Video Article

Utilizing Transcranial Magnetic Stimulation to Study the Human Neuromuscular System

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Abstract

Transcranial magnetic stimulation (TMS) has been in use for more than 20 years [1], and has grown exponentially in popularity over the past decade. While the use of TMS has expanded to the study of many systems and processes during this time, the original application and perhaps one of the most common uses of TMS involves studying the physiology, plasticity and function of the human neuromuscular system. Single pulse TMS applied to the motor cortex excites pyramidal neurons transsynaptically [2] (Figure 1) and results in a measurable electromyographic response that can be used to study and evaluate the integrity and excitability of the corticospinal tract in humans [3]. Additionally, recent advances in magnetic stimulation now allows for partitioning of cortical versus spinal excitability [4,5]. For example, paired-pulse TMS can be used to assess intracortical facilitatory and inhibitory properties by combining a conditioning stimulus and a test stimulus at different interstimulus intervals [3,4,6-8]. In this video article we will demonstrate the methodological and technical aspects of these techniques. Specifically, we will demonstrate single-pulse and paired-pulse TMS techniques as applied to the flexor carpi radialis (FCR) muscle as well as the erector spinae (ES) musculature. Our laboratory studies the FCR muscle as it is of interest to our research on the effects of wrist-hand cast immobilization on reduced muscle performance[6,9], and we study the ES muscles due to these muscles clinical relevance as it relates to low back pain[8]. With this stated, we should note that TMS has been used to study many muscles of the hand, arm and legs, and should iterate that our demonstrations in the FCR and ES muscle groups are only selected examples of TMS being used to study the human neuromuscular system.

Video Link

The video component of this article can be found at https://www.jove.com/video/3387/

Protocol

1. Single and Paired-Pulse TMS of the FCR and ES Muscles

1. **Basic Safety Precautions:** Prior to performing TMS on a human subject it is necessary to first screen them for basic safety precautions as it pertains to exposure to a magnetic field. In our laboratory we follow the screening guidelines set forth by the Institute for Magnetic Resonance Safety, Education and Research [10]. In our laboratory we also routinely exclude individuals with a family history of epilepsy seizures. We also require subjects undergoing TMS of the ES muscles to wear earplugs and a mouth guard due to the less focal and stronger stimulation intensities.
2. **Electrical Recordings:** To examine TMS responses in the motor system it is necessary to record electromyographic (EMG) signals from skeletal muscles. For the FCR muscle we place surface electrodes on the forearm using a bipolar electrode arrangement located longitudinally over the muscle on shaved and abraded skin as we have previously described [7,11]. For the erector spinae muscles we use a similar electrode arrangement located longitudinally over the muscles at the L3-L5 vertebral level on shaved and abraded skin [8].
3. **TMS Coil Orientation:** To predominantly activate corticospinal neurons transsynaptically it is necessary to position the TMS coil appropriately [12]. For the FCR muscles we place a 70-mm figure-of-eight TMS coil tangential to the scalp and 45-degrees to the midline so that the induced current flows in a lateral-posterior to medial-anterior direction. For the ES muscles we use a double-cone coil that has greater penetration depth and is needed due to the representation of these muscles being deeper in the homunculus. Here, the coil is positioned such that the current flows in an anterior to posterior direction. We have custom-modified our coil with a laser attachment system to assist us in subsequent re-positioning of the double cone coil.
4. **Identifying 'Hotspot'**: It is necessary to determine the stimulation location that elicits the largest motor evoked potential. For the FCR muscle we do this by subtly moving the TMS coil around in very small increments and determining where we observe the largest motor evoked potential amplitude. Once located we note this area with indelible ink on either the scalp or a lycra cap. TMS of the ES muscles is considerably more uncomfortable to human subjects than TMS of upper limb muscles. Accordingly, we have streamlined our TMS protocol for the ES muscles to increase it's tolerability and feasibility. Here, instead of locating the "hotspot" we use anthropometric measurements to identify the vertex of the skull. Specifically, we identify the vertex as the intersection of the skull in the sagittal (between the nasinon and inion) and coronal (between the tragus) planes.

5. **Biomechanical Positioning:** In our laboratory when we perform TMS of the FCR muscles subjects are seated with the arm resting in an extended position in a BioDex System 4 Dynamometer (Figure 2). However, we should note that this is only an example of one possible set-up for measuring exerted forces. For the ES muscles subjects are asked to sit with an upright posture while their hands rest in their lap (Figure 3). They are seated in a swivel base chair with the thigh at 90° relative to the trunk, the lower leg at ~45° relative to the thigh, and the lumbar spine in a neutral posture8.
6. **Quantifying Motor Threshold**: For the FCR, we determine motor threshold (MT) by delivering single pulses at gradually increasing stimulation intensities until motor evoked potentials have peak-to-peak amplitudes greater than 50 microvolts in more than 50% of trials (Figure 4). To streamline the TMS protocol and increase tolerability and feasibility we do not determine motor threshold in the ES muscles with the same precision as when we test the upper limb musculature. Rather, we begin the TMS protocol by delivering an initial single pulse at 50% of the maximum stimulator output to determine if this stimulus intensity is above or below motor threshold. If an MEP is observed at this stimulus intensity-defined as discernable MEP relative to the level of the background EMG-the intensity is reduced to 40% of stimulator output to determine if this stimulus intensity is sub- or supra-threshold [8].
7. **Quantifying MEP Amplitude using Single-Pulse TMS**: To examine the motor evoked potential amplitude of the FCR we deliver a single TMS pulse to the 'hotspot' at an intensity equal to 130% of motor threshold, and calculate the peak-to-peak amplitude. Generally, we normalize this outcome to the maximal compound muscle fiber action potential observed following supramaximal electrical stimulation of the median nerve. We should note that the MEP size is very dependent on the degree of cortical excitability. Accordingly, when the TMS pulse is delivered during a background contraction, when cortical excitability is increased, the MEP size will dramatically increase. For the ES muscles, we deliver a single TMS pulse to the vertex at an intensity 40 or 50% above the sub-motor threshold intensity [8]. Unfortunately, because peripheral nerves innervating the ES muscles are not accessible to electrical stimulation we are not able to normalize these motor evoked potentials to the compound muscle fiber action potential.
8. **Quantifying Silent Period Duration using Single-Pulse TMS**: When a TMS pulse to the cortex is delivered during a muscle contraction it will produce a motor evoked potential followed by electrical quiescence before activity resumes that is indicative of corticospinal inhibition and commonly referred to as the silent period [13] (Figure 5). To quantify the silent period we deliver a single TMS pulse to the 'hotspot' at an intensity equal to 130% of motor threshold while the study participant is performing a wrist flexion muscle contraction at 15% of their maximal strength. We have not previously quantified the silent period duration of the ES muscles; however, we should note that we have anecdotally observed its existence in this muscle group when the TMS pulse id delivered during a background contraction.
9. **Quantifying Intracortical Facilitation using Paired-Pulse TMS:** We use paired-pulse TMS to quantify intracortical facilitation [6,7] (Figure 6 and 7 represents this measurement for the FCR and ES muscles, respectively). For the FCR muscle we first determine the stimulus intensity needed to elicit a motor evoked potential that is between 0.5-1.0 mV. Next, we deliver a subthreshold conditioning pulse-which in our laboratory is commonly set equal to 70% of motor threshold- 15-msec before the suprathreshold test pulse. This conditioning pulse delivered at this time period prior to the test pulse will increase, or facilitate, the amplitude of the motor evoked potential more than a single unconditioned pulse of the same intensity. For the ES muscle group the conditioning pulse intensity is set to the observed sub-motor threshold intensity (either 40% or 50% of stimulator output) and the test pulse intensity is set to 40% above the sub-motor threshold level (80% or 90% of stimulator output)[8]. We should note that the intensity of the conditioning pulses could be varied depending on the objective of the study. Similarly, the pulse intervals can vary depending on the muscle and its location relative to the cortex.
10. **Quantifying Short-Interval Intracortical Inhibition using Paired-Pulse TMS:** We also use paired-pulse TMS to quantify short-interval intracortical inhibition [6,7] (Figure 6 and 7 represents this measurement for the FCR and ES muscles, respectively). Here, for both the FCR and ES muscles, the procedures are the same as described for measuring intracortical facilitation with the exception that the interstimulus interval between the two pulses is reduced to 3 msec. This conditioning pulse delivered at this time period prior to the test pulse will decrease, or inhibit, the amplitude of the motor evoked potential more than a single unconditioned pulse of the same intensity.
11. **Quantifying Long-Interval Intracortical Inhibition using Paired-Pulse TMS:** Delivering two identical suprathreshold test pulses that are separated by 100 milliseconds can also be used to assess long-interval intracortical inhibition [6,7]. In this case-for the FCR muscle- the motor evoked potential associated with the second pulse will be smaller, or inhibited more, than that associated with the first (Figure 8). We have not previously quantified long-interval intracortical inhibition in the ES muscles due to concerns over subject tolerability.

2. Representative Results:

Following the delivery of a suprathreshold TMS pulse, the muscles being stimulated should demonstrate an easily observable EMG response (the MEP) (illustrated in Figures 4-8). The latency between the stimulus onset and the MEP will vary between the muscle groups being examined, but for the FCR it is generally 16-19 msec (Figure 6) and for the ES it is 17-22 msec (Figure 7; although it should be noted that in some subjects definitive MEP onset in the ES muscles is more difficult to visually identify). It should be noted that when testing the ES muscle group several other muscle groups are also visibly and dramatically stimulated concomitantly (including the muscles of the lower extremity, which are represented within the same general region of the homunculus). During the measurement of intracortical facilitation the MEP amplitude is generally larger than that observed with a single unconditioned pulse (Figure 6 and 7). However, it is our experience that the degree of facilitation varies between muscles groups with some muscle groups-such as the FCR- showing only modest facilitation in many subjects. For the measurement of short-interval and long-interval intracortical inhibition a decrease in the MEP amplitude is generally observed in comparison to a single unconditioned pulse of the same intensity (Figures 6-8).

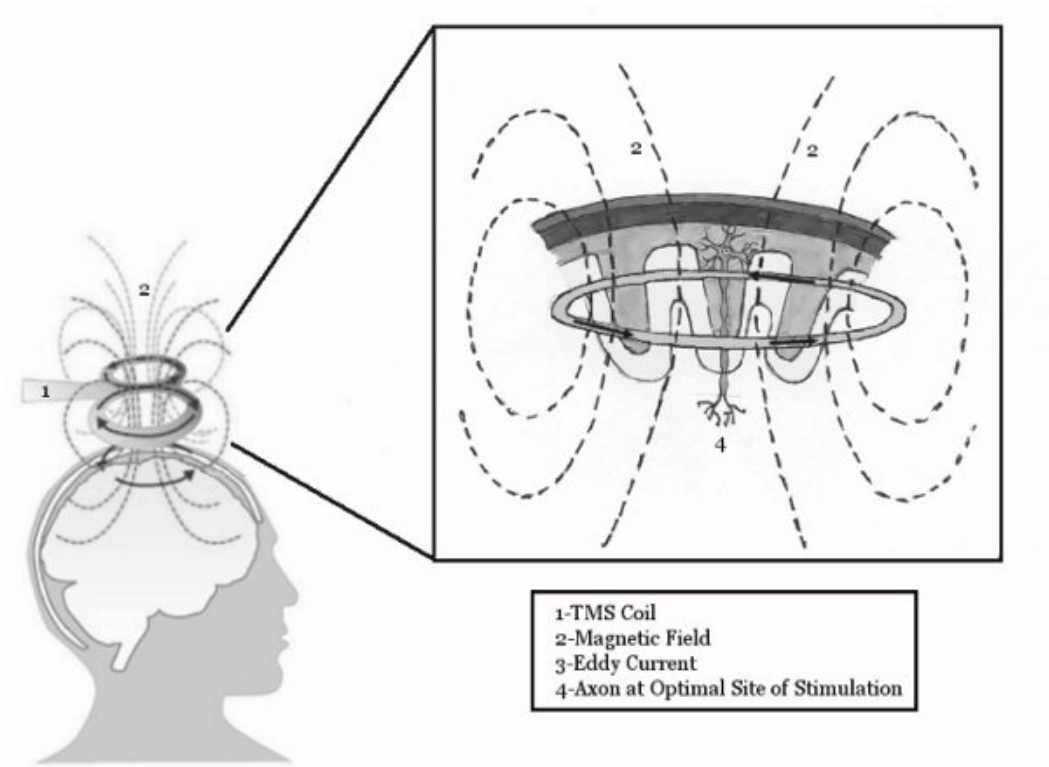

Figure 1. The basic mechanisms of TMS. The TMS coil induces a magnetic field, which penetrates the scalp and induces an Eddy current within the motor cortex. This eddy current is then able to stimulate neurons within the brain. Figure reprinted from McGinley and Clark, In Press[14].

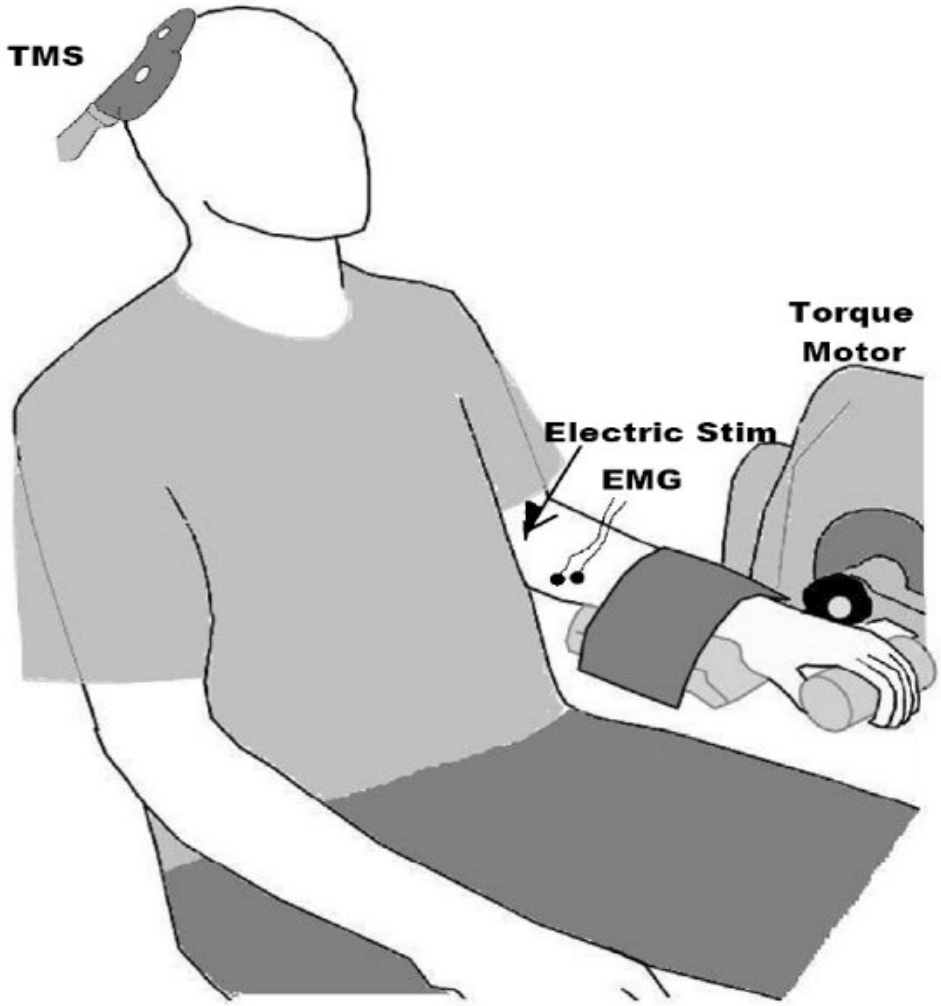

Figure 2. Setup for performing TMS on the FCR muscle. Note the recording of electromyogram (EMG) signals from the forearm, and the TMS paddle over the motor cortex. We generally also record muscle forces, and use electrical peripheral nerve stimulation to obtain the maximal compound muscle fiber action potential, as this is useful in interpreting amplitude values (e.g., one can express and MEP relative to the maximal muscle response as opposed to a absolute mV value which can be heavily influenced by non-physiologic factors such as subcutaneous adipose tissue). Figure reprinted from the following: Clark et al. 2008[9], Clark et al., 2010[6], and McGinley et al. 2010[7].

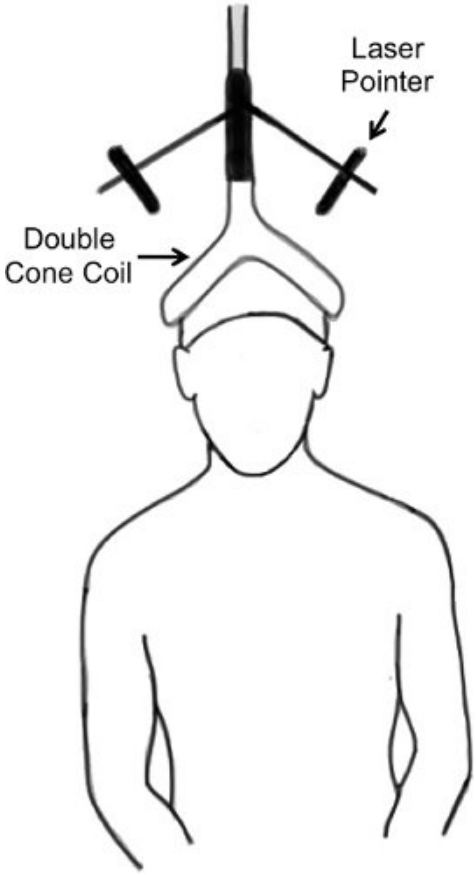

Figure 3. Setup for performing TMS on the erector spinale muscles. Figure reprinted from Goss et al. 2011[8].

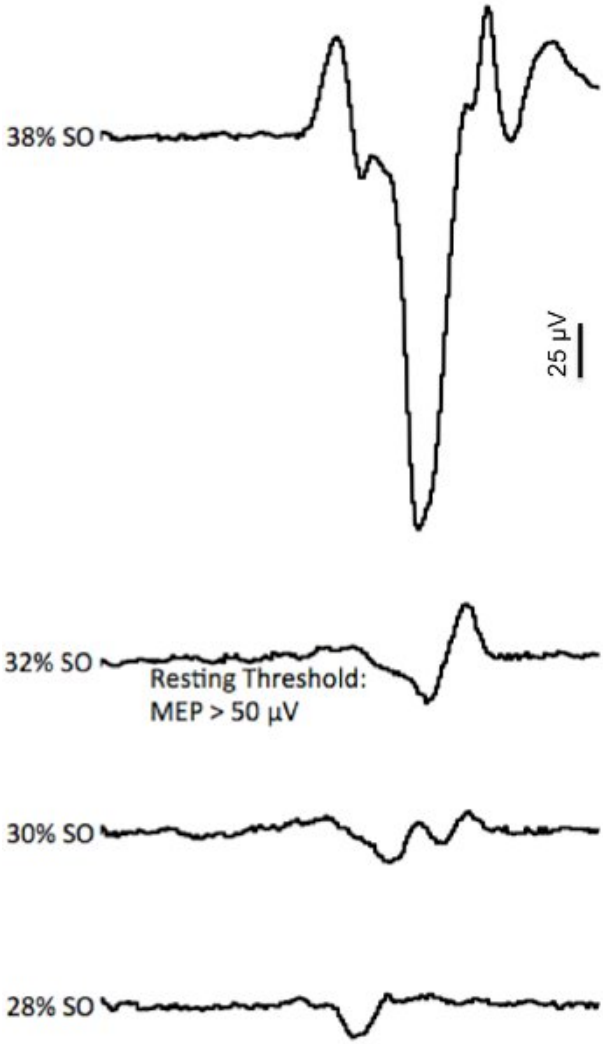

Figure 4. Example of the motor threshold determination. The EMG traces represent the motor evoked potential (MEP) response to gradually increasing stimulus intensities (represented as a percentage of stimulator output (SO)). Note that at the lower intensities (28-30% of SO) very small MEPs were elicited (sub-threshold), but that at 32% SO a MEP was elicited that reached motor threshold (typically defined as an MEP with a p-p amplitude > 50 µV).

Figure reprinted from McGinley and Clark, In Press[14].

Figure 5. TMS during a contraction: motor evoked potential & silent period. The silent period is observed when a subject performs a slight contraction and a single stimulus is applied to the motor cortex. The first part of the silent period is due to spinal cord inhibition and the latter part is attributed to cortical inhibition, specifically $GABA_B$ receptors. There is no consensus way to quantify the duration of the silent period, but our findings indicate that either defining it from stimulus onset or MEP onset to the return of the voluntary interference electromyogram signal is the most reliable[15].

Figure reprinted from Clark and Quick, 2011[16], and McGinley and Clark, In Press[14].

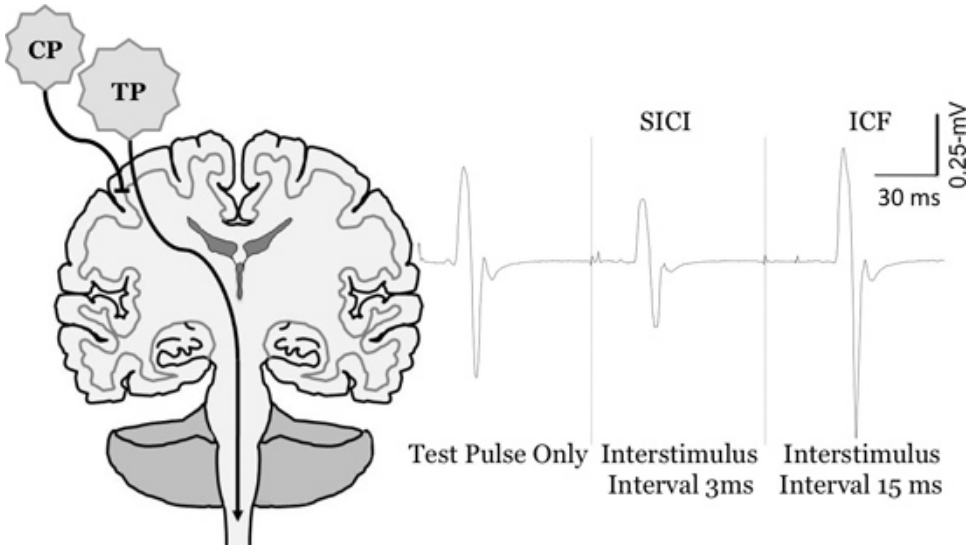

Figure 6. Change in motor evoked potential sized ith paired pulse TMS of the FCR muscle. Measurement of short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF). To quantify SICI and ICF the conditioning pulse (CP) is set below motor threshold, and the test pulse (TP) is set to evoke MEP's between 0.5-1 mV. At short interstimulus intervals (e.g., 3-msec) the CP inhibits the MEP in comparison to the TP only (SICI), whereas at longer interstimulus intervals (e.g., 15-msec) it facilitates the MEP (ICF).

CP: conditioning pulse, TP: test pulse Figure reprinted from Clark et al., 2010[6], McGinley et al. 2010[14], Clark and Quick, 2011[16], and McGinley and Clark, In Press[14].

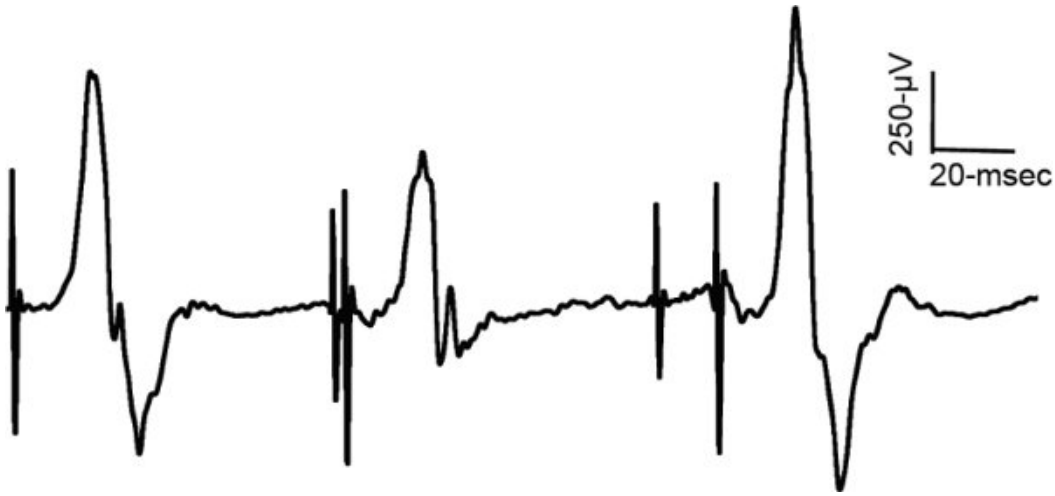

Figure 7. Change in motor evoked potential sized with paired pulse TMS of the ES muscle. Example of EMG traces from the erector spinae muscles and the measurement of short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF).
Figure reprinted from Goss et al. 2011[8].

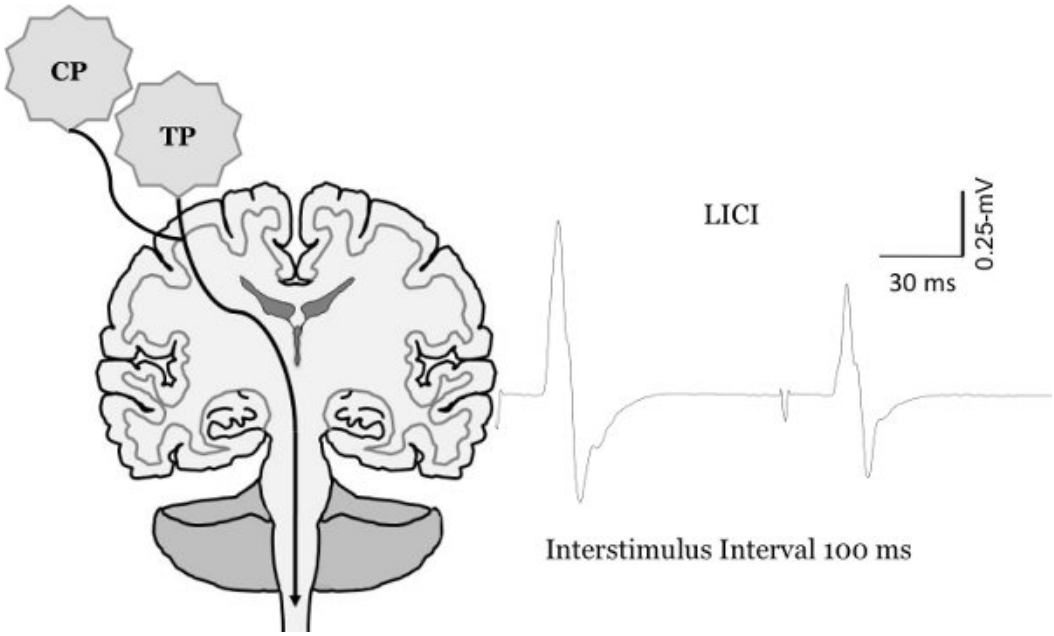

Figure 8. Change in motor evoked potential sized with paired pulse TMS. Measurement of long-interval intracortical inhibition (LICI). To quantify LICI two test pulses are delivered at an interstimulus interval of 100-msec. This results in the second MEP being inhibited in comparison to the first MEP.
Figure reprinted from Clark et al., 2010[6], McGinley et al. 2010[7] and McGinley and Clark, In Press[14].

Discussion

The overall goal of this article is to provide scientists and clinicians a visual account of our laboratories use of transcranial magnetic stimulation. However, in addition to providing a visualization of these experiments, below we discuss basic issues to consider when performing TMS in this manner, provide a brief overview of the physiology of TMS responses, and also discuss our use of TMS with regards to the usage of others.

General Issues To Be Aware of When Performing TMS As Described in the Article

There are several issues to be aware of when performing paired-pulse TMS. For instance, the Magstim BiStim[2] system—likely the most popular TMS equipment line— offers the potential to combine two Magstim 200[2] units and permit paired-pulse stimulation through a single stimulating coil. However, it should be noted that when one is evoking MEPs with the unconditioned pulse it is best to set one of the MagStim units to "0%" and still indicate an interpulse interval (e.g., 100 msec) as opposed to turning the unit off. Reason being is that the BiStim[2] system when one of the units is not on summates the two single pulses provided by the Magstim stimulators to produce a single high power pulse equal to 113% of a single Magstim 200[2]. Thus, when one is using the unconditioned pulse for normalization to potentials elicited with paired-pulse TMS it is critical that the test pulse intensities be held constant in this regard.

Issues To Be Aware of When Performing TMS On The Erector Spinae Muscles

With regards to the TMS procedures for the ES muscle group there are several specific issues and limitations to mention. For instance, the pulse intensities used in our protocol are not being expressed relative to motor threshold. In single- and paired-pulse TMS studies in appendicular muscles it is common for motor thresholds to be defined within a relatively small range (e.g., 1–3% of stimulator output), and conditioning and test pulses being expressed relative to threshold levels (e.g., conditioning pulses equal to 70% of motor threshold) [17]. We generally choose not to perform a protocol of this nature due to the additional number of pulses that would be required to precisely determine motor threshold. TMS of upper extremity muscles is generally highly tolerable, and isolates the twitch response to the targeted limb segment. Conversely, TMS of the lumbar paraspinal muscles is considerably less tolerable. We have previously reported that our streamlined protocol is bearable to most subjects (~5 on a scale of 0–10 with 10 being intolerable). Similarly, we generally choose to further limit the total number of TMS pulses for the ES muscle group by stimulating directly over the vertex to permit recording of bilateral responses. This stimulation site has been used in previous TMS studies of the lumbar paraspinal muscles [18-22]. However, we should note that vertex stimulation may not be the optimal site for evoking lumbar MEPs as recent findings indicate that the optimal site for evoking responses in the contralateral lumbar paraspinal muscles is situated 1-cm anterior and 4-cm lateral to the vertex [23]. Lastly, we should note that it is our experience that tightly controlling for biomechanical/postural positioning of the lumbar spine is critical to obtaining reliable TMS data from the ES muscle group. In our pilot work we examined responses in many different postural positions, but found that our best responses were obtained with the subject seated as illustrated in the video article.

Physiology of Single Pulse TMS Outcome

Single pulse TMS, as the name implies, involves the delivery of one magnetic pulse to the brain and recording and examining the resultant EMG response. The method has proven incredibly useful for testing the integrity of the entire neuromuscular tract. In general this method is used to deduce variables such as motor threshold, motor evoked potential amplitude, and silent period duration which all give insight into the excitability

of the neuromuscular system. Although this technique has allowed researches to understand a great deal about the neuromuscular system it does have some disadvantages, which will be addresses throughout this section.

Motor threshold is defined as the lowest intensity needed to evoke a MEP in the contralateral muscle group of interest when a single pulse is applied to the motor cortex [3]. After the "hotspot" (the location where the largest MEP is observed) has been found, MT is determined by slowly increasing the intensity of the pulse applied to the motor cortex until an MEP is reliably elicited. In general, most investigators define threshold of resting muscles as the stimulus intensity needed to evoke MEP's with a peak-to-peak amplitude that is greater than 50 μV in 50% of trials (e.g., in 5 of 10 trials) [3]. This value can also be defined during contraction ('active MT') if state–dependent measures are of interest. Here, MT is generally defined as a given proportion of the background EMG activity (e.g., 2x above background), or an absolute amplitude (e.g., 300 μV). Resting MT is influenced by the orientation, density, and electrical susceptibility of the cortical neurons. As such, alterations in resting MT can reflect changes at a variety of levels [i.e., the neural membrane, axonal electronic properties, the structure and number of excitatory projections onto the primary motor cortex, or upregulation of receptors in this region [24] and hence represents a global assessment of the membrane excitability of pyramidal neurons [24,25]. With regards to active MT, voluntary contraction results in a reduction in motor threshold compared with resting conditions, which is thought to be indicative of the magnitude of voluntary motor drive to the corticomuscular pathway [26].

MEP amplitude is another outcome measure indicative of excitability. When TMS is applied to the motor cortex at an intensity above MT, high-frequency indirect waves (I waves) are elicited in the corticospinal tract [27], which are modifiable by many mechanisms including neurotransmitters (i.e., glutatmate, GABA), modulators of neurotransmission (i.e., acetylcholine, norepinephrine, and dopamine) [25], and interneurones contacted by corticospinal tract cells [28] with the actual efficacy of the corticomotoneuronal synapse itself demonstrating some activity-dependent changes [29] all functioning to influence the amplitude of the MEP. As such, the amplitude of the signal can be modulated at both the cortical and spinal levels it is difficult to parse out specifically where, spatially within the nervous system, a change has occurred or a difference exists. Reduced or increased MEP amplitudes can be indicative of changes within the neuromuscular system and can be associated with specific disease processes [3]. Another way of assessing corticospinal excitability via single pulse TMS is through the development of a recruitment curve (or an input-output curve). Here, stimulus intensity is gradually increased and the resultant change in MEP amplitude is plotted. This curve indicates that there is a core group of neurons that are needed for motor threshold, but there are additional neurons that can be recruited to increase the response in the muscle [30].

Another relatively common outcome derived from single pulse TMS is the corticospinal silent period. Delivering a magnetic pulse to the cortex during a muscle contraction assesses the silent period. This pulse produces the characteristic MEP as previously mentioned followed by electrical quiescence before activity resumes that is indicative of corticospinal inhibition and commonly referred to as the corticospinal silent period. While there is some controversy about the best method of quantifying the silent period [31], it has proven to be a useful scientific tool for understanding physiological mechanisms along with clinical diagnostic potential [32-34]. The physiological mechanisms underlying the silent period are not fully understood, but include inhibition in both the motor cortex and spinal cord. The first part of the silent period (50-60 ms) is attributed to mechanisms within the spinal cord such as activation of Renshaw cells [3,35]; whereas the latter portion has been attributed to cortical mechanisms, specifically γ-aminobutyric acid (GABA) type B receptor mediated inhibition. Data for these physiologic underpinnings are based on findings that the administration of tiagabine, an inhibitor of GABA uptake from the synaptic cleft into neurons, results in a shortening of the silent period [35]. Accordingly, these findings suggest that blockage of GABA within the motor cortex leads to decreased inhibition. Although the silent period is a useful measurement of inhibition it does have some pitfalls. The greatest downfall of measuring the silent period is that if changes are discovered their spatial localization is difficult to ascertain as it contains both cortical and spinal components. Despite the inability to use this value to localize plastic adaptations or lesions it is still a good reflection of inhibition within the neuromuscular tract.

Physiology of Paired Pulse TMS Outcomes

Similar to single pulse TMS, paired-pulse TMS can be used to ascertain excitatory and inhibitory properties of the neuromuscular system. The main difference between paired and single pulse techniques are that paired-pulse experiments are generally thought to more precisely measure intracortical properties. The primary values that are evaluated are short intracortical inhibition (SICI), long intracortical inhibition (LICI), and intracortical facilitation (ICF). In paired pulse TMS two stimuli are applied to the motor cortex and depending on the interstimulus interval and stimulus intensity various excitatory and inhibitory responses will be observed. In addition, paired-pulse TMS can be used to investigate interhemispheric inhibition and facilitation using a similar paradigm.

After the hotspot and motor threshold have been determined, SICI is elicited by applying a subthreshold pulse (e.g., 70-95% below threshold), and 2-4 ms later applying a suprathreshold pulse. The advantage of this technique is that the first stimuli activates intracortical neurons, but does not activate lower motor neurons in the spinal cord. The average amount of inhibition observed is 20-40% of the unconditioned MEP [3]. Based on a variety of pharmacological studies it is suggested that the underlying mechanism of SICI is $GABA_A$ mediated inhibition. For example, administration of $GABA_A$ agonists (e.g.,lorazepam) increase SICI, and administration of GABA re-uptake inhibitors (e.g., tiagabine) decrease SICI [25]. SICI has an advantage over the single pulse measurement of the silent period because the level of inhibition can be localized to the primary motor cortex.

The measurement of ICF is virtually identical to that used in assessing SICI, except that the interstimulus interval is longer (e.g., 10-25 msec). By simply increasing the inerstimulus interval the second MEP evoked is facilitated 20-30% above an unconditioned suprathreshold stimulus MEP [3], although it is our experience that the degree of facilitation varies between respective muscle groups being examined. ICF represents a balance or combination of increased facilitatory and decreased inhibitory properties. Pharmacological studies have observed that both N-methyl-D-aspartate (NMDA) antagonists and $GABA_A$ agonists decreased ICF [25]. These findings indicate that ICF is mediated by glutamate facilitation via NMDA receptors, but this process is tempered through $GABA_A$ inhibition, suggesting that SICI and ICF are not mutually exclusive.

LICI is another indicator of intracortical inhibition, but this paired pulse paradigm has two major differences in comparison with SICI and ICF. Not only is the interstimulus interval increased (e.g., 50-200 msec), but both pulses are suprathreshold. Similar to SICI, the physiological mechanism is mediated through GABA, but in LICI the inhibition is thought to occur mainly thru $GABA_B$ receptors as opposed to $GABA_A$ receptor inhibition as seen in SICI. Pharmacological studies administering baclofen, a $GABA_B$ agonist, have observed an increase in LICI and a decrease in SICI indicating they are mediated by different receptors, but are interrelated [36]. It has been proposed that LICI increases from activation of

post-synaptic $GABA_B$ receptors and SICI is decreased from activation of pre-synpatic $GABA_B$ receptors that decrease the release of GABA [36]. Therefore, these findings suggest the LICI and the latter portion of the silent period are mediated by similar mechanisms, $GABA_B$.

Comparing and Contrasting Our Use of TMS to That of Others

In this article we have demonstrated single and paired pulse TMS applied to study the muscles of the forearm and lumbar spine; however, we should note that many scientists and clinicians (including our own group) have used TMS to study other muscles of the hand, upper arm, leg, etc. So, the visual presentation herein is simply meant to be an example of methodological approaches used in TMS research, as opposed to a comprehensive overview of its uses. Similarly, TMS can be used to assess other parameters not presented in this article. Some of these are presented and discussed below.

Interhemispheric Facilitation and Inhibition: A different application of paired-pulse TMS involves a subthreshold stimulus being applied to the motor cortex and then a suprathreshold stimulus applied to the opposite motor cortex, which allows for the investigation of interhemispheric interactions. Both interhemispheric facilitation (IHF) and interhemispheric inhibition (IHI) can be observed, but IHI is a stronger response. IHF does not have a well-defined protocol or mechanism, but it has been observed at interstimulus intervals of 4-8 ms [4]. IHI can be elicited at a wide range of interstimulus intervals (6-50ms), and it is partially mediated by $GABA_B$. Pharmacological studies administering baclofen, a $GABA_B$ agonist, specifically suggest that longer interval IHIs were mediated by postsynaptic $GABA_B$ receptors [4]. In general, the paired pulse technique can be used to study a large variety of variables that provide insight into intracortical and interhemispheric properties.

Repetitive TMS: Repetitive TMS (rTMS) can also be used to study the human neuromuscular system. This uses the same basic experimental set-up as single pulse TMS, but a series of stimuli at a fixed intensity are applied to the motor cortex and the effects on variables such as MEP amplitude and silent period are measured. The parameters for rTMS can be manipulated by changing the intensity, frequency, number, and length of the stimuli. In general there are two types of frequencies, high (>1Hz) or low (<1 Hz), which are associated with two types of post-synaptic, long-term plasticity [37]. High frequency pulses are generally given intermittently over a period of time (e.g., 100 trains at 100 Hz every 10 s for ten trials); whereas low frequency stimulation is given continuously over a period time (e.g., 1Hz for 20-30 minutes) [34]. When the stimuli are applied repetitively to the cortex it results in a temporal summation leading to a greater change in cortical activity than a single pulse [38]. rTMS has a great deal of potential in clinical situations that will discussed further in the clinical application section. The physiological mechanisms behind high and low frequency TMS are poorly defined, but are generally thought to reflect long term potential (LTP) and long term depression (LTD) respectively. One study by Chen and colleagues indicated that rTMS at low frequencies (900 pulses at 1 Hz) in humans resulted in changes in MEP amplitude, motor threshold, and excitation spread reflecting a depression of cortical excitability [39]. Another study using hippocampal slices from rats observed that high-frequency rTMS (10 trains of 20 pulses at 100Hz with 1 s intervals, 5 repetitions with 10 s intervals, OR 3 trains of 100 pulses at 100Hz with 20 s intervals) induced LTP changes that were directly correlated with NMDA activity [40]. In general it is thought that NMDA receptor activation, postsynaptic depolarization, increased intracellular calcium concentration, and GABA mediate LTP and LTD [34,39,40], but more research is needed to fully define the mechanism of rTMS.

Cervicomedullary Evoked Potentials. Magnetic stimulation applied over the back of the head using a double-cone magnetic stimulator can be used to activate the spinal tracts and evoke motor responses. The motor responses, commonly referred to as cervicomedullary evoked potentials (CMEPs), are of particular interest to scientists interested in segmental behavior of the motor pathway as they have a large monosynaptic component and as such can be used to test alpha-motorneuron excitability [41].

Eliciting CMEPs is technically challenging, as the evoked responses are relatively small in amplitude. In general, responses are best seen with the coil positioned with its central section over or near the inion and with the current directed downward [41]. However, in some individuals CMEP responses are not observed most likely due to anatomical differences resulting in the limit of effective stimulation not being achievable as the magnetic pulse intensity decays by the square root of the distance. However, with appropriate training and skills, laboratories experienced with performing cervicomedullary junction stimulation have reported high levels of day-to-day reliability (r=0.87) [42]. Coupling two magnetic stimulators in series will allow for a stronger overall pulse, which can be advantageous when attempting to elicit CMEPs. Additionally, using voluntary contraction to increase the excitability of the alpha-motorneuron pool can enhance the probability of obtaining responses. It should be noted that while cervicomedullary magnetic stimulation is considerably less painful than electrical stimulation, it does activate the muscles in the head and neck and some subjects find this experience to be uncomfortable.

Cortical Mapping. Since 1991, TMS evoked motor responses have also been used to map brain functions in a direct stimulus/evoked response manner previously only possible during invasive surgery when the surface of the brain was exposed [43-45]. During cortical mapping, a grid is placed on the scalp (e.g., a swim cap with a grid pattern) and the MEP amplitudes evoked at numerous sites are determined and the values are plotted to create a 3-dimensional representation between spatial location (x and y axis') and MEP amplitude (z-axis) [46]. These cortical maps provide three pieces of information: the total *area* on the scalp from which MEP's for the target muscle were recorded, the "hot spot" for a muscle, and the amplitude weighted *center of gravity* (*COG*) [47]. The COG corresponds to the center of the TMS map or the scalp location/ topography where the most neurons can be activated for a muscle or a movement, which may or may not be equivalent to the hot spot [46,48]. Shifts in the location of COG (medial lateral or anterior posterior directions) are commonly suggested to demonstrate cortical reorganization or plasticity in response to injury, spontaneous recovery, or due to rehabilitation intervention [48,49].

These cortical maps, while insightful, need to be interpreted cautiously. Although the stimulation protocol is similar to the principles used by Penfield, it is important to recognize that the maps created using this technique do not compare in precision to maps created using intracortical microstimulation [46,48]. Animal studies have demonstrated that individual corticospinal neurons innervate several motor neuron pools and thus different muscles and corticospinal neurons that innervate a particular muscle are distributed among other corticospinal neurons projecting to different muscle combinations [50,51]. This mosaic somatotopy of the cortex and the overlapping spinal cord projections in combination with the lack of stimulus precision with TMS means that multiple muscles will respond to a single TMS pulse delivered at one point on the scalp matrix [46]. The maps usefulness can be further confounded by electrode placement that permits cross talk, or signals evoked at the same time from other muscles, to interfere with the specificity and quality of the recorded MEP [47].

Conduction Time. Central motor conduction time is defined as the latency difference between the MEPs induced by stimulation of the motor cortex and those evoked by spinal (motor root) stimulation. It is calculated by subtracting the latency of the potential induced by spinal stimulation

from that of cortical stimulation [3]. When a TMS coil is placed over the back of the neck or lumbosacral spine, the magnetic pulse will stimulate spinal roots but not the descending spinal tracts themselves [3]. Accordingly, central motor conduction time likely includes the true time for central motor conduction plus at least one synaptic delay at the spinal level and time from the proximal root to the intervertebral foramen.

Paired Associative Stimulation. Paired associative stimulation (PAS) is a technique that involves stimulation of a peripheral nerve, and TMS stimulation of the motor cortex [30,52]. The two stimuli are applied at a regular interval so that they result in a synchronous response in the motor cortex. Depending on the length of the interval the stimuli will either facilitate or inhibit each other [30,52]. For example, when a stimulus is applied at the median nerve and then 25 ms later at the motor cortex the stimuli facilitate each other resulting in a long-term potentiation (LTP) like response [30]. Conversely if the stimulus interval is only 10 ms the TMS stimulus inhibits the peripheral nerve stimulation resulting in a long-term depression (LTD) response [30]. Due to these responses, PAS is often used to help model brain plasticity. Furthermore, studies using NMDA receptor antagonists showed that the LTP type responses in PAS can be blocked, which further supports its use as a plasticity model [52]. PAS also has a few clinical applications, such as stroke rehabilitation, but is not currently used as widely as rTMS [52].

Clinical Applications. TMS also has clinical utility for diagnosing and treating selected neuromuscular conditions. Techniques such as single and paired pulse techniques are being used by researchers to further understand the pathophysiology of a variety of diseases and many with the hope of finding new diagnostic criteria. Similarly, TMS is being used to aide in the diagnostic process by helping clinicians and researchers differentiate between diseases with similar presentations. Finally, a great deal of research is focused on investigating the utility of rTMS as a therapeutic strategy. This section will discuss the clinical uses of TMS focusing on idiopathic Parkinson's disease, stroke, primary dystonia, amytotrophic lateral sclerosis (ALS), and multiple sclerosis (MS).

There are a variety of single and paired pulse TMS values that have the potential to be used in the diagnosis of a variety of neuromuscular disorders. Each neuromuscular disorder has a distinctive set of TMS findings that may be useful in further elucidating pathophysiology, diagnosis, and differentiating disorders with similar clinical presentations. Although there have been no definitive findings, there is potential for TMS to help distinguish between Parkinsonian conditions (eg. Parkinson's disease, corticobasal degeneration), and primary and secondary dystonia [34]. Similarly, TMS has the potential to help determine the prognostic outcome for some neuromuscular conditions. For example, a good prognostic factor following stroke is the presence of MEPs in the paretic limb when the affected hemisphere is stimulated [33,52]. In general, a great deal of research still needs to be conducted to determine the utility of TMS in the diagnostic process, but current findings suggest it has potential.

In addition to diagnostic possibilities, a great deal of attention has been given to rTMS as a potential therapeutic tool. One of the most studied diseases is Parkinson's disease. A few studies observed an improvement in the Unified Parkinson's Disease Rating Scale (UPDRS) after sub-threshold rTMS at high frequency to the motor cortex [30,34]. These findings ranged from a 15% to 50% improvement in measured outcome that lasted up to 1 month [34]. Unfortunately, the current research is inconclusive because there is a great deal of variability in protocols that makes it difficult to elucidate the true value of rTMS as a therapeutic method [3,32,34]. A handful of studies have investigated the affects of rTMS on dystonia with promising results. Most of these studies used 1Hz rTMS applied to the primary motor cortex and observed improvement in symptoms that lasted for a couple of hours to months after a single session [30,34,53]. Although these are promising results, more research needs to be conducted to confirm these findings and investigate the potential of multiple-session rTMS.

There have been several rTMS approaches in stroke rehabilitation. Studies have stimulated both the affected and unaffected hemispheres in hopes of facilitating recovery of the affected hemisphere. In most of these studies there was significant improvement on disability scores and an overall short-term improvement in motor function [3,30,52,54]. As with most rTMS methods, larger scale, controlled, and long-term studies need to be performed to fine tune the protocol and determine the therapeutic potential. However, the promise demonstrated in this brief review of rTMS as a therapeutic tool, warrants the need for these large-scale studies to assess its efficacy.

Conclusions

In summary, in this article we have sought to first provide a visual account of basic TMS procedures—at least as employed by our laboratory. Additionally, we have sought to highlight and discuss other scientific and clinical uses of TMS at it relates to the human neuromuscular system. As TMS is growing exponentially in popularity—and hopefully as research continues— new uses and techniques will be implemented to further our understanding of the neuromuscular system.

Disclosures

No conflicts of interest declared.

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