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Central excitab[illegible]y contributes to supramaximal volitional contractions in [illegible]man incomplete spinal cord injury

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Non-technical summary Individuals with a motor incomplete spinal cord injury (SCI) present clinically with partial control of muscles below the site of the injury, but experience profound weakness which can limit the ability to perform functional tasks such as walking. Interestingly, when individuals with an incomplete SCI are asked to maximally and repeatedly contract their quadriceps muscles, they demonstrate an *increase* in the peak force generated; individuals without SCI experience a decline in force, or 'fatigue'. Following these repeated maximal efforts, reflex responses to electrical stimulation over the quadriceps muscle elicited amplified and prolonged, involuntary motor activity. Such responses were not observed prior to the maximal contractions, and were not observed in neurologically intact subjects. This finding suggests that increases in spinal excitability following these maximal efforts may enhance force generating capacity, and provides insight into possible novel therapeutic interventions to restore function following SCI.

Abstract 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 five 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 one, three and five 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 protocols to elicit repeated muscular contractions resulted in a significant decrease in evoked torque generation, suggesting limited the muscular contributions to the observed phenomenon. To evaluate potential central mechanisms underlying the augmented torques, non-linear 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.

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Abbreviations CAR, central activation ratio; LEMS, lower extremity motor score; MH, medial hamstrings; MVE, maximum volitional effort; NMES, neuromuscular electrical stimulation; PIC, persistent inward current; RF, rectus femoris; SCI, spinal cord injury; VL, vastus lateralis; VM, vastus medialis.

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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 fibre 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.* 2009). 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 mechanism 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, non-linear 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_{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 is 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 motoneurones (Gorassini *et al.* 2002a). 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 (PICs), defined as a non-inactivating Na^+ and Ca^{2+} currents intrinsic to the motoneurone (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 behaviours 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 behaviours (Bennett *et al.* 1999; Gorassini *et al.* 2004), although little is known regarding the contribution of PICs to volitional motor behaviours in human SCI (Zijdewind & Thomas, 2003).

Methods to assess motoneurone 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. 2nd 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 require prolonged central drive (Collins *et al.* 2002; Fig. 10), and remain consistent with spinal motoneurone 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 MVE 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.

Methods

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 year) spinal lesions above the T10 neurological level (see Table 1), 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.

Experimental set-up

Experiments lasted approximately 1–1.5 h. 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 six degree of freedom load cell (ATI, Apex, NC, USA) 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 ($\times$1000), band pass filtered (20–450 Hz) and sampled at 1000 Hz simultaneously with the torque data.

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 s each with >1 min 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; Grass S48, external isolation; Grass Technologies, West Warwick, RI, USA) was delivered to the knee extensors through 3 inch $\times$ 5 inch self-adhesive, stimulating electrodes (ConMed Corp., Utica, NY, USA) 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 three bouts of contractions, each consisting of one, three, or five consecutive MVEs (5 s on, 5 s off), with verbal encouragement provided during each effort. A 5 min rest period was given between bouts of repeated MVEs (Hornby *et al.* 2009). 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 Fig. 1.

In 10 subjects (10 legs) with SCI, maximal M-waves and twitch torques were elicited using a single (1 ms duration)

Table 1. Subject demographics

Subject no.	Sex	Age (years)	Level of injury	Time since injury (months)	Knee extensor modified Ashworth	LEMS	Baseline MVE (N m)	CAR
1a/b	M	44	C5–6	266	3/3	44	59/81	0.52/0.32
2	F	40	C5	40	1	14	10	0.11
3	M	42	C6–7	51	3	37	107	0.58
4	M	40	C5	85	1	21	24	0.14
5a/b	M	31	C6	87	3/0	44	103/164	0.65/0.80
6	F	61	T6	63	1	50	58	0.57
7	M	43	C6–7	85	0	29	26	0.30
8a/b	M	35	C6–7	109	3/4	31	39/18	0.35/0.21
9a/b	M	49	C2–4	70	1/2	36	44/113	0.49/0.63
10	M	55	C6	18	3	47	109	0.54
11a/b	M	44	T5–7	133	3/3	47	137/107	0.79/0.84
12	M	33	C5–7	93	0	23	16	0.20
13	M	22	C6	50	2	48	124	0.75
14	M	58	T7	404	0	21	27	0.14
15	M	35	C4	30	4	43	114	0.63

Abbreviations: M, male; F, female; C, cervical; T, thoracic; LEMS, lower extremity motor score; MVE, maximal voluntary effort; CAR, central activation ratio.

pulse from a constant current stimulator (Digitimer Ltd, Welwyn Garden City, 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

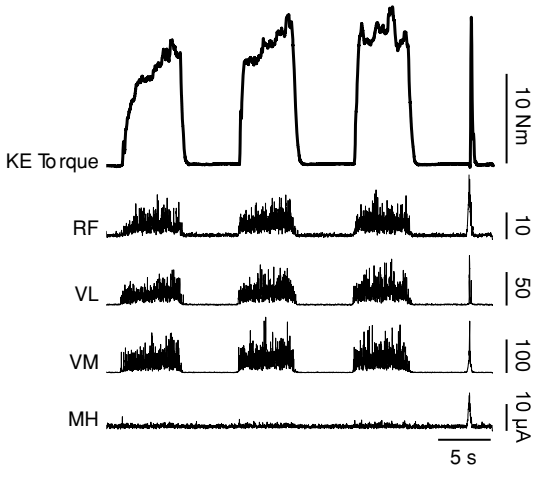

Figure 1. Torque and EMG responses during three repeated MVE contractions of KE followed by supramaximal stimulation of femoral nerve in an individual with incomplete SCI
The reserve of volitional force generation is observed in the knee extensors of an individual with motor incomplete SCI during 3 repeated 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 mutiple forms of peripherial stimulation prior to (not shown) and 5 s following a bout of repeated contractions (a single stimulus to assess changes in twitch and M-wave responses is shown here).

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_{max}. Twitch torques were measured from the torque response resulting from M_{max} stimulation.

In 13 subjects (18 legs) with SCI and nine 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, five 1 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 s of stimulation divided into three 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 nine 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, electrical 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 μs duration, 30 Hz; Grass S48, external isolation) was found

by delivering increasing levels of stimulation for 1 s of stimulation through 3 inch × 5 inch 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 min 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.

Data collection and analysis

Data were acquired and analysed using custom LabView software (National Instruments, Austin, TX, USA). 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 ±50 ms was then averaged to represent peak torque. The largest torque elicited during the three 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 100 ms 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 analysed to assess alterations in antagonist activity during repeated MVEs.

Twitch torque and M-wave parameters were analysed 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 analysed 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* was determined by calculating peak-to-peak non-rectified amplitude of waveform, and *M-wave area* was 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 analysed 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 second 25 Hz stimulation by the averaged torque produced during the final 500–2000 ms of the first 25 Hz stimulation (note: the 1st 500 ms of each 25 Hz stimulation was not analysed to obviate electro-mechanical 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 s 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 means ± standard deviation, and in figures presented with standard error of the mean. All statistical analyses were performed using computer software (Statview; SAS Institute Inc., Carey, NC, USA) with $\alpha = 0.05$. Data were assessed for normality using the Kolmogorov–Smirnov test, 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 one, three, or five 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 Student's unpaired *t* test or the Mann–Whitney *U* test 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.

Results

Participants' demographic and clinical data are presented in Table 1. Baseline assessments demonstrate substantial weakness of knee extensors (74 ± 47 N m) secondary to central activation deficits (CAR values = 0.48 ± 0.24). In individuals with incomplete SCI, peak knee extensor torques during five 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 three bouts of five 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.* 2009).

Changes in twitch torques and M-waves

Single pulse stimulation of the femoral nerve applied at supramaximal current intensity prior to and following one, three or five MVEs revealed variable changes in neuromuscular responses for individuals with SCI. A single subject example of changes in twitch responses is shown in Fig. 2*A* prior to and following three 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 N m) in twitch torque following a single MVE only (Fig. 2*B*), with non-significant increases following three or five 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

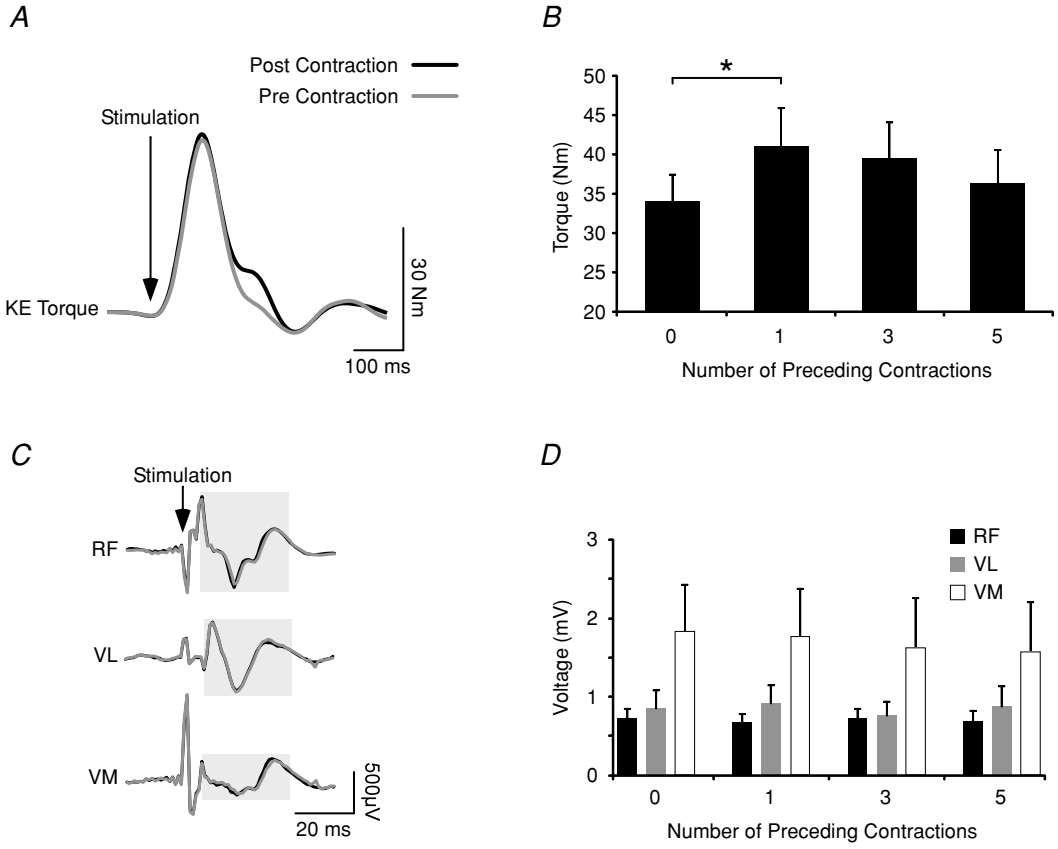

Figure 2. Torque and EMG responses to stimulation of the femoral nerve prior to and following three repeated MVEs of the knee extensor in human incomplete SCI
A, individual knee extensor torque response to stimulation of femoral nerve following 3 repeated MVEs. *B*, group data indicates significant increases 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 analysed. *D*, group data pooled across muscles indicate no change in M-wave amplitude following repeated MVEs.

little change in M-wave characteristics from each muscle assessed. Figure 2*C* depicts these changes in a single subject prior to and following three repeated MVEs (same trial used for Fig. 2*A*). M-wave amplitudes and integrated areas were not significantly different for pooled or individual muscle M_{max} recordings ($P = 0.38$ for pooled response, Fig. 2*D*), with a mean increase of 3% from baseline across all knee extensor muscles following a single MVE.

High-amplitude NMES-elicited repeated 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.* 2009). To test this hypothesis, repeated high-amplitude NMES was performed using a stimulation protocol similar to the timing of repeated MVE contractions (5 s on, 5 s off) performed over 10 min. 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 Fig. 3*A*. Analysis of the first five contractions revealed a significant decline in knee extensor torques at the fifth contraction (decreased to 88 ± 11% of the first

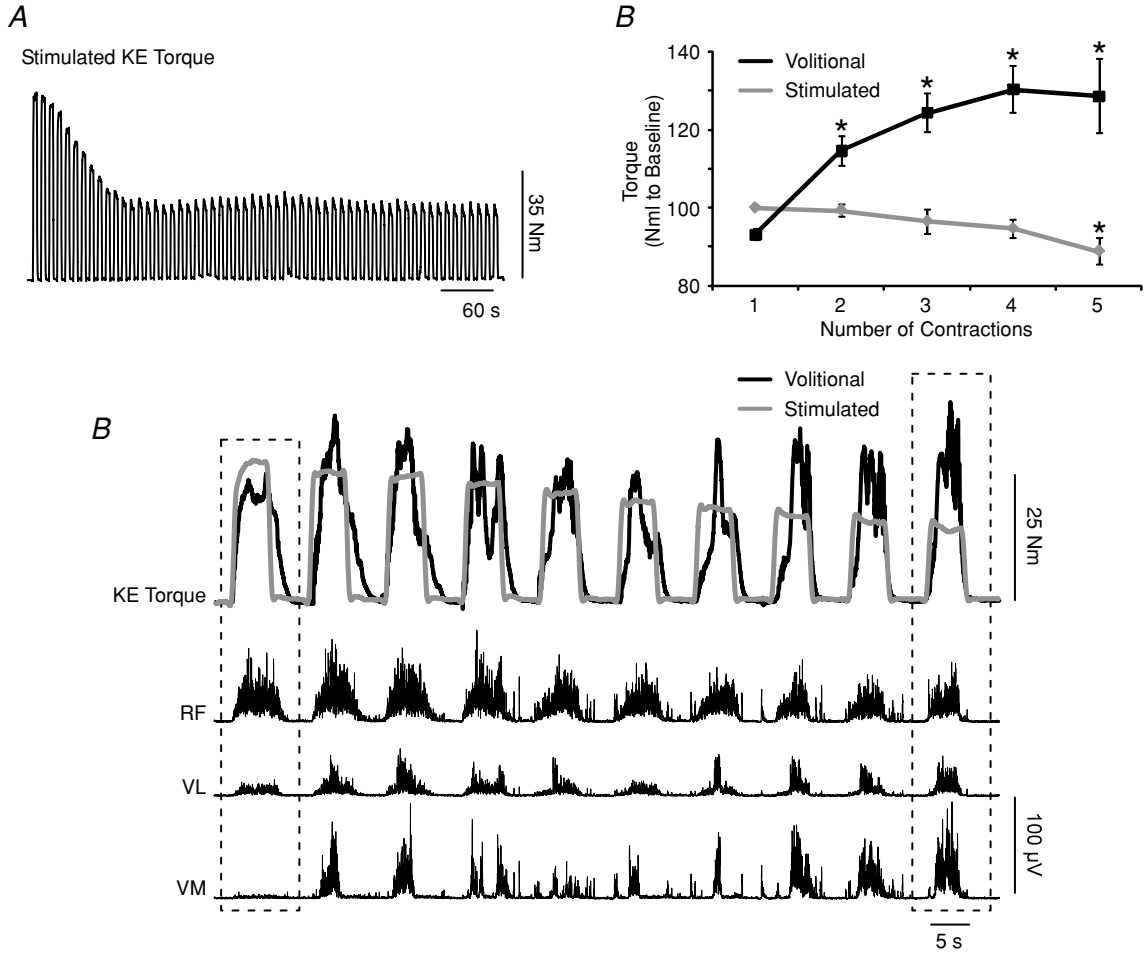

Figure 3. Repeated high-amplitude NMES-elicited contractions (5 s on, 5 s off) produced a decline in force generation
A, torque responses from knee extensor in one subject during 10 min of high-amplitude NMES. A sharp decline in force generation is observed with repeated high-amplitude NMES (Gerrits *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.

NMES-elicited torque, Fig. 3*B*). Eight subjects produced the maximal torque on the first stimulation, with all forces in the other two subjects <15% greater than torques generated on the first NMES-elicited contraction. In contrast, during five repeated MVEs, peak torques of the same subjects increased up to 28 ± 20%, with significant differences between the first and second to fifth MVEs ($P < 0.01$, Fig. 3*B*). 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 Fig. 3*C*, indicating the divergence in responses between the two testing conditions.

Responses to low-amplitude variable-frequency NMES

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 ± 5.1 mA. An example of typical torque and EMG responses to low-amplitude variable-frequency NMES prior to and following three repeated MVEs is demonstrated in Fig. 4. In this example the torque generated during the second 25 Hz (i.e., following 100 Hz stimulation) was greater than torque during the first 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.

Such behaviours were consistent across all 13 subjects (18 limbs tested), with the exception of one of the 13 subjects (subject 6) who consistently demonstrated flexion withdrawal reflexes (i.e., flexor spasms) during the first 25 Hz and 100 Hz epochs of the variable-frequency NMES. Figure 5 demonstrates these responses, with flexor reflexes denoted by the downward torque deflections. These data were difficult to quantify and were not included in the group analysis, despite the consistent observation of prolonged EMG and torque responses following stimulus termination.

Quantitative analysis of augmented and prolonged torques across the remaining 12 subjects with SCI (17 limbs tested) and nine healthy control subjects is presented in Fig. 6*A*–*C*. During low-amplitude variable-frequency NMES applied to individuals with SCI, small increases in knee extensor torque were evident during the second *versus* first 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 three 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 N m) and following three repeated MVEs (1.53 ± 2.91 N m), 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 three and five 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.

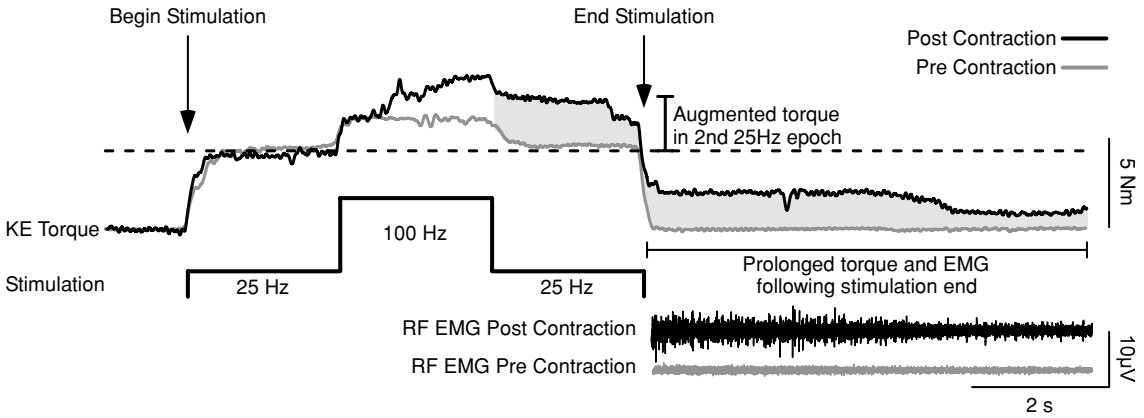

Figure 4. Torque and EMG response to low-intensity variable-frequency NMES prior to and following three MVE contractions in an individual with incomplete SCI
Prior to MVE contractions (grey line), there is minimal augmentation of torque during the 2nd 25 Hz epoch and minimal prolonged torque and EMG 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.

In control subjects, during low-amplitude (22.3 ± 10.3 mA) variable-frequency NMES, low levels of augmented torques were evident during the second *versus* first 25 Hz stimulation during baseline (pre-MVE) testing ($21 \pm 9\%$ increase), with non-significant decrease following single or repeated MVEs ($P = 0.93$; 6% decrease following three contractions *versus* resting conditions). Torque and EMG activity following stimulation followed a similar trend, with relatively low baseline values (0.47 ± 0.20 N m 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 three contractions *versus* resting conditions in healthy controls. Unpaired comparisons between responses to variable-frequency NMES in SCI and control subjects at each of the four preceding conditions (0, 1, 3 and 5 contractions) were not significant (P values range from 0.06 to 0.48).

Associations between quantitative and clinical measures with variable-frequency NMES

Greater augmented and prolonged torques during low-amplitude variable-frequency NMES following three and five 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.* 2009). Correlation analyses were performed to examine potential associations between peak torque increases during the bout of one, three or five 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 one to five MVEs and augmented torques during the second 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_{max} 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 second 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.

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.

Alterations at the peripheral motor excitability with repeated MVEs

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_{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

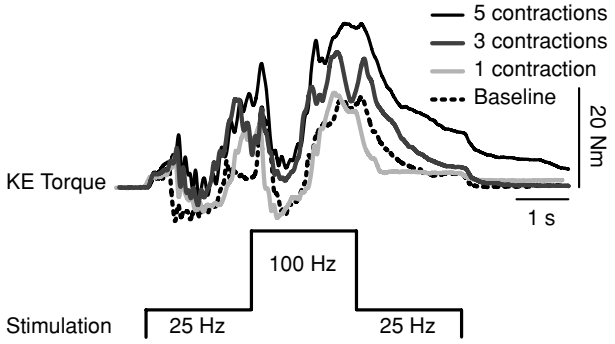

Figure 5. One individual with SCI demonstrates spastic flexion responses to variable-frequency NMES and was not included in the group analysis
Above is an overlay of torque response of this individual at baseline and following 1, 3 and 5 MVE contractions. Despite flexion reflexes, a clear wind up of responses following MVE contractions is observed.

our individuals with incomplete SCI. Such a reduction in central activation suggests that many motor units were likely not to have been 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.* 2009).

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 demonstrate substantial differences between torque responses to repeated NMES and repeated MVEs. Rapid, significant decreases in NMES-elicited torques were observed by the fifth repeated contraction, consistent with published observations in complete SCI (Gerrits *et al.* 1999). While 2/10 subjects demonstrated increased torques during the first five 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 fibres, particularly following SCI (Godfrey *et al.* 2002). Additionally, the lack of antidromic collision during submaximal stimulation may allow for reflexive Ia activation of the motoneurone 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.* 2009) suggest a greater central (rather than peripheral) contribution to the increased volitional torques.

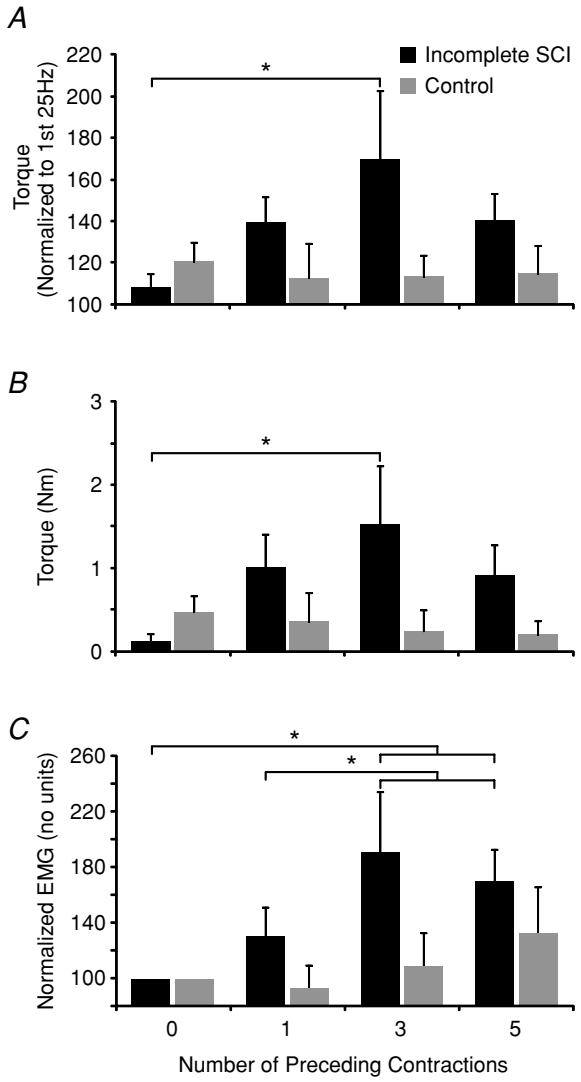

Figure 6. Response to variable-frequency NMES is quantified using augmented torque response during the second 25 Hz epoch (*A*), and prolonged torque (*B*) and EMG responses (*C*) following termination of stimulation
Group data from individuals with motor incomplete SCI indicate an increase in augmented 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 ($n = 9$).

Potential alterations in spinal excitability with repeated MVEs

Low-amplitude variable-frequency NMES has previously been employed to evaluate spinal motoneurone excitability in humans (Collins *et al.* 2001, 2002), although the modulation of the resultant motor behaviours 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 protocol 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 second 25 Hz stimulation (Frigon *et al.* 2011). That is, testing at approximately 90 deg 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 to be 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 behaviours 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.* 2006) 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 first 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 behaviour 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.* 2009).

While warm-up of intrinsic motoneurone 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 noradrenaline) inputs elicit substantial alterations in intrinsic spinal properties, including augmentation of PICs in spinal motoneurones (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 protocol 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 motoneurones 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.

Additional potential sites of increased central excitability

Though our results appear consistent with motoneurone 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 motoneurones 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.* 2000). 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 to be mediated by alterations in reflexes, which are hyperexcitable in the subject population tested (spasticity scores), and may indeed have a common origin at the motoneurone. Further experimentation is necessary to determine the extent of cortical contributions to the increased torques with repeated MVEs and responses to variable-frequency NMES.

Clinical significance

Associations between increased volitional torques with repeated MVEs with underlying PIC-like behaviour 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, 2001) and human studies (Hornby *et al.* 2003, 2006; Gorassini *et al.* 2004) have provided evidence to suggest that spasticity/spasms may be meditated by motoneurone PIC activity. While a previous study had linked the influence of PIC activity to volitional activity in human incomplete SCI (Zijdewind & Thomas, 2003), 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 motoneurone PIC activity. Involuntary spastic motor behaviours are thought to be a negative consequence of SCI, although individuals with incomplete SCI often report utilizing their spastic motor activity for functional behaviours (Gittmann, 1963; Dietz, 2008). It is likely that alterations in voluntary and involuntary motor activity following SCI possess similar underlying mechanisms (i.e. PIC activation of the spinal motoneuron; 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.

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Author contributions

All experiments were performed at the Rehabilitation Institute of Chicago. C.K.T., M.D.L. and T.G.H. contributed to the conception and design of the experiments. All authors contributed to the collection, analysis and interpretation of the data. All authors approved the final manuscript.

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