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EFFECT OF STIMULUS WAVEFORM ON TRANSCRANIAL MAGNETIC STIMULATION METRICS IN PROXIMAL AND DISTAL ARM MUSCLES

Christopher Lynch

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A thesis submitted in partial fulfillment of the requirement for the degree of Master of Science in Biomedical Engineering at Virginia Commonwealth University.

by

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B.S. Biomedical Engineering, North Carolina State University, 2017

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List of Abbreviations

Physiological Abbreviations

Cl^-	Calcium
K^+	Potassium
Na^+	Sodium
CNS	Central nervous system
FDI	First dorsal interosseous
M1	Primary motor cortex

General Abbreviations

AMT	Active motor threshold
EMG	Electromyography
MEP	Motor evoked potential
MSO	Maximum stimulator output
nMEP	Normalized motor evoked potential
RMT	Resting motor threshold
rTMS	Repetitive transcranial magnetic stimulation
SCI	Spinal cord injury
TMS	Transcranial magnetic stimulation
MVIC	Maximum voluntary isometric contraction
PA	Posterior-Anterior
AP	Anterior-Posterior

Abstract

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Transcranial magnetic stimulation (TMS) is useful for modulating neural activity when applied repetitively or assessing neural pathway excitability when applied in single or dual pulse paradigms. TMS devices generate one of two types of stimulus waveforms, monophasic or biphasic, which have dissimilar neuronal activation and therefore different impacts on stimulation effectiveness. Efficacy can be quantified via motor evoked potential (MEP) amplitudes in response to suprathreshold stimuli that represent corticospinal excitability and resting motor thresholds (RMTs), and active motor thresholds (AMT) that represent motor cortical excitability. MEPs vary based on the waveform and direction of the current induced in the brain, being either anterior-to-posterior (AP) or posterior-to-anterior (PA) direction. For instance, studies targeting distal muscles of the upper limb, such as the first dorsal interosseous (FDI), have demonstrated greater efficacy of monophasic stimulation that induces a PA current in the brain relative to biphasic stimulation that induces a PA then AP ($bi_{PA\text{-}AP}$) current in the brain by showing reduced RMT with monophasic PA ($mono_{PA}$) stimulation. The

effect of stimulation waveform on TMS metrics have been extensively studied for hand muscles. However, induced current effects on more proximal muscles of the upper limb, such as the biceps brachii, remains to be fully elucidated. Thus, the primary objective of this study was to determine the effect of stimulation type on TMS metrics in the biceps, and in the FDI to provide context of our cohort in light of previous findings. A second objective was to determine the test-retest reliability of TMS metrics for each waveform and muscle. Individuals participated in two sessions. Surface electromyogram (EMG) electrodes were placed over the primary target muscles (biceps and FDI). Maximum voluntary isometric contractions (MVICs) were recorded to normalize MEPs (nMEPs) to the maximum EMG and to determine the AMT during 20% effort. RMT was determined as the lowest stimulus intensity that induced MEPs of ≥ 50 μV in at least 5 of 10 consecutive stimuli with the target muscle fully relaxed. AMT was determined during an isometric contraction of 20 ± 2.5% of their target muscle MVIC as the stimulus intensity that elicited a MEP ≥ 200μV in at least 5 of 10 consecutive stimuli. Ten MEPs were recorded at 120% of RMT for each waveform. TMS stimulation was applied via a Magstim® BiStim^2 stimulator (monophasic) and Magstim® Super Rapid^2 Plus^1 stimulator (biphasic), both using a Magstim® D70 Alpha flat (uncoated) coil. RMTs and AMTs were lower for mono$_{PA}$ stimulation compared to bi$_{PA-AP}$ stimulation for both the biceps and the FDI, and demonstrated high test-retest reliability. Normalized MEP amplitudes were greater with mono$_{PA}$ than bi$_{PA-AP}$ in the FDI, but presented no difference in the biceps. Test-retest reliability of FDI nMEP amplitudes was poor, while moderate reliability was seen in the biceps. This study suggests that current TMS waveform research on upper limb distal muscles is translatable to proximal muscles for motor thresholds but not for MEPs, and test-retest reliability and nMEPs are sensitive to differences in the cortical representations of distal and proximal muscles of the upper limb and lack intermuscular reliability. While further research is needed to elucidate the effect of these two waveforms, this study provides a framework for expected TMS metrics in distal and proximal muscles of the upper limb.

Chapter 1: Introduction

1.1 Neurophysiology of the Central Nervous System

The central nervous system (CNS) is a complex network, composed of the brain and spinal cord, responsible for processing and controlling the body's movements [42,51]. The CNS receives information about changes in the external environment and body movement which is processed by the brain. Utilizing this information, and past memories, the brain determines the most appropriate course of action and transmits this decision to the rest of the body via the spinal cord [42,51]. The cells that contain and transport these decisions from the brain, through the spinal cord, and to the rest of the body are referred to as neurons [42,51].

1.1.1 Neurons

Neurons are specialized cells that function as the body's commutation system (Figure 1). They respond to external changes (i.e., stimuli) by generating electrical signals known as action potentials, which they transmit to other cells throughout the body [42,51]. Action potential propagation begins at the post-synaptic terminal where neurotransmitters act on membrane receptors and alter the membrane potential. If the neurotransmitters induce a negative change in membrane potential relative to its resting state, the cell hyperpolarizes via an inward flow of Cl^- and outward flow of K^+ [15]. Whereas if the neurotransmitters induce a positive change in membrane potential relative to its resting state, the cell depolarizes via an inward flow of Na^+ [15]. Should the membrane sufficiently depolarize and reach the threshold potential, the neuron will generate an action potential and simultaneously increase the membranes conductance to K^+ to return the membrane potential to its resting state [15]. The action potential rapidly propagates down the axon towards the pre-synaptic terminal at the other end of the neuron. Each end of the neuron, i.e., the site of communication between two neurons, is known as a synapse [51]. When the action potential reaches the synapse, the neuron secretes a chemical referred to as a neurotransmitter which diffuses across to the post-synaptic terminal of the next neuron where this neuron is either stimulated or inhibited [42,51]. The neurotransmitter secreted by the pre-synaptic cell will determine whether the post-synaptic cell is stimulated or inhibited [15,42,51].

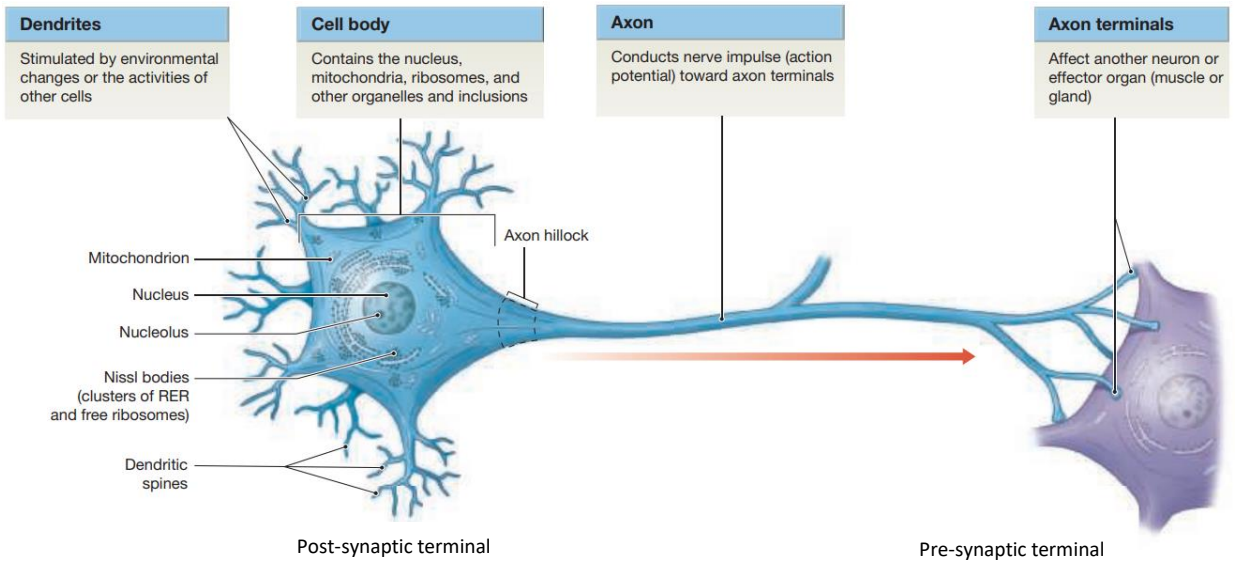

Figure 1: Anatomy of the neuron depicting the direction of signal propagation [31].

There are three types of neurons which can be determined via their synapse: sensory neurons, interneurons, and motor neurons [42]. Sensory neurons (located in the peripheral nervous system (PNS)) detect external stimuli and transmit information to neurons in the CNS [42]. Interneurons (located completely in the CNS) receive information from sensory neurons, process the information, and transmit the body's intended response to the external stimuli to the motor neurons [42]. Motor neurons (located in the PNS) receive the decision of the body's response to the stimuli and transmit that signal predominately to muscle and gland cells [42]. There are two different types of motor neurons: upper motor neurons and lower motor neurons. Upper motor neuron cell bodies are located within the CNS, particularly in the cerebral cortex of the brain [31]. Lower motor neuron cell bodies are located within the motor nucleus of the brainstem and spinal cord [31]. The axons from upper motor neurons project to either interneurons in the spinal cord or directly to the lower motor neurons, which are connected skeletal muscle fibers [31]. Lower motor neurons are only capable of exciting skeletal muscle tissue, making them the only link between the brain and the muscles.

1.1.2 The Brain and its Motor Cortex

The brain, the most complex organ in the human body, contains roughly 20 billion neurons each of which simultaneously receive and process information from thousands of synapses [31]. The cerebral cortex amounts for nearly 40% of the mass of the brain and contains roughly 14-16 billion neurons [42]. Approximately 75% of the brains total neurons are located in the cerebral cortex because it is the part of the brain that performs the most complicated neural functions, and analytical and integrative processing of this magnitude requires vast amounts of neurons [31]. Within the cerebral cortex, each primary region is paired with an association area (Figure 2a) [31,42].

The primary motor region receives input from sense organs or the brainstem, and transmits motor command output to motor nerve fibers in the brainstem for distribution to the cranial and spinal nerves [42]. On the other hand, the motor association (premotor) area involves the interpretation of sensory or motor input, planning of motor output, cognitive processing, and the storage and retrieval of memories [31,42]. First, the motivation for movement is developed in the premotor area. This is where a motor plan is created which details the degree and sequence of muscle activation. When finalized, the motor plan is then transmitted via interneurons to upper motor neurons in the primary motor area [42]. The primary motor area is topographically organized by cortical representations of voluntary muscles (Figure 2b), the size of which are represented unequally for each muscle [51]. The amount of cortical area for a given muscles corresponds to the degree of complexity in the movements each muscle performs. For instance, the hands require more skilled, complex, and delicate movements, thus have a much larger cortical representation than the feet [51]. However, the boundaries between these cortical areas are not precisely defined and there is overlap between adjacent areas [42]. Finally, the upper motor neurons then relay the motor plan down to the lower motor neurons in the brainstem and spinal cord via descending pathways, resulting in muscle activation [42].

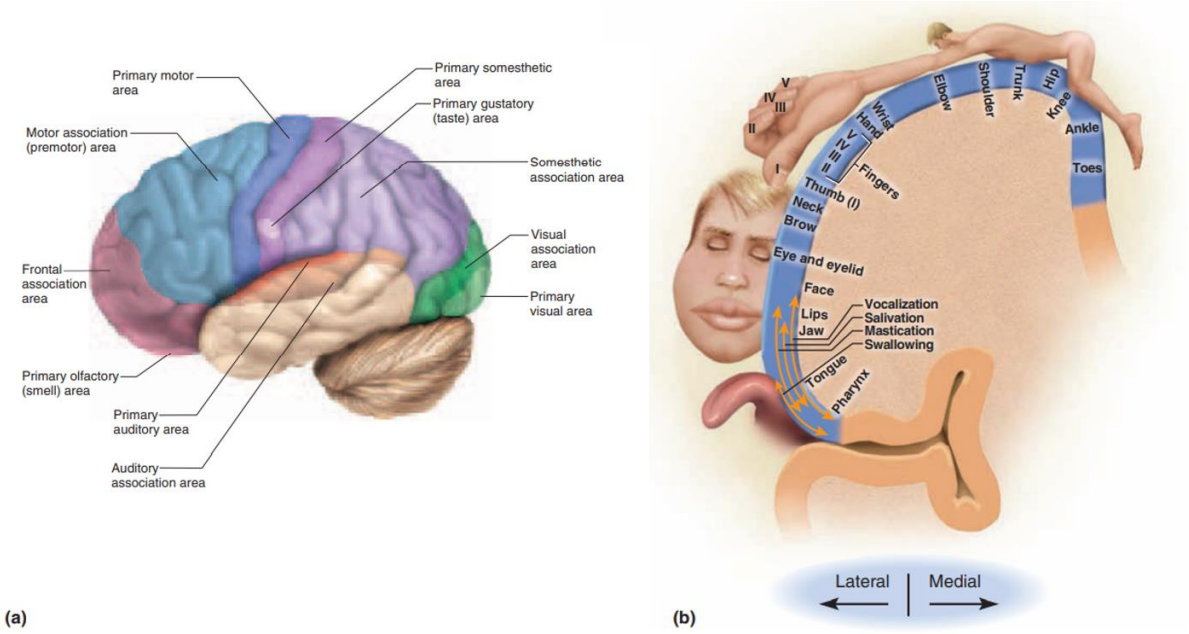

Figure 2: (a) Functional regions of the cerebral cortex [41]. **(b)** Topographical representation of the primary motor cortex (M1) [42].

1.1.3 The Spinal Cord

The spinal cord is imperative to the CNS as it serves as the bridge that connects the brain to the rest of the body below the neck. This is accomplished via ascending sensory tracts and descending motor tracts. Ascending tracts transmit sensory information from the base of the spinal cord up to the top and into the cerebral cortex of

the brain (Figure 3a). There are three specific neurons that carry out this task: first-order neuron, second-order neuron, and third-order neuron [31,42,51]. The first-order neuron is at the base of the spinal cord and conducts a signal when it detects a stimuli, the second-order neuron receives the signal from the first-order neuron and carries it to the upper end of the brainstem where it passes the signal to the third-order neuron, which carries the signal the rest of the way to the cerebral cortex [31,42,51]. Descending tracts, on the other hand, transmit motor information from the cerebral cortex down to the bottom of the spinal cord (Figure 3b). There are two specific neurons that carry out this task: upper motor neurons and lower motor neurons [31,42]. The upper motor neuron begins in the cerebral cortex or brainstem and carries the motor signal out of the brain where the axon termites on a lower motor neuron within the brainstem or spinal cord, in which the lower motor neuron carries the motor signal the rest of the way to the target [42]. The upper motor neurons extend to the lower motor neurons via direct and indirect pathways. Direct pathways provide signals to the lower motor neurons directly from the cerebral cortex, while indirect pathways provide signals to the lower motor neurons via motor centers in the brainstem [42,51]. The direct pathways, also known as the pyramidal pathways, consist of axons descending from pyramidal cells in the primary and premotor area which transmit action potentials propagated in the cerebral cortex directly to the lower motor neurons [31,51]. Upper motor neurons that have pyramid-shaped bodies are pyramidal neurons. The corticospinal tract, a part of the pyramidal tract, contains axons that travel down upper motor neurons and terminate of lower motor neurons in the spinal cord which transmit information for precise, coordinated, voluntary movement [31,42,51].

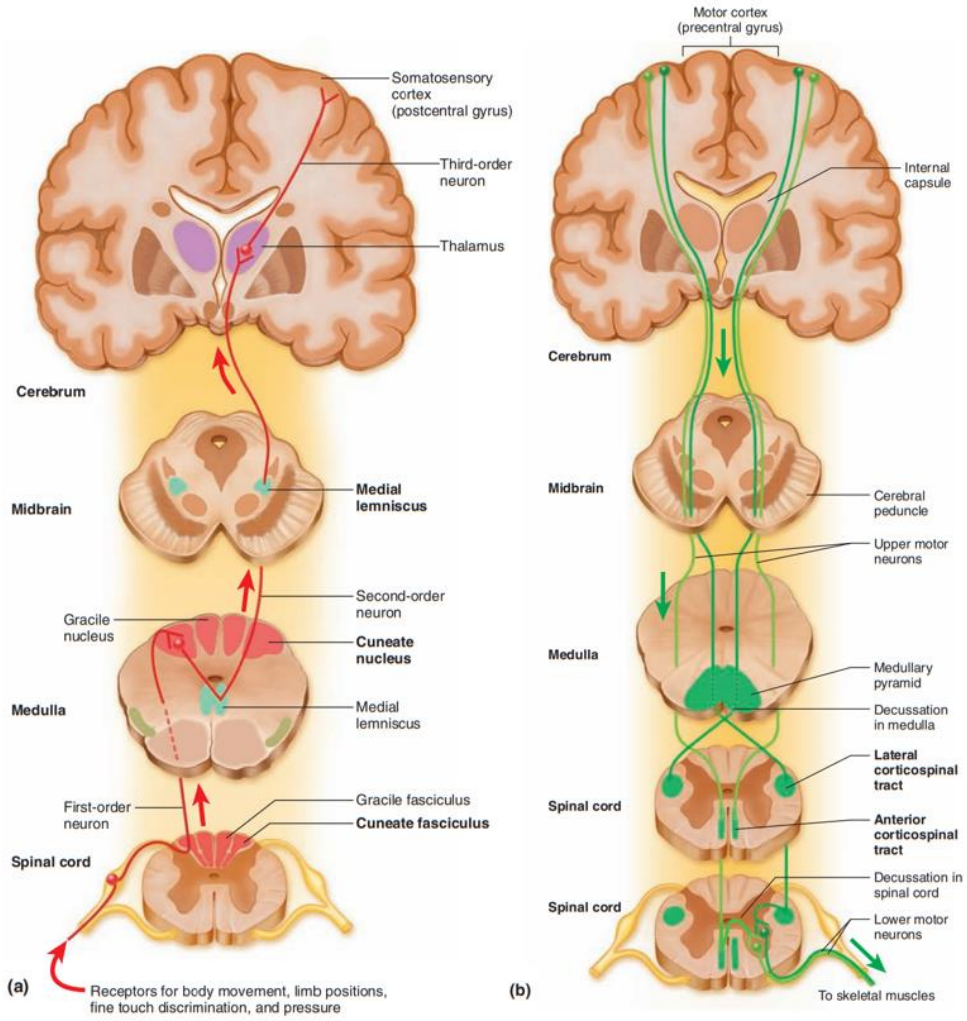

Figure 3: (a) Ascending Tracts of the CNS. Nerve signals, such as sensory, carry information from the bottom of the spinal cord to the cerebral cortex at the top. **(b) Descending Tracts of the CNS.** Nerve signals, such as motor commands, that originate in the cerebral cortex are carried down the spinal cord to skeletal muscles [42].

1.2 Transcranial Magnetic Stimulation

Transcranial magnetic stimulation (TMS) is a non-invasive form of brain stimulation in which a rapid change in magnetic field (i.e., a pulse) generates an electrical current in the brain through electromagnetic induction. The electrical current can be targeted in a specific region of the brain. Should the electrical field cause depolarization of neurons an action potential is generated and motor neurons are recruited causing an invoked muscle twitch, which can be recorded as a motor-evoked potential (MEP) [36]. The type and quantity of neurons that are recruited during an action potential are determined by the induced current generated via the current passed through the coil [1,5,12,27].

1.2.1 Stimulation Characteristics

The basic functionality of a magnetic stimulator (including the coil) can be reduced to three elements in a simple circuit: capacitor, inductor, switch. The capacitor (stimulation unit) stores the high voltage energy while the switch is open, but when the switch closes the circuit is complete and the energy is discharged from the capacitor and flows to the inductor (stimulation coil) [54]. Additional components are added to the simplified circuit to prevent the energy from continuously oscillating after a single pulse, however, this is how different pulse types are formed.

There are two main types of pulse configurations that are generated within a magnetic stimulator: monophasic and biphasic (Figure 4). Monophasic pulses are dampened after the first quarter-cycle of a sine wave resulting in a strong, short duration current flow in one direction followed by a weak, non-stimulating, current flow in the opposite direction [7,12]. Monophasic pulses have a single phase of stimulation and are thus mainly used to measure cortical excitability with single and paired pulses. Biphasic pulses are allowed to flow for a full sine cycle thus consisting of two half segments of equal amplitude and opposite direction current flow [7,12]. The reverse (2nd) phase reduces the neuronal excitation inhibited from the initial phase as well as activates different neurons that are more sensitive to current flow in that particular direction [49]. During the full sine wave of a biphasic pulse, energy in the inductor returns to the capacitor rather than being dissipated like with the monophasic pulse [54]. The return of energy to the capacitor decreases the charge time and allows for stimulations to be repeated more than 50 times per second [7,49]. Due to the high stimulation frequency, biphasic stimulation is mainly used to measure cortical excitability with repetitive pulses [7,49].

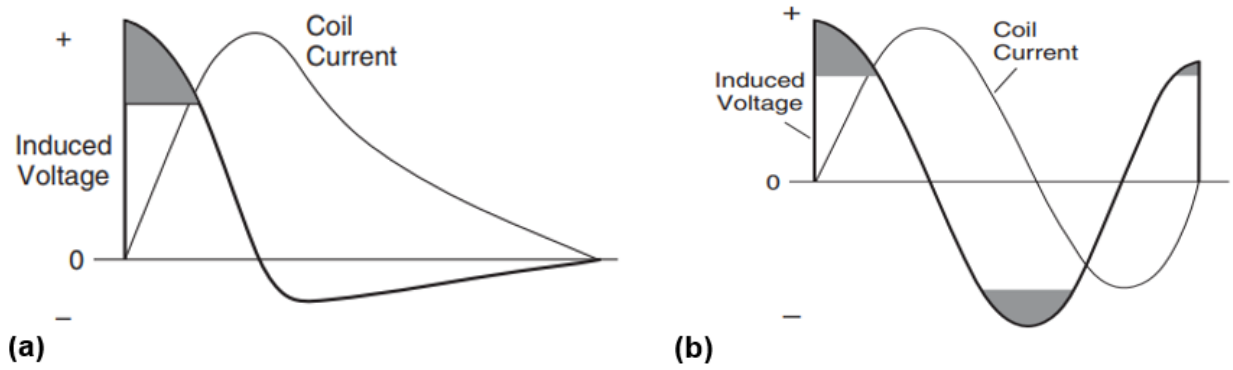

Figure 4: (a) Monophasic TMS Waveform. (b) Biphasic TMS Waveform. (a-b) The lighter curve represents current in the stimulation coil. The darker curve represents the voltage in the stimulation coil and the induced voltage in the brain. The shaded areas are peak voltages when neuronal membranes are most likely to depolarize [54].

The effect each waveform has on depolarization can be further varied depending on the direction induced current flows within the brain, which can be determined by coil placement. A figure-8 coil, with current flowing posteriorly to anteriorly, will generate a magnetic field that penetrates into the brain inducing electrical current that flows at right

angles with respect to the coil orientation (Figure 5) [54]. Therefore, when the coil is oriented perpendicular to the central sulcus and current in the coil is flowing posterior to anterior, the resulting induced current in the brain is in the posterior-anterior (PA) direction. To induce current in the brain in the anterior-posterior (AP) direction, either the coil can be turned 180 degrees or the current in the coil can be changed to flow anterior to posterior. Along with waveform pulses, the direction of induced current flow in the brain can determine the types and quantity of activated neurons. Monophasic pulses produce earlier and more focal activity when current flows PA, compared to current flowing AP [49].

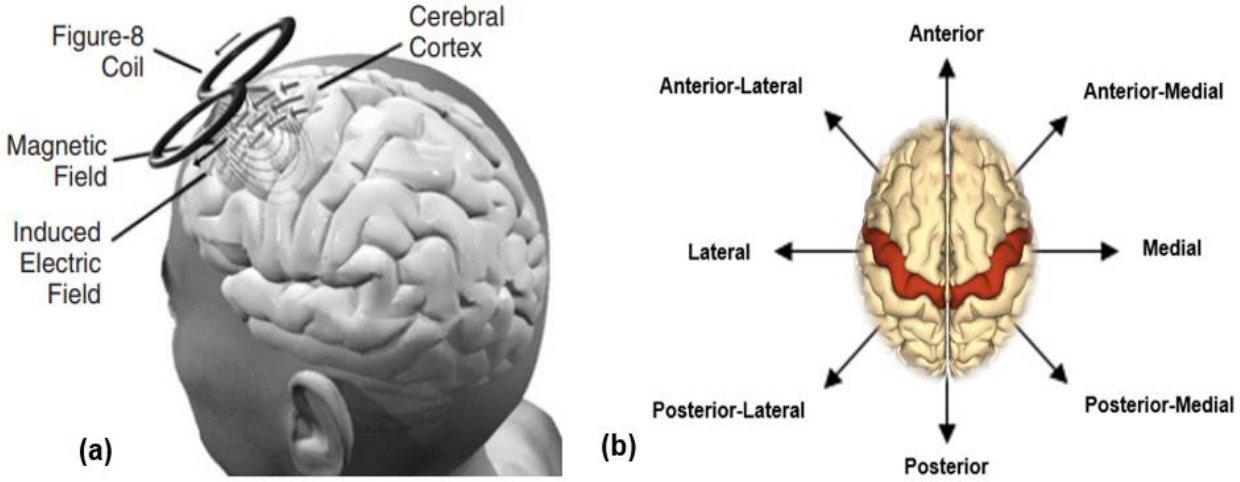

Figure 5: (a) Magnetic and electric field produced by figure-eight TMS coil. Black arrows depict current direction within the coil. Small grey arrows forming circles depict the magnetic field generated from the coil. Large grey arrow depict induced electrical current in the brain [54]. **(b) Superior view of anatomical directions.** The central sulcus is highlighted in red.

In addition to pulse configuration, focality of the TMS coil also affects neuronal activation. Focality, defined by the half-value electrical field spread ($S_{1/2}$), measures the electrical field spread tangentially across the brains surface, while accounting for depth of electrical field penetration [9]. The shape and size of the TMS coil (Figure 6) affects the focality and depth of stimulation (Figure 7). Each TMS is subject to a depth-focality tradeoff, where lower $S_{1/2}$ represents higher focality. While the half-value depth ($d_{1/2}$) of electrical field penetration is similar for both circular (1.0-3.5 cm) and figure-8 (0.9-3.4 cm) coils, focality is higher in the figure-8 coils (5-261 cm^2) than the circular coils (34-273 cm^2) [9]. Thus, figure-8 coils are more commonly used due to their higher focality.

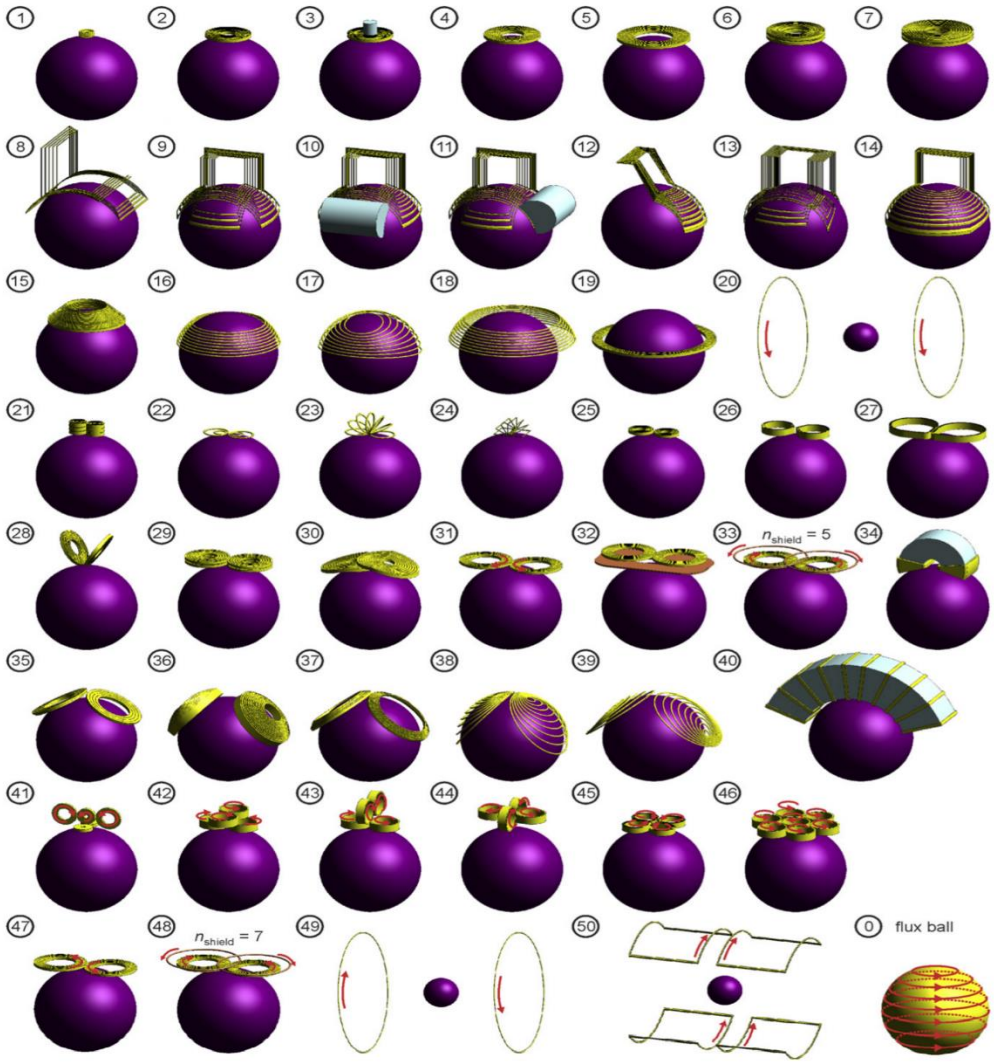

Figure 6: Simulation models of 50 TMS coil configurations: (1) Animal mini-coil, (2) Magstim 50 mm circular coil (P/N 9999), (3) 50 mm circular coil with iron core, (4) Magstim 70 mm circular coil (P/N 3192), (5) Magstim 90 mm circular coil (P/N 3192), (6) Magstim animal MST coil, (7) Magstim human MST coil (S/N MP39), (8) Brainsway H coil, (9) Brainsway H1 coil, (10) H1 coil with frontal iron core, (11) H1 coil with lateral iron core, (12) Brainsway H1L coil, (13) Brainsway H2 coil, (14) Brainsway HADD coil, (15) Magstim cap coil, (16) crown coil, (17) crown coil with back-splayed winding, (18) crown coil with back-spaced winding, (19) supraorbital halo coil, (20) MRI z-gradient coil in parallel-current (Helmholz) mode, (21) 3-layer double coil, (22) double butterfly, (23) circular slinky-7 coil, (24) rectangular slinky-7 coil, (25) Magstim 25 mm figure-8 (P/N 1165), (26) Cadwell Corticoil, (27) Cadwell B-shaped coil, (28) 50 mm V-coil, (29) MagVenture C-B65 butterfly coil, (30) MagVenture MC-B70 butterfly coil, (31) Magstim 70 mm figure-8 coil (P/N 9925, 3190), (32) 70 mm figure-8 with shielding plate, (33) 70 mm figure-8 with active shield (5 turns), (34) Neuronetics iron core figure-8 coil (CRS 2100), (35) MagVenture D-B80 butterfly coil, (36) MagVenture MST twin coil, (37) Magstim double cone coil (P/N 9902), (38) eccentric double cone coil with center-dense windings, (39) eccentric double cone coil with center-sparse windings, (40) stretched C-core coil, (41) 3-D differential coil, (42) 3-D coil array #1, (43) 3-D coil array #2, (44) 3-D coil array #3, (45) Cadwell cloverleaf coil, (46) circular coil array, (47) Magstim 70 mm figure-8 coil in reversed-current mode, (48) 70 mm figure-8 with active shield (7 turns), (49) MRI z-gradient coil opposing-current (Maxwell) mode, and (50) MRI x- (or y-) gradient (Golay) coil. The last configuration,

labeled “0”, is the ideal “flux ball” coil whose windings are parallel to the circles of latitude of the spherical model and cover the whole head [9].

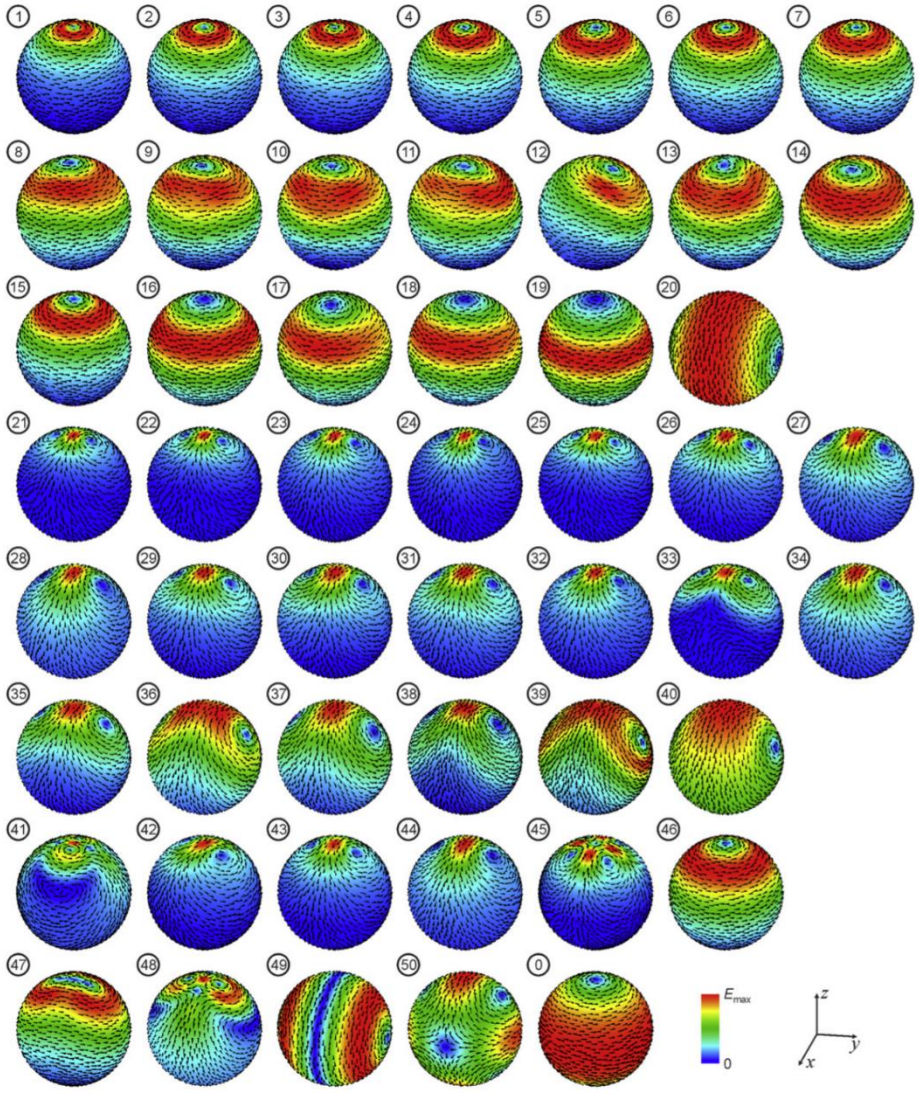

Figure 7: Induced electric field distribution on the brain surface by the 50 TMS coils from Figure 6. The electric field magnitude is plotted with a color map normalized to the field maximum in the brain, for each coil. The arrows indicate the electric field direction [9].

1.2.2 Measuring Corticospinal Excitability

1.2.2.1 I-Waves & D-Waves

A TMS pulse targeted over the primary motor cortex (M1) can induce corticospinal descending activity composed of a series of high-frequency waves termed D-waves and I-waves [6,20,56]. The earliest wave that is recorded following cortical activation, the D-wave, originates from direct activation of cortical layer-V pyramidal tract neurons (PTNs), while I-waves have a greater latency and originate from indirect trans-synaptic activation of layer-V PTNs [5,13,56]. Furthermore, the current direction

(PA or AP) induced in M1 from the TMS coil influences the activation of different types of I-waves [5,6,13,20,56]. At low intensity, $mono_{PA}$ current evokes a monosynaptic descending wave consisting of early I-waves (I_1 wave) that reflects the indirect activation of local cortico-cortical connections projecting to PTNs in cortical layers II-III [5,6,13,20]. At high intensity, $mono_{PA}$ current evokes polysynaptic chains that begin to consist of later I-waves (I_2-I_5 waves) in layers II-III [5,13]. A further increase in intensity evokes D-waves, reflecting the direct activation of PTNs in cortical layer V [11,13]. Meanwhile, $mono_{AP}$ current evokes later I-waves with greater latencies that reflects the indirect activation of horizontal cortico-cortical connections from surrounding regions that project to PTNs in cortical layers II-III [5,6,13,20]. On the other hand, biphasic pulses (PA-AP and AP-PA) evoke a more complex pattern of I-waves and D-waves where stimulus intensity affects neuronal recruitment and the combination of current direction has competing effects [5,6]. The descending volley resulting from neuronal depolarization induced by a TMS pulse targeted over M1, ultimately recruits motor units eliciting a muscle response, or MEP, on the contralateral side of the body [20] (Figure 8). MEP amplitudes indirectly measure changes in corticospinal motor pathway activation (corticomotor excitability) using electromyography (EMG) on the target muscle [7,49,36].

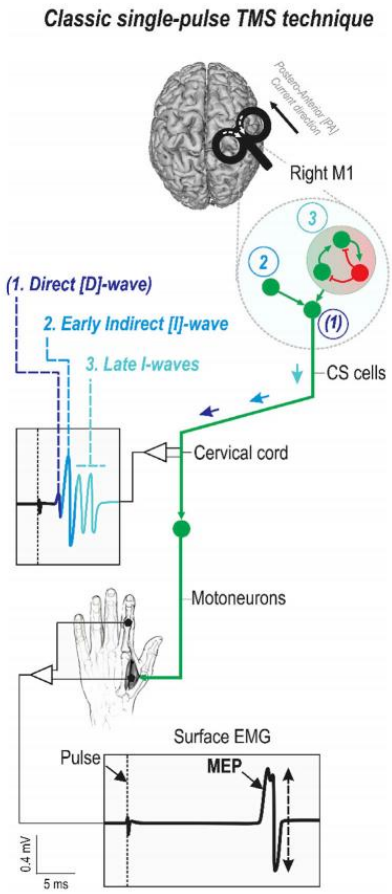

Figure 8: Classic single-pulse TMS technique [10].

1.2.2.2 Stimulus Intensity & Motor Thresholds

The intensity of TMS used to elicit MEPs in M1 is determined based on individual cortical thresholds because M1 is the only region where excitation effects have a directly measurable physiological effect (i.e. MEPs) [26]. Motor thresholds are defined as the minimum stimulation intensity required to elicit a muscle response, which is determined by a MEP of minimal amplitude. The minimum TMS stimulation intensity required to elicit MEPs of at least 50 µV peak-to-peak amplitude in roughly 50% of 5 to 10 trials in a fully relaxed muscle is referred to as the resting motor threshold (RMT) [27,39]. The active motor threshold (AMT) is determined as the minimum TMS stimulation to elicit MEPs of at least 200 µV peak-to-peak amplitude in roughly 50% of 5 to 10 trials in a slightly contracted muscle, typically 20% of the maximum voluntary isometric contraction (MVIC) [28,39]. RMT reflects the neuronal membrane threshold, while AMT reflects the quantity of axons near firing threshold [22].

Chapter 2: Objectives and Methods

2.1 Objectives of the Study

The effect of stimulation waveform on TMS metrics have been extensively studied for hand muscles [5,7,33,35,49]. However, induced current effects on more proximal muscles of the upper limb, such as the biceps brachii, remains to be fully elucidated. Induced current effects on TMS efficacy may be different in proximal relative to distal muscles. In order to optimize the utility of TMS for clinical neurorehabilitation, an understanding of whether the effect of stimulation waveform on TMS metrics differs according to the target muscle is needed.

Despite the many studies investigating the effects of stimulus waveform on TMS metrics [5,7,33,35,49], no prior work has compared the effect of $mono_{PA}$ and $bi_{PA\text{-}AP}$ stimulation on TMS metrics of a proximal arm muscle, such as the biceps brachii. Elucidating the effect of these two waveforms is needed considering $bi_{PA\text{-}AP}$ is the most common waveform used to deliver rTMS protocols that aim to promote neuroplasticity [29,50], and $mono_{PA}$ is the most common waveform used to assess changes in excitability after rTMS [29,50]. As clinicians and researchers design rTMS protocols targeting the biceps, it is important to know whether assessment of corticomotor excitability may differ by $mono_{PA}$ or $bi_{PA\text{-}AP}$ stimulation, given that many rTMS devices only deliver biphasic stimulation. Use of a single stimulator to deliver rTMS and assess TMS metrics is advantageous regarding study design and clinical feasibility.

Thus, the primary objective of this study was to determine the effect of stimulation type ($mono_{PA}$ vs $bi_{PA\text{-}AP}$) on RMT, AMT, and MEP amplitudes in the biceps. We also tested the effect of stimulation type on RMT, AMT, and MEP amplitudes in the FDI to provide context of our cohort in light of previous findings [5,7,33,35,49]. A second

objective was to determine the test-retest reliability of RMT, AMT, and MEP amplitudes for each waveform ($mono_{PA}$ and bi_{PA-AP}) and muscle (biceps and FDI).

2.2 Methods

2.2.1 Participants

Twelve individuals aged 24.5 ± 3.5 years (5 female, 7 male) were recruited for this study which was approved by the Institutional Review Board (IRB) at Virginia Commonwealth University (VCU). Data from two participants were excluded because their RMT with bi_{PA-AP} stimulation could not be determined within the maximum stimulator output (MSO) range of our system. Inclusion criteria were non-impaired participants that were free of contradictions for TMS. Exclusion criterion were individuals with neurologic and/or musculoskeletal disorder or injury. Individuals participated in two sessions. For all protocols, participants were seated in a semi-reclined chair with the arm supported against gravity (Figure 9).

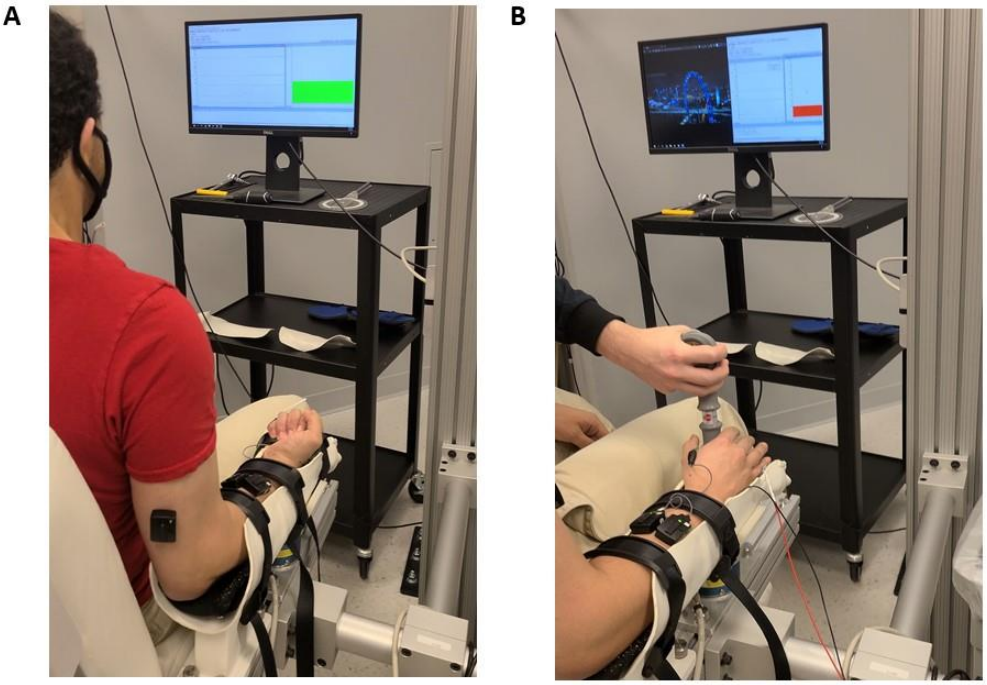

Figure 9: Participants were seated upright with an elbow angle of 90° and straps were used to secure the arm. (A) The arm was supinated for biceps measurements. (B) The arm was pronated for FDI measurements and an additional device was placed against the inner wall of the cast for FDI contractions.

2.2.2 Surface electromyogram

Surface electromyogram (EMG) electrodes were placed on the dominant arm after the skin was shaved and cleaned with alcohol wipes. Surface EMG electrodes (Trigno™ Wireless System; Delsys, Natick, MA) were located over the primary target

muscles (biceps and FDI). Trigno™ Avanti sensors were located on the biceps brachii, while the Trigno™ Mini sensors were located on the FDI. EMG signals were amplified (x909) and bandpass-filtered at 20-450 Hz prior to analog to digital conversion (Micro 1401 MkII, Cambridge Electron Design, Cambridge, UK). All EMG data were sampled at 2 kHz with Spike2 software (Cambridge Electronic Design, Cambridge, UK).

2.2.3 Maximum voluntary isometric contraction

We recorded maximum voluntary isometric contractions (MVICs) to normalize MEPs to the maximum EMG [19] and to determine the AMT during 20% effort [39]. To establish the MVIC, participants were asked to maximally flex their elbow (biceps) or abduct their index finger (FDI) and hold for five seconds. To encourage maximum contraction, participants were motivated by the researchers and were provided visual feedback of the EMG signal as a bar graph (Figure 9). Participants completed three maximum contraction trials separated by one minute of rest for each muscle. The greatest root mean squared (RMS) value was computed from these trials over a 500 ms window at the maximum amplitude [45].

2.2.4 Transcranial magnetic stimulation

To determine the effect of stimulus waveform on RMT, AMT, and normalized MEP (nMEP) amplitudes, monophasic and biphasic pulses were delivered in separate blocks, each for the biceps and FDI resulting in four blocks. Each of the four blocks consisted of the same structure; only the stimulus waveform ($mono_{PA}$ or $bi_{PA\text{-}AP}$) and muscle (biceps or FDI) differed across each block where waveform order was randomized each session and biceps measurements always preceded FDI measurements. TMS stimulation was applied via a Magstim® BiStim^2 stimulator (monophasic) and Magstim® Super Rapid^2 Plus^1 stimulator (biphasic), both using a Magstim® D70 Alpha flat (uncoated) coil. The coil was oriented perpendicular to the central sulcus (or about 45° from a parasagittal plane aligned with the corpus callosum) as to induce a PA then AP current in the brain with $bi_{PA\text{-}AP}$ stimulation or a PA current in the brain with $mono_{PA}$ stimulation (Figure 10). During each session participants were blinded to the type of stimulation.

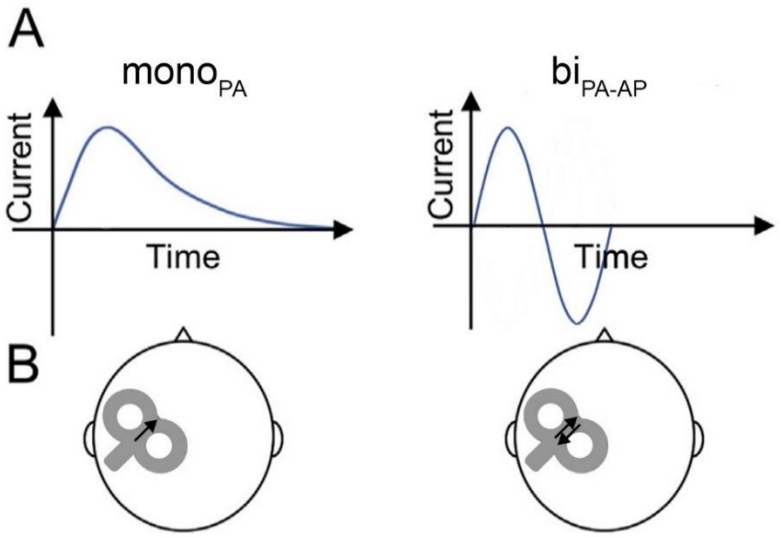

Figure 10: TMS pulse waveforms and corresponding induced current directions in the brain tested in this study. (A) Diagram showing mono$_{PA}$ and bi$_{PA-AP}$ magnetic waveforms generated by the stimulators. (B) Location of the TMS coil over the motor cortex, perpendicular to central sulcus, and arrows depicting induced current direction(s) in the brain.

2.2.5 TMS metrics

Single-pulse TMS was delivered to the motor cortex contralateral to the target arm while at rest. Using the vertex as a reference, the coil was positioned perpendicular to the central sulcus (or about 45° from a parasagittal plane aligned with the corpus callosum). Through a motor mapping procedure, the motor hotspot was identified as the location that evoked the largest peak-to-peak MEP amplitude in the target muscle using the lowest stimulation intensity. This hotspot location was marked on a cap secured on the participant's head and all subsequent stimulations were applied to this hotspot. All biceps measurements were recorded when the biceps motor hotspot was determined, prior to determining the FDI motor hotspot and recording all FDI measurements. RMT was determined as the lowest stimulus intensity that induced MEPs of ≥ 50 μV in at least 5 of 10 consecutive stimuli with the target muscle fully relaxed [38]. With visual feedback of their effort provided (Figure 9), participants sustained an isometric contraction of 20 ± 2.5% of their target muscle MVIC during which the AMT was determined as the stimulus intensity that elicited a MEP ≥ 200μV in at least 5 of 10 consecutive stimuli [39]. Stimulus intensity was determined using an adaptive parameter estimation by sequential testing (PEST) software [2]. Lastly, ten single pulse MEPs were recorded at 120% of RMT for each waveform [5,7].

2.2.6 Data and statistical analysis

MEP amplitudes were calculated from the biceps and FDI EMG data using custom-written MATLAB (MathWorks (2020)) code. Peak-to-peak MEP amplitudes were normalized to the RMS of the participant's MVIC recorded during the same session, with the ratio multiplied by a scaling factor of 100. Normalized MEPs served as our measure of corticomotor excitability. MEP latencies were calculated by measuring the

time from the triggered stimulus to the onset of the MEP, accounting for offsets in EMG hardware and Spike2 software.

A Kolmogorov-Smirnov test revealed that all TMS metrics are normally distributed ($p<0.01$). The following were compared between stimulation type and muscle using two-tailed student's t-tests: RMT, AMT, nMEP amplitudes, and MEP latencies. To examine test-retest reliability of the motor thresholds across two sessions, Wilcoxon matched-pairs tests were calculated [17]. Intraclass correlation coefficients (ICCs) were calculated with the ICC(A,1) formula to examine the reliability of nMEPs [32]. ICC values were interpreted as high (ICC ≥ 0.75), moderate (0.50 ≤ ICC < 0.75), low (0.25 ≤ ICC < 0.50), and very low to none (ICC < 0.25) [37]. Statistical analyses were performed using MATLAB and GraphPad Prism. Statistical significance was set at $p<0.05$.

2.2.7 Post-hoc analysis

When RMTs are large enough that suprathreshold MEPs (i.e., 120% of RMT) would be recorded at greater than 100% MSO, the MEPs may be considered under stimulated [5]. This under stimulation can increase the variability in MEP amplitude [27]. Sixty percent of biceps RMT values determined with $bi_{PA\text{-}AP}$ stimulation were larger than 84% MSO resulting in under stimulation. A two-tailed student's t-test comparing biceps $bi_{PA\text{-}AP}$ nMEPs larger than 84% RMT and less than 84% RMT determined whether under stimulation affected our results. Since our nMEP reliability was inconsistent with previous findings in the FDI [4,5,30] and in the biceps [43], we calculated a two-sample F-test [21] to determine if our variance across sessions was consistent with our reliability findings [43].

Chapter 3: Effect of Stimulus Waveform on Transcranial Magnetic Stimulation Metrics in Proximal and Distal Arm Muscles

3.1 Introduction

Transcranial magnetic stimulation (TMS) is a non-invasive form of brain stimulation that is useful for modulating neural activity when applied repetitively or assessing neural pathway excitability and inhibition when applied in single or dual pulse paradigms [27]. Commercial TMS devices generate either a monophasic waveform or a biphasic waveform [49]. Each of these waveforms can be distinguished via the length and duration of the first and second phase of the pulse. The biphasic waveform results from a full sine-wave which generates two pulses of equal amplitude and opposite polarity that facilitate the production of more than 50 pulses per second [7]. Due to its high frequency, biphasic stimulation is used in repetitive TMS (rTMS) protocols to promote or dampen cortical activity that lasts beyond the stimulation period [7]. In contrast, monophasic waveforms result from the dampening of a sine-wave after the first or second quarter cycle which results in a loss of energy and generates a unidirectional stimulation current [7]. The monophasic waveform is primarily used with

single or paired-pulse TMS protocols to examine direction specific effects of neural pathway excitability [7].

Stimulation waveform and the resulting current induced in the brain alters the effectiveness of stimulation [33]. Efficacy can be quantified via motor evoked potential (MEP) amplitudes in response to suprathreshold stimuli that represent corticospinal excitability and resting motor thresholds (RMTs), and active motor thresholds (AMT) that represent motor cortical excitability [27]. The RMT is the minimum stimulus intensity necessary for generating a ≥ 50 μV MEP [38], and the AMT is the minimum stimulus intensity necessary for generating a ≥ 200 μV MEP during a sustained 20% isometric contraction [39]. MEPs indirectly measure changes in corticospinal motor pathway activation (corticomotor excitability) via an electromyography (EMG) sensor on the target muscle [7]. MEPs vary based on the waveform and direction of the current induced in the brain, being either anterior-to-posterior (AP) or posterior-to-anterior (PA) direction [33]. For instance, studies targeting distal muscles of the upper limb, such as the first dorsal interosseous (FDI), have demonstrated greater efficacy of monophasic stimulation that induces a PA current in the brain relative to biphasic stimulation that induces a PA then AP current in the brain by showing reduced RMT with monophasic PA stimulation [7,35,49]. Hereafter, $mono_{PA}$ refers to stimulation that induces a PA current in the brain and $bi_{PA\text{-}AP}$ refers to stimulation that induces a PA then AP current in the brain. The reduced efficacy of $bi_{PA\text{-}AP}$ stimulation is a result of the second (reverse) phase. The first PA phase depolarizes the neuronal membrane; the second AP phase generates current opposing the direction of the first phase, thereby activating neurons more sensitive to that particular current direction and hyperpolarizing the membrane. The second phase has a greater effect on neuronal activation than the first phase [18], and given that facilitatory corticospinal pyramidal neurons are more sensitive to the PA current direction [35], the efficacy of the $bi_{PA\text{-}AP}$ is less than $mono_{PA}$ when the FDI motor hotspot is stimulated.

In order to optimize the utility of TMS for clinical neurorehabilitation, an understanding of whether the effect of stimulation waveform on TMS metrics differs according to the target muscle is needed. The effect of stimulation waveform on TMS metrics have been extensively studied for hand muscles [5,7,33,35,49]. However, induced current effects on more proximal muscles of the upper limb, such as the biceps brachii, remains to be fully elucidated. Induced current effects on TMS efficacy may be different in proximal relative to distal muscles because distal muscles have a higher density of corticospinal neurons in the motor cortex [52], larger cortical motor map areas [43], differences in spontaneous motor outflow [30], and are less innervated by other pathways (e.g., cortical-reticulospinal) [23]. These characteristics correlate to corticospinal pathways to distal hand muscles being more excitable (as measured by MEPs) and less variable in response to TMS relative to proximal arm muscles when the same stimulus waveform is applied [53]. Understanding the effect of the TMS induced current in the brain on TMS metrics is critical for establishing a basis to track and predict TMS metrics in rehabilitation and the design of interventions involving TMS. For example, the presence of MEPs can indicate or predict functional recovery [44] and proximal recovery often precedes distal recovery in individuals who have suffered a spinal cord injury (SCI) [40] or stroke [43]. Also, proximal muscle neurophysiology and

function is important to monitor during recovery since proximal muscles tend to compensate for poor dexterity [3].

Despite the many studies investigating the effects of stimulus waveform on TMS metrics [5,7,33,35,49], no prior work has compared the effect of $mono_{PA}$ and $bi_{PA\text{-}AP}$ stimulation on TMS metrics of a proximal arm muscle, such as the biceps brachii. Elucidating the effect of these two waveforms is needed considering $bi_{PA\text{-}AP}$ is the most common waveform used to deliver rTMS protocols that aim to promote neuroplasticity [29,50], and $mono_{PA}$ is the most common waveform used to assess changes in excitability after rTMS [29,50]. Further, the intensity of rTMS is commonly set as a percent of AMT, where AMT may be determined with either $bi_{PA\text{-}AP}$ or $mono_{PA}$ stimulation [50]. As clinicians and researchers design rTMS protocols targeting the biceps, it is important to know whether assessment of corticomotor excitability may differ by $mono_{PA}$ or $bi_{PA\text{-}AP}$ stimulation, given that many rTMS devices only deliver biphasic stimulation. Use of a single stimulator to deliver rTMS and assess TMS metrics is advantageous regarding study design and clinical feasibility.

Thus, the primary objective of this study was to determine the effect of stimulation type ($mono_{PA}$ vs $bi_{PA\text{-}AP}$) on RMT, AMT, and MEP amplitudes in the biceps. We also tested the effect of stimulation type on RMT, AMT, and MEP amplitudes in the FDI to provide context of our cohort in light of previous findings [5,7,33,35,49]. A second objective was to determine the test-retest reliability of RMT, AMT, and MEP amplitudes for each waveform ($mono_{PA}$ and $bi_{PA\text{-}AP}$) and muscle (biceps and FDI). We hypothesized that for each muscle (biceps and FDI) the $mono_{PA}$ stimulation would result in lower RMT and AMT compared to the $bi_{PA\text{-}AP}$ stimulation [49] and the $mono_{PA}$ stimulation would result in greater MEPs compared to the $bi_{PA\text{-}AP}$ stimulation [8]. Furthermore, we hypothesized that the test-retest reliability of the FDI metrics would be greater relative to the biceps metrics [30,43], and that metrics taken via $mono_{PA}$ stimulation would have greater test-retest reliability relative to $bi_{PA\text{-}AP}$ stimulation in both muscles [5].

3.2 Results

3.2.1 Effect of Stimulus Waveform on TMS Metrics

RMTs were lower for $mono_{PA}$ stimulation compared to $bi_{PA\text{-}AP}$ stimulation for both the biceps (respectively: 60 ± 9.06 %MSO, 84 ± 11.02 %MSO, $p<0.01$, Figure 11) and the FDI (respectively: 48 ± 8.21 %MSO, 48 ± 8.21 %MSO, $p<0.01$, Figure 11). Similarly, AMTs were lower for $mono_{PA}$ stimulation compared to $bi_{PA\text{-}AP}$ stimulation for both the biceps (respectively: 39 ± 9.32 %MSO, 61 ± 11.36 %MSO, $p<0.01$, Figure 11) and the FDI (respectively: 34 ± 11.74 %MSO, 56 ± 14.71 %MSO, $p<0.01$, Figure 11). Normalized MEPs in response to single pulse $mono_{PA}$ and $bi_{PA\text{-}AP}$ stimulation were not different for the biceps (respectively: 185.84 ± 193.08 %MVC, 182.00 ± 244.22 %MVC, $p=0.86$, Figure 12). Normalized MEPs were greater with $mono_{PA}$ stimulation compared to $bi_{PA\text{-}AP}$ stimulation in the FDI (respectively: 302.33 ± 276.69 %MVC, 236.19 ± 218.53 %MVC, $p=0.01$, Figure 12). There was no difference in MEP latencies in response to $mono_{PA}$ compared to $bi_{PA\text{-}AP}$ stimulation for both the biceps (respectively: 25.55 ± 2.31 ms, 25.50 ± 1.80 ms, $p=0.82$, Figure 12) and the FDI (respectively: 33.98 ± 1.54 ms, 33.38 ± 5.51 ms, $p=0.14$, Figure 12). Even though 60% of our biceps RMTs with $bi_{PA\text{-}AP}$

stimulation were larger than 84% MSO, the post-hoc analysis determined that under stimulation did not affect our MEP data (p=0.18).

3.2.2 Effect of Target Muscle (Biceps or FDI) on TMS Metrics

RMTs were greater for biceps compared to FDI with both single pulse $mono_{PA}$ ($p<0.01$) and bi_{PA-AP} ($p<0.01$) stimulation. AMTs were not different between the biceps and the FDI for both $mono_{PA}$ ($p=0.15$) and bi_{PA-AP} ($p=0.21$) stimulation. Normalized MEPs were greater for the FDI compared to the biceps for both $mono_{PA}$ ($p<0.01$) and bi_{PA-AP} ($p<0.05$) stimulation. MEP latencies were longer for the FDI compared to the biceps for both $mono_{PA}$ ($p<0.01$) and bi_{PA-AP} ($p<0.01$) stimulation.

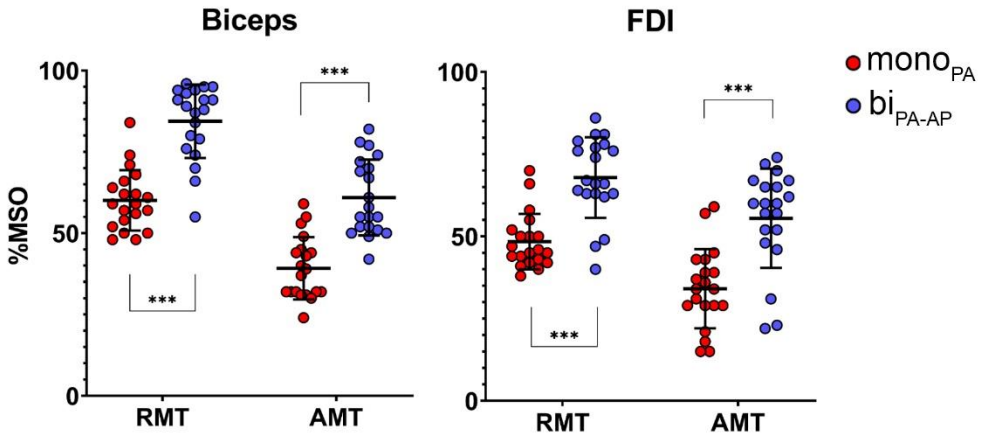

Figure 11: Motor thresholds were lower with $mono_{PA}$ stimulation compared to bi_{PA-AP} stimulation in both muscles. Asterisks represent statistical difference ($p<0.01$) and error bars represent one standard deviation.

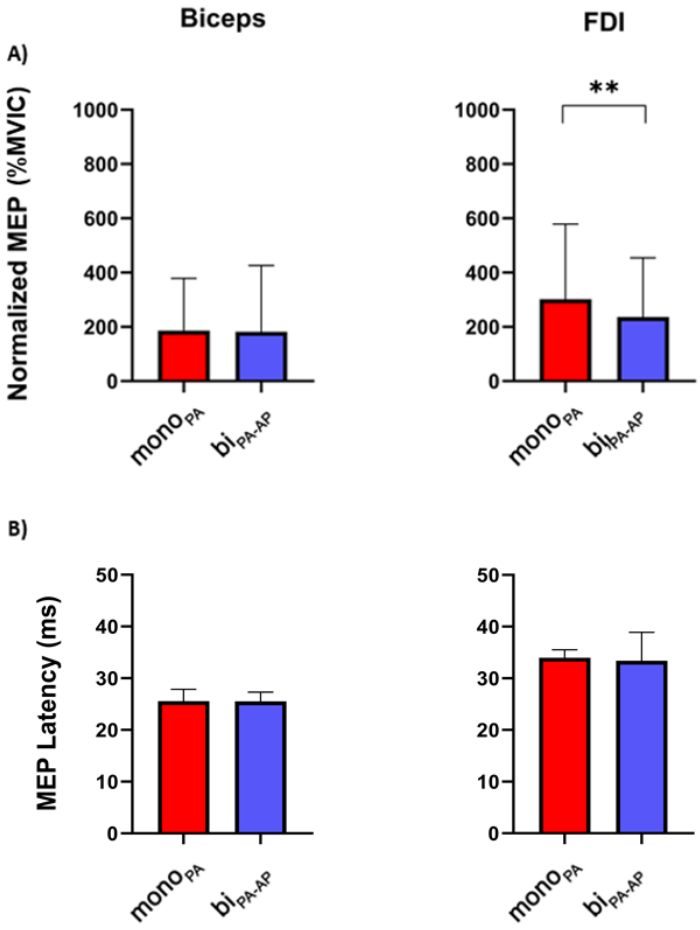

Figure 12: (A) Normalized MEPs did not differ between waveforms for the biceps while nMEPs were greater for FDI when obtained with $mono_{PA}$ compared to bi_{PA-AP} stimulation. Asterisks represent statistical difference ($p<0.01$) and error bars represent one standard deviation. (B) Motor evoked potential latencies did not differ by stimulation type. Asterisks represent statistical difference ($p<0.01$) and error bars represent one standard deviation.

3.2.3 Test-Retest Reliability of TMS Metrics

The Wilcoxon matched-pairs test revealed that the RMTs and AMTs were not different between sessions ($p<0.01$). Thus, the RMTs and AMTs were reliable across sessions. The ICCs for nMEPs indicated very low to no reliability for $mono_{PA}$ (0.07) and bi_{PA-AP} (0.10) in the FDI, and moderate reliability for $mono_{PA}$ (0.72) and bi_{PA-AP} (0.56) in the biceps. Motor thresholds have equal variance ($p>0.05$) between sessions and nMEP amplitudes have unequal variance ($p<0.05$) between sessions, as per the two-sample F-test. Motor thresholds and average nMEP amplitudes are presented in Table 1 and Table 2, respectively.

Table 1: Motor threshold metrics for each participant and session

		Biceps				FDI			
		$mono_{PA}$	bi_{PA-AP}	$mono_{PA}$	bi_{PA-AP}	$mono_{PA}$	bi_{PA-AP}	$mono_{PA}$	bi_{PA-AP}
Participant	**Session**	**RMT (%MSO)**		**AMT (%MSO)**		**RMT (%MSO)**		**AMT (%MSO)**	
1	1	61	89	24	58	42	66	29	60
	2	68	80	32	51	40	49	29	22
2	1	64	94	45	74	43	63	34	46
	2	56	93	44	67	38	47	21	23
3	1	59	87	53	69	58	76	15	70
	2	57	94	55	82	44	81	29	72
4	1	74	91	43	61	42	63	36	48
	2	84	55	59	42	70	40	57	31
5	1	62	79	40	55	44	64	37	52
	2	50	66	32	50	47	62	15	57
6	1	59	88	31	52	52	79	18	65
	2	52	91	32	52	50	81	29	65
7	1	48	74	31	50	55	86	39	74
	2	48	70	32	49	41	63	34	60
8	1	54	76	30	55	45	67	31	52
	2	50	84	32	55	45	76	39	62
9	1	66	91	49	77	50	66	45	60
	2	71	95	37	78	66	74	59	57
10	1	62	96	44	70	46	78	43	67
	2	57	95	39	72	50	77	43	67

Table 2: Average nMEP amplitudes for each participant and session

		Biceps		FDI	
		$mono_{PA}$	bi_{PA-AP}	$mono_{PA}$	bi_{PA-AP}
Participant	**Session**	**nMEP Amplitudes (%MVIC)**			
1	1	424.3	462.6	298.0	390.2
	2	649.7	909.0	332.5	38.4
2	1	346.0	128.5	811.0	103.2
	2	539.4	548.0	184.3	115.7
3	1	64.1	61.3	573.7	89.4
	2	126.2	198.9	42.2	600.8
4	1	19.4	17.3	119.9	116.1
	2	18.0	20.0	230.4	204.6
5	1	123.7	212.9	70.9	76.5
	2	209.0	238.0	391.7	131.9
6	1	59.8	58.6	86.3	129.7
	2	51.6	77.1	138.3	311.2
7	1	76.3	101.1	226.6	121.6
	2	129.7	84.6	127.3	290.6
8	1	68.1	66.6	149.9	165.9
	2	78.1	68.1	185.0	178.7
9	1	112.1	41.5	596.2	210.1
	2	115.6	81.0	75.9	216.6
10	1	298.2	157.8	747.3	834.0
	2	207.5	107.1	659.2	398.6

3.3 Discussion

The primary objective of this study was to determine the effect of stimulation type ($mono_{PA}$ vs bi_{PA-AP}) on RMT, AMT, and MEP amplitudes in the biceps in nonimpaired individuals. We also tested the effect of stimulation type on RMT, AMT, and MEP amplitudes in the FDI to provide context of our participant cohort in light of previous findings. We hypothesized that for each muscle (biceps and FDI), $mono_{PA}$ stimulation would result in lower RMT and AMT compared to the bi_{PA-AP} stimulation and the $mono_{PA}$ stimulation would result in greater nMEPs compared to the bi_{PA-AP} stimulation. This hypothesis was supported in the FDI, and is consistent with existing literature [5,7,33,35,49]. This hypothesis was partially supported in the biceps; RMTs and AMTs for the biceps were lower with $mono_{PA}$ stimulation compared to bi_{PA-AP} stimulation, but biceps nMEP amplitudes did not differ between the waveforms. Regarding our second objective of investigating reliability between two sessions, we hypothesized that the test-retest reliability of the FDI metrics would be greater relative to the biceps metrics. This hypothesis was not supported; there was no difference in reliability in motor thresholds due to muscle, and the biceps nMEP amplitudes had greater reliability than the FDI nMEPs. We also hypothesized that TMS metrics recorded via $mono_{PA}$ stimulation would have greater test-retest reliability relative to bi_{PA-AP} stimulation. This hypothesis was

partially supported in the biceps; $mono_{PA}$ stimulation had greater reliability relative to bi_{PA-AP}, albeit both were in the range of moderate reliability, for nMEP amplitudes, but equivalent reliability for motor threshold metrics. This hypothesis was not supported in the FDI; $mono_{PA}$ stimulation had similar reliability relative to bi_{PA-AP} for nMEP amplitudes and equivalent reliability for motor thresholds. Our results provide a framework for expected TMS metrics obtained with common stimulus waveforms when assessing the FDI or biceps. Although the FDI and biceps vary in cortical representation and have different volume and density of corticospinal neurons, our results suggest that current TMS waveform research on upper limb distal muscles is translatable to proximal muscles for motor thresholds but not for MEPs, and test-retest reliability and nMEPs are sensitive to difference in muscles and lack intermuscular reliability.

3.3.1 The effect of waveform on RMT, AMT, and nMEPs

Stimulus waveform affected the biceps and FDI motor thresholds similarly. RMTs and AMTs were lower with $mono_{PA}$ stimulation in both the biceps and the FDI compared to bi_{PA-AP}, suggesting that cortico-cortical connectivity in these regions have similar responses to stimulation [57]. However, biceps nMEPs were unaffected by the waveform type whereas FDI nMEPs were greater with $mono_{PA}$ compared to bi_{PA-AP} stimulation. This suggests that the neural architecture of instantaneous excitement [30] and corticospinal tract responsiveness [57] differs by motor control region.

3.3.2 The effect of waveform on MEP latencies

We reported the effect of stimulus waveform on MEP latencies because MEP latencies are typically less variable relative to MEP amplitudes [47] and latencies represent conduction velocity, which is relevant to neurorehabilitation. We found no effect of stimulus waveform on biceps MEP latencies, although many biceps MEPs were not assessed at suprathreshold intensities. However, even though our MEP latencies (average: 25 ms for proximal, 33 ms for distal) are slightly larger than current literature (average: 14 ms for proximal. 22 ms for distal), our distributions and difference in latencies between proximal and distal muscles are similar to previously reported [16,47,55]. Furthermore, our findings complement previous work [24] by showing differences in the effect of a PA current and a more complex waveform that stimulates multiple neuron pools [49]. While AP currents are associated with longer MEP latencies [24], a biphasic waveform activates both AP and PA-sensitive [49]. Therefore, the neuron pools associated with sensitivity to specific current direction could play a role in conduction velocity of the overall pathway. There was also no waveform effect on the MEP latencies in the FDI; $mono_{PA}$ and bi_{PA-AP} stimulation generated similar latencies within each muscle. However, there has been mixed results when comparing current direction and waveform effects on MEP latencies [11]. For example, no waveform effects were seen in MEP latencies when comparing $mono_{AP}$ and bi_{AP-PA} stimulation [7]. The mixed results, specifically for the bi_{PA-AP} waveform, is likely a result of the initial PA phase. Even though the second phase in biphasic stimulation is the strongest [18,49], the threshold is lower for PA current, relative to AP, given that facilitatory corticospinal pyramidal neurons are more sensitive to PA current [35]. The reduced threshold provides the PA current with the opportunity to stimulate cortical neurons quicker and

have a more prominent effect than the AP phase, even when the AP current is induced prior to PA current in bi_{AP-PA} stimulation [11,49]. Thus, generating mixed waveform effect results. Our MEP latencies between muscles were expected [14]; the FDI demonstrated longer latencies compared to the biceps for both $mono_{PA}$ and bi_{PA-AP} stimulation.

3.3.3 The effect of waveform on test-retest reliability

Test-retest reliability of TMS metrics has previously been studied on upper limb distal muscles comparing waveforms [5] and monophasic stimulation comparing upper limb distal and proximal muscles [43]. Our motor thresholds were consistent with previously reported results that demonstrated strong reliability between sessions for the FDI [4,5,30] and the biceps [43]. Our nMEPs, showing poor reliability in the FDI and moderate reliability in the biceps, were inconsistent with previous findings that demonstrated moderate to high reliability in the FDI [4,5,30], and poor reliability in the biceps [43]. Furthermore, our nMEPs had similar reliability between waveforms for both muscles, whereas opposing reliability between waveforms has been seen in the FDI [5]. It is well established that MEPs are highly variable [5,43] and current direction also influences the variability of MEPs [33]. This was supported by our post-hoc, in which nMEPs had unequal variance between sessions, whereas the more reliable motor thresholds exhibited equal variance between sessions for both muscles and waveforms. Additionally, similar to our results, reliability in the FDI has been reported to be less reliable than the biceps [25]. Potential contributions to the variability of MEPs across studies are differences in number of sessions [7,25], number of subjects [21,25], navigated stimulation [5,7], and suprathreshold intensity (i.e., determined via fixed portion of MSO [48] or via specific percentage of RMT [7]), to name a few. Our data suggests that target muscle is a major factor in the reliability of TMS metrics while at rest, while the direction of induced current in the brain does not significantly affect intramuscular reliability.

Preliminary investigation, while promising, suggests that current TMS waveform research on upper limb distal muscles is translatable to proximal muscles for motor thresholds but not for MEPs, despite differences in cortical representation, matching conventional expectations in both targets [5,35,43,49]. Additionally, test-retest reliability and nMEPs are more sensitive to these differences, and lack intermuscular reliability, resulting in conventional expectations not translating from the FDI to the biceps [5,35,43,49]. These findings can aid future, larger studies in optimizing TMS parameters to maximize the efficacy and reliability of TMS for clinical use. The efficacy of TMS for clinical use will be most notable when there is a deeper understanding in the effects waveforms and induced neuronal current directions have on outcome metrics and their role in monitoring recovery.

Chapter 4: Conclusions and Future Directions

Despite the many studies investigating the effects of stimulus waveform on TMS metrics, no prior work has compared the effect of $mono_{PA}$ and bi_{PA-AP} stimulation on

TMS metrics of a proximal arm muscle, such as the biceps brachii. Elucidating the effect of these two waveforms is needed considering bi_{PA-AP} is the most common waveform used to deliver rTMS protocols that aim to promote neuroplasticity, and $mono_{PA}$ is the most common waveform used to assess changes in excitability after rTMS. Further, the intensity of rTMS is commonly set as a percent of AMT, where AMT may be determined with either bi_{PA-AP} or $mono_{PA}$ stimulation. As clinicians and researchers design rTMS protocols targeting the biceps, it is important to known whether assessment of corticomotor excitability may differ by $mono_{PA}$ and bi_{PA-AP} stimulation, given that many rTMS devices only deliver biphasic stimulation. Use of a single stimulator to deliver TMS and assess TMS metrics is advantageous regarding study design and clinical feasibility.

4.1 Work Completed

The primary objective of this study was to determine the effect of stimulation type ($mono_{PA}$ vs bi_{PA-AP}) on RMT, AMT, and MEP amplitudes in the biceps and the FDI. A second objective was to determine the test-retest reliability of RMT, AMT, and MEP amplitudes for each waveform ($mono_{PA}$ and bi_{PA-AP}) and muscle (biceps and FDI). Twelve individuals were recruited for this study, but data from two participants were excluded because their RMT with bi_{PA-AP} stimulation could not be determined within the MSO range of our system. Participants were tested across two sessions, each consisting of both waveforms and both muscles. The expectation was that for each muscle (biceps and FDI) the $mono_{PA}$ stimulation would result in lower RMT and AMT compared to the bi_{PA-AP} stimulation and the $mono_{PA}$ stimulation would result in greater MEPs compared to the bi_{PA-AP} stimulation. Furthermore, we expected the test-retest reliability of the FDI metrics would be greater relative to the biceps metrics, and that metrics taken via $mono_{PA}$ stimulation would have greater test-retest reliability relative to bi_{PA-AP} stimulation in both muscles.

4.2 Key Takeaways

Stimulus waveform affected the biceps and FDI motor thresholds similarly. RMTs and AMTs were lower with $mono_{PA}$ stimulation in both the biceps and the FDI compared to bi_{PA-AP}, suggesting that cortico-cortical connectivity in these regions have similar responses to stimulation [57]. However, biceps nMEPs were unaffected by the waveform type whereas FDI nMEPs were greater with $mono_{PA}$ compared to bi_{PA-AP} stimulation. This suggests that the neural architecture of instantaneous excitement [30] and corticospinal tract responsiveness [57] differs by motor control region.

We found no effect of stimulus waveform on biceps MEP latencies, although many biceps MEPs were not assessed at suprathreshold intensities. However, even though our MEP latencies (average: 25 ms for proximal, 33 ms for distal) are slightly larger than current literature (average: 14 ms for proximal. 22 ms for distal), our distributions and difference in latencies between proximal and distal muscles are similar to previously reported [16,47,55]. There was also no waveform effect on the MEP latencies in the FDI; $mono_{PA}$ and bi_{PA-AP} stimulation generated similar latencies within

each muscle. Even though the second phase in biphasic stimulation is the strongest [18,49], the threshold is lower for PA current, relative to AP, given that facilitatory corticospinal pyramidal neurons are more sensitive to PA current [35]. Thus, generating mixed waveform effect results. Our MEP latencies between muscles were expected [14]; the FDI demonstrated longer latencies compared to the biceps for both $mono_{PA}$ and $bi_{PA\text{-}AP}$ stimulation.

Our motor thresholds reliability were consistent with previously reported results that demonstrated strong reliability between sessions for the FDI [4,5,30] and the biceps [43]. Our nMEPs, showing poor reliability in the FDI and moderate reliability in the biceps, were inconsistent with previous findings that demonstrated moderate to high reliability in the FDI [4,5,30], and poor reliability in the biceps [43]. Furthermore, our nMEPs had similar reliability between waveforms for both muscles, whereas opposing reliability between waveforms has been seen in the FDI [5]. Similar to our results, reliability in the FDI has been reported to be less reliable than the biceps [25]. Potential contributions to the variability of MEPs across studies are differences in number of sessions [7,25], number of subjects [21,25], navigated stimulation [5,7], and suprathreshold intensity (i.e., determined via fixed portion of MSO [48] or via specific percentage of RMT [7]), to name a few.

The work from this thesis showed two significant findings. First, current TMS waveform research on upper limb distal muscles is translatable to proximal muscles for motor thresholds but not for MEPs. Second, test-retest reliability and nMEPs are more sensitive to the differences in cortical representation between proximal and distal muscles of the upper limb and lack intermuscular reliability, resulting in conventional expectations not translating from the FDI to the biceps. These findings can aid future, larger studies in optimizing TMS parameters to maximize the efficacy and reliability of TMS for clinical use.

4.3 Limitations

One limitation of our study is that 60% of our biceps $bi_{PA\text{-}AP}$ RMT values were larger than 84% MSO, therefore suprathreshold $bi_{PA\text{-}AP}$ stimulation of 120% RMT to the biceps was not always attainable [3,4]. However, a post-hoc analysis determined that under stimulation did not affect our MEP data. Additionally, we did not record I-waves/D-waves which are a much more informative measurement regarding the effect of stimulus waveform [6]. However, measuring these is an invasive procedure adding to the complexity of the study. Furthermore, MEPs showed that our test-retest reliability contradicted previous reliability findings in the FDI [4,5,30] and the biceps [43]. We collected MEPs as they are a widely used noninvasive metric of corticomotor excitability, conventionally elicited from a suprathreshold stimulus that scales from a percentage of RMT [7,27,43,44]. It is well established however that MEPs are highly variable [5,43]. Efforts to reduce MEP variability are ongoing, with studies suggesting: the most ideal number of MEPs to record is 30 [46], removing the first few MEPs in any train [21], and recording MEPs during a slight voluntary contraction [43]. These

measures were not accounted for in this study, though, as they are in the early stages of proposal.

4.4 Future Directions

The findings from this study provide several opportunities for future research. First, additional studies are needed to help understand the variable responses that are associated with MEPs. These could be conducted through computational modeling or additional non-impaired studies which aim to develop a universal standard for the recording the most reliable MEPs. Several factors contribute the variability including: number of sessions [7,25], navigated stimulation [5,7], suprathreshold intensity (i.e., determined via fixed portion of MSO [48] or via specific percentage of RMT [7]), and various percentages of RMT [7,39,43]. While MEPs are highly variable, epidural recordings of I-waves and D-waves could provide a more informative measurement regarding the effect of stimulus waveform [6]. This could be of great benefit to the research community and clinicians if we can reduce the variability of MEPs across individuals and studies.

Furthermore, this study only utilized single pulse stimulation whereas future studies should research waveform effects on other types of stimulation including paired-pulse and rTMS on proximal muscles. Induced current direction in the brain has been shown to affect the plastic after-effects of rTMS [50] as well as inhibition and facilitation of paired-pulse stimulation [5], however this research is majorly studied in distal muscles of the upper limb. Traditionally, biphasic stimulation is used for delivering rTMS [48] and monophasic is used for paired-pulse protocols [5]. Additionally, monophasic is the most common waveform to assess changes in excitability following rTMS. Being that paired-pulse and rTMS are primarily implemented with one particular waveform, this future research would be beneficial because use of a single stimulator is advantageous regarding study design and clinical feasibility.

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Appendix A: Conference Abstracts

SB³C2021
Summer Biomechanics, Bioengineering and Biotransport Conference
June 14-18, Virtual

EFFECT OF STIMULUS WAVEFORM ON TRANSCRANIAL MAGNETIC STIMULATION METRICS IN PROXIMAL AND DISTAL ARM MUSCLES

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INTRODUCTION

Transcranial magnetic stimulation (TMS) is useful for modulating neural activity when applied repetitively or assessing neural pathway excitability when applied in single or dual pulse paradigms [1]. TMS devices generate one of two types of stimulus waveforms, monophasic or biphasic, which have dissimilar neuronal activation and therefore different impacts on stimulation characteristics [2]. To date, research on the effects of waveform has been conducted on upper limb distal muscles, such as the first dorsal interosseous (FDI). Research in the FDI has shown greater efficacy in the monophasic stimuli (when the induced brain current is in the posterior-anterior direction) demonstrated via reduced motor threshold's (MTs) [2]. This is likely a result of the 2nd phase in biphasic stimulation as the 2nd phase not only reduces the activation time and thus the effectiveness of the initial phase, but activates different neurons that have different sensitivity to each current direction [2]. Additionally, FDI research has shown that MTs and motor evoked potential's (MEPs) are inversely correlated due to their role in measuring intrinsic and synaptic plasticity, respectively [3]. However, the FDI has more available motor neurons than the biceps brachii (BB) at equivalent force levels [4]. Therefore, conclusions regarding neuroplastic effects drawn from the numerous TMS studies using upper limb distal muscles may not necessarily apply to other muscle groups, particularly upper limb proximal muscles. This presents a clinical need as TMS is increasingly being utilized for neurorehabilitation as an adjunct to conventional physical therapy, especially spinal cord injury (SCI) which affects entire extremities [5].

The purpose of this study is to determine the effect of TMS waveforms (monophasic vs biphasic) on important TMS metrics and determine if muscle groups (i.e. upper limb proximal vs distal) have any effect on these outcome metrics. We hypothesize that monophasic stimulation will result in lower MTs, both resting MT (RMT) and active MT (AMT), compared to biphasic [2], however the opposite will occur for normalized MEPs (nMEPs) resulting in greater nMEPs for monophasic compared to biphasic stimulation, for both muscles [3]. Furthermore, we hypothesize that an upper limb proximal muscle (BB) will have statistically different MTs, RMT and AMT, as well as nMEPs compared to the upper limb distal muscle (FDI) for both waveforms.

METHODS

Experimental Set-up: Ten individuals aged 25.0 ± 3.22 years (3 female, 7 male) were recruited for this study which was approved by the Institutional Review Board (IRB) at Virginia Commonwealth University. Surface electromyogram (EMG) electrodes were located over the primary target muscles (BB and FDI) before determining the cortical hotspot, maximum voluntary contraction (MVC), RMT, and AMT. TMS stimulation was applied via a Magstim® BiStim² stimulator (monophasic) and Magstim® Super Rapid² Plus¹ stimulator (biphasic), both via a Magstim® D70 Alpha flat (uncoated) figure-eight coil. The coil was oriented similarly for each waveform (i.e. perpendicular to the central sulcus) as to induce a biphasic posterior-anterior/anterior-posterior (PA/AP) current and a monophasic PA current in the motor cortex. RMT was determined as the lowest stimulus intensity that induced MEPs of ≥ 50 μV in at least 5 of 10 consecutive stimuli with the target muscle fully relaxed. With visual feedback provided, participants sustained an isometric contraction of 20 ± 2.5% MVC of the target muscle during which the AMT was determined as the stimulus intensity that elicited a MEP ≥ 200μV in at least 5 of 10 consecutive stimuli. Lastly, 10 single pulse MEPs were recorded at 120% RMT for each waveform.

Data and Statistical Analysis: Statistical analyses were performed using MATLAB (MathWorks (2020)) and GraphPad Prism. RMT, AMT, and nMEPs were compared between monophasic and biphasic waveforms using a two sampled t-test with unequal variance. Additionally, RMT, AMT, and nMEPs were compared between BB and FDI using an ANOVA with interactions.

RESULTS

BB RMTs were significantly lower for monophasic (60.1±9.06 %MSO) than for biphasic (84.4±11.02 %MSO) ($p<0.01$). FDI RMTs were also significantly lower for monophasic (48.4±8.2 %MSO) than for biphasic (67.9±11.94 %MSO) ($p<0.01$). BB AMTs were significantly lower for monophasic (39.2±9.3 %MSO) than for biphasic (60.9±11.4 %MSO) ($p<0.01$). FDI AMTs were also significantly lower for monophasic (34.1±11.7 %MSO) than for biphasic (55.5±14.7 %MSO) ($p<0.01$) (Figure 1).

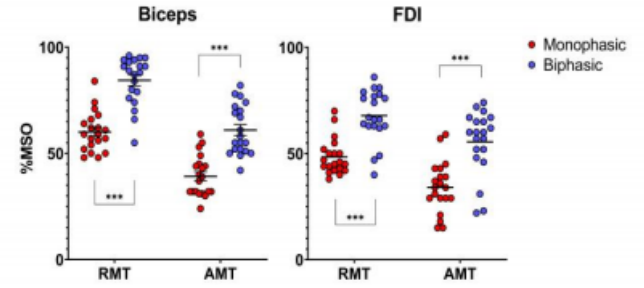

Figure 1: Left: Biceps MTs, are significantly greater for biphasic compared to monophasic. Right: FDI MTs, are significantly greater for biphasic compared to monophasic. Asterisks represent statistical difference ($p<0.01$) and error bars represent standard the error of the mean (SEM).

BB nMEPs present no significant difference between monophasic (6.20±19.55 µV%MVC) and biphasic (6.56±21.38 µV%MVC) ($p=0.88$). However, FDI nMEPs were significantly greater for monophasic (3.02±2.37 µV%MVC) compared to biphasic (2.36±1.91 µV%MVC) ($p=0.01$) (Figure 2).

An ANOVA revealed a statistical difference between BB and FDI for RMT ($p<0.01$) and nMEP ($p<0.01$), however, there was no significant difference for AMT ($p<0.01$). Additionally, there was no significant interaction amongst waveform and muscle for RMT ($p=0.31$), AMT ($p=0.95$), and nMEP ($p=0.65$).

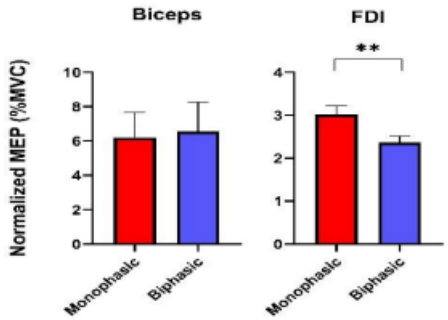

Figure 2: Left: Biceps nMEPs are not significantly different between monophasic and biphasic. Right: FDI nMEPs are significantly greater for monophasic compared to biphasic. Asterisks represent statistical difference ($p<0.01$) and error bars represent standard the error of the mean (SEM).

DISCUSSION

There were two key outcomes of this study. First, in agreement with the literature [2], using a monophasic stimulus led to lower MTs, both RMT and AMT, for the FDI and the BB. The difference in MT is likely related to the general organization of inhibitory and facilitatory corticospinal pyramidal tract neurons and the facilitatory neurons having higher sensitivity to the PA current direction [6]. However, this also contributes to the increased MT of the biphasic PA/AP stimulation. The 2nd AP phase not only reduces the activation time and thus the effectiveness of the initial phase, but activates different neurons that have different sensitivity to each current direction [2]. Additionally, the 2nd AP phase hyperpolarizes the membrane and reduces the number of sodium ion-channels available for depolarization [6]. While MTs reflect sodium ion-channels activity which may indicate intrinsic plasticity due to long-lasting changes in neuronal excitability, analyzing MEPs provides information about synaptic plasticity, caused by long-lasting attenuation of synaptic transmission [3].

Second, while we found a significant difference in FDI nMEP amplitude between waveforms, with monophasic having greater nMEPs than biphasic [3], we did not find a significant difference in BB nMEP amplitudes between waveforms. A possible explanation of this finding could be that more distal muscles not only have larger cortical areas, but the cortical areas are also denser than proximal muscles [7]. This, in combination with proximal muscles having weaker monosynaptic input [8], could lead to relatively greater biphasic nMEPs in the BB compared to the FDI. Furthermore, Turton, et al., discovered that at lower force levels a larger number of available motor neurons are recruited for upper limb distal muscles compared to proximal muscles where greater force levels were required for the same level of neuronal activation [4]. Being that our MEPs were recorded while at rest, this is another potential reasoning for the unexpected lack of significant difference between waveforms for BB nMEPs.

One limitation of our study is the small sample size. In order to fully understand these effects for the purpose of neurorehabilitation, this study would need to be further investigated in individuals with neurological impairment. However, our preliminary data suggests that regardless of the waveform, the neuroplasticity findings from FDI research, may not necessarily be applicable to all muscles, specifically the BB. This is rationale that further investigation needs to be conducted on the intrinsic and synaptic plasticity effects on additional muscles where TMS neurorehabilitation could be advantageous.

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Vita

Christopher Steven Lynch was born October 26th, 1993 in Durham, North Carolina where he was raised. He graduated from C.E. Jordan High School in 2012 and went on to attend Virginia Commonwealth University to pursue a bachelor's degree in engineering. Concluding his second year at VCU, he transferred to North Carolina State University to pursue a bachelor's in Biomedical Engineering. During his undergraduate studies, Chris worked on a new clinical solution to preexisting endotracheal tube cuff's which can leak during long-term intubation causing ventilator associated pneumonia. After graduating in spring 2017, Chris worked full time for then next year in the Brain Injury Translational Research Center at Duke University working on developing new approaches to patient monitoring that will integrate patient physiologic monitoring with brain activity recorded by electroencephalography (EEG). In fall 2018, Chris began attending VCU as a master student pursuing a degree in Biomedical Engineering. He joined the Rehabilitation Engineering to Advance Ability Lab (REALab) under Dr. Carrie Peterson to study the effects of stimulus waveform on transcranial magnetic stimulation metrics in proximal and distal arm muscles. While completing his graduate degree and research, Chris worked part time in VCU's Physiology & Biophysics Department as a lab/research technician assistant performing animal tissue histology, collecting images from an electron microscope, and image processing. He hopes to use these experiences in the future to aid him in developing innovative devices or software for neurorehabilitation.