MODULATION OF CORTICOSPINAL EXCITABILITY DURING ARM CYCLING IN HUMANS

by

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Abstract

Animal studies have shown that the basic pattern for locomotor activities are generated via neural networks found in the spinal cord, referred to as central pattern generators (CPGs) (Grillner, 1981). In humans, accumulating research evidence suggests that primates, including man, have a similar locomotor centre as animals that controlled by CPGs (Petersen et. al., 1998). It's indicative that CPGs are sufficient to enable locomotion in quadrupeds; however a more extensive cortical input is involved in the production of locomotion and/or cycling in humans (Zehr et. al 2004). Advanced methods such as transcranial magnetic stimulation (TMS) and transmastoid electrical stimulation were implemented to examine supraspinal and spinal excitability, and bridge the gap between animal and human research. Therefore, this thesis set out to determine changes in corticospinal excitability in biceps brachii during different motor outputs, including those generated by spinal CPGs.

The major findings from the present study suggest that corticospinal excitability is enhanced, in biceps brachii, during the initiation of the flexion phase of arm cycling when compared to an intensity matched contraction. The results also proposed that spinal mechanisms are the dominant factors which drive task- and phase-dependent modulation of corticospinal excitability during arm cycling.

Keywords

Central Pattern Generators (CPGs), Locomotion, Cycling, Supraspinal, Spinal, Transcranial Magnetic stimulation (TMS), Transmastoid Electrical Stimulation, Corticospinal Excitability, Biceps Brachii, Task-and Phase-Dependent

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Table of Contents

Certificate of Examination II
Abstract III
Kewords III
Acknowledgements IV
Table of Contents V
List of Figures VII
List of Appendices VIII
List of Abbreviations Used IX
Section 1: Literature Review 1
Introduction 2
Neuroanatomy 2
I. The Primary Motor Cortex 2
II. The Corticospinal Tract 3
Neural Control of Locomotion: Animals to Humans 3
I. Changes in spinal motoneurone properties during rhythmic motor output in the cat 4
II. Corticospinal excitability during rhythmic motor output 4
III. Task- and state-dependent changes 6
IV. Changes in Force Threshold 7
V. Changes in Firing Frequency 8
Studying Corticospinal Excitability: Translation from animal to human research 8
I. Transcranial Magnetic Stimulation 9
II. Transmastoid Electrical Stimulation 11
III. Nerve Stimulation 13
IV. Electromyography (EMG) 14

Significance of the study 14
References 15
Section 2: Manuscript 19
Modulation of Corticospinal Excitability During Arm Cycling in Humans 20
Abstract 21
Introduction 22
Methods 24
Results 28
Discussion 33
Conclusion 35
References 36
Section 3: Appendices 38
Appendix 1: TMS Safety Checklist 39
Appendix 2: Consent Form 42
Appendix 3: Statement of Confidentiality form 47

List of Figures

Figure 1. Schematic illustration of the experimental setup 25

Figure 2. Individual MEP and CMEP responses during cycling and tonic 29

Figure 3. Group supraspinal background EMG.....30

Figure 4. Group spinal background EMG.....30

Figure 5. Group task-dependant supraspinal changes.....31

Figure 6. Group task-dependant spinal changes.....31

Figure 7. Individual MEP and CMEP responses during cycling 32

List of Appendices

Appendix 1: TMS safety checklist

Appendix 2: Consent Form

Appendix 3: Statement of Confidentiality Form

List of Abbreviations Used

AHP: Afterhyperpolarization

CMEP: Cervicomedullary Motor Evoked Potential

CPG: Central Pattern Generators

EMG: Electromyography

FCR: Flexor Carpi Radialis

M1: Primary Motor Cortex

MEP: Motor Evoked Potential

M-max: Maximal Muscle Response

MSO: Maximum Stimulator Output

M-wave: Muscle Response

MVC: Maximal Voluntary Contraction

SP: Silent Period

TMS: Transcranial Magnetic Stimulation

Vth: Voltage Threshold

Section 1: Literature Review

Introduction

Recent work in adult decerebrate cats have demonstrated that spinal motoneurone excitability is enhanced throughout rhythmic and alternating motor outputs generated by spinal circuitry, referred to as central pattern generators (CPGs) (Krawitz et al. 2001; Power et al. 2010). Whether similar changes in motoneurone excitability occur in humans during motor outputs driven in-part by spinal CPGs is not well-understood. While in humans it is generally accepted that spinal CPGs contribute to locomotion and cycling, it is also thought that these motor outputs rely more on input from the cortex than quadrupeds whereby spinally generated motor outputs are possible. The primary objective of this research project is to determine whether motoneurone excitability is enhanced throughout arm cycling, a motor output generated in part by a spinal CPG. Because CPG-mediated motor outputs in humans also rely on supraspinal input, we will also assess changes in cortical excitability. The brain is known to influence motor output through descending connections terminating in the spinal cord. The main descending pathway activated during voluntary movement is the corticospinal tract. The following sections discuss the role of the structures involved in the motor pathway involved in human movement. The sections also review changes in the electrical properties of spinal motoneurones during motor output in recent animal studies. This is followed by a discussion of changes in corticospinal excitability in humans during different motor outputs, including those generated by spinal CPGs.

Neuroanatomy

The primary motor cortex initiates voluntary movement through descending connections found in the spinal cord. The lateral corticospinal tract originates from the motor cortex and is the main pathway activated during movement. This section discusses the role of the main structures that enable movement in the human body.

I. The Primary Motor Cortex

The primary motor cortex (M1) lies in Brodmann area 4 and is located anterior to the central sulcus in the precentral gyrus. The primary motor cortex contains a

somatotopic representation of the different body parts called the motor homunculus (Latash, 2007). The body parts on the cortex are proportional not to their size, but rather to the complexity of the movements that they can perform (Snell, 2009). Hence, the areas for the hand and face are especially large compared with those for the rest of the body. The main role of the primary motor cortex is to generate neural impulses that pass down to the spinal cord and control the execution of movement. The primary motor cortex contains large output cells (Betz cells) which sends an axon down the corticospinal tract to synapse onto the interneuron and motoneurone found in the spinal cord (Latash, 2007).

II. The Corticospinal Tract

The corticospinal tract contains about one million axons, half of which originate from the motor cortex (Latash, 2007). The corticospinal tract is made up of two separate tracts: the lateral corticospinal tract and the anterior corticospinal tract, which decussates (cross over to the other side of the body) at the level of the medulla (Snell, 2009). Since the cross over takes place at the brainstem, most of the axons from right hemisphere travel on the left side of spinal cord and innervate muscles of the left limb, while most axons that form a tract from the left hemisphere travel on the right side of the spinal cord and innervate muscles of right limb (Magill, 2007). The lateral corticospinal tract is the largest and the most central part of the corticospinal tract and is responsible for the control of the distal musculature. On the other hand, the anterior corticospinal tract is responsible for the control of the proximal musculature (Purves et. al. 2004). In the spinal cord, the upper motoneurone from motor cortex synapses onto the lower motoneurone in the anterior horn, which which innervates multiple skeletal muscles involved in movement (Snell, 2009).

Neural Control of Locomotion: Animals to Humans

Animal studies have found that spinal networks known as central pattern generators (CPGs) contribute to the control of locomotion (Sherrington, 1910). Based on these findings, researchers were able to translate animal-model of

locomotion to humans and found the involvement of subcortical circuits in locomotor-like activities, but with a greater input from cortex (Porter and Lemon, 1993).

I. Changes in spinal motoneurone properties during rhythmic motor output in the cat

The electrical properties of spinal motoneurones are modulated quickly and reversibly when going from a resting state to motor output. These changes include a hyperpolarization of the voltage threshold (Vth) for action potential initiation and a reduction in the amplitude of the afterhyperpolarization (AHP). Vth is the membrane potential at which the inward sodium (Na+) current outweighs the outward potassium (K+) current an action potential is initiated (Gardiner, 2011). AHP is the prolonged hyperpolarization period of a neurone's action potential which is facilitated by calcium-activated potassium channel (Gardiner, 2011).

Power, McCrea and Fedirchuk (2010) examined changes in motoneurone Vth, AHP amplitude, and the emergence of voltage-dependent depolarizations during ipsilateral scratch in both spinal intact and acutely spinalized decerebrate cats. Some significant results show a decrease or hyperpolarization of Vth and an increase in motoneurone excitability during fictive scratch following a spinal transection at C1, which disconnected all descending input from the brainstem. This supports the role of central pattern generators (CPG) found in rhythmic movements in many invertebrate and vertebrate species.

AHP limits how fast a motoneurone can fire, and its removal from an action potential event gives the nerve impulses a higher firing rate by bringing the spikes closer together. The second state-dependant change shown in the study by Power and colleagues (2010) was a decrease in AHP amplitude, which enabled high firing rates during scratch or rhythmic activity in decerebrate cats.

II. Corticospinal excitability during rhythmic motor output

The initiation of motor output is characterized by changes in the excitability of many structures within the central nervous system. In humans, accumulating research evidence suggests that primates, including man, have similar locomotor centre as animals that controlled by CPGs (Petersen et. al., 1998). It's indicative that subcortical circuits are sufficient to enable locomotion in quadrupeds; however a more extensive cortical input is involved in the production of locomotion and/or cycling in humans (Zehr et. al., 2004).

Evidence from transcranial magnetic stimulation (TMS) studies show some direct role from the motor cortex by examining the ankle muscles during treadmill walking, and thus suggesting that there is a relative involvement of supraspinal mechanisms to the motor pattern of human leg movement (Capaday et al. 1999; Christensen et al. 2001; Petersen et al. 1998). Subthreshold TMS during cycling and static contraction provided evidence that the motor cortex actively drives the motoneurones of the leg muscles examined during cycling (Sidhu et. al. 2011). Previous work by Peterson & colleagues (2001) has shown the activation of intracortical inhibitory circuits followed by EMG suppression via weak magnetic stimulation. This decreases excitability of cortical cells and reduces output from the motor cortex during walking. Subthreshold TMS during cycling evoked suppression of background EMG during cycling in the lower limb muscles and inhibition occurred 10 ms after facilitation which lasted for 7 ms (Sidhu et. al. 2011). On average, the amplitude of EMG suppression was greater during static contractions compared with that during cycling. If the surpraspinal centers were not involved in the production of muscle activity during cycling, then subthreshold TMS would have no effect on that background EMG. In addition to examining the role of the supraspinal centers in the production of lower limb cycling, Sidhu et. al. (2011) also examined spinal excitability. Responses from the motor cortex, motor evoked potentials (MEPs), and cervicomedullary junction, cervicomedullary motor evoked potentials (CMEPs), were modulated similarly during cycling. This suggests that the observed changes in the MEPs were driven mainly by changes at the spinal level

(Sidhu et. al, 2011). However, there were subtle differences between normalized (to EMG) MEP vs. CMEP sizes prior to the EMG burst. Thus, it is possible that the excitability of cortical neurons increases briefly prior to start of the burst, but this was difficult to see with the methods and temporal resolution of the analysis used in the study (Sidhu et. al. 2011). There were also showed muscle dependent changes in the major thigh muscles (ie. rectus femoris, vastus lateralis, and biceps femoris). Cortical excitability increased prior to muscle activation in vastus lateralis, but not in rectus femoris and biceps femoris, which shows intermuscle differences in phase-dependent changes in corticospinal excitability during locomotion (Sidhu et. al. 2011).

Given that the cortex has more monosynaptic connections with the motoneurones controlling upper limb musculature as compared to the lower limb, it may be that the corticospinal control of upper limb musculature is very different than those in the legs. Zehr et. al. (2004) examined the role of the motor cortex and reflex pathways in the generation of rhythmical motor output in the FCR during cycling and suggested that CPGs contribute to the control of rhythmic arm movement. For example, Carroll et. al. (2006) found decreased corticospinal excitability (i.e. decrease MEP and H-reflex amplitude) during the flexion phase of rhythmic arm movement when compared to a tonic contraction. Carroll et. al. (2006) suggested that the decrease in MEPs during arm cycling was due to spinal mechanisms, and that alternative circuits (e.g., CPGs or spinal reflex pathways) provide a proportionally greater contribution to the control of rhythmic arm movements than of tonic contraction in humans. They also found a facilitation of spinal reflexes, shown via subthreshold TMS, during tonic contraction but not during arm cycling which shows task-dependent changes of corticospinal excitability.

III. Task- and state-dependent changes

Motoneurones show little to no excitability during a resting state when compared to a state (ie. movement) in which motoneurones can be readily activated to initiate and maintain muscle contraction. In animals, Vth hyperpolarization and AHP are

state-dependent changes in motoneurone excitability in induced motor output (i.e. scratch and stance). In decerebrate cats, motoneurone excitability is enhanced during rhythmic motor outputs such as locomotion via the hyperpolarization of Vth and decrease in AHP, which are both important in motoneurone recruitment in locomotion (Dai et al. 2002; Krawitz et al. 2001, Power et. al. 2010). In neonatal rat, evidence show reduced motoneuronal AHP during locomotion, independent to supraspinal influences, suggesting the activation of spinal cord locomotor circuits (Schmidt, 1994). Thus, these observed state-dependent changes that alter motoneurone excitability during fictive scratch and locomotion are also proven to be task-dependent.

Similar results have been discerned in humans, using non-invasive techniques such as magnetic stimulation of the motor cortex and electrical stimulation of the transmastoid process. Changes in corticospinal excitability, with contributions from the motor cortex, during upper and lower body cycling demonstrate a greater excitability during rhythmic movement of the major limbs tested (Carroll et. al. 2006). In the study by Carroll et. al. (2006), there was a decrease in corticospinal excitability during the flexion phase of rhythmic arm movement, and a facilitation of spinal reflexes, shown via subthreshold TMS, during tonic contraction but not during arm cycling (Carroll et. al. 2006), which shows task-dependent changes of corticospinal excitability. Additionally, evidence from task- and phase-dependency of reflexes in arm muscles during cycling suggest that CPG networks contribute to the control of rhythmic arm movement, either by directly acting on the motoneuronal pools or indirectly via interneuronal reflex networks (Zehr et. al. 2004).

Studying Corticospinal Excitability: Translation from animal to human research

Animal studies were originally done to examine basic alternating extensor-flexor rhythm underlying locomotion that is generated by a local network found in the spinal cord, referred to as central pattern generators (CPGs) (Grillner, 1981).

However, little was known about the central control of human locomotion, but accumulating evidence suggests that primates, including man, have similar locomotor centre that are a lot more difficult to activate pharmacologically and electrically (Petersen, Christensen and Nielsen, 1998). Advanced methods such as transcranial magnetic stimulation (TMS) and electrical stimulation at the cervicomedullary junction were implemented to evaluate corticospinal excitability, and build a bridge between animal and human research.

I. Transcranial Magnetic Stimulation

In 1985, Anthony Barker and colleagues successfully completed a transcranial magnetic stimulation (TMS) study, a non-invasive method that activates the human motor cortex through the skull (Barker, Jalinous, and Freeston, 1985). The magnetic stimulation of the motor area occurs by a rapid discharge of current elicited through a coil placed over the scalp, which generates a magnetic field oriented perpendicular to the coil (Rothwell, Thompson, Day, Boyd and Marsden, 1991). The rapidly changing magnetic field then induces stimulation of the interneurons, neural tissue in the brain, that synapse onto the neurons of the motor cortex. The magnetic coil then causes depolarization of the neurons to activate the descending pathway involved in motor output of the specific muscle being stimulated.

There are different types of magnetic coils that produce different magnetic field patterns. A round coil is the original TMS coil; a figure-eight coil, also known as a butterfly coil, results in a focal pattern of activation; a double-cone coil conforms to shape of head which is useful for deeper stimulation; and a four-leaf coil is used for focal stimulation of peripheral nerves. During a TMS study, a figure-eight coil is known to be an ideal one as it activates more superficial muscles. This coil consists of two separate round coils placed side-by-side with the currents being discharged in opposite directions. The stimulated electric fields add up so the maximal current are at the junction between the two coils (Centre for Cognitive Neuroimaging, 2012). However, the study in this thesis used a round coil.

TMS of the motor cortex induces D-waves (direct) or I-waves (indirect). D-waves represent the direct stimulation of the corticospinal axons at either the initial segment of the neuron or at the proximal internodes in the subcortical white matter. Alternatively, I-waves represent the trans-synaptic activation of corticospinal neurons, following D-waves at intervals of approximately 1.5ms, labeled as I1, I2, and I3 waves, which is in order of their latency (Rothwell, 1997). Patton and Amassian (1954) suggested that I-waves reflect repetitive firing of pyramidal tract neurones due to excitatory postsynaptic potentials found in a reverberating neuronal circuit in the motor cortex. Furthermore, Philips (1987) added that the effectiveness of I-waves is synchronized by the tendency of pyramidal neurones to fire repetitively at high frequency during sustained depolarizing inputs. Latencies of I-waves are longer than D-waves and are thus thought to appear via trans-synaptic activation of pyramidal tract neurons within the motor cortex. Rothwell (1997) stated that I-waves are more commonly evoke during TMS, whereas D-waves are more readily activated by transcranial electrical stimulation, which involves the direct activation of corticospinal fibers.

Marsden, Merton & Morton (1983) first demonstrated that electrical stimulation of the motor cortex in man produces a muscle twitch followed by a silence of EMG activity. Cortical silent period (SP) corresponds to the suppression of muscle activity for a short period after a muscle response to TMS. Other studies using transcranial electrical and magnetic stimulation in hand muscles to examine the physiological mechanisms associated with silent period have demonstrated that the silent period is comprised of both a cortical and spinal component. The first part of the silent period (50-60 ms) is due to descending inhibitory influences, whereas inhibitory mechanisms in the motor cortex contributes to most of the suppression in the ongoing voluntary EMG activity (Rothwell, 1997).

The homunculus has an overrepresentation of the upper limb and hand region of the body, and is therefore often used in TMS experiments. The descending lateral corticospinal tract is a collection of axons that travel between the cerebral cortex of

the brain and the spinal cord. When TMS evokes the area of the motor cortex that controls the muscle studied, it sends a stimulus down the motor pathway, activating the corticospinal tract. Following this neural activity, an action potential is discharged which produces a motor evoked potential (MEP) in the muscle, which is the electrical activity of the muscle generated by the nervous system following a TMS.

The MEP can be recorded using electromyography (EMG) which helps measure the peak-to-peak amplitude of the response at the optimal site for the muscle, and thus provides an indication of cortical excitability. If the peak-to-peak amplitude has decreased, we can say the excitability anywhere in the pathway (brain, spinal or muscle) reduced. If MEP amplitude increased, the excitability anywhere in the pathway from the cortex (cortical, spinal or muscle) increased.

II. Transmastoid Electrical Stimulation

In 1980, Merton and Morton developed the first noninvasive technique to activate the motor cortex via a transcranial high-voltage electrical stimulus, and thus enabling researchers to examine the role of significant motor pathways. Additionally, Ugawa et. al. 1991 established that motor responses could be induced in muscles by passing an electrical pulse either across the spinal cord between electrodes on the mastoid process, or along the spinal cord between electrodes in the midline over the skull and upper cervical vertebrae. Stimulating the cervicomedullary junction evokes large, short latency motor responses, because the axons at the level of the pyramidal decussation are more susceptible to stimulation (Taylor and Gandevia, 2004). The review by Taylor and Gandevia in 2004, studied CMEPs for the following reasons:

1) To provide an intermediate site of stimulation,

2) To examine directly the behavior of the corticospinal pathway and the motoneurone pool,

3) To determine whether changes in the cortically evoked motor evoked potential (MEP) are cortical or spinal in origin.

Stimulation at the cervicomedullary junction elicits a single volley in the descending axons which activates motoneurones of the chosen muscle synaptically and produces a short-latency excitatory response (Taylor, 2006). In order to determine which descending motor tracts were activated by subcortical stimulation, Ugawa et. al. (1991) conducted a series of collision experiments. The responses from right hand muscle (first dorsal interosseous) were recorded after electrical stimulation was elicited at the brainstem as well as the left motor cortex, with both shocks given simultaneously during a voluntary contraction of the muscle. The cortical response was seen to be suppressed due to the collision of the descending cortical volley with an antridromic volley from the stimulation at the brainstem. The researchers used small transcranial electrical stimulus which mainly evokes a single descending volley, therefore the collision of the impulses indicates that the responses to both these stimuli travel in the same axons (Taylor, Petersen, Butler and Gandevia, 2002). A study done in 1991 by Thompson, Day and Crockard, deduce that the first descending volley is recorded at latencies about 1.9 to 2.1 milliseconds at the pyramidal decussation which lies at the cervicomedullary junction. In the collision experiment, stimulation at the motor cortex produced descending volleys in the pyramidal tract that reached the brainstem level after 1.8 milliseconds; with the brainstem shock given 0.2 milliseconds after the first descending volley passed the site of stimulation. As the cervicomedullary stimulation and transcranial electrical stimulation were delivered at this interstimulus interval, the antidromic volley collided with the cortical stimulation and occluded the response completely (Taylor, 2006). When the cortical shock was given 3 milliseconds before the brainstem, the brainstem stimulus would have occurred 1.2 milliseconds after transmission of the cortical volley. The absolute refractory period of the pyramidal tract axons would be over, and the brainstem stimulus would evoke a second descending volley. The EMG response was greatly facilitated, probably by temporal summation of the two synaptic inputs (one volley from the cortical stimulus and the other from the brainstem) at the level of the spinal motoneurones (Ugawa, et. al, 1991). Thus, proving that electrical stimulation at the cervicomedullary junction activates the corticospinal tract.

Furthermore, Taylor (2006) highlighted that in biceps, a single motor unit response show narrow peaks which are similar in width to those elicited by stimulation of Ia afferent and are consistent with monosynaptic activation. In 2002, Petersen and colleagues tested the theory of monosynaptic response to the stimulation of the corticospinal tract of the human biceps by looking at latency changes of CMEP between rest and contraction. During a contraction, the conduction velocity of descending axons increase which would decrease the latency, activating the motoneurones earlier than those at rest. However, Petersen et. al. (2002), found minimal changes in latency between rest and contraction CMEP which suggested that only one synapse in involved in the fastest pathway from the site of stimulation to the motoneurone. As CMEPs primarily produce a monosynaptic response to the stimulation of corticospinal axons, it allowed researchers to examine motoneurones during and after tasks that involve strong voluntary contractions. The discovery of CMEPs can enable researchers in the motor control field to study brief or long-lasting changes in motoneurone excitability, and help identify any changes taking place in the motor pathway.

CMEPs can be recorded using electromyography (EMG) which helps to measure the peak-to-peak amplitude of the response at the optimal site for the muscle, and thus provides an indication of spinal motoneurone excitability. A decrease in CMEP amplitude sets a less excitable spinal or muscle state, whereas an increase in CMEP amplitude shows the excitability anywhere in the pathway from the cortex to the muscle increased.

III. Nerve stimulation

M-wave is a muscle or motor response, which indicates the strength of peripheral excitability, a measure from nerve to muscle. It represents the electrical event in muscle fibers which is from the neuromuscular junction to the action potential propagation along sarcolemma and t-tubules. When the corticospinal tract is stimulated at the pyramidal junction, it elicits a motor evoked potential response

from the muscle being studied. However, since the stimulus travels down the descending tract to the peripheral nerves of the muscle, the excitability seen could either be at the spinal or muscle level. Taking muscle or peripheral excitability into account, CMEPs are made relative to muscle response to see if the change in excitability was in spinal or muscle.

IV. Electromyography (EMG)

EMG an experimental technique concerned with the development, recording and analysis of myoelectric signals that are formed by physiological variations in the state of muscle fiber membranes (Konrad, 2005). The basic signal is a measure of changes in electrical potential across the muscle fiber. A resting membrane potential is ≈ -90mv and with sufficient stimulation, the potential inside cell rises to ≈ 30-40mv. The change in action potentials from multiple fibers in a motor unit are simultaneously recorded using EMG. The small biological electrical activity from the muscle goes to an amplifier which amplifies that signal which then goes through an A-to-D board that translates amplitude and polarity of a sampled signal into a digital format, ie. turns biological signal into digital so the computer can read it (Konrad, 2005).

Significance of the study

The results of this study will provide evidence as to the role of supraspinal and spinal mechanisms in generating rhythmic upper-body motor output in humans. It is essential to understand the mechanisms that help alter or produce motor output and further investigate the regulation of spinal CPGs during various movements. Ultimately, the understanding and knowledge built on spinal networks and movement will enable researchers to develop rehabilitation interventions for spinal cord injuries.

References

Barker, A.J., Jalinous, R. and Freeston, I.L. (1985) Non-invasive stimulation of human motor cortex. Lancet, II: 1106–1107.

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

Capaday, C., Lavoie, B. A., Barbeau, H., Schneider, C., and Bonnard, M. (1999). Studies on the corticospinal control of human walking. I. Responses to focal transcranial magnetic stimulation of the motor cortex. J Neurophysiol 81: 129–139.

Carroll, T. J., Baldwin, E. R. L., Collins, D. F. and Zehr, E. P. (2006). Corticospinal Excitability Is Lower During Rhythmic Arm Movement Than During Tonic Contraction. J Neurophysiol, 95, 914-921.

Centre for Cognitive Neuroimaging in Glasgow. (2012). Transcranial Magnetic Stimulation (TMS).

Christensen, L. O. D., Andersen, J. B, Sinkjòr, T., and Nielsen, J. (2001). Transcranial magnetic stimulation and stretch reflexes in the tibialis anterior muscle during human walking. Journal of Physiology 531.2, pp. 545—557.

Dai, Y., Kelvin E. Jones, Brent Fedirchuk, David A. McCrea and Larry M. Jordan (2002). A modelling study of locomotion-induced hyperpolarization of voltage threshold in cat lumbar motoneurones. Journal of Physiology, 544 (2), 521–536.

De Luca, C. J. (1985). Control Properties of Motor Units. J. exp. Biol. 115, 125-136.

Duysens, J., and Van de Crommert, H. W.A.A. (1998). Neural control of locomotion; Part 1: The central pattern generator from cats to humans. Gait and Posture 7, 131–141.

Gardiner, P. F. (2011). Advanced Neuromuscular Exercise Physiology. Human Kinetics, Sheridan Books

Grillner, S. (1981). Control of locomotion in bipeds, tetrapods, and fish. In: Handbook of Physiology. The Nervous System. Motor Control. Bethesda, MD: Am.

Konrad, P. (2005). The ABC of EMG – A Practical Introduction to Kinesiological Electromyography. Noraxon Inc. USA.

Krawitz, S., Fedirchuk, B., Dai, .Y, Jordan, L. M. and McCrea, D. A. (2001). State-dependent hyperpolarization of voltage threshold enhances motoneurone excitability during fictive locomotion in the cat. J Physiol 532, 271–281.

Latash, M. (2007). Neurophysiological Basis of Movement. Human Kinetics; Second Edition.

Magill, R. (2007). Motor Learning and Control: Concepts and Applications. New York: McGraw-Hill.

Marsden, C. D., Merton, P. A. & Morton, H. B. (1983). Direct electrical stimulation of corticospinal pathways through the intact scalp in human subjects. In Motor Control Mechanismsin Health and Disease, ed. DESMEDT, J. E., pp. 387-391. Raven Press, New York.

Masakado, Y. (1994). Motor Unit Firing Behaviour in Man. Keio J Med, 43 (3), 137-142.

Patton, H.D. and Amassian, V.E. (1954) Single and multiple unit analysis of the cortical stage of pyramidal tract activation. J. Neurophysiol. 17: 345–363.

Petersen, N. T., Christensen L. O. D., and Nielsen, J. B. (1998). The effect of transcranial magnetic stimulation on the soleus H reflex during human walking. J Physiol. 513, 599-610.

Petersen, N. T., Butler, J. E., Marchand-Pauvert, V., Fisher, R., Ledebt, A., Pyndt, H. S., Hansen, N. L., and Nielsen, J. B. (2001). Suppression of EMG activity by transcranial magnetic stimulation in human subjects during walking. J Physiol 537: 651–656.

Petersen, N. T., Taylor, J. L., and Gandevia, S. C. (2002). The effect of electrical stimulation of the corticospinal tract on motor units of human biceps brachii. J Physiol (Lond) 544:277 – 284.

Petrofsky, J. S. (1978). Control of the recruitment and firing frequencies of motor units in electrically stimulated muscles in the cat. Medical and Biological Engineering and Computing, 16 (3), 302-308.

Phillips, C.G. (1987) Epicortical electrical mapping of motor areas in primates. In Motor Areas of the Cerebral Cortex. Ciba Foundation Symp. 132., Wiley, Chichester, pp. 5–15.

Porter, R. and Lemon, R. N. (1993). Corticospinal Function and Voluntary Movement. Oxford, UK: Clarendon.

Power, K. E., McCrea, D. A .and Fedirchuk, B. (2010). Intraspinally mediated state-dependent enhancement of motoneurone excitability during fictive scratch in the adult decerebrate cat. J Physiol 588(15), 2839–2857.

Purves, D., Augustine, G. J., and Fitzpatrick, D. (2004). Neuroscience. Sinauer Associates; Third Edition.

Rothwell, J. C., Thompson, P. D., Day, B. L., Boyd, S. and Marsden, C. D. (1991). Stimulation of the human motor cortex through the scalp. Experimental Physiology 76, 159-200.

Rothwell, J. C. (1997). Techniques and mechanisms of action of transcranial stimulation of the human motor cortex. Journal of Neuroscience Methods 74, 113–122.

Schmidt, B. J. (1994). Afterhyperpolarization modulation in lumbar motoneurons during locomotor-like rhythmic activity in the neonatal rat spinal cord in vitro. Exp Brain Res, 99, 214-222.

Sherrington, C. S. (1910). Flexion-reflex of the limb, crossed extension reflex and the stepping reflex and standing. J Physiol. 40:28 – 121

Sidhu, S. K., Hoffman, B. W., Cresswell, A. G., and Carroll, T. J. (2011). Corticospinal contributions to lower limb muscle activity during cycling in humans. J Neurophysiol 107: 306–314.

Snell, R. S. (2009). Clinical Neuroanatomy. Lippincott Williams & Wilkins; Seventh Edition.

Taylor, J. L., Petersen, N. T., Butler, J. E., and Gandevia, S. C. (2002) Interaction of transcranial magnetic stimulation and electrical transmastoid stimulation in human subjects. J Physiol (Lond) 541:949 – 958.

Taylor, J. L., and Gandevia, S. C. (2004). Noninvasive stimulation of the human corticospinal tract. J Appl Physiol 96, 1496-1503

Taylor, J.L. (2006). Stimulation at the cervicomedullary junction in human subjects. Journal of Electromyography and Kinesiology 16, 215 – 223.

Thompson, P. D., Day, B. L, Crockard, H. A, Calder, I., Murray, N. M. F., Rothwell, J. C., Marsden, C. D. (1991). Intra-operative recording of motor tract potentials at the cervico-medullary junction following scalp electrical and magnetic stimulation of the motor cortex. Journal of Neurology, Neurosurgery, and Psychiatry;54:618-623

Ugawa, Y., Rothwell, J. C., Day, B. L., Thompson, P. D, and Marsden, C. D. (1991). Percutaneous electrical stimulation of corticospinal pathways at the level of the pyramidal decussation in humans. Ann Neurol. 4, 418-27.

Zehr, P. E., Carroll, T. J., Chua, R., Collins, D. F., Frigon, A., Haridas, C., Hundza, S. R., and Thompson, A. K. (2004). Possible contributions of CPG activity to the control of rhythmic human arm movement. Can. J. Physiol. Pharmacol. 82: 556–568.

Section 2: Manuscript

Modulation of Corticospinal Excitability During Arm Cycling in Humans

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Abstract

The purpose of the current study was to examine corticospinal contributions to upper-limb muscle activity during arm cycling in humans. Motor evoked potentials (MEPs) in response to transcranial magnetic stimulation and cervicomedullary evoked potential (CMEPs) in response to transmastoid electrical stimulation were used to examine task- and phase-dependent modulation of corticospinal excitability between arm cycling and tonic contraction. Responses from the biceps brachii muscle were compared between arm cycling and tonic contraction at three different positions (i.e. 3, 6 and 12 o'clock, relative to a clock face), while participants generated equal amounts of muscle activity. Average MEP and CMEP responses for both tasks were made relative to M-wave. When compared to an intensity matched tonic contraction, both MEPs and CMEPs were significantly larger during arm cycling at the 3 o'clock position (MEPs: $P = 0.033$; CMEPs: $P = 0.007$) which corresponds to the end of the extension and beginning of the flexion phase of arm cycling. MEPs and CMEPs were also similarly modulated at all positions while arm cycling. The data indicate that transmission through the corticospinal pathway is enhanced during the initiation of the flexion phase of arm cycling due in part to enhanced spinal excitability. Because MEPs and CMEPs were modulated similarly throughout arm cycling, it appears that spinal mechanisms are the dominant factors driving the phase-dependent modulation of corticospinal excitability.

Introduction

Animal studies have demonstrated that the basic pattern for locomotor activities is generated via neural networks found in the spinal cord, referred to as central pattern generators (CPGs) (Grillner, 1981). In humans, indirect evidence suggests that CPGs play a role in locomotor-like activities (locomotion or cycling) (Zehr et. al. 2004). Although decerebrate preparations in cats indicate that subcortical circuits are sufficient to enable locomotion in quadrupeds, a more extensive cortical input is involved in the production of locomotion (Porter and Lemon, 1993, as discussed in Petersen et. al. 2003) and/or cycling in humans (Zehr et. al 2004).

Evidence from transcranial magnetic stimulation (TMS) studies show some direct role for the motor cortex in the generation of rhythmic motor output like cycling (Sidhu et. al. 2011). For example, Sidhu et. al. (2011) demonstrated that subthreshold TMS during cycling evoked suppression of background EMG in the lower limb muscles. This means that subthreshold TMS activates an intracortical inhibitory circuit which projects to and inhibits the motor cortical neurones, resulting in the suppression of the EMG signal. If the surpraspinal centers were not involved in the production of muscle activity during cycling, then subthreshold TMS would have no effect on that background EMG. In addition to examining the role of the supraspinal centers in the production of lower limb cycling, Sidhu et. al. (2011) also examined spinal excitability. They used transmastoid stimulation to examine phase and muscle dependent changes of corticospinal excitability in the major thigh muscles (ie. rectus femoris, vastus lateralis, and biceps femoris). Responses from the motor cortex (MEPs) and cervicomedullary junction (CMEPs), both absolute and normalized to background EMG, were modulated similarly across all phases of cycling, with MEPs mainly driven by changes at the spinal level. In contrast, there was an increase in cortical excitability prior to muscle activation in vastus lateralis, but not in rectus femoris and biceps femoris, which shows intermuscle differences in phase-dependent changes in corticospinal excitability during locomotion (Sidhu et. al. 2011). Thus, supraspinal centers are directly involved in the generation of

cycling and that spinal factors dominate phase-dependant modulation of corticospinal excitability.

Given that the cortex has more monosynaptic connections with the motoneurones controlling upper limb musculature as compared to the lower limb and that corticospinal excitability of lower limb muscles appears to be muscle dependent, it may be that the corticospinal control of upper limb musculature is very different than those in the legs. Zehr and colleagues (2004), have studied extensively the role of the motor cortex and reflex pathways in the generation of rhythmical motor output in the FCR during cycling. They suggest that CPG networks contribute to the control of rhythmic arm movement, either by directly acting on the motoneuronal pools or indirectly via interneuronal reflex networks (Zehr et. al. 2004). For example, corticospinal excitability is decreased during arm cycling in the FCR muscle when compared to a tonic contraction, suggesting that reflex and CPG networks contribute to the control of rhythmic arm movement (Carroll et. al. 2006). There was a decrease in corticospinal excitability (i.e. decrease MEP amplitude) during the flexion phase of rhythmic arm movement, and a facilitation of spinal reflexes, shown via subthreshold TMS, during tonic contraction but not during arm cycling (Carroll et. al. 2006), which shows task-dependent changes of corticospinal excitability.

Unlike the study by Sidhu et al. (2011), a direct measure of motoneurone excitability was not made using transmastoid stimulation. Instead, Carroll et. al. (2006) indirectly measured motoneurone excitability by examining the H-reflex pathway. Changes in the H-reflex pathway (i.e. H-reflex amplitude) however can be due to changes in either motoneuronal and/or pre-motoneuronal excitability. This is an important distinction given that recent work in the adult decerebrate cat indicates the spinal motoneurone excitability is altered during rhythmic motor output, void of any descending influence (Power et al. 2010).

The purpose of the current study was to examine corticospinal contributions to bicep brachii activity during arm cycling in humans, using transmastoid electrical stimulation and transcranial magnetic stimulation.

Methods

Participants

Twelve healthy men; aged 20 – 23 years without any known neurological deficits, participated in the experiment, which consisted of two conditions – arm cycling and tonic contraction. All participants signed a consent form and completed TMS safety checklist prior to commencing the experiment. Participants with any known contraindications to magnetic stimulation were excluded from the study. The data collected was confidential and stored via a coded system, making the data anonymous.

Experimental Set-up

Both conditions, cycling and tonic contraction were performed on the same arm cycle ergometer (Monark Rehab Trainer 881 E) as shown in fig. 1. The participants were instructed to sit upright with their shoulders at the same level as the axis of rotation of the crank and slightly away from the ergometer with their hands gripping on the handles. A brace was worn to restrict movement at the right wrist joint. The position of the right arm was specified relative to a clock face (12, 3 and 6 o'clock). For example, in fig. 1, the right arm is at 12 o'clock. Stimulation of the motor cortex and corticospinal tract were elicited at each of the three positions during cycling and at an intensity matched to that of a tonic contraction.

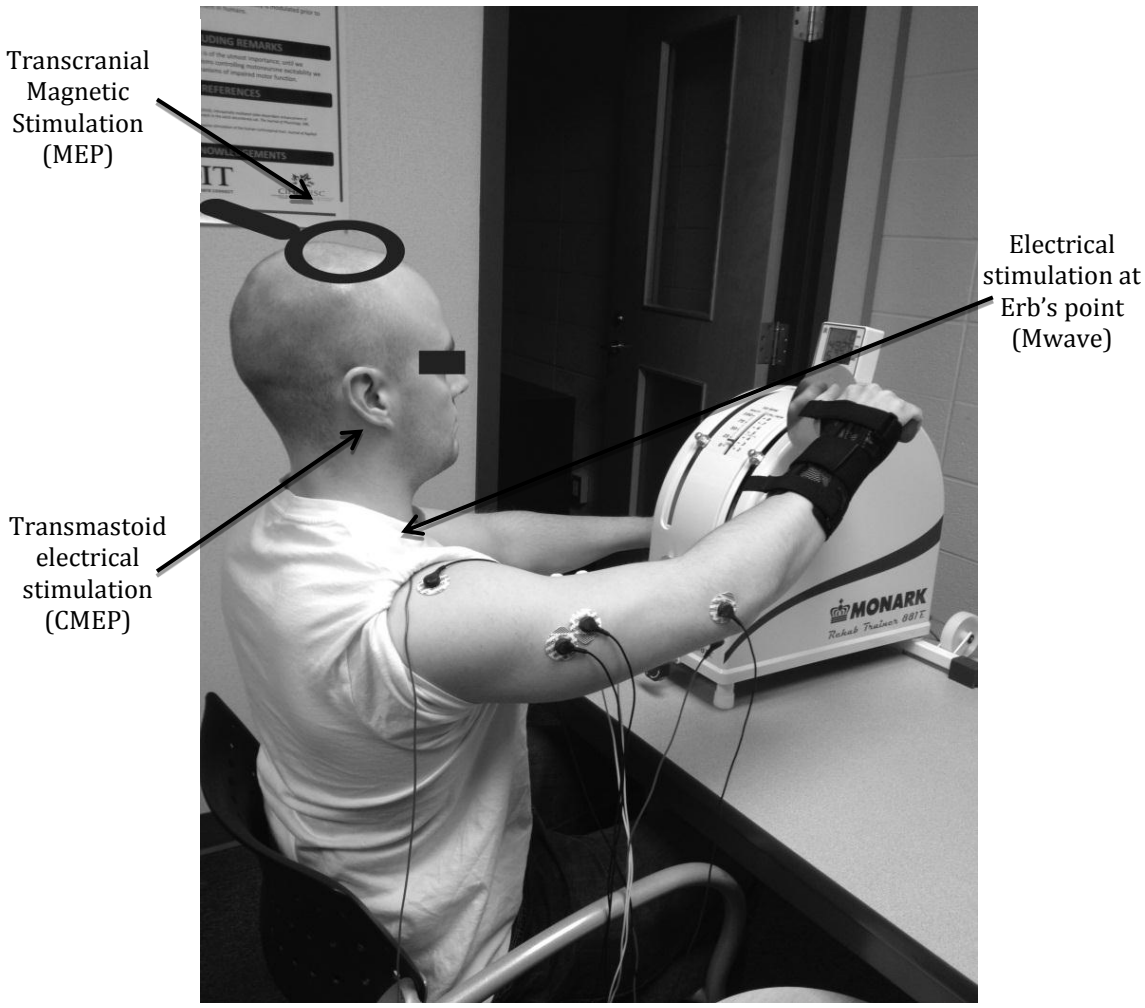

Fig. 1: Schematic illustration of the experimental setup

General Procedures

Max Muscle Response

Mmax was determined at rest by increasing the stimulation intensity gradually until the size of M-wave failed to increase with further increases in intensity. The level of intensity was then increased by 20% and 5 M-max were elicited at this supramaximal intensity. Peak-to-peak amplitude was measured from the average of 5 frames. MEPs and CMEPs were made equal to 5 to 10% of M-max to standardize the data for different individuals and to target the same pool of motoneurones.

Max EMG Cycle

Participants wore a wrist support on the right arm and cycled at 60RPM for 60 seconds (3 frames – 20 seconds each frame) with the erogometer set at 75W. Peak-

to-peak amplitude of the 6 bursts (between 6 and 12 seconds) from the second frame was measured. A visible horizontal line was set equal to 20% of the EMG cycle amplitude to normalize MEP and CMEP sizes to background EMG, as variations in EMG activity across the rhythmic activity could account for any phase-dependant changes. The participants then performed an isometric contraction against a force transducer while having their transmastoid process and motor cortex stimulated. Once MEPs and CMEPs were equal to either 5 or 10% of M-max, we used that stimulus intensity for the cycling and tonic experiments.

Arm Cycling

Participants cycled at 60RPM with the ergometer set at 25W. TMS and transmastoid electrical stimulation were delivered 10 times and nerve stimulation was delivered 5 times, pseudo-randomly at each 3 position (i.e. 3, 6 and 12 o'clock). 10 frames with MEPs and CMEPs, and 5 frames with M-wave, were tagged and averaged to measure peak-to-peak amplitude and the mean background EMG (50 ms prior to stimulation) for the tonic experiment.

Tonic Contraction

A visible horizontal line, equal to the mean EMG of cycling for each position (i.e. 3, 6 and 12 o'clock), was set on the screen. Participants contracted at this line, with their arms at each position to match the intensity produced during cycling for the biceps muscle. Same stimulus paradigms and intensities from the cycling experiment were used. All stimulated frames for MEP, CMEP and M-wave were tagged and averaged to measure the peak-to-peak amplitude.

Electromyography (EMG)

EMG signals were recorded from the right biceps brachii using pairs of Ag-AgCl surface electrodes (MeditraceTM 130 ECG conductive adhesive electrodes) placed 2 cm apart (centre to centre). Ground electrodes were placed on medial and lateral epicondyles. Thorough skin preparation for all recording electrodes included removal of dead epithelial cells with abrasive (sand) paper around the designated areas followed by cleansing with an isopropyl alcohol swab. An inter-electrode impedance of < 5 kOhms was obtained prior to recording to ensure an adequate

signal-to-noise ratio. Data was collected on-line at 2 KHz for off-line analysis using the CED 1401 interface and the Signal 4 (Cambridge Electronic Design Ltd., Cambridge, UK) software program. Signals were amplified (CED 1902) and filtered using a 3-pole Butterworth with cutoff frequencies of 10-1000 Hz.

Transcranial Magnetic Stimulation (TMS)

Stimulation of the left motor cortex was applied at vertex using a Magstim 200 stimulator (Magstim, Dyfed, UK), equipped with a round coil. To locate vertex, the distances from nasion to inion, and from tragus to tragus were measured and marks were placed halfway directly over the scalp for both measurements. The intersection for both marks was defined as vertex. The coil was held parallel to the floor for the remainder of the study. Stimulation intensity varied subject to subject and ranged from 20 to 45% MSO. The same stimulation intensity was used for both conditions in each subject.

Transmastoid electrical stimulation

The cathode surface electrode was placed on the left side, just below the mastoid process in the "groove", while the anode was placed on the right side. The stimulator (model DS7AH, Digitimer Ltd, Welwyn Garden City, UK) pulse duration was set to 100 μs and stimulation intensity ranged from 130 to 220 mA.

Nerve Stimulation

The cathode surface electrode was placed on Erb's point, while the anode was placed on the acromion process, with the stimulator (model DS7AH, Digitimer Ltd, Welwyn Garden City, UK) pulse duration set at 200 μs. Stimulation intensity ranged from 120 to 300 mA.

Measurements

Average peak-to-peak amplitude was calculated for MEPs, CMEPs and Mwave to study changes in supraspinal, spinal and peripheral excitability for both tasks at the three phases (12, 3 and 6 o'clock). The average rectified EMG, 50 ms prior to the

stimulus, was calculated to measure the background EMG to determine the muscle contraction for arm cycling and tonic contraction at all angles. Data was analyzed using Signal 4 software (CED, UK).

Statistical analysis

A two-way repeated-measures ANOVA using IBM SPSS Statistics Version 19 was used to determine whether statistical significant differences occurred in MEP, CMEP, M-wave, and background EMG amplitudes between cycling and tonic contraction conditions at each arm position (i.e., 3, 6, and 12 o'clock). Separate paired t-tests were utilized to determine changes in excitability for each arm position between arm cycling and intensity matched tonic contraction. Statistical significance was set at a p-value at $p < 0.05$.

Results

Background EMG

Background EMG for bicep brachii at 3 arm positions (i.e. 3, 6 and 12 o'clock) was measured 50 ms prior to stimulus artifact (i.e. before the stimulus was delivered) for arm cycling and tonic contraction to examine the intensity of the muscle contraction when MEPs and CMEPs were elicited. Differences in background EMG would indicate that the amount of effort to produce a given contraction was more or less than the other condition. It was important to ensure that the intensity of the muscle contractions during both conditions were similar because MEPs and CMEPs are drastically altered based on background activity of the neuromuscular system (i.e. small increases in background EMG would substantially increase the amplitude of evoked potentials).

Average MEP and CMEP responses of 12 participants for both rhythmic and tonic contraction conditions were made relative to M-wave to control for individual differences (absolute MEP peak-to-peak amplitude over absolute M-wave peak-to-peak amplitude).

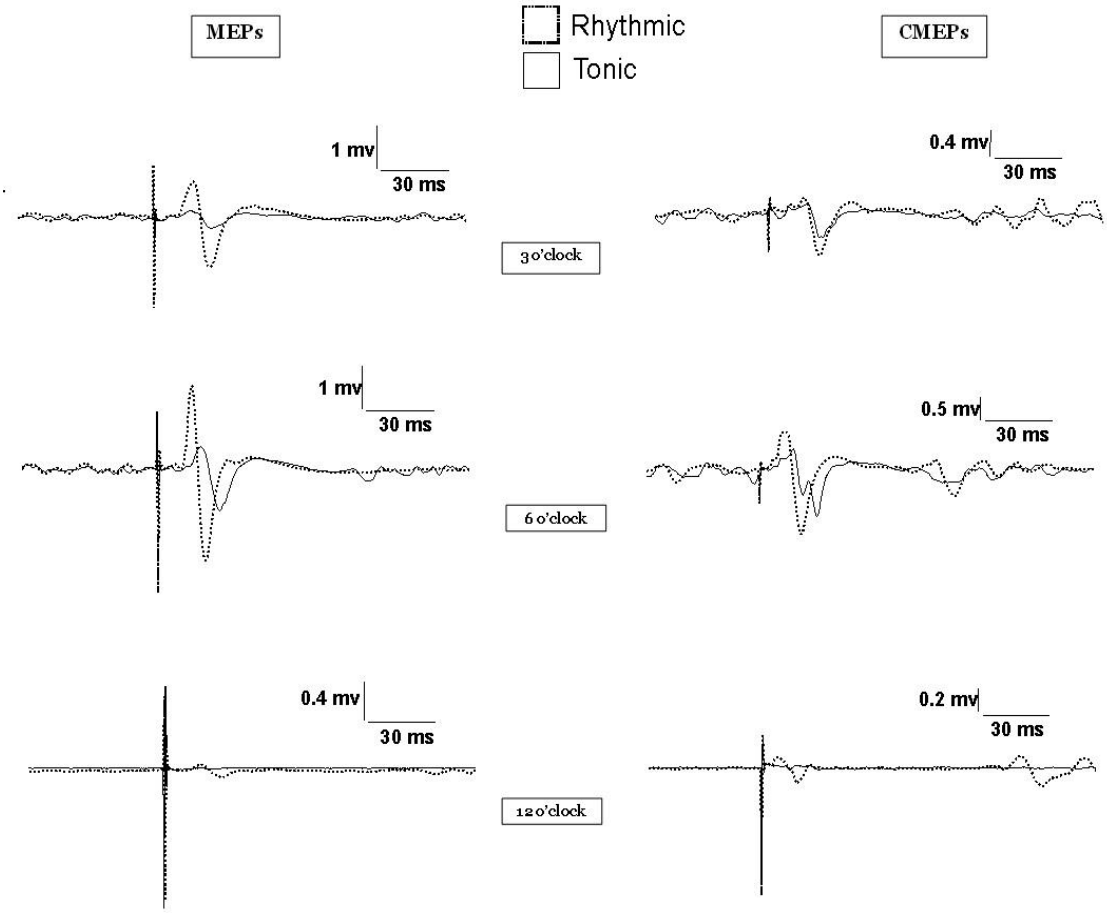

Fig. 2: Individual MEP and CMEP responses during cycling and tonic. The left column presents MEP responses, and the right column presents CMEP responses. The dashed trace corresponds to rhythmic movement, and the solid trace corresponds to intensity matched tonic contraction. Fig. 6 displays larger MEP and CMEP responses for arm cycling in comparison to tonic contraction at 3 and 6 o'clock. However, the intensity of the contraction is larger during cycling at the 6 o'clock at the cortical level, as shown in fig. 3. In contrast, when comparing CMEPs during arm cycling and tonic contraction, the values were statistically insignificant at 6 o'clock (*p = 0.196),* as shown in fig. 6. MEPs and CMEPs were statistically significant at the 3 o'clock position (*P = 0.007*)* which ascribes to a larger response during cycling. Lastly, the size of MEP and CMEP responses at the 12 o'clock position was similar and statistically insignificant at both cortical and spinal levels *(p = 0.688; p = 0.223).*

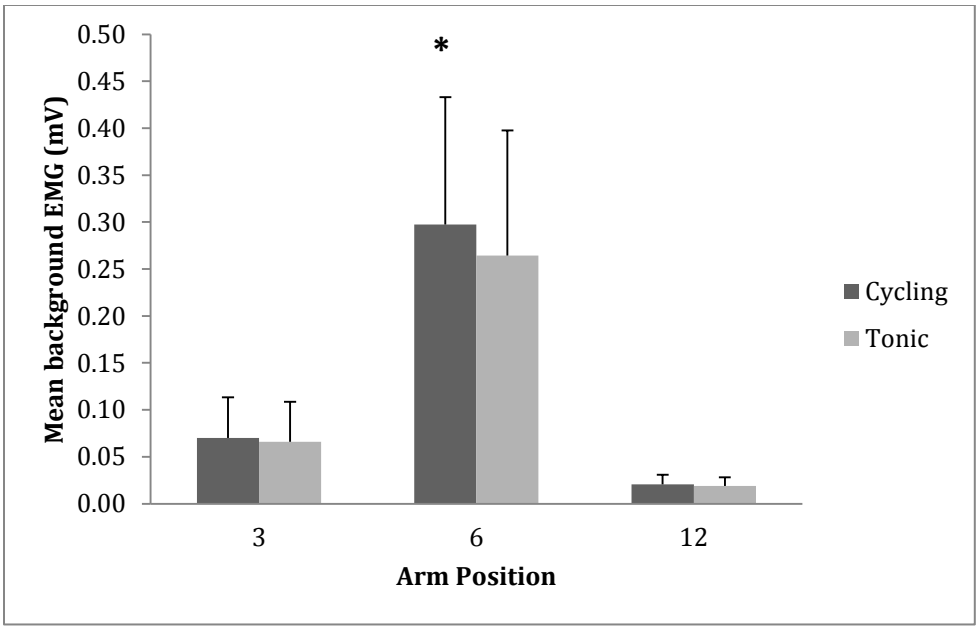

Fig. 3: Group supraspinal background EMG. For MEPs, the intensity of the muscle contraction were the same during cycling and tonic at the 3 and 12 o'clock position *(p=0.449; p=0.102, respectively)*, but was larger for cycling at the 6 o'clock position *(p=0.046*).* Thus, we were unable to compare supraspinal excitability for cycling and tonic contraction at the 6 o'clock position, as shown in fig. 3. Asterisks denote statistically significant differences between arm cycling and tonic contraction conditions.

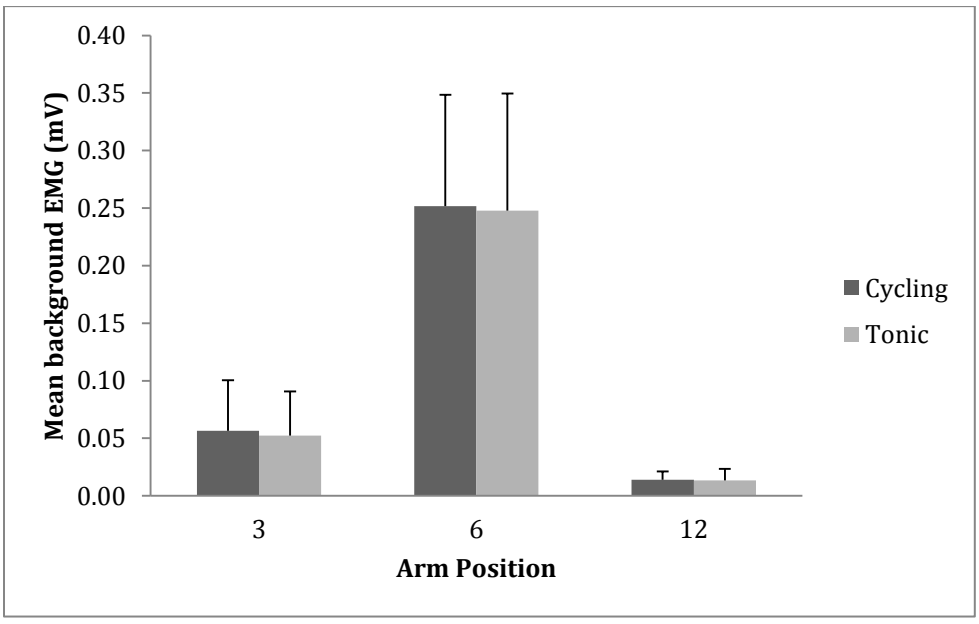

Fig. 4: Group spinal background EMG. For CMEPs, the intensity of the muscle contraction were the same during cycling and tonic at 3, 6 and 12 o'clock *(p= 0.132; p= 0.775; p= 0.603, respectively),* and were therefore able to compare spinal excitability for the two tasks at each arm position, as shown in fig. 4.

Task-dependent Changes in Corticospinal Excitability

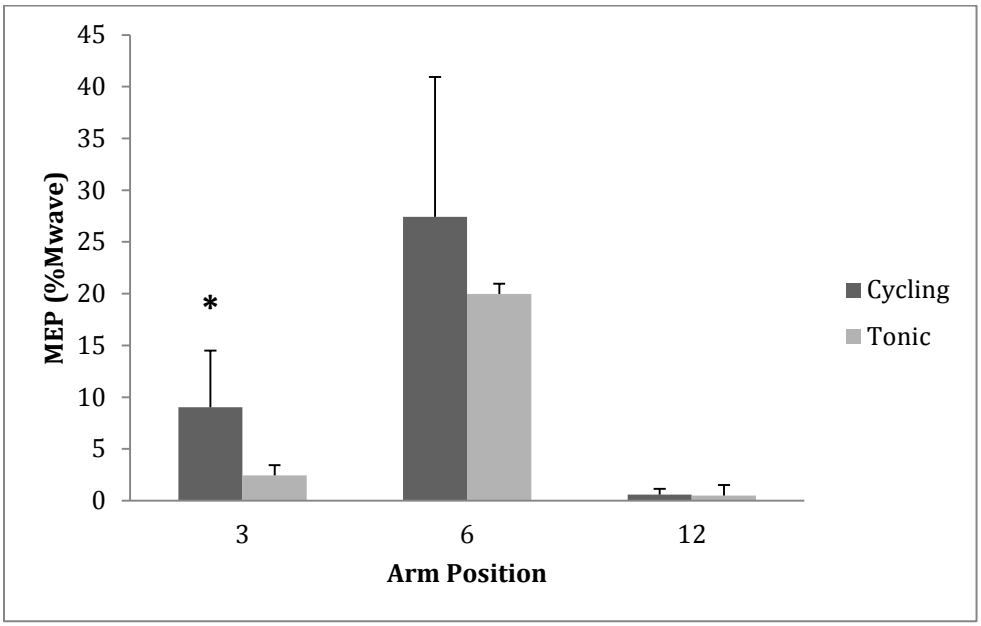

Fig. 5: Group task-dependant supraspinal changes. MEPs were larger at 3 o'clock during arm cycling when compared to tonic contraction *(p = 0.001*).* At 6 o'clock, MEPs were larger during cycling and were also statistically significance *(p = 0.002*)* but the background EMG was larger for cycling at this phase, which may explain the large response for the cycling condition. The size of MEPs was similar between arm cycling and tonic contraction at the 12 o'clock position *(p = 0.688).* Asterisks denote statistically significant differences between arm cycling and tonic contraction conditions.

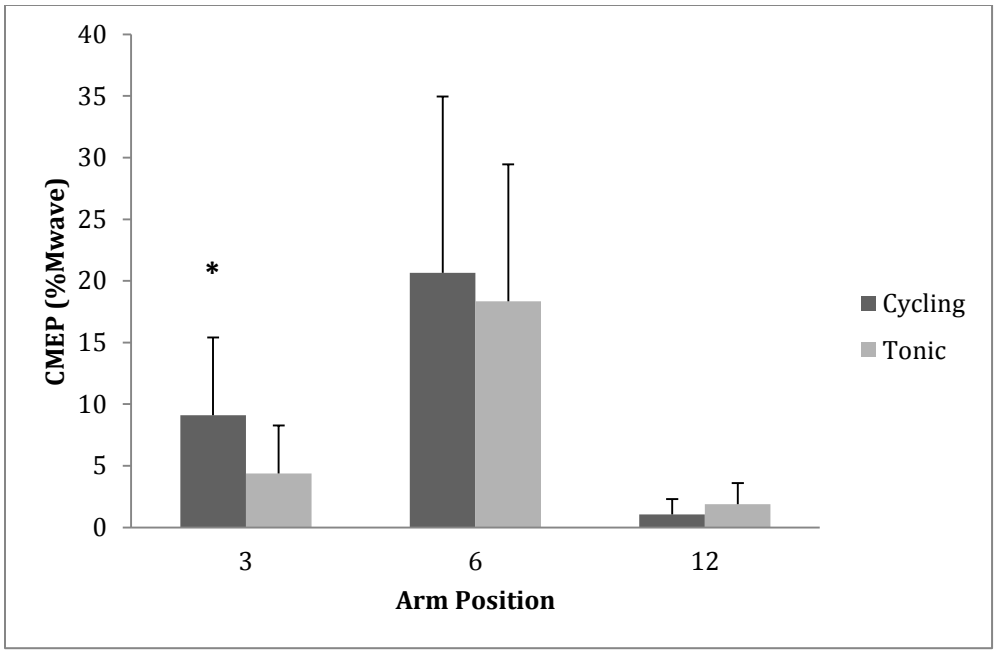

Fig. 6: Group task-dependant spinal changes. CMEPs were larger during arm cycling when compared to tonic at 3 o'clock and were statistically significant *(p = 0.007*)* at this phase. The size of CMEPs was similar between arm cycling and tonic contraction at the 6 and 12 o'clock position (*p = 0.196; p = 0.223, respectively).* Asterisks denote statistically significant differences between arm cycling and tonic contraction conditions.

Phase-dependent

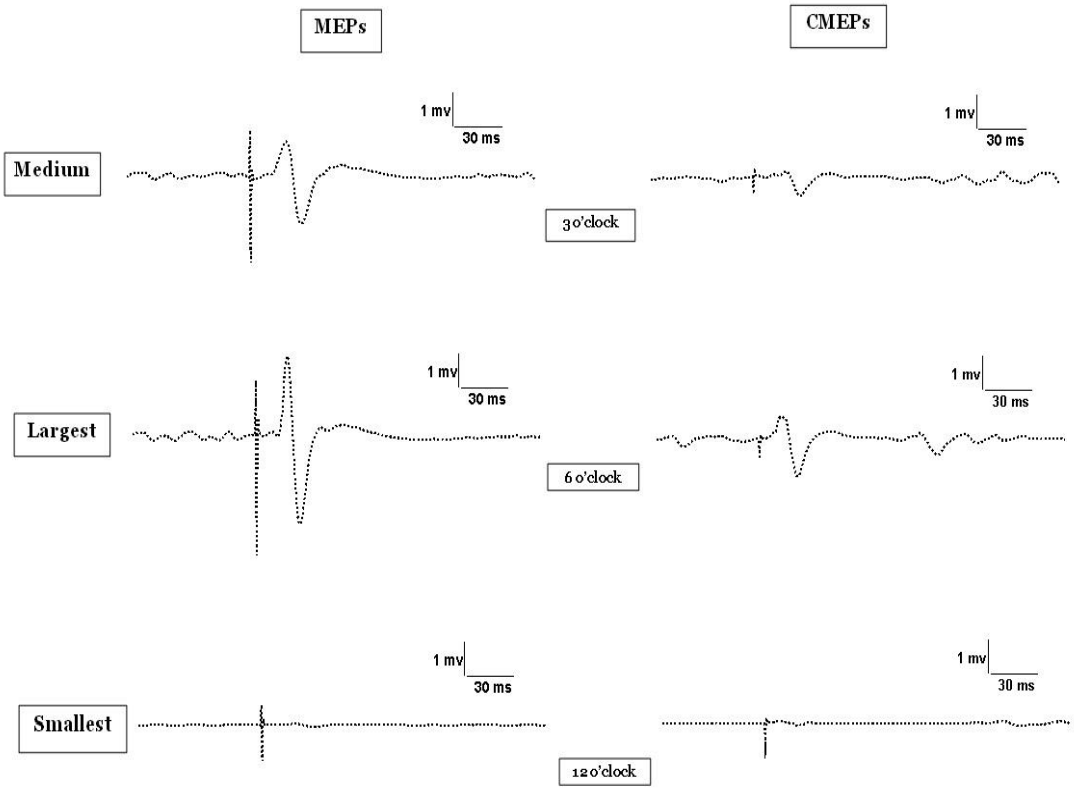

Fig. 7: Individual MEP and CMEP responses during cycling. The left column presents MEP responses, and the right column presents CMEP responses. Both figures demonstrate similar modulation of supraspinal and spinal excitability across all phases (i.e. 3, 6 and 12 o' clock) during cycling. When looking at the 3 phases, at 3 o'clock, the size of MEPs and CMEPs are at a medium size, largest at the 6 o'clock position and smallest at the 12 o'clock position.

Discussion

This study used transmastoid electrical stimulation and transcranial magnetic stimulation, to examine corticospinal contributions to the bicep brachii muscle during arm cycling in humans. Responses elicited by transcranial magnetic stimulation (TMS) could be due to for changes in cortical or spinal excitability as it is a measure of the corticospinal tract as a whole. An increase in MEP amplitude during cycling could therefore be due to changes at the supraspinal or spinal level. Because CMEP amplitude increased along with MEP amplitude, the results suggest that an enhanced spinal excitability contributed to the increase in MEP amplitude. Our findings suggest that spinal mechanisms are the dominant factors driving task- and phase-dependant modulation of corticospinal excitability during arm cycling.

Supraspinal Excitability is Enhanced During Arm Cycling

When compared to an intensity matched tonic contraction, motor evoked potentials were significantly larger during arm cycling at the 3 o'clock position (MEPs: $p = 0.033$) which corresponds to the end of the extension and beginning of the flexion phase of arm cycling. Previous work by Carroll et. al. (2006), found a *decrease* in MEPs (i.e. supraspinal excitability) at 6 o'clock during arm cycling in the FCR muscle when compared to a tonic contraction. Using a similar experimental paradigm, the present study demonstrated an *increase* in MEP amplitude of the biceps brachii at the 3 o'clock position during arm cycling as compared to an intensity-matched tonic contraction. One of the differences between the study by Carroll et. al (2006) and the present study was the muscle investigated. Carroll et. al. (2006) examined the FCR muscle while the current study examined the biceps brachii. Biceps brachii flex the arm, whereas the FCR flex the wrist. Thus, it may be that corticospinal excitability is muscle-dependant as in the lower limb. For example, Sidhu et. al. (2011) demonstrated an increase in cortical excitability prior to muscle activation in vastus lateralis, but not in rectus femoris or biceps femoris.

Spinal Motoneurone Excitability is Enhanced During Arm Cycling

Carroll et. al. (2006) demonstrated a *decrease* in H-reflex amplitude at the 6 o'clock position in the FCR during arm cycling when compared to a tonic contraction. In the present study, we demonstrated an *increase* in CMEP amplitude at the 3 o'clock position in the bicep brachii during arm cycling when compared to a tonic contraction.

H-reflexes measures the efficacy of synaptic transmission as the stimulus travels in afferent (Ia sensory) fibers through the motoneurone pool of the corresponding muscle to the efferent (motor) fibers (Brooke et. al., 1997). Thus, a reduction in H-reflex amplitude could be due to a decrease in afferent input to the motoneurone pool via presynaptic inhibition or a decrease in motoneurone excitability. In contrast, we used electrical transmastoid stimulation, which activates the descending corticospinal tract which has been shown to have a large monosynaptic connection to the bicep motoneurone pool. Corticospinal axons are also free from presynaptic inhibition. Thus, transmatoid stimulation has been suggested to be a method suitable for directly assessing motoneurone excitability (Taylor and Gandevia, 2004). Thus, barring in the intermuscle differences in spinal excitability (i.e. FCR vs. biceps brachii), the decrease in H-reflex amplitude demonstrated by Carroll et. al. (2006) may have been mainly due to reduced afferent input to the motoneurone pool. The present work suggests that the spinal motoneurone excitability is increased during cycling. Transmission in the afferent pathway was not examined. Enhanced motoneurone excitability during cycling is similar to the results demonstrated fictive scratch in the adult decerebrate cat (Power et al. 2010).They demonstrated a hyperpolarization of the voltage-threshold for action potential initiation and a decrease in afterhyperpolarization amplitude (i.e. enhanced of motoneurone excitability) (Power et. al. 2011). These changes in motoneurone properties were the opposite that occurred in the same motoneurone during stance, a tonic contraction. If the same changes that occur in spinal motoneurones during scratch in cat occur during arm cycling in humans, it could account for the increased CMEP amplitude during cycling. For example, a lowering

of the voltage-threshold in the motoneurone pool would allow more motoneurones to be activated by the transmastoid stimulation, thus increasing CMEP amplitude.

Although the findings from the current study were different from the study done by Carroll et. al (2006), both studies determined that the change in MEPs during arm cycling was driven mainly by changes at the spinal level. Consequently, the data from the current study indicate that spinal mechanisms are the dominant factors driving task-dependent modulation of corticospinal excitability during arm cycling which is consistent with the suggestion that spinal circuits contribute to the control of rhythmic arm cycling.

Phase-Dependent Modulation of Corticospinal Excitability

When comparing the 3 phases during arm cycling, at 3 o'clock, the size of MEPs and CMEPs are at a medium size, largest at the 6 o'clock position and smallest at the 12 o'clock position (fig. 7). This indicates that both supraspinal and spinal excitability were modulated similarly across phases during cycling. This is in agreement with the findings of Sidhu et. al. (2011). They suggested that the modulation of corticospinal excitability during lower limb cycling in humans was generated in large part to the changes in the excitability in the spinal factors. Sidhu et. al. (2011) found that the MEP and CMEP responses (absolute and normalized to background EMG) were modulated similarly in the leg muscles. Increased MEP amplitude could be at the supraspinal or spinal level. Because CMEP amplitude is increased along with MEP amplitude, this suggests that an enhanced spinal excitability contributed to an increase in MEP amplitude.

Conclusion

The present study used transmastoid electrical stimulation, a direct method to evaluate spinal motoneurone excitability, and transcranial magnetic stimulation to examine corticospinal contributions to bicep brachii activity during arm cycling. Our data indicate that corticospinal excitability is enhanced, in biceps brachii, during the initiation of the flexion phase of arm cycling when compared to an intensity

matched tonic contraction. The results also demonstrate similar modulation of MEPs and CMEPs throughout arm cycling across all phases (i.e. 3, 6 and 12 o'clock). The results from this study suggest that spinal mechanisms are the dominant factors driving task- and phase-dependent modulation of corticospinal excitability during arm cycling which is consistent with the proposition that spinal circuits contribute to the control of rhythmic arm cycling.

References

Basmajian, J. V., and Deluca,C. J. Muscles Alive: Their functions revealed by electromyography 5th ed. Will-iams and Wilkins, Baltimore, 1986.

Brooke, J. D., Cheng, J., Collins, D. F., McIlroy, W. E., Misiaszek, J. E., AND Staines, W. R. (1997) Sensori-sensory afferent conditioning with leg movement: gain control in spinal reflex and ascending paths. Prog Neurobiol 51: 393– 421.

Capaday, C., Lavoie, B. A., Barbeau, H., Schneider, C., and Bonnard, M. (1999). Studies on the corticospinal control of human walking. I. Responses to focal transcranial magnetic stimulation of the motor cortex. J Neurophysiol 81: 129–139.

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

Carroll, T. J., Baldwin, E. R. L., Collins, D. F. and Zehr, E. P. (2006). Corticospinal Excitability Is Lower During Rhythmic Arm Movement Than During Tonic Contraction. *J Neurophysiol,* 95, 914-921.

Christensen, L. O. D., Andersen, J. B, Sinkjòr, T., and Nielsen, J. B. (2001). Transcranial magnetic stimulation and stretch reflexes in the tibialis anterior muscle during human walking. Journal of Physiology 531.2, pp. 545—557.

Grillner, S. (1981). Control of locomotion in bipeds, tetrapods, and fish. In: Handbook of Physiology. The Nervous System. Motor Control. Bethesda, MD: Am.

Krawitz, S., Fedirchuk, B., Dai, .Y, Jordan, L. M. and McCrea, D. A. (2001). State-dependent hyperpolarization of voltage threshold enhances motoneurone excitability during fictive locomotion in the cat. J Physiol 532, 271–281.

Petersen, N. T., Christensen, L. O. D., and Nielsen, J. B. (1998). The effect of transcranial magnetic stimulation on the soleus H reflex during human walking. J Physiol. 513, 599-610.

Porter, R. and Lemon, R. N. (1993). *Corticospinal Function and Voluntary Movement.* Oxford, UK: Clarendon.

Power, K. E., McCrea, D. A .and Fedirchuk, B. (2010). Intraspinally mediated state-dependent enhancement of motoneurone excitability during fictive scratch in the adult decerebrate cat. *J Physiol* 588(15), 2839–2857.

Rothwell, J. C., Thompson, P. D., Day, B. L., Boyd, S. and Marsden, C. D. (1991). Stimulation of the human motor cortex through the scalp. Experimental Physiology 76, 159-200.

Sidhu, S. K., Hoffman, B. W., Cresswell, A. G., and Carroll, T. J. (2011). Corticospinal contributions to lower limb muscle activity during cycling in humans. *J Neurophysiol* 107: 306–314.

Taylor, J. L., and Gandevia, S. C. (2004). Noninvasive stimulation of the human corticospinal tract. *J Appl Physiol* 96, 1496-1503.

Zehr, P. E., Carroll, T. J., Chua, R., Collins, D. F., Frigon, A., Haridas, C., Hundza, S. R., and Thompson, A. K. (2004). Possible contributions of CPG activity to the control of rhythmic human arm movement. Can. J. Physiol. Pharmacol. 82: 556–568.

Section 3: Appendices

Appendix 1: Magnetic Stimulation safety checklist

*Please answer the following questions by checking off either **YES or NO***

Questions	Yes	No
1) Do you suffer from epilepsy, or have you ever had an epileptic seizure?		
2) Does anyone in your family suffer from epilepsy		
3) Do you have any metal implant(s) in any part of your body or head? (Excluding tooth fillings)		
4) Do you have an implanted medication pump?		
5) Do you wear a pacemaker?		
6) Do you suffer any form of heart disease?		
7) Do you suffer from reoccurring headaches?		
8) Have you ever had a skull fracture or serious head injury?		
9) Have you ever had any head surgery		
10)Are you pregnant?		
11)Do you take any medication? **Note if taking medication, check list for contraindicated medication on next page.*		
12)Do you suffer from any known neurological or medical conditions?		

Comments: _____

_____

Name: _____

Signature: _____

Date: _____

Medications contraindicated with magnetic stimulation

1) Tricyclic antidepressants

Name	Brand
Amitriptyline (& butriptyline)	Elavil, Endep, Tryptanol, Trepiline
Desipramine	Norpramin, Pertofrane
Dothiepin hydrochloride	Prothiaden, Thaden
Imipramine (& dibenzepin)	Tofranil
Iprindole	-
Nortriptyline	Pamelor
Opipramol	Opipramol-neuraxpharm, Insidon
Protriptyline	Vivactil
Trimipramine	Surmontil
Amoxapine	Asendin, Asendis, Defanyl, Demolox, Moxadil
Doxepin	Adapin, Sinequan
Clomipramine	Anafranil

2) Neuroleptic or Antipsychotic drugs

A. Typical antipsychotics

• Phenothiazines: • Thioxanthenes:
- o Chlorpromazine (Thorazine) o Chlorprothixene
- o Fluphenazine (Prolixin) o Flupenthixol (Depixol and Fluanxol)
- o Perphenazine (Trilafon) o Thiothixene (Navane)
- o Prochlorperazine (Compazine) o Zuclopenthixol (Clopixol and Acuphase)
- o Thioridazine (Mellaril) • Butyrophenones:
- o Trifluoperazine (Stelazine) o Haloperidol (Haldol)
- o Mesoridazine o Droperidol
- o Promazine o Pimozide (Orap)
- o Triflupromazine (Vesprin) o Melperone
- o Levomepromazine (Nozinan)

B. Atypical antipsychotics

- Clozapine (Clozaril)
- Olanzapine (Zyprexa)
- Risperidone (Risperdal)
- Quetiapine (Seroquel)
- Ziprasidone (Geodon)
- Amisulpride (Solian)
- Paliperidone (Invega)

C. Dopamine partial agonists

- Aripiprazole (Abilify)

D. <u>Others</u>

- Symbyax -A combination of olanzapine and fluoxetine used in the treatment of bipolar depression.
- Tetrabenazine (Nitoman in Canada and Xenazine in New Zealand and some parts of Europe
- Cannabidiol One of the main psychoactive components of cannabis

Appendix 2: Consent Form

Title of Research Study: *Modulation of Corticospinal Excitability During Rhythmic Motor Output in Humans*

This study (# REB 12-008) has been reviewed by the University of Ontario Research Ethics Board and has been approved as of September 17th, 2012.

You are invited to participate in a research study. It is up to you to decide whether to be in the study or not. Before you decide, you need to understand what the study is for, what risks you might take and what benefits you might receive. This consent form explains the study. Please read this form carefully, and feel free to ask any questions you might have*. If you have any questions about your rights as a participant in this study, please contact the Compliance Officer at 905 721 8668 ext 3693 or compliance.uoit.ca.*

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1) Introduction/Background:
The motor cortex is the region of the brain that is involved in the planning, control and execution of movement. The axons of the motor cortex descend to the spinal cord to relay information to its motor neurons which are directly connected to the muscles, allowing them to contract. Thus, Sherrington (1906) identifies spinal motoneurones as the "final common path", and states that they the ultimate units that enable movement.

2) Purpose:
The purpose of this study is to examine motoneurone excitability in the cortex and spinal cord during rhythmic upper-body movement in humans.

3) Description of the study procedures:
The participants will undergo the following procedures during each experiment:

a) Trial Session:
A short trial session to familiarize participants with the testing procedures (i.e. nerve stimulation, force production and magnetic stimulation) at different intensities.

b) Experiment session:
This will be randomized to examine motoneurone excitability during rhythmic upper-body movement through the following recordings:

i. Electrical stimulation of the nerve that activates the muscles to record the muscle response.
ii. Magnetic stimulation of the motor cortex to record the muscle response from the brain.
iii. Electrical stimulation at the cervicomedullary junction (back of the neck close to the bottom of the skull) that will activate the spinal cord and present activity of the muscle.

Muscular responses from the biceps and triceps will be assessed throughout different phases of the rhythmic movement – i.e. arm position at 12, 6, and 3 o'clock during upper body cycling.

4) Potential Benefits:
Participants will just have the benefit of learning more about the function of their nervous system. The scientific community will benefit by learning more about the role of the spinal cord in movement. This is has important potential applications for spinal cord injury rehabilitation.

5) Potential Risk or Discomforts:
Surface EMG techniques used have low risks such as skin irritation from an alcohol swab or electrode gel which can be managed by cleansing the area and applying anti-histamine cream.
These reactions are very uncommon and have never lasted more than a few hours, however if a reaction persists we advise you to seek medical attention.

Magnetic stimulation of the cortex and cervicomedullary junction may cause mild discomfort and twitching of the neck muscles, causing a jerky movement of the individual's head. If however you feel that the stimulation is painful, it will be stopped immediately. Some people may also experience nausea or a mild headache. Both these reactions are uncommon and not serious. If you experience any of these effects for longer than 24 hours after the experiment please contact the principal investigator.

Nerve stimulation will be used to test the excitability of the nerve-muscle connection. The stimulation will cause a twitching of the muscle and mild discomfort, but is not painful. The stimulation may cause delayed muscle soreness, similar to that following exercise, but is not serious in nature.

6) Storage of Data:
Data will be stored on a password protected hard drive accessible only to the study investigators.

7) Confidentiality:
All data collected will be confidential and stored via a coded system, making the data anonymous.

8) Right to Withdraw:
You are free to withdraw from the study at any time without prejudice. There will be no academic or personal consequences associated with the withdrawal. Participants will have no effect on their grades or performance in any course, or experience any conflict of interest.

9) Debriefing and Dissemination of Results:
The data from this research will be submitted to scientific conferences and peer reviewed journals. At the completion of the study, you will be sent a summary of the research findings and any place where the data has been published. All published data will be coded so that your data is not identifiable.

10) Questions:
Thank you very much for your time and for making this study possible. If you have any questions or wish to know more please contact:

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Signature Page
Please read the following before signing the consent form and remember to keep a copy for your own records.

Title of Research Study: *Modulation of Corticospinal Motoneurone Excitability During Rhythmic Motor Output in Humans*

Name of principal investigator: **Amita Raj**

To be filled out and signed by the participant. Please check as appropriate.

I have read the consent [and information sheet].	Yes { } No { }
I have had the opportunity to ask questions/to discuss this study.	Yes { } No { }
I have received satisfactory answers to all of my questions.	Yes { } No { }
I have received enough information about the study.	Yes { } No { }
I have spoken to Amita Raj or a qualified member of the study team.	Yes { } No { }
I understand that I am free to withdraw from the study	Yes { } No { }

- at any time
- without having to give a reason
- without prejudice

I understand that it is my choice to be in the study	Yes { } No { }
I agree to take part in this study.	Yes { } No { }

_____ _____
Signature of participant Date

_____ _____
Signature of witness Date

To be signed by the investigator:
I have explained this study to the best of my ability. I invited questions and gave answers. I believe that the participant fully understands what is involved in being in the study, any potential risks of the study and that he or she has freely chosen to be in the study.

_____ _____
Signature of investigator Date

Signing this form gives us your consent to be in this study. It tells us that you understand the information about the research study. When you sign this

form, you do not give up your legal rights. Researchers or agencies involved in this research study still have their legal and professional responsibilities

Appendix 3: Statement of Confidentiality for Student PI and Research Assistants

Title: Modulation of Corticospinal Excitability During Rhythmic Motor Output Humans

Name (please print):

_____

PLEASE READ

An important part of conducting research is having respect for privacy and confidentiality. *Respect for human dignity also implies the principles of respect for privacy and confidentiality. In many cultures, privacy and confidentiality are considered fundamental to human dignity. Thus, standards of privacy and confidentiality protect the access, control and dissemination of personal information. In doing so, such standards help to protect mental or psychological integrity. Further, they are consonant with values underlying privacy, confidentiality and anonymity.* [Tri-Council Policy Statement on Ethical Conduct for Research Involving Humans, 1998].

Out of respect for human dignity and people's right to privacy we ensure our research participants both anonymity and confidentiality. There will be no individual information used to prevent any form of recognition. During data collection, participant names will be replaced with numeric codes, and will be stored on a password encrypted computer and/or external hard drive for back up. The findings of this study may be presented at conferences and also in peer-reviewed publications. As the data is coded in a manner that ensures confidentiality, and prevents any identification of the individuals who participated in the study.

In signing below you are agreeing to respect the participant's right to privacy and that of other people possibly identified through the data collection and/or analysis process. As a Co-Student PI, or a Research Assistant, all information shall not be shared in a public environment or with friends or family members to respect the confidentiality and anonymity rights that the participants deserve. The study and its participants are to be discussed only during research meetings.

In signing below you are indicating that you understand the following:

- I understand the importance of providing anonymity and confidentiality to research participants;
- I understand that while I do not know the name of the participant, the raw data may contain references to the individual and/or other individuals. I understand that this information is to be kept confidential;
- I understand that the raw data are not to be discussed outside of research meetings;
- I understand that data files (electronic and hard copy) are to be secured at all times (i.e., not left unattended). Further, data files will be stored as outlined in the Letter of Information and Consent approved by the UOIT Research Ethics Board.

In signing my name below, I agree to the above statements and promise to ensure the participants in this study anonymity and confidentiality.

Signature:

_____Date:_____