Wind-Up of Spinal Neurons Contributes to
Supramaximal Volitional Torque in Human Spinal Cord Injury

BY

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THESIS

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DEDICATION

To my sister, Michelle
ACKNOWLEDGMENTS

First, I would like to thank my wife, Rachel. I cannot imagine seeing this process from the outside, it is humbling to have such an amazing wife – I am madly in love you. And my parents, Richard and Nancy, I could not have fathomed any of this without your love and support.

I would also like to thank my committee members: Dr. Corcos, the time you spent working on my grant applications meant the world to me and changed how I view science. Dr. Hasan, your clarity of thought and presentation style is inspirational and I will forever strive to emulate this. Dr. Heckman, I truly appreciate your support and enthusiasm, and I am looking forward to the future. Dr. Rymer, you have founded an amazing research center and I am so grateful I was given the opportunity to benefit from your hard work. And Dr. Hornby, this work could not have happened without your support. Any future success I may have is a direct reflection of your efforts.
To the reader: the following work represents my attempt to describe the finding of increased torque production during fatiguing contractions (i.e. supramaximal volitional torque generation) in patients with chronic motor incomplete spinal cord injury. This involved synthesizing multiple content areas including motor control, fatigue and potentiation, and spinal cord injury. At the outset of this process, it quickly became apparent that these fields were not speaking the same language and this hindered my ability to synthesize these ideas at a very basis level. In an attempt to overcome this, I sought to unify these concepts under a single framework. I look forward to revising and extending this framework over the coming years and would appreciate any feedback you may have.
“In my opinion, there are no universally accepted definitions of the segmental motor system.”

Dr. Douglas Stuart

Personal communication
June 23, 2011
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<tr>
<td>ADP</td>
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<td>AMPA</td>
<td>alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid</td>
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<td>ANOVA</td>
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<td>ATP</td>
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<td>SERCA</td>
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<td>SSRI</td>
<td>Selective Serotonin Reuptake Inhibitor</td>
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<td>T</td>
<td>Thoracic</td>
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<tr>
<td>TA</td>
<td>Tibialis Anterior</td>
</tr>
<tr>
<td>TES</td>
<td>Transcranial Electric Stimulation</td>
</tr>
<tr>
<td>TMS</td>
<td>Transcranial Magnetic Stimulation</td>
</tr>
<tr>
<td>US</td>
<td>United States of America</td>
</tr>
<tr>
<td>VL</td>
<td>Vastus Lateralis</td>
</tr>
<tr>
<td>VM</td>
<td>Vastus Medialis</td>
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Spinal cord injury (SCI) is a debilitating disease process that results in profound sensorimotor impairments. Deficits in volitional motor control generation are particularly devastating as the severity of impairments in torque generating capability, particularly of the knee extensors, are a primary determinant of walking ability in individuals with motor incomplete SCI. In contrast to previous research indicating greater fatigue following SCI using electrically stimulated contractions, recently published data indicates individuals with motor incomplete SCI are able to generate supramaximal torque during repeated maximal volitional effort contractions, with little evidence of fatigue. Such findings could potentially augment volitional force and contribute to gait improvements, although the mechanisms are poorly understood. As multiple sites across the segmental motor system may contribute to fatigue and/or potentiation, the goal of this thesis is to understand the mechanisms that contribute to this supramaximal volitional torque generation in humans with incomplete SCI.

To this end, three electrophysiological and/or pharmacological experiments were performed on a total of 20 individuals with incomplete SCI. Data from the first experiment suggest supramaximal volitional torque generation is associated with increases in spinal neuron excitability, rather than alterations within peripheral structures, including neuromuscular transmission or excitation-contraction coupling. The second experiment demonstrates how a history of volitional activation will modulate both supramaximal volitional torque generation and increase short latency tendon reflexes. The third experiment demonstrates how serotonergic medications produce widespread alterations in both reflexive
and volitional activation of the motor system, though serotonin induced co-contraction may limit volitional force production.

These findings are consistent with the windup of spinal neurons during repeated maximal effort contractions in humans with incomplete SCI, possibly through intrinsic neuronal properties and/or their modulation by descending monoaminergic pathways. This work provides new insight on how people with incomplete SCI produce volitional force. It is proposed that this will provide a basis for novel rehabilitative interventions to overcome the profound impairments in volitional force generation that occurs following human incomplete SCI.
I. Introduction

The generation of force is essential to movement. Humans utilize the neuromuscular system to generate force. The conceptual framework of muscle force generation is presented in Figure 1 and contains three functional units of force generation: 1) the motor unit (i.e., the motoneuron and the muscle fibers it innervates), 2) supraspinal drive from cortical and brainstem centers, and 3) reflexive/proprioceptive inputs relaying directly to motoneurons or to selected interneuronal networks. Each of these units can be subdivided into different components, although the basic organization as presented in Figure 1 is well accepted in the field.

Using this framework, volitional force generation begins as a set of command signals from various brain centers. This supraspinal drive converges onto spinal neurons and will excite functional groups of motoneurons, termed motor pools. Sufficient activation of the motor pool results in a contraction of muscle fibers, thereby resulting in force generation. This generation in force will activate various reflex pathways which will feed back onto the spinal circuitry.

This is a dynamic framework with complex interactions that occur over various timeframes. Short term adaptations, such as potentiation and fatigue, and long term structural changes, which occur following long-term exposure to various stimuli or damage to descending pathways (as occurs in spinal cord injury), will have a strong influence on force generation. The main finding in this proposal focuses on a novel short term adaptation within a motor system that has undergone long term structural changes: the potentiation of
force generation during fatiguing contractions in individuals with chronic incomplete spinal cord injury (SCI).
Figure 1: The segmental motor system. The segmental motor system is defined as the set of neuromuscular structures that have ‘direct’ actions to and from the motoneuron. This includes the actions intrinsic to the motor unit, reflexive input and supraspinal drive. Last-order, excitatory (green) and inhibitory (red) pathways which have ‘direct’ actions on the motoneuron are shown. Specific structures are described in Section 1a.
The organization of this dissertation warrants a brief mention. Chapter 1 utilizes the segmental motor system to help provide a common framework in which to describe motor control, fatigue/potentiation, and spinal cord injury. The introduction contains four sections: Section 1 contains a brief review of the segmental motor system; Section 2 describes fatigue and potentiation of the segmental motor system; Section 3 details the disease process following SCI; Section 4 discusses fatigue and potentiation following spinal cord injury. Chapters 2-4 detail the rationale, methodology, and results of 3 experiments, suitable for publication, which attempt to describe the physiology underlying the paradoxical increase in volitional force generation during fatiguing contractions following spinal cord injury. Chapter 5 consists of a brief summary of the 3 experiments and points to remaining questions regarding the physiology and rehabilitation of the motor system following spinal cord injury.
a. The segmental motor system: ‘direct’ actions to and from the motoneuron

Investigations into the motor system have produced seminal finding in neuroscience. For instance, the description of the giant motor axon of the squid (Young, 1936) allowed for the detailed electrophysiological findings of Drs. Hodgkin and Huxley (conclusion manuscript, Hodgkin & Huxley, 1952). These experiments provided a revolutionary understanding of the electrochemical basis of ionic transmission, common to all excitable membranes (Goldman, 1943). Additionally, the neuromuscular junction has been particularly valuable in understanding synaptic transmission. Findings from the motor endplate have: 1) established the processes of transmitter release via pre synaptic vesicular exocytosis (Heuser et al., 1979), 2) established the quantal release of neurotransmitter (Katz & Miledi, 1972), and 3) provided the first structural analysis of post-synaptic membrane receptor proteins (Unwin et al., 1988).

Similarly, the motor reflex response of the Alpasia has become an incredibly important framework to investigate the neurobiology of memory (Reviewed in Kandel & Schwartz, 1982). Findings from the motor system have become standard principles throughout neuroscience.

The study of motor control often utilizes what is termed the segmental motor system. Published descriptions of the segmental motor system often: point to its conceptual utility, provide a list of particular structures, and are written with the assistance of Dr. Doug Stuart. For example, Stuart and Enoka (1990) state, “the term [segmental motor system] is a convenient rubric for spinal (or brainstem) motor circuitry, motor units, muscle receptors, and the segmental connections of muscle joint and cutaneous receptors.” (for additional examples see; Callister et al., 1995; Hornby, 2000). Investigations of the segmental motor
system have produced foundational information on motor behaviors ranging from descriptions of simple reflexes to coordinated locomotion. The overwhelming majority of the science discussed below comes from studies of the segmental nervous system, and further investigations in this area will yield important discoveries for the foreseeable future (Boulenguez et al., 2010; Murray et al., 2010). The importance of the segmental nervous system cannot be overstated.

This importance notwithstanding, investigations into the segmental nervous system do not typically include supraspinal influences. This makes translation from segmental models to humans particularly difficult as, outside of complete spinal transection, supraspinal drive has a variable but profound effect on the segmental motor system through direct activation and indirect modulation. Supraspinal control of the segmental nervous system was noted in the early 20th century; rapid advances in the supraspinal control of segmental motor system began with advances in animal preparations. For example, the decerebrate cat preparation allows for investigations of supraspinal brainstem drive in the unanesthetized animal. Using the decerebrate cat, the threshold for elicitation of the flexion reflex was markedly decreased following spinal transection (Sherrington & Sowton, 1915). It was later observed that brainstem centers elicit tonic inhibition of many reflex pathways (Ib, II and III; Eccles & Lundberg, 1959). Further work in the supraspinal control of spinal neurons has revealed a critical interconnectedness at a premotoneuronal level between reflexive input and supraspinal drive (reviewed in Baldissera et al., 1981). As the choice in animal preparation profoundly influences the actions of the spinal cord, for true functional relevance, it is critical that results devoid of supraspinal effects be placed in the context of
supposed supraspinal actions. Because of this difficulty in translation, the addition of supraspinal processes including descending direct and excitatory pathways and a description of effort, defined here as the biophysical processes of will and its perception, are included in the current definition of the segmental motor system (Stuart, personal communication).

i. **Motor unit**

A motor unit is defined as the motoneuron and the muscle fibers which it innervates. Each motoneuron innervates multiple muscle fibers. The muscle is the force generating component of the motor system.

1. **Muscle**

   a. **Structure**

Skeletal muscle is organized in a hierarchical manner with each structure surrounded by a membrane. The muscle cell is termed a fiber and is surrounded by an endomysium. Muscle fibers are grouped together in bundles, ensheathed by a perimysium and called a fascicle. Fascicles are grouped together and surrounded by an epimysium to form the whole muscle.

Each skeletal muscle fiber is comprised of a series of sarcomeres. The sarcomere is the active force generating component of the muscle and can be considered the functional unit of the muscle. The sarcomere is comprised of a ribbed pattern of proteins, in particular actin and myosin. Given the proper environment, these proteins undergo a conformational change to generate linear force (excitation-contraction coupling). The force generated by the contraction of the sarcomere is added within and among the muscle fibers to generate torque about a joint.
i. **Actin**

Actin is a thin double helical polymer that contains periodic myosin binding sites. These sites have a high affinity for myosin. The rate of the actin-myosin binding will be proportional to the force generated. The affinity of this binding is regulated by two proteins, tropomyosin and troponin. Tropomyosin has a similar structure as actin and physically blocks the myosin binding site. Troponin is a complex of three molecules TnT, TnC and TnI, each of which are named for the particular function. TnT is bound to tropomyosin; TnC can bind Ca$^{2+}$ and TnI inhibits the actin-myosin binding in the absence of Ca$^{2+}$.

ii. **Myosin**

Myosin is a helical protein structure composed of a light and heavy chain and commonly depicted in the shape of a golf club. The functional actin binding site is located on the myosin head. One of the factors regulating the rate of force development is the myosin light chains, which performs the ‘power stroke’ of the myosin head. The release of the myosin head from actin is an ATP dependent process, a reaction which occurs on the heavy chain. Both the myosin light and heavy chains have multiple isoforms allowing for a continuum of force kinetics.

iii. **Additional proteins**

Other proteins that comprise muscle include titin, nebulin, and obscurin. These proteins are thought to provide stability to actin and myosin (Kontrogianni-Konstantopoulos *et al.*, 2009). Addition functional roles will surely emerge with continued investigations.
b. **Excitation contraction coupling**

The generation of muscle force follows a discrete time course of three events; the electrical activation of the muscle, the release of Ca\(^{2+}\), and the generation of force. One of the best early examples of this comes from observations of a single muscle fiber that has been injected with Ca\(^{2+}\) light-reactive dye. Using this approach, Ashley & Ridgway (1970) demonstrated that the endplate potential produces a large influx of intracellular Ca\(^{2+}\) which is correlated with force production.

Intracellular Ca\(^{2+}\) concentrations are regulated by the sarcoplasmic reticulum. The conductance of the sarcoplasmic reticulum is increased in response to the endplate potentials. For this change in conductance to occur, the endplate potential travels along the sarcolemma and down the T-tubules located deep in the muscle fiber through voltage gated Na\(^{+}\) channels. Along the T-tubules are voltage sensitive dihydropyridine (DHP) receptors, which are complemented by ryanodine receptors on the sarcoplasmic reticulum. The conformational change of the DHP receptor triggers the release of Ca\(^{2+}\) from the sarcoplasmic reticulum via activation of the associated ryanodine receptors.

During high levels of intracellular Ca\(^{2+}\), the myosin binding sites (on actin) become exposed. The myosin head becomes strongly bound to the actin, the light chain undergoes its ‘power stroke’ and force is generated. For active force production to change (either increase or decrease), it is necessary for the myosin head to detach from the actin and allow the tropomyosin to once again block the myosin binding sites. For this unbinding to occur, energy is required in the form of ATP. For force production to cease, intracellular Ca\(^{2+}\) concentrations are lowered via Ca\(^{2+}\) being pumped back into the sarcoplasmic reticulum via
sarco/endoplasmic reticulum Ca\(^{2+}\) ATPase proteins (SERCA pumps). The SERCA pump is an ATP dependent process as the movement of Ca\(^{2+}\) is against its electrochemical gradient.

**c. Force relationships**

**i. Force-frequency**

As demonstrated by the work of Ashley & Ridgway (1970), force generation is a Ca\(^{2+}\) dependent process. The events of Ca\(^{2+}\) release and reuptake have relatively slow time constants, particularly as compared to electrical excitation. This timing allows for the Ca\(^{2+}\) concentration to summate with repeated activation. The level of intracellular Ca\(^{2+}\) is proportional to measures of force generation. Therefore the frequency of activation will determine the amount of Ca\(^{2+}\) release and be proportional to the force generated. Indeed the force generated by intraneural microstimulation of human motor axons is highly dependent on the frequency of stimulation (Fuglevand et al., 1999).

**ii. Length-tension**

Mechanical properties depend on the sarcomere length and will vary with given distances, thus producing an ‘optimal’ muscle length for force generation. Lieber, Loren and Fridén (1994) used intraoperative laser diffraction to suggest 2.8 µm as the optimal length of the *in vivo* sarcomere of the human wrist extensor and that active force generation will be lessened at both shorter and longer lengths. These actions are consistent with the underlying crossbridge mechanics; at longer lengths the sarcomere has less binding sites will be available and a shorter lengths there may be collision of actin molecules.
iii. Force-velocity

The speed of contraction also influences muscular force generation. The original work of Edman (1988) on a single muscle fiber of the tibialis anterior of the frog shows the influence of contraction speed (concentric, isometric and eccentric) on the maximal force generation. The two arms of this curve can be explained by 2 distinct mechanisms: 1) the force generated by the muscle will be proportional to the number of active cross-bridge formations and 2) the crossbridge cycle is a time dependent process. The faster the muscle moves in the direction of shortening (concentric), the faster the cross bridges will be cycling and the fewer the number of formed cross bridges at any given time.

2. Motoneuron

The motoneuron is the only means to activate the muscle fibers and generate force and has been termed the “final common pathway” (Sherrington, 1906, Lecture IV). All processing of the CNS that result in activation of the muscle must converge on the motoneuron. For this reason, and due to the relative ease of investigation, the research community has placed much interest on the study of the motoneuron. While the motoneuron was once considered a passive follower of synaptic input, it has since been demonstrated that motoneurons possess active dendritic conductances that actively shape motor output.

a. Structure

The motoneuron consists of a large soma with an even larger dendritic field, spanning a few segments, and a single axon. The motoneurons are situated in the ventral horn of the spinal cord and are organized in specific pools, such that a pool of motoneurons will innervate multiple fibers from a single muscle or synergists. Motoneurons have one of three targets:
muscle fibers, muscle spindle or both muscle fibers and spindles; these motoneurons are termed alpha, gamma and beta respectively.

b. **Excitable membrane**

i. **Resting membrane potential**

The motoneuron consists of an excitable membrane. This excitable membrane has a resting membrane potential somewhere near -70 mV and is regulated by the electrochemical gradient caused by the separation of ions through the selectively permeable membrane of the motoneuron. This separation produces a relatively high concentration of intracellular K⁺ while there is a relatively high concentration of extracellular Na⁺. A large and modifiable component of the resting membrane potential is the conductance of resting K⁺ leak channels. A number of these channels are open in the basal state of the motoneuron; this allows K⁺ to travel along its concentration gradient (outward) producing a negative voltage across the membrane.

ii. **Post-synaptic potential**

The resting membrane potential is altered in response to a change in conductance for selective ions. Selective permeability of ion channels occurs as particular channels are sensitive to specific chemical, electrical or mechanical stimuli. The change in chemical ionic balance will alter the membrane potential by either making the voltage more positive (depolarize) and more negative (hyperpolarize). For example a voltage gated Na⁺ channel will remain closed until a certain membrane voltage is reached, once the voltage threshold is crossed the channel will open, and Na⁺ will flow across its concentration gradient, this is typically a inward current which depolarizes the cell producing an excitatory post synaptic
potential (EPSP). Similarly, a ligand gated glycine receptors will, in the presence of receptor activation, allow for entry of Cl⁻ during resting conditions and will result in an inhibitory post-synaptic potential (IPSP) via hyperpolarization of the cell membrane or increase in conductance (Hubner et al., 2001). In humans, it may be possible to estimate the net post-synaptic potential through analysis of spike timing using intramuscular electrodes (Turker & Cheng, 1994; Miles, 1997; Powers & Turker, 2010).

iii. **Action potential**

The action potential is an all or nothing depolarization event which is a primary means of neural communication within the motor system. An action potential occurs when the soma of the motoneuron is sufficiently depolarized to a threshold level. At this time, near simultaneous changes in the conductance of Na⁺ and K⁺ occur. Voltage gated Na⁺ open in a positive feedback manner resulting in an extremely quick depolarization; the Na⁺ channels inactivate in less than 1 ms afterwards. Near simultaneous with the Na⁺ events, K⁺ channels open allowing K⁺ to flow out of the cell. The action potential travels down the axon using saltatory conduction. The action potential ends in the presynaptic terminal where voltage gated Ca²⁺ are located. This depolarization allows for an inward flux of presynaptic Ca²⁺ current. This Ca²⁺ will bind to specific proteins and allow for quantal transmitter release via vesicular exocytosis.

iv. **Plateau potential**

One aspect of active motoneuron properties, which is particularly salient to the thesis, is the generation of a long lasting depolarization termed plateau potentials. An example of these intrinsic motoneuron properties is the presence of voltage-sensitive, persistent (non-
inactivating), inward Ca\(^{2+}\) and Na\(^{+}\) currents (PICs), which amplify and prolong the effects of brief synaptic inputs (Hornby et al., 2002). These currents give rise to lasting depolarization/discharge, also called plateau potentials (Bennett et al., 2001a). The classic demonstration of motoneuron PIC activity is shown in a series of papers from Dr. Hultborn’s group (Conway et al., 1988; Crone et al., 1988; Hounsgaard et al., 1988). Here, intracellular (IC) recording of a motoneuron in a decerebrate cat demonstrated prolonged discharge following a brief electrical stimulus delivered to nerve (see Figure 1 in Hounsgaard et al., 1988). Such activity is modulated substantially by descending serotonergic (5HT) projections, as acute spinalization abolishes this behavior and application of 5HT precursors restores motoneuron PIC activity (see Figure 6b in Crone et al., 1988).

The functional role of PICs, and even their existence in humans, is controversial. One complicating factor is the lack of direct measurements of PIC in humans. Instead indirect measures of motoneuron PIC activity in humans have been suggested. For example the net excitation to a motor pool can be estimated through the firing frequency of a single motor unit. When this information is combined with activity of additional, simultaneously firing units, estimates of PIC activity have been inferred (Gorassini et al., 2002a)

c. **Frequency and recruitment relationships**

The generation of muscle force is regulated by the frequency at which the motoneuron is firing and the number of motoneurons firing. There are specific relationships which govern this rate coding (Monster & Chan, 1977) and recruitment (Henneman, 1957) of motoneurons. This is important for a variety of reasons, however one aspect which deserves further elaboration is the role in which these spinal motoneuron properties decrease the
burden on the central nervous system. De Luca and Erim (1994) suggest a simple ‘hydraulic’ model to help explain the properties of rate coding and recruitment of motoneurons with a common drive from the cortex. This simplified model explains how a common drive to a motoneuron pool would first activate low threshold motor units. Increasing drive to the pool will increase the firing rate of recruited units and eventually recruit higher threshold units. This process of increasing firing rate of already active units and recruiting additional motor units is repeated until maximal force generation is reached.

i. **Current – frequency relationship**

The amount of current into the cell is related to the frequency at which the cell will fire. Granit et al (1963) demonstrates a near linear relationship between the current injected into the motoneuron and the rate at which the cell will fire. This injected current to the soma was previously thought to be similar to the synaptic current delivered to the dendrites; however active dendritic processes (i.e. plateau potentials) may be minimized with injected current (Bennett et al., 1998b). Nonetheless, the linear relationship between the current and firing frequency may contain various ‘ranges’ of firing relationships. Schwindt and Crill (1982) suggested two firing ranges (primary and secondary) while work from Dr. Bennett’s laboratory suggests the plateau potential activation serves as an additional ‘tertiary’ range of firing (Li et al., 2004; Elbasiouny et al., 2006). In humans, rate limiting behavior of motor unit discharge is observed such that motor units recruited early in a contraction saturate in their firing while the later recruited motor units continue to fire. This selective saturation of motor unit firing is often observed in human experiments (Monster & Chan, 1977) but noticeably absent during current injections in the soma (Schwindt & Crill, 1984). While the precise
mechanisms remain unclear, two hypotheses have been used to explain this behavior. First, it has been suggested that this ‘crossover organization’ may be a result of interactions between reflexive input and supraspinal drive (Heckman & Binder, 1993). Second, this saturation may arise from the increased conductance derived from dendritic plateau potential generation (Li et al., 2004)

ii. **Orderly recruitment of motoneurons**

Recruitment of motoneurons is controlled in large part by the input resistance of the cell. In turn, the input resistance of the cell is determined in large part by the K+ leak channels. Using the hydraulic model described above, synaptic current is delivered to the motoneuron pool equally to each of the motoneuron. The motoneurons with the highest input resistance (i.e. smaller motoneurons) will respond with a larger depolarization and begin to fire first. With increasing levels of current provided to the motoneuron pool, the motoneurons will be further depolarized. Already firing cells will increase their rate of fire and progressively larger motoneurons will be recruited.

3. **Neuromuscular junction**

An additional component of the motor unit is the junction between the motoneuron and the muscle fibers it innervates. The structural components of the neuromuscular junction are rarely discussed in motor unit physiology (Sanes & Lichtman, 2001). However, this junction is critically important, as it ensures transmission from the motoneuron to the muscle fiber.
ii. **Reflex input**

1. **Spindle afferents**

The muscle contains a large number of muscle spindles (Kokkorogianiss, 2004) located in parallel with the muscle fibers. Muscle spindles transduce information regarding length and velocity, in addition to other forces (e.g. Coriolis forces; Lackner & DiZio, 2000), through group Ia and II fibers. Spindle activation can either produce EPSPs or IPSPs in motoneurons dependent upon the monosynaptic or polysynaptic pathways. The large diameter afferents are thought to have direct monosynaptic connections to the innervating motoneuron and are used frequently as a methodological framework to guide the study of spinal cord physiology, particularly in humans.

The structure and function of muscle spindles has been expertly reviewed by P. B. C. Matthews (1981). Briefly, the spindle is composed of either bag or chain fibers, whose names are descriptive of the shape of the receptor rich polar region. Bag fibers are further categorized as dynamic or static and termed bag_1 and bag_2 respectively. The kinetics of the mechanical deformation are dependent on the shape of the fiber, and these subtypes relay different information regarding muscle length. Bag fibers appear to be more responsive to alterations in muscle length where as the chain fibers will respond best to constant length. Group Ia afferents appear to contain information from all three organs while group II fibers contain information unique to chain fibers. As such Group Ia fibers more faithfully encode change in length or velocity where group II afferents are more responsive to length. The muscle spindle is unique due to the gain regulation provided contractile elements controlled by γ/β motoneurons. γ motoneurons are divided in such a manner that allow for regulation
of static and dynamic responses; $\gamma_d$ motoneurons increases bag₁ response while $\gamma_s$ activates bag₂ and chain organs. Activation of $\gamma_d$ and $\gamma_s$ is thought to be controlled independently and do so to meet functional task demands (Prochazka, 1996).

Group Ia afferents terminate in lamina VI, VII, and IX (Ishizuka et al., 1979) while II afferents terminate more dorsally in lamina II, IV-VI (Hoheisel et al., 1989). This anatomical distribution supports the notion that group Ia pathways have strong monosynaptic projections to homonymous motoneurons and disynaptic inhibitory pathways to antagonists; in contrast, group II pathways have strong polysynaptic connections. In humans, the monosynaptic Ia afferent pathway is assessed using electrical or mechanical stimulation to activate Ia afferents (see however, Burke et al., 1984). Indeed Ia afferents have polysynaptic connections, the most common of which is the delivery of an IPSP to the antagonist motoneuron pool, termed reciprocal inhibition (Crone, 1993). As Ia afferents have a large axonal diameter and a low threshold for electrical stimulation, selective Ia activation is relatively easy to obtain in humans and has been studied extensively using peripheral electrical stimulation. This technique has been termed the H-reflex, named after Paul Hoffman (Hoffmann, 1910; via Zehr, 2002). The underlying theory is that during increasing amplitude of electrical stimulation applied over axons, the large diameter Ia axons are selectively excited at lower amplitudes and evoke a reflex response. At slightly higher amplitudes, motor axons will be excited and serve as a useful control of stimulus consistency. Due to its likely monosynaptic connections to the motoneuron and relative ease to elicit in humans, the H-reflex has been extensively used to examine human spinal cord circuitry and various conditioning protocols have been developed to assess multiple neural
pathways (Pierrot-Deseilligny & Burke, 2005). Unfortunately, this ease of experimentation has likely led to common misunderstandings regarding this technique.

Group II afferents may have some monosynaptic connections to homonymous motoneurons (Kirkwood & Sears, 1975; Stauffer et al., 1976). However, it is likely that the majority of group II effects are via polysynaptic pathways (Lundberg et al., 1977a). Classically, group II afferents have been shown to inhibit extensor motor pools and excite flexor motor pools (Lundberg et al., 1975), however more recent work has seen that such organization is likely not accurate and the actions of group II interneurons are largely dependent on the modulation from descending pathways (Jankowska et al., 2000). Selective activation of group II afferents may be accomplished through changes in muscle length during muscle vibration (Kanda & Rymer, 1977), however conditioned stimulation protocols are more commonly used to assess group II activation. In humans it seems as if the connections of group II afferents are mainly excitatory, and potentiation of the heteronymous H-reflex at latencies consistent with group II pathways is observed during a tonic contraction (Marchand-Pauvert et al., 2005).

2. **Golgi tendon organ afferents**

The musculotendinous junction contains a number of Golgi tendon organs, located in series with the muscle fibers. The receptor organ is interwoven with cartilaginous bundles. Over various levels of force, the cartilaginous bundles will deform the receptor membrane causing changes in conductance and signaling. The pooled response of these receptors allows detection of very small changes in muscle force over a wide range of forces (Crago et al., 1982). At the spinal level, Ib afferents innervate the motoneuron through di- and tri-synaptic
Ib interneurons (Lundberg et al., 1977b). Ib interneurons demonstrate complex, state dependent processing; for example during resting conditions, Ib interneurons will inhibit motoneurons while during stance Ib afferents will excite motoneurons in both agonists and synergists in the free moving cat (Pratt, 1995). Such changes are under fine control as the excitatory and inhibitory action of the Ib pathway on flexors and extensors will alternate within a step cycle (Gossard et al., 1994). In humans, facilitation of the conditioned H-reflex is observed at latencies consistent with Ib pathways during static contractions (Pierrot-Deseilligny et al., 1982). In humans selective electrical stimulation of Ib afferents may be accomplished using nerve stimulation slightly above Ia activation after decreasing the efficacy of the Ia reflex pathway through prolonged tendon vibration (Coppin et al., 1970; Heckman et al., 1984; Hayward et al., 1986).

3. **Group III/IV muscle afferents**

Small diameter afferents carry signals regarding metabolites back into the spinal cord. Group III/IV afferents are chemosensitive and will respond to a variety of endogenous pain producing chemicals (Mense, 1977; Rotto & Kaufman, 1988), which are produced during fatiguing contractions (e.g. lactic acid; Sinoway et al., 1993). These afferents are thought to branch onto interneurons and have complicated actions on the motoneuron. For example, group III/IV activation tend to excite flexors and inhibit extensors in the spinal, deafferented, decerebrate cat (Kniffki et al., 1979).

4. **Renshaw afferents**

In 1941 it was demonstrated that antidromic activation of motor axons in a deafferented cat will often produce short term inhibition of neighboring motoneurons (Renshaw, 1941). This
effect is produced by axon collaterals which synapse onto spinal neurons termed Renshaw cells (Eccles \textit{et al}., 1954; Van Keulen, 1981). Most motoneurons have these axon collaterals onto Renshaw cells (see however, Cullheim & Kellerth, 1978; Horner \textit{et al}., 1991) and, of unique interest, it appears motoneurons may release glutamate to excite Renshaw cells, in addition to the acetylcholine released at the neuromuscular junction (Nishimaru \textit{et al}., 2005). Though Renshaw afferents may be preferentially activated by larger motor units, recurrent inhibition increases linearly with force (Hultborn \textit{et al}., 1988) and may provide an efferent copy of force generation (Windhorst, 1996). Methods to assess recurrent inhibition in humans rely mainly upon the conditioned reflex response (Katz & Pierrot-Deseilligny, 1999) and selective activation of Renshaw cells in humans is improbable. Though it has been stated, that “given the simplicity of the circuit, and its direct proximity to motor outflow, our failure to clarify the function of the Renshaw neuron is an embarrassment” (Cited in Windhorst, 1996, pg.520), recent advances in experimental methods have increased the pace of research in this spinal pathway (Alvarez & Fyffe, 2007).

5. **Additional peripheral afferents**

In addition to the Ia, Ib, II, III/IV and renshaw afferents derived from the motor unit, the spinal cord receives information from a host of other peripheral receptors which deserves a brief mention. Some of these receptors encode information regarding pressure, stretch and vibration among other stimuli. This information is signaled through large diameter fibers. Other receptors respond to various forms of pain and temperature and transmit through small diameter fibers. In the most general of terms the large diameter cutaneous afferents may have little effect in terms of short latency actions, which is suggestive of possible
transcortical contributions. On the other hand, small diameter cutaneous afferents may access the well-studied flexor reflex afferent pathway.

6. **Spinal interneurons**

Based upon their functional connectivity, Ramon y Cajal (1909) proposed spinal neurons to be classified as afferent, efferent and those that ‘interpose’ between them (i.e. interneurons; from Jankowska, 1992). Jankowska (1992) has proposed further subdivision of interneurons into three categories: 1) segmental interneurons that contain strict local connections to neighboring segments, 2) propriospinal neurons that project to several segments, or 3) ascending tract neurons that project information to the brain. More recent work has considered the molecular and genetic morphology of interneurons, and such work has provided a means to investigate many additional subtypes of interneurons (Goulding, 2009).

Spinal interneurons likely receive multiple excitatory and inhibitory inputs from multiple sources and play an important role in integration of motor output. In addition to allowing modification of reflexive pathways, spinal interneurons play an active role in the shaping of coordinated movement. For example, using the deafferented, spinalized, decerebrate cat, Brown (1911) demonstrated coordinated and reciprocal movements of the tibialis anterior and gastrocnemious. As the theory suggesting that motor output is a result of a chain of reflexes (Sherrington, 1906, pg 182) was popular in the early 20th century, the suggestion that the spinal cord could produce coordinated movements in the absence of sensory information was a contentious shift in thinking at the time.
iii. **Supraspinal drive**

1. **Specific command**

The specific command consists of supraspinal pathways which have near-direct fast synaptic (ionotropic) connections to the motoneuron. The specific command is provided by four main pathways: cortico-, reticulo-, vestibulo- and rubrospinal projections (Baldissera et al., 1981). Further, it is commonly argued that the interneurons integrate inputs from the specific command in addition to reflex input (Fetz & Cheney, 1990).

Despite these two commonalities, each of these pathways differ in anatomical structure (Haines, 2006) and physiological function (Shapovalov, 1972). It is generally considered that corticospinal projections are active during willful, volitional movements while the brainstem, or bulbospinal, pathways contribute to more automatic (but skillful) behaviors, such as locomotion (Shik & Orlovsky, 1976). These bulbospinal projections are part of the extrapyramidal motor system and can be divided into two functional subunits termed Group A and Group B. Axons that form Group A tend travel bilaterally and innervate interneuron at multiple spinal levels. Group B axons tend to be contralateral, innervate interneurons at a single level and may have monosynaptic connections to the motoneuron.

In addition to functional differences, the pathways comprising the specific command will change over time. On an evolutionary timescale the anatomical significance of these pathways has shifted. For instance, phylogenetically older vertebrates tend to have dense brainstem projections onto interneuronal circuits (Grillner, 1985), while higher mammals may have more monosynaptic corticospinal projections – thus the proper amount of caution
should be used when interpreting across animal models. Additionally, the anatomical and functional development of these pathways will differ across the lifespan (Stanfield, 1992), whose time course may differ for each animal model. Caution should be observed when comparing suprapsinal pathways across the lifespan; nevertheless, generalizations regarding the physiological function of these pathways are evident.

a. **Corticospinal projections**

With regards to volitional contraction in humans, the primary motor cortex is particularly salient. In higher mammals, layer V pyramidal cells have direct excitatory actions on spinal motoneurons (Isa et al., 2007); the anatomical and functional projections of these cells have been extensively studied in terms of motor control and multiple relationships have emerged. Electrical activation of these cells demonstrates a diffuse somatotopic manner (Penfield & Rasmussen, 1950; via Schieber, 2001). The firing frequency of individual corticospinal neurons is associated with force production (Cheney & Fetz, 1980). Additionally, the pattern of firing of subpopulations of motor cortex corresponds to the direction of the movement (Georgopoulos et al., 1982).

As eluded to above, there is debate on the functional connectivity of the corticospinal interneurons. It has been suggested that corticospinal interneurons have private access to motoneurons and act in a manner which is relatively independent of reflexive input (Kostyuk & Vasilenko, 1979); such view is echoed in the work of Sherrington (Burke, 1985 cited in Stuart & Hultborn, 2008). This view is in contrast to the views of Lundberg, which suggests sensory information strongly influences corticospinal interneurons likely innervates spinal motoneurons through interneurons (Pierrot-Deseilligny, 2002).
b. **Reticulospinal projections**

Reticulospinal projections originate from the caudal pontine reticular nuclei and the gigantocellular reticular nucleus. These nuclei receive input from corticoreticular and spinoreticular pathways, which may represent an important site of interaction between nocioception and activation of the motoneuron. Motor projections from both nuclei contain multiple axon collaterals and exert actions on multiple spinal levels. Axons from the corticoreticular pathways travel ipsilaterally along the medial (pontine) reticulospinal tract while axons from the spinoreticular pathways travel bilaterally along the lateral (medullary) reticulospinal tract.

Bilateral ionotropic activation of motoneurons has been shown to be mediated by both direct (monosynaptic) and indirect (interneuronal) connections, which allows for either excitation or inhibition of target motoneuron (Jankowska et al., 2003). In the upper extremity, these bilateral connections have been shown to be active in “double reciprocal output organization patterns” during functional activities (Davidson et al., 2007). Buford and colleagues further suggest that brainstem projections organize to represent functional, flexion/extension synergies between and across muscles in humans (Herbert et al., 2010).

In humans, it has been suggested that reduced inhibition of these pathways occur after stroke and contribute to abnormal ‘synergies’ (Dewald et al., 1995). Further, analysis of spike timing of human motor units (e.g. synchronization and coherence techniques) suggests such tonic brainstem drive may contribute to the spastic, spontaneous firing of motor units in this population (Mottram et al., 2010). Preliminary data suggests nocioceptive pathways may facilitate volitional motor output in individuals with incomplete SCI; nocioceptive
activation of either ionotropic or metabotropic reticulospinal projections contribute to this behavior (Jayaraman et al., 2009).

c. Vestibulospinal projections

Vestibulospinal projections transduce unique information from the fastigial and purkinje nuclei in the cerebellum in addition to the vestibular sensory organ – thus the medial and lateral vestibular nuclei are isolated from direct cortical projections. Projections from these nuclei descend both bilaterally and ipsilaterally and both will terminate at extensor motor pools at multiple spinal levels. Paradigms have been developed to assess functional relevance of vestibulospinal projections. For instance, observations of walking on an incline/decline with and without head tilts reveal vestibulospinal projections may generally excite spinal neurons during functional tasks (Matsuyama & Drew, 2000). In humans, a vestibular-induced soleus H reflex facilitation may be mediated by increased activation via vestibulospinal projections (Knikou & Rymer, 2003). Clinically, specific head movements can be used to assess the integrity of various components of the vestibular motor pathways – labyrinthine hyper-, hypo- and dys-function will each result in unique stereotypical clinical signs (Boyle, 2001).

d. Rubrospinal projections

Corticorubral and cerebelorubral pathways activate descending (magnocellular) projections from the red nucleus. These projections terminate at specific segmental levels in the cervical spinal cord and produce EPSPs in contralateral proximal limb flexor motoneuron pools. Such pathways are anatomically small in humans; as such its potential role in fast-synaptic transmission may be limited. Although the rubrospinal system may currently be considered
“a supplement to the corticospinal system” (pg, 387; Haines, 2006). Nevertheless ease of plasticity of this system would be well suited component in a motor learning process (Ito & Oda, 1994). It is likely rubrospinal projections will have some influence to motor output in humans (Yang et al., 2011a).

2. **Excitability command**

In addition to these ionotropic connections, the brainstem pathways also directly mediate the excitability of spinal neurons through neuromodulators. Specifically the raphe nuclei and the locus ceruleus of the brainstem synthesize and release serotonin (5-HT) and norepinephrine (NE), respectively, with substantial, yet diffuse projections to spinal circuits (Bjorklund & Skagerberg, 1982). These descending monoaminergic connections are thought to release 5HT during motor activity (Gerin et al., 1995; Veasey et al., 1995). Monoaminergic drive appears to be related to intensity such that increasing levels of intensity correlate with increasing levels of monoaminergic drive (Jacobs & Fornal, 1999; Jacobs et al., 2002). 5HT and NE agents are described as having a facilitative effect on spinal neurons, eliciting PICs of the motoneurons.

Conversely, reflex pathways may be suppressed by descending brainstem projections. In particular, group Ib, II and III reflexes are tonically inhibited in a decerebrate preparation where as such inhibition is not observed in spinalized animals (Eccles & Lundberg, 1959). Pharmacological investigations reveal such inhibition may be mediated by monoamines. For example, serotonin and norepinephrine agonists will often inhibit the actions of group I/II spinal interneurons, however some subpopulations of these interneurons are excited by such
chemicals (Jankowska et al., 2000; Hammar & Jankowska, 2003). Similar modulatory effects are observed in the dorsal horn pain processing interneurons (Belcher et al., 1978).

The concept of modulatory control of the motor system via supraspinal drive is critically important to recent advances in motor control. The 30 year old discovery of the modulatory control of the intrinsic properties of spinal neurons (Schwindt & Crill, 1982) heavily influences the current concept of modulation of intrinsic excitability of spinal neurons (Hounsgaard & Kiehn, 1989). These active properties have been shown to be essential components of the neural circuitry for motor control across multiple motor systems including locomotion of the lamprey (Wallen et al., 1989b), flight of locust (Parker, 1995) and peristaltic contractions of the crustaceans’ somatogastric network (Christie et al., 1995). With respect to the modulation of mammalian locomotion, the cat has been a particularly valuable model of the pharmacological modulation of spinal neurons (Barbeau & Rossignol, 1991). As animal models (particularly cat and rat) of spinal cord injury appear to have strong correlations with human injury (Rossignol et al., 2002), this work may have strong clinical applications. Rehabilitative strategies based upon supplementation of metabotropic agonists, such as quipazine, have been progressed using the rat model of SCI (Courtine et al., 2009). Preliminary data from human incomplete SCI suggests similar pharmacological strategies may be effective when combined with training (Hornby et al., 2009a).

3. **Effort**

Effort is defined here as the biological events which generate and perceive motor cortical output. The volitional generation of motor cortical output is often discussed in philosophical terms regarding ‘free will’ (Hallett, 2007) however a discussion of effort is necessary for most
any investigation of volitional behavior. Here, we will not discuss the upstream cause of effort.

In this context, effort is typically assessed using an individuals’ subjective report of perceived in which force/torque production is anchored to a numeric rating scale. There exists a somewhat intuitive relationship between a given effort and the force that is generated. In other words, a small effort will produce a small force – with increasing amounts of efforts there will be in an increase in the amount of force produced (Pincivero et al., 2003). Effort is correlated with increase in agonist EMG amplitude, resting twitch and volitional activation, and when a submaximal force is maintained the subjects rating of effort will increase though the behavior may stay fairly constant (Sogaard et al., 2006). It is thought that both peripheral receptors and corollary discharges from the motor cortex can contribute to this kinesthetic sense of effort (McCloskey, 1978).
b. **Fatigue and potentiation of the segmental motor system**

The response of the motor system to a particular input is not constant over time. Acute changes occur throughout the motor system that serve to either decrease or increase the motor response to a given inputs this concept is important for a variety of reasons. From a basic motor control standpoint, motor systems must compensate for the current state of the system by altering the relative strength of synaptic inputs to accomplish a motor task. For example, from a sport performance or rehabilitation perspective, accommodation or habituation at the peripheral neuromuscular apparatus will require augmented central drive to ensure adequate force generation. Conversely, augmentation of neural or muscular excitability through various mechanisms can enhance the desired motor output. This latter augmentation may prove to be a useful rehabilitation strategy to overcome the weakness associated with a variety of pathological conditions.

As mentioned above, either decreases or increases in motor response can be observed, although more rigorous definitions are required. Fatigue is defined as any exercise-induced reduction in the ability of a muscle to generate force or power to a given input. As a corollary, potentiation is defined as any exercise-induced increase in the ability of a muscle to generate force or power to a given input. These definitions are modeled from those of Simon Gandevia, although slight differences in language are considered necessary in this context. Specifically, Dr. Gandevia defines fatigue as “any exercise induced reduction in force generating capacity” (2001; pg. 1732). The definition used in this context will add the term “to a given input” at the end of this definition, as this latter statement has additional implications. Namely, this addition allows for a clear description of compensatory changes.
that occur despite constant motor output. For example, fatiguing processes at the level of the muscle may occur during submaximal contractions, however, despite this a steady state force can be maintained through increasing central (i.e., neural) drive. Addition of the statement “to a given input” allows for fatigue to be considered despite constant force production, if for instance, a measure of increased cortical drive is observed with no change in force production. Similarly, this definition allows for potentiation to be considered on more equal footing with fatigue, because short-term facilitation of neuromuscular or neural excitability can occur rapidly, which is likely accommodated by the central commands to execute a desired motor task.

While there are short-term alterations across the segmental motor system that account for the general phenomena of fatigue and potentiation, the loci of such alterations have been generally categorized as ‘central’ and ‘peripheral.’ Central refers primarily to the central nervous system, and peripheral encompasses peripheral nerves, the neuromuscular junction, and muscular factors. However, such terms can be confusing, as the motoneuron soma and dendritic arborizations would be considered central and its axon and synaptic terminal are considered part of the peripheral system. As such, the segmental motor system framework provides a common language to discuss sources of fatigue and potentiation. Indeed, the phenomena of fatigue and potentiation can arise from changes at nearly all levels of the segmental motor systems, including the motor unit, reflexive input and supraspinal drive. Table I provides an abbreviated list of potential sites of fatigue and potentiation at these three levels, which is identified schematically on Figure 2. Each of these potential sources of short term adaptations is described below.
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<td><strong>Supraspinal Drive</strong></td>
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Figure 2: Potential sites which contribute to fatigue and potentiation in the human segmental motor system. See Table I.
i. **Fatigue**

Fatigue is commonly observed as a decrease in force generation over time. Historically fatigue has been considered to be a change at the level of the muscle, however more recent evidence clearly demonstrates central factors are critically important. Peripheral fatigue can occur at sites ranging from the transmission of the action potential along the axon to the interaction of the crossbridges, where as central fatigue results from either a decrease in excitatory input to the motoneuron, an increase in inhibitory input to the motoneuron, or a decrease in the excitability of the motoneuron.

Common techniques used to assess peripheral sites of fatigue involve various forms of electrical stimulation of the peripheral axon under resting conditions. These electrical and force responses may be used delineate mechanisms relating to failure of neuromuscular propagation and/or failure in excitation contraction coupling. On the other hand, central fatigue is clearly demonstrated when maximal stimulation to the neuraxis produces additional force when delivered on top of a maximal volitional effort contraction. The fact that the muscle is able to generate additional force is a clear indicator that the activation of the muscle is less than maximal. While there are straight forward methods to best assess and interpret peripheral sites of fatigue, the methods to assess central fatigue, such as supramaximal stimulation superimposed during volitional contractions, are less clear and the interpretation is often debated (de Haan et al., 2009; Taylor, 2009; see response to these articles).

As a caveat, short and long term decrease in firing rates of motoneurons, irrespective of cause, should not immediately be taken as a sign of fatigue. Such changes have been
suggested to optimize the neuromuscular system. For example, a longer interpulse interval (e.g. decreased instantaneous firing frequency) between successive motor unit activation may serve to enhance motor output (Marsden et al., 1983). Further, during sustained maximal contractions a decrease in maximal firing rates is observed. It has been suggested that this may provide a means to optimize force generation during the leftward shift in the force frequency properties during fatigue and minimize unnecessary excitation (Bigland-Ritchie et al., 1983; see however Fuglevand & Keen, 2003).

1. **Motor unit**

   a. **Changes in excitation contraction coupling**

   Excitation contraction coupling includes the following processes to transduce electrical stimuli to mechanical action: 1) action potential propagation along sarcolemma, 2) action potential propagation along t-tubule, 3) activation of dihydropyridine receptors, causing an interaction with ryanodine receptors resulting in a change in Ca$^{2+}$ conductance, 4) release of Ca$^{2+}$ from the sarcoplasmic reticulum, 5) reuptake of Ca$^{2+}$ via SERCA pumps, 6) Ca$^{2+}$ binding to troponin causing an unmasking of the myosin binding site on actin, 7) interaction of crossbridges. Detailed studies of isolated muscle have indicated that metabolic byproducts from the muscle contraction may impair particular aspects of this process. While many metabolites have been hypothesized to contribute to impairment in excitation-contraction couple, three specific by-products have been implicated in multiple studies. Inorganic phosphate (Pi) has been shown to decrease the release of Ca$^{2+}$ by modification of channel conductance and decreased force per crossbridge. In addition, excess ADP decreases the velocity of contraction. Elevated H$^+$ ion concentration is considered the classic metabolic
pathway implicated in fatigue, although role of muscle acidification is controversial (Stackhouse et al., 2001) and decreases in force generation may be explained by excess Pi.

The dissociation of the M-wave recovery and the recovery of the twitch force is a clear indicator of a decrease in at least one of the steps in the excitation contraction coupling process. Additionally, because there are associated changes in the speed of contraction associated with excitation contraction coupling fatigue, a detailed analysis of rate of force production and relaxation following a twitch can be used to indicate fatigue in the steps of excitation contraction coupling. For example, a fatigued muscle will demonstrate a decreased rate of relaxation, which this could result from either prolonged Ca\(^{2+}\) transients or delayed cross bridge detachment. It has long been considered that Ca\(^{2+}\) concentrations are associated with twitch relaxation time (Sandow, 1965). However, in an isolated muscle, both fatigue and pharmacologically induced decreases in ATP concentration increased the twitch relaxation time, suggesting prolonged cross bridge attachment (Edwards et al., 1975). Similar kinetics of twitch force in humans may indicate this prolonged cross bridge attachment.

b. **Alterations in neuromuscular transmission/propagation**

Neuromuscular transmission/propagation consists of synaptic transmission across the neuromuscular junction and propagation of the action potential across the sarcolemma. As reviewed by Sieck and Prakash (1995), changes in transmission include alterations in Ca\(^{2+}\) balance and/or sensitivity at the presynaptic terminal, decrease in synaptic vesicles and/or decrease in quantal size. Diminished responses could also occur due to desensitization of
endplate receptors. Further, propagation along the sarcolemma may also be influenced by altered membrane excitability.

Failure in anyone of these sites can be observed with supermaximal stimulation of the peripheral nerve and recording the EMG response (M-wave). Maximal M-waves has been shown to decrease following sustained submaximal volitional contractions in healthy controls (Fuglevand et al., 1993) and after stimulated contractions in individuals with chronic motor incomplete SCI (Thomas, 1997; Shields et al., 1998; see however Klein et al., 2006).

Alterations in maximal M-wave can be measured by latency, amplitude, duration and area, and changes in these parameters can be used to help infer the specific site of failure. For example, an increased latency of M-wave onset would likely indicate a delay in the conduction velocity of the axon, whereas a decrease in M-wave amplitude with no change in area would indicate a slowing of the propagation of the endplate potential along the sarcolemma. Changes in both amplitude and area are particularly difficult to interpret as this could indicate branch point failure, reduced vesicle release and neurotransmitter depletion in addition to changes in the sarcolemma. It is therefore fortunate that in the above studies the M-wave amplitude decreased with minimal changes in area, thereby allowing for some assertions regarding a possible decrease in excitability of the sarcolemma membrane. Another similarity in the above studies was the time course of M-wave recovery – typically the M-wave recovers relatively soon after contraction whereas the force of the motor response (twitch) remains depressed.

From this dissociation of the M-wave and the twitch force, it may be difficult to claim that a decrease in M-wave activity contributes to fatigue. Fuglevand et al. (1993) overcame
this limitation using a model which incorporated the time course of 3 fatiguing and 2
potentiating processes. From this model they were able to conclude that the reduction in the
M-wave does contribute to decrease motor output.

c. **Axonal conduction block of peripheral neurons**

It has been shown that action potentials are not faithfully transmitted along theaxon under
certain conditions. Following prolonged activity, the local ionic balance surrounding ion
channels will undergo changes. In healthy motor axons, this change occurs prior to expected
influences due to alter electrochemical gradients and therefore may be due to alterations in
inward Na\(^+\) channel kinetics and accumulation of extracellular K\(^+\), but Na\(^+\)/K\(^+\) ATPase
activity may play a larger role in chronic pathology. This alteration in ionic balance will
hyperpolarize the axon which will increase the size of the action potential but also increase
current needed to excite the axons with axonal stimulation. Such changes may reflect an
inability for submaximal axonal stimulation to activate a consistent number of motor units –
this may be viewed as an artificial type of fatigue, however the clinical use of electrical
stimulation is rarely used at maximal levels and the subsequent hyperpolarization and decline
in force generation would be noted as fatigue (decrease in force generation to a given input).

Changes in local ionic balance may result in failed transmission particularly at axonal
branches. Branch point failure is most observed during high frequency stimulation (~50 Hz).
This is more readily observed at large bifurcations rather than smaller divisions (Grossman et
al., 1979) though shifts in ionic concentrations are more pronounced in smaller axons
(Smith, 1983). Further, conduction block is observed in human motor axons during
volitional contractions to a greater extent than sensory fibers (Vagg et al., 1998). Conduction
block through changes in ionic balance has been associated with fatigue in a variety of patients with chronic demyelination (Cappelen-Smith et al., 2000; Kaji et al., 2000; Cappelen-Smith et al., 2003), such changes are rarely observed in healthy controls (see however Kuwabara et al., 2002). Following neurological injury, such as SCI, the death of motoneurons and subsequent reinnervation from surviving motoneurons produces additional branching that likely increases the suitability of branch point failure and may contribute to decreases in force generation (Thomas & Zijdewind, 2006).

d. Late adaptation of the motoneuron

Late adaptation refers to the decrease in motoneuron firing rate to sustained input. A classic example of late adaptation comes from Kernell and Monster (1982) who demonstrated that intracellular current injection eliciting constant, sustained discharge of a cat motoneuron produces an initial rapid then more gradual decrease in firing rate during the first 30 s. For a variety of reasons this is difficult to assess in humans, two of which are most prominent: first, a sustained drive to the motoneuron must be obtained – during a submaximal contraction, increased drive to the motoneuron could compensate for any adaptation and therefore a maximal contraction must be used. Second, reflexes are known to alter motoneuron firing patterns (as described in detail below) and therefore must be accounted for or removed.

In an attempt to assess motoneuron firing rates to constant synaptic drive in humans, Macefield et al. (1993) overcame both these difficulties by using microneurography in combination with a distally located peripheral nerve block of the same nerve. Using this technique, this group was able to record from the axon of the motoneuron during a
maximal sustained contraction in the absence of reflexive input. In general, they observed a decrease in maximal firing rate as compared to normally innervated motor units with little change in minimal firing rates. Additionally, during sustained maximal contractions of the “deafferented motoneuron,” firing rates were shown to decrease only slightly during a sustained maximal contraction and rates could return to maximal levels with an ‘extra effort’ contraction. However, in the absence of controlling supraspinal input, the relative role of motoneuron adaptation to the decay in force generation with fatiguing efforts is not clear (Nordstrom et al., 2007).

2. Reflexive input

a. Decreased Ia excitation

During a contraction the agonist muscle is contracted and spindles are activated resulting in EPSP in the contracting motoneuron. While this spindle support to the contracting muscle will help reinforce the activity of the motoneuron, any decline in this spindle support would cause decrease excitation of the motoneuron and may contribute to fatigue.

Macefield and colleagues (1991) assessed the decline in spindle activity during a contraction in humans. To accomplish this, spindle recordings were obtained via axon recordings of the peripheral nerve using microneurography. Spindle responses were confirmed via mode of activation (muscle stretch), and discriminated from GTO and cutaneous afferents. During low level sustained contractions up to 50% MVE, a decrease in firing rate of muscle spindles was observed early (10s) in the contraction. This decrease likely contributes to decreased motor unit discharge and may partially explain the lower firing rates in the deafferented motoneuron described previously (Macefield et al., 1993).
Two lines of evidence suggest that Ia afferents may facilitate motoneuron firing. First, microneurographic recordings performed proximal to a peripheral nerve block can allow for recordings of volitional motoneuron activity in the absence of afferent feedback. Without reflex support, motoneurons demonstrate \( \sim 30\% \) decrease in firing rates, which may be in part due to lack of Ia feedback (Gandevia et al., 1990). Second, rigorous animal experiments demonstrate that a partial block of a peripheral nerve will produce a reversible deafferentation of gamma motoneurons, selectively blocking spindle pathways (Matthews & Rushworth, 1957, 1958). Hagbarth et al. (1986) demonstrated a partial nerve block will decrease firing frequency of a voluntary motor unit in humans. Further vibratory manipulation of excitatory and inhibitory Ia input will modulate volitional motor unit activity and force generation in kind. Caution is needed in the interpretation of partial nerve blocks as selective gamma deafferentation may be more technically difficult to obtain in humans where less stringent controls can be implemented (Mackenzie et al., 1975).

Both of these results are consistent with the idea that ongoing spindle support reinforces a volitional contraction. If, during a fatiguing contraction, the Ia supports declines, the excitability of the motoneuron would be decreased and may contribute to fatigue. Recent work suggests this decline in spindle support may not be a factor in fatigue as increases in Ia support via tendon vibration does not alter the responsiveness of the motoneuron during a sustained maximal effort contraction (McNeil et al., 2011).
b. **Post activation depression**

As outlined in the preceding section, Ia afferent pathways may serve to facilitate motoneuron firing; as such any decrease in spindle support would be observed as a disfacilitation of motoneuron firing and may contribute to fatigue. With repetitive Ia activation there is a time dependent decrease in EPSP response of the motoneuron (Curtis & Eccles, 1960). This decrease in EPSP response is likely due to alterations in transmitter release probabilities (Lev-Tov & Pinco, 1992). The decrease in synaptic efficacy may be observed and would contribute to fatigue using a similar ‘disfacilitation’ argument as expressed in the preceding section.

c. **Increased group III/IV inhibition**

Since the early 1980s (and likely before) it has been proposed that metabolically sensitive muscle afferents inhibit the motoneuron of the contracting muscle. This was thought to be a mechanism that couples the firing of the motoneuron to the ability of the muscle during fatigue (i.e. muscle wisdom; Bigland-Ritchie et al., 1983). It is thought that the metabolites produced during a contraction can remain in the muscle if blood flow to the muscle is occluded following a contraction – therefore group III and VI afferents will continue to fire during temporary occlusion (long occlusion will eventually impair neural transmission).

Butler et al (2003) combined this concept with noninvasive corticospinal simulation, which, when controlled for by M-waves, allow for assessment of corticospinal transmission and motoneuron excitability. The response to corticospinal stimulation is measured by surface EMG and is called cervicomedullary motor evoked potential (CMEP). Alterations in the CMEP during vascular occlusion following a fatiguing contraction were then used to
assess the role of group III/IV inhibition on elbow flexor motoneurons. During fatigue of the elbow flexors, the CMEP decreased. However, during vascular occlusion following the contraction, the CMEP quickly returned to baseline levels. This indicates that group III and IV afferents do not directly inhibit motoneuron excitability or corticospinal transmission in the elbow flexors.

In a subsequent study (Martin et al., 2006), this finding was confirmed for the elbow flexors. However the situation was reversed with elbow extensors. During vascular occlusion following fatigue of the elbow extensors, triceps CMEPs remained low. Further, during vascular occlusion following fatigue of the elbow flexors, triceps CMEPs also remained low. This indicates that for the elbow extensors, small diameter metabolite sensitive muscle afferents from both agonist and antagonist directly inhibit motoneuron excitability and/or corticospinal transmission. Therefore, group III/IV inhibition, may directly inhibit motoneuron excitability but only for some muscles.

d. Role of other afferent pathways

Previous studies have investigated the potential effects of other reflex pathways on fatigue-related decline in muscle force, including altered Ib input from the Golgi tendon organ, (Lafleur et al., 1992; Gregory et al., 2002), recurrent inhibition. (Kukulka et al., 1986; Loscher et al., 1996), and presynaptic inhibition. Though few studies have definitively identified their contribution to fatigue, it is theoretically possible that each could contribute to a decrease in firing of the motoneuron and further work on these areas is necessary.
3. **Supraspinal drive**

   a. **Axonal conduction of corticospinal neurons**

   Peripheral axons of the motoneuron may undergo activity dependent conduction block via axonal hyperpolarization which could contribute to fatigue. Similar conduction block may be observed in the central nervous system in demyelinated axons during extremely high rates of stimulation (>300 Hz; McDonald & Sears, 1969). Indeed, individuals with multiple sclerosis demonstrate an inverse correlation between conduction times and twitch force in response to TMS which is not observed in healthy controls (van der Kamp *et al.*, 1991). While this is consistent with conduction block of corticospinal pathways, TMS produces a high frequency train of action potentials along corticospinal axons (~ 500 Hz; Day *et al.*, 1987) which may preferentially elicit conduction block which may not be observed during physiologically relevant firing frequencies.

   b. **Decreased cortical excitability**

   TMS induces a current in the cortex which (directly and indirectly) activates pyramidal cells. Following precise stimulation of the motor cortex, a motor evoked response can be detected via EMGs in the target muscle. With stringent controls and assumptions, this can be used to assess cortical excitability. Typically the MEP is controlled for my normalizing to a maximal M-wave of the target muscle. Additionally controls may employed such as the normalization to a submaximal M-wave as changes in the sarcolemma may be masked by the size difference in the maximal M-wave versus CMEP (Butler *et al.*, 2003).

   Samii *et al* (1996) demonstrated a decrease in MEP response following a series of 30 s contractions of wrist extensors at half maximum levels until task failure. This decrease in
MEP response was observed for a few minutes following the final contraction. As increases in TMS induced silent period increased with no measurable change in TES silent period suggests increased intracortical inhibition is observed during sustained contractions (Sacco et al., 1997). However, protocols using paired TMS at short intervals found a decrease in short interval cortical inhibition during sustained maximal contractions, suggesting a compensatory mechanism (Maruyama et al., 2006). Decreases in cortical excitability may play a role in fatiguing volitional contractions, however a concurrent potentiation of cortical excitability is often observed early during the performance of a fatiguing contraction and may mask decreases in MEP (Samii et al., 1996).

c. **Decreased monoaminergic support of the motoneuron**

Monoamines increase the gain of the motoneuron pool by increasing motoneuron excitability. Any decrease in modulatory drive would therefore decrease the excitability of the motoneuron and would be deflected as a decrease in firing frequency or a complete derecruitment of motor units. Indeed, a decrease in firing of presumed serotonergic neurons in descending raphe pathways (obscurus and pallidus) may decrease with sustained motor activity (Fornal et al., 2006). Further, spinal concentrations of 5HT assessed using fast cyclic voltammetry confirm decreases in 5HT concentrations with sustained activity (Xie et al., 2009). Interestingly, microdialysis assays of spinal 5HT concentrations demonstrate a similar decrease over time (Gerin et al., 1995).
d. **Plasma levels of branched-chain amino acids and free fatty acids**

Outside of the alterations in spinal excitability, serotonin has been implicated in arousal and sleep. As such metabolic factors from prolonged exercise may impinge upon these regulatory centers and decrease physical performance through mental fatigue. Increased concentrations of serotonin at the level of the hypothalamus are observed during exercise (Blomstrand et al., 1989). Newsholme and Blomstrand (1995) suggest that metabolic energy usage during exercise could contribute to these increased serotonin levels. Their theory states that during exercise the ratio of plasma concentrations of branched-chain amino acids to free fatty acids will decrease resulting in increases in plasma concentrations of the serotonin precursor, tryptophan. High plasma levels of tryptophan will increase serotonin synthesis, causing interactions with arousal and sleep centers in the brain resulting in mental fatigue.

Animal experiments are consistent with this hypothesis. For example, a decrease in time to exhaustion during treadmill running is observed with serotonin receptor agonist (Bailey et al., 1992) where as an increase in running time is observed with serotonin antagonist (Bailey et al., 1993). A similar decrease in time to exhaustion could be observed following SSRI administration in humans (Wilson & Maughan, 1992; see however; Thomas & Smith, 2006). Additionally supplementation of branched-chain amino acids during exercise may improve exercise performance, the theory being that this will increase the ratio of branched-chain amino acids to free fatty acids, decreasing plasma tryptophan and decreasing serotonin production (Blomstrand, 2006; Newsholme & Blomstrand, 2006).
e. **Compensatory cortical drive**

In general, signs of peripheral failure can be observed as an increased need for central drive during a sustained submaximal contraction. During a submaximal contraction, increases in agonist EMG activity is observed without changes in the level of force (Fuglevand & Keen, 2003). Further, recent studies using fMRI suggest that increased drive from the motor cortex may be responsible for this increasing central drive (Liu et al., 2003). Specifically, increases in contralateral motor cortex activity during submaximal fatiguing contractions are consistently observed. In addition to contralateral motor cortex activity, researchers found increased in ipsilateral motor cortex activity and activity of other brain areas including the cerebellum and the prefrontal cortex. This increased brain activity did not result in activation of antagonists nor activation of contralateral muscles (Liu et al., 2003). This suggests the recruitment of ipsilateral corticospinal and/or ionotropic support from these other brain centers. From this increased need for central drive during low level contractions, one can deduce this is a result of a decreased ability to generate force from peripheral mechanisms.

f. **Increased sense of effort**

Given a constant level of effort, the force generated will decrease as a function of time (Stevens, 1969; Jones & Hunter, 1983). As a corollary, given a constant force contraction, the sense of effort will increase over time. This increase in effort during a fatiguing task is reflected as in increase in subjective report of effort (Stevens, 1969) or changes in weight estimation (McCloskey et al., 1974). During submaximal contractions, this increasing effort may be associated with an increase in EMG (see Figure 1, Fuglevand et al., 1993). It has been
suggested that increases in sense of effort, rather than perception of force, may contribute to fatigue during prolonged motor activity (Jones, 1995).

**ii. Potentiation**

Potentiation is defined here as any exercise induced increase in force generating capacity to a given input. As with fatigue, the motor unit, reflexive input and supraspinal drive may contribute to an increase in motor output to the same stimuli.

1. **Motor unit**

   a. **Twitch potentiation**

   A classic example of increased motor output is the potentiation of the muscle response to a single pulse of electrical stimulation. Here the stimulation is delivered to a peripheral nerve prior to and following a brief volitional and or stimulated contraction of the tested muscle. The post-contraction twitch is generally larger than the pre-contraction twitch. The mechanisms are thought to be due to increases in Ca$^{2+}$ sensitivity of troponin through the phosphorlation of myosin light chains (Zhi *et al.*, 2005). Garner *et al.* (1989) provided a text book example of early and late twitch potentiation both during and following electrical stimulation. To further confirm this process was in the muscle, a record of stable M-waves was provided. As outlined earlier this indicates potentiation along the events of excitation contraction coupling. In a separate manuscript, Vandervoort *et al.* (1983) demonstrates twitch potentiation is greatest following brief, high intensity contractions.

   b. **Doublet potentiation and catch-like properties**

   Following 2 pulses of electrical stimulation with very close inter-pulse intervals (doublet), there is a tendency for the nonlinear summation of force (Burke *et al.*, 1970). Using the
gastrocnemius of the cat Sandercock and Heckman (1997) examined the sites of potentiation within the excitation contraction coupling process by lengthening the muscle during or soon after the doublet. Doublet potentiation was influenced by length in a time dependent manner. Changes in length immediately after the doublet continue to result in muscle potentiation, while stretches delivered 100 ms after the doublet abolished the extra force. From this, the authors suggest that the initial, position-independent increase in force of the doublet is developed via augmented Ca$^{2+}$ release, where as the sustained, position-dependent force is maintained by a persistent attachment of the crossbridges. Further, Binder-Macleod & Clamann (1989) suggests that these two mechanisms may underlie the augmented force produced on the descending limb of a train of stimulation which increases and decreases linearly in stimulation frequency. This doublet potentiation can be observed in humans and may be used as a method to sustain force output during fatiguing stimulated contractions (Binder-Macleod & Barker, 1991).

c. **Increased motoneuron excitability**

Increases in motoneuron excitability may also contribute to motor output. For example, in intact humans, repeated contractions decrease the force at which a motor unit is recruited (Suzuki et al., 1990; Gorassini et al., 2002b). Originally, this observation was attributed to a facilitative effects of spindle input on motor unit recruitment during repeated low-level volitional contractions (Suzuki et al., 1990). However, it was later observed that spindle discharge declines during contractions (Macefield et al., 1991). More recently, this observation was thought to be due to intrinsic motoneuron properties, particularly the PICs. Such PICs are time and voltage-dependent and give rise to sustained depolarization (i.e.
plateau potentials) or discharge with minimal or absent synaptic input. In humans, PIC activity can be inferred using paired recordings of motor units (Gorassini et al., 2002b). Using this technique, synaptic drive to the motoneuron pool is estimated using the firing frequency of a low-threshold ‘control’ unit. During phasic contractions, the recruitment and derecruitment of a higher threshold ‘test’ unit is plotted against the firing frequency of the ‘control’ unit. Motoneuron excitability would be observed as a decrease in synaptic drive necessary to cause the motoneuron to fire (e.g. a lower derecruitment vs. recruitment threshold). Indeed a decrease in derecruitment threshold during a contraction and a decrease in re-recruitment threshold are observed during phasic contractions in intact humans.

2. Reflexive input

   a. Facilitation of the monosynaptic reflex

   There is some evidence to suggest that the Ia monosynaptic reflex is potentiated following a history of contractions. As discussed above, the H-reflex appears to decrease following a history of repeated contractions. Contrary to this, the tendon reflex (elicited by percussion of the tendon) appears to increase following a history of repeated contractions (Enoka et al., 1980; Biro et al., 2007). Originally, this discrepancy was considered to be a measure of increased spindle sensitivity. However, at this time, the difference between these two reflex measures is not sufficient to ascribe to changes in the fusimotor system because the afferent volley is different between the two reflexes. Namely, the afferent volley produced by the H-reflex is a synchronous 5 ms volley where as the tendon reflex may induce multiple volleys of action potentials over 25 ms (Burke et al., 1983). It has been suggested that the tendon reflex allows for activation of interneuronal (or oligosynaptic) pathways (Burke et al., 1984),
and can possibly facilitate presynaptic transmitter release in a nonlinear fashion (paired-pulse facilitation; Stuart & Redman, 1991), and may contribute to the reduces the effects of concurrent presynaptic inhibition as compared to the H-reflex (Morita et al., 1998). Potentially, the extended duration of the afferent volley during tendon reflexes may be sufficiently long to activate time-dependent, long-lasting motoneuron conductances (PICs).

The H- and tendon reflexes also appear to activate different pathways. For example, electrical stimulation of the peripheral nerve may alter transmission of Ib inhibitory interneurons (Marchand-Pauvert et al., 2002). This would limit the size of the reflex response to electrical stimulation, leaving the response to mechanical stimulation unchanged. Further, mechanical percussion of the tendon will likely activate group II afferents and cutaneous afferents. Additionally, spindle activation of remote muscles may occur as a result of mechanical transmission of the percussion through the body (reflex irradiation; Lance & Degail, 1965).

b. Muscle specific group III and IV potentiation

As mentioned previously, Martin et al (2006) demonstrated using vascular occlusion and cervicomedulary stimulation, elbow flexors are not inhibited by group III and IV afferents during fatigue where as the extensors are inhibited by group III and IV afferents. In a further experiment this group demonstrated that motoneuron excitability of the elbow flexors is potentiated by group III and IV afferents from the elbow extensor. Group III and IV afferents may also generate plateau potentials in dorsal horn interneurons (Morisset & Nagy, 1999). Such activation may contribute to the windup of the flexion reflex (Hornby et al., 2003)
3. Supraspinal drive

a. Facilitation of cortical excitability

Multiple groups have shown that the MEP in response to TMS is enhanced following contractions. The methods to assess are similar to that of the twitch potentiation. Data from both Norgaard (2000b) and Samii (1996) demonstrate that the MEP is facilitated following a wide range of volitional efforts (~20% MVE to 100% MVE). Interestingly, discrepancies in the time course of potentiation range from <30s to ~5 min.

One difference between these two studies which warrants further elaboration is the methods used to suggest cortical excitability rather than other sites along the neuraxis. Samii (1996) used transcranial electric stimulation (TES) to electrically induce an action potential in the corticospinal axon prior to and following a voluntary contraction. Since no potentiation was observed following TES, the response to TMS was concluded to be cortical. Norgaard (2000b) used peripheral nerve stimulation to elicit an H-reflex prior to and following a volitional contraction. Since no potentiation was observed in the H-reflex, the response to TMS was concluded to be corticomotoneuronal in origin.

b. ‘Super–effort’

During sustained maximal effort contraction both forces and EMG will decrease (Bigland-Ritchie & Lippold, 1979). This is due to changes at the level of the motor unit; reflexive input and supraspinal drive as outlined above. There are lines of evidence to suggest that effort decreases over time during maximal contractions, which can be overcome by a ‘super-effort’ (Macefield et al., 1993) or “extra efforts” (Thomas et al., 1989). Ikai & Steinhaus (1961) demonstrated that during a fatiguing protocol consisting of brief MVEs of the elbow...
flexors a subject could generate higher forces (sometimes greater than maximal) if the contractions was preceded by a gunshot or yelling. From this the authors concluded that maximal volitional force generation was limited by “psychologically induced inhibition.” A similar observation is noted when a deafferented motoneuron is maximally (volitionally) activated over a period of time. Here the firing rate of the motoneuron gradually declines over a period of a few minutes but is restored to baseline levels when verbal encouragement is provided to the subject (Gandevia et al., 1993; Macefield et al., 1993). This “super-effort” contraction overcomes the decline in firing rates observed with prolonged maximal contractions of the deafferented motoneuron.
c. **Spinal cord injury**

Spinal cord injury (SCI) is a debilitating disease process which produces profound deficits in volitional strength and the presence of involuntary motor activity, including spasms and spasticity (Lance, 1980). It has been suggested that impairments in volitional force generating capability will predict functional mobility in both the acute (Crozier et al., 1992; van Middendorp et al., 2011), subacute (Zorner et al., 2010), and chronic (Saraf et al., 2010; Yang et al., 2011b) SCI population. Further, interventions which target volitional force generating capability have been shown to improve functional mobility. This dissertation focuses on the newly described increase in volitional torque generation during repeated maximal effort contractions in individuals with motor incomplete SCI. An understanding of this supramaximal torque generation will provide insight on how individuals with incomplete SCI generate force and form the basis of an effective rehabilitation program.

i. **Prevalence and etiology**

Approximately 12,000 new cases of SCI occur each year, with a prevalence of approximately 265,000 individuals in the US alone (2010a). However, a recent survey conducted by the Christopher and Dana Reeve Foundation reported a prevalence of 1,275,000 individuals with paralysis associated with SCI (2010b). This survey used a very broad and functional definition of paralysis and relied on reports from individuals and family members, not patient medical records. Nonetheless, the substantial differences between the two estimates imply that there are considerably more people living with SCI than previously thought.

The incidence of SCI is much greater in males (80.8%). A large percentage of patients with SCI are Caucasians (67.3%) and African-Americans (22.6%), with smaller percentages
of Hispanic-Americans (9.3%) or Asian-Americans (1.6%). When these racial/ethnic categories are taken in the context of the racial/ethnic makeup of the US population, the incidence rate of SCI is highest among non-whites. Motor vehicle accidents are the leading cause of SCI for individuals less than 60 years of age. For individuals older than 60, falls are the leading cause of SCI. SCI resulting from sports, violence and motor vehicle accidents declined while falls increased with advancing age. The mean age at injury has increased steadily over the past 30 years and is currently 40.2 years. Importantly, improved medical care initially following SCI and continuing treatment in the chronic stage post-injury have increased survival rates substantially. The average life expectancy of individuals with chronic SCI is also increasing, and approaching that of able-bodied individuals, but is strongly dependent on the level and completeness of injury. (National Spinal Cord Injury Statistical Center, 2010a)

ii. **Disease process**

The vast majority of spinal injuries involve mechanical trauma to the spinal cord. This mechanical trauma produces a specific disease process. This disease process involves temporal sequence of events which result from the initial injury and subsequent adaptations of the motor system. The result of this pathological process is a fairly consistent presentation of motor behaviors. Nearly 80% of individuals with SCI experience spasticity (Maynard et al., 1990; Skold et al., 1999) while 50% with SCI possess some form of residual volitional motor control distal to the lesion (DeVivo, 2007). Understanding this disease process will help explain voluntary and involuntary motor behaviors in individuals with chronic SCI. This
understanding should be used to develop rehabilitation strategies. The effects of a spinal cord injury on the segmental motor system can be found in Table II and Figure 3

1. **Initial injury**

The physical injury to the spinal cord is typically considered in 2 stages, the primary and secondary injury. In this model the primary injury is a direct result of the trauma while the secondary injury is caused by the body’s response to the trauma, resulting in further damage.

   a. **Primary injury**

   Durmont et al (2001a) describes 4 common mechanisms of primary injury to the spinal cord: 1) impact with continued compression, 2) impact without continued compression, 3) distraction, and 4) transection. In response to the primary injury, physical disruption of both the white and grey matter is evident. Further, the disruption of blood flow may further preferentially damage the grey matter through hypoxia, ischemia, and edema due to its high vascularity (Taoka et al., 1997).

   b. **Secondary injury**

   The secondary injury consists of interrelated sequelae of events that follow from the primary injury. Responses by the nervous, vascular and immune systems may result in further damage to the spinal cord. The proper medical management of this secondary injury is thought to limit the progression of damage, however efficacy trials of various neuroprotective medication have had limited success (Hall & Springer, 2004; Taricco et al., 2006; Baptiste & Fehlings, 2007; Rachevsky et al., 2011).
i. **Nervous system**

Following SCI a period of neurogenic shock ensues, where both nervous and non-nervous systems become disregulated. For example, cardiovascular disregulation is commonly observed following SCI, where altered blood pressure control, decreased cardiac output and reflexive increases in heart rate are observed, which contribute to myocardial injury (Guha & Tator, 1988). However, the major focus of the present discussion will be towards the alteration in neural excitability following injury.

Initially, the damage to local neural structures allow for excess glutamate release. This hyperconcentration of glutamate produces a series of events termed ‘excitotoxicity’ (Olney, 1978), which is thought be due in part to increased depolarization through AMPA and specifically NMDA receptor activation, the latter of which allows extracellular calcium entry. Glutamate-dependent injury is attenuated with NMDA-receptor antagonists in animal models of stroke, however the translation to human stroke has been slow (Rothman & Olney, 1995). After years of supportive animal research, pre-efficacy human safety trials of NMDA-antagonists seemed promising as a neuroprotective agent following stroke (Grotta et al., 1995). However, subsequent efficacy trials were twice suspended due to increased mortality in the NMDA-antagonist group (Hoyte et al., 2004), and unfortunately other compounds have shown little signs of neuroprotection following stroke (Sacco et al., 2001; Muir et al., 2004). Animal studies investigating the use of NMDA-antagonists to prevent secondary injury following SCI are once again promising (Faden et al., 1988; Gaviria et al., 2000; Esposito et al., 2011), however, human translation is limited at this time.
ii. **Vascular system**

Damage to the local vascular system produces both hemorrhagic and ischemic conditions. The microcirculation surrounding the area of injury appears to be more susceptible to damage than the larger vessels. The hemorrhaging from this microcirculation results in petechiae and eventual necrosis of surrounding tissue (Dumont *et al.*, 2001a) with vasospasms and thrombosis (Koyanagi *et al.*, 1993a; Koyanagi *et al.*, 1993b). The combined damage produces local ischemia to the surrounding tissue. With hyperperfusion following restoration of blood flow, the damage produces a cascade of oxidant species (Phillis & Sen, 1993). These short-lived oxidant species produce oxidative stress on surrounding cells which will eventually damage lipids, proteins and DNA (Lewen *et al.*, 2000).

There is evidence to suggest that removal of these oxidant species may attenuate damage. For example, there is a depletion of endogenous antioxidants following SCI, and exogenous supplementation may assist in preventing peroxidation (Dumont *et al.*, 2001b). Acute delivery of exogenous oxidative scavengers has been shown to be safe following brain injury (Muizelaar *et al.*, 1993), however human efficacy trials have yet to demonstrate a neuroprotective effect following brain injury (Young *et al.*, 1996). This may still be a promising area of research for neuroprotection following SCI as high dose methylprednisone may act through antioxidant pathways (See below; Bracken *et al.*, 1990; Bracken *et al.*, 1997). Additionally, a small open-label human trial of free radical scavengers demonstrated positive results (Chen *et al.*, 2005).
iii. **Immune system**

In addition the response of the nervous system and vascular system, the response of the immune system may contribute to the secondary injury following an SCI. In the hours after injury neutrophils are observed in the area of injury and may remain elevated for ~10 days (Fleming *et al.*, 2006). Neutrophils release enzymes which damage basement membranes (Zimmerman & Granger, 1990) and have been implicated in further hemorrhaging following SCI (Taoka *et al.*, 1997). CD68 immunoreactive microglia and macrophages appear 24 hours after injury and may last for months (Fleming *et al.*, 2006). In addition to phagocytosis, these cells release a cascade of cytokines including interleukin-1 and interleukin-6 which, among other things, act as endogenous pyrogens, creating a non-permissive environment (Rothwell, 1994). In addition to neutrophils and microglia/macrophages, T-leukocytes may be present in the weeks and months following SCI (Fleming *et al.*, 2006; Chang, 2007; see however Schmitt *et al* 2000).

Interventions targeted at the immune system have proven to be a delicate process, as some of these processes may in fact be beneficial for functional recovery. For example, some data suggest that T-leucocytes may be beneficial to functional recovery (Hauben *et al.*, 2000; see however Gonzalez *et al.*, 2003; Jones *et al.*, 2004). Over the course of multiple large scale human efficacy trials (Bracken *et al.*, 1984; Bracken *et al.*, 1990; Bracken *et al.*, 1997), some clinicians consider the use of high dose methylprednisolone as the current standard of care for acute spinal cord injuries. Methylprednisolone is a steroid which “alter[s] the expression of critical inflammatory mediators and growth factors, with resultant anti-inflammatory, immunosuppressive, and antiproliferative properties” (Gomes *et al.*, 2005). Steroids have
been shown to improve functional recovery in animals in the vast majority of published studies (Sayer et al., 2006), and 2 large human efficacy trials have demonstrated a positive effect (Bracken et al., 1990; Bracken et al., 1997). As such, the use of methylprednisolone is common following acute spinal cord injury, however this remains controversial. Detractors of this treatment criticize the original study design of the efficacy trials, the lack of independent confirmation, the lack of functional benefits and the possibility of added harm (Hurlbert, 2000; Walker & Criddle, 2001; Hugenholtz, 2003; Hurlbert & Hamilton, 2008; Miller, 2008; Rozet, 2008). This has led some to conclude that the current widespread use of methylprednisolone is due to peer pressure and fear of litigation rather than evidence (Hurlbert, 2006).

2. Adaptation of the segmental motor system

Following the initial injury, substantial specific and time-ordered effects on the segmental motor system result in continued alterations in sensorimotor integration. In general, there is an immediate loss of motor function (both reflexive and volitional) with a gradual return of reflexive and, at times, volitional motor control. Table II and Figure 3 outline the changes in the segmental motor system following spinal cord injury. In the hours and days following a spinal trauma there is likely to be a complete disruption of supraspinal drive (including both disruption of the specific command and the excitability command). This disruption of supraspinal drive reduces motoneuron excitability, which ultimately leads to minimal reflex and/or, if possible, volitional motor output. In the weeks and years following spinal cord injury, there is a progressive increase in reflex activity thought to be due the recovery of
motoneuron excitability. In those with incomplete SCI, restoration of function of spared descending pathways may lead to improve volitional motor control.
Table II: Some adaptations of the segmental motor system following spinal cord injury

<table>
<thead>
<tr>
<th>Motor Unit</th>
<th>Reflex input</th>
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<td>8. Alterations in transmitter release</td>
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<td>3. Peripheral nerve hypoxcitability</td>
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<td>4. Excitatory receptor upregulation</td>
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<td>5. GABA down/up regulation</td>
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<td>6. Alterations in persistent and constitutive currents</td>
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<td>14. Sprouting of corticospinal pathways</td>
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<td>15. Release of tonic inhibition</td>
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<td>16. Sprouting of metabotropic pathways</td>
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<td>17. Cortical alterations</td>
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<td>18. Increased sense of effort</td>
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Figure 3: Some adaptations of the segmental motor system following spinal cord injury. See Table II.
The homeostatic response of the segmental motor system has been characterized clinically, although there is still debate as to the precise processes underlying clinical presentation. It is common clinical practice to describe the progression of motor recovery following SCI in two stages: immediate spinal shock, followed by long-term adaptations (acute and chronic). Later, it was proposed that there may be three (Little et al., 1999) or even four (Ditunno et al., 2004) stages following spinal cord injury. Rather than attempt to describe these changes in a temporal sequence, the changes will instead be described at various points throughout the segmental motor system emphasizing the chronic adaptations.

a. **Motor unit**
   
   i. **Atrophy**

One of the most striking aspects of SCI on muscle is the decrease in muscle mass and specifically the size of muscle fibers (i.e., atrophy). In rats following complete SCI, a significant 36% decrease in muscle cross sectional area after 5 days is observed (Dupont-Versteegden et al., 1998). This decrease in muscle cross sectional area decreases to 44% of control values at 3 months and may increase to 55% of control at 6 months (Otis et al., 2004). In humans, complete SCI results in a similar extent of atrophy (~45% decrease; Castro et al., 1999). Following incomplete SCI this atrophy is observed to a lesser extent (~20% decrease in rat, Hutchinson et al., 2001; ~33% decrease in humans, Gorgey & Dudley, 2007).

As muscle cross sectional area is correlated with force generating capability (Maughan et al., 1983), a functional consequence of SCI is decreased force generating capability. Indeed individuals with complete SCI (Gerrits et al., 1999) and incomplete SCI (Hornby et al., 2009b)
demonstrate severe decreases in force generation as compared to healthy controls using electrically evoked and volitional contractions, respectively.

**ii. Molecular transformation**

In addition to atrophy there may be changes in the fiber type composition of muscles distal to the lesion. Though there is much disorganization in muscle composition, in general there is a trend towards fast fatigable fibers following SCI. One of the main molecules involved with speed of the muscle contraction is the myosin heavy chain (MHC) molecule as data suggest a general trend in decreased expression of MHC I and in increase in MHC IIa, x, b in animal models following chronic spinal cord transection (Talmadge *et al.*, 1999; Talmadge *et al.*, 2002). The appearance of the hybrid MHC I/IIx isoform suggests there is not an orderly transition between MHC isoforms. Following animal models of incomplete SCI these changes may not be as readily observed (Hutchinson *et al.*, 2001).

In addition to changes in MHC isoforms, SCI also induces a disregulation in oxidative and glycolytic enzymes in animal models. Otis *et al.* (2004) demonstrated increases in the glycolytic enzyme, α-glycerophosphate dehydrogenase, in the weeks following spinal transection; this would be expected from the MHC II isoform transformation. Surprisingly, in the weeks following SCI there is a simultaneous increase observed in oxidative enzymes, specifically succinate dehydrogenase and citrate synthase. In the months following injury, both oxidative and glycolytic enzymes decrease below normative control values.

In human complete SCI, alterations in MHC isoforms in response to SCI are more varied, but likely follow a similar trend towards fast isoforms as do animal models. Again, the hybrid MHC I/II isoform fibers may be observed in human SCI (Burnham *et al.*, 1997).
Such changes have been demonstrated in individuals with incomplete SCI (Stewart et al., 2004). Alterations in MHC expression likely begin sometime past 4 months post injury and reach a steady state before 70 months, calling in to question the criteria for chronic SCI (Biering-Sorensen et al., 2009; see however Ditor et al 2004).

These changes in MHC isoforms and oxidative/glycolytic enzymes will alter the speed contraction, force frequency relationship and fatigability of the muscle. This is readily observed in animal models (Talmadge et al., 2002).

iii. Peripheral nerve hypoexcitability

In addition to changes at the soma of the motoneuron, the peripheral axon undergoes stereotypical changes in the days and weeks following SCI. These changes result in complex changes to the peripheral axon (Lin et al., 2007). Longitudinal investigations suggest that these changes in the first few weeks following SCI and returns towards uninjured values during the first month (Boland et al., 2011). It is unknown if peripheral nerve hyperpolarization following SCI contributes to motor behaviors via either voluntary or reflexive activation.

iv. Excitatory receptor upregulation

Outside of the spike in glutamate, the motoneuron is devoid of the majority of both reflex and supraspinal pathways in the acute stages following SCI. As such excitatory receptors may increase in efficacy. For example, 24 hours after injury there are increases in NMDA receptor subunit expression at and distal to the site of the lesion, some of which may persist for more than weeks (Grossman et al., 2000). This may contribute to increased synaptic
efficacy from descending pathways if present and intact, or from other pathways, including interneuronal and afferent inputs, which can lead to augmented reflex activity.

v. **GABA down/up regulation**

GABA receptor activation increases Cl⁻ conductance, hyperpolarizing the cell. The GABA receptors can indirectly contribute to motoneuron depolarization through downregulation. In the neonatal rat, alterations in GABA receptor are dependent on the serotonergic system (Sadlaoud *et al.*, 2010) – it is plausible that this 5-HT sensitive down regulation of GABA receptors occurs following adult onset SCI. Regardless of age on onset, in the weeks and months following SCI there is an upregulation of GABA receptors (Kroin *et al.*, 1993). In the intact nervous system this GABA upregulation may result in inhibition of cell firing, however changes in Cl⁻ conductance in the motoneuron may allow Cl⁻ induced EPSPs in the adult nervous system.

vi. **Alterations in persistent and constitutive currents**

The motoneuron is a dynamic participant in the generation of muscle force. The monoaminergic modulation of dendritic PICs is a means to actively regulate the gain of the motor output. In response to the loss of monoaminergic drive following SCI, recovery of motoneuron and reflex excitability is evident in the weeks to months (i.e., chronic stages) following injury, resulting in manifestation of spastic reflexes mediated by a rebound of PIC activity (Bennett *et al.*, 2001a). Increased motoneuron PIC activity in chronically spinalized motoneurons and resulting spasticity are likely a result of supersensitivity to 5HT (Li *et al.*, 2001).
2007) and conformational changes of receptors to a constitutively active state (Murray et al., 2010; Rank et al., 2011).

Involuntary motor output following human incomplete SCI (i.e. spasticity) is thought to be mediated in part by PIC-like activity (Hornby et al., 2003; Gorassini et al., 2004; Hornby et al., 2006a), and PIC-like activity using the low intensity, variable frequency NMES has been demonstrated in this population (Nickolls et al., 2004). However there is a paucity of research attempting to identify the influence of PICs in volitional motor activity following incomplete SCI, with only one report providing indication of PIC-like activity (Zijdewind & Thomas, 2003).

vii. **Reorganization of Cl- transporter**

Following spinal cord injury there is a reorganization of Cl- homeostasis, via down regulation of the potassium-chloride co-transporter-2 (KCC2). This shifts the reversal potential positive by 10 mV and, as a result, GABA and glycinergic input from may produce an EPSP in the motoneuron (Boulenguez et al., 2010). Such change in the actions of inhibitory input to the motoneuron is an exciting avenue of research, as this finding redefines the role and extent of inhibition following SCI (Crone et al., 2003).

b. **Reflex input**

Following SCI “our knowledge about plastic changes within the spinal cord is rather limited”(Maier & Schwab, 2006). Nevertheless, human electrophysiological data demonstrates increase excitation and a decrease in inhibition. Pierrot-Deseilligny and Burke (2005) provide an excellent description of the methods used to suggest increases in group II excitation, changes in Ib pathways and decreases in post activation depression, in addition to
changes at the motor unit, contribute to motor dysfunction following SCI. It is likely the alterations below contribute to the observed changes in reflex activity.

i. **Synaptic growth**

Without experimental intervention, it is unlikely that long range synaptic growth occurs in the CNS following SCI (Schnell & Schwab, 1990). However, it has been known for some time that local growth or sprouting readily occurs following SCI (McCouch *et al.*, 1958). While synaptic growth of descending pathways is typically considered a positive event which correlates with functional recovery, synaptic growth of reflex pathways and interneurons is thought to contribute to sensory motor deficits following SCI including autonomic dysreflexia (Weaver *et al.*, 2001), alterations in pain sensation (Gwak *et al.*, 2003) and changes in motor reflexes (Calancie *et al.*, 2005) and bladder control (de Groat, 1995; Leung *et al.*, 2007). In the weeks following injury, there is an increase in sprouting of reflexive inputs and interneurons, most of which does not enter the ventral horn (Krenz & Weaver, 1998). The lack of motoneuron connectivity via local sprouting suggests increased weight of spinal interneuronal networks contributing to motor output following SCI.

It has been suggested that there is an activity-dependent, competitive reorganization between sprouting of reflex and descending pathways which may shape recovery (Little *et al.*, 1999). If so, a means to regulate sprouting may be a strategy to promote motor output and decrease unwanted sensorimotor responses (Hagg, 2006). Indeed there is evidence to suggest training may prevent deleterious afferent sprouting (Singh *et al.*, 2011).
ii. **Alterations in transmitter release**

With repetitive Ia activation there is a time dependent decrease in EPSP response of the motoneuron (Curtis & Eccles, 1960). This depression is observed to a lesser extent in the months following SCI (Nielsen et al., 1993; Nielsen et al., 1995). This is taken as evidence to suggest these changes in post activation depression is due to increases in pre-synaptic Ia transmitter release following SCI, however other pre- and post-synaptic mechanism could be involved (Zucker & Regehr, 2002).

iii. **Unmasking of silent pathways**

Not all synapses are the same and as such there are varying degrees of synaptic strength – some signals with be faithfully transmitted while others will rarely evoke a post-synaptic EPSP, this latter event is termed silent or latent pathway. It has long been suggested that the CNS increases the strength of these silent pathways in response to a lesion of the nervous system (Jacobs & Donoghue, 1991).

A similar event occurs in corticospinal neurons as demonstrated through facilitation of phrenic output following intermittent hypoxia. Here a high cervical hemisection severs the corticospinal inputs into the phrenic nerve, resulting in unilateral paralysis of the diaphragm and functional defects in breathing. These deficits are restored through acute intermittent hypoxia. The pathway of long term facilitation has been shown to be initiated via 5HT and dependent on brain derived neurotrophic factor (BDNF; Baker-Herman & Mitchell, 2002; Baker-Herman et al., 2004). It is yet to be seen if acute intermittent hypoxia will prove to have similar benefits to the somatic motor system.
iv. **Receptor upregulation/supersensitivity**

In a similar manner to motoneurons receptor plasticity, receptors in dorsal horn and interneurons may upregulate in the weeks following SCI. In the dorsal horn, upregulation of metabotropic glutamate receptor (Gwak & Hulsebosch, 2005) and supersensitivity to 5HT (Hains et al., 2003b) is observed in the weeks following SCI. These changes are implicated in the presence of central pain following SCI (Hulsebosch et al., 2009).

v. **Presence of reciprocal facilitation**

In a healthy nervous system, activation of the Ia afferents will inhibit the antagonist and in some cases this pathway may be tonically active. However, following SCI, the appearance of reciprocal facilitation is often observed (Crone et al., 2003; Xia & Rymer, 2005; see however Lance & Degail, 1965). Reorganization of primary afferent polysynaptic pathways (reviewed in Rossignol & Frigon, 2011), prolongation of synaptic activation of the motoneuron (Norton et al., 2008) and alterations in inhibition via Cl- transporters (Boulenguez et al., 2010) may contribute to the reflex reversal following SCI.

vi. **Reorganization of neurotransmitter transporters**

Glial cells play a critical role in the regulation of glutamate in the synaptic cleft of spinal neurons (Danbolt, 2001). As described previously, immediately following injury there is an influx of glutamate. This local, high concentration of glutamate contributes to the secondary injury through an excitotoxicity cascade. In the hours following SCI there is increased expression of glutamate transporters (Vera-Portocarrero et al., 2002). In the weeks following SCI there is a decrease observed in glutamate transporters (Olsen et al., 2010; Kim et al.,...
2011). As this transporter regulates the synaptic concentration of glutamate, alterations in glutamate transporters will modulate the efficacy of synaptic transmission.

c. **Supraspinal drive**

i. **Sprouting of corticospinal pathways**

Following SCI, axotomized corticospinal axons begin a process of dieback followed by sprouting (Schwab & Bartholdi, 1996). In the weeks following incomplete spinal lesion, corticospinal pathways begin a process of sprouting which continues for months (Hill et al., 2001). This sprouting likely occurs in three areas. First, the spared axons may increase their connections to spinal circuits (Weidner et al., 2001). Second, axotomized corticospinal neurons may form new synaptic connection to long propriospinal neurons proximal to the site of the lesion, effectively bridging the lesion. It is then the case that propriospinal neurons reform on to the original targets of the axotomized corticospinal neurons (Bareyre et al., 2004). Third, axotomized corticospinal neurons may form new synaptic connection to motoneurons proximal to the site of the lesion, for example hindlimb motoneurons may become functional forelimb motoneurons following spinal injury (Ghosh et al., 2010).

ii. **Release of tonic inhibition**

It has been suggested that in the uninjured system, tonic descending cortical projections inhibit the activity of excitatory interneurons. Norepinephrine, specifically the alpha2 receptor, has been shown to depress group II transmission in the ventral horn of the cat (Bras et al., 1989; Bras et al., 1990). Similar results have been observed in humans (Marque et al., 2005). Following SCI, this tonic descending inhibition would be removed. This disinhibition of excitatory interneurons would result in increased motor output – indeed
following acute spinal transection group Ib, II and III interneurons are less inhibited (Eccles & Lundberg, 1959).

iii. Sprouting of metabotropic pathways

Following SCI there is a disruption of descending monoaminergic pathways. As described above, the segmental nervous system adapts to these changes through changes in receptors, transporters, and changes in synaptic growth. There is debate as to the possible changes in the descending monoaminergic projections. Following a thoracic hemisection in animal models, there is an immediate decrease in 5HT concentrations in the lumbar region of the hemisected side of the spinal cord. Ipsilateral 5HT concentration recovers slightly in the weeks following spinal cord injury, and is accompanied by growth of contralateral serotonergic fibers through lamina X, however this concentration of 5HT fibers remained well below sham control values distal to a spinal lesion (Saruhashi et al., 1996; Hains et al., 2002). Similar, but more robust, increases in local 5HT sprouting may occur in response to deafferentation (Polistina et al., 1990). Following spinal cord transaction, there is likely no regrowth of 5HT fibers (Bregman, 1987).

iv. Cortical alterations

Though the original insult is at the level of the spinal cord, cortical reorganization occurs in response to SCI and likely contributes to the sensorimotor deficits. Animal models have suggested neurons in the primary motor cortex undergo apoptosis (Hains et al., 2003a). In humans with complete SCI, anatomical changes including decreases in motor cortex grey matter and fiber loss are observed in the years following SCI (Wrigley et al., 2009). While anatomical changes to the motor cortex may occur in the months following SCI, functional
changes may occur much quicker. For example, in the minutes following either complete spinal transection or reversible anesthetic block of thoracic spinal transmission, the brain demonstrates increases in slow wave activity with less spontaneous activity (Aguilar et al., 2010). Following human SCI, chronic changes in motor cortex representations are observed (Henderson et al., 2011).

v. **Increased sense of effort**

An increased sense of effort during motor activity is a common finding in individuals with chronic diseases processes of the central nervous system (Thomas & Zijdewind, 2006). Though not formally assessed in the SCI population, based upon similar disease processes, it has been suggested that individuals with SCI need more effort to produce a given level of force as compared to a healthy control individual. Twitch interpolation during sustained MVE demonstrates significant declines in central drive during a sustained maximal contraction (Latash et al., 1996)

iii. **Rehabilitation of the motor system**

1. **Delivering optimal physical rehabilitation**

As with any training programs, there is likely an optimal set of interventions to maximize functional motor recovery. While particular interventions will be dependent on the extent of motor impairments following SCI, it is my belief that a unified framework will apply to all functional retraining of the neuromuscular system following neurological injury. Kleim and Jones (2008) have written an excellent review outlining this framework. It is the belief of many that principles such as specificity, repetition and intensity are general tenants which
should be included in the rehabilitation of the motor system following any injury of the central nervous system (Hornby et al., 2011).

2. **Impairments correlate with activity limitations**

Despite rehabilitative efforts, SCI is likely to produce severe impairments in strength which limit an individual’s independent functional mobility. Individuals with motor incomplete SCI on average possess less than half the volitional torque generating ability as compared to healthy age-, gender-, height- and body weight matched controls. (Jayaraman et al., 2006) These deficits in strength are particularly devastating to mobility as torque generating capability of lower extremity muscles are a primary determinant of walking ability in this population. (Crozier et al., 1992; Saraf et al., 2010; Zorner et al., 2010; van Middendorp et al., 2011; Yang et al., 2011b)

This loss of mobility has a deleterious effect on physical activity and promotes a cycle of further deconditioning. This decrease in physical activity and sedentary lifestyle leads to a host of chronic disease conditions including cardiovascular disease (Groah et al., 2001), diabetes and related endocrine disorders (Imai et al., 1996), obesity (Gupta et al., 2006) and osteoporosis (Giangregorio & McCartney, 2006). These chronic disease conditions often result in hospitalization (Jaglal et al., 2009; Krause & Saunders, 2009; Waddimba et al., 2009), poor quality of life (Lidal et al., 2008) and may cost millions of dollars over the lifespan of the individual (2009b).
3. Rehabilitation of impairments will improve activity

Limitations

Exercise has been thought to increase strength, help regain mobility and promote health in individuals with SCI. Various studies have looked at providing exercise interventions in individuals with incomplete SCI with mixed results. For instance, a case series of 3 individuals with SCI who underwent a 12 week lower extremity progressive resistance and plyometric training program demonstrated increases in peak torque production in addition to increases in walking speed (Gregory et al., 2007). Likewise, a 6 month multimodal intensive (meaning, time spent not effort) exercise program in individuals with SCI demonstrated improvements in clinical measures of strength and function (Harness et al., 2008). While these studies suggest improvements in strength, recent studies have suggested that 8 weeks of progressive resistance training may not improve strength or function in individuals with incomplete SCI (Glinsky et al., 2008).

In all, studies attempting to improve strength in individuals with SCI via exercise interventions have had mixed results. However it is important to note that when these modest improvements in strength are observed, it is often accompanied by measurable gains in function. It is noteworthy that this quantitative research is mirrored by the subjective analysis of barriers of exercise in individuals with SCI. According to individuals with SCI who do not exercise, a major barrier to exercise is due to a ‘limited return on investment’ (Kehn & Kroll, 2009) – at this point in time, these ‘limited return on investments’ may indeed be true. Finding an effective strength training program would be an important finding in the lives of individuals with incomplete SCI.
Figure 4: Potential sites of fatigue and potentiation correspond with physiological changes following chronic spinal cord injury.
d. **Acute changes in force generation following chronic spinal cord injury**

Fatigue is a common complaint in individuals following SCI. A large percentage of patients with SCI (50% - 70%) complain of fatigue (Suzuki *et al.*, 2007; Anton *et al.*, 2008; Fawkes-Kirby *et al.*, 2008; Wijesuriya *et al.*, 2012). The impact of this fatigue is substantial and is inversely correlated with activity levels (Tawashy *et al.*, 2009) and quality of life (Jensen *et al.*, 2007). Despite this substantial number of complaints and the seriousness of the consequences, the potential mechanisms of fatigue in the population are unclear. Much work has focused on alterations in excitation contraction coupling, though this mechanism may not explain the subjective reports of fatigue in this population.

i. **Fatigue**

1. **Electrically evoked contractions**

A common means to assess fatigability is using repeated trains of electrical stimulation. When tested in this manner, both animal models of and individuals with chronic SCI, the muscle become more fatigable as compared to healthy muscle (Thomas, 1997; Gerrits *et al.*, 1999). This increased fatigability to evoked contractions is not observed in individuals with acute SCI (Shields, 1995). Alterations in muscle fiber types towards more fatigable isoforms likely contribute to this decrease in force generation during electrically evoked contractions. Further the recovery from fatiguing stimulated contractions appears to be longer in individuals with SCI (Mahoney *et al.*, 2007). These changes are thought to be associated with deficits in excitation contraction coupling as minimal change in the maximal M-wave is observed during electrically evoked contractions (Shields *et al.*, 1998; Klein *et al.*, 2006; Pelletier & Hicks, 2011; see however Ollivier-Lanvin *et al.*, 2009)
2. Exhaustion of neuronal activity

Dietz & Muller (2004) demonstrate individuals with chronic complete SCI generate decreases in lower extremity EMG during robot assisted treadmill walking over a course of 5-10 min. This exhaustion of EMG during assisted walking was not observed in healthy controls or individuals with acute SCI. Though speculative, the authors suggest that this exhaustion is due to the degradation of interneuronal circuitry following chronic motor complete SCI.

3. Sense of effort

Patients with a chronic neurological disease often report fatigue as a clinical symptom. In this context, fatigue is often defined as “perceived, […] subjective, […] not the as muscle weakness, depression, or muscle fatigability” (Chaudhuri & Behan, 2004, pg. 978). This fatigue is generally thought to limit function; as such questionnaires such as the Fatigue Severity Scale have been developed to quantify clinical reports of fatigue following neurological injury (Krupp et al., 1989). Since this time the Fatigue Severity Scale has been often used to quantify patients’ reports of fatigue. Recently, this scale has been shown to have excellent psychometric properties in the SCI population (Anton et al., 2008).

It is possible that the subjective report of fatigue may not be a result of the injury but instead a factor of particular treatments. For example, poly-pharmacy is a major issue following SCI, where the average patient being prescribed 8 different medications (Krause et al., 2009). Medications to treat pain or spasticity are indeed associated with the amount of self-reported fatigue in this population (Lee et al., 2010). The role of medication induced fatigue may be over shadowed by the fact the amount of prescription medication used is
inversely related to function (Kohout et al., 2011), and positively related to need for rehospitalization (Krause, 2010) and mortality (Krause et al., 2009).

ii. **Potentiation**

1. **Twitch potentiation**

Animal models of spinal cord hemisection suggest that twitch potentiation occurred to a lesser extent in both hemisected and transected animals compared to both control and sham animals (Celichowski et al., 2006; MacIntosh et al., 2008). Whereas in human complete SCI, twitch potentiation is observed to a greater extent in the SCI subjects as compared to healthy controls (Shields et al., 2006; Dudley-Javoroski et al., 2008). The discrepancy between animal models of SCI and humans with SCI is unclear, however differences may be able to be explained by differential alterations in the muscle between the two (Biering-Sorensen et al., 2009).

2. **Doublet potentiation and catch-like properties**

There appears to be an increase in catch like properties following spinal cord injury (MacIntosh et al., 2008). Such changes may be explained by the changes in fiber type composition as described above. As electrically stimulation tends to produce greater fatigue in paralyzed muscles, the use of these catch-like properties may be used to minimize fatigue with electrical stimulation (Scott et al., 2005; Scott et al., 2007). This increase in catch-like properties may not be common to muscles and/or stimulation protocols (Bickel et al., 2004; Chou et al., 2008)
3. Windup of reflex activity

Increases in motor output to repeated reflexes have been observed following SCI. This includes the windup of both the stretch (Hornby et al., 2006a) and flexor (Hornby et al., 2003) reflexes following chronic incomplete SCI. This is thought to be due to increases in PIC mediated motorneuron excitability; however windup of dorsal horn interneurons may also contribute.

4. Supramaximal torque generation

It was recently observed that repeated maximal volitional effort (MVE) contractions can elicit a progressive increase in maximal torque-generation in humans with incomplete SCI (Hornby et al., 2009b). This finding of supramaximal torque-generation is in stark contrast to longstanding research indicating a substantial decrease in repeated force-generating capability (i.e., fatigue) during evoked contractions in humans with complete SCI using percutaneous NMES, indeed it appears volitional effort is required for the observed increase in torque (Thompson et al., 2009). It is important to note this supramaximal torque-generation is not due to learning, as similar torque profiles are observed when the bout of repeated MVE contractions is performed within and between testing sessions.

e. Summary of introduction

In all, the segmental motor system has proven to be a powerful conceptual framework from which to investigate motor control. In the context of this thesis, the segmental motor system will be used to help explain the mechanisms underlying supramaximal torque generation in human chronic incomplete spinal cord injury (see Section 1.d.4 above). Therefore, this introduction was a convenient place to describe the short term and long term alterations in
motor output of the segmental motor system. Specifically, 23 potential sites of short term alterations in motor output (fatigue or potentiation) were described (see Table I and Figure 2). Additionally, 18 long term alterations in the segmental motor system were described for chronic incomplete spinal cord injury (see Table II and Figure 3). Of these 18 chronic adaptations, 5 adaptations have been shown to influence the short term adaptations of the motor system (see Figure 4).

From this introduction, we will now move towards experiments which seek to understand why people with SCI increase maximal force generation. The end goal of this line of investigation is to use this knowledge to develop a novel rehabilitation program for individuals with SCI, however it my hope that work will also help elucidate spinal cord physiology in both healthy and diseased conditions.

Three experiments will be described. The first experiment (Chapter 2) will describe our attempts to establish a spinal locus of this increase in volitional force generation using various electrophysiological techniques. The second experiment (Chapter 3) will describe how this increase in volitional force generation is modulated by intensity of preceding contractions and rest between successive contractions. The third experiment (Chapter 4) will attempt to understand the specific neuromodulators involved in this behavior by using medications with increase or decrease the effectiveness of specific neurotransmitters. It is hoped that this knowledge will allow for the development of specific physical and pharmacological interventions which may help overcome the debilitating deficits in volitional force generation found in individuals with incomplete spinal cord injury.
II. Segmental mechanisms – Experiment 1

a. Summary

Despite greater muscle fatigue in individuals with spinal cord injury (SCI) when compared to neurologically intact subjects using neuromuscular electrical stimulation (NMES) protocols, few studies have investigated the extent of volitional fatigue in motor incomplete SCI. Using an established protocol of 20 repeated, intermittent, maximal volitional effort (MVE) contractions, we previously demonstrated that subjects with incomplete SCI unexpectedly demonstrated a 15% increase in peak knee extensor torques within the first 5 MVEs with minimal evidence of fatigue after 20 contraction. In the present study, we investigated potential segmental mechanisms underlying this supramaximal torque generation. Changes in twitch properties and maximum compound muscle action potentials (M-waves) were assessed prior to and following 1, 3, and 5 MVEs, revealing a significant 17% increase only in maximum twitch torques after a single MVE. Despite this post-activation potentiation of the muscle, use of conventional NMES-paradigms to elicit repeated muscular contractions, suggesting limited muscular contributions to the observed phenomenon. To evaluate potential central mechanisms underlying the augmented torques, nonlinear responses to wide-pulse width (1 ms), low-intensity, variable-frequency (25-100 Hz) NMES were also tested prior to and following repeated MVEs. When variable-frequency NMES was applied following the repeated MVEs, augmented and prolonged torques were observed and accompanied by sustained quadriceps electromyographic activity often lasting >2s after stimulus termination. Such data suggest a potential contribution of elevated spinal excitability to the reserve in volitional force generation in incomplete SCI.
b. **Introduction**

Spinal cord injury (SCI) is a debilitating disease process which results in profound sensorimotor deficits. In individuals with motor complete SCI, the muscles below the lesion level can experience rapid and progressive atrophy (Castro et al., 1999) and potential fiber type conversion (Dudley-Javoroski & Shields, 2008). These changes can contribute to decreased force generating capacity and greater fatigue (Gandevia, 2001) as compared to neurologically intact subjects when elicited by high-amplitude neuromuscular electrical stimulation (NMES) (Gerrits et al., 1999). Similar muscular adaptations are known to occur in individuals with motor incomplete SCI (Stewart et al., 2004; Shah et al., 2006). Despite these muscular changes, individuals with motor incomplete SCI can sustain low-level volitional forces for a longer duration than able-bodied individuals (Thomas & del Valle, 2001). More recent data obtained during repeated, intermittent, maximal volitional effort (MVE) contractions of the knee extensors have shown that individuals with motor incomplete SCI demonstrate short-term 15% increases in volitional torques with corresponding increases in knee extensor electromyographic (EMG) activity (Hornby et al., 2009b). The term supramaximal volitional torque generation will be used to describe this increase in torque generation, as the level of torque generation is above what is commonly accepted as maximal torque generation (i.e. single effort performed in isolation of other contractions). The precise mechanisms underlying this supramaximal volitional torque generation in human incomplete SCI remains unknown.

Within the segmental motor system, at least three distinct loci of excitability may contribute to this increase in force generation during repeated MVEs in human incomplete
SCI. First, nonlinear summation of sarcoplasmic Ca$^{2+}$ release (Duchateau & Hainaut, 1986) and/or myosin light chain phosphorylation (Tubman et al., 1997) may contribute to augmented twitch responses following a muscle contraction (i.e., post-activation potentiation) (Brown & von Euler, 1938). Post-activation potentiation is greater in fast twitch muscles (Brown & Loeb, 1998) and varies with both duration and magnitude of preceding contractions, with maximal potentiation observed following brief, high intensity efforts (Vandervoort et al., 1983).

Second, alterations in neuromuscular transmission and propagation may augment force generation via increased transmitter release (Kalkstein & Magleby, 2004) and/or augmented Na$^+$/K$^+$-ATPase activity at the sarcolemma (Hicks & McComas, 1989). In neurologically intact individuals, maximum compound muscle action potentials ($M_{\text{max}}$) can increase following volitional contractions of various muscle groups, particularly following brief high intensity efforts (Hicks et al., 1989; Zijdewind et al., 1999; Hamada et al., 2003).

A third potential segmental mechanism underlying increased torques may be a change in reflex sensitivity and/or excitability of spinal circuits. During sustained, low-level contractions in intact individuals, decline in spindle feedback are thought to contribute to decreased motor output (Macefield et al., 1991). However, other data suggest facilitative effects of spindle input on motor unit recruitment during repeated low-level volitional contractions (Suzuki et al., 1990). These latter observations were more recently attributed to increased excitability of spinal motoneurons (Gorassini et al., 2002b). Specifically, the decrease in synaptic input to re-recruit motor units during repeated low-level contractions has been attributed to the presence of persistent inward currents (PIC), defined as a non-
inactivating Na\(^+\) and Ca\(^{2+}\) currents intrinsic to the motoneuron (Heckmann et al., 2005). PIC activation results in augmented and prolonged depolarization from brief synaptic inputs (i.e., plateau potentials) (Hounsgaard et al., 1984) resulting in increased and/or sustained motor output. Further, PIC behaviors demonstrate a progressive increase in neuronal excitability following brief repeated excitation, or warm-up, (Svirskis & Hounsgaard, 1997) and are sensitive to metabotropic modulation (Hounsgaard et al., 1988). Data from both animal models and humans with SCI suggest that PIC activity may contribute to involuntary (spastic) motor behaviors (Bennett et al., 1999; Gorassini et al., 2004), although little is known regarding the contribution of PICs to volitional motor behaviors in human SCI (Zijdewind & Thomas, 2003b).

Methods to assess motoneuron PIC activity in humans are necessarily indirect. One method of inferring PIC-like activity involves elicitation of augmented and prolonged torques and EMG following a train of low-amplitude, wide-pulse width (1-ms duration), variable-frequency (25-100-25 Hz) NMES in resting subjects. The resulting augmented and prolonged motor activity is indicative of spinal PIC-like activity, as this response occurs in individuals without conscious awareness (Collins et al., 2001, 2002), in subjects with complete SCI (Nickolls et al., 2004), and is absent following peripheral nerve block. More recent human and animal work has suggested that the augmented torque produced during the low-frequency stimulation (i.e. 2\(^{\text{nd}}\) 25 Hz) is strongly regulated by muscular mechanisms, similar to staircase potentiation (Frigon et al., 2011). Specifically the augmented torque is dependent on the length of the muscle, and observed predominantly at shorter versus longer muscle lengths. The length dependence of the augmented torque is evident in both cat
muscle surgically isolated from nervous system and human muscle distal to a peripheral nerve block. While both neural and muscular mechanisms may contribute to increased torque produced during the stimulation, the prolonged torque and accompanying EMG activity following termination of neuromuscular stimulation requires prolonged central drive (Collins et al., 2002; Fig. 10), and remains consistent with spinal motoneuron PIC activity.

To elucidate potential segmental mechanisms underlying increased force generation with repeated MVEs in human SCI, we investigated changes in segmental motor system excitability prior to and following single and repeated MVEs contractions. Understanding potential mechanisms underlying the acute increase in volitional torque with repeated MVEs can facilitate development of targeted therapies that harness this reserve of excitability for functional gains.

c. **Methods**

i. **Subjects**

Individuals with motor incomplete SCI were recruited from the outpatient clinics of the Rehabilitation Institute of Chicago. Experiments were performed on 15 subjects (13 males) with chronic (> 1 yr.) spinal lesions above the T10 neurological level (see Table III), with five subjects tested bilaterally (20 legs total). Nine neurologically intact control subjects were also recruited. Multiple experiments were performed on separate days, and not all participants were tested on all procedures. Participants with SCI were classified as either C or D using the American Spinal Injury Association Impairment Scale (Maynard et al., 1997) and demonstrated residual volitional knee extensor strength in at least one limb, with evidence of normal or hyperactive reflexes (Priebe et al., 1996). Exclusion criteria included medical
history of multiple CNS lesions, history of lower limb peripheral nerve injury, or orthopaedic injury which may limit knee extensor contractions. Clinical examination performed prior to testing included assessment of responses to passive limb movement of the knee (Modified Ashworth: range 0-5; Bohannon & Smith, 1987) and lower extremity motor score (LEMS: range 0-5; Marino & Graves, 2004). None of the subjects were using anti-spasticity medication at the time of the study, and all had previous experience using the testing apparatus. All subjects were aware of the study protocol to assess volitional fatigue, but were unaware of the preliminary data or hypothesis regarding the experimental procedures. All procedures were conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Northwestern University.
Table III: Subject demographics

<table>
<thead>
<tr>
<th>Subject</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Level of Injury</th>
<th>Time Since Injury (Months)</th>
<th>Knee Extensor Modified Ashworth</th>
<th>LEMS</th>
<th>Baseline MVE (Nm)</th>
<th>CAR</th>
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<td>C5-6</td>
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ii. **Experimental setup**

Experiments lasted approximately 1-1.5 hr. Subjects were seated in the adjustable height chair of the testing apparatus (System 3®; Biodex Medical Systems, Shirley, NY, USA) with the hips flexed to 80-90 deg and the knee positioned at 90 deg. The distal shank was secured to the dynamometer arm, which was coupled to a 6 degree of freedom load cell (ATI, Apex, NC) used to assess knee extensor torques. Torque signals were low-pass filtered at 200 Hz and collected at 1000 Hz. Surface EMG was recorded using active bipolar electrodes (Delsys, Boston, MA, USA applied over the vastus lateralis (VL), vastus medialis (VM), rectus femoris (RF) and medial hamstrings (MH). EMG signals were amplified (×1000), band passed filtered (20-450 Hz) and sampled at 1000 Hz simultaneously with the torque data.

iii. **Experimental protocol**

Torque and EMG data were collected on the more impaired limb (if tested unilaterally), as determined during clinical evaluation and confirmed by differences in central activation ratios (CARs) between limbs. Experiments began with subjects performing three baseline MVEs lasting 3-8 seconds each with > 1 minute between attempts. Subjects were instructed to produce an isometric contraction by attempting to extend the knee as hard and as fast as possible, with vigorous verbal encouragement but no visual feedback. During each baseline MVE, a brief train of electrical stimulation (10 pulses, 600 µs duration, 100 Hz, 135V; S48 Grass, external isolation, West Warwick, RI) was delivered to the knee extensors through 3” X 5” self-adhesive, stimulating electrodes (ConMed Corp, Utica, NY) placed over the distal VM and the proximal VL. The stimulation was triggered manually by the experimenters.
when the knee extensor torque appeared to reach a plateau during MVEs (Miller et al., 1999). The electrically elicited torque superimposed on the maximum volitional torque was used to estimate voluntary knee extensor activation calculated using the central activation ratio (CAR). CAR was calculated by dividing the mean voluntary torque produced 100 ms before the stimulation onset by the peak electrically elicited KE torque.

Following baseline contractions, subjects performed up to 3 bouts of contractions, each consisting of 1, 3, or 5 consecutive MVEs (5 s on, 5 s off), with verbal encouragement provided during each effort. A 5 minute rest period was given between bouts of repeated MVEs (Hornby et al., 2009b). Maximal M-waves, twitches or variable-frequency NMES (each described below) were collected prior to and 5 s following volitional contractions when torques had returned to baseline levels. A single subject example is demonstrated in Figure 5.

In 10 subjects (10 legs) with SCI, maximal M-waves and twitch torques were elicited using a single (1 ms duration) pulse from a constant current stimulator (Digitimer Ltd, Hertfordshire, UK) from a custom-made stainless-steel bipolar bar electrode placed superficially over the femoral nerve. Maximal M-waves were found simultaneously in the VL, RF and VM by probing for the optimal site of the femoral nerve near the inguinal outlet and increasing current until no subsequent increase in M-wave response in any muscle group was elicited; stimulation during testing was then delivered at 120% of this current to elicit $M_{\text{max}}$. Twitch torques were measured from the torque response resulting from $M_{\text{max}}$ stimulation.

In 13 subjects (18 legs) with SCI and 9 healthy control subjects (9 legs), low-amplitude variable-frequency NMES was delivered using the constant current stimulator through the
same stimulating electrodes used to obtain CAR values. Testing began by finding the torque response to brief constant frequency trains of stimulation elicited at maximum stimulator output (100 mA, 5 ms duration pulses at 100 Hz). Stimulating currents were then reduced to elicit twitch amplitudes which produced 7-10% of maximal torque and remained at this intensity for subsequent variable-frequency NMES (Collins et al., 2001). The variable-frequency NMES consisted of 6 seconds of stimulation divided into 3 frequency epochs; 2 s at 25 Hz, 2 s at 100 Hz, and 2 s at 25 Hz. This variable-frequency NMES experiment was repeated in 9 neurologically intact subjects.

Ten subjects (10 legs) with SCI participated in a separate set of experiments based on findings of post-activation potentiation (see Results). Here we sought to determine whether repeated high-amplitude, electrically stimulation of the knee extensors in the absence of volitional drive would result in similar increases in knee extensor torques as observed during repeated MVEs in individuals with incomplete SCI. Following baseline testing as described above, subjects with SCI underwent an electrically stimulated fatiguing protocol of the knee extensors (Gerrits et al., 1999). Maximal evoked torque (200 ms duration, 30 Hz; S48 Grass, external isolation, West Warwick, RI) was found by delivering increasing levels of stimulation for 1 s of stimulation through 3” x 5” electrodes (placed over quadriceps as described above) until no further increases in torque was observed. Stimulation intensity was determined by the voltage that produced 30-33% of the maximal evoked torque and used as baseline (Gerrits et al., 1999). Subjects underwent a 10 minute high-intensity NMES protocol similar to the timing of MVEs (5 s on, 5 s off), with calculated stimulation parameters.
Torque produced during the high-intensity NMES was normalized to maximal baseline stimulated torque. For these tests only, torque data were collected at 100 Hz.
Figure 5: Motor responses during three repeated maximal contractions followed by supramaximal stimulation of femoral nerve. The reserve of volitional force generation is observed in the knee extensors of an individual with motor incomplete SCI during 3 repeated MVE contractions (5 s on, 5 s off). Increased EMG activity is observed in knee extensor muscles with little change in antagonist muscles. Potential segmental mechanisms underlying this reserve of volitional force generation were assessed using multiple forms of peripheral stimulation prior to (not shown) and 5 seconds following a bout of repeated contractions (a single stimulus to assess changes in twitch and M-wave responses is shown here).
iv. Data collection and analysis

Data was acquired and analyzed using custom LabView® software (National Instruments Austin, TX). Torque signals were low-pass filtered at 10 Hz using a Butterworth filter (4 pole, zero-phase lag). Peak torque was identified for each contraction and the period corresponding to +/- 50ms was then averaged to represent peak torque. The largest torque elicited during the 3 baseline MVEs was used to normalize the subject’s knee extensor torques during subsequent testing.

EMG signals during volitional contractions were notch filtered (58–62 Hz, zero-phase lag, 4 pole Butterworth), full-wave rectified and smoothed using a low-pass filter (10 Hz, zero-phase lag, 4 pole Butterworth) to create an envelope for further analysis. EMG activity during the repeated contractions was also normalized to the mean EMG activity present 100ms prior to the peak torque found during maximal baseline effort. Pooled extensor EMG activity was calculated as the average of the normalized VL, VM and RF activity. MH EMG activity was analyzed to assess alterations in antagonist activity during repeated MVEs.

Twitch torque and M-wave parameters were analyzed from neuromuscular responses to supramaximal, single pulse stimulation to the femoral nerve. Peak twitch torques, contraction times and half-relaxation time were measured from torque responses to femoral nerve stimulation filtered at 50 Hz. M-waves were analyzed from each knee extensor muscle (RF, VL, and VM) independently using the EMG signal prior to notch filtering. Following the stimulation artifact, a 30 ms window was used to assess M-wave parameters. M-wave amplitude determined by calculating peak-to-peak non-rectified amplitude of waveform, and M-wave area determined by calculating integrated area of rectified M-wave. M-wave values were
normalized to peak values from the baseline trials, and presented independently for each muscle and averaged across muscles. Raw twitch torque and M-wave values were also analyzed and used for illustrative purposes.

Analysis of torque and EMG responses to variable-frequency NMES (25-100-25 Hz stimulation for 2 s each) was performed to determine whether augmented and prolonged motor activity indicative of PIC-like activity was enhanced following repeated MVEs. Augmented torques were defined as an increase in torque responses from the first to the second 25 Hz stimulation period as described previously (Dean et al., 2007). Prolonged torques and EMG activity were defined as sustained motor activity above resting, pre-stimulation values for 2 s following NMES termination. Torque signals were analysed in 500 ms bins during the 6 s stimulation and 2 s following stimulus termination. Augmented torques were calculated by normalizing averaged torque response produced during the final 500-2000 ms of the 2nd 25 Hz stimulation by the averaged torque produced during the final 500-2000 ms of the 1st 25 Hz stimulation (note: the 1st 500 ms of each 25 Hz stimulation was not analyzed to obviate electromechanical delays). Prolonged torques were determined by calculating the average torque above baseline from 500 ms to 2000 ms following the end of stimulation.

Stimulation artifact during low-amplitude variable-frequency NMES precluded analysis of EMG activity, although prolonged EMG activity following stimulation was determined. Step response ringing associated with the Butterworth filter was minimized using a 50 Hz low-pass Bessel filter. Prolonged EMG activity was determined for up to 2 seconds following stimulus termination. EMG off-time was determined when rectified, smoothed
EMG signals crossed below 1 SD above resting, pre-stimulation values for ≥50 ms (Hodges & Bui, 1996). Integrated area was found by calculating the rectified EMG area from stimulus off-time to the EMG off-time. To assess between-subject variations, the integrated EMG was normalized to baseline conditions and expressed as a percentage.

Data in the text are presented as mean ± standard deviation, and in figures presented with standard errors. All statistical analyses were performed using computer software (Statview®; SAS Institute, NC, USA) with α = 0.05. Data were assessed for normality using Kolmogorov-Smirnov tests, with non-parametric tests used for non-normally distributed data. One-way and two way repeated measures ANOVAs were used to assess consistency of torque responses and differences in responses to specific neuromuscular stimuli from baseline to either 1, 3, or 5 repeated MVEs. Post-hoc Tukey-Kramer analyses were used as appropriate to determine individual differences among multiple comparisons. Comparisons between SCI and control subjects were made using an unpaired t-test or Mann-Whitney U using the combined responses to variable frequency NMES following MVE contraction for each group. Correlations between key variables were determined using Pearson product moments or Spearman rho coefficients as appropriate.

d. Results

Participants’ demographic and clinical data are presented in Table III. Baseline assessments demonstrate substantial weakness of knee extensors (74±47 Nm) secondary to central activation deficits (CAR values = 0.48±0.24). In individuals with incomplete SCI, peak knee extensor torques during 5 repeated MVEs (averaged across bouts) revealed an increase of 23±25% from initial baseline MVEs (p < 0.01, n=20 limbs tested in 15 subjects).
Seven subjects who performed at least 3 bouts of 5 repeated MVEs in the same experimental sessions demonstrated consistent increases in torque production (significantly increased at the 3rd, 4th and 5th contractions compared to the baseline; p<0.01), with no differences observed between repeated bouts (p=0.99) and no interaction (p=0.41), supporting the robust nature of the phenomenon (Hornby et al., 2009b).

i. **Changes in twitch torques and M-waves**

Single pulse stimulation of the femoral nerve applied at supramaximal current intensity prior to and following 1, 3 or 5 MVEs revealed variable changes in neuromuscular responses for individuals with SCI. A single subject example of changes in twitch responses is shown in Figure 6A prior to and following 3 MVEs, with little changes in response characteristics. Peak twitch torques from the knee extensors revealed small but significant increases following MVE contractions (n=10, p<0.01). Post-hoc comparisons revealed a significant 17% increase (from 33.9±11.1 to 41.0±15.8 Nm) in twitch torque following a single MVE only (Figure 6B), with non-significant increases following 3 or 5 repeated MVEs. No significant differences in contraction time (baseline = 92.9±30.7 ms) or half-relaxation time (baseline = 72.3±28.51 ms) were observed (p=0.57 and 0.09, respectively).

Supramaximal femoral nerve stimulation applied prior to and following single or repeated MVEs revealed little change in M-wave characteristics from each muscle assessed. Figure 6C depicts these changes in a single subject prior to and following 3 repeated MVEs (same trial used for Figure 6A). M-wave amplitudes and integrated areas were not significantly different for pooled or individual muscle M_{max} recordings (p = 0.38 for pooled
response, Figure 6D), with a mean increase of 3% from baseline across all knee extensor muscles following a single MVE.
Figure 6: Motor responses to stimulation of the femoral nerve prior to and following three repeated maximal effort contractions of the knee extensors. (A) Individual knee extensor torque response to stimulation of femoral nerve following 3 repeated MVEs. (B) Group data indicates significant increases in baseline twitch torque values when stimuli are delivered following 1 MVE. (C) M-wave response of VL, VM, and RF to femoral nerve stimulation following 3 repeated MVE contraction (same subject as in 3A). Grey boxes indicate the 30 ms window in which the M-wave was analyzed. (D) Group data pooled across muscles indicate no change in M-wave amplitude following repeated MVEs.
ii. **Repeated electrically-evoked contractions**

The observed potentiation of peak twitch torques following a single MVE suggested that peripheral mechanisms could account for some of the increased torque responses with repeated MVEs, independent of changes in central excitability (Hornby *et al.*, 2009b). To test this hypothesis, repeated high-amplitude NMES was performed using a stimulation paradigm similar to the timing of repeated MVE contractions (5 s on, 5 s off) performed over 10 minutes. NMES intensity was selected to generate 30-33% of maximum stimulated torque to improve subject tolerance, representing 59±6.5% of their peak knee extensor torque during baseline MVEs elicited using 8.7±2.1 V; similar intensities and durations of knee extensor contractions have been used previously in patients with complete SCI (Gerrits *et al.*, 1999). In the 10 subjects tested, repeated high-amplitude NMES-elicited knee extensor torques demonstrated a rapid and marked decline from initial levels; a single subject example is shown in Figure 7A. Analysis of the 1st 5 contractions revealed a significant decline in knee extensor torques at the 5th contraction (deceased to 88±11% of the first NMES-elicited torque, Figure 7B). Eight subjects produced the maximal torque on the 1st stimulation, with all forces in the other 2 subjects < 15% greater than torques generated on the 1st NMES-elicited contraction. In contrast, during 5 repeated MVEs, peak torques of the same subjects increased up to 28±20%, with significant differences between the 1st and 2nd-5th MVEs (p < 0.01, Figure 7B). Despite differences in average baseline NMES-elicited and volitional knee extensor torques, a comparison between protocols observed in a single subject with nearly equivalent baseline torques is shown in Figure 7C, indicating the divergence in responses between the two testing conditions.
Figure 7: Repeated high-amplitude electrically-evoked contractions produced a decline in force generation. (A) Torque responses from knee extensor in one subject during 10 minutes of high-amplitude NMES. A sharp decline in force generation is observed with repeated high-amplitude NMES (Gerrits et al., 1999). (B) Comparison of 1st 5 stimulated contractions and 5 repeated MVEs revealed significant differences. (C) Overlaid torque data from an individual with motor incomplete SCI in which the first NMES-elicited contraction generated a similar torque to the first MVE contraction, although torque changes differed with repeated contractions. EMGs from volitional efforts indicate central changes involved in increased torque generation.
iii. **Responses to low-amplitude variable-frequency stimulation**

Stimulation intensities used during low-amplitude variable-frequency NMES elicited 7.2±2.8% of torque produced at maximal stimulator output assessed with brief stimulation trains (5 pulses, 100 Hz) at a stimulation intensity of 15.9±5.1mA. An example of typical torque and EMG responses to low-amplitude variable-frequency NMES prior to and following 3 repeated MVEs is demonstrated in Figure 8. In this example the torque generated during the 2nd 25 Hz (i.e., following 100 Hz stimulation) was greater than torque during the 1st 25 Hz stimulation, although only following repeated MVEs. Following stimulus termination, EMG activity from knee extensor muscles was apparent and contributed to prolonged torque responses.
Figure 8: Motor response to low-intensity variable-frequency stimulation prior to and following three maximal effort contractions. Prior to MVE contractions (grey line); there is minimal augmentation of torque during 2nd 25 Hz epoch and minimal prolonged torque and EMG following the end of stimulation. Following 3 repeated MVEs (black line) there is a substantial increase in torque during the 2nd 25 Hz and a prolonged torque and EMG response following the end of stimulation, suggestive of PIC-like behaviour of spinal neurons.
Such behaviors were consistent across all 13 subjects (18 limbs tested), with the exception of 1 of the 13 subjects (subject 6) who consistently demonstrated flexion withdrawal reflexes (i.e., flexor spasms) during the 1st 25 Hz and 100 Hz epochs of the variable-frequency NMES. Figure 9 demonstrates these responses, with flexor reflexes denoted by the downward torque deflections. This data was difficult to quantify and was not included in the group analysis, despite the consistent observation of prolonged EMG and torque responses following stimulus termination.
Figure 9: One individual demonstrates spastic flexion responses to variable-frequency stimulation and was not included in the group analysis. Above is an overlay of torque response of this individual at baseline and following 1, 3 and 5 MVE contractions. Despite flexion reflexes, a clear wind up of responses following MVE contractions is observed.
Quantitative analysis of augmented and prolonged torques across the remaining 12 subjects with SCI (17 limbs tested) and 9 healthy control subjects is presented in Fig 10A-C. During low-amplitude variable-frequency NMES applied to individuals with SCI, small increases in knee extensor torque were evident during the 2nd versus 1st 25 Hz stimulation during baseline (pre-MVE) testing (7.9±27% increase). This augmented torque increased significantly following repeated MVEs. Repeated measures ANOVA revealed significant differences across contraction number (p = 0.03), with post-hoc assessment revealing a significant 57% increase between baseline (8.0±27.5%) and following 3 repeated MVEs (69.5±136%).

Analysis of prolonged torques during 500-2000 ms following the variable-frequency NMES train revealed significant differences across contraction number (p=0.01). Post-hoc assessment indicated significant differences only between the baseline assessment (0.12±0.34 Nm) and following 3 repeated MVEs (1.53±2.91 Nm), which represents a > 10-fold increase from resting conditions. Prolonged torques were generated by sustained knee extensor EMG activity, as integrated EMG increased significantly above baseline values after 3 and 5 repeated MVEs (p=0.02; 92 and 71% increases from baseline conditions). No apparent differences in prolonged EMG responses were observed between individual knee extensor muscles.

In control subjects, during low-amplitude (22.3±10.3 mA) variable-frequency NMES, low levels of augmented torques were evident during the 2nd versus 1st 25 Hz stimulation during baseline (pre-MVE) testing (21±9% increase), with non-significant decrease following single or repeated MVEs (p=0.93; 6% decrease following 3 contractions versus resting
conditions). Torque and EMG activity following stimulation followed a similar trend, with relatively low baseline values (0.47±0.20 Nm and 4.9±0.90 mV·ms respectively). Non-significant changes in prolonged torque and EMG were also observed following single or repeated contractions (p=0.82 and p=0.71 respectively). This represents a 49% decrease in prolonged torque and 9% increase in prolonged EMG following 3 contractions versus resting conditions in healthy controls. Unpaired comparisons between responses to variable frequency NMES in SCI and control subjects at each of the 4 preceding conditions (0, 1, 3, and 5 contractions) were not significant (p-values range from 0.06 to 0.48).
Figure 10: Group response to variable-frequency electrical stimulation. Responses are quantified using (A) augmented torque response during 2nd 25 Hz epoch, and prolonged (B) torque and (C) EMG responses following termination of stimulation. Group data from individuals with motor incomplete SCI indicates an increase in augmented torque during stimulation and prolonged torque and EMG following repeated MVE contractions (n=17). No change is observed in intact control subjects following an identical protocol (n=9).
iv. **Associations between quantitative and clinical measures**

Greater augmented and prolonged torques during low-amplitude variable-frequency NMES following 3 and 5 MVEs in individuals with SCI mirrors the increases in peak knee extensor torques with repeated MVEs in the present and previous investigations (Hornby *et al.*, 2009b). Correlation analyses were performed to examine potential associations between peak torque increases during the bout of 1, 3 or 5 MVEs (normalized to peak baseline values) and EMG/torque parameters determined with variable-frequency NMES. Across all trials (n=51), there were low to moderate, but significant, correlations between peak torque during the repeated 1-5 MVEs and augmented torques during the 2nd 25 Hz (r=0.31, p=0.02), prolonged torques post-stimulation (r=0.57, p<0.01), and prolonged EMG post-stimulation (r=0.50, p<0.01). There were no associations with augmented twitch response (n=30, r=-0.35, p=0.06) or M<sub>max</sub> responses (n=30, r=0.27, p=0.16).

Additionally we investigated potential associations between clinical measures of spasticity to the observed responses. The largest peak torque increases and augmented and prolonged motor responses following single or repeated MVEs were used to assess the relationship with the modified Ashworth scores from the tested knee extensor. Correlation coefficients between peak percentage knee extensor torque increases and modified Ashworth scores of the tested limb were not significant (n=20, r=0.34, p=0.18). In contrast, low to moderate correlations between spasticity and augmented and prolonged responses to variable-frequency NMES were demonstrated. Significant correlations were observed between tested knee extensor spasticity scores and augmented torques during the 2nd 25Hz stimulation
(n=17, r=0.50, p=0.04) and prolonged torques (n=17, r=0.53, p = 0.04), but not prolonged EMG (n=17, r=0.42, p=0.09) following stimulus termination.

e. Discussion

The present study investigated potential mechanisms underlying the reserve of volitional force generation with repeated MVEs in individuals with incomplete SCI. Though post-activation potentiation was observed following a single MVE, repeated high-amplitude NMES-elicited contractions did not produce similar torque increases. In contrast, augmented and prolonged motor activity observed in response to low-amplitude variable-frequency NMES following repeated MVEs suggests increased central excitability may play a role in the reserve of volitional force generation in individuals with incomplete SCI.

i. Alterations in peripheral motor excitability with repeated contractions

Post-activation potentiation following repeated MVEs was expected based on data from neurologically intact humans (Vandervoort et al., 1983). The observed mean increase (16%) in twitch torques was substantially smaller, however, than intact subjects following 10 s MVE contractions of the knee extensors (70% increase) (Hamada et al., 2003). For $M_{\text{max}}$, ~20% increases have been observed in hand muscles of intact subjects following repeated, intermittent MVEs (Hicks et al., 1989), although no increase was observed in the present study. Differences between studies may be due to deficits in central activation for our individuals with incomplete SCI. Such a reduction in central activation suggests that many motor units were likely not recruited during single or repeated MVEs which could limit the observation of changes in neuromuscular contractile and electrical properties. Nonetheless,
the increase in twitch torques after a single contraction could account for a portion of the augmented volitional torques during repeated MVEs (Hornby et al., 2009b).

Repeated, high-amplitude NMES has been previously utilized to bypass volitional activation in an attempt to delineate central and peripheral mechanisms of fatigue (Bigland-Ritchie et al., 1986). Our data clearly demonstrates substantial differences between torque responses to repeated NMES and repeated MVEs. Rapid, significant decreases in NMES-elicited torques were observed by the 5th repeated contraction, consistent with published observations in complete SCI (Gerrits et al., 1999). While 2/10 subjects demonstrated increased torques during the 1st 5 NMES contractions, all increases were transient and < 15% of the initial torques. In addition, it is likely that the submaximal versus maximal stimulation used in the present study underestimates the loss in force generation during electrically evoked contractions as submaximal stimulation may recruit fatigue resistant muscle fibers, particularly following SCI (Godfrey et al., 2002). Additionally, the lack of antidromic collision during submaximal stimulation may allow for reflexive Ia activation of the motoneuron pool which can facilitate force generation, as is thought to occur with variable frequency NMES. Differences between electrically evoked and volitional contractions could be evidence for lack of meaningful peripheral potentiation, although there are numerous differences between these modes of activation, most notably the fixed number of recruitment motor units with electrical stimulation (Gregory & Bickel, 2005), and further work is necessary to strengthen this assertion. Nevertheless, the present data and previous demonstration of significant associations between EMG and torques during
repeated MVE (Hornby et al., 2009b) suggest a greater central (rather than peripheral) contribution to the increased volitional torques.

ii. **Potential alterations in spinal excitability with repeated contractions**

Low-amplitude variable-frequency NMES has previously been employed to evaluate spinal motoneuron excitability in humans (Collins et al., 2001, 2002), although the modulation of the resultant motor behaviors following repeated MVE contractions in intact and SCI subjects had not been explored. Consistent with previous work, variable frequency NMES tested on subjects with incomplete SCI during resting conditions demonstrated very small increases in augmented and prolonged motor activity, particularly as compared to healthy controls (Nickolls et al., 2004). Importantly, however, variable-frequency NMES applied in our control subjects also elicited very small augmented and prolonged torques, which differs from previous reports using similar techniques (Dean et al., 2007). Our observation of relatively smaller increases in EMG and torque may be due to the choice of muscles tested, as most published studies assessed lower leg responses to variable frequency NMES. Nevertheless, this stimulation paradigm has produced similar responses in multiple lower and upper extremity muscles (Baldwin et al., 2006), although response amplitudes may be different between muscle groups (Blouin et al., 2009). Additionally the length of the tested muscle could also be responsible for the augmented torque response during the 2\textsuperscript{nd} 25 Hz stimulation (Frigon et al., 2011). That is, testing at approximately 90\textdegree knee flexion could provide sufficient muscle elongation to minimize muscular contributions to augmented torques during the stimulation, particularly as compared to previous work (Collins et al., 2002; Fig. 1). This explanation does not account for the prolonged torques and associated
EMG following termination of variable-frequency NMES, which requires sustained central activation.

In the context of these previous results, the present data suggest that marked increases in motor responses to variable-frequency NMES following repeated MVEs are likely secondary to central mechanisms, but only in individuals with SCI. The observed changes in patients with SCI were much greater than pre-MVE values and larger than published data from similar patient populations (Nickolls et al., 2004). The increases in EMG/torque responses following repeated MVEs were coincident with the increases in peak knee extensor torques during MVEs, and significant correlations between the behaviors suggest a link between these phenomena.

Similar observations of progressive increases of involuntary and voluntary motor activity with repeated activation (i.e. wind-up) have been reported. In individuals with SCI, repeated electrocutaneous stimuli at the foot (Hornby et al., 2003; Schmit et al., 2003) and plantarflexor stretch perturbations (Hornby et al., 2006a) reveal augmented and prolonged flexor and stretch reflex responses, respectively. Similarly, during repeated low-force isometric contractions in intact subjects, warm-up of motor unit recruitment (i.e., decreases in threshold) was observed during subsequent contractions performed within a specific duration following the 1st effort (Gorassini et al., 2002b). These results were attributed to wind-up of PIC activity in underlying spinal circuits, as the time constant of both the motor unit re-recruitment threshold in intact subjects and flexor reflexes in SCI subjects was 4-5 s, consistent with PIC behavior in reduced preparations (Svirskis & Hounsgaard, 1997). The interval between repeated MVEs used in the present study (5 s) would allow such time-
dependent PIC facilitation, although precise characterization of the exponential time course of altered excitability is difficult with repeated MVEs in patients with slowed volitional force generation (Hornby et al., 2009b).

While warm-up of intrinsic motoneuron properties may partly account for increased force-generating capacity with repeated MVEs, modulatory influences from bulbospinal pathways may also contribute to the present results. Brainstem-derived monoaminergic (serotonin and norepinephrine) inputs elicit substantial alterations in intrinsic spinal properties, including augmentation of PICs in spinal motoneurons (Hounsgaard et al., 1988; Miller et al., 1996). Greater modulatory drive does occur with specific stimuli such as arousal and increased volitional activity (Gerin et al., 1995; Veasey et al., 1995), which is consistent with the current experimental paradigm of repeated MVEs. Metabotropic modulation of spinal circuits with repeated MVEs could facilitate torque production during volitional efforts, even with similar descending ionotropic synaptic inputs. Additionally, this modulation of spinal motoneurons or interneurones would also contribute to increased reflex excitability following termination of the descending drive, observed as augmented and prolonged responses to variable frequency NMES. Increased sensitivity of spinal circuits to monoamines following SCI (Harvey et al., 2006; Lee et al., 2007; Murray et al., 2010) can account for the stark differences in volitional and reflex responses observed in SCI compared to intact subjects.

While increases in EMG can indicate changes in central excitability, it may be important to note that use of a notch filter to remove 60 Hz noise also occludes a portion of the EMG signal. If there is a disproportionate shift in 60 Hz activity, it may be detected as a change in
the amplitude of the EMG envelope. We believe such a significant frequency shift during brief bouts of repeated MVE contractions is unlikely. Nonetheless, an assessment of motor unit activity during and following supramaximal torque generation is warranted.

iii. **Additional potential sites of increased central excitability**

Though our results appear consistent with motoneuron PIC activation, other potential sites of central excitability may contribute. For example, ventral interneurones demonstrate PIC-like activity (Hounsgaard & Kjaerulff, 1992; Dougherty & Kiehn, 2010), are modulated by descending monoaminergic inputs (Zhong et al., 2010), and could contribute to augmented motor responses as described for motoneurons above. Differentiating between increases in interneuronal versus motoneuronal excitability is not possible in the present study.

Altered excitability of descending cortical circuits with repeated MVEs may also contribute to the present observations. While previous data suggest decreased corticospinal transmission with repeated MVEs (Di Lazzaro et al., 2003; Petersen et al., 2003), recent evidence suggests this depression may be muscle specific (Giesebrecht et al., 2010). Intact subjects may instead demonstrate increases in excitability of some central pathways with high intensity volitional contractions (Samii et al., 1996; Norgaard et al., 2000b). Recent preliminary data further suggest a potential cortical contribution to the self-sustained firing of motor-units in human subjects elicited though vibration or electrical stimulation (Collins et al., 2010). In the present study, however, all subjects reported attempts to relax following performance of repeated MVEs and during and following all stimulation protocols. All subjects stated they did not intervene volitionally. Differences in the ability to volitionally
suppress motor responses in individuals with SCI as compared to control subjects have long been considered spinally mediated (i.e., changes below the lesion level). Similar instructions were provided to control subjects, with very small responses to variable-frequency NMES. The prolonged responses to variable frequency NMES following MVEs in SCI subjects are likely mediated by alterations in reflexes, which are hyperexcitable in the subject population tested (spasticity scores), and may indeed have a common origin at the motoneuron. Further experimentation is necessary to determine the extent of cortical contributions to the increased torques with repeated MVEs and responses to variable-frequency NMES.

iv. **Clinical significance**

Associations between increased volitional torques with repeated MVEs with underlying PIC-like behavior may be of clinical interest. The notion that PIC activity is an integral part of motor activity across species has been discussed previously (Kiehn & Eken, 1998; Hornby et al., 2002; Heckman et al., 2009). Data from both animal (Bennett et al., 1999; Bennett et al., 2001a) and human studies (Hornby et al., 2003; Gorassini et al., 2004; Hornby et al., 2006a) have provided evidence to suggest that spasticity/spasms may be mediated by motoneuron PIC activity. While a previous study had linked the influence of PIC activity to volitional activity in human incomplete SCI (Zijdewind & Thomas, 2003a), the present investigation suggests that the generation of supramaximal volitional torque in human incomplete SCI may also be associated with augmented spinal excitability, potentially due to motoneuron PIC activity. Involuntary spastic motor behaviors are thought to be a negative consequence of SCI, although individuals with incomplete SCI often report utilizing their spastic motor activity for functional behaviors (Gittmann, 1963; Dietz, 2008). It is likely that alterations in
voluntary and involuntary motor activity following SCI possess similar underlying mechanisms (Murray et al., 2010). Rehabilitative strategies used to augment volitional forces through the mechanisms described above may serve to augment strength gains and facilitate functional improvements (Crozier et al., 1992; Saraf et al., 2010) whereas therapies used to depress excitability of spinal circuitry may limit the potential utility of this rehabilitation strategy.
III. Endogenous optimization – Experiment 2

a. Summary

Individuals with motor incomplete SCI are able to produce an initial increase in volitional torque generation during a ‘fatiguing’ protocol of intermittent 5 s, maximal volitional effort (MVE) contractions separated by a 5 s rest duration. While both facilitative and depressive mechanisms likely underlie the alterations in motor output during repeated MVEs, the specific parameters which optimize torque generation in patients with incomplete SCI are unknown. In the present study, we investigated the contribution of intensity of volitional contractions and duration of rest between MVEs which maximize peak volitional torque output. Under isometric conditions, the role of intensity was assessed by having subjects perform single MVEs following two preceding contractions performed at varying intensities (5-100% MVE, 5 s intervals). In a similar manner, the duration of rest was assessed by having the patient perform a bout of 5 MVEs performed at variable durations between repeated efforts (5-115 s intervals, 100% MVE). Peak torque generation was found to be greatest when the MVE effort was preceded by high intensity efforts (>62% MVE) performed at brief rest intervals (<6.5 s). These increases in volitional torque generation were mirrored by intensity- and time- dependent increases in the short latency quadriceps tendon reflex responses following volitional efforts. These findings suggest a functional role for increased spinal neuron excitability following volitional contractions in individuals with SCI and provide the framework for novel rehabilitation interventions.
b. Introduction

Lower extremity strength is a primary impairment in patients with motor incomplete spinal cord injury (SCI), and is a major determinant of ambulatory function (Crozier et al., 1992; Saraf et al., 2010; Zorner et al., 2010; van Middendorp et al., 2011). While many rehabilitation interventions focus on improving function through the use of task-specific training (e.g. gait training), there are few studies which focus on the use of strengthening exercises to minimize functional limitations. Long-standing data in neurologically intact individuals suggest that specific strength training regimens can increase peak torques during maximum volitional effort (MVE) contractions (ACSM, 2009a), although few studies have demonstrated similar benefits in paretic lower extremities in patients with incomplete SCI (Gregory et al., 2007; Harness et al., 2008; Harvey et al., 2010).

Recent data indicate that performance of repeated MVE contractions can elicit a progressive increase in maximal torque generation in humans with motor incomplete SCI, defined as supramaximal volitional torque generation (Hornby et al., 2009b; Thompson et al., 2011b). In this previous work, subjects with chronic motor incomplete SCI performed a series of intermittent MVE contractions of the knee extensors (KE; 5 s duration and 5 s rest). During the first 5 repeated MVEs, individuals with SCI consistently demonstrate 15-23% increases in peak volitional torques with concomitant increases in pooled quadriceps electromyographic (EMG) activity. These data contrast sharply with data from neurologically intact control subjects, who demonstrate an immediate decrease in peak torque during a similar protocol.
In patients with SCI, supramaximal volitional torque generation appears to be driven by increases in central, possibly spinal, excitability rather than changes at the peripheral neuromuscular apparatus (Thompson et al., 2011b). Strength training programs which incorporate supramaximal volitional torque may be able to harness this augmented spinal excitability to produce long-term increases in volitional force generation and maximize functional gains individuals with incomplete SCI. However, there is little information regarding the optimal patterns of activation needed to maximize short-term increases in volitional torque generation. Accordingly, the goal of this manuscript is to quantify changes in peak volitional torques during single or repeated MVEs following variable intensities of preceding contractions and rest intervals. As SCI produces profound weakness which results in difficulty or inability to perform functional tasks, understanding the capacity of volitional force generation in individuals with incomplete SCI may help guide treatment strategies in this population.

c. **Methods**

i. **Ethical approval**

All procedures were approved by the Institutional Review Board of Northwestern University and performed in accordance with the Declaration of Helsinki.

ii. **Subject selection and informed consent**

Individuals with motor incomplete SCI were recruited from a non-public registry housed within the Rehabilitation Institute of Chicago. Experiments were performed on 11 male participants with chronic (> 1 yr.) spinal lesions above the T10 neurological level. Three experiments were performed on separate days; all subjects participated in the first two
experiments, a subset of these subjects participated in the third experiment. Participants were classified as either C or D using the American Spinal Injury Association Impairment Scale (Maynard et al., 1997). All subjects demonstrated residual volitional KE strength as determined by the lower extremity motor score (Marino & Graves, 2004). Exclusion criteria included medical history of multiple CNS lesions and diagnosis of lower limb peripheral nerve injury or orthopaedic injury which may limit maximal effort during KE contractions. None of the subjects were using anti-spasticity medication at the time of the study. All had previous experience using the testing apparatus and performing the repeated MVE protocol, however they were unaware of the hypotheses regarding the experimental procedures.

iii. Experimental setup

The three experiments were performed at least 5 days apart. Each session lasted approximately 1-1.5 hours and the order of the first two experiments was pseudo-randomized while the third experiment was always performed last. For all experiments, subjects were seated in the adjustable height chair of the testing apparatus (System 3®; Biodex Medical Systems, Shirley, NY, USA) with the hips flexed to 80-90 deg and the knee positioned at 90 deg. KE torques and surface electromyographic (EMG) data was collected on all subjects on the more impaired limb as determined during clinical evaluation, with the same limb being tested during all sessions. The distal shank of the tested limb was secured to the dynamometer arm, which was coupled to a 6 degree of freedom load cell (ATI, Apex, NC) used to assess KE torques. A custom made handheld reflex hammer instrumented with a piezoelectric load cell (Kistler Instrument Corp., NY, USA) (Xia & Rymer, 2005) was used for reflex testing. Torque/force signals were low-pass filtered at 200 Hz and collected at
1000 Hz. Surface EMG was recorded using active bipolar electrodes (Delsys, Boston, MA, USA) applied over the vastus lateralis (VL), vastus medialis (VM), rectus femoris (RF) and medial hamstrings (MH). EMG signals were amplified (×1000), band passed filtered (20-450 Hz) and sampled at 1000 Hz simultaneously with the torque data.

iv. **Experimental protocols**

Experiment 1 examined the role of intensity of preceding contractions on the generation of supramaximal volitional torque generation in human incomplete SCI. Following a brief warm-up period, subjects performed 3 baseline MVEs lasting 3-8 seconds each with > 3 minutes between attempts. During all MVE contractions, subjects were instructed to extend the knee as hard and as fast as possible; vigorous verbal encouragement and visual feedback of KE torques was provided on a computer monitor. The peak torque generated from these single MVE contractions was defined as “100% intensity” for subsequent Experiment 1 testing. Following baseline assessments, subjects performed 6 bouts of 3 consecutive, 5 s KE contractions with 5 s rest between contractions. The first two contractions were performed at 1 of 6 torque intensities (5, 10, 25, 50, 75 or 100% of baseline MVE torques) while the third contraction was a MVE contraction. Custom designed software was implemented to provide computerized visual feedback of the target torques during the contractions. The order of testing at different torque intensities was pseudo-randomized, with a rest of no less than 5 minutes provided between testing bouts.

Experiment 2 examined the contribution of the duration between MVE contractions on the generation of peak torque generation in human incomplete SCI. Following a brief warm-up period, subjects performed 8 bouts of 5 consecutive MVEs. Each MVE was maintained
for 5 seconds with a rest period of 5, 10, 15, 25, 35, 55, 75, or 115 seconds between consecutive attempts. Vigorous verbal encouragement during each MVE attempt and visual feedback was provided. The 8 bouts of contractions were delivered in a pseudo-random order and a rest period of no less than 5 minutes was provided between bouts of repeated MVEs. Due to the duration of this experiment, single baseline MVEs were not performed as in Experiment 1; rather the first contraction of each bout was isolated and the average maximum torque produced during these 8 contractions (1st MVE of each time interval tested) was defined as baseline.

Experiment 3 examined the intensity and time dependent alterations in the KE tendon reflex following volitional contractions in human incomplete SCI. Follow a brief warm-up period, subjects performed 3 baseline MVEs as in experiment 1. The optimal location to elicit reflexes was found through manual assessment of tendon reflexes with the instrumented reflex hammer and, following no less than 5 minutes of rest, 2 min of baseline tendon reflexes were elicited at a rate of ~0.2 Hz. Identical baseline reflex testing was repeated following a rest of no less than 5 minutes. Following baseline assessments, subjects performed 6 bouts of 2 consecutive, 5 second KE contractions with 5 second rest between contractions at 1 of 6 torque intensities (5, 10, 25, 50, 75 or 100% of baseline MVE torques), with KE tendon responses elicited after the 2nd contraction tendon reflexes at.2 Hz for 1 min. The order of testing at different torque intensities was pseudo-randomized, with a 5 minute rest period between testing bouts.
v. **Data collection and analysis**

Kinetic and EMG data was acquired and analyzed using custom LabView® software (National Instruments Austin, TX). Offline volitional torque signals were low-pass filtered at 10 Hz using a Butterworth filter (4 pole, zero-phase lag). Maximum torque was identified for each contraction and the period corresponding to +/- 50ms was then averaged to represent peak torque. The highest peak torque elicited during baseline MVEs was used to normalize the subject’s KE torques during subsequent MVE.

Offline, EMG signals were notch filtered (58–62 Hz, zero-phase lag, 4 pole Butterworth), full-wave rectified and smoothed using a low-pass filter (20 Hz, zero-phase lag, 4 pole Butterworth) to create an envelope for further analysis. For each muscle, EMG activity during the repeated contractions was normalized to the mean EMG activity present 100ms prior to the peak torque found during baseline MVEs. Pooled extensor EMG activity was calculated as the average of the normalized VL, VM and RF activity (Hornby *et al.*, 2009b). MH EMG activity was analyzed to assess alterations in antagonist motor activity.

Rate of torque development during MVE contractions were assessed by calculating the time needed to produce 20-80% of the maximum torque \( T_{20-80} \) produced for each individual MVE contraction (Gregory *et al.*, 2007). Similar methods were used to calculate the rate of EMG increase. For each MVE contraction, the maximal EMG value for each muscle was found. EMG\(_{20-80}\) is defined as the average time needed to reach 20-80% of the maximal signal and pooled across the RF, VL and VM.

Tendon reflexes were analysed using the unfiltered EMG signals. Offline, the peak tap force for each tap was found and defined as tap onset \( t_0 \). For each muscle, the onset and
peak-to-peak amplitude of the tendon reflex was calculated. This amplitude was plotted against tap force to construct a baseline reflex response curve for each muscle. Tendon reflexes obtained following volitional contractions were normalized to baseline reflex response curve by averaging each test reflex to a sliding window (7-12) of consecutive baseline responses chosen within a linear region of the reflex response curves. The normalized RF, VL, and VM tendon reflexes are pooled to represent KE tendon reflexes.

vi. Statistics

Data in the text are presented as mean ± standard deviation, and in figures presented with standard errors. As a manipulation check, 95% confidence intervals (CI) were calculated for the independent variables in Experiments 1 and 2. For variable intensity efforts, 95% CIs of peak torques during the two targeted intensity contractions were averaged at each contraction level and compared between the different intensity levels to ensure no overlap. Similarly, for rest interval assessments, 95% CIs of the duration between contractions of the two targeted intervals were calculated and compared across the different targeted rest intervals. All statistical analyses were performed using computer software (Statview®; SAS Institute, NC, USA) with α = 0.05. Data were assessed for normality using Kolmogorov-Smirnov tests. A paired t-test was used to assess for differences in baseline torque generation between experiments 1 and 2. One-way and two-way repeated measures ANOVAs were used to assess MVE torques and reflex responses as appropriate for each experiment. Post-hoc Tukey-Kramer analyses were used to reveal individual differences among multiple comparisons. Intensity and time constants were estimated from the two separate single
declining exponential models (nlinfit function; Matlab, Mathworks, Natick, MA).

Correlations between torque and EMG were determined using Pearson product moments.

d. Results

The torque generated during baseline MVE contractions of the knee extensors was similar between Experiments 1 and 2 (59 ± 33 and 62 ± 34 Nm respectively; p=0.55); this torque is substantially less than maximal KE torques generated by healthy controls (> 200 Nm) but very similar to previous reports of baseline KE MVEs in this population (Jayaraman et al., 2006; Gregory et al., 2007; Hornby et al., 2009b; Thompson et al., 2011b).

i. Intensity of preceding contractions

Peak torques generated during submaximal contractions for all 6 different intensities was analyzed in all 11 subjects. During submaximal attempts, subjects tended to produce slightly more torque than asked of them, despite visual feedback, however there was no overlap between the actual torques generated at the different contraction levels (Table IV). Further, despite efforts to ensure consistency of contraction intensity, greater torque was produced on the second contraction as compared to the first for the two highest intensities. For the 75% MVE trials, the two preceding contractions were 73.8 ± 5.2% MVE and 77.9 ± 5.5% MVE respectively (p < 0.05); for 100% intensity contractions, the two preceding contractions were 98.2 ± 5.2% MVE and 103.7 ± 9.4% MVE (p < 0.05).
Table IV: Intensity of preceding contractions prior to a MVE contraction in human incomplete SCI

<table>
<thead>
<tr>
<th>Target Intensity</th>
<th>Actual Intensity</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>5.8 ± 1.7</td>
<td>5.1 – 6.6</td>
</tr>
<tr>
<td>10</td>
<td>11.4 ± 2.6</td>
<td>10.3 – 12.6</td>
</tr>
<tr>
<td>25</td>
<td>26.5 ± 2.4</td>
<td>25.5 – 27.5</td>
</tr>
<tr>
<td>50</td>
<td>52.7 ± 4.8</td>
<td>50.7 – 54.7</td>
</tr>
<tr>
<td>75</td>
<td>75.9 ± 5.6</td>
<td>73.5 – 78.2</td>
</tr>
<tr>
<td>100</td>
<td>100.1 ± 7.9</td>
<td>97.7 – 104.3</td>
</tr>
</tbody>
</table>

a All data is in % MVE and presented as Mean ± SD

b Confidence interval (95% CI) between actual intensity
Supramaximal torques were generated during single MVE contractions across the subject population only when preceded by high intensity contractions. Figures 1a and b demonstrate a single subject’s torque and EMG data when a MVE contraction is preceded by two lower intensity (25%) contractions, and when an MVE contraction is preceded by two high intensity (100%) contractions. Group data reveals a significant increase in torque generation (125.1 ± 10.6% MVE) when a single MVE contraction is preceded by 100% MVE contractions as compared to all other intensities (≤ 75% MVE; p < 0.0001). Further, single MVEs preceded by 75% MVE contractions were significantly different from 5 and 10% MVE contractions (Figure 1c).

Increases in torque generation were mirrored by increases in quadriceps EMG activity. Muscle activity was on average higher than baseline contractions following any level of preceding contractions. Group data revealed significant differences in pooled extensor EMG activity during single MVE contractions when preceded by two 100% contractions (p < 0.01; Figure 1d) as compared to contractions ≤ 25% of baseline MVEs. A significant correlation was observed between increases in mean torques and increased pooled extensor EMG values (pooled data r=0.45, p < 0.0001). Analysis of MH EMG activity revealed non-significant increases during single MVEs preceded by high intensity contractions (p=0.07), although a low but significant correlation between increased KE torques and MH EMG values was observed (r=0.26; p < 0.05).

A faster rate of torque development (T_{20-80}) was observed when single MVEs were preceded by higher intensity contractions. Group data reveals significant decreases in T_{20-80} (p<0.01; Figure 1e) with preceding contractions of variable intensity, with specific
differences following 100% intensity efforts as compared to both 5 and 10% efforts, and between 75% and 10% efforts. Analysis of EMG\textsubscript{20-80} revealed similar trends as KE torque data, but differences only approached statistical significance (p=0.08).
Figure 11: **Intensity of preceding contractions alters supramaximal volitional torque generation.** Single subject example of a single KE MVE preceded by (A) 2 contractions at 25% MVE or (B) 2 contractions at 100% MVE. KE torque and agonist EMG (VL shown) is increased during MVE contractions when MVE is preceded by high intensity contractions, with little change at lower intensities. (C) Normalized group data (n=11) confirms single-subject data, where MVEs preceded by high intensity contractions elicit greater torques (75 and 100% MVE; p<0.0001). Increases in torque were mirrored by (D) increases in pooled extensor EMG and (E) decreases in rate of torque development (both p < 0.01).
To better describe the relationship between increases in peak torques and pooled quadriceps EMG, the intensity of preceding contractions is plotted against subsequent single MVE torques (Figure 12), demonstrating the exponential decay of torques responses described by the equation:

\[
\text{Torque} = 0.2539 \times e^{-0.0263 \times (100 - \text{Intensity})}
\]

The intensity constant for supramaximal torque generation was estimated to be 38% below the baseline MVE or 62% MVE. Similar analyses were performed for pooled KE EMG activity, with decay constant estimated to be 72.5% below the baseline MVE or 27.5% MVE.
Figure 12: Estimated intensity constant of supramaximal volitional torque. Generation of supramaximal torque is dependent on intensity of preceding contractions. Maximum torque produced during MVE (normalized to baseline) is plotted against the intensity of the preceding contractions across all subjects (5–100% MVE; as in Figure 12). Data were fit with an exponential regression line revealing an intensity constant of 38.0% below the baseline MVE or 62.0% MVE.
ii. **Rest time between contractions**

The effects of duration between repeated MVEs on peak torque and EMG responses were determined on a separate day; here subjects were asked to generate five repeated volitional contractions at variable durations of rest intervals (5-115 s). Experimentally, intervals of rest between repeated MVE contractions were defined using a threshold of 12.5% of peak torque generated during single MVEs (Norgaard et al., 2000a), and all subjects were able to produce less than this threshold between all contractions. The average duration between repeated MVE was slightly shorter than asked of them, although there was no overlap between testing conditions (Table V).
Table V: Rest times between repeated MVE contractions in human incomplete SCI

<table>
<thead>
<tr>
<th>Target Time</th>
<th>Actual Time</th>
<th>95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>4.2 ± 0.4</td>
<td>4.1 – 4.3</td>
</tr>
<tr>
<td>10</td>
<td>8.9 ± 0.7</td>
<td>8.7 – 9.1</td>
</tr>
<tr>
<td>15</td>
<td>13.9 ± 0.6</td>
<td>13.7 – 14.1</td>
</tr>
<tr>
<td>25</td>
<td>24.0 ± 0.6</td>
<td>23.8 – 24.2</td>
</tr>
<tr>
<td>35</td>
<td>33.8 ± 0.9</td>
<td>33.5 – 34.1</td>
</tr>
<tr>
<td>55</td>
<td>54.0 ± 0.7</td>
<td>53.8 – 54.2</td>
</tr>
<tr>
<td>75</td>
<td>73.9 ± 0.7</td>
<td>73.7 – 74.1</td>
</tr>
<tr>
<td>115</td>
<td>113.7 ± 0.9</td>
<td>113.4 – 113.9</td>
</tr>
</tbody>
</table>

\[ a \text{ All data is in seconds and presented in Mean ± SD} \]

\[ b \text{ Confidence interval (95% CI) between actual time} \]
By varying the duration between repeated MVEs, supramaximal torques were generated only with very brief rest periods between efforts. Figures 13a and b demonstrate torque and EMG responses from a single subject during 5 repeated MVEs with brief (5 s) and long (75 s) rest intervals. Group data reveals a significant increase in torque generation when repeated MVEs are separated by rest intervals of 5, 10 and 15 s with the torque produced during the 3rd, 4th and 5th contractions significantly greater than the 1st contraction (p<0.0001). Across all subjects, maximum torque generation during these repeated MVE was 122.5 ± 24.7% MVE which was not significantly different from torque produced during Experiment 1 (p=0.695). When contractions 2 through 5 were averaged for each rest condition, a similar trend was observed (increased with rest periods of 5, 10 and 15 s; p<0.0001; Figure 13c). Conversely, small differences were observed at intervals > 15 s.

Increases in volitional torques were mirrored by increases in EMG activity. Group data reveals an increase in pooled extensor EMG activity during MVE contractions separated by a brief rest period of ≤ 15 s with the pooled extensor EMG produced during the contractions 2 through 5 (p<0.0001; Figure 13d). A significant correlation between mean torque and pooled extensor EMG values was observed (r=0.740; p<0.0001). Interestingly, increases in MH EMG were observed during repeated MVEs, but only at the shortest interval rest (p<0.01). A significant positive correlation between mean torque and MH EMG values was also observed across all testing conditions (r=0.651; p<0.0001).

A faster rate of torque development (smaller T_{20-80}) was observed during MVEs which were separated by brief rest periods ≤ 10 s (p<0.0001; Figure 13e). Analysis of EMG_{20-80}...
produced a similar trend which was significant for rest periods \( \leq 10 \) s \((p<0.01)\). No differences were observed in rate of torque or EMG development at longer rest intervals.
Figure 13: **Timing of preceding contractions alters supramaximal volitional torque generation.** Single subject KE torque and VL EMG data demonstrating (A) increased motor output with 5 repeated MVE contractions with a 5 s rest interval and (B) minimal alterations at 75 s rest intervals. (C) Normalized group data (n=11) confirms increased volitional KE torques at brief intervals (5, 10 and 15 s; p<0.0001). Increases in torque were mirrored by (D) increases in pooled extensor EMG and (E) decreases in rate of torque development (both p < 0.0001). (note: values in panels C-E contain average values for contractions 2-5 for each bout).
The relationship of duration between repeated MVEs and the relative (percentage) increase in peak torque responses is plotted in Figure 14, and an exponential decay of torque responses is described by the equation:

\[ \text{Torque} = 0.2259 * e^{-0.1549 \times \text{Duration}} \]

The time constant for supramaximal torque generation was estimated to be 6.5 s. Similar analyses performed on the supramaximal EMG responses revealed a time constant of 16.4 s.
Figure 14: Estimated time constant of supramaximal volitional torque.
Generation of supramaximal torque is dependent on duration of rest between repeated MVE contractions. Torque produced during 2nd, 3rd, 4th and 5th MVE (normalized to baseline) is plotted against the duration of rest between contractions across all subjects (5–115 s; as in Figure 13). Data was fit with an exponential regression line revealing a time constant of 6.5 s.
Throughout experiments 1 and 2, subjects produced a wide range of peak torques during submaximal and supramaximal volitional contractions (torque ranged from 2.9 to 191.0% MVE). Figure 15 plots all the torques and pooled extensor EMGs for all 638 contractions performed in experiments 1 and 2. There was a strong correlation between pooled extensor EMG and torque throughout both experimental sessions (r=0.8632; p<0.0001), with a slightly greater fit using a 2nd order polynomial fit (r =0.8703). The positive coefficient of the degree two term of the polynomial fit indicates a greater relatively increase in pooled extensor EMG as compared to torque at higher intensities. This finding was observed in 8/11 subjects tested.
Figure 15: Relationship between knee extensor torque and muscle activity during a wide range of volitional efforts. Normalized torque and EMG data over a range of intensities (5-100% MVE) and rest intervals between contractions (5-115 s) collected from experimental sessions are plotted (638 volitional contractions). Comparison of torque output (2.9–191.0% MVE) and EMG (6.0–333.1% MVE) revealed a strong curvilinear relationship (r=0.8703; p<0.0001).
iii. **Tendon reflex following contractions**

Spinal contributions to increased torque generation following repeated MVEs at variable intensities and rest durations were assessed by delivering patellar tendon taps prior to and following various combinations of volitional efforts. Manual percussion of the patellar tendon produced short latency tendon reflexes in 6/7 patients tested. Tendon reflexes from the 7th patient were unable to be elicited; this data were excluded from group analyses and discussed below.

During baseline testing, the minimal force delivered to the tendon ranged between 0.38 and 2.83 N while the maximal force delivered to the tendon ranged between 2.54 and 12.70 N. Average forces delivered to the tendon following volitional contractions corresponded to 50.0 ± 12.0% of maximal tap force (Table VI). The amplitudes of KE tendon reflexes were positively correlated with tap forces; as a general trend for all muscles, a 2nd order polynomial fit tends to describe the relationship of the reflex response curves (r= 0.77±0.12). The latency of the reflex remained consistent over a wide range of tap forces and following volitional efforts; the mean latencies of baseline tendon reflexes are presented in Table VI.
Table VI: Baseline amplitude and latency of reflex response to quadriceps tendon tap in human incomplete SCI

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Peak Amplitude</th>
<th>Latency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>IQR</td>
</tr>
<tr>
<td>RF</td>
<td>648</td>
<td>300 – 1174</td>
</tr>
<tr>
<td>VL</td>
<td>774</td>
<td>540 – 1081</td>
</tr>
<tr>
<td>VM</td>
<td>993</td>
<td>844 – 1297</td>
</tr>
<tr>
<td>MH</td>
<td>92</td>
<td>74 – 111</td>
</tr>
</tbody>
</table>

a Amplitude is represented in millivolts and latency in milliseconds

b Latency amplitude presented as Mean ± SD

c Baseline MH reflex response was observed in 5/7 patients
Despite the variation in tapping force on reflex output, the amplitude of tendon reflexes was strongly dependent upon the history of volitional contractions. For example, Figure 16 demonstrates increases in tendon reflex amplitude in response to the first two tendon following two 100% effort contractions, but not following two 5% effort contractions in an individual with incomplete SCI. Group data revealed immediately increased amplitudes of the pooled KE reflex response during high intensity contractions (p < 0.01; Figure 17A). Additionally, a brief potentiation of reflex amplitude is observed following 100% effort contractions (p < 0.01; Figure 17B). When reflex responses are assessed across all conditions, a significant interaction between time and intensity on the potentiation of the pooled KE tendon reflex is observed (p < 0.05; Figure 17C).
Figure 16: Patellar tendon reflexes following volitional contractions. Patellar tendon reflexes elicited in a single subject following either two 100% KE MVE contractions or two 5% MVE contractions. (A) Amplitudes of the post-contraction RF reflex response are plotted against a wide range of baseline (pre-contraction) responses and (A) normalized to neighboring baseline values. (c) Such potentiation is observed in multiple muscles (including MH) and dependent on the intensity and timing of preceding contractions.
Figure 17: Patellar tendon reflexes following volitional contractions in a group of individuals. Group data (n=6/7) demonstrate potentiation of the tendon reflexes (A) immediately following high but not low intensity efforts (p<0.01). (B) Such potentiation diminishes over time (p<0.01). (C) Three-dimensional plot of both intensity and time-dependent of reflex responses, indicating a significant interaction (p<0.05).
Reflex activation of the hamstrings in response to percussion of the patellar tendon was observed in 5 of the tested subjects. The reciprocal MH reflex response was smaller in amplitude and occurred on average 6 ms later than responses of RF, VL, and VM (Table VI). The MH reflex response also displayed a significant interaction between time and intensity in response to volitional contractions (p < 0.01).

Lastly, tendon reflexes were unable to be elicited on the tested side in one patient with incomplete SCI. Further clinical testing revealed absent stretch reflex activity. Despite severely diminished stretch reflex activation, Figure 18 demonstrates the patient can generate time and intensity dependent supramaximal torque in a manner similar to what is observed in other individuals with incomplete SCI. Also of note, the supramaximal volitional efforts clearly demonstrate a strong 6.7 Hz oscillation which is invariant within and between days, but tends to decrease with repeated contractions to 6.0 Hz (see also Hornby et al., 2009b).
Figure 18: Volitional contractions in an individual with severely diminished stretch reflex activation. Tendon reflexes were unable to be elicited on the tested side in one subject (confirmed with clinical absence of stretch reflexes). Supramaximal torque responses were nonetheless dependent on history of activity in a manner consistent with the other subjects.
e. **Discussion**

The primary findings in the present study are that generation of supramaximal torques in patients with chronic incomplete SCI is strongly dependent on the magnitude and timing of preceding contractions. High intensity contractions separated by a brief rest periods are necessary to maximize volitional output following chronic SCI. Increases in volitional quadriceps EMGs and short latency tendon reflexes suggest this increase in maximal volitional torque generation is driven by short-term alterations in spinal neuron excitability. These findings contrast from similar behaviors in healthy individuals and aid in our understanding of volitional force generation in this patient population.

i. **Alterations in volitional torque generation**

The role of intensity of preceding contractions on peak volitional torque generation in human SCI is in direct contrast to previous fatigue data from healthy individuals. High intensity sustained or intermittent volitional contractions performed by individuals without neurological injury has long been known to result in more rapid decline in the ability to maintain forces. Using NMES evoked contractions of the quadriceps, fatigue was found to be greater with stimulation intensities which evoked 50% MVE as compared to stimulation which evoked 20% MVE (Binder-Macleod et al., 1995). During a sustained submaximal volitional contraction, higher intensity contractions often decrease time to task failure and reduce maximal force output (Maluf et al., 2005; see however, Dolmage and Cafarelli 1991; Egana & Green, 2007).

The current findings in relation to the timing of preceding contractions on peak volitional torque generation in human SCI is in direct contrast to the temporal paramentrs of
fatigue from healthy individuals. For example, repeated tetanic stimulation (900 ms) fast-twitch extensor digitorum longus in the mouse demonstrates substantial fatigue at intervals of \( \leq 30 \) s (Barclay, 1992). This general concept is mirrored in human experiments using electrically evoked (Packmanbraun, 1988; Holcomb et al., 2006) and volitional (de Salles et al., 2009; Mendez-Villanueva et al., 2009) contractions.

Following neurological injury, the mode of muscle activation (electrically evoked or volitional) strongly influences the pattern of force generation over time. Following chronic SCI electrical stimulation of the muscle will demonstrate greater fatigue relative to healthy individuals. The substantial decrease in force generation during evoked contractions is likely due to peripheral alterations, in particular muscle fiber type transformation towards fast isoforms. Following chronic SCI volitional activation of the muscle will produce less fatigue and may generate supramaximal volitional torques relatively to healthy individuals. (Hornby et al., 2009b). While the pattern of activation is quite different between the two conditions of muscle activation, these combined results may explain the disproportionate increase in quadriceps EMG activity relatively to volitional torques with higher intensity contractions. However, increases in antagonist co-contraction may also contribute to this disproportionate increase.

ii. **Alterations in the tendon reflex**

In neurologically intact individuals, the tendon reflex induces a dispersed, 2-5 pulse afferent volley lasting \( \sim 25 \) ms (Burke et al., 1983). This volley is sensitive to the gain of the muscle spindle and activates the motoneuron through mono- and polysynaptic pathways through direct activation of group Ia, II, Ib and cutaneous afferents (Burke et al., 1984) and/or
through convergence onto common spinal interneurons (Lundberg et al., 1977b). In response to synaptic activation through tendon percussion, human motoneurons are thought to produce a sustained postsynaptic potential for ~65 ms (Turker et al., 1997). Following chronic neurological injury, there is evidence to suggest minimal changes in afferent volley in response to stretch (Wilson et al., 1999; see however, Nielsen et al., 1995). However, reorganization of primary afferent polysynaptic pathways (reviewed in Rossignol & Frigon, 2011) and prolongation of synaptic activation of the motoneuron (Norton et al., 2008) may contribute to alterations in reflex activation following SCI. Further, these alterations very well account for the appearance of reciprocal facilitation of the tendon reflex observed here and elsewhere (Crone et al., 2003; Xia & Rymer, 2005; see however Lance & Degail, 1965).

The increase in tendon reflex immediately following high intensity effort contractions in human SCI was anticipated. Patients with SCI consistently demonstrate decreased rate-dependent depression of the Hoffman reflex and windup of the stretch and flexor reflexes. While some of these alterations may be due to alterations in transmitter release (Nielsen et al., 1993; Nielsen et al., 1995), it is likely that altered spinal neuron excitability contributes to the windup of reflex responses following SCI (Hornby et al., 2006a). Furthermore, volitional potentiation of the tendon reflex is routinely observed in neurologically intact individuals (Enoka et al., 1980; Biro et al., 2007). Such changes in reflex are often thought to be driven by pre-synaptic mechanisms including increased spindle sensitivity (Hagbarth et al., 1985), post-contraction sensory discharge (Hutton et al., 1973), and alterations in pre-synaptic inhibition (Hultborn et al., 1987). Short term potentiation of the stretch reflex circuitry may also be
accounted for by postsynaptic mechanisms, including increases in motoneuron excitability (Bennett et al., 1998a; Gorassini et al., 2002b).

iii. The contributions of spinal neurons to volitional efforts

While multiple sites along the neuraxis may contribute to this increase in volitional force generation, the impact of impaired supraspinal drive and alterations in spinal neurons are particularly salient. Following incomplete SCI there is partial damage to spinal tracts near the site of injury. Damage to corticospinal projections reduces the central drive to the motoneuron pool and is observed clinically and in research protocols as decreased efferent drive to maximally activate the peripheral neuromuscular system (decreased CAR); such reduced drive may act to prevent neuromuscular depression which occurs in response to maximal activation. Furthermore, chronic SCI produces a disregulation of membrane receptors and ion transporters of spinal neurons (Boulenguez et al., 2010; Murray et al., 2010). These postsynaptic alterations result in hyperexcitable motoneurones, which may be less responsive to inhibition.

Previous work has suggested that supramaximal torque generation in human SCI may be due to modulation of spinal motoneuron excitability. Such modulation may be secondary to time-dependent activation of persistent inward currents (PICs) with repeated contractions, and/or via increased excitatory modulation of motoneurons with increased descending monoamnergic input during high intensity efforts (Thompson et al., 2011b). Four lines of evidence from this data are consistent with this hypothesis. First, the role of intensity is consistent with increased monoaminergic drive. Greater modulatory drive from brainstem pathways occurs with specific stimuli such as arousal and increased volitional activity (Gerin
et al., 1995; Veasey et al., 1995), and would be expected from high intensity contractions used in the current experimental paradigm. These brainstem-derived monoaminergic (serotonin and norepinephrine) inputs elicit short term alterations in intrinsic spinal properties, including augmentation of PICs in spinal motoneurons (Hounsgaard et al., 1988; Miller et al., 1996). Further, increased sensitivity of spinal circuits to monoamines following SCI (Harvey et al., 2006; Lee et al., 2007; Murray et al., 2010) when combined with a reduction in central activation (Hornby et al., 2009b) can account for the stark differences in responses observed in SCI compared to intact subjects.

Second, a widespread increase in volitional and reflexive motor activity was observed. Increases in coactivation during fatiguing contractions has been reported, though typically during sustained, low intensity contractions (Psek & Cafarelli, 1993). Increased coactivation is observed during high intensity volitional contractions in individuals with incomplete SCI, specifically, an increase in MH EMG is oftentimes observed during repeated knee extension. As brainstem monaminergic projections are diffuse and target multiple spinal circuits (Bjorklund & Skagerberg, 1982), widespread PIC-mediated excitability of motor output would be expected under levels of high monoaminergic drive (Heckman et al., 2008).

Third, the prolonged time course of supramaximal torque generation is similar to that of PIC mediated windup of spinal neurons observed in reduced preparations (Svirskis & Hounsgaard, 1997; Bennett et al., 1998a) and the windup of flexion reflexes human SCI (Hornby et al., 2003). Though the time constants underlying torque and EMG activity are longer than reported previously, establishing the precise time constants are hindered by the inability to precisely control the timing of repeated MVEs in SCI subjects, particularly rests
of very short durations. Longer time courses may be due to increased modulatory drive with repeated efforts, thereby augmenting the duration of the observed efforts.

Lastly, common increases in the short latency reflexes and supramaximal volitional torques suggest volitional efforts are potentiated at the level of the spinal reflex. Interestingly, data from an individual with absent tendon and stretch reflexes suggests such changes in volitional force generation may be generated independent from reflex activation. This finding is consistent with increased spinal neuron excitability. Though stretch mediated facilitation of volitional contractions has been inferred (Gandevia et al., 1990) brief vibration superimposed on a weak contraction can recruit and sustain the firing of additional motor units (Kiehn & Eken, 1997), recent data suggest spindle discharge may have little effect during high intensity contractions (McNeil et al., 2011). The data presented in Figure 18 suggests supramaximal torque generation can still be observed despite severely diminished stretch reflex activation.

iv. **Potential supraspinal contributions**

The locus of changes in central excitability may also be distributed throughout the neuraxis. For example, use of transcranial magnetic stimulation to elicit motor evoked potential in target muscles has been utilized to assess potentiation of cortical circuits following volitional contractions in neurologically intact individuals. Though differing in the reported time course of facilitation of the motor evoked potential (few seconds to several minutes), it is clear that a wide range of intensities (10-100% MVE) will produce a similar MEP facilitation in contractions in both the upper (Samii et al., 1996) and lower (Norgaard et al., 2000b) extremities. These results are in contrast to the above data which suggests only high intensity
contractions >60% MVE are necessary to observe the supramaximal torque generation (>25% MVE for EMG activity) in human incomplete SCI. Further, it is unclear how alterations in corticospinal transmission following SCI will influence this activity dependent alterations in response in human SCI to cortical stimulation (Smith et al., 2000).

v. **Clinical implications**

SCI is a debilitating disease process which produces among other things profound weakness. In individuals with residual volitional motor control distal to the lesion, this impairment in force generation, particularly of the KEs, has been shown to correlate with walking ability (Crozier et al., 1992; Saraf et al., 2010; Zorner et al., 2010; van Middendorp et al., 2011). While selected strength training protocols have demonstrated efficacy in improving volitional activation and walking ability in this population (Gregory et al., 2007), it is unclear if the exercise parameters were optimal to maximize strength gains. Development of an exercise protocol based upon the above short-term increases in torque generating capacity may provide a rational and possibly effective means to maximize volitional force generation with repeated training. Indeed, preliminary data indicates that a training intervention based upon supramaximal torque generation will demonstrate greater functional improvements as compared to progressive resistance training in individuals with incomplete SCI (Jayaraman et al., 2010). The data provided in the above experiments should guide the requisite intensity and rest needed to observe supramaximal torque generation in human incomplete SCI.
IV. Exogenous modulation – Experiment 3

a. Summary

Individuals with motor incomplete SCI are able to produce volitional torques well above their one repetition maximum during a bout of repeated maximal volitional effort contractions. This windup of supramaximal volitional torque generation is consistent with short term increases in spinal neuron excitability through descending brainstem modulation via serotonin (5HT). In a double-blinded, randomized study design, we assessed the potential role of 5HT modulation on volitional strength and reflex motor behaviors. Agents utilized include a 5HT antagonist/inverse agonist (cyproheptadine) and a selective serotonin reuptake inhibitor (SSRI; escitalopram). Results indicate cyproheptadine produces significant decreases in volitional torque generation and reflex amplitude, where as escitalopram produces little change in volitional torque generation, possibly secondary to large increases in co-contraction and reflex amplitude were observed. These findings suggest that 5HT has widespread actions on volitional and reflexive motor output following SCI. Such findings suggest 5HT has an endogenous role in volitional torque generation following SCI. Understanding the contribution of monoaminergic mechanisms underlying motor control following human motor incomplete SCI may help guide current and future therapies.
b. Introduction

Incomplete spinal cord injury (SCI) initiates a disease processes which leads to profound impairments in motor control. Two prominent, functionally relevant impairments include weakness, defined as deficits in force generation during maximal voluntary effort contractions, and hyperactive reflexes which encompasses both spasticity, defined as velocity dependent increases in stretch reflex excitability, and spasms, defined here as sustained, involuntary activation of muscles across single or multiple joints. A variety of pharmacological, physical and/or surgical interventions have been directed towards improving function thorough amelioration of these impairments, although their relative efficacy is unclear.

The mechanisms underlying spasticity and spasms following SCI are multifaceted and include changes in both afferent pathways and spinal neurons. One mechanism underlying spastic motor behaviors that has gained considerable recent support is the role of altered spinal motoneuron excitability following SCI. Under healthy conditions, motoneuron behavior is influenced by persistent inward (Ca$_{2+}$ and Na$^+$) currents (PICs), which amplify and sustain synaptic depolarization. This motoneuron PIC activation, and the resulting motor output, is strongly influenced by spinal concentrations of endogenous neuromodulators, in particular serotonin (5HT; reviewed in Heckman et al., 2003). Substantial changes to these 5HT receptors occur in the weeks to months following initial SCI, allowing for PIC activation with minimal residual descending 5HT (Bennett et al., 1999; Gorassini et al., 2004; Murray et al., 2010). The disregulation of PIC activation is thought to contribute to the presence of both spasticity and spasms; accordingly, medications which
block 5HT activity may decrease spasticity/spasms (Murray et al., 2010), whereas medications which augment 5HT bioavailability may increase spasticity/spasms (Stolp-Smith & Wainberg, 1999).

Volitional motor output may also be dependent on motoneuron PIC activity. Under conditions where descending spinal pathways are intact, volitional motor tasks produce variable PIC amplification through activity dependent 5HT release from brainstem centers (Gerin et al., 1995; Veasey et al., 1995). Following incomplete SCI, the loss of descending corticospinal pathways contribute to decreased force generating capacity, although residual 5HT pathways may still act to modulate volitional motor output. For example, PIC activation may help explain the observations of increased time to task failure during sustained motor activity (Thomas & del Valle, 2001) and increases in peak volitional torque generation during maximal effort contractions (Hornby et al., 2009b) in patients with incomplete SCI. Further, medications which decrease 5HT may decrease volitional strength (Wainberg et al., 1990) and functional performance (Murray et al., 2010) in individuals with motor incomplete SCI whereas preliminary data suggests medications which augment 5HT may increase strength (Hornby et al., 2006b; Thompson et al., 2011a).

The current study assesses acute alterations in volitional knee extensor (KE) torque generation and KE tendon reflex activity following single dose administration of serotonergic medications. The primary hypotheses were that administration of a 5HT antagonist (cyproheptadine) would depress volitional and reflexive motor function, whereas an agent that augmented 5HT bioavailability such as a selective serotonin reuptake inhibitor (SSRI; escitalopram) would increase volitional and reflex function. Such information is
important for understanding the motor consequences of these medications, which are typically utilized for non-motor disease processes, and help shed light on the mechanisms of impairments that result from SCI.

c. Methods

i. Ethical approval

All procedures were approved by the Institutional Review Board of Northwestern University and performed in accordance with the Declaration of Helsinki.

ii. Subject selection and informed consent

Individuals with motor incomplete SCI were recruited from a non-public registry housed within the Rehabilitation Institute of Chicago. Experiments were performed on 7 male participants with chronic (> 1 yr.) spinal lesions above the T10 neurological level. Participants were classified as either C or D using the American Spinal Injury Association Impairment Scale (Maynard et al., 1997). All subjects demonstrated residual volitional KE strength as determined by the lower extremity motor score (Marino & Graves, 2004).

Exclusion criteria included medical history of multiple CNS lesions, known reaction to study medications and diagnosis of lower limb peripheral nerve injury or orthopaedic injury which may limit maximal effort during KE contractions. All subjects underwent a 14 day washout period for all anti-depressant, anti-spastic, and other medications with known interactions to the study agents. All had previous experience using the testing apparatus and performing the repeated MVE protocol, however they were unaware of the hypotheses regarding the experimental procedures.
iii. **Study medication**

Two study medications were used: an SSRI (20 mg escitalopram oxalate, Lexapro, Forest Pharmaceuticals Inc.) and a serotonin antagonist/inverse agonist (8mg cyproheptadine, Periactin, Merck Inc.). Medications were delivered in a double blinded randomized fashion with similar overencapsulation of each agent as performed by a licensed pharmacist. The pharmacist was not involved with the data collection or analysis, and blinding was removed following completion of all analyses.

iv. **Experimental setup**

Two experiments were performed at least 5 days apart. Each experiment lasted approximately 9 hours with 2.5 hours of testing in the morning, a 4 hour delay for medication to reach peak plasma concentrations and an identical 2.5 hours of testing in the afternoon. All subjects completed the two days of testing. For all experiments, subjects were seated in the adjustable height chair of the testing apparatus (System 3®; Biodex Medical Systems, Shirley, NY, USA) with the hips flexed to 45 deg and the knee positioned at either 60 or 90 deg and consistent for each subject. KE torques and surface electromyographic (EMG) data was collected on all subjects on the more impaired limb as determined during clinical evaluation, with the same limb being tested during all sessions. The distal shank of the tested limb was secured to the dynamometer arm, which was coupled to a 6 degree of freedom load cell (ATI, Apex, NC) used to assess KE torques. Torque signals were low-pass filtered at 200 Hz and collected at 1000 Hz.

A custom robotic reflex hammer was developed using a linear motor (NTI AG/LinMot, Spreitenbach, Switzerland). This motor could deliver forces up to 137 N with
high repeatability of position (± 0.05 mm) at peak velocities of 5.3 m/s and accelerations in excess of 4 m/s². A load cell (Omegadyne, OH, USA) was attached in series at the end of the neodymium slider and used to record forces associated with the tendon percussion. During testing, the motor was secured to a rigid frame and bolted to the floor. A position control strategy was employed to alter forces delivered to the tendon – forces were varied by moving the initial position of the tapper relative to the tendon using a constant 30 mm stroke. Force signals were low-pass filtered at 220 Hz and collected at 1000 Hz simultaneously with the torque data.

Surface EMG was recorded using active bipolar electrodes (Delsys, Boston, MA, USA) applied over the vastus lateralis (VL), vastus medialis (VM), rectus femoris (RF), medial hamstrings (MH), lateral hamstring (LH), medial gastrocnemius (MG), soleus (Sol) and tibialis anterior (TA). EMG signals were amplified (×1000), band passed filtered (20-450 Hz) and sampled at 1000 Hz simultaneously with the torque/force data.

v. Experimental protocols

Volitional torque generation was assessed during single and repeated MVE contractions of the KEs. During all MVE contractions, subjects were instructed to extend the knee as hard and as fast as possible; vigorous verbal encouragement and real-time visual feedback of KE torques was provided on a computer monitor. Following a brief warm-up period, experiments began with subjects performing three baseline MVEs lasting 3-8 seconds each with a minimum of 5 minutes rest was provided between attempts. During each baseline MVE, a train of electrical stimulation (10 pulses, 600 µs duration, 100 Hz, 140V; S48 Grass, external isolation, West Warwick, RI) was delivered to the knee extensors through 3” X 5”
self-adhesive, stimulating electrodes (ConMed Corp, Utica, NY) placed over the distal VM and the proximal VL. The stimulation was triggered manually by the experimenters when the knee extensor torque appeared to reach a plateau during MVEs (Miller et al., 1999). The electrically elicited torque superimposed on the maximum volitional torque was used to estimate voluntary knee extensor activation calculated using the central activation ratio (CAR). Thirty seconds following each of the baseline MVEs an electrically evoked contraction was elicited from the resting muscle using a train of electrical stimulation as described above. Repeated MVEs were performed as above, but without electrical stimulation, at a rate of 5s on 5s off for a total of 5 contractions.

Assessment of KE tendon reflex responses was performed using the position-controlled robotic reflex hammer. The hammer was optimally aligned perpendicular to the quadriceps tendon as determined during clinical testing prior to set-up. Reflex threshold was identified as the minimum distance necessary to elicit EMG responses of at least one KE muscle. The force applied to the tendon was controlled by delivering taps (constant 30 mm stroke distance) at 11 different positions ranging from 1 mm further away from the starting position to elicit a minimal reflex response (i.e., 0 mm) and moved progressively closer to the tendon in randomly ordered 1mm steps such that 10 responses at different starting positions were elicited. The minimum rest between tendon reflexes was 20 s, and responses were elicited 2-3 times at each position. To examine potential rate-dependent changes in tendon reflex responses, the hammer was positioned at an initial starting position of 1-2 mm above the reflex threshold position, and bouts of 5 taps were delivered with 5, 2, 1, or 0.5 s rest between taps. A minimum of 20 s was provided between bouts of taps.
vi. **Data collection and analysis**

Kinetic and EMG data was acquired and analyzed using custom LabView® software (National Instruments Austin, TX). Offline volitional torque signals were low-pass filtered at 50 Hz using a Butterworth filter (4 pole, zero-phase lag). Maximum torque was identified for each volitional contraction and the period corresponding to +/- 50ms was then averaged to represent peak torque. The highest peak torque elicited during baseline MVEs was used to normalize the subject’s KE torques during subsequent MVE. CAR values were calculated by dividing the mean voluntary torque produced 100 ms before the stimulation onset by the peak electrically elicited KE torque.

Offline, EMG produced during volitional contractions full-wave rectified and smoothed using a low-pass filter (20 Hz, zero-phase lag, 4 pole Butterworth) to create an envelope for further analysis. For each muscle, EMG activity during the repeated contractions was normalized to the mean EMG activity present 100ms prior to the peak torque found during baseline MVEs. Pooled extensor EMG activity was calculated as the average of the normalized VL, VM and RF activity (Hornby et al., 2009b). Similarly, MH and LH EMG activity were pooled to represent KF EMG activity and used to assess alterations in antagonist motor activity.

Tendon reflexes were analysed using the unfiltered EMG signals. Offline, the peak tap force for each tap was found and defined as tap onset (t0). For each muscle, the onset and peak-to-peak amplitude of the tendon reflex was calculated. To assess the gain of the reflex response, the reflex amplitudes at the 11 different tap conditions was plotted against tap force to construct a reflex response curve for each muscle. Tendon reflexes were fit with
a 3\textsuperscript{rd} order polynomial. Baseline reflexes were normalized such that the peak of the reflex response curve was equal to 1.0 and post medication values were normalised to baseline values. Normalized RF, VL, and VM tendon reflexes were pooled and used to construct the KE reflex response curve; a similar KF reflex response curve was calculated from the normalized LH and MH values. For both the KE and KF reflex response curves, the tap force necessary to produce 20, 50, 80 and 100\% of baseline peak reflex was assessed for each day. Additionally, the peak reflex response and force needed to elicit this response was calculated for each of the post medication conditions.

To examine the effects of rate-dependent changes in tendon reflex responses, the first response of each bout was identified during baseline testing and used to normalize subsequent responses of the particular bout. The KE and KF values were constructed using these normalized responses. Values obtained post medications were normalized to both the first reflex of the bout and to the first reflex of the respective baseline bout.

At times, the persistent activity of single motor units could be readily distinguished through the surface EMG record. Such trials were noted and units were decomposed through template matching software (Spike2 v7.03a; Cambridge Electronic Design, Cambridge, ENG) and spike timing was established. The instantaneous firing frequency was calculated as the reciprocal of the inter-spike interval.

\textbf{vii. Statistics}

Data are presented as mean ± standard error. All statistical analyses were performed using computer software (Statview\textsuperscript{®}; SAS Institute, NC, USA) with \( \alpha = 0.05 \). A paired t-test or one-way repeated measures ANOVA was used to assess for differences in motor output.
between baseline conditions. One-way and two-way repeated measures ANOVAs were used to assess MVE torques and reflex responses as appropriate for each experiment. Post-hoc Tukey-Kramer analyses were used to reveal individual differences among multiple comparisons.

d. Results

i. Evoked contractions

During electrically evoked contractions, patients with SCI were able to generate 112 ± 35 and 103 ± 33 Nm during baseline testing of burst stimulation; this was not different between days (p > 0.05). Rate of torque development and half relaxation time were also similar between days (Table VII; p > 0.05). Oral administration of either cyproheptadine or escitalopram produces minimal alterations in electrically evoked torque and the rate of torque development. Significant differences were observed in the half relaxation time between post medication conditions (89 ± 41 and 120 ± 37 ms; p < 0.05).
Table VII: Alterations in evoked and volitional motor output in response to serotonergic medications

<table>
<thead>
<tr>
<th></th>
<th>Cyproheptadine</th>
<th>Escitalopram</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Electrically Evoked Torque</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Twitch (Nm)</td>
<td>112 ± 35</td>
<td>114 ± 11</td>
</tr>
<tr>
<td>Contraction Time (ms)</td>
<td>156 ± 39</td>
<td>159 ± 38</td>
</tr>
<tr>
<td>½ Relaxation Time (ms)</td>
<td>109 ± 33</td>
<td>89 ± 41</td>
</tr>
<tr>
<td>Baseline MVE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Torque (Nm)</td>
<td>76 ± 15</td>
<td>67 ± 15*</td>
</tr>
<tr>
<td>CAR</td>
<td>0.48 ± 0.10</td>
<td>0.40 ± 0.09*</td>
</tr>
<tr>
<td>KE EMG (nml to pre)</td>
<td>-</td>
<td>96 ± 9.7</td>
</tr>
<tr>
<td>KF EMG (nml to pre)</td>
<td>-</td>
<td>111 ± 15</td>
</tr>
<tr>
<td>Repeated MVE (ave)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Torque (Nm)</td>
<td>117 ± 4.8</td>
<td>87 ± 15</td>
</tr>
<tr>
<td>KE EMG (nml to pre)</td>
<td>153 ± 31</td>
<td>130 ± 35</td>
</tr>
<tr>
<td>KF EMG (nml to pre)</td>
<td>201 ± 69</td>
<td>167 ± 55</td>
</tr>
</tbody>
</table>

* Asterisks indicates significance at p<0.05
ii. **Single maximal volitional effort contractions**

During baseline testing of volitional torque generation, subjects were able to produce 76 ± 15 and 74 ± 16 Nm during pre-cyproheptadine and pre-escitalopram testing, respectively (p > 0.05). This torque was driven by CARs of 0.48 ± 0.10 and 0.47 ± 0.09 (p > 0.05).

Study medications significantly affected the subject’s ability to generate volitional torque. Figure 19 demonstrates a single subjects’ baseline MVEs for each condition. Group data demonstrates cyproheptadine produces a significant decrease in volitional generation (to 67 ± 15 Nm (p < 0.05), whereas a non-significant decrease was observed following escitalopram. These changes were mirrored by a significant 0.08 ± 0.02 point decrease in CAR following cyproheptadine (p < 0.05) and a non-significant 0.04 ± 0.03 point decrease following escitalopram. (Figure 20)
Figure 19: Baseline contractions prior to and following acute administration of serotonergic medication. During baseline testing a subject with SCI was able to produce a similar amount of volitional torque and at times persistent activity observed in MG. Following cypeoheptadine, a decrease in volitional torque is observed. Following escitalopram minimal changes in volitional torque is observed, where as noticeable increases in agonist and antagonist is observed; persistent motor unit firing is observed in multiple muscle groups.
Figure 20: Group data of baseline volitional contractions prior to and following serotonergic medication. (A) cyproheptadine produces a significant decrease in volitional generation and (B) CAR of the tested KEs; non-significant decrease are observed following escitalopram. Increases in (C) agonist and (D) antagonist EMG were observed following escitalopram with minimal change following cyproheptadine.
The EMG activity underlying these contractions appears to follow a different pattern. Following cyproheptadine, normalized KE EMG showed a non-significant decrease, however, following escitalopram, KE EMG significantly increased to $134 \pm 14\%$ MVE ($p<0.05$). Co-contraction, measured by normalized KF EMG, followed a similar trend; no significant changes were observed following cyproheptadine, whereas following escitalopram, baseline KF EMG increased to $180 \pm 29\%$ MVE ($p < 0.01$; Figure 20). Similar trends in EMG activation were observed in the TA, MG and Sol, with TA and MG showing significant differences between post-cyproheptadine and post-escitalopram conditions ($p < 0.05$).

### iii. Repeated maximal volitional effort contractions

No difference in torque produced during repeated MVEs between days was observed ($p < 0.05$) with average peak torque produced during repeated MVE contractions equal to $117 \pm 4.8$ and $134 \pm 13\%$ baseline MVE. Figure 21 demonstrate repeated MVE contractions in a single subject prior to and following serotonergic medication. Study medications failed to significantly alter torque generation during repeated MVE contractions, though trends in torque generation were observed (Figure 22). Following cyproheptadine, subjects demonstrated a decrease windup of volitional force generation to $87 \pm 15\%$ MVE, whereas following escitalopram there was a trend to increase the windup of volitional force generation $162 \pm 40\%$ MVE.
Figure 21: Repeated contractions in an individual prior to and following serotonergic medication. During baseline testing, an individual with incomplete SCI is able to produce supramaximal volitional torque during repeated MVE contractions of the KE. Following cyproheptadine a noticeable reduction in volitional torque generation is observed with little change following escitalopram. Widespread decreases in EMG activity are noted in some muscles following cyproheptadine; widespread augmented and prolonged EMG activity is observed following escitalopram.
Figure 22: Group data of repeated contractions prior to and following serotonergic medication. Group data demonstrates trends in KE torque and EMG; cyproheptadine appears to depress motor activity whereas escitalopram appears to increase such activity (A, C, E). When contractions 2-4 are averaged (B, D, F), similar trends are observed and a significant increase in KE EMG is noted.
This trend in volitional torque generation is supported by significant increase in normalized KE EMG during rMVE contractions following escitalopram (216 ± 52% MVE) as compared to cyproheptadine (130 ± 35% MVE; p < 0.05). A similar, though non-significant, trend was observed in the KF EMG signals.

iv. **Gain and amplitude of the knee extensor tendon reflex**

The range of forces delivered to the tendon was similar between days. The average minimum and maximum tap forces during pre-cyproheptadine testing were 15 ± 3.8 N and 97 ± 12 N; this was not statistically different from values delivered during pre-escitalopram testing (9.9 ± 1.8 N and 96 ± 9.3 N).

Single subject responses to tendon reflex testing prior to and following serotonergic medication are shown in Figure 23. Among all subjects, the normalized gain of the pooled KE tendon reflex was similar between pre-cyproheptadine and pre-escitalopram testing and was characterized by 3rd order polynomial, where average r² values were similar between days (0.72 ± 0.07 and 0.77 ± 0.05). The corresponding 100% baseline reflex response was 99 ± 0.8 and 98 ± 0.6 % of baseline max and occurred at similar levels of force. There were no differences in the tap force needed to produce 20, 50, 80 and 100% baseline reflex response between pre-cyproheptadine and pre-escitalopram testing (p > 0.05; Figure 24).

Different ranges of force were necessary to elicit tendon reflexes following study medications. Specifically, post-cyproheptadine required significantly more tap force (35 ± 12 versus 15 ± 3.8 N; p < 0.05) to elicit reflex responses; negligible changes in threshold tap force were observed post-escitalopram. Maximal force delivered to the tendon was similar.
among conditions with post-cyproheptadine being tested at the highest average maximal force (103 ± 7.3 N) among the three conditions (see Figure 24A).

Study medications had strong effects on the tapping force required to elicit a set percentage of the maximum baseline reflex response. The force needed to elicit 20% of maximum baseline reflex responses was significantly higher post-cyproheptadine than all other conditions (p < 0.0001). Despite increases in tapping force, the reflex responses remained diminished post-cyproheptadine, with only 3/7 subjects able to produce EMGs ≥50% baseline reflex amplitude and only 1/7 able to reach 80% baseline reflex amplitude. None of the subjects were able to reach 100% maximum baseline reflex amplitude post-cyproheptadine. Conversely during post-escitalopram testing, similar force was needed to elicit 20% baseline reflex response, however significantly less force is needed to reach 100% baseline levels as compared to pre-medications conditions (p < 0.05).

The peak reflex amplitude was also significantly altered following study medications. Post-cyproheptadine the peak tendon reflex decreased to 54.7 ± 7.6% baseline reflex (range: 25.2 – 82.4%) whereas post-escitalopram peak tendon reflexes increased to 229.0 ± 66.3% baseline reflex (range: 91.9 – 594.0%; p < 0.01). Post-cyproheptadine, the force required to elicit the peak reflex increased by 15.1 ± 7.1 N as compared to baseline conditions; whereas post-escitalopram, peak tendon reflex amplitude was elicited with -9.2 ± 7.0 N less force (p < 0.05). The combined changes in the threshold and amplitude reflex responses are demonstrated in Figure 24C.
Figure 23: Tendon reflex in an individual following acute administration of serotonergic medication. (A) Tendon reflexes of the agonist and antagonist are presented over a wide range of tap forces. (B) When the normalized EMG is plotted against the tap force, the reflex recruitment curves are similar between days. (C) Following cyproheptadine reflexes are depressed and (D) following escitalopram reflexes are increased.
Figure 24: Tendon reflex in a group of individuals following acute administration of serotonergic medication. (A) Cumulative sum of tap forces delivered across all experiments suggests less force is delivered to the tendon during post-escitalopram assessments and more force is delivered during post-cyproheptadine assessments. (B) Increases in peak reflex responses were observed following escitalopram administration. (C) The gain of the reflex response curve is similar across days during baseline testing. Following cyproheptadine there is a rightward shift in bias where as following cyproheptadine there is a leftward shift in gain.
v. **Rate dependent windup of the knee extensor tendon reflex**

Rate dependent windup of the KE tendon reflex was assessed by eliciting 5 consecutive taps separated by rest intervals of 5, 2, 1 or 0.5 s. The average force delivered to the tendon across days was 44.9 ± 9.8 N. The force delivered to the tendon was not consistent during the series of 5 taps; a significant decrease in force delivered to the tendon was observed over the series of taps (p < 0.0001) and demonstrated a significant interaction different tapping rates (p < 0.05). The most modest decrease in tap force was found at rest intervals of 5 s, where the initial force of 43.9 ± 10.0 N decreases to 42.6 ± 9.6 N. The largest rate-dependent decline in tap force was observed at rest intervals of 1 s, where the initial force of 44.5 ± 10.3 N decreases to 39.8 ± 10.5 N.

Despite this decrease in delivered tapping force with repeated taps, patients demonstrated increased reflex responses from the first reflex response. Across all four conditions (5, 2, 1, 0.5 s rest) there was a significant increase in pooled KE reflex response with repeated taps (p < 0.01). Within each condition significant increases in reflex responses were observed in each of the 4 conditions (p < 0.05), with the exception of 0.5 s rest. While the reflex responses at different tapping rates were not significant, a clear difference was observed. At rest intervals of 5 s, KE reflex response demonstrated a modest average increase of 129.4 ± 9.2% of initial reflex response. At 2 s intervals a substantial average increase of 210.4 ± 47.6% was generated. At higher rates of tapping, there appeared to be saturation in reflex response such that the average increase in KE reflex response was 168.2 ± 24.2 and 154.6 ± 40.6 for 1 s and 0.5 s intervals respectively.
Study medications appear to have an effect on the rate dependent wind-up of the tendon reflex. When reflexes are normalized to the first response of each testing conditions, both cyproheptadine and escitalopram demonstrate negligible differences in the magnitude of increased KE reflex responses. Differences are observed, however, when reflex responses are normalized to the initial (pre-drug) baseline conditions. Post-cyproheptadine resulted in initial reflex responses were roughly equivalent to baseline conditions (100.3 ± 26.4% baseline KE reflex) this is in spite of the fact 24.6 ± 8.3% more force was being delivered to the tendon. Conversely, with post-escitalopram, the initial reflex responses were much higher (486.7 ± 161.6% baseline KE reflex) despite 18.4 ± 14.6% less force being delivered to the tendon.
Figure 25: Windup of tendon reflexes individuals following acute administration of serotonergic medication. (A) During baseline testing there is a rate dependent windup of tendon reflexes, which is maximal at 0.2 Hz. Following administration of either study medications, a decrease in windup is observed when normalized to the first tap of the post medication series (B, D). When post medication data is normalized to the premedication responses minimal change is observed post-cyproheptadine, large increases are observed post-escitalopram.
vi. **Persistent motor unit activity**

It was not uncommon for volitional, electrical and/or mechanical activation of the motor system to produce prolonged activation of the motor system despite the subjects being instructed to “relax completely.” In some cases, persistent motor unit activity could be observed and decomposed.

In three subjects, repeated tendon taps were able to elicit persistent motor unit activity of various muscle groups in the tested leg. Such motor unit activity was only observed in the post-escitalopram condition. In one subject taps delivered with 5 sec rest elicited a small amount of persistent activity in all three quadriceps with RF demonstrating clear motor unit activity. For this unit, the recruitment threshold was 8.8 pps, peak firing was 13.5 pps with derecruitment occurring at 5 pps.

e. **Discussion**

The current investigation sought to understand the influences of 5HT on volitional and reflexive motor function on humans with incomplete SCI through the pharmacological manipulation of 5HT systems. Following a double-blinded administration of a 5HT antagonist/inverse agonist (cyproheptadine), significant decreases in volitional torque generation and reflex amplitude was observed. Following administration of an SSRI (escitalopram), little change in volitional torque generation was noted while large increases in reflex amplitude were observed. These findings suggest that 5HT has a strong role in volitional and reflexive motor output following SCI.
i. **Actions of serotonin on the segmental nervous system**

In addition to direct ionotropic actions, brainstem pathways modulate the activity of spinal neurons through metabotropic agents. The raphe nuclei of the brainstem synthesize 5HT and have substantial projections to spinal neurons (Bjorklund & Skagerberg, 1982). In the ventral horn, 5HT projections make direct synaptic connections to motoneurons, a large percentage of which (> 95%) occur in the dendritic region (Alvarez et al., 1998). These descending monoaminergic projections release 5HT during the normal waking state (Gerin et al., 1995; Veasey et al., 1995) and are tightly coupled with the intensity of motor activities, such that increasing levels of intensity correlate with increasing levels of monoaminergic drive (Jacobs & Fornal, 1999; Jacobs et al., 2002).

Descending 5HT elicits multiple interrelated effects on spinal neurons which, in general, serve to increase motoneuron excitability. In the presence of 5HT agonists, motoneurons exhibit a depolarization of resting membrane potential and accompanied increases in input resistance (Elliott & Wallis, 1992; Hsiao et al., 1997), decreased duration of the post spike afterhyperpolarization (Wallen et al., 1989a; White & Fung, 1989; Hsiao et al., 1997) and hyperpolarizes the voltage threshold for spike activation (Fedirchuk & Dai, 2004; Harvey et al., 2006; Power et al., 2012). Lastly, modulation of PIC activity through direct and indirect activation of Ca\(^{2+}\) and Na\(^{+}\) conductances can facilitate the amplitude and duration of plateau potentials to produce self-sustained firing and enhanced warm-up/wind-up with repeated activation (Bennett et al., 1998a). Such changes modulate the input-output properties of motoneurons towards a more excitable state.
In the present investigation, the input-output properties of motoneurons were assessed via the tendon tap-reflex response relationship prior to and following the application of pharmacological manipulation of 5HT in humans with SCI. Following cyproheptadine, a rightward shift in this relationship was observed, with minimal change in gain, while escitalopram produced an increase in gain of the reflex response curve with minimal change in bias or threshold (Figure 24). Similar changes in the short latency reflex response have been demonstrated in animal models despite potential alterations in presynaptic actions (Barasi & Roberts, 1974; Fung & Barnes, 1989; Machacek et al., 2001; see below). These findings suggest endogenous 5HT receptor activation contributes to motor output during resting conditions in humans with incomplete SCI and such activity can be augmented with increased bioavailability of 5HT. This finding is also consistent with the disregulation of PIC activation in spinal neurons known to occur following SCI (Bennett et al., 1999; Murray et al., 2010). Although alterations in other properties mentioned above may also contribute to this behavior (see however, Bennett et al., 2001b).

Alterations in tendon reflex responses observed might be accounted for by 5HT-induced alterations in interneuronal circuits. However, group Ib, II and III reflexes are tonically inhibited in a decerebrate preparation with intact descending brainstem projections whereas such inhibition is not observed in spinal animals (Eccles & Lundberg, 1959). Direct application of 5HT agonists will often inhibit the actions of group I/II spinal interneurons, however some subpopulations of these interneurons are excited by such chemicals (Jankowska et al., 2000; Garraway & Hochman, 2001a; Hammar & Jankowska, 2003). Similar modulatory effects are observed in the dorsal horn pain processing interneurons (Belcher et
al., 1978) in which EPSP amplitude is attenuated, resulting in a decrease in transmitter release (Garraway & Hochman, 2001b; see however Machacek et al., 2001). As such, it is unclear how changes in interneuron activity contribute to the potential changes in volitional or reflex responses.

ii. **Actions of serotonin on volitional force generation**

Of particular interest in the present study is the potential functional role of 5HT induced alterations in spinal neurons. In the current investigation, individuals with motor incomplete SCI demonstrated a decrease in volitional force generation following cyproheptadine administration, whereas widespread increases in agonist and antagonist EMG was observed following escitalopram administration with little change in volitional torque generation. Such findings are consistent with the segmental actions of 5HT on spinal neurons described above. While SSRI medication has been shown to increase reflex activity and may also increase volitional force generation, the present findings indicate that unintended increases in antagonist EMG activity may have limited the potential for increases in volitional force generation, particularly given then observed significant increase in agonist EMG activity. In addition to increases in antagonist EMG, activity of non-associated muscle groups of the tested leg, particularly TA and MG, activated during volitional contractions decreased following cyproheptadine and increased following escitalopram. Such widespread activation of non-associated muscle groups has been previously observed in individuals with incomplete SCI (Sherwood et al., 1996; Alexeeva et al., 1997) and has been attributed to propriospinal interneurons (Dimitrijevic et al., 1983). Modulation of non-associated muscle activity by 5HT agents is consistent with their wide spread actions on spinal neurons.
Further, 5HT has been shown to increase the receptive field of spinal neurons allowing for an unmasking of polysynaptic pathways (Shay et al., 2005; see however, Hyngstrom et al., 2008), which could augment non-associated muscle responses. Conversely, mechanical stimulation/vibratory oscillation of the tendon taps via tissue may directly activate afferents with no need for interneuronal connections (Lance & Degail, 1965). It is therefore unclear what role spinal interneurons have in the activation of non-associated muscles or their modulation.

iii. **Windup of motor behaviors**

One of the key features of PICs is their tendency to “wind-up”, in which repeated activation at intervals < 4-6 s progressively facilitates efferent activity (Bennett et al., 1998a). This mechanism may contribute the presence of supramaximal torque generation in individuals with incomplete SCI with repeated MVEs (Hornby et al., 2009b; Thompson et al., 2011b) and the windup of stretch (Hornby et al., 2006a) and flexor (Hornby et al., 2003) reflex activity following SCI. The current findings extend this work by demonstrating the rate-dependent windup of the short latency tendon reflex. Here, tendon taps delivered to the patellar tendon demonstrate an increase in reflex amplitude at frequencies > 0.2 Hz, with maximal reflex responses observed at 0.5 Hz. Rate dependent increase in the stretch reflex has been observed in individuals with SCI during large amplitude stretches (30 deg; Hornby et al., 2006a). However with brief inputs to the motoneuron, such as H-reflex or small amplitude stretches (6 deg), do not produce facilitation per se – instead a reduction in reflex depression is often observed (Trimble et al., 1998; Grey et al., 2008; Shields et al., 2011).
While 5HT strongly modulates PIC activity and presumably would augment responses to repeated activation (Perrier et al., 2002), we observed a decrease in the wind-up of reflex responses following both cyproheptadine and escitalopram administration. This depression following cyproheptadine is consistent with initial hypotheses, although the responses following escitalopram were largely unexpected. In this latter condition, however, the reflex amplitudes post-escitalopram were >4 times larger than baseline conditions, and the lack of further windup of reflex responses may be due to a rate-limiting behavior underlying PIC activity (Fuglevand, 2010) or saturation of excitability (Lee & Heckman, 2000). Further work is required to assess the role of 5HT on the PIC mediated windup of spinal neurons.

iv. Potential therapeutic utility

Following human SCI oral administration of cyproheptadine and escitalopram produces nearly opposite alterations in both reflex and volitional motor behaviors. As hyperactive reflexes and decreased volitional force generating capability are primary impairments following SCI, understanding how medications alter these behaviors is expected to have therapeutic utility. Cyproheptadine administration produces a decrease in both the short latency tendon reflex and long lasting spastic reflex activity in individuals with incomplete SCI (Murray et al., 2010). As spasms may at times be painful and interfere with functional activities, the use of 5HT antagonists/inverse agonists may prove to be a viable means to decrease this unwanted activities. Unfortunately, cyproheptadine appears to decrease volitional strength and may interfere with walking ability. Further, patients with SCI often report using their spasms for functional activities (e.g. locomotion); the impact of decreasing these “functional spasms” is unclear.
In contrast, preliminary data has shown that SSRIs increases volitional strength in individuals with incomplete SCI. Unfortunately, the present data also suggest that SSRIs increase co-contraction during volitional efforts and spasms. In the current investigation 20 mg of escitalopram produced large increases agonist EMG activity; it is likely the large increases in antagonist co-contraction limited the ability for this efferent activity to be reflected in volitional torque generation. Previous preliminary data suggests that 20 mg of escitalopram increases the volitional force generation of the plantarflexors to a greater extent than both 10 mg of escitalopram and placebo in individuals with incomplete SCI (Hornby et al., 2006b). It is possible any torque produced by co-contraction of the dorsiflexors had less of an impact on volitional force generation as do the knee flexors in these current experiments. It may be that multiple medications are necessary to fine tune the precise behaviors (Fung et al., 1990).

v. **Conclusion**

Blinded, oral administration of either cyproheptadine or escitalopram produces large changes in volitional and reflex motor behaviors following human incomplete SCI. Such changes are consistent with the disregulation of 5HT sensitive spinal neurons. Understanding how endogenous and exogenous modulation of spinal neurons contributes to motor output will likely have a therapeutic value in alleviating impairments following human SCI.
V. Conclusion

The preceding work is an attempt to understand the mechanisms of supramaximal volitional torque generation in individuals with incomplete SCI. For this, the concept of the segmental motor system was defined as the set of neuromuscular structures which have ‘direct’ actions to and from the motoneuron and a framework for motor control was built upon this definition. This framework was used to delineate potential sites of fatigue and potentiation commonly described in healthy conditions. A review of impairments observed following SCI was provided and the resulting alterations in the segmental motor system were described. This provided a common basis on which to discuss alterations in fatigue and potentiation in individuals with incomplete SCI.

Though individual conclusions are provided at the end of the 3 experiments, the following section provides an overview of the major themes which emerged from the experiments. Additionally, areas of future investigation are provided.

a. Synopsis of reported experiments

From the experiments outlined above, the proposed mechanism of supramaximal volitional torque generation in humans with incomplete SCI is that high intensity volitional effort contractions enhance brainstem mediated release of serotonin (5HT) onto spinal neurons. This endogenous release of 5HT increases the excitability of spinal motoneurons, perhaps in individuals with SCI to a greater extent than healthy controls. Because individuals with incomplete SCI are unable to fully activate their muscles during a volitional contraction, this increased spinal neuron excitability serves to amplify the descending command producing augmented motor output on additional efforts for a relatively short period of time.
The data presented in this thesis are consistent with this hypothesis. Though further work is necessary to delineate the precise mechanisms, conclusions can be made regarding volitional motor output in individuals with incomplete SCI.

i. **Supramaximal torque is consistently observed**

Throughout all experiments, patients with incomplete SCI were able to increase volitional force generation well beyond what is typically observed during maximal volitional contractions performed in isolation. This is consistent with the initial findings of Hornby *et al* (2009b) in which individuals with incomplete SCI were able to repeatedly generate supramaximal torque both within and between days. Figure 26 demonstrate the average torque produced during a series of 5 repeated MVE contractions separated by a rest of 5 s. Though alterations in subject population and experimental conditions may explain minor differences, the consistent observation of supramaximal torque generation suggests this finding is not due to the subjects ‘learning’ how to perform the task of maximal knee extension, but instead to short term alterations within the segmental motor system.
Figure 26: Supramaximal volitional torque generation observed across all three experiments. During the course of Experiments 1, 2 and 3, a consistent increase in volitional force generation was observed during repeated MVE contractions of the knee extensors in individuals with incomplete SCI.
ii. **Spinal neuron excitability contributes to volitional force generation**

The experiments described in this thesis investigate the potential mechanisms underlying the initial findings described above, and generally demonstrate this increase in volitional force generation is associated with increases in reflex activation. In Experiment 1, long lasting reflexes were evoked with surface electrical stimulation in patients with SCI, but only after repeated volitional efforts. Similarly in Experiment 2, potentiation of the short latency tendon reflex was observed with maximal responses noted immediately following high intensity efforts. Alterations in peripheral structures were assessed using supramaximal axonal stimulation and changes in neuromuscular transmission/propagation or excitation contraction coupling were suggested to contribute minimally to the behavior. These alterations in reflex activity are consistent with short term increases in spinal neuron excitability following high intensity volitional efforts. This increased spinal neuron excitability likely contributes to supramaximal volitional torque generation in human incomplete SCI.

iii. **Activation history modulates motor output**

Data from the Experiment 2 also describes the importance of intensity of volitional efforts and duration between maximal effort contractions on volitional and reflex activity in individuals with incomplete SCI. By altering both the intensity and timing of preceding contractions, it was apparent that supramaximal volitional torque generation was best observed during high intensity contractions separated by brief periods of rest. Such findings are important for future investigations, as it defines a washout period during which
decreased spinal excitability which likely contribute to net volitional torque generation. Such data may provide parameters for an optimal strength training interventions.

iv. **Serotonin has strong effects on motor output**

The primary hypothesis suggests endogenous 5HT modulates spinal neuron excitability during volitional effort contractions. This hypothesis was assessed in the third experiment through the use of serotonergic medication; indeed 5HT has a profound effect on motor output in individuals with incomplete SCI. In general 5HT antagonists/inverse agonist depressed volitional and reflexive motor output where as SSRI medications increase volitional and reflexive motor output, though increases in co-contraction limited observable increases in volitional torque. Further work is necessary to fine tune the actions of serotonergic medications on the segmental motor system and delineate the possible modulation other non-serotonergic medications.

While the motoric consequences of these agents are of substantial interest, the data also highlight the potential for poly-pharmacy in individuals with SCI. For example, individuals with chronic SCI are approximately 4 times more likely to have symptoms of depression as compared to neurologically intact individuals (Fuhrer et al., 1993; Kemp & Krause, 1999). As such, a large number (>70%) of these individuals will be prescribed anti-depression medication (Smith et al., 2007) whose primary action is to increase 5HT bioavailability. As SSRI medications will increase clinical measures of spasticity, anti-spastic medications will conceivably be prescribed to counteract the motoric side effects of SSRIs. This becomes a broader concern when one recognizes that poly-pharmacy is a major issue following SCI, where the average patient being prescribed 8 different medications (Krause et
al., 2009). This is particularly alarming as the amount of prescription medication used is inversely related to function (Kohout et al., 2011), and positively related to need for rehospitalization (Krause, 2010) and mortality (Krause et al., 2009). Possible poly-pharmacy could be avoided if SSRI-induced increases in spasticity/spasms were accounted for in the medical management of both the psychological and motor consequences of SCI. This highlights the need for coordinated interdisciplinary medical care following SCI.

b. **Further experimentation needed to assess supramaximal torque generation**

The experiments described above provide a strong foundation on which to further clarify the mechanisms underlying the observed behaviors, how these results translate to other types of contractions (i.e., dynamic), and their potential clinical impact towards improving function in individuals with incomplete SCI.

i. **Additional experiments needed to clarify mechanisms**

1. **Motor unit activity**

A general limitation of the detailed experiments was the use of the amplitude of surface electromyographic (EMG) signals to assess muscle activation. Though surface EMG provides a general estimate of muscle activity, various limitations, such as crosstalk between muscles, may hinder the ability to properly assess differences between individual muscles. Other limitations include placement considerations, where normalization is required to compare EMG across testing sessions, but may also introduce errors of the processed signals. Further, artifact from movement and electrical noise may contain overlap in frequency with the true EMG signals. Also changes in sarcolemma excitability may alter the
magnitude of the EMG envelope. Taken together these limitations could result in erroneous claims regarding efferent drive.

To overcome these limitations and provide further information regarding the neural mechanisms underlying supramaximal volitional torque generation, fine-wire electrodes can be inserted intramuscularly or subcutaneously and provide selective recordings of motor unit action potentials. These signals can be decomposed using template matching software and provide information on motor unit firing rates and recruitment. Figure 27 demonstrates intramuscular recordings during supramaximal volitional torque generation in humans with incomplete SCI.
Figure 27: Motor unit activity during supramaximal volitional efforts in two individuals. Fine wire recordings from VL during the generation of supramaximal torque are shown in 2 individuals with incomplete SCI. For each subject, torque traces are shown with corresponding surface and intramuscular EMG – instantaneous firing frequency is calculated from the decomposed signal. Both patients demonstrate increases in peak firing rate during the generation of supramaximal volitional torque – additionally, patient 1 (right) demonstrates recruitment of additional ‘supramaximal motor units’.
2. **Actions of spinal interneurons**

Though our results appear consistent with motoneuron PIC activation, alterations in spinal interneurons could contribute substantially to the observed behaviors, although their role is almost completely unclear. For example and similar to motoneurons, ventral interneurons demonstrate PIC-like activity (Hounsgaard & Kjaerulff, 1992; Dougherty & Kiehn, 2010), are modulated by descending monoaminergic inputs (Zhong *et al.*, 2010), and could contribute to augmented motor responses as described for motoneurons above. Differentiating between increases in interneuronal versus motoneuronal excitability is not possible in the present study.

Current techniques to assess spinal interneurons in humans are limited and rely mainly on careful interpretation from detailed methods of reflex activation. One proposed means to overcome these limitations is to attempt to quantify correlated and synchronous activity of paired motor unit recordings. Methods such as cross-correlation, coherence and short term synchronization of motor unit pairs are routinely used in humans to assess the activity of common last order inputs into the motoneuron pool, but rarely consider the role of spinal interneurons. Detailed animal work is necessary to delineate the role of spinal interneurons to avoid over-interpretation of such data. After the contributions of spinal interneurons to correlated motor unit activity is better established in animal models, such techniques could be applied to volitional contractions performed by humans.

3. **Supraspinal contributions**

Increases in cortical drive could possibly account for some of the present findings. While previous data suggest decreased corticospinal transmission with repeated MVEs (Di Lazzaro
et al., 2003; Petersen et al., 2003), recent evidence suggests this depression may be muscle specific (Giesebrecht et al., 2010). Intact subjects may instead demonstrate increases in excitability of some central pathways with high intensity volitional contractions (Samii et al., 1996; Norgaard et al., 2000b). Repeated activation may increase output from the motor cortex; indeed increases in cortical activity are observed during submaximal fatiguing contractions in healthy individuals (Liu et al., 2003).

The current experiments were not designed to assess cortical contributions to supramaximal volitional torque generation. It is proposed that supraspinal contributions will be quite difficult to assess. For example, imaging approaches may not provide a true representation of supraspinal drive as motor and sensory areas are tightly linked (Schieber, 2001); increases in brain activity would be expected from increases in sensory afferents with repeated contractions. Electrophysiological techniques have been used to attempt to distinguish between cortical and spinal excitability. These are technically rigorous experiments, as stable TMS, CMEP and M-wave responses should be considered. However, even with faithful recordings, these assessments may not activate the motoneuron in the same manner (i.e. 500 Hz burst with TMS vs. single synchronous pulse with CMEP). It is unknown what, if any, effect this may have at the level of the motoneuron – though nonlinear summation of activation is noted throughout the segmental motor system.

In an attempt to avoid these technical issues it may be possible to gain indirect assessments of supraspinal drive through alterations of a learned submaximal effort. In healthy individuals, a post contraction overestimation of submaximal forces (5% MVE) is observed and attributed to potentiation of spinal proprioceptive pathways (Hutton et al.,
In individuals with incomplete SCI similar, short term (< 1 min) overestimation of a learned submaximal effort (Figure 28). Such findings are consistent with the spinal augmentation of a constant cortical drive.
Figure 28: Short term increases in torque produced during performance of a learned submaximal effort. Individuals with incomplete SCI can learn to consistently generate a prescribed submaximal effort without visual feedback. (a) Torque trace reveals consistent submaximal force generation with and without visual feedback corresponding to 80% MVE. Increased force generation is observed during a 80% effort performed 5 sec following 2 MVEs but returns to baseline levels following 1 min. (b) Group data (n=5) indicated this short term force overestimation is observed over a wide range of submaximal efforts. (c) The 118% MVE value predicted through the extrapolation of magnitude submaximal force overestimation corresponds with reported values of supramaximal volitional torque generation.
ii. **Further behavioral investigations**

The current investigations have assessed isometric contractions of KEs in individuals with incomplete SCI. Though the knee extensors are an important muscle with regards to the ability of the individual to walk, efferent drive during repeated maximal activation of other muscles is largely unknown. Further, it is unclear if such volitional torque generation is observed during different types of contractions (concentric and eccentric) which may replicate more closely the modes of contractions used in functional tasks. Such understanding is important for future development of rehabilitation interventions to improve strength and functional performance of various motor tasks (i.e., walking, transfers).

1. **Supramaximal volitional torque in other muscles**

The current investigations focused on the presence of supramaximal volitional torque generation in the knee extensors. Though the knee extensors are strongly correlated with functional mobility following SCI (Crozier *et al.*, 1992; Saraf *et al.*, 2010; Zorner *et al.*, 2010; van Middendorp *et al.*, 2011), the generation of supramaximal torque in other muscles would provide benefits in terms of further physiological and rehabilitative investigations. Investigations are underway to assess the presence of supramaximal volitional torque generation flexion and extension the hip, knee and ankle in a large number of individuals (~100) with incomplete SCI.

2. **Contraction type**

Functional activities often require the production of dynamic muscle contractions. As strength training may be contraction specific (Higbie *et al.*, 1996; Roig *et al.*, 2009), it is
important to understand if supramaximal volitional torque generation is dependent on contraction type. Figure 29 demonstrates supramaximal torque generation during performance of concentric knee extension.
Figure 29: Supramaximal volitional torque generation during concentric contractions. A subject with motor incomplete SCI performs a series of 5 isokinetic contractions at 20 deg/s over a 80 deg range (4 s on, 6 s off). Increases in gravity corrected KE torque and RF EMG is observed. Following a 1 min rest, torque and EMG returns to baseline levels.
3. **Actions from remote muscles**

When individuals with incomplete SCI generate volitional torque, activity of antagonist and non-associated muscles tends to increase (See Figures 19 and 21). For multiple reasons it would be important to know if contraction of remote muscles, instead of the agonist, would potentiate the agonist during an MVE contraction. Such alterations in motor activity following MVE contractions of remote muscles (e.g. Jendrassik's maneuver; JM) would be expected from the hypothesized widespread actions of brainstem neuromodulators. Previous data in intact subjects suggest that release of presynaptic inhibition is the primary mechanism underlying the increase in reflex responses following performance of a JM (Dowman & Wolpaw, 1988; Zehr & Stein, 1999). Performance of JMs will also increase spinal levels of endogenous 5HT from brainstem projections and modulate spinal PIC activity. This modulation of spinal neuron excitability in human incomplete SCI could be responsible for increased efferent output following JM. Indeed, the preliminary data are consistent with this hypothesis; in a single subject, performance of repeated JMs of the upper extremities resulted in supramaximal volitional torque in the knee extensors (Figure 30).

Once this is further established, the role of effort may be able to be further assessed by paralyzing the remote muscle (Frigon *et al.*, 2011) and having the patient perform JMs of this paralyzed muscle. This would help obviate concerns of the reflexive consequences of contractions of remove muscles.
Figure 30: Supramaximal volitional torque generation following contractions of remote muscles. (a) Single subject with incomplete SCI produces supramaximal volitional of the KE during three repeated MVE contractions. (b) The same subject is able to produce supramaximal torque when remote contractions (upper extremity Jendrassik maneuver; JM) followed by 1 MVE of KE. Similar increases in KE force-generating capability were observed (44 vs. 39 Nm) irrespective of location of repeated MVE contractions.
iii. **Clinical translation**

Exercise has been thought to increase strength, help regain mobility and promote health in individuals with SCI (Jacobs & Nash, 2004), although the multiple studies which have evaluated the effects of exercise interventions in individuals with incomplete SCI have had mixed results. For instance, a case series of 3 individuals with SCI who underwent a 12 week lower extremity progressive resistance and plyometric training program demonstrated increases in peak torque production in addition to increases in walking speed (Gregory *et al.*, 2007). Likewise, a 6 month multimodal intensive (meaning, time spent not effort) exercise program in individuals with SCI demonstrated improvements in clinical measures of strength and function (Harness *et al.*, 2008). While these studies suggest improvements in strength, recent studies have suggesting that progressive resistance training may not improve strength or function in individuals with incomplete SCI (Glinsky *et al.*, 2008; Harvey *et al.*, 2010).

While the results are inconsistent, it is important to note that when these modest improvements in strength are observed, it is often accompanied by measurable gains in function. It is noteworthy that this quantitative research is mirrored in the subjective analysis of barriers of exercise in individuals with SCI. According to individuals with SCI whom do not exercise, a major barrier to exercise is due to a ‘limited return on investment’ (Kehn & Kroll, 2009) – at this point in time, these ‘limited return on investments’ may indeed be true.

An intuitive application of supramaximal volitional torque generation is to develop a strength training protocol based upon repeated maximal volitional effort contractions. In a small sample of individuals with motor incomplete SCI (n=5), a 4 week training program consisting of repeated MVE contractions produced significant gains in volitional torque
generation as compared to a similar conventional training program that follows ACSM guidelines (Figure 31). Such findings may represent an effective means to overcome the deficits in volitional strength which limit function in this population. It is important to continue with the experiments outlined in the above section to fine tune a protocol which maximizes supramaximal volitional torque generation prior to a large scale clinical trial.
Figure 31: Preliminary increases in volitional torque generation following 4-week training program based upon supramaximal volitional torque generation. Five patient with chronic incomplete SCI performed 4 weeks of either 3 sets of 10 repeated MVE contractions or 3 sets of 10 traditional strength training exercises for flexion and extension at the knee and ankle over 4 weeks in a randomized crossover manner (4 months between protocols). Immediately following the respective protocol, a 12% increase in volitional torque generation was observed whereas a 62% increase was observed following supramaximal strength training.
c. **Concluding remarks**

Supramaximal volitional torque generation is a novel finding that fits well within the theoretical framework outlined throughout this thesis. Though further work is necessary, it is my hope that this work will eventually be used to improve the lives of patients with SCI.
VI. References


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Individuals with incomplete SCI increase their maximal volitional torque generation during a fatiguing protocol. This project will 1) describe the volitional behaviors which elicit and 2) the motor unit activity which underlies this supramaximal torque generation in preparation for a clinical training protocol.

PUBLICATIONS
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**ABSTRACTS**

**Oral Presentations**


**Poster Presentations**


