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Awake behaving electrophysiological correlates of forelimb hyperreflexia, weakness and disrupted muscular synchronization following cervical spinal cord injury in the rat

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Abstract

Spinal cord injury usually occurs at the level of the cervical spine and results in profound impairment of forelimb function. In this study, we recorded awake behaving intramuscular electromyography (EMG) from the biceps and triceps muscles of the impaired forelimb during volitional and reflexive forelimb movements before and after unilateral cervical spinal cord injury (cSCI) in rats. C5/C6 hemicontusion reduced volitional forelimb strength by more than 50% despite weekly rehabilitation for one month post-injury. Triceps EMG during volitional strength assessment was reduced by more than 60% following injury, indicating reduced descending drive. Biceps EMG during reflexive withdrawal from a thermal stimulus was increased by 500% following injury, indicating flexor withdrawal hyperreflexia. The reduction in volitional forelimb strength was significantly correlated with volitional and reflexive biceps EMG activity. Our results support the hypothesis that biceps hyperreflexia and descending volitional drive both significantly contribute to forelimb strength deficits after cSCI and provide new insight into dynamic muscular dysfunction after cSCI. The use of multiple automated quantitative measures of forelimb dysfunction in the rodent cSCI model will likely aid the search for effective regenerative, pharmacological, and neuroprosthetic treatments for spinal cord injury.

Keywords

Spinal cord injury; Electromyography; Hyperreflexia; Spasticity; Strength; Forelimb

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Author disclosure

The authors declare that no competing financial interests exist.

1. Introduction

Spinal cord injury (SCI) currently affects 276,000 individuals in the U.S [1]. Cervical spinal cord injury (cSCI) accounts for 55% of all SCIs, which are commonly incomplete injuries resulting from a contusive insult [2]. SCI can chronically affect multiple functions that impair quality of life, including reduced arm and hand function, hyperreflexia and increased muscle activation variability [3–10]. The anatomical and molecular changes associated with these post-injury states are well studied [11,12]. However, the dynamic electrophysiological changes that contribute to dysfunction after SCI are not well understood.

Forelimb function depends upon the transfer of sensorimotor information via multiple circuits to and from the central nervous system [13–16]. Forelimb strength involves muscular coordination across multiple spinal segments and is commonly impaired following cSCI [17]. Prior to injury, force generation is associated with synchronized activation within and across muscles [18–20]. Muscular synchronization is thought to be anatomically mediated by branched descending inputs and segmental interneuronal networks in the spinal cord [21,22]. Disruption of these networks may arise from spinal insult and affect forelimb sensorimotor function.

SCI can promote maladaptive sensorimotor circuit formation and hyperreflexia through aberrant spinal plasticity [23–29]. Hyperreflexia and spasticity can also interfere with function [10,30]. Several animal studies have used awake behaving electrophysiological recordings associated with forelimb function [31–34]. Electrophysiological measurements of hyperreflexia after SCI are usually performed in the anesthetized or restrained state [35,36]. Awake behaving electrophysiological recordings of muscle function across multiple assessments are needed to determine whether these hyperreflexive states contribute to diminished sensorimotor function or are an independent source of sensorimotor impairment.

In the current study, we assessed muscular dysfunction following unilateral cSCI using awake behaving electromyography (EMG) simultaneously recorded from the biceps and triceps of the impaired forelimb. Forelimb EMG was recorded before and after injury during a volitional forelimb strength task [37] and during reflexive forelimb withdrawal from a thermal stimulus [38].

2. Materials and methods

2.1. Subjects and experimental design

All procedures performed in the study were approved by the University of Texas at Dallas Institutional Animal Care and Use Committee. Adult female Sprague Dawley rats (N = 8) used in this study were housed one per cage (12 h light/dark cycle). All assessments were performed during the dark period of the cycle. Rats received chronically implanted EMG electrodes into the long head of the biceps brachii and the long head of the triceps brachii of the trained forelimb to assess muscular dynamics during the isometric pull task and forelimb withdrawal assessment. Before injury, rats were food deprived Monday–Friday (ad libitum access to water) and trained to proficiency on the isometric pull task using only the right forelimb. Pre-lesion baseline limb withdrawal metrics were recorded during the final stage

of isometric pull training. Once proficient at the isometric pull task, rats were subjected to a right side cervical spinal contusion at spinal level C5/C6 to impair the trained forelimb. After a 7 day recovery period, forelimb function and EMG was assessed each week for 4 weeks during the isometric pull task and forelimb withdrawal assessment.

2.2. Chronic electromyography (EMG) implant surgery

Prior to training on the isometric pull task, rats received chronically implanted intramuscular electrodes to monitor forelimb EMG. Rats were first deeply anesthetized using an injection (i.p.) of ketamine hydrochloride (50 mg/kg), xylazine (20 mg/kg) and acepromazine (5 mg/kg). Rats were then placed in a stereotaxic frame and the cranium was exposed and cleaned. Bones screws were inserted into the cranium and were covered in acrylic (4 for anchoring and 1 for EMG ground; ground screw location = 2 mm lateral & 2 mm caudal to Bregma). A 10 channel female strip header (Adafruit Industries, P/N 1544) was then secured with additional acrylic atop the bone screws for interfacing with individual EMG leads. Two muscles of the right (i.e. trained) forelimb were implanted in each animal: the long head of the biceps brachii (referred to herein as biceps; elbow flexion, supination and shoulder flexion) and the long head of the triceps brachii (referred to herein as triceps; elbow extension). Each muscle was implanted with a twisted pair of wires with each wire containing one recording site (1 mm uninsulated surface), similar to previous studies (stainless steel; 7 stranded; Teflon coated; AM-Systems Inc.; Sequim, WA) [39]. Only one recording site was recorded from and the other served as a backup. A small incision was made over the right humerus, and a single twisted pair of EMG leads (one for each muscle) were subcutaneously tunneled and intramuscularly implanted into the belly of each respective muscle. EMG leads were knotted at the insertion and exit sites of the implant site to secure the leads within the muscle. EMG leads were finally inserted into the connector on the cranium and covered in additional acrylic. The incised skin over the forelimb and cranium was closed with suture and cleaned with isopropyl alcohol and iodine. Rats were given an injection (s.c.) of Baytril (10 mg/kg) and Ringer's solution (5 mL), placed on a heating pad and monitored until sternally recumbent. EMG rats were given at least 7 days to recover from surgery. Brief stimulation was performed under anesthesia through the recording electrode pair producing movement about the elbow during the cSCI surgery (below) and after the study to confirm the location of the EMG implants (1–1.6 mA; 50 Hz; 0.2 s duration; 0.2 micro pulse width).

2.3. Isometric pull task training and post-Injury assessment

All rats in the study were trained to proficiency on the isometric pull task prior to cSCI. The isometric pull task is an automated and quantitative means to measure multiple parameters of volitional forelimb force generation [37]. Identical parameters of the behavioral chamber and pull handle force transducer were used in this study (MotoTrak Device, Vulintus, Inc.; Dallas, TX). Custom software was used to display and record experimental data during the performance of the task. A microcontroller board (Vulintus, Inc.) sampled the force transducer every 10 ms and relayed information to custom MATLAB software for offline analysis.

Initial training sessions consisted of two 30 min sessions (separated by at least 2 h) five days per week, similar to previous studies [37,40]. Rats were initially shaped by the experimenters to promote association of the pull handle with a food reward (45 mg chocolate-flavored pellets, Bio-Serv; Flemington, NJ). The pull handle was initially fixed at 0.75 inches outside of the behavioral chamber to encourage reaching. A trial was initiated when the rats exerted at least 10 g of force on the pull handle. A trial window of 2 s started after trial initiation where the animal could receive a reward by pulling with a force exceeding a given “pull success” threshold (Fig. 1A). After initial shaping and successful association, an adaptive pull success threshold was used to encourage pulling of the handle. This moving pull success threshold was initialized after 10 trials and was defined as the median peak force of the previous 10 pull trials. Over successive training sessions (accompanied by increasing force generation), rats were finally introduced to training sessions using a static force threshold of 120 g. Once on this stage (PRE-SCI), rats achieved isometric pull task proficiency when able to successfully pull 120 g of force on 85% of trials for 10 consecutive training sessions, similar to previous studies [37,40]. During PRE-SCI, rats rapidly progressed to proficiency on the task after 21.1 ± 2 sessions. After reaching isometric pull task proficiency, rats were given a cervical SCI. Each post-SCI assessment time point on the isometric pull task started approximately 7 (Wk1), 14 (Wk2), 21 (Wk3) and 28 (Wk4) days post-SCI. Each post-SCI assessment time point consisted of four 30 min sessions across 2 consecutive days to assess forelimb strength (2 thirty minute sessions per day). Day 1 consisted of two sessions with an adaptive force threshold (minimum force threshold: 10 g; maximum force threshold: 120 g). Day 2 consisted of one session with a static force threshold (fixed 120 g) and one session with an adaptive force threshold. On a given assessment day, the two 30 min sessions were separated by at least two hours. We calculate and report the Success Rate (proportion of trials >120 g), Peak Force (maximum force generated in a trial) and Pull Speed (grams/10 ms in a trial of all rising phases of isometric force profiles) across rats for all assessment time points.

2.4. Forelimb withdrawal assessment

All rats were habituated to the testing chamber (acrylic chamber; L: 10 in., W: 4.75 in., H: 12 in.; atop a glass floor) before baseline assessment (minimum of 4 h). Baseline forelimb withdrawal metrics were measured before cSCI during PRE-SCI testing, and once per week following cSCI. By 7 days post-SCI, all rats exhibited plantar placement with weight support on all four paws, similar to previous studies [41]. Signs of thermal hyperalgesia were measured for all four paws using the Ugo Basile Plantar Heat test (Comerio VA, Italy) [38]. After at least 1 h of acclimation to the testing chamber, an infrared heat stimulus was applied to the glabrous surface of a given paw and the Limb Withdrawal Latency was recorded. A total of 5 trials per limb were recorded during a given assessment with at least a 30 s delay between trials (an additional 5 trials for the right forelimb, see below). Assessment was always performed at least 2 h after the final 30 min isometric pull task session for that day (if applicable).

2.5. Cervical spinal cord injury (cSCI) surgery

After achieving isometric pull task proficiency, rats received a right side (i.e. side of trained limb) C5/C6 spinal cord contusion. Rats were anesthetized with ketamine (50 mg/kg),

xylazine (20 mg/kg), and acepromazine (5 mg/kg). Similar to previous studies [41], after a right side dorsal C5 laminectomy the vertebral column was stabilized using spinal microforceps rostral and caudal to the C5 vertebral body. An impactor probe (1.25 mm diameter) was then lowered to within 2 mm of the middle of the right hemicord at C5/C6 using the Infinite Horizon Impact Device (Precision Systems and Instrumentation; Lexington, KY). The spinal cord was then rapidly contused with a force of 200 kilodynes (no dwell time), resulting in tissue displacement to a depth of 1600–1800 μm . The skin overlying the exposed vertebrae was then closed in layers and the incised skin closed using surgical staples. All rats received Buprinex (s.c., 0.03 mg/kg, 1 day post-op), Baytril (s.c., 10 mg/kg, daily for 3 days) and Ringer's solution (s.c., 5 mL) following surgery. All rats were given 7 days to recover after SCI surgery before any post-SCI assessment(s).

2.6. EMG data acquisition

2.6.1. Common method—Rats received chronically implanted EMG electrodes into the biceps and triceps of the trained forelimb to assess muscular dynamics during the isometric pull task and forelimb withdrawal testing. The animal was tethered via its headcap into a rotating commutator located at the top of the chamber during testing in each respective chamber. All rats freely navigated about the chamber and rarely interacted with the tether. EMG signal from a given muscle was passed to a Medusa pre-amplifier (sampling frequency: 6 kHz; Tucker Davis Technologies; Alachua, FL) and then to a RZ5 signal processor for filtering and signal conditioning. The signal from each muscle was band-pass filtered (2nd order Butterworth filter; 40–1000 Hz) and notch (2nd order Butterworth filter; Center Frequency: 60 Hz) filtered online (RPvdsEX; Tucker Davis Technologies).

2.6.2. Isometric pull task—Again, a trial was initiated during the isometric pull task when the rats exerted at least 10 g of pull force on the handle. A TTL pulse sent from the task microcontroller interface synchronized force and EMG signal recordings at the approximate time of each pull trial initiation. Custom MATLAB software was used to record fore-limb EMG activity for offline analysis. Biceps EMG, triceps EMG and the isometric force profile were recorded simultaneously from 1 s before to 4 s after a pull trial initiation.

2.6.3. Forelimb withdrawal assessment—The testing chamber described above was placed on the glass platform during testing. Again, after 1 h of acclimation to the testing chamber an infrared heat stimulus was applied to the glabrous surface of a given paw and the Limb Withdrawal Latency was recorded. The event signal generated during a limb withdrawal event was acquired from the Plantar Heat test apparatus for all trials and transferred to a custom Arduino interface (Arduino, LLC; Torino, Italy). Custom MATLAB software was used to record the limb withdrawal event signal simultaneously with forelimb EMG activity for offline analysis. This was done for 10 non-consecutive trials (for the right forelimb) and 5 non-consecutive trials (each for the remaining right hindlimb and left forelimb and hindlimb), with at least a 30 s delay between trials.

2.7. EMG data analysis

2.7.1. Common method—We used signal processing approaches similar to previous studies on EMG and force [4,17,22]. Biceps and triceps EMG recordings were full wave rectified offline using custom MATLAB algorithms (Mathworks; Natick, MA). The linear envelope of the full wave rectified EMG was generated using a low-pass filter (5th order Butterworth filter; 50 Hz). We chose to perform Peri-Event Time Histograms (PETH) and Cross-Correlation (CC) based analyses for forelimb EMG around events during the isometric pull task (event = pull trial initiation) and forelimb withdrawal testing (event = right forepaw withdrawal). Right forelimb EMG was therefore analyzed during right forelimb events only.

2.7.2. Peri-Event time histogram analysis—EMG Peri-Event Time Histograms (PETH's) were generated as follows for both the isometric pull task and forelimb withdrawal testing. The linear envelope of the EMG was first de-meant (mean of a single session's EMG matrix; rows = pull trials, columns = time) and binned (5 ms bins). For a given assessment and muscle, a grand PETH was calculated as the mean of each bin for all trials (isometric pull EMG PETH = total of 600 bins, from -1 to +2 s around a pull trial initiation; limb withdrawal EMG PETH = total of 400 bins, from -1 to +1 s around a limb withdrawal). PETH bins were considered significant if exceeding a 99% confidence interval, which was calculated from the mean of the whole session's de-meant EMG matrix. For single testing sessions across rats and assessment time points we measured: Response Magnitude (total EMG activity over 99% confidence interval; units = μ Volts) and First Bin Latency (time of first significant bin; units = s) (Fig. 1B, exemplar EMG PETH). All EMG Response Magnitudes were normalized within rats and reported as Percent of PRE-SCI (i.e. baseline).

2.7.3. Mean similarity analysis—To assess trial to trial motor output variability, we calculated the Mean Similarity Index for volitional biceps EMG, volitional triceps EMG and isometric pull force profiles similar to previous studies [34]. For a given recording during the isometric pull task or forelimb withdrawal testing, the force profile or EMG matrix was row normalized so that a group of trials did not dominate the overall matrix (rows = trials; columns = time). The median vector of the session's recording was then calculated. On a trial by trial basis, the Pearson's correlation coefficient was calculated between a single trial (row vector) and the median vector for the overall session. This resulted in the mean similarity index for the given recording, which ranges from 0 to 1.

2.7.4. Cross-correlation analysis—To assess synchrony between biceps and triceps, EMG Cross-Correlograms (CC's) were generated as follows for the isometric pull task. We evaluated potential cross-talk between the simultaneously recorded biceps and triceps EMG prior to CC analysis using approaches similar to Kilner et al. [80]. Biceps and triceps EMG recordings were first processed using “blind signal separation” described in previous studies [80,81]. This approach has been used in EMG studies assessing EMG synchrony [22,80]. Our preliminary EMG assessment suggests a $14.9 \pm 6.5\%$ contribution of potential cross-talk to EMG CC strength (see below). Therefore, cross-talk contribution was minimal.

The linear envelope of the EMG was de-meaned (mean of the whole session's EMG matrix; rows = pull trials, columns = time) and binned (5 ms bins). For single trials, EMG activity was normalized to its respective peak amplitude, yielding scaled values from 0 to 1. We did this to ensure that a single muscle did not dominate the single trial vector, similar to previous studies (e.g. EMG amplitude can be affected by electrode placement in the muscle, signal-to-noise ratio and muscle size) [34]. CC analysis therefore assessed synchrony based on the pattern of muscular activation irrespective of amplitude. EMG CC analysis was performed for single pull trials (from -1 to +2 s around a pull trial initiation). CC histograms were generated using the cross-correlation of the biceps and triceps discrete-time vectors. CC histograms were normalized using the biased correction (where x = biceps vector; y = triceps vector; m = given time lag; N = EMG vector length):

$$\hat{R}_{xy,bias}(m) = \frac{1}{N} \hat{R}_{xy}(m)$$

For single testing sessions across rats and assessment time points we measured: CC strength (the maximum of the CC histogram) and CC Time Lag (time index of maximal CC strength; units = milliseconds). CC strength was considered significant if exceeding a 99% confidence interval, which was calculated from the mean of the CC histogram. All EMG CC Strengths were normalized within rats and reported as Percent of PRE-SCI (i.e. baseline).

2.7.5. cSCI lesion quantification—Rats were sacrificed using an injection (i.p.) of sodium pentobarbital (Beuthenasia-D; 1 mL) and were transcardially perfused with buffered 0.1 M PBS (60 mL; pH 7.5) followed by buffered fixative (4% PFA in 0.1 M PBS; 120 mL; pH 7.5). The spinal tissue was then removed and select spinal roots were kept intact for lesion localization reference. Tissue was post-fixed overnight in the same buffered fixative and cryo-protected (30% sucrose in 0.1 M PBS) until the tissue sank. The tissue was then embedded and frozen in Shandon M1 embedding matrix (Thermo Fisher Scientific; Waltham, MA). Transverse sections were sliced at 50 μ m thickness using a cryostat and slide mounted. Slides were then stained for Nissl substance and Myelin to allow for lesion quantification, similar to previous studies [41,42]. Bright field photomicrographs were taken of Nissl and Myelin stained tissue above, through, and below the level of the spinal hemiconfusion. cSCI lesion metrics were quantified using Image J software. The lesion extent was expressed as the proportion of spared tissue of the right (i.e. injured) hemicord with respect to the left (i.e. uninjured) hemicord at 600 μ m increments rostral and caudal to the hemiconfusion epicenter.

2.7.6. Statistical analysis—All data are reported in text and figures as the mean $\pm$ standard error of the mean (SEM). Statistical normality was assessed for all tests prior to analysis (SPSS; IBM). Tests of Sphericity were performed prior to Repeated Measures analyses. If Sphericity was violated, the Greenhouse-Geiser corrected omnibus statistic was used to assess significance. An alpha of 0.05 was considered significant for omnibus measures.

Effect of cSCI on isometric pull task variables, EMG PETH, CC and Mean Similarity metrics was assessed using one-way repeated measures ANOVAs. The independent variable

was Time with 5 levels (PRE-SCI, Wk1–Wk4). Differences across Time were assessed using Simple Contrasts with PRE-SCI serving as the reference. All differences across Time were Bonferroni corrected for multiple comparisons ($\alpha = 0.05/\text{number of comparisons}$) if needed. Regressions were performed using Pearson's correlation analysis (SPSS; IBM), with post-cSCI single animal means serving as the independent sample.

3. Results

3.1. Cervical SCI lesion quantification

Spinal cord tissue was assessed for lesion size and location after C5/C6 hemicontusion, similar to previous studies [41]. Animals exhibited a lateralized spinal cord lesion spanning a rostrocaudal length of 3.6 ± 0.4 mm. At the cSCI epicenter, an average of $25.8 \pm 1.6\%$ of the right hemicord tissue was spared (Fig. 1C and D). The spinal lesion led to consistent gray and white matter damage. Therefore, cervical spinal hemicontusion resulted in a repeatable lesion to the spinal tissue across animals.

3.2. Cervical SCI promotes forelimb withdrawal hyperreflexia

SCI at lower spinal levels can promote hyperreflexia of the lower limbs [7,43], but it is not well known whether cervical SCI engenders upper limb hyperreflexia. To test this, we recorded awake behaving biceps and triceps EMG from the forelimb during reflexive withdrawal from a thermal stimulus before and after cSCI (Fig. 2A and B). PETH-based analysis indicates that the response magnitude for biceps and triceps showed significant increases after cSCI (Fig. 2C; biceps: $F(4,4) = 4.6$, $p = 0.005$; post-hoc vs. PRE-SCI: Wk1 ($p = 0.048$), Wk2 ($p = 0.029$), Wk3 ($p = 0.008$) and Wk4 ($p = 0.046$); triceps: $F(4,4) = 3.5$, $p = 0.018$; post-hoc vs. PRESCI: Wk1 ($p = 0.046$), Wk3 ($p = 0.009$) and Wk4 ($p = 0.009$)). This increased activation of biceps and triceps during forelimb withdrawal is indicative of withdrawal hyperreflexia after cSCI, similar to lower limb hyperreflexia observed after lower level SCI in previous studies [7,43]. There were no significant changes in limb withdrawal latency for the ipsilesional or contralesional limbs after cSCI, indicating an absence of thermal hyperalgesia (Fig. 3A; Right Forelimb: No Significant Difference (NSD), $F(4,4) = 0.9$, $p = 0.447$; Right Hindlimb: NSD, $F(4,4) = 0.5$, $p = 0.603$; Fig. 3B; Left Forelimb: NSD, $F(4,4) = 2.8$, $p = 0.059$; Left Hindlimb: NSD, $F(4,4) = 0.6$, $p = 0.657$). There were also no significant changes in first bin latency for biceps or triceps at any post-lesion time point, in spite of significant increases in the magnitude of muscle activation (Fig. 3C; biceps: NSD, $F(4,4) = 0.2$, $p = 0.935$); triceps: NSD, $F(4,4) = 0.5$, $p = 0.682$).

3.3. Cervical SCI chronically reduces volitional forelimb strength

As expected, volitional forelimb strength was significantly reduced following cSCI (Figs. 4 A and 5 A). Peak force, success rate, and pull speed were significantly reduced compared to PRESCI at all post-injury time points (Fig. 4B; Peak force: $F(4,9) = 191.1$, $p < 0.001$; Fig. 5B; Success rate: $F(4,9) = 772.5$, $p < 0.001$; Fig. 5C; Pull speed: $F(4,9) = 76.8$, $p < 0.001$). A modest recovery of peak force and pull speed was observed in the weeks following injury (Bonferroni corrected for multiple comparisons; Peak force and pull speed: Wk2–Wk4 vs. Wk1, $p < 0.01$). Similar to previous studies following forebrain lesions, there was a transient decrease in trials per session following cSCI at Wk1, which returned to pre-lesion levels for

the remainder of the assessment time points (Fig. 5D; $F(4,9) = 4.1$, $p = 0.009$; Wk1 vs. PRE-SCI: $p = 0.006$) [37,40]. These data indicate that cSCI produces a chronic reduction of volitional forelimb strength, which is a common impairment seen after human cSCI [4].

We next performed correlation analyses to assess the interaction between forelimb hyperreflexia and volitional forelimb strength. There was no significant correlation between week to week changes in hyperreflexia EMG response magnitude and week to week changes in post-cSCI isometric force generation (NSD, biceps: $R = 0.34$, $p = 0.019$; NSD, triceps: $R = 0.24$, $p = 0.197$). Biceps, but not triceps, hyperreflexia EMG response magnitude was significantly negatively correlated with post-cSCI isometric force generation (Fig. 4C; biceps: $R = -0.69$, $p = 0.028$; triceps: $R = -0.36$, $p = 0.189$). This indicates that flexor hyperreflexia significantly contributes to reduced strength after cSCI.

3.4. Cervical SCI reduces volitional triceps EMG

We recorded awake behaving biceps and triceps EMG from the forelimb during the isometric pull task to assess the contribution of volitional forelimb EMG to volitional forelimb strength before and after cSCI. Before injury, biceps and triceps EMG profiles showed repeatable activations (Fig. 6A and B). The EMG for both muscles showed greater activation during a pull success, reaching a maximum around the time of the pull initiation (Fig. 6C). For single pulls, the average biceps and triceps EMG response magnitude was significantly positively correlated with the area under the curve of isometric force profiles during PRE-SCI, similar to previous studies on the relationship between EMG activity and isometric force generation ($R = 0.55$, $p = 0.018$, $N = 13,936$ pulls) [17]. Therefore, at the time of isometric pull task proficiency, isometric EMG profiles were consistent and repeatable. Biceps and triceps EMG generally preceded isometric force generation, with higher isometric forces relating to higher levels EMG activation.

cSCI disrupted volitional muscle activation for triceps, but not biceps during isometric pulls. The response magnitude for triceps, but not biceps, showed a significant decrease at all post-cSCI assessment time points compared to PRE-SCI (Fig. 7A; triceps: $F(4,4) = 27.3$, $p < 0.001$; Wk1–Wk4 all $p < 0.001$ vs. PRE-SCI; biceps: NSD, $F(4,4) = 1.1$, $p = 0.358$). This differential change in EMG activation during volitional forelimb movement may be due to the location of alpha motor neurons innervating each muscle in reference to the location of the cSCI, with the biceps motor neurons above and triceps motor neurons below the level of the lesion [44]. There was no significant effect of cSCI on first bin latency for triceps or biceps, suggesting that cSCI disrupts muscle activation magnitude but not activation timing (Fig. 6D; triceps: NSD, $F(4,4) = 2.4$, $p = 0.072$; biceps: NSD, $F(4,4) = 0.7$, $p = 0.561$).

3.5. Volitional biceps EMG is significantly correlated with reduced forelimb strength

We next assessed the contribution of volitional forelimb EMG to volitional forelimb strength. There was a significant negative correlation between week to week changes in biceps, but not triceps, EMG response magnitude during the isometric pull task and week to week changes in post-cSCI isometric force generation (biceps: $R = 0.42$, $p = 0.019$; NSD, triceps: $R = 0.18$, $p = 0.197$). The response magnitude of biceps EMG during the isometric pull task was significantly positively correlated with post-cSCI isometric force generation

(Fig. 7B; $R = 0.73$, $p = 0.018$). Alternatively, triceps EMG response magnitude was not significantly correlated with post-cSCI isometric force generation ($R = -0.11$, $p = 0.786$). Therefore, our results support the hypothesis that biceps muscle activation plays a significant role in residual volitional forelimb strength following cSCI. Biceps, but not triceps, EMG activation during the isometric pull task was significantly negatively correlated with its activation during withdrawal hyperreflexia assessment (biceps: $R = -0.7$, $p = 0.026$; triceps: $R = -0.41$, $p = 0.154$). This result supports the hypothesis that there is a relationship between the magnitude of hyperreflexia and descending drive, which has been proposed in previous SCI studies [45].

3.6. Cervical SCI increases volitional EMG and isometric force profile variability

Motor output variability is a common effect of SCI, and can contribute to impairment of volitional motor function [10]. We calculated the mean similarity index to assess motor output variability for volitional forelimb EMG and isometric force profiles (see 2.7.3). There was a significant decrease in triceps and biceps EMG mean similarity index at all post-lesion assessment time points compared to PRE-SCI indicating increased muscle activation variability (Fig. 8A; triceps: $F(4,4) = 6.2$, $p = 0.015$; post-hoc vs. PRE-SCI: Wk1 ($p = 0.004$), Wk2 ($p = 0.020$), Wk3 ($p = 0.0019$), Wk4 ($p = 0.011$); biceps: $F(4,4) = 7.6$, $p = 0.004$; post-hoc vs. PRE-SCI: Wk1 ($p = 0.020$), Wk2 ($p = 0.007$), Wk3 ($p = 0.005$), Wk4 ($p = 0.003$)). Lastly, there was a significant decrease in the mean similarity index of pull force profiles at all post-lesion assessment time points compared to PRE-SCI indicating increased force output variability (Fig. 8B; $F(4,4) = 29.1$, $p < 0.001$; post-hoc vs. PRE-SCI: Wk1 ($p < 0.001$), Wk2 ($p < 0.001$), Wk3 ($p = 0.012$), Wk4 ($p < 0.001$)). We next assessed the role of motor output variability to residual forelimb strength. There was no significant correlation between week to week changes in the mean similarity index of EMG or pull force profiles and week to week changes in post-cSCI isometric force generation (NSD, biceps: $R = 0.05$, $p = 0.395$; NSD, triceps: $R = 0.34$, $p = 0.051$; NSD; pull force profile: $R = 0.31$, $p = 0.066$). The mean similarity index of pull force profiles, but not biceps or triceps EMG, was significantly correlated with post-cSCI isometric force generation (Fig. 8C; biceps: $R = 0.25$, $p = 0.274$; triceps: $R = 0.04$; $p = 0.460$; Fig. 8D; pull force profile: $R = 0.75$, $p = 0.014$). This result indicates that increased endpoint force variability, and not muscle activation variability, significantly contributes to reduced volitional strength.

3.7. Cervical SCI chronically reduces the magnitude, but not the timing, of forelimb EMG synchrony

Muscular synchrony can facilitate joint stability and force output before injury [18,20], and can be interrupted following injury. We used cross-correlation based analyses to assess the contribution of biceps and triceps EMG synchrony to forelimb strength before and after injury (Fig. 9A). During PRE-SCI, biceps and triceps were generally synchronously active during an isometric pull (Fig. 6A–C). The magnitude of this synchrony was significantly higher for pull successes compared to failures (Fig. 9B; $p < 0.05$). cSCI significantly reduced the maximal biceps and triceps EMG synchrony at all post-lesion time points compared to PRE-SCI (Fig. 10A; $F(4,4) = 11.4$, $p = 0.004$; post-hoc vs. PRE-SCI: Wk1 ($p = 0.046$), Wk2 ($p < 0.001$), Wk3 ($p < 0.001$), Wk4 ($p < 0.001$)). cSCI did not significantly

affect the time lag of maximal EMG synchrony, indicating that cSCI disrupts EMG synchrony magnitude but not synchrony timing (Fig. 10B; NSD, $F(4,4) = 0.05$, $p = 0.906$).

We next assessed the contribution of intermuscular forelimb synchrony to residual forelimb strength following cSCI. There was no significant correlation between week to week changes in biceps and triceps EMG synchrony and week to week changes in post-cSCI isometric force generation (NSD, $R = 0.06$, $p = 0.387$). The magnitude of biceps and triceps EMG synchrony was not significantly correlated with post-cSCI isometric force generation (Fig. 10C; $R = -0.26$, $p = 0.261$). Therefore, cSCI chronically reduced strength related intermuscular synchrony, but this reduction is unlikely to significantly contribute to residual forelimb strength following cSCI.

4. Discussion

In the present study, we show that cSCI chronically reduced volitional forelimb strength. Reflexive and volitional EMG activation of the biceps, but not triceps, was significantly correlated with residual forelimb strength after cSCI. Reduced EMG synchrony and increased EMG activation variability was not significantly correlated with residual forelimb strength. cSCI did not have a significant impact on EMG latency metrics throughout the study, which suggests that cSCI significantly impacts muscle activation magnitude but not activation timing. Our results support the hypothesis that biceps hyperreflexia and descending volitional drive both significantly contribute to forelimb strength deficits after cSCI. These findings represent new insight into volitional and reflexive muscular dysfunction after cSCI in the awake behaving animal using automated quantitative assessments.

4.1. The anatomical pathways associated with forelimb dysfunction following cervical SCI

Our C5/C6 cSCI would be expected to disrupt volitional triceps, but not biceps, EMG activation due to their respective alpha motor neuron pool locations below and above the level of the lesion (Fig. 11A) [44]. Several descending motor tracts have been shown to project to the biceps and triceps motor neuron pools [46–49], and lesion studies suggest their significant involvement in fore-limb function [14,16,50–53]. Although volitional biceps activation was preserved following injury, reactive sprouting of spinal afferents and aberrant circuit rewiring can result in both hyperreflexia and volitional motor output variability due to the close proximity of biceps sensorimotor circuitry to the lesion [7,43,54–56]. Additionally, biceps hyperreflexia can be mediated by reduced pre-synaptic inhibition and increased motor-neuronal excitability [10,29,57–60]. These potential mechanisms are not mutually exclusive, and all may contribute to the changes in volitional and reflexive biceps EMG dynamics.

Triceps EMG dynamics showed opposite effects compared to biceps during volitional activations (Fig. 11A). Interrupting descending motor pathways can explain the reduced volitional triceps EMG activation seen in this study, similar to previous studies after human cervical SCI [4]. Although sprouting of spinal afferents and sensory response increases have been seen below a SCI [24,61], acute spinal shock due to deafferentation may also contribute the decrease in volitional triceps motor output [8,62]. Additionally, interruption of the

descending raphe- and coeruleo-spinal projections may also contribute to decreases in the intrinsic excitability of premotor- and motor-neuronal cell groups [63–66]. In spite of reduced volitional EMG activation, triceps EMG increased during reflexive forelimb withdrawal indicating hyperreflexia. Previous work using anesthetized or restrained preparations have shown electrophysiological signatures of hyperreflexia in muscle groups below a SCI in-vivo [35,36]. Additional studies using spinal invitro preparations confirm that the observed hyperreflexia in-vivo is significantly contributed to by increased plateau potentials and monoamine supersensitivity [58,59]. These pre- and post-synaptic circuit modifications may all contribute to the changes in volitional and reflexive triceps EMG dynamics.

4.2. The contribution of forelimb hyperreflexia to forelimb weakness following cervical SCI

Biceps, but not triceps, hyperreflexia during withdrawal from a thermal stimulus was significantly correlated with post-injury forelimb strength, indicating that flexor withdrawal hyperreflexia likely contributes to volitional sensorimotor dysfunction. Our results are in agreement with previous studies on flexor withdrawal hyperreflexia in the lower extremities using noxious stimulation before and after injury in humans [67,68]. Importantly, the radiant heat source used during forelimb withdrawal assessment does activate both thermal and cutaneous afferents [69]. Primary afferents carrying thermo-cutaneous information are synaptically connected to premotor interneurons involved in mediating volitional motor activation [70,71]. Therefore, these reflexive and volitional circuits overlap and may interact. As such, this stimulus is not fully independent of ones encountered during the isometric pull task and can activate similar circuits across the reflexive and volitional forelimb assessments.

Previous studies suggest that hyperreflexia could interfere with volitional muscular control due to the interaction between proprioception, pre-motor regulation, and motor output [10,54]. In the lower extremities following incomplete SCI, hyperreflexia increases while volitional strength decreases over weeks following injury, suggesting that hyperreflexia may interfere with recovery of strength [10]. Following Brown-Sequard SCI, lack of afferent input to local spinal circuits prevented hyperreflexia and allowed significant residual volitional strength [72]. Our results support the hypothesis that volitional motor output is significantly affected by hyperreflexive states following cSCI. Future studies may aim to assess the role of hyperreflexia in recovery of volitional forelimb function following cSCI.

4.3. Cervical SCI chronically reduced biceps and triceps synchronization associated with isometric force generation

Before cSCI, we observe that the magnitude of biceps and triceps synchronization is associated with higher levels of isometric force generation. This is consistent with previous studies on muscular synchrony and volitional strength [18,19,73,74]. This synchronous activation of biceps and triceps likely acts to stabilize the elbow during the production of isometric force, thus allowing controlled application of force [31,32]. It should be noted that the biomechanical restraints of the isometric pull task play a central role in our findings on muscular dynamics before and after cSCI. Previous reports have shown that flexors and extensors can have dynamic activation patterns outside of classical flexion and extension

[20]. Other tasks which permit a greater degree of forelimb movement during force application may reveal different effects on EMG dynamics.

Following cSCI, the chronic reduction of biceps and triceps synchrony is likely due to the disruption of multiple pathways mediating volitional drive and synchronization of the forelimb. Several studies have suggested that the degree of multi-segmental motor unit synchronization is proportional to the amount of shared axonal inputs from supraspinal centers [21]. EPSP amplitude, motor unit inter-spike-interval variability, motor unit spike rates, and the excitability of the motor neurons also play considerable roles in motor synchronization [75]. Future studies may aim to assess the role of EMG synchronization associated with recovery of strength following injury and disambiguate the mechanisms that underlie aberrant synchrony after injury.

4.4. Future directions using partially or fully automated behavioral assessments after CNS injury

The isometric pull task has been shown to be a sensitive measure of impaired volitional forelimb strength in previous studies on ischemic stroke and traumatic brain injury [37,40,76]. It is a quantitative and automated task that allows for many trials of forelimb behavior and therefore statistical power, without necessitating manual video analysis or live scoring of behavior. Previous studies have provided valuable preclinical data regarding the effect of cSCI alone or with therapeutic interventions on various forelimb sensorimotor behaviors [16,52,53,77,78]. We extend these findings on forelimb dysfunction following cSCI to volitional forelimb strength, which has received little attention and is a common deficit seen in humans [3–6,17]. In addition, we utilized the defined events within the isometric pull task and reflex assessment to trigger EMG recordings from multiple muscles. These tools allowed for unbiased, quantitative forelimb measurements in the awake behaving rats. Accurate, thorough assessment of forelimb function is critical for the development and evaluation of therapies to restore fore-limb function, as recovery of upper limb use is a top priority for subjects with a cSCI [79]. These tools may be used in future studies to trigger recordings of other measures (e.g. cortical recordings or kinematics) after SCI or other preclinical models of CNS insult.

5. Conclusions

In the present study, we assess the muscular dynamics associated with chronically reduced volitional forelimb strength following cSCI. Our results support the hypothesis that biceps hyperreflexia and descending volitional drive both significantly contribute to forelimb strength deficits after cSCI. We also highlight the contribution of altered forelimb muscular synchronization and motor output variability to chronically reduced volitional fore-limb strength. These long term electrophysiological assessments were performed in the awake behaving animal using automated quantitative measurements. Future studies may utilize the electrophysiological state of the injured system to identify spared sensorimotor function to inform the design of therapeutic interventions to promote recovery.

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Abbreviations

EMG	electromyography
cSCI	cervical spinal cord injury
SCI	spinal cord injury
PETH	peri-event time histograms
CC	cross correlation

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HIGHLIGHTS

- Cervical spinal cord injury chronically reduced volitional forelimb strength up to one month post-injury.
- Forelimb electromyography indicates a significant contribution of flexor hyperreflexia and volitional drive to chronic forelimb weakness.
- Cervical spinal cord injury also promoted increases in forelimb motor output variability and decreased muscular synchronization.
- Electrophysiological and behavioral assessments are performed in the awake behaving animal using automated and quantitative measures.

Fig. 1.

Forelimb assessments and cSCI lesion quantification. (A) Example of isometric force profiles at PRE-SCI during a single trial (4 separate pulls marked with red numbers). Horizontal green line indicates the pull trial initiation threshold. Horizontal red line indicates the PRE-SCI pull success threshold. (B) Exemplar EMG Peri-Event Time Histogram (PETH) recorded during forelimb withdrawal assessment at PRE-SCI with metric notations (FBL = First Bin Latency; Black Shaded PETH = Response Magnitude; dashed line = 99% confidence interval). (C) Transverse tissue section through cervical hemicontusion epicenter (grey scale Nissl and Myelin stain; 20x magnification; scale bar = 2 mm). (D) Rostro-caudal extent of unilateral cervical hemicontusion expressed as a proportion of the spared tissue of the lesioned hemicord relative to the contralateral hemicord (negative mm = caudal to lesion epicenter; positive mm = rostral to lesion epicenter). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

REFLEXIVE EMG

Fig. 2. cSCI promotes forelimb withdrawal hyperreflexia. Exemplar PETHs of biceps (A) and triceps (B) EMG activity around right forepaw withdrawal before and after cSCI. (C) cSCI promoted significant increases in response magnitude for the biceps (at all post-cSCI time points) and triceps (at Wk1, Wk3 and Wk4) indicating forelimb withdrawal hyperreflexia. Results are from within animals using one-way repeated measures ANOVAs. Different from PRE-SCI at * $p < 0.05$, ** $p < 0.01$.

Fig. 3.

Limb withdrawal latency and EMG activation metrics. No significant differences in limb withdrawal latency were seen for the ipsilesional (A) or contralateral (B) limbs at any post-cSCI time point, indicating a lack of thermal hyperalgesia following cSCI (RFP = right forepaw; RHP = right hindpaw; LFP = left forepaw; LHP = left hindpaw). (C) There was no significant effect of cSCI on first bin latency for both muscle's EMG during forelimb withdrawal assessment. Results are from within animals using one-way repeated measures ANOVAs.

Fig. 4. cSCI chronically reduced volitional forelimb strength, Which is correlated to flexor withdrawal hyperreflexia. (A) Mean isometric pull force profiles before lesion and 1–4 weeks after lesion (mean $\pm$ 95% confidence interval). (B) cSCI produced a significant decrease in isometric pull peak force at Wk1–Wk4 compared to PRE-SCI. Result is from within animals using a one-way repeated measures ANOVA. Different from PRE-SCI at *** $p < 0.001$. (C) The response magnitude for biceps, but not triceps, EMG during forelimb withdrawal assessment was significantly negatively correlated with isometric peak force following cSCI. Each point in the correlation is a single animal's value for the given muscle. Values represent the mean across Wk1–Wk4.

Fig. 5. cSCI chronically reduced multiple parameters of volitional forelimb strength. (A) 3D peak force histogram showing the distribution of isometric pull peak forces at PRE-SCI, Wk1–Wk4 (5 g bins). cSCI produced a significant decrease in success rate (B) and pull speed (C) at Wk1–Wk4 compared to PRE-SCI. (D) cSCI produced a transient decrease in trials per session at Wk1 compared to PRE-SCI. Results are from within animals using one-way repeated measures ANOVAs. Different from PRE-SCI at ** $p < 0.01$; *** $p < 0.001$.

Fig. 6.

Volitional forelimb EMG. Exemplar biceps (A) and triceps (B) EMG heat matrix at PRE-SCI for a single session (rows = single pulls; columns = 5 ms bins; -0.2 s before to 0.1 s after a pull; pull initiation = vertical lime green dashed line) and the corresponding median EMG profile for the given session (right). (C) Differential EMG PETH (EMG PETH for pull Successes minus EMG PETH for pull Fails) at PRE-SCI for biceps and triceps (mean $\pm$ 95% confidence interval). (D) There was no significant effect of cSCI on first bin latency for both muscle's EMG during the isometric pull task. Result is from within animals using a oneway repeated measures ANOVA.

Fig. 7.

Effect of cSCI on volitional forelimb EMG. (A) cSCI produced a significant decrease in response magnitude for triceps EMG, but not biceps, during the isometric pull task at all post-cSCI time points indicating reduced volitional drive to the triceps. Results are from within animals using one-way repeated measures ANOVAs. Different from PRE-SCI at *** $p < 0.001$. (B) The response magnitude for biceps EMG, but not triceps, during the isometric pull task was significantly positively correlated with isometric peak force following cSCI. Each point in the correlation is a single animal's value for the given muscle. Values represent the mean across Wk1–Wk4.

Fig. 8.

cSCI increases volitional motor output variability. (A) cSCI promoted significant decreases in the mean similarity index for both biceps and triceps volitional EMG at all post-cSCI time points indicating increased muscle activation variability. (B) cSCI also promoted significant decreases in the mean similarity index for isometric pull force profiles at all post-cSCI time points indicating increased force output variability. Results are from within animals using one-way repeated measures ANOVAs. Different from PRE-SCI at * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (C) The mean similarity index for biceps and triceps EMG during the isometric pull task was not significantly correlated with isometric peak force following cSCI. (D) The mean similarity index for isometric force profiles was significantly positively correlated with isometric peak force following cSCI. Each point in the correlations (C and D) is a single animal's value. Values represent the mean across Wk1–Wk4.

Fig. 9.

Pre-cSCI forelimb EMG synchrony. A.) Exemplar EMG cross-correlogram (CC) around a single pull attempt (i.e. time 0 s) during PRE-SCI (CC Peak = maximum value of CC histogram; CC time lag is at 0 ms; dashed line = 99% confidence interval). B.) At PRE-SCI, normalized CC Strength between biceps and triceps during pull Fails is significantly lower compared to pull Successes (CC strength for pull Fails normalized to CC strength for pull Successes within animals). Result is from a paired t-test. Different at * $p < 0.05$.

Fig. 10.

cSCI disrupts the magnitude, but not the timing, of forelimb EMG synchrony. (A) cSCI significantly decreased the magnitude of EMG synchrony between the biceps and triceps during the isometric pull task. (B) There was no effect of cSCI on the time of maximal EMG synchrony. Results are from within animals using oneway repeated measures ANOVAs. Different from PRE-SCI at * $p < 0.05$; *** $p < 0.001$. (C) Biceps and triceps EMG synchrony during the isometric pull task was not significantly correlated with isometric peak force following cSCI. Each point in the correlation is a single animal's value. Values represent the mean across Wk1–Wk4.

Fig. 11. Overview of the effects of cSCI on reflexive and volitional forelimb EMG. (A) Cartoon representation of the location of biceps and triceps alpha motor neuron pools and the C5/C6 cSCI with a summary of the observed effects during reflexive and volitional forelimb EMG assessments.