differ significantly between sessions (1.38 ± 1.07 mV vs. 1.21 ± 0.92 mV; P=0.29). Grid points along the grid abscissa showed the smallest change in normalized MEP amplitude (ratio 0.81 ± 0.45 to 1.25 ± 0.57). Grid points N1, N2, NE, and SE showed the largest change in amplitude and largest variation in MEP amplitude between sessions (ratio 1.71 ± 2.18 to 2.75 ± 2.87).

We analyzed subsets of points of the grid to investigate whether different topographies of the map showed greater test-retest reliability, and thus greater sensitivity to detect change during intervention trials (Figure 3.3). Grid subsets included the center 3x3 matrix of 9 points directly adjacent to (and including) the FDI hotspot, points along the grid abscissa (W3→E3, 7 points), and central points on the abscissa (W2→E2, 5 points). Comparison of the reliability of the mean amplitude of the grid is shown in Table 3.1. The mean motor map MEP amplitude decreased during the second session in all combinations (-11.0 to -15.1%), though no grid subsets differed significantly between

sessions. Mean MEP amplitudes showed significant correlations in each subset (0.82-0.87). ICC coefficients ranged from 0.90-0.92 indicating high test-retest reliability.

Stimulus-Response Recruitment

Ratios of MEP amplitude derived at the motor hotspot during the recruitment procedure are shown in Figure 3.3. Mean MEP amplitude by subject decreased between sessions for 3 of the 4 intensities (see Table 3.2); however, none of the differences reached statistical significance. MEP amplitude between sessions was significantly correlated at 100, 140, and 160 %RMT (r = 0.83, 0.94, and 0.93 respectively, $P < 0.05$), but not at 120% RMT (r = 0.44, P = 0.38).

Stimulation amplitudes of 100, 140, and 160% RMT showed high between-session reliability (ICC = 0.88-0.95) while reliability at 120% RMT was moderate (ICC = 0.54).

Center of Gravity

Graphical representation of CoG movement between sessions is shown in Figure 4. The mean distance of CoG shift was 2.79 ± 1.3 mm. The mean scalar distance of the x- and y-coordinates was 1.86 ± 1.2 and 1.90 ± 1.1 mm, respectively. The ICC coefficients of the x-and y-coordinates were 0.91 and 0.39, respectively, indicating high reliability between sessions for CoG position along the grid abscissa, but low reliability for the ordinal position.

Discussion

The use of transcranial magnetic stimulation to study plasticity parameters is becoming increasingly widespread. Hand and forearm muscles are easily accessible for studying the physiological behavior of the motor cortical representation in individuals with and without neurological lesions. The main purpose of this study was to investigate the test-retest reliability of a short-duration mapping procedure using a fixed grid as an

indicator of transient and long term changes in cortical excitability of a representative muscle in the hand.

The main findings of this study were: 1) FDI mean MEP amplitude was highly reliable between sessions across the mapping grid. Grid points distant to the hotspot on the ordinal axis showed the greatest MEP amplitude variability between sessions, 2) Recruitment curves showed no significant difference in curve shape between sessions, and 3) the location of the grid map CoG was highly stable across sessions. The x-coordinate showed greater between-session reliability than the y-coordinate.

Motor Mapping

The motor cortex is organized in terms of movements associated with complex interactions of different muscles under the influence of multiple brain centers. Thus, it is not unreasonable to expect that reproducibility may vary at different points across the map topography. While mean MEP amplitude of the entire 15-point map was highly reproducible, the ordinal periphery of the map showed greater percent change in amplitude than those points situated closer to the estimated axis of the central sulcus. Mortifee et al. (134) measured lower between-session coefficients of reliability in MEP amplitude primarily (though not exclusively) along the periphery of the adductor pollicis brevis and abductor digiti minimi somatotopic motor maps. Most prior mapping studies have measured the area of the map as the number of points at which a response was evoked in the target muscle (133, 134, 194). We kept the area of the motor map fixed in the present study in the interest of session brevity and to minimize potential variability at the periphery of the map. One limitation of constructing maps using fixed grids is that some active cortical sites may not be stimulated, thus underestimating map dimensions near active but unstimulated sites. Cicinelli and colleagues used a similar model of 11 stimulation sites covering an 8 x 8 cm region of the abductor digiti minimi (ADM) to measure interhemispheric differences in motor representation (33). Maximal “hotspot”

activity in both hemispheres was centered primarily around 3 adjacent points approximately 2 cm apart. The same group reported greatest MEP activity along a vector corresponding to the central sulcus alignment including "anomalous hot spots" located outside the locus of ADM representation in patients with hemispheric stroke (192). In the thenar and hypothenar eminences, Mortifee et al. noted individual stimulation points that had a low coefficient of reliability (≤ 25%) intermingled in an apparently random arrangement with stimulating sites that had high coefficients of reliability (≥ 75%)(134). The authors presumed that sites with high coefficients of reliability correspond to those parts of the cortical map having the lowest thresholds, citing the reverse relationship between threshold and MEP amplitude. The variable reliability in the present study may then reflect a similar fractionated nature of the FDI distribution.

Though not significantly different, the mean MEP amplitude of the comprehensive map was lower between sessions in 4 of 6 subjects. This may be due in part to greater subject familiarity with the procedure and thus potential reduced anxiety about the magnetic stimulation during repeat mapping. At the same time, this finding supports that the injection of magnetic energy during the procedure did not itself increase overall excitability, an important consideration for future studies investigating the effects of therapeutic interventions on cortical excitability. The locations of the grid map that show the least variation were located primarily on the x-axis itself. The current study did not have imaging reference points to verify the orientation of the coil with respect to the central sulcus and motor strip; however, with the 45° oblique angle of the coil, it is suggested that movement along the ordinal axis represented a movement perpendicular to the primary motor strip.

Stimulus-Response Recruitment

Overall, reliability during the recruitment procedure was high (ICC = 0.85-0.92) with the exception of 120% of resting motor threshold (ICC = 0.44, MEP %change = -

36.5%). This is in contrast to high measured reliability (ICC = 0.95, MEP %change = -13.5%) of MEPs acquired at the identical location and stimulation intensity during the mapping procedure. It is possible that MEP may have been influenced by the preceding pulses, although intensities during recruitment were presented in pseudorandom order, arguing against an ordering effect. However, given the differences in reliability between the mapping and recruitment procedures, a greater number of stimulus pulses may be necessary to lessen the influence of the natural within-subject variation of the MEPs.

A potential limitation of the mapping protocol in this study is reduced sensitivity to expansion of the motor map. For this reason we measured the stimulus-response characteristics across progressively increasing stimulus intensities. Growth in the map area may be cautiously inferred by the input-output relationship. Ridding and Rothwell observed during acute (ischemic) and chronic (amputation) modulation of upper extremity cortical maps that increases in recruitment curve slope qualitatively mirrored increases in motor map area of the biceps brachii and forearm flexors, suggesting that recruitment curves can be used to detect changes in motor maps. The authors cautioned that while recruitment curves will detect changes in the area of cortical maps, they do not readily detect changes in the distribution of excitability or uneven expansion of the cortical map (157).

Center of Gravity

The CoG represents the amplitude-weighted center of the motor map. A shift in the CoG position is frequently used to represent functional or architectural reorganization in the motor cortex with skill acquisition (121) or after neurological lesions (33). In the present study, the absolute distance of CoG movement was 2.7 mm between sessions, slightly less than prior studies reporting average shifts of 3-4 mm (133, 194). To examine whether the larger dimensions of the grid along the abscissa compared to the ordinate influenced position of the CoG, we analyzed the shift in CoG position using only

the 3 x 3 stimulation point matrix of points immediately surrounding (and including) the FDI hotspot. The magnitude and direction of the CoG did not differ between the 3x3 matrix and the full 15-point map.

Separate reliability analyses were performed for the CoG x- and y-coordinates. Absolute mean CoG movement was nearly identical between the x- and y-coordinates (1.86 ± 1.2 mm and 1.90 ± 1.1 mm, respectively); however, the x-coordinate location (ICC = 0.91) showed high reliability, whereas the y-coordinate reliability was low (ICC = 0.39). It should be emphasized that the y-axis in this study was aligned parallel to the orientation of the coil. As such, the y-axis matched the direction of current flow across the cortex. The y-coordinate variability may be influenced by the parallel direction of the induced current to a greater degree than in studies reporting the y-axis as parallel to the nasion-inion line. Motor maps are often elongated along the axis of the coil (204), thus the increased variability in MEP amplitude and lower reliability in CoG location in the y-axis may be due in part to the 45° oblique orientation of the coil. The present study benefitted from use of a 3-D tracking camera to monitor coil location and orientation with a high level of precision. In general, coil position error was < 1 mm from the defined stimulation point, and within 1-2° of the original planar orientation. Moreover, reapplication of the head reference marker between sessions introduced no more than 2 mm of spatial error. For this reason we are confident that the inherent variation in the TMS parameters was not a function of coil position changes that may alter the pool of neuron stimulated during the experiment.

Functional Considerations

A primary goal of the present study was to investigate the reliability of a short-duration method to estimate of global cortical excitability changes. The present data suggest that while locations across the somatotopic representation responded differently, group mean MEP amplitudes were highly reliable at all combinations of the gridmap,

despite the greater variation along the ordinal axis. As such, the present data support the use of the entire grid map as a reliable means to characterize changes in M1 excitability in the upper extremity. Moreover, the use of the entire map to estimate excitability allows for reliable measurement of directional shifts in the CoG.

Table 3.1 Reproducibility of motor map and subsets (n = 6).

Grid Subset	**MEP %Change**	**P value**	***r* value**	**P value**	**ICC**
Full Grid	-11.0	0.13	0.87	0.023*	0.93
3x3 Center Grid	-15.1	0.10	0. 88	0.022*	0.92
Hotspot	-13.5	0.14	0.94	0.005*	0.94
W3 →E3 X-axis	-13.1	0.31	0.82	0.043*	0.90
W2 →E2 X-axis	-13.0	0.34	0.83	0.040*	0.90

Note: *indicates significant Pearson correlation.

Table 3.2 Reproducibility of MEP by recruitment amplitude (n = 6).

Recruitment Procedure Intensity (%RMT)	**MEP %Change**	**P value**	***r* value**	**P value**	**ICC**
100	1.7	0.95	0.83	0.042*	0.88
120	-36.5	0.26	0.44	0.384	0.56
140	-14.7	0.27	0.94	0.006*	0.95
160	-18.0	0.31	0.93	0.007*	0.92

Note: *indicates significant Pearson correlation.

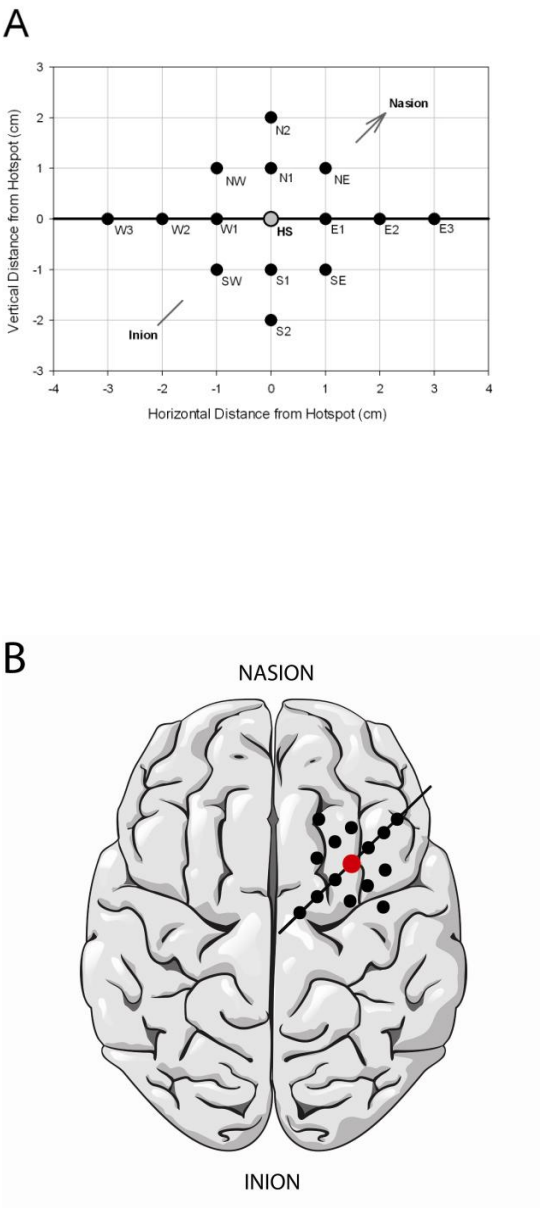

Figure 3.1 Mapping grid geometry (A) Two-dimensional diagram of the mapping grid. Stimulation points were spaced at 1 cm intervals with the grid origin centered at the FDI motor hotspot. Grid loci are identified by the cardinal compass point and distance from hotspot. Ordinal axis corresponds to the vertical axis of the TMS coil. (B) Representation of the mapping grid overlying M1. The black reference line corresponds to the grid abscissa aligned along the approximate axis of M1.

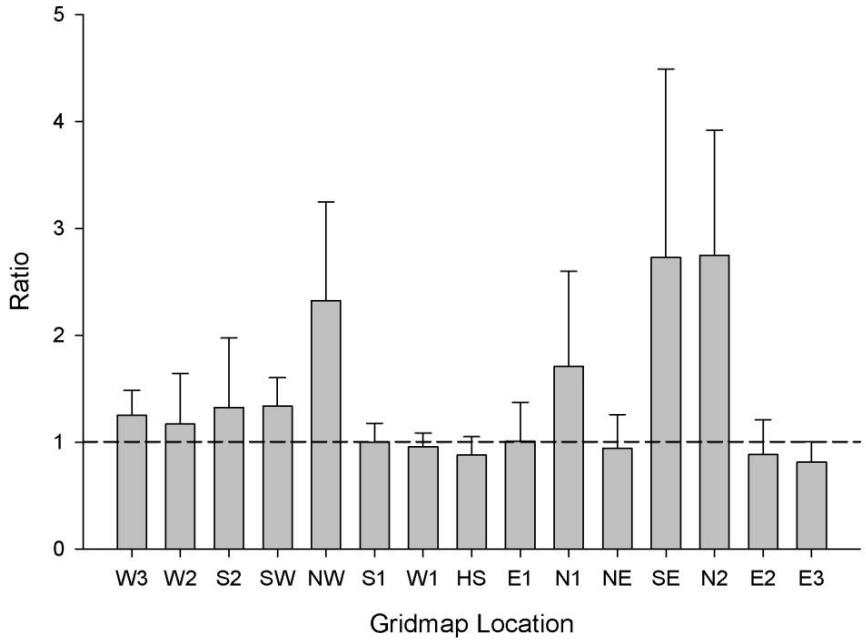

Figure 3.2 Mean MEP amplitude ratios measured between sessions corresponding to mapping grid loci. Ratios represent mean MEP amplitude of Time 2/Time 1 by grid point. Grid loci are identified as the cardinal compass point and distance from the FDI motor hotspot (see Figure 1 for reference).

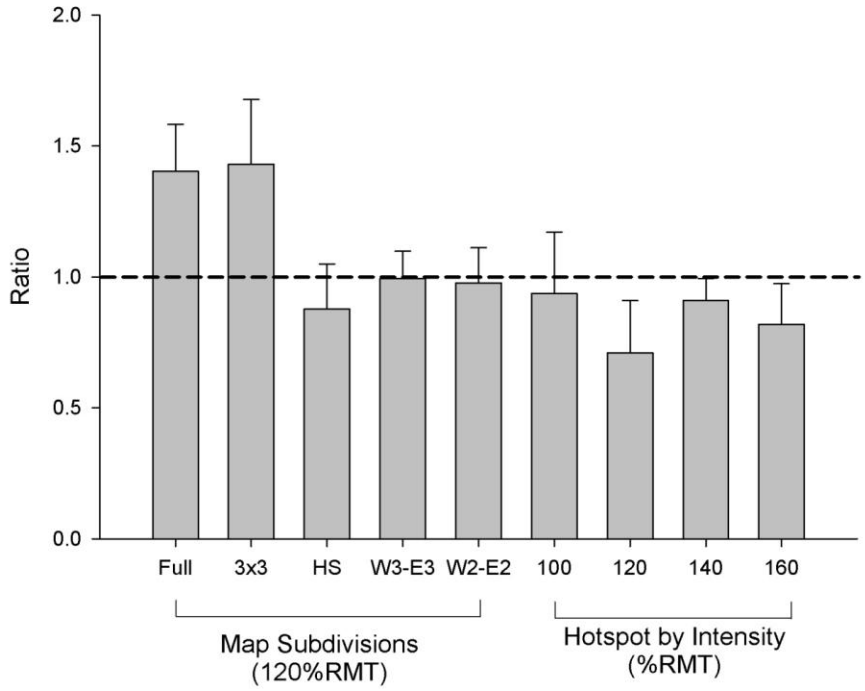

Figure 3.3 MEP amplitude Time2/Time 1 ratios for mapping grid subregions (mean ± SE). All MEPs during the mapping procedure were obtained at 120% RMT. MEP amplitude ratios shown at the right of the figure correspond to stimulus intensity during the recruitment procedure. All recruitment MEP ratios were obtained at the motor hotspot.

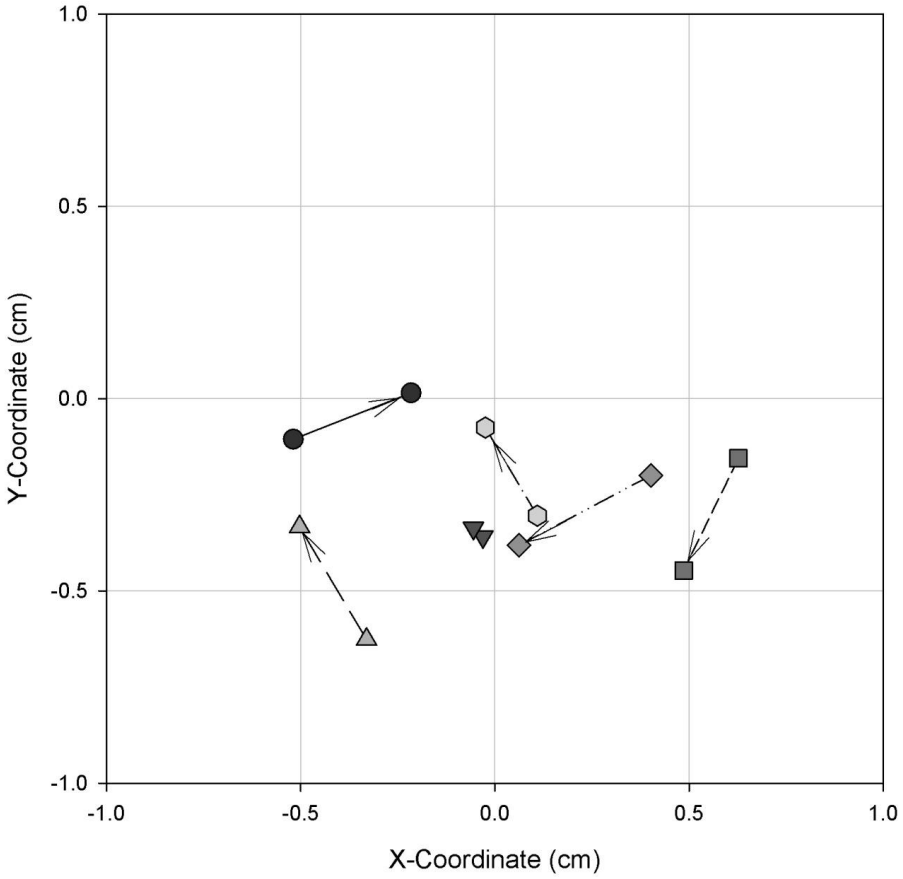

Figure 3.4 2-D representation of CoG movement (cm) between Time 1 and Time 2 for each participant. The COG was calculated using mean MEP amplitude of all 15 grid points of the FDI motor map. Arrows represent the direction of movement between sessions.

CHAPTER IV

THE INFLUENCE OF WHOLE BODY HEAT STRESS ON MOTOR CORTICAL EXCITABILITY, MOTOR LEARNING, AND COGNITIVE PERFORMANCE

Introduction

Regular physical activity is known to help prevent or reduce the incidence of numerous chronic age-related physical impairments (113). A growing body of literature now supports that exercise also facilitates a variety of molecular and cellular processes that enhance central nervous system (CNS) plasticity. In animal models, moderate intensity exercise influences availability of brain metabolic enzymes (51, 191), long-term potentiation of hippocampal neurons (59), and expression of brain-derived neurotrophic factor (BDNF) (13) and insulin-like growth factor-1 (IGF-1)(193). Synaptic facilitation is prevalent across the primary motor cortex (M1), cerebellum, and hippocampus suggesting that systemic physiological stress in appropriate doses may have a general priming effect for CNS performance (for reviews, see: (111, 179, 197). Indeed, BDNF upregulation and synaptogenesis observed with systemic exercise are associated with greater acquisition and retention of complex learning tasks in both young and adult animals (174, 196, 198). Recent evidence from human studies supports exercise as a means to enhancing M1 representational plasticity (34), improve fine motor task accuracy (8), and ameliorate cognitive decline (57, 111, 199). As such, systemic activity represents a powerful intervention to optimize public health across the lifespan. Recent American Heart Association (AHA) guidelines recommend 5-7 days per week of exercise be performed at an intensity that induces profuse sweating (80). However, only 27% of the adult population in the United States engages in exercise at the recommended level that would provide protection against chronic diseases (182). As the healthcare costs of the age-related chronic diseases rise, it is important to explore novel interventions that

can serve as alternatives or supplements to exercise for those who choose not to engage in vigorous activity or who cannot do so due to disability.

Aerobic exercise induces sweating in response to active hyperthermia up to or greater than a 1° C increase in core body temperature. Similar to exercise, passive exposure to high ambient temperatures stimulates the sympathetic nervous system (166), resulting in increased heart rate (185) and concentrations of serum catecholamines (114, 115). This raises the question of whether systematic passive heat stress, in the absence of muscular exertion, can enhance CNS plasticity and performance in humans. Prior work from our lab has demonstrated that 30 minutes of whole-body heat stress increased heart rate to approximately 65% of age-predicted maximum and core temperature by 0.82° C. Serum catecholamine hormones (norepinephrin and epinephrine) increased approximately 60% and prolactin, an indirect marker of serotonergic neurotransmitter activity, by nearly three-fold (92). Pharmcological manipulation of norepinephrine agonists is associated with training-dependent improvements in motor skill acquisition and evoked motor cortex excitability in the upper extremity (155, 156). Likewise, administration of serotonin reuptake inhibitors improves accuracy with extended practice of visual tracking tasks using the hand (123). From these findings, we hypothesized that the cascade of neurochemical factors observed with heat stress may enhance cortical excitability and motor learning.

Acute enhancement of cognitive function and sense of well-being is also observed in some exercise models, believed to result from increases in arousal, cerebral blood flow, and neurotransmitter availability that occur within 20 minutes of starting exercise (75, 132). Cognitive enhancement has been typically observed with moderate intensity activity lasting less than 60 minutes [for review, see (18, 39)], most frequently within the cognitive domain of executive function (111, 112). Performance frequently follows an inverted U-shaped pattern of facilitation, with greatest enhancement at moderate intensities between 40-70% of maximal work capacity (28). The relationship between

hyperthermia and cognitive function is still in question. Wide variety in methods used to induce hyperthermia, the domain of cognition tested, and timing of testing have yielded equivocal results (9, 10, 31, 50, 85, 86). When clear performance decrement is observed, induced stress is typically high and associated with significant dehydration (32), high perceived exertion (143), or fatigue from long-duration exercised-induced hyperthermia (142). The influence of passive heat stress in doses that induce cardiovascular and hormonal responses similar to moderate intensity activity is not fully understood and has not been examined. As such, the influence of short-duration heat stress with relative euhydration on cognitive performance is a novel component of this investigation.

The potential benefits of passive heat stress as a therapeutic intervention are intriguing. In light of the similarities in long lasting hormonal, neurotransmitter, and stress protein responses that follow aerobic exercise and passive heating, we postulated that elevation of core temperature through a measured dose of heat stress will increase cortical excitability and improve performance on a precision motor learning task. A secondary hypothesis was that heat stress would enhance acute performance on cognitive function tests and perceptions of mood. To test these hypotheses, we performed two experiments, the first investigating cortical excitability measured via transcranical magnetic stimulation after a 30 minute dose of passive heat stress and the second investigating motor learning performance on a precision tracking task using the index finger with or without heat stress. Accordingly, the main purposes of this study are to determine 1) whether 30 minutes of passive whole-body heat stress increases excitability of the motor cortex of the hand, 2) whether heat stress improves motor learning of a precision upper extremity tracking, and 3) whether passive heat stress acutely enhances cognitive performance and mood.

Methods

Subjects

Descriptive statistics for all subjects are shown in Table 4.1. Eleven healthy right-handed individuals (5 female) were recruited for Experiment 1 (cortical excitability). Subjects had no known cardiovascular or neurological disorders, history of seizures, implanted electrodes or pacemaker, or non-dental metal in the head. Subjects completed a TMS safety inventory to screen for potential contraindications to TMS (103). Subjects were excluded if they regularly participated in heat stress (sauna), or had known long-term specialized use of the hands such as regular playing of a musical instrument (159). For Experiment 2 (motor learning), 20 healthy male subjects were recruited to assess motor learning after heat or no heat stress. Subjects were randomly assigned to the Heat (n=10) or Control (n=10) groups. Three subjects from Experiment 1 participated in Experiment 2 (2 Heat, 1 Control). Pilot data from our laboratory showed that the learning effect of the upper extremity tracking task was partially retained 7 days after the initial learning session, necessitating separate subject cohorts for each condition for the Heat and Control groups. All subjects gave written informed consent in accordance with the University of Iowa Human Subjects Institutional Review Board. Subjects were instructed to refrain from exhaustive exercise and consumption of caffeine and alcohol during the 24 hours prior to testing. Subjects logged dietary intake to maintain similar nutritional and hydration status prior to each session. General activity level of Heat and Control subjects in Experiment 2 was assessed using the Marx Activity scale (129) and the Baecke physical activity questionnaire (7).

Heat Stress Intervention

Whole-body heat stress was induced in a custom-designed environmental heat chamber (Saunatec Inc., Cokato, MN). The temperature of the chamber was thermostatically regulated to 73°C at face level (relative humidity 10-15%). Subjects'

body weight was recorded 5 minutes prior to entering the chamber to quantify fluid mass lost through dehydration. Subjects sat in the chamber for 30 minutes, though were permitted to exit the chamber prior to the targeted 30-minute dose if unable to tolerate the entire duration. After exiting the chamber, subjects cooled for 15 minute prior to the TMS or motor learning procedures. All subjects were given water and encouraged to drink an amount equal to or greater than fluid loss (by weight) prior to post-heat measurements.

Tympanic temperature, heart rate, and thermal sensation

Tympanic temperature was measured using a tympanic membrane infra-red sensor (ThermoScan IRT 4520 Braun, Kronberg, Germany). The tympanic membrane shares the same blood supply with the hypothalamus (74), and thus may be more representative of brain temperature in the thermoregulation centers, particularly when it is expected to change rapidly during or after the heat intervention. Tympanic temperature rises in a near linear manner over 30 minutes in the current heating protocol (92), thus readings were taken only prior to entering the chamber, immediately upon exiting, and at 5 minute intervals for 15 minutes thereafter. Our pilot study showed that the skin temperature in the hand decreases quickly taking less than 10 min to return to baseline temperature). Heart rate was measured throughout heat stress via a thoracic Polar heart rate monitor (Polar Electro Inc., Woodbury NY) transmitted to a wireless data recorder (MSR Electronisc GmbH, Winterthur, Switzerland) beginning 5 minutes prior to entering the heat chamber until 15 minutes after exiting. Subjects rated their subjective thermal sensation on a 13-point scale (1=So Cold I am Helpless; 7 = Comfortable; 13 = So Hot I am Sick and Nauseated) during the heat intervention at 5 minute intervals, starting 5 minutes prior to heating until 15 minutes after exiting the chamber (87).

Transcranial Magnetic Stimulation

Stimulation of the non-dominant right motor cortex was delivered by a Magstim 200^2 stimulator (Magstim Company Ltd., Whitland, Dyfed, UK) equipped with a 70 mm diameter figure-of-eight coil as described in Chapter 2. This stimulator/coil combination produces a monophasic magnetic pulse with a 100μs rise time (1000μs duration). The coil was positioned tangentially to the skull surface and held at a 45° angle to the sagittal plane with the handle oriented posterolaterally creating a posterior-to-anterior current flow over the cortical surface (16, 203). The coil was moved in 0.5-1 cm increments systematically across the scalp surface until locating the site eliciting the largest MEPs in response to a suprathreshold intensity pulse (50-60% maximal stimulator output), also known as the motor "hotspot". The optimal coil position over the hotspot was recorded by a Polaris infrared 3-D positional tracking camera (Northern Digital, Inc., Waterloo, Ontario, Canada). Coil position was referenced to a 3-D head marker affixed to the subject's forehead that was digitized to four anatomical landmarks (ear tragi, tip of nose, and skull vertex). Subsequent coil positioning during stimulation was maintained within maximum error tolerance of 2 mm of tangential translation and 2° of planar deviation (pitch/roll) and coil rotation (yaw).

The resting motor threshold (RMT) was determined by stimulating over the hotspot with a suprathreshold magnetic pulse. Intensity was decreased in 1-2% increments of maximal stimulator output (MSO) until the stimulus became sub-threshold (162). RMT was defined as the minimum intensity sufficient to elicit MEPs with amplitude ≥ 50 μV on at least 4 of 8 consecutive pulses. All magnetic stimulus intensities were normalized as a percentage of each subject's respective RMT.

Electromyographic (EMG) Recordings

Motor evoked potentials were recorded from the first dorsal interosseus (FDI) muscle of the left hand with bipolar Ag-AgCl electrodes (8mm diameter with 20 mm

inter-electrode distance). The common ground electrode was affixed to the anterior tibia of the ipsilateral leg. EMG signals were preamplified on-site by a factor of 35 before being differentially amplified. The differential amplifier had an input impedance of 15MΩ at 100Hz, a frequency response of 15-1000 Hz, a common mode rejection ratio of 87 dB at 60 Hz and gain of 500-10K times. EMG data were amplified (1-5k), filtered (20-4000Hz), digitally sampled at 2 KHz, and stored on a microcomputer. Analog EMG signals were digitized for offline analysis using Datapac 2K2 ver. 3.18 (Run Technologies Inc, Laguna Hills, CA). MEPs were quantitated as the peak-to-peak MEP amplitude taken from a time window 20-50ms after the magnetic stimulus pulse onset.

MEPs from the FDI were collected over the cortical representation surrounding the motor hotspot using the 15-point motor map described in Chapter 3. The TMS protocol to measure MEP properties between sessions shows high reliability for mean amplitude of the entire map and hotspot (ICC = 0.90-0.93). Five MEPs were recorded at each map locus at stimulus intensity equal to 120% RMT (75 MEPs total). MEP intensity curves were obtained at the motor hotspot by delivering 5 pulses at 80, 100, 120, 140, and 160% of RMT intensity (25 MEPs total). Upon completion of the pre-heat mapping and recruitment procedures, the FDI surface electrode, ground, and head reference marker were removed for the heat stress intervention (or control) to prevent burning of the subjects' skin. The electrodes and reference marker were replaced according to skin site markings approximately 20 minutes after the subject had exited the chamber. Error in head marker position relative to the digitized anatomical landmarks after replacement averaged 1.01 ± 0.48 mm and 1.09 ± 0.61 mm for the Control and Heat conditions, respectively. Maximum acceptable positional error of head reference replacement was set a priori at 2 mm.

Motor Learning Task

In Experiment 2, we utilized the visually-guided fine motor tracking task described previously in Chapter 2. Briefly, subjects sat facing a computer screen with the left hand stabilized palm down on a custom metal frame. The index finger was attached to a pulley housing a potentiometer by a cuff and weighted lanyard which resisted abduction at ~ 5% of isometric maximum voluntary contraction force (MVC). Custom computer software (126, 177) generated a progressive sine wave trace across the screen that served as the tracking target. Subjects controlled the vertical position of a cursor using abduction (downward cursor displacement) and adduction (upward displacement) of the index finger, attempting to overlay the cursor trace over the target trace. End points of the sinusoidal target reflected approximately 15 degrees of abduction/adduction of the metacarpal phalangeal joint (MCP). Both traces were simultaneously generated in real time, such that subjects did not have prior visual feedback of the target position. A numerical absolute error score was displayed on the computer screen after each trial, providing the subject with knowledge of results. Reduction in absolute and variable tracking error over 10 blocks constituted the magnitude of motor learning induced by the task.

Experimental Protocol

Schematic timelines for both experiments are shown in Figure 4.1. In Experiment 1, subjects participated in 2 sessions, separated by at least 7 days and performed at a similar time of the day for each subject. Subjects underwent cortical mapping and recruitment procedures prior to and following a 30-minute heat stress intervention (Heat) or 30 minutes sitting in the chamber at ambient room temperature (Control). Eight subjects underwent Heat prior to the control session, while 3 underwent the Control session first. Procedures were identical between the Heat and Control condition sessions except for the temperature of the environmental heat chamber. In Experiment 2, subjects

received 30 minutes of passive heat stress and then performed 10 training blocks of the tracking task, each comprising 5 sinusoidal movement cycles at 0.4 Hz, with one minute rest given between trials.

Cognitive Function and Mood

A subset of subjects from each experiment completed two tests of cognitive function: the Stroop Test and the Trail-Making Test. The Stroop Color-Word Test (Victoria version) (181) is a cognitive test of selective attention and conflict resolution to assess response inhibition. In the Stroop test, subjects are shown a page with names of colors printed in incongruently colored inks (e.g. the word "blue" printed in red ink). Subjects are asked to name the ink color in which the words are printed, ignoring the word itself (*Color-Word* test). The time taken to read 25 words is recorded and used for statistical analysis. Faster times indicate better response inhibition. Secondary components of the Stroop task include color naming (*Color* test) in which the pattern XXXX was printed in various ink colors with subjects required to read aloud the color of ink and word naming (*Word* test) where subject read the names of colors printed in black ink. The amount of time (in seconds) required to read each set is recorded and used for statistical analysis. Faster times are indicative of better test performance.

The Trail Making Test (Parts A and B) assesses the cognitive domain of set shifting (Spreen 1998). Part A consists of a page with encircled numbers from 1-25. Subjects are instructed to draw a line as quickly and accurately as possible sequentially from circle 1 to circle 25. Part B consists of a page of encircled numbers (extending from 1-13) and letters (A-L). Subjects are instructed to draw a line as quickly and accurately as possible from 1 to A, A to 2, 2 to B, B to 3, and so on until all numbers and letters are connected. The amount of time (in seconds) is recorded and used for statistical analysis. Faster times are indicative of better test performance.

Subjects rated mood using the Positive and Negative Affect Schedule (PANAS) general dimension scale (201) prior to and following the experimental procedures in both experiments. The PANAS comprises 20 affective descriptors (10 positive; 10 negative) for which subjects rate the extent to which they have experienced each term on a 5-point scale during the past month (1 = *very slightly or not at all*, 2 = *a little*, 3 = *moderately*, 4 = *quite a bit*, and 5 = *very much*. Young, healthy subjects show internal consistency of 0.89 and 0.89 for the positive and negative affective components, respectively, for this time period (202). Subjects completed the Stroop test, Trail-Making Test, and PANAS inventory prior to commencement of the experimental protocols in each experiment and repeated tests following the 15-minute cooling interval after exiting the chamber in the Heat and Control conditions.

Data Analysis

Three tympanic temperatures and two heart rate measures were averaged for each time interval collected. Cortical excitability was quantified by the mean MEP amplitude of the 15-point motor map. Five MEPs were collected at each of the 15 map loci and averaged by map locus. MEP map amplitude represented the mean MEP amplitudes of the 15 points. MEP amplitude was normalized to the rectified EMG signal of each subject's maximal voluntary contraction (MVC) and expressed as a percent of MVC for analysis. For statistical analysis, the MEP amplitudes ratio of the post-heat MEP amplitude divided by pre-heat MEP amplitude. MEP amplitude ratios were calculated at each intensity of the recruitment procedure.

Tracking task learning was quantified by the reduction in absolute and variable error between Block 1 and Block 10. Absolute error represented the mean absolute value of the difference in displacement between subject finger trace and the target trace every 100 ms of the task. Variable error represented the standard deviation of absolute error within a trial every 100 ms of the task. Error scores were normalized as a percentage of

Block 1 error. Cognitive tests scores were quantified by time (in seconds) necessary to complete the test, with decreased times indicating of improved performance. Test times were averaged across subjects.

Statistical Analyses

One-way repeated measures analysis of variance (ANOVA) was performed for time effects relative to baseline on temperature, heart rate, and thermal comfort during the heat stress procedure. Student's T tests were used to test for group differences in age, body weight, body fat percentage, body mass index (BMI), and activity scores. Two-way Analysis of Variance (ANOVA) with repeated measures was used to test for cortical excitability change between groups (heat vs. control) and time (pre vs. post), for learning (error) within and between subject groups (learning vs. control) and for cognitive test performance between groups (heat vs. control) and time (pre vs. post). Two-way ANOVA with repeated measures of Block were used to test for differences between the Heat and Control groups for the learning task. Correlational analysis was performed to test the association of stress variables (HR, temperature, thermal sensation, fluid loss), subject demographics (body weight, body fat %) with learning success and cortical excitability. In the text, results are expressed as mean ± standard deviation (SD), whereas in the figures, the error bars represent standard error (SE). After testing for significant interaction, main effects and simple effects analyses were carried out. Results of all analyses were considered significant at $P \leq 0.05$. All statistical analyses were performed using SPSS 19 for Windows software package.

Results

Responses to Heat Stress

All subjects in the Heat condition completed 30 minutes of passive heat stress without ill effects. Heart rate, tympanic temperature, and thermal sensation responses are

presented in Table 4.2. Tympanic temperature increased rapidly during heat stress, by approximately 2° C by the end of the 30-minute session. Mean tympanic temperature was still significantly elevated 15 minutes after exiting the chamber ($P < 0.01$) in Experiment 1, but prior investigation using this protocol showed subjects return near baseline (< 0.25° C difference) by 25-30 minutes after leaving the chamber (92), the approximate time of the mapping and recruitment procedures. HR increased to 126-128 beats/min during after 30 minutes of heat, or approximately 65% of age-predicted maximum. HR did not differ from the pre-heat baseline 15 minutes after exiting the chamber prior to the TMS study. After sitting in the heat chamber for 30 minutes, subjects felt "hot" to "very hot", with mean thermal sensation rating reaching 10.8 ± 0.75 (11 = very hot) . Fifteen minutes after exiting the chamber, thermal sensation did not differ from baseline, decreasing to 7.1 ±0.38 (7 = comfortable). Males and females did not differ in maximum tympanic temperature (p=.37), heart rate (p=.08), or thermal comfort (p=0.43) during the heat stress protocol.

In Experiment 2, tympanic temperature, HR, and thermal comfort responded similarly to Experiment 1. After 30 minutes of heat, temperature increased from 36.7 ± 0.2 to 38.6 ± 0.51° C (1.9° C increase from control), HR from 67.0 ± 7.4 to 126.1 ± 16.0 beats/minute. Subjects rated thermal comfort sensation as hot to very hot (10.7 ±1.3). All measures returned to baseline level 15 minutes after exiting the heat chamber.

Cortical Map Excitability

The mapping and recruitment procedures commenced approximately 25 minutes after subjects left the chamber accounting for cooling time and replacement of recording electrodes. Representative examples of motor evoked potentials collected from a single male subject during the TMS mapping and recruitment procedures are shown in Figure 4.2. The primary wave shape was similar between pre and post conditions collected before and after no heat stress or with heat stress despite removal of the electrode

between the pre and post-heat measurements. Latency of the MEP in the Heat condition was significantly shorter after heat stress, decreasing from 22.5 ± 2.0 ms to 21.7 ± 1.7 ($F_{(1,87)}$=44.52, P<0.001). MEP latency did not differ in the Control condition.

Baseline MEP amplitude of the motor map of the Heat and Control sessions showed high reliability (ICC = 0.80). Mean peak-to-peak MEP amplitude of the motor map decreased in the Control condition from 139.1 ± 120.0% MVC to 115.9 ± 91.6% MVC for the pre and post measurements, respectively, but increased in the Heat condition from 111.62 ± 118.9% MVC to 142.26 ± 146.8% MVC. There was a significant Group (Heat vs. Control) x Time (pre vs. post) interaction of MEP amplitude ratio ($F_{(1,10)}$=10.23, P = 0.01). MEP amplitude ratio in Heat subjects increased significantly after heat stress than after no heat stress (P < 0.05). The mean MEP amplitude ratio was 0.96 ± 0.45 in the Control condition and 1.48 ± 1.1 for the heat condition, indicating a mean 48% increase in MEP amplitude as a result of heat stress compared to a 4% decrease with no heat stress (Figure 4.3A).

We performed a comparison of the magnitude of change displayed by each subject between conditions. Figure 4.3B displays a comparison of the magnitude of change in the Heat condition relative to the Control condition (Ratio of Change) for each subject. For example, a Ratio of Change = 2 indicates that MEP amplitude ratio in the Heat condition was twice that observed in the Control condition. A value of 1 represented no difference in MEP amplitude ratios between conditions. Six subjects (5 male) showed greater than 49% increase in MEP amplitude with heat stress compared to no heat stress. To assess for differential responses to heat stress between sexes, we tested for Group (male vs. female) by Condition (Heat vs. Control). Male MEP amplitude ratios were 2.02 ± 1.3 and 1.07 ± 0.48 for the Heat and Control conditions, respectively, while female MEP amplitude ratios were 0.84 ± 0.43 and 0.84 ± 0.35 for Control and Heat, respectively. There was a significant Group x Condition interaction ($F_{(1,9)}$ = 5.89, P

= 0.038), where males showed significantly greater increase in MEP amplitude ratio in the Heat condition (P = 0.02) but not in the control condition (P = 0.61).

For the MEP recruitment procedure, there was no significant Condition by Intensity interaction ($F_{(4,40)}$ = 1.45, P = 0.47) or significant main effects of Intensity (F(4,40) = 0.617, P = 0.67) or Condition (F (1,10) = 9.63, P = 0.056) though MEP amplitude ratio showed a trend to increase after heat stress . MEP amplitude ratio was 1.63 ± 0.86 for the Heat group and 1.04 ± 0.56 for the Control group. Separate analysis of males and females revealed a significant main effect of Condition in male subjects, (F(1,5) = 7.66, P = 0.039) wherein MEP amplitude ratio was significantly greater after heat stress. No significant interaction or main effect was present in female subjects.

Pre and post-heat stress coordinates of the center of gravity (CoG) of the motor map were calculated to determine whether heat stress exerted a directional influence on the distribution of excitability in M1. Mean CoG shift was 2.46 ± 1.2 mm and 2.68 ± 1.2 mm for the Control and Heat conditions, respectively. In the Control condition, reliability of the CoG was high for the X-coordinate (ICC = 0.83) and low to moderate for the Y-coordinate (ICC = 0.32). In the Heat condition, X-coordinate reliability was high (ICC = 0.92) and moderate for the Y-coordinate (ICC = 0.62). We have previously demonstrated high reliability of this measurement in the current protocol with mean CoG shift of 2.79 ± 1.3 mm in control subjects (see Chapter 3). There was no significant shift in CoG position following heat stress.

Motor Learning Task

All subjects showed rapid learning of the tracking task. Absolute error decreased by 50.1% and 55.4% from Trial 1 to Trial 10 for the Control and Heat groups, respectively, while variable error decreased by 33.6% and 40.8% for control and heat groups, respectively (Figure 4.4). No significant Group x Block interaction was found for either absolute ($F_{(9,199)}$ = 0.54, P < 0.85) or variable error ($F_{(9,199)}$ = 1.21, P < 0.29).

There was a significant main effect of Block for absolute error ($F_{(9,199)} = 45.2$, $P < 0.001$) and variable error ($F_{(9,199)} = 39.14$, $P < 0.001$). Follow-up tests showed absolute and variable error during Trials 2-10 were significantly less than Trial 1 ($P < 0.001$) but did not decrease after Trial 7 for absolute error and after Trial 5 for variable error.

Cognitive Tests/Mood

Cognitive test scores are shown in Table 4.3. For the Stroop Color test, there was no significant interaction between group or time ($F_{(1,15)} = 1.90$, $P = 0.19$) or main effect of group ($P = 0.12$) or time ($P = 0.11$) There was a significant effect of time for the Stroop Word test ($F_{(1,15)} = 7.70$, $P = 0.014$) but scores did not differ between groups after heat stress. There was no significant interaction of group and time for the Stroop Color-Word ($F_{(1,15)} = 7.66$, $P = 0.91$), but a significant main effect of time was present ($F_{(1,15)} = 7.67$, $P = 0.014$) where mean scores were lower on repeat testing independent of group. On the Trail-Making Test-A, the Heat group score decreased from 21.51 ± 9.5 to 19.37 ± 7.3 s compared with 16.46 ± 3.6 to 17.12 ± 4.2 s for the control group. The group x time interaction showed a trend for heat subjects to decrease time needed to complete the test after heat stress but did not reach statistical significance ($P = 0.063$). For the Trail-Making Test-B, there was no significant group x time interaction ($F_{(1,14)} = 0.168$, $P = 0.69$) or main effect of Time ($F_{(1,14)} = 0.151$, $P = 0.70$) or group ($F_{(1,14)} = 0.578$, $P = 0.46$). The Heat and Control groups did not differ by group or time (pre-post) in response to heat stress on the PANAS-Positive affect component. There was a significant main effect of time for the PANAS-Negative scale ($F_{(1,24)} = 9.44$, $P = 0.005$) in which both groups showed lower scores after the intervention (heat or no heat).

Influence of physical stressors on excitability and learning

No significant correlations were found between change in subject stress responses (HR, tympanic temperature, or thermal sensation) and change in MEP amplitude of the motor map, MEP intensity curve, or magnitude of motor learning. Separate analysis of

male subjects who showed significant MEP potentiation after heat stress also showed no significant association between stress responses and cortical excitability. No significant correlations were found between change stress responses and cognitive function. Neither change in MEP amplitude (Experiment 1) nor magnitude of motor learning (Experiment 2) was significantly correlated with cognitive test performance.

Discussion

Maintaining central nervous system health and performance across the lifespan is an important public health care goal. Low compliance or limited participation in many individuals suggests the need for interventions that supplement physical activity to achieve this goal. Whole body heat stress may serve as a palatable means to reap certain benefits of exercise. The primary aim of this study was to determine whether whole-body heat stress acutely enhanced CNS performance. The main findings were: (1) a single 30-minute bout of heat stress increased resting excitability of the FDI motor cortex. Males showed significantly greater M1 excitability after heat stress than females, (2) MEP stimulus-response amplitudes were significantly increased after heat stress, but did not differ according to stimulus intensity between groups. Males showed significantly greater MEP enhancement across stimulus intensities than female subjects, (3) motor learning error did not differ between subjects undergoing heat stress or no heat stress; however, subjects that underwent heat stress showed a general trend for lower tracking error (4) Performance on the Stroop and Trail-Making tests did not differ after heat stress. Subjects reported a decrease in negative affect after both the heat and control conditions.

Cardiovascular, Heart Rate and Subjective Response to Heat Stress

The increase in HR with heat stress in the present study was consistent with previous findings from our lab using the same heating protocol (92). The increase in HR

is a compensatory response to avoid a large drop in mean arterial blood pressure, so that the cardiac output can be relatively stable even with reduced stroke volume (105). HR for subjects in both experiments increased to roughly 65% of age-predicted maximum HR (220 – age), which is considered moderate in exercise (71). Tympanic temperature rose rapidly by approximately 2.0° C. This change in temperature is roughly equivalent to a rectal temperature of 0.8° C(92). By the end of the heat stress, subjects felt "very hot" on average. All three variables returned to baseline by 15 minutes after exiting the chamber, with the exception of mean tympanic temperature during Experiment 1; however, due to set-up time, the TMS procedures were performed approximately 30 minutes after exiting the chamber by which time all subjects returned to baseline tympanic temperature. Importantly, these cardiovascular findings were similar in magnitude to our previous investigation reporting increased serum concentrations of prolactin and catecholamine hormones (92).

Motor Cortical Excitability after Heat Stress

The mean MEP amplitude of the 15-point cortical map increased approximately 48% in response to 30 minutes of heat exposure. This contrasts with a 4% decrease in MEP amplitude measured during the control condition. The minimal change in mean MEP amplitude observed in the control condition supports that excitability after heat stress was not significantly influenced by the TMS procedures themselves. MEPs after heat stress showed a global pattern of increased excitability without an obvious directional effect. The mean shift in map CoG after heat was within the range of natural variability (~2.7 mm) in the mapping protocol established previously for control subjects in Chapter 3. The absence of a shift in CoG after heat stress suggests there was no redistribution of center of cortical excitability in response to the intervention that might be observed if excitation and inhibition modulated differently among adjacent M1 regions.

Somewhat unexpectedly, males showed significantly greater increase in MEP amplitude despite no differences in mean increase of tympanic temperature, heart rate, or perceived level of thermal sensation between sexes. Five of six male subjects showed MEP enhancement of 49-150% after heat stress compared to the control condition. Only 1 of 5 female subjects showed similar MEP enhancement. The cause of the difference is not entirely clear. Cerebral blood flow decreases during passive heat stress (139, 205); though blood flow differences have not been detected between sexes (139) or were established for male subjects only (205). Moreover, these studies measured cerebral blood flow with core temperature increases of approximately 2.0° C, more than twice the estimated core temperature in the present study. Ross et al. recently evoked motor potentials from the vastus lateralis measured in parallel with cerebral blood flow velocity (CBFv) during progressive passive hyperthermia to 39° C (160). CBFv and cortical voluntary drive both decreased with core temperatures elevated 0.5-2.0°C; however, corticospinal excitability measured by the MEP response to TMS was unaltered. This study differed from the present investigation in that core temperature was elevated over a period of 160 minutes (40 minutes per 0.5° C of temperature increase). Measures were obtained in the hyperthermic state, such that brain temperature likely differed between the studies. We obtained all post-heat measures more than 15 minutes after heat stress terminated and tympanic temperature had returned to or near baseline. However, the lack of MEP modulation during acute stress detected by Ross et al. suggests that MEP amplitude in the present investigation was unlikely to be negatively affected by cerebral vascular factors.

It has been shown in our lab and others that young females are more fatigue resistant than young males in certain muscular fatigue tasks (89, 92, 128). Though subjects did not perform muscular exertion during the study, heat stress is implicated in the development of central fatigue (188) wherein loss of performance develops secondary to reduced neural output, independent of the force generating capacity of a muscle [for

review see (67)]. The onset of central fatigue is influenced by interaction of serotonin, dopamine, and norepinephrine. We demonstrated that prolactin, an indirect marker of serotonin availability, increases nearly four-fold in the present heat stress protocol (92). Todd et al. reported central fatigue measured by twitch interpolation of the elbow flexors during hyperthermia to 38.5° C (188). In the present study, cortical excitability measures were taken at rest rather than during voluntary activation thus it is not possible to determine whether excitability changes resulted from decreased cortical drive upstream from the motor cortex or at the spinal level. The neurochemical responses of this protocol suggest that serotonin would still be elevated in all subjects during testing. However in the present study it is not possible to determine whether modulations in the MEP are due to purely cortical or a combination of cortical and spinal circuitry.

Heat Stress and Motor Learning

Movement or muscle specific learning interventions are associated with an increase in excitability or local expansion of the cortical representation of the muscle of interest (35, 146). Facilitation of MEP amplitude observed during task acquisition and practice is considered to be a key component of early motor skill acquisition (136, 169). In Chapter 2, we determined that the tracking task induced significant potentiation of MEP amplitude during resting recruitment using TMS. For this reason we hypothesized that the pre-existing increases in motor cortex excitability seen after heat stress might translate to greater motor learning. Based the significantly greater response in cortical excitability in males observed in Experiment 1, we selected a second cohort of male subjects to assess motor learning after heat stress in order to increase the likelihood of responders. Both heat and control subjects demonstrated significant reduction of error on the motor tracking task. Subjects learned rapidly, reaching a steady state of error reduction by the 5-7th trial; however, the lack of interaction indicates that the rate of error reduction or consistency of performance did not differ between groups when error was

normalized to Trial 1 performance. When tracking error was analyzed using the non-normalized absolute error, the heat group showed similar rate of error reduction to the control group, but absolute error trended lower across all trials ($P = 0.107$). We observed a similar trend in our lab measuring tracking task performance during a single limb squat task (unpublished data). The magnitude of learning over 10 training blocks did not differ between subjects undergoing heat stress and controls, though heat subjects displayed lower initial error and faster rate of error reduction. In the present study, heat subjects did not differ from controls in the rate of learning over 10 blocks or over the first 6 blocks when error reduction was most rapid.

Though no acute enhancement of motor learning was present, the potentiation of MEPs seen in males in Experiment 1 may have important ramifications for long term retention and learning. Positive correlations between motor learning success and the magnitude of MEP facilitation have been reported in the upper extremity (135, 137, 180, 209); with significant increases induced by ballistic contractions using concentric biceps brachii flexion (209) or isometric pinch force (135). In the latter study, no relationship was found with graded ramp contractions using visual feedback of the EMG signal, similar to the position feedback of the present study. At the same time, early enhancement of MEPs is implicated in motor skill consolidation, wherein newly acquired motor skills become resistant to contextual interference over time (136, 137, 169). Facilitation of MEPs during the initial stages of learning may lead to increased learning retention over time. In fact, mild systemic stress in mice facilitates spatial learning retention over 1 week and upregulates BDNF gene expression in the hippocampus (4). Future investigations of heat stress influence on learning should include within subject comparison of M1 excitability and learning success to direct comparison of heat induced excitability changes and magnitude of task learning.

Cognitive Tests

Stroop Word and Color-Word scores were lower upon repeat testing but the effect was present in both Heat and Control subjects suggesting that heat stress did not acutely enhance test performance. There was also no clear effect when comparing test performance to the magnitude of motor learning or change in M1 excitability. The lack of differences was also present between subjects in Experiment 1 who showed a significant increase in M1 excitability versus non-responders. Subjects in both groups were primarily young adults enrolled in intensive university graduate programs, and as such, likely functioning at very high baseline levels of cognitive ability. In this event, test duration may partly explain the lack of differences between groups. The Stroop test (Victoria version) components used in this study consisted of 25 items. Other commonly used versions not available in the public domain contain 100 items on the Color-Word test. Klein and colleagues showed that young adults perform the first half of the 100-item Stroop test rapidly but become slower during the second half, in contrast to older adults who showed the reverse effect (107). Thus, the shorter Victoria version may not offer sufficient sensitivity in a young healthy population.

No group differences were detected for the Trail Making test. The pre-intervention TMT-B times reported in this study (35.6-42.4 s) were lower than previously published reports of middle aged adults by 15-20 seconds (28). Their lower initial test scores likely provided less room for acute improvement after an intervention than may exist for older adults or individuals with pre-existing cognitive impairment.

Significant cognitive improvements from stress-based interventions have been most readily observed with long term training (39, 179). Future studies of heat stress and cognitive function should evaluate the influence of repeated heat exposure as a priming stimulus for cognitive performance across multiple domains of cognition.

Conclusions

Whole body heat stress is an intriguing novel intervention for individuals with limited activity to derive some of the benefits of exercise. Establishing the acute effects of passive heat stress on CNS excitability and cognitive function is an important preliminary step to toward quantifying the effectiveness of systematic hyperthermia to enhance long-term CNS health and performance. Whole-body heating acutely increased motor cortex excitability globally across the architectural distribution of a hand intrinsic muscle. Future studies should examine the effect of whole body heat stress training in healthy individuals and those with CNS impairments to determine whether this novel intervention is a plausible strategy to enhance CNS plasticity.

Table 4.1 Descriptive statistics of subject characteristics. Subjects in Experiment 1 participated in both Heat and Control conditions. Separate subject cohorts comprised the Heat and Control groups in Experiment 2. Three subjects from Experiment 1 participated in Experiment 2 (2 Heat, 1 Control). P values are presented for variables measured between Heat and Control subjects in Experiment 2.

	Experiment 1	**Experiment 2**		
Variable	**(n=11, 5 female)**	**Heat (n = 10)**	**Control (n=10)**	**P value**
Age	25.2 ± 5.8	26.90 ± 6.17	31.00 ± 7.26	0.19
Weight (kg)	75.6 ± 19.2	86.02 ± 11.6	88.50 ± 13.0	0.66
BMI	24.6 ± 3.2	26.88 ± 4.89	27.75 ± 5.37	0.71
%Body fat	21.8 ± 5.6	21.32 ± 7.16	21.07 ± 8.42	0.94
Baecke *Total*	--	8.09 ± 0.96	8.43 ± 1.20	0.50
Work	--	2.11 ± 0.25	2.38 ± 0.88	0.35
Sport	--	3.03 ± 0.61	2.90 ± 0.91	0.72
Leisure	--	2.95 ± 0.70	3.15 ± 0.47	0.46
Marx	--	7.40 ± 5.00	7.1 ± 5.60	0.88

Note: Data are expressed as mean ± standard deviation.

Table 4.2 Physiological responses to heat stress. Measurements were taken immediately prior to entering the chamber (baseline), immediately after exiting (Post0) and 15 minutes after exiting to ambient room temperature (Post15).

	Experiment 1			**Experiment 2**		
Variable	**Baseline**	**Post0**	**Post15**	**Baseline**	**Post0**	**Post15**
HR (beats/min)	69.4 ± 12.3	128.8 ± 15.1**	81.7 ± 12.2	67.0 ± 7.4	126.1 ± 16.0**	75.9 ± 9.3
Temp (° C)	36.7 ± 0.26	38.7 ± 0.41**	37.2 ± 0.59*	36.7 ± 0.18	38.6 ± 0.51**	36.8 ± 0.24
Thermal Sensation	6.8 ± 0.87	10.8 ± 0.75**	7.1 ± 0.38	6.8 ± 0.42	10.7 ± 1.3**	7.1 ± 0.33
Body Weight (kg)	75.6 ± 19.2	75.1 ± 19.1	--	86.0 ± 11.6	85.4 ± 11.6	--
Body Weight (%change)	--	0.69 ± 0.24	--	--	0.74 ± 0.29	--

Note: *= significantly increased from baseline ($P < 0.05$); **= ($P < 0.01$

Table 4.3 Cognitive test scores. Stroop test and TMT scores are reported in seconds. There was a significant main effect of time for the Stroop Color-Word test. Color-Word score was significantly lower across Control and Heat groups during the post condition. No interaction ($P > 0.05$) for group x time was found for any comparisons. The Control and Heat groups represent different subject cohorts.

	Control (n=7)			Heat (n=10)		
	Pre	Post	% Change	Pre	Post	%Change
STROOP						
Color	9.95 ± 1.5	9.79 ± 1.4	1.08 ± 8.0	12.56 ± 3.6	11.05 ± 2.5	8.96 ± 17.4
Word	9.57 ± 2.71	9.06 ± 2.3*	3.52 ± 14.3	10.17 ± 3.1	8.93 ± 2.7*	11.61 ± 9.7
Color-Word	17.32 ± 3.1	15.9 ± 2.8*	7.49 ± 10.9	19.25 ± 6.8	18.0 ± 6.5*	6.25 ± 11.5
TMT-A	16.46 ± 3.6	17.18 ± 4.2	2.91 ± 12.9	21.51 ± 9.5	19.37 ± 7.3	7.45 ± 12.2
TMT-B	35.62 ± 7.7	38.47 ± 11.5	10.57 ± 36.8	42.41 ± 17.9	42.33 ± 17.1	5.94 ± 24.0

Note: Data expressed are mean ± standard deviation. *= ($P < 0.05$).

Table 4.4 Mood Assessment. Control and Heat groups represent different subject cohorts.

	Control (n=14)		Heat (n=12)	
	Pre	Post	Pre	Post
PANAS-Pos	33.29 ± 4.9	32.93 ± 5.0	36.67 ± 4.3	37.08 ± 6.2
PANAS-Neg	14.79 ± 5.8	13.00 ± 2.8*	14.92 ± 2.5	13.41 ± 2.6*

Note: * (p<0.05).

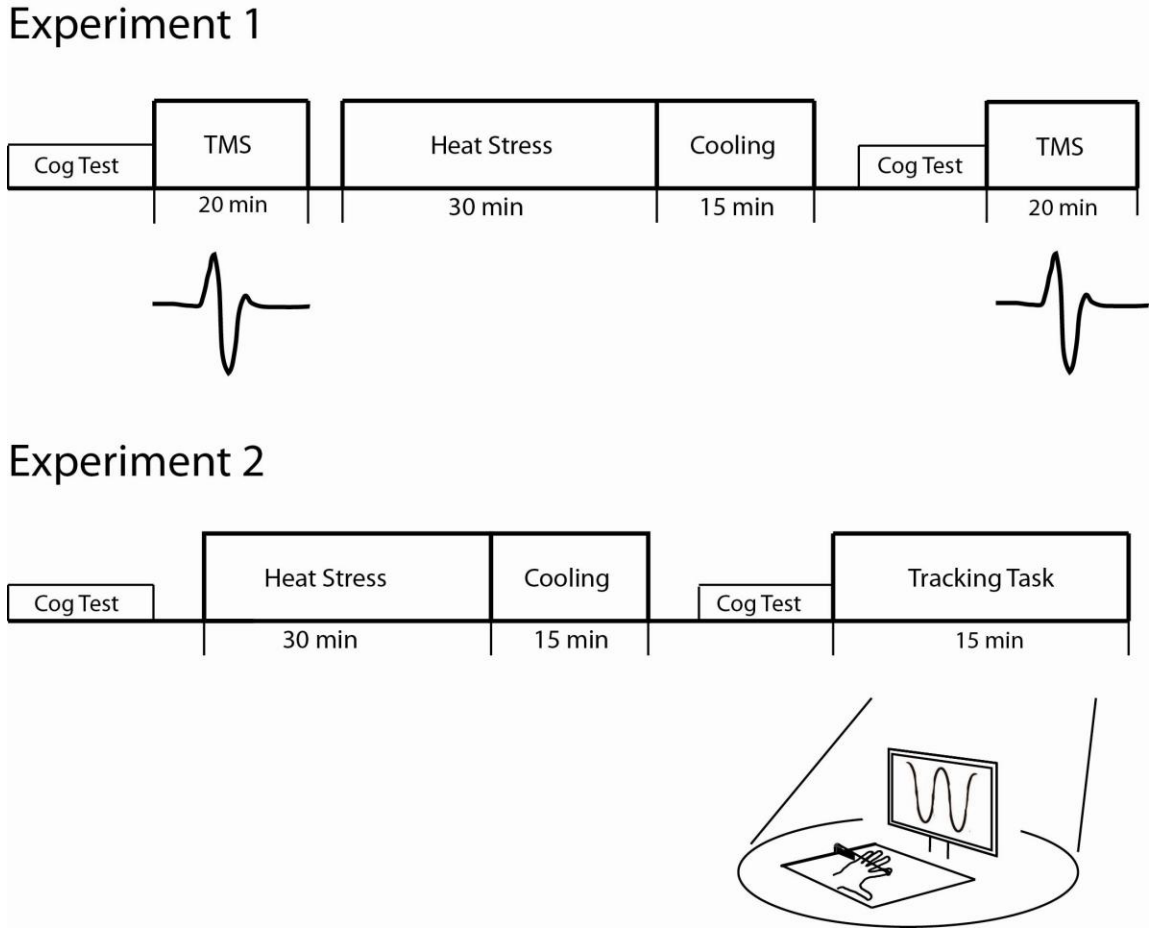

Figure 4.1 Schematic timeline of experimental protocols.

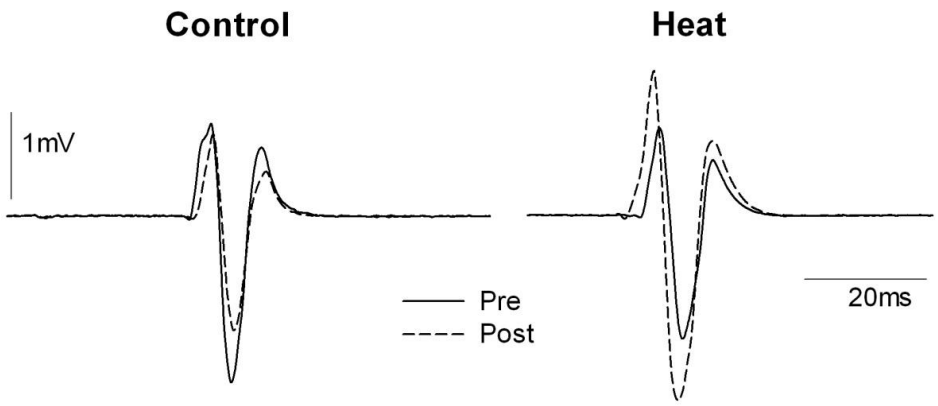

Figure 4.2 Representative motor evoked potentials to transcranial magnetic stimulation (TMS) collected from a single male subject during the control and heat conditions. MEPs were recorded at the motor hotspot at intensity of 120% RMT.

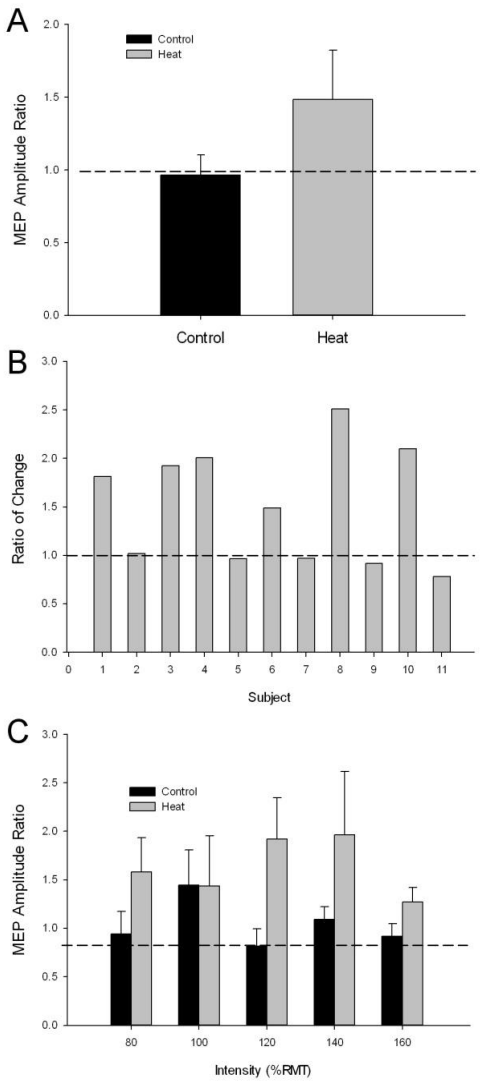

Figure 4.3 MEP responses to heat stress. (A) Ratio of motor map mean MEP amplitude measured before and after no heat stress (Control) or before and after 30 minutes of whole body heat stress (Heat). (B) Ratio of percent change in MEP amplitude during the heat condition to percent change in MEP amplitude of the control condition. MEP change in amplitude between the MEP amplitude ratios for the Control and Heat conditions listed by subject. Six of eleven subjects (5 male) showed 49% or greater increase in MEP amplitude ratio for the heat condition compared to the control condition. (C) MEP amplitude ratio shown by intensity of the stimulus-response recruitment procedure. There was no significant interaction of condition and intensity during the recruitment procedure, but showed a strong trend of greater MEP amplitude in the Heat condition, ($P = 0.056$).

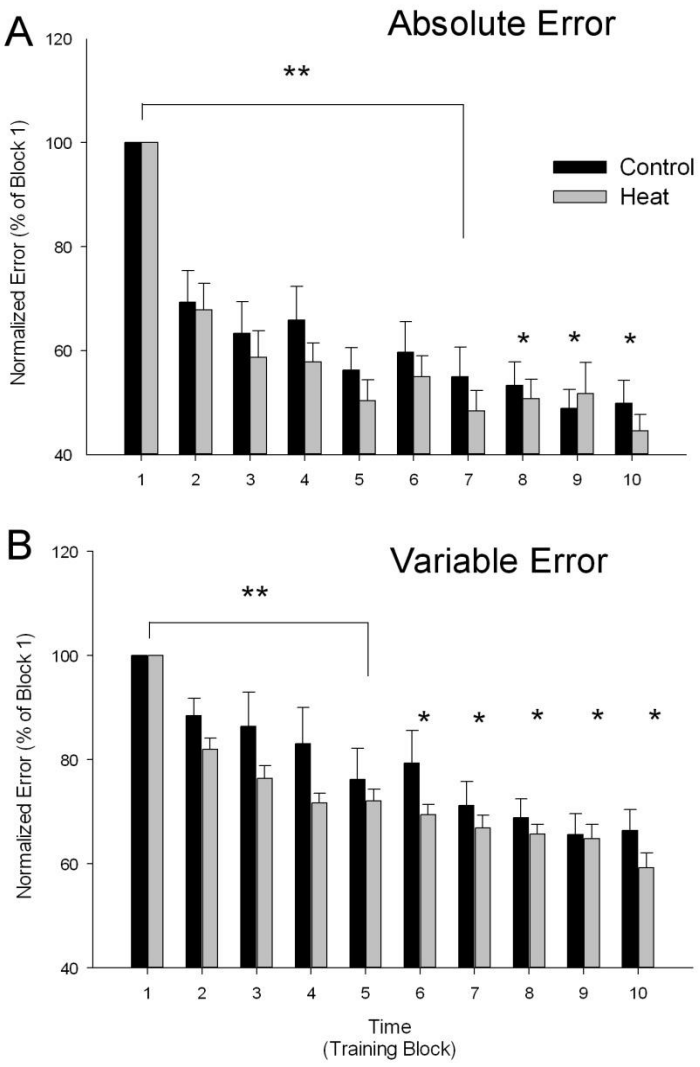

Figure 4.4 Tracking task error. (A) Absolute error reduction by training block for the Control (black bars) and Heat (gray bars) groups. Error values are normalized as the percent of Block 1 error. (B) Variable error reduction by training block for the Control (black bars) and Heat (gray bars) groups. Error did not differ between groups at any given training block for either error type. The respective error scores did not differ between the concentric (shortening) and eccentric (lengthening) phases of the tracking cycle and thus are shown as pooled errors within each condition. Error bars are standard errors. **indicates Block error was significant reduced from baseline (P <0.01). Blocks marked with * did not differ from each other.

CHAPTER V

CONCLUSIONS

Novel sensorimotor experiences stimulate neuronal networks to modify their intrinsic excitability and spatial connectivity within and between CNS structures specific to the nature of the task or intervention. The development of interventions to optimize motor and cognitive performance requires at thorough understanding of the influence of activity-based and systemic stressors on neuroplasticity variables. A large body of research has been devoted to motor learning effects on cortical plasticity; however, relatively few studies have examined how changes neuroplasticity variables in the motor cortex are manifested with voluntary motor output. The first specific aim contributes further findings to this field. The second specific aim provides a template for future investigation of cortical plasticity, with particular relevance for examination of cortical excitability changes induced by systemic stress. The third specific aim investigates the novel use of whole-body heat stress as a therapeutic modality to enhance central nervous system performance. The purpose of this study was to establish the acute effects of physiological stressors on CNS plasticity. Each study employed a novel technique or analysis not previously reported in the literature. Each of the specific aims was achieved. The hypotheses of the studies, as originally stated, along with the conclusions from each study are discussed individually.

Specific Aims and Hypotheses

While each of the specific aims was achieved, not all of the original hypotheses were supported by the results of the studies. The specific aims and hypotheses, as originally stated, along with the conclusions from each study are discussed individually.

Hypothesis 1a: Motor learning, defined as the reduction in error during a precision tracking task, will lead to a significant increase of the mean MEP amplitude of the resting MEP intensity curve when compared to pre-training. This finding supports

that motor learning enhances cortical excitability during this novel dynamic motor learning task using the FDI.

In support of this hypothesis, subjects showed a significant increase in MEP amplitudes measured during resting recruitment at stimulation intensities greater than 110% of resting motor threshold, consistent with prior studies of reporting MEP enhancement and expansion of the motor cortex representation in response to motor learning tasks(102, 122, 146, 153) . Significant increase in excitability was accomplished after only ten blocks comprising 50 cycles of finger movement. These results support that acute enhancement of M1 excitability is present after short duration training.

Hypothesis 1b: The mean MEP amplitude, superimposed on various backgrounds of volitionally active muscle, will be increased as a function of volitional background force after learning a dynamic motor learning task. The silent period (SP) will be significantly reduced after the learning task as a function of volitional background force after learning. This finding supports that excitatory and inhibitory cortical inputs shape cortical modulations involved in motor learning.

Our results did not support this hypothesis. Despite increased cortical excitability measured at rest after task training, expected increases in excitability during voluntary activation were not observed. MEP amplitude was decreased at 20% MVC suggesting that cortical inhibition shaped the motor output to a greater extent at low levels of activation than MEP facilitation. This finding argues that modulations in cortical excitability during voluntary activation were likely specific to the force demands of the task. Cortical inhibition was increased across all levels of activation as evidenced by the significant increase in SP duration. This finding was in contrast to prior studies investigating SP duration as a function of task complexity or stimulus intensity with showed either shortening or no change in SP duration (109, 150, 187).

Hypothesis 1c: There will be an association between those who showed the greatest learning and enhancement of the mean MEP of the intensity curve and cortical

silent period. This will show that motor learning is scaled to measured changes in cortical electrophysiological measures of excitability and inhibition.

Our findings partially support this hypothesis. Despite the unexpected increase in SP duration induced by the learning task, the magnitude of learning was significantly correlated to the increase in SP duration at 20% MVC. As such, the level of inhibition showed greater relationship to the learning outcomes than the level of excitability. Similar to the MEP at 20% MVC, the association appears to be specific to the lowest level of voluntary activation. To our knowledge, ours is the first investigation to relate the magnitude of change in cortical excitability to the degree of motor learning success of a precision task.

Specific Aim 2 (Chapter 3): To determine the reproducibility of a primary motor cortex mapping and recruitment procedure evoked by single pulse TMS of the first dorsal interosseus muscle in healthy subjects.

Hypothesis 2a: The mean MEP amplitude of a 15-point map will show high reliability with intra-class correlation coefficients (ICC) > 0.8 between 2 mapping sessions separated by 30 minutes. Mean peak-to-peak amplitude will be closely associated across activation sites (>0.8) before and after mapping sessions. The mean MEP amplitude of the recruitment curve will show no systematic change between the two testing sessions.

Mean MEP amplitudes of the motor map were highly reliable between testing sessions. Intraclass correlations of the motor map were ≥ 0.90 for all combinations of grid points of the motor map. Under the current protocol, comparison of MEP amplitudes as the mean of the entire map, the hot spot only, or along the X-axis of the map present equally reliable options for analysis. Importantly, this study validates the use of a map of fixed size to reliably reflect changes in MEP amplitudes due to therapeutic interventions.

Hypothesis 2b: The absolute movement of the individual X- and Y-coordinates will not exceed 4 mm between two mapping sessions separated by 30 minutes. The x-coordinate will show greater reliability than the y-coordinate because of the elongation of the map in the direction of induced current flow, where larger map area provides a greater area over which the y-coordinate may fall.

Our results showed that mean shift of the CoG in the absence of any intervention was < 2.7 mm. This finding is in line with prior published reports (127, 133, 206) and fully support the hypothesis. There was no systematic movement of the CoG to suggest that the testing protocol influenced the center of excitability in resting conditions. Also consistent with prior published reports, the X-coordinate showed greater reliability than the Y-coordinate. A unique feature of this study was relating the abscissa to the approximate axis of the central sulcus. As a result, the Y coordinate reflected movement toward or away from the primary sensory cortex.

Specific Aim 3 (Chapter 4): To determine if passive whole-body heat stress affects motor cortex excitability, motor performance, and cognitive function.

Hypothesis 3a: Passive heat stress will increase cortical excitability as evident by an increase in MEP amplitude of the FDI motor map. The mean MEP peak-to-peak amplitude will not differ significantly after no heat stress. Passive heat stress will lead to a significant increase of the mean amplitude of the resting MEP intensity curve when compared to no heat stress.

In support of this hypothesis, 30 minutes of passive heat stress led to increased M1 excitability. Moreover, this effect was present greater than 25-30 minutes after cessation of heat stress, suggesting the mechanisms underlying the increase due to heat were long lasting. Males showed a significantly greater increase in MEP amplitude of the FDI motor map and also the MEP intensity curve compared to females. The reason for the difference between sexes is not clear.

Hypothesis 3b: Passive heat stress will cause individuals to perform a dynamic movement task with less absolute and variable error compared to control subjects who do not receive heat stress, supporting that heat stress improves acute motor learning.

Subjects demonstrated learning of the motor task as evidenced by significant reduction in absolute and tracking error; however, no difference in the change in error or rate of learning was found between the heat and control groups. Thus, this hypothesis was not supported by the study findings. Although no acute learning changes were evident between groups, previously documented increases in protective chaperones, catecholamines, and activity of the serotonergic-dopaminergic pathways induced by heat stress may serve as a priming mechanism to increase motor learning with repetitive practice over longer durations.

Hypothesis 3c: Passive heat stress will improve performance on two tests of cognitive function. Components of the tests measuring executive function will show comparatively greater improvement after heat stress. This finding will support that improvements in cognitive performance observed with moderate doses of exercise may also be induced by systemic thermal stress.

Subjects showed a significant improvement in the Stroop Color test upon repeat testing; however, the effect was seen with or without heat stress. No significant improvements were found in the Stroop Color-Word test or Trail Making Test-B that are more likely to reflect changes in the executive function domain. The possibility remains that heat stress may differentially influence various domains of cognitive performance not represented in these tests.

Summary

The studies presented in this thesis were carried out to broaden our understanding of the influence of physical activity on central nervous system plasticity influencing cortical excitability, motor learning and cognition performance. The first study examined

how a learning specific task influenced cortical excitability and the manifestation of these changes in during voluntary activation. The findings supported that parallel excitatory and inhibitory processes shaped motor output after task acquisition. Inhibitory influences showed a significant relationship with the magnitude of learning. Moreover, the relationship was most evident at levels of muscle activation closely resembling those experienced during the training task, supporting a task specific modulation of the motor cortex. The following studies demonstrated that whole body heat stress as a form of systemic physiological loading increased motor cortical excitability similar to that observed during motor learning. The immediate modulations in the motor cortex did not fully translate into acute functional performance changes. However, modulations in cortical excitability may serve as preliminary processes to induce long term change. Whole body heat stress is an intriguing novel intervention that may help individuals unable to exercise to reap some of the benefits of physical activity. Future studies are critical to determine the utility of whole-body heat stress as a means to enhance CNS performance and the mechanisms by which initial modulations in CNS plasticity lead to long-term structural and physiological change.

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