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Posteroanterior cervical transcutaneous spinal stimulation targets ventral and dorsal nerve roots

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HIGHLIGHTS

- We report a novel posteroanterior approach to cervical spinal stimulation.
- This approach easily activates arm and hand muscles on both sides simultaneously.
- A mix of afferent and efferent nerve roots is activated across stimulus intensities.

ABSTRACT

Objective: We aim to non-invasively facilitate activation of spared neural circuits after cervical spinal cord injury (SCI) and amyotrophic lateral sclerosis (ALS). We developed and tested a novel configuration for cervical transcutaneous spinal stimulation (cTSS).
Methods: cTSS was delivered via electrodes placed over the midline at ~T2-T4 levels posteriorly and ~C4-C5 levels anteriorly. Electromyographic responses were measured in arm and hand muscles across a range of stimulus intensities. Double-pulse experiments were performed to assess homosynaptic post-activation depression (PAD). Safety was closely monitored.
Results: More than 170 cTSS sessions were conducted without major safety or tolerability issues. A cathode-posterior, 2 ms biphasic waveform provided optimal stimulation characteristics. Bilateral upper extremity muscle responses were easily obtained in subjects with SCI and ALS. Resting motor threshold at the abductor pollicis brevis muscle ranged from 5.5 to 51.0 mA. As stimulus intensity increased, response latencies to all muscles decreased. PAD was incomplete at lower stimulus intensities, and decreased at higher stimulus intensities.
Conclusions: Posteroanterior cTSS has the capability to target motor neurons both trans-synaptically via large-diameter afferents and non-synaptically via efferent motor axons.
Significance: Posteroanterior cTSS is well tolerated and easily activates upper extremity muscles in individuals with SCI and ALS.

1. Introduction

Regaining hand function represents the highest priority for individuals with cervical SCI (Anderson, 2004). Nearly all spinal cord injuries (SCI) are anatomically incomplete (Bunge et al., 1993; Kakulas, 1999). Activating spared nerve circuits, whether via physical activity, drug administration, or electromagnetic stimulation, can augment neural plasticity (Ziemann et al., 2006; Brus-Ramer et al., 2007; Maier et al., 2008; Carmel et al., 2014). Electrical stimulation over the epidural surface of the lumbar spinal cord has produced dramatic improvements in motor and cardiovascular function (Harkema et al., 2011; Angeli et al., 2014, 2018; Gill et al., 2018; Harkema et al., 2018; Wagner et al., 2018). However, invasive stimulator implantation carries surgical risks, which are

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significantly higher in the cervical than lumbar spine. We and others therefore aim to use non-invasive cervical stimulation to activate spinal circuits involved in hand function (Einhorn et al., 2013; Murray and Knikou, 2017; Gad et al., 2018; Inanici et al., 2018; Milosevic et al., 2019). In this study, we demonstrate the concept of a novel electrode configuration for delivering non-invasive phasic cervical transcutaneous spinal stimulation (cTSS). The anode is placed over the midline of the anterior surface of the neck, several segments rostral to the cathode placed posteriorly.

To our knowledge, only one other publication has applied phasic cTSS using a posteroanterior configuration across the neck similar to ours (Milosevic et al., 2019). No safety data were reported in that study, which focused exclusively on healthy young volunteers. Electrical stimulation targeted at the cervical cord may unintentionally activate other vital structures in close proximity, such as the trachea, laryngeal muscles, carotid and vertebral arteries, carotid baroreceptors, and vagus nerve (ter Laan et al., 2010; Antonino et al., 2017; Brock et al., 2017; Gad et al., 2018). Individuals with cervical SCI are at increased risk of experiencing autonomic dysreflexia, a potentially dangerous combination of hypertension, cerebral vasodilation, and bradycardia (Biering-Sørensen et al., 2018). Therefore, our initial testing of this cervical stimulation configuration in individuals with SCI and amyotrophic lateral sclerosis (ALS) in addition to able-bodied volunteers necessitated careful monitoring of safety, cardiovascular, and pulmonary status. In addition to safety and demonstration-of-concept, we conducted experiments to determine motor thresholds, distribution of muscle activation, and underlying neural transmission pathways for this stimulation configuration across a range of stimulus intensities. We hypothesized that posteroanterior cTSS could elicit upper extremity muscle responses safely and easily in participants with a range of neural impairment, and that muscle responses could be elicited by activation of both sensory and motor fibers.

2. Methods

2.1. Design

This manuscript reports safety and preliminary circuit mechanism data from a study that was pre-registered at clinicaltrials.gov (NCT02469675). All procedures were approved by the Institutional Review Board of the James J. Peters VA Medical Center, Bronx, NY. All applicable institutional and governmental regulations concerning the ethical participation of human volunteers were followed during the course of this research.

2.2. Participants

Individuals between ages 21 and 65 without neurological injury (able-bodied or AB), those with chronic spinal cord injury, and those with amyotrophic lateral sclerosis were eligible for participation. For SCI participants, inclusion criteria included duration of injury greater than 12 months, level of injury between levels C2-C8, and incomplete paresis of intrinsic muscles in either hand. For ALS participants, inclusion criteria included diagnosis of definite or probable ALS. All participants required detectable F-wave responses of left or right abductor pollicis brevis (APB) muscle to median nerve stimulation and detectable motor evoked potentials (greater than 50 μV) in left or right APB muscle to transcranial magnetic stimulation (TMS). Exclusion criteria included ventilator dependence, open tracheostomy site or other open lesions over the neck, shoulders, or arms, multiple sclerosis, hemorrhagic brain injury, seizures, medications that increase seizure risk, recurrent spontaneous bouts of symptomatic autonomic dysreflexia, significant coronary artery or cardiac conduction disease, bipolar disorder, active psychosis, pregnancy, or implanted electrical or ferromagnetic devices (Rossi et al., 2009). Demographic data for enrolled participants are listed in Table 1. Participant ID numbers were assigned in order of study enrollment. Note that some participants enrolled but did not undergo testing – these participants are not included in the table.

2.3. General protocol

Sessions were performed at a consistent time of day per subject, with attempts to maintain consistent timing of caffeine intake. Stimulation was delivered with subjects in seated upright position in an adjustable TMS chair (Magventure), or for one participant (39), in her own cushioned wheelchair. For participants without neurological injury, TMS was targeted toward the dominant arm. For those with SCI or ALS, TMS was targeted toward the arm with lower motor thresholds and more reliable electrophysiological responses. The APB of the target arm was the main outcome muscle for all experiments. Arms and hands were pronated and relaxed on a pillow cushion placed in the participant's lap. Blood pressure, heart rate, pulse oximetry, and symptoms were monitored and recorded every three minutes during cTSS, and no less than every 15 minutes during other portions of the protocol. Subjective symptoms were assessed according to questions suggested by the International Federation of Clinical Neurophysiology (Rossi et al., 2011). Additionally, during comparison of different cTSS delivery polarities and waveforms, discomfort at 150% of resting motor threshold (RMT) was recorded on a 0 to 10 scale. Heights and neck circumferences were obtained retrospectively from AB and SCI participants, but we were unable to successfully reach all subjects.

2.4. cTSS

The surface electrode configuration comprises four 5 × 10 cm electrodes (Natus 019-422200). One electrode is placed longitudinally over the posterior midline with the cephalad edge ~4 cm caudal to the C7 spinous process, corresponding to the T2-T4 vertebral levels posteriorly. Another electrode is placed horizontally over the anterior midline with the caudal edge ~2–3 cm superior to the sternal notch, corresponding to the C4-C5 levels anteriorly (Fig. 1). Two 5 × 10 cm electrodes over the distal clavicles are connected to a common ground.

Stimulation was delivered using constant-current peripheral nerve stimulators (Digitimer DS7A or DS8R). Intensity-response curves were collected using the following waveforms: anode-posterior 2 ms biphasic, cathode-posterior 2 ms biphasic, cathode-posterior 1 ms biphasic, cathode-posterior 1 ms monophasic, and cathode-posterior 2 ms biphasic waveforms. Sets of pulses at intensities ranging between 80% to 200% of RMT were delivered at 0.2 Hz in pseudorandom order. Response latencies and peak-to-peak amplitudes at all recorded muscles were averaged from 5-6 repetitions per intensity. For the majority of experiments in this manuscript, including determination of resting motor threshold (RMT), biphasic 2 ms pulses with posterior cathode were used. RMT was determined as the intensity (in mA) required to elicit a potential in the abductor pollicis brevis (APB) muscle of at least 50 μV in 5 out of 10 repetitions.

To measure post-activation depression (PAD), pairs of cTSS pulses (40 ms interstimulus interval) were delivered at intensities ranging between 100% to 200% of RMT (or 175% RMT for SCI and ALS subjects). Each pulse of a pair was delivered at equal intensity.

2.5. Transcranial magnetic stimulation (TMS)

TMS was used as a screening tool for study entry. A MagPro R30 or X100 system (Magventure) with 80 mm winged coil (D-B80) was used. The magnet was oriented at a 45-degree angle from

Table 1
Participant demographics. (**A**) **SCI**. DOI – duration of injury; LOI – neurological level of injury; ISNCSCI – International Standards for the Neurological Classification of SCI. (**B**) **ALS**. Onset – Time since ALS symptom onset; ALSFRS – score on ALS Functional Rating Scale-Revised (Cedarbaum et al., 1999). (**C**) **Able-bodied**. mA – milliamperes; %MSO – percent of maximal stimulator output; nd – not determined.

A – SCI Demographics:

ID	Gender	Age	Height (cm)	Neck Circumf (cm)	Trauma?	DOI (yr)	LOI	ISNCSCI Grade	cTSS Threshold (mA)	TMS Threshold (%MSO)
1	M	29	193	40	T	3	C8	C	32.0	47.0
2	M	52	173	39	T	17	C8	C	36.0	37.0
18	M	64	180	40	T	14	C4	D	10.0	60.0
23	M	57	168	nd	T	12	C4	D	42.0	57.0
27	M	40	185	nd	T	14	C4	D	24.5	65.0
28	M	43	185	nd	T	14	C5	D	29.0	58.0
31	M	54	173	38	T	6	C5	C	32.0	42.0
34	F	52	152	36	T	16	C4	B	10.0	76.0
39	F	22	160	35	NT	1	C5	C	5.5	36.0
30	*M*	*35*	*180*	*nd*	*T*	*12*	*C4*	*B*	*51.0*	*71.0*
32	*M*	*36*	*168*	*nd*	*T*	*11*	*C3*	*C*	*46.0*	∞
40	*M*	*49*	*185*	*47*	*T*	*13*	*C6*	*B*	*7.5*	*80.0*
41	*F*	*61*	*165*	*nd*	*T*	*20*	*C2*	*D*	*5.5*	∞

B – ALS Demographics

ID	Gender	Age	Onset (yr)	ALSFRS	cTSS Threshold (mA)	TMS Threshold (%MSO)
3	M	52	3	37	34.0	81.0
7	M	60	4	30	27.0	59.0
21	M	58	1.5	35	19.0	51.0
37	F	63	2	31	23.0	64.0

C – AB Demographics

ID	Gender	Age	Height (cm)	Neck Circumf (cm)	cTSS Threshold (mA)	TMS Threshold (%MSO)
5	M	25	177	39	24.0	32.0
8	M	27	178	42	37.5	39.0
9	M	44	178	40	22.0	39.0
11	F	22	165	34	20.5	47.0
12	F	23	151	36	23.0	36.0
14	M	44	192	38	32.0	33.0
15	M	45	160	38	22.5	38.0
16	M	24	185	39	20.0	46.0
22	M	58	175	42	45.5	64.0
24	M	53	nd	nd	40.0	50.0
25	M	55	173	40	44.5	37.0
29	M	48	nd	nd	34.0	45.0
36	M	23	178	39	30.0	31.0
38	M	22	193	46	13.0	58.0

Note, neck circumferences and heights were not collected for ALS participants.

the sagittal plane, centered over the hand motor cortex hotspot for maximal APB response. The first 11 participants wore reusable cloth headcaps upon which the hotspot was labeled with a marker in relation to the vertex. Participants 12 and onward wore a headband with passive markers detected using an optical infrared tracking system (Vicon) integrated with a Brainsight neural navigation system (Rogue Research). RMT was determined as the percent of maximal stimulator output required to elicit a potential in the APB muscle of at least 50 μV in 5 out of 10 repetitions.

2.6. Peripheral nerve stimulation (PNS)

Stimulation was delivered using constant-current peripheral nerve stimulators (Digitimer DS7A or DS8R) and dual surface electrodes (Natus 019-429400) placed over the median and ulnar nerves at the wrist. Monophasic 0.2 ms duration pulses were delivered at supramaximal intensity 20 times at 0.5 Hz to record both direct (M-wave) and late (F-wave) responses. F-wave latency was calculated as the average response latency across 20 stimuli. Peripheral motor conduction time (PMCT) was calculated as $(\text{Latency}_M + \text{Latency}_F - 1) \div 2$ (Robinson et al., 1988).

2.7. Electromyography (EMG)

EMG was recorded using surface sensors with 300x preamplification, 15–2,000 Hz bandwidth, and internal grounding (Motion Lab Systems Z03-002). EMG was collected at a sample rate of 5,000 Hz via digital acquisition board and customized LabVIEW software (National Instruments USB-6363). Muscles recorded included abductor pollicis brevis (APB), abductor digiti minimi (ADM), flexor carpi radialis (FCR), and/or biceps brachii.

2.8. Analysis

The APB muscle was the primary outcome muscle for all experiments. All stimulus intensities were expressed as a percentage of each subject's target APB RMT obtained using the cathode-posterior biphasic 2 ms configuration. Several sources of partially-missing data arose: due to discomfort at the highest stimulation intensities, some subjects completed testing across an incomplete range of intensities (See 3.3). Several subjects with SCI or ALS had frequent spontaneous muscle activity that prevented reliable interpretation of recordings over some muscles. Due to technical artifacts, some biceps recordings were unusable. To handle missing data, linear mixed modeling was performed using the lmer package in R. Models were fit using a restricted maximum likelihood (REML) approach. Fixed and interaction effects were subjected to analysis of variance (ANOVA) using Satterthwaite's method.

To determine if cTSS elicits similar responses bilaterally, linear mixed modeling was performed to analyze response amplitude with fixed effects: target (On vs Off) and stimulus intensity; and random effects: subject.

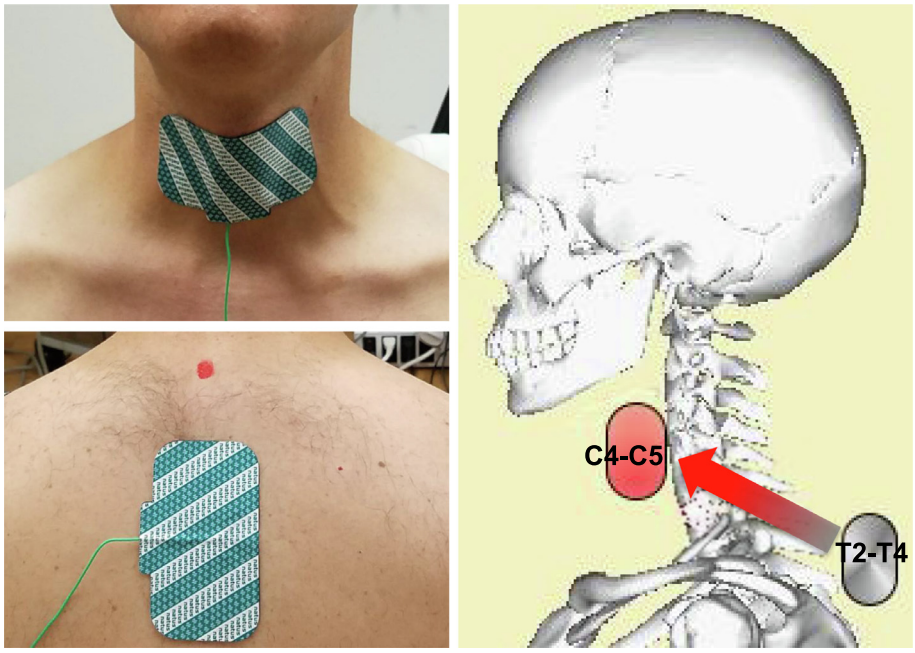

Fig. 1. Posteroanterior cTSS electrode configuration. 5 × 10 cm electrodes are placed over ~T2-T4 posteriorly and ~C4-C5 anteriorly as described in Methods. The red mark on the subject's back represents the location of the C7 spinous process. cTSS: cervical transcutaneous spinal stimulation.

To determine if cTSS response latency at the APB muscle changed across stimulus intensity, each participant's PMCT was subtracted from cTSS response latency at each stimulus intensity. Linear mixed modeling was performed to analyze relative response latency with fixed effects: group (AB vs SCI) and stimulus intensity; and random effects: subject.

To determine if cTSS transmission pathways changed from synaptic to non-synaptic across stimulus intensity, PAD was measured at each muscle. Response amplitude to the second stimulus of each pair was normalized to the amplitude of the first response. PAD was calculated as 100 – (normalized second response amplitude). Linear mixed modeling was performed to analyze relative PAD with fixed effects: group (AB vs SCI) and stimulus intensity; and random effects: subject.

The small number of ALS participants in this study served as a demonstration of principle and safety. No statistically meaningful inferences were attempted for the ALS group.

Excel (Microsoft), SPSS Version 23 (IBM) and R (The R Foundation for Statistical Computing) were used for all statistical analysis.

3. Results

3.1. Subjects

31 individuals (14 AB, 13 SCI, 4 ALS) underwent cTSS as part of this study (Table 1). Participants ranged in age from 22 to 64 years old. 25 males (11 AB, 11 SCI, 3 ALS) and 6 females (2 AB, 3 SCI, 1 ALS) participated. Of the 13 cervical SCI participants, all except one had traumatic SCI, and all except three had grade C or D according to the International Standards for the Neurological Classification of Spinal Cord Injury. Three SCI participants (32, 40, 41) screened out of further participation due to inability to obtain median nerve F-wave responses or hand muscle motor evoked potentials in response to TMS. One SCI participant screened out of further participation due to complete plegia of finger muscles (30). ALS subjects ranged between scores of 30–37 on the revised ALS Functional Rating Scale (Cedarbaum et al., 1999).

The italic participants in Table 1A were ineligible for further participation due to insufficient electrophysiological responses or motor function as detailed in the Methods.

3.2. cTSS configuration and waveform comparison

Initial testing was performed to compare three aspects of cTSS configuration: orientation; waveform; and stimulus duration. In the first several AB and SCI subjects, the cathode-posterior orientation elicited far larger responses at lower intensities than the anode-posterior orientation. This agreed with other published cTSS configurations which have all used a cathode-posterior orientation (Sabbahi and Sengul, 2012; Einhorn et al., 2013; Milosevic et al., 2019). Therefore, further tests were done only in the cathode-posterior orientation, using monophasic or biphasic waveforms of either 1 ms or 2 ms duration. Intensity-response curves were compared across configurations at intensities between 80% and 200% of RMT (Fig. 2). No significant differences were found in participant perception of discomfort, except for a trend toward lower discomfort with 1 ms than 2 ms duration pulses (Table 2). In the cathode-posterior orientation, all subjects had easily obtainable cTSS responses, whereas the cathode-anterior orientation elicited minimal or small responses in most subjects. Surprisingly, three SCI subjects (18, 27, 39) showed robust responses to anode-posterior (cathode-anterior) stimulation even at lower perithreshold intensities. Unsurprisingly, longer-duration pulses elicited higher responses than shorter duration pulses. In contrast to monophasic pulses, charge-balanced biphasic pulses reduce the risk of net charge injection and tissue damage (Hofmann et al., 2011). Therefore, further experiments were performed using cathode-posterior 2 ms biphasic pulses.

3.3. Safety and tolerability

Participants underwent over 170 sessions of cTSS without procedure-related serious adverse events. Three AB subjects (24, 25, 29), 3 SCI subjects (18, 23, 28), and 1 ALS subject (3) expressed

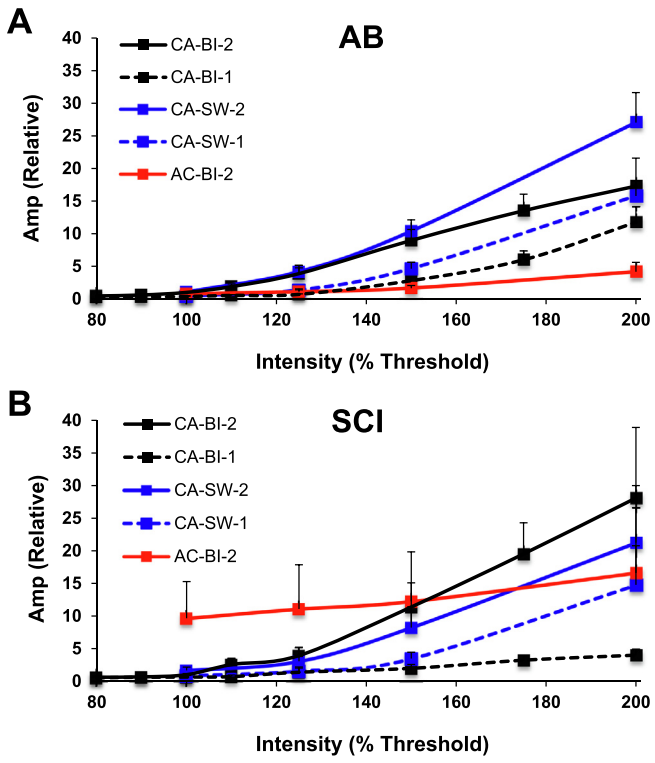

Fig. 2. Comparison of cTSS configuration and waveforms across range of intensities in able-bodied (AB) volunteers. cTSS was delivered in Anode-posterior/Cathode-anterior ('AC') or Cathode-posterior/Anode-anterior ('CA') orientation using monophasic (square-wave 'SW') or biphasic ('BI') pulses of 1 ms or 2 ms duration in 13 AB participants (**A**) or 9 SCI participants (**B**). Relative amplitudes at the target APB were normalized to the response at 100% resting motor threshold using cathode-posterior, biphasic 2 ms pulses (CA-BI-2). Error bars represent SEM. cTSS: cervical transcutaneous spinal stimulation. SEM: standard error of the mean. APB: abductor pollicis brevis. SCI: spinal cord injury.

Table 2
Analog scale for discomfort with different cTSS waveforms. No significant differences were found in the level of discomfort (0 to 10) with different waveforms. AC, Anode-posterior/Cathode anterior orientation; CA, Cathode-posterior/Anode anterior orientation; SW, Square-Wave (monophasic) waveform; BI, biphasic waveform; 1 ms or 2 ms duration.

Waveform	AS (SD)
CA-SW-1	3.77 (2.26)
CA-SW-2	4.54 (2.37)
CA-BI-1	3.52 (2.04)
CA-BI-2	4.21 (2.16)
AC-BI-2	4.06 (2.21)

discomfort at the highest stimulation intensities (150%–200% of motor threshold), so completed testing across an incomplete range of intensities. Seven participants reported mild side effects possibly related to cTSS, including incidents of light headedness, feeling flushed, nausea, metallic taste, a sensation of "sharp" breathing, neck pain, and sore throat. All mild events self-resolved within minutes or less. We closely monitored autonomic stability during cTSS. Seven participants with SCI demonstrated sustained (15 minutes or more) incidents of 20% or greater increase in mean arterial pressure from baseline. Two participants with SCI demonstrated sustained (15 minutes or more) incidents of 20% or greater decrease in mean arterial pressure. One participant with SCI demonstrated a sustained (15 minutes or more) incident of 20% or greater increase in heart rate. Eight participants (7 with SCI, 1 able-bodied) demonstrated sustained (15 minutes or more) incidents of 20% or greater decrease in heart rate. With the exception of one participant, these episodes were entirely asymptomatic. One participant with SCI experienced mild headache and facial flushing during an episode of sustained BP elevation, which raised concern for autonomic dysreflexia. The session was terminated early, and it was later revealed that she had a new urinary tract infection that day, with hematuria. The incident was deemed unlikely related to cTSS. Out of an abundance of caution, the investigating team and institutional review board agreed to abort her further participation in the study.

3.4. cTSS thresholds and distribution

cTSS evoked responses simultaneously in both arms in all subjects (Fig. 3). There was no statistical difference between muscle activation in the target or off-target hand in able-bodied subjects for either the APB (target:intensity interaction $F = 2.831$ ($p = 0.095$)) or ADM muscles (target:intensity interaction $F = 2.162$ ($p = 0.144$)). Resting motor threshold (RMT) at the target abductor pollicis brevis (APB) muscle averaged 29.2 ± 7.8 mA (range 13.0–45.5 mA) in AB participants, 26.0 ± 7.2 mA (range 5.5–51.0 mA) in SCI participants, and 25.8 ± 12.9 mA (range 19.0–34.0 mA) in ALS participants (Table 1). There was no obvious correlation between cTSS RMT values and anthropometric features such as age, neck circumference, SCI level, or severity of SCI or ALS. Thresholds were mostly stable when measured across multiple sessions on different days (coefficient of variation 12.6% in AB participants, 24.1% in SCI participants, and 11.5% in ALS participants).

3.5. cTSS response latencies shorten with increasing stimulus intensity.

Relative latencies corresponded to the distance of each recording electrode from the cathode. At 125% RMT, the bicep responded first (AB: 7.41 ± 0.59 ms; SCI: 7.74 ± 0.89 ms; ALS: 9.07 ± 0.15 ms), followed by the FCR (AB: 9.58 ± 0.95 ms; SCI 11.09 ± 1.00 ms; ALS: 11.94 ± 0.47 ms), and then the APB (AB: 16.92 ± 0.30 ms; SCI: 17.81 ± 0.74 ms; ALS: 21.11 ± 4.60 ms) (Table 3).

Notably, response latencies decreased as cTSS intensity increased (Table 3, Figs. 4 and 5). Several subjects did not tolerate

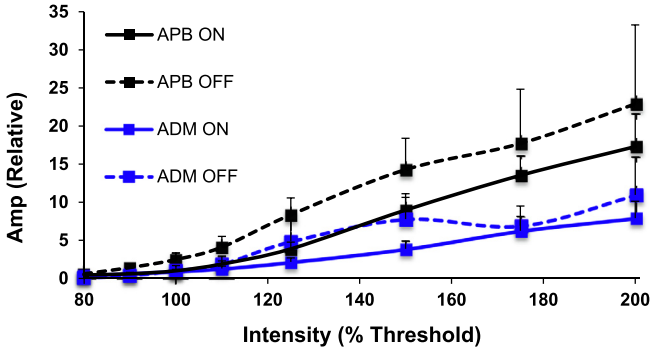

Fig. 3. Comparison of bilateral cTSS responses. cTSS was delivered in cathode-posterior orientation using biphasic pulses of 2 ms duration in able-bodied subjects (n = 12 for on-target, n = 8 for off-target). Relative amplitudes were normalized to the response at 100% resting motor threshold of the target APB muscle. There was no statistical difference between muscle activation in the target or off-target hand in able-bodied subjects for either the APB (target:intensity interaction $F = 2.831$ ($p = 0.095$)) or ADM muscles (target:intensity interaction $F = 2.162$ ($p = 0.144$)). Error bars represent SEM. cTSS: cervical transcutaneous spinal stimulation. SEM: standard error of the mean. APB: abductor pollicis brevis. ADM: abductor digiti minimi.

Table 3
Latency in ms (SEM) from cTSS impulse to target arm muscle responses.

	% RMT	AB	SCI	ALS
APB				
	100	**17.84** (0.51)	**19.29** (0.85)	
	110	**17.67** (0.41)	**17.55** (0.66)	**22.46** (2.82)
	125	**16.92** (0.30)	**17.81** (0.74)	**21.11** (4.60)
	150	**16.20** (0.29)	**17.06** (0.59)	**21.02** (2.73)
	175	**15.97** (0.24)	**16.49** (0.59)	**20.01** (2.79)
	200	**15.58** (0.31)	**16.61** (0.93)	**19.71** (2.85)
FCR				
	100	**10.35** (2.09)	**14.72** (1.18)	
	110	**9.38** (0.82)	**12.47** (1.13)	**12.35** (0.28)
	125	**9.58** (0.95)	**11.09** (1.00)	**11.94** (0.47)
	150	**9.26** (1.01)	**11.11** (0.55)	**12.95** (0.88)
	175	**8.64** (0.47)	**10.28** (0.58)	**11.67** (0.14)
	200	**8.43** (0.54)	**10.82** (0.78)	**11.39** (0.22)
Biceps				
	100	**8.23** (0.65)	**7.74** (0.38)	
	110	**7.63** (0.65)	**7.37** (0.43)	**9.43** (1.64)
	125	**7.41** (0.59)	**7.74** (0.89)	**9.07** (0.15)
	150	**6.74** (0.27)	**6.97** (0.48)	**8.79** (0.58)
	175	**6.52** (0.49)	**6.46** (0.25)	**8.26** (0.59)
	200	**6.44** (0.48)	**6.82** (0.15)	**7.56** (0.54)

pulses at 175–200% of RMT. Proximal muscles did not always respond at 100% of APB RMT. Therefore, the number of subjects/-muscles measured at each intensity level varied between 9–13 and 4–5 in hand and arm muscles, respectively, in AB subjects, and between 5–9 and 2–8 in hand and arm muscles, respectively, in SCI subjects, and between 2–4 in all muscles in ALS subjects. At the target APB, the average response latency at 200% RMT was

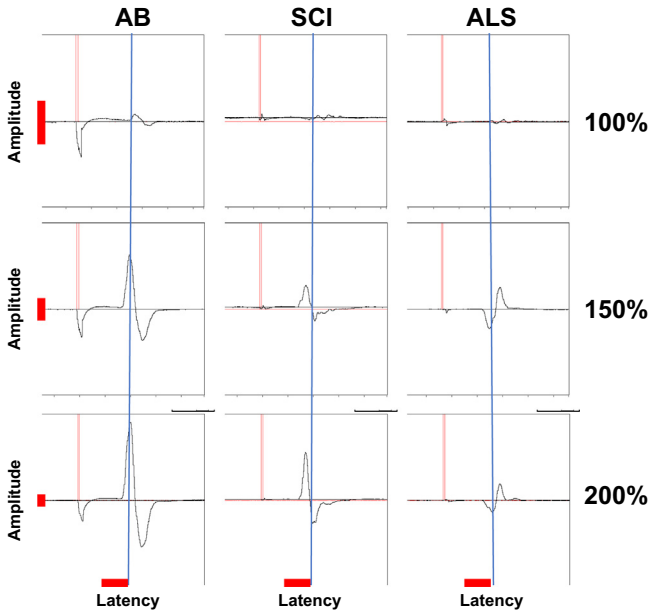

Fig. 4. Representative EMG traces across stimulus intensities. cTSS was delivered in cathode-posterior orientation using biphasic pulses of 2 ms duration. Representative cTSS responses of the target APB muscle at 100%, 150%, and 200% of resting motor threshold (RMT) are shown for an AB, SCI, and ALS subject (9, 27, and 37, respectively). Red line indicates stimulus onset. Blue line indicates response onset at 100% RMT. Latency decreases at higher stimulus intensity in all groups. Amplitude is generally lower in SCI and ALS subjects than AB subjects. Y-axis scale bar: 0.25 mV (note different zoom in each row). X-axis scale bar: 10 ms. cTSS: cervical transcutaneous spinal stimulation. APB: abductor pollicis brevis. AB: able-bodied. SCI: spinal cord injury. ALS: amyotrophic lateral sclerosis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

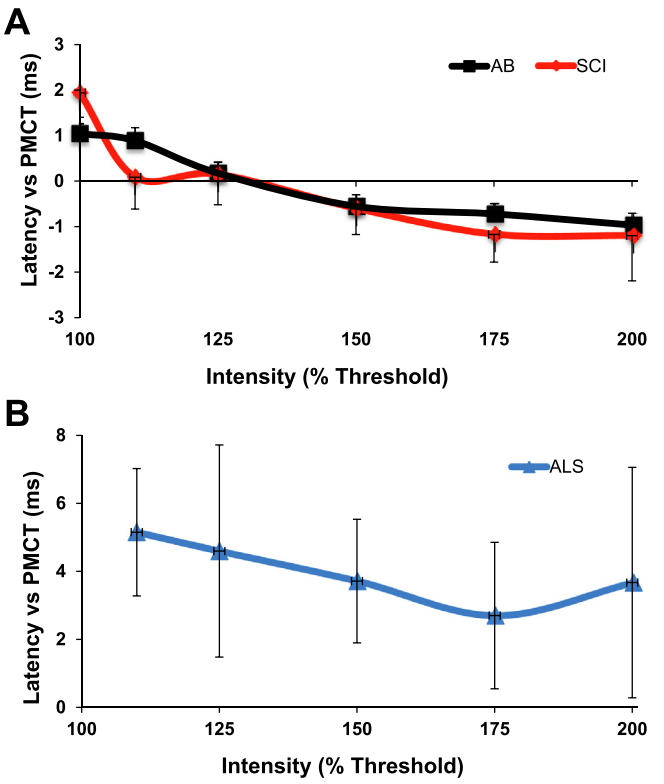

Fig. 5. cTSS response latency at target APB muscle decreases as stimulus intensity increases. (**A**) Able-bodied (n = 13) and SCI (n = 9) subjects. The effect of stimulus intensity on normalized APB response latency was highly significant ($F = 143.633$ ($p < 0.0001$)), with no significant effect of AB or SCI group ($F = 0.448$ ($p = 0.505$)). (**B**) ALS (n = 3) subjects. At each stimulus intensity, APB response latencies were normalized to each participant's PMCT (value = 0 on Y-axis). Error bars represent SEM. cTSS: cervical transcutaneous spinal stimulation. SEM: standard error of the mean. APB: abductor pollicis brevis. PMCT: peripheral motor conduction time. AB: able-bodied. SCI: spinal cord injury. ALS: amyotrophic lateral sclerosis.

2.09 ms, 0.94 ms, and 2.75 ms shorter than the latency at 110% RMT in able-bodied, SCI, and ALS participants respectively. When normalized to each individual's APB peripheral motor conduction time (PMCT), average cTSS response latencies in AB and SCI but not ALS subjects dropped below average PMCT at stimulation intensities at and above 150% RMT (Fig. 5). The effect of stimulus intensity on normalized APB response latency was highly significant ($F = 143.633$ ($p < 0.0001$)), with no significant effect of AB or SCI group ($F = 0.448$ ($p = 0.505$)). These data suggest that the transmission route between cTSS stimulus and muscle response shortens as stimulus intensity increased.

3.6. Post-activation depression is incomplete and decreases with increasing stimulus intensity.

Pairs of cTSS pulses (40 ms interstimulus interval) were delivered at intensities ranging between 100% to 200% of RMT (or 175% RMT for SCI and ALS subjects). Each pulse of a pair was delivered at equal intensity. FCR and biceps were not always recorded during this experiment. One AB subject (24) did not tolerate paired pulses at any intensity. Several other subjects did not tolerate paired pulses at 175–200% of RMT. Therefore, the number of subjects measured at each intensity level varied between 8–12 and 3–6 in hand and arm muscles, respectively, in AB subjects, and between 5–8 and 2–4 in hand and arm muscles, respectively, in SCI subjects. At peri-threshold intensity (~100–120% RMT), the response amplitude of the target APB to the second pulse was rel-

atively inhibited compared to the first response. At higher stimulus intensities, the second pulse was less inhibited (Fig. 6). The effect of stimulus intensity on APB PAD was highly significant ($F = 21.186$ ($p < 0.0001$)), with no significant effect of AB or SCI group ($F = 0.099$ ($p = 0.754$)). One AB subject (22) showed no PAD at lower intensities. One AB subject (38) showed no decrement in PAD at higher intensities. Interestingly, these AB subjects had the two widest neck circumferences of all subjects tested (Table 1). One SCI subject (39) showed a paradoxical increase in PAD with increasing intensities. Removing these outliers from the PAD data resulted in a stronger average PAD at lower intensities and less PAD at higher intensities (Fig. 6B). Notably, the trend for PAD to be higher at low stimulus intensities was less consistent in the ADM muscle and in SCI subjects (**Supplementary Table**).

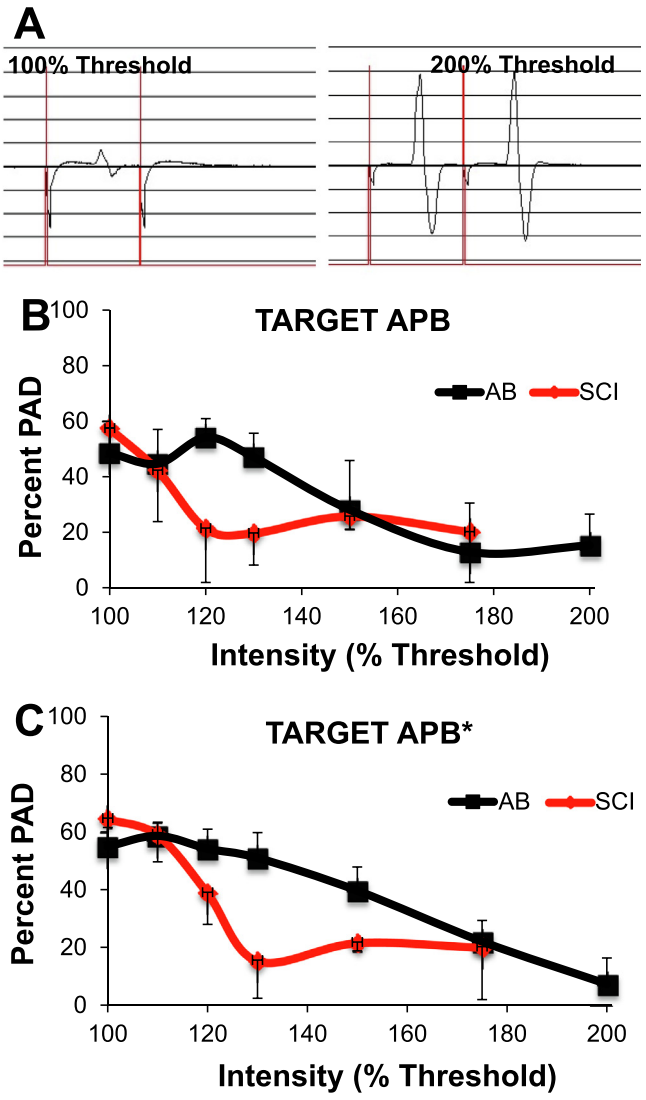

Fig. 6. Post-activation depression decreases as stimulus intensity increases. Paired cTSS pulses were delivered with a 40 ms interstimulus interval. (**A**) Representative examples are shown for stimulus intensities 100% and 200% of resting motor threshold (RMT). Relative suppression of the response to the second pulse represents post-activation depression (PAD). (**B**) The effect of stimulus intensity on APB PAD was highly significant ($F = 21.186$ ($p < 0.0001$)), with no significant effect of AB or SCI group ($F = 0.099$ ($p = 0.754$)). (**C**) Same data with asterisk indicating that 2 outliers were removed from AB and 1 outlier removed from SCI. Error bars represent SEM. cTSS: cervical transcutaneous spinal stimulation. SEM: standard error of the mean. APB: abductor pollicis brevis. AB: able-bodied. SCI: spinal cord injury.

4. Discussion

Electrical stimulation is a promising intervention in rehabilitation for the SCI and possibly ALS populations. Targeted stimulation of the spinal cord, as demonstrated with lumbar epidural stimulation (Harkema et al., 2011; Angeli et al., 2014, 2018; Gill et al., 2018; Wagner et al., 2018), may recruit endogenous circuitry that leads to re-expression of natural movement synergies (Giszter, 2015; Wenger et al., 2016). Labeled with many different names by different research groups, non-invasive transcutaneous spinal stimulation (TSS) has also been shown to activate spinal circuits, likely through similar large-fiber afferent nerve circuits as those activated by epidural stimulation (Hofstoetter et al., 2018). In the current study, we describe initial safety and mechanistic results of a relatively novel phasic cTSS paradigm applied in individuals with SCI, ALS, and without neurological injury or disease.

Our stimulation configuration uses a cathode directly over posterior elements of the spinal canal, similarly to most other TSS approaches. However, our cathode is larger (5×10 cm) than that used by most other groups for cervical stimulation, and is placed more caudally (over ~T2-T4) (Einhorn et al., 2013; Gad et al., 2018; Inanici et al., 2018; Milosevic et al., 2019). Furthermore, the anode in our configuration is centered directly over anterior elements of the cervical spinal canal, as opposed to anodes over the clavicles, abdomen, or iliac crests commonly used in other approaches. Interestingly, unlike any AB subject or the majority of other SCI subjects, three SCI subjects (18, 27, 39) showed robust responses to anteroposterior stimulation (cathode anterior, anode posterior). In each of these cases, the anterior cathode was situated roughly at the lesion level (Table 1). However, other SCI participants with similar lesion level did not show similarly robust response to anteroposterior stimulation. Two of the subjects who responded to anteroposterior stimulation had previous spine surgery with internal fixation. But six other SCI participants with prior spine surgery did not show a similarly robust response to anteroposterior stimulation. Therefore, this phenomenon remains unexplained.

To our knowledge, two other studies have used a similar posteroanterior cervical electrical stimulation paradigm in able-bodied human subjects: Donges et al. used direct-current stimulation (DCS) with a cathode over C6-T1 and anode over the cervicomental angle (Dongés et al., 2017). DCS is applied at very low intensities (3 mA in the Donges study). They found no change in muscular responses to transcranial stimulation or cervicomedullary stimulation after 20-minute sessions of cervical DCS. As mentioned earlier, Milosevic et al. applied cTSS using a 5×5 cm cathode over the C7-T1 area, a 7.5×10 cm anode over the anterior neck (not specified if the electrode was placed vertically or horizontally), and a 2 ms monophasic waveform (Milosevic et al., 2019). Our results in the current study largely agree with theirs, as will be detailed below. In rats, Zareen et al. applied cervical DCS with the cathode over C4-T2 and anode over the anterior chest (Zareen et al., 2017). Finite Element Modeling of the electric fields produced by this configuration projected the greatest current density directly between the cathode and anode. We speculate a similar 'hotspot' of current density along the axis between our cathode–anode configuration, projecting across the C5-T1 spinal segments and associated roots (Fig. 1).

4.1. Safety

Safety was not reported in the Donges or Milosevic studies. A cTSS study in able-bodied subjects by Sabbahi and Sengul using cathodal stimulation over C7-T1 posteriorly with the anode over the left acromion observed no significant electrocardiographic

changes during stimulation at up to 100 mA (Sabbahi and Sengul, 2012). Placing the anode over the anterior neck may create an electric field that intersects vital off-target structures such as the vagus and phrenic nerves, the trachea, and the carotid arteries. During careful monitoring, we have observed no serious safety issues over more than 250 sessions conducted in our laboratory to date. Several subjects showed asymptomatic increases in blood pressure during cervical stimulation, but we have also noted comparable increases during other stimulation paradigms in our lab, such as peripheral nerve stimulation over the wrist. Therefore, this was likely to have been a non-specific response to pain or discomfort. Furthermore, we are collecting more detailed electrocardiographic and beat-to-beat blood pressure data as part of a separate ongoing cTSS study and have not observed any consistent changes during cTSS. The large (5 × 10 cm) cathode reduces charge density and discomfort (Roy et al., 2014). Overall tolerability was high, with discomfort at 150% of motor threshold rated at roughly 4 on a 0–10 visual analog scale (Table 2). Across other cervical and lumbar TSS studies, safety has not always been explicitly presented, but there have been no serious incidents to our knowledge.

4.2. Effective widespread muscle activation

We observed activation of multiple upper extremity muscles bilaterally at lower stimulus intensities than reported using other cTSS configurations. APB motor thresholds were achieved at intensities ranging from 5.5 to 51 mA in participants with chronic SCI, ALS, and able-bodied volunteers. Although we did not formally measure threshold at other muscles, we often observed activation of proximal arm and shoulder muscles at *lower* intensities than required for APB. Milosevic et al. achieved polymuscular activation at stimulation intensities between 50–90 mA. Using the C7-T1 to left acromion configuration described above, Sabbahi et al. noted arm muscle thresholds of roughly 40 mA (Sabbahi et al., 2014). Einhorn et al. performed cTSS in 13 able-bodied volunteers with a 5 × 10 cm cathode over the C4-T1 area, two 5 × 10 cm anodes over the clavicles, and 1 ms monophasic pulses (Einhorn et al., 2013). Motor thresholds at elbow and wrist muscles ranged between 77–228 mA in that study. Gad et al. used 2 small electrodes over C3-C4 and C6-C7 posteriorly, with two larger anodes over the iliac crests (Gad et al., 2018). They applied 1 ms monophasic or biphasic pulses with a 10,000 Hz carrier frequency to eight individuals with chronic cervical SCI, reporting activation thresholds at biceps and wrist muscles between 10–200 mA but not individually specified. Using a very similar paradigm (biphasic pulses only), Inanici et al. have observed motor thresholds between 30–60 mA in upper extremity muscles (Inanici et al., 2018) (and personal communication). Lumbar TSS studies have generally used intensities in the range of 40–300 mA or ~25 V to achieve effective muscular activation (Minassian et al., 2007; Krenn et al., 2013; Knikou, 2014; Gerasimenko et al., 2015; Hofstoetter et al., 2018; Murray and Knikou, 2019).

4.3. cTSS in ALS

This is the first study to report cTSS in individuals with ALS. Like SCI, ALS comprises a mixture of degenerating and spared motor circuits in the spinal cord (in addition to neurodegeneration in layer V of the motor cortex). Unlike SCI, ALS features diffuse, ongoing lower motor neuron degeneration. The combination of diffuse upper and lower motor neuron degeneration reduces motor responses in individuals with clinically established ALS using non-invasive stimulation techniques such as TMS and peripheral nerve stimulation (Chervyakov et al., 2015; Shibuya et al., 2017). Likewise, we have observed very high or unobtainable TMS thresholds, as well as difficulty obtaining F-responses using peripheral nerve stimulation, in many of the ALS subjects we have tested to date among various studies in our laboratory. Therefore, it is striking how easily motor responses have been obtained in ALS individuals using cTSS. Including other ongoing studies in progress in our laboratory, cTSS responses have now been easily obtained in all 9 subjects with ALS that have undergone cTSS to date, whereas suitable TMS and peripheral nerve stimulation responses have been obtained from just 7 of 15 ALS subjects to date. This suggests that cTSS can trigger motor responses via afferent sensory circuits, which are spared in ALS – likely paralleling the hyperreflexic responses to tendon stretch so often seen in these patients on clinical examination.

4.4. Circuit mechanism

Two observations suggest that the cathode-posterior, anode-anterior cTSS configuration reported here is able to activate both dorsal afferent and ventral efferent root fibers. First is the intensity-dependent change in muscular response latency. Second is the partially intensity-dependent change in post-activation depression (PAD).

4.4.1. Response latency

At intensity levels near RMT, we observed that APB muscle response latencies were ~2.5 ms longer than at intensity levels near 200% of RMT. The same trends were noted at more proximal wrist and elbow flexor muscles. Similar findings of reduced response latency with increased stimulus intensity have been observed with application of lumbar TSS (Minassian et al., 2007; Gerasimenko et al., 2015; Sayenko et al., 2015). In fact, at the highest stimulation intensities used in our study, response latencies averaged 1 ms shorter than PMCT as calculated by peripheral F-wave latencies. This closely correlates with the findings of a seminal mechanistic study by Mills and Murray (Mills and Murray, 1986). In that study, small 1 cm electrodes were used for cTSS, with the cathode placed over C7-T1, and the anode 6 cm rostrally in the posterior midline. All stimuli were delivered at intensities sufficient to elicit maximal compound muscle responses. Through various latency comparisons and collision experiments, they convincingly demonstrated that this form of cTSS excited efferent nerve roots 2 to 4 cm distal to motor neuron cell bodies, independent of synapses.

4.4.2. PAD

Homosynaptic PAD occurs when presynaptic large-diameter afferents are unable to release sufficient neurotransmitter when fired in quick succession (Hultborn et al., 1996). Therefore, high levels of PAD imply activation of motor neurons transsynaptically via large-diameter afferents. Low levels of PAD imply activation of efferent motor axons non-synaptically. Multiple lumbar TSS studies have observed strong leg muscle PAD at interstimulus intervals of 30–50 ms, but without systematically varying stimulus intensity (Minassian et al., 2007, 2016; Roy et al., 2014; Hofstoetter et al., 2018; Murray and Knikou, 2019). Using a 40 ms interstimulus interval over a range of intensities, we observed that APB PAD percentages were 30–35% higher at intensity levels near RMT than at intensity levels near 200% of RMT. Even at lower intensities, PAD was incomplete, averaging roughly 45% in able-bodied and SCI individuals. Interestingly, the trend of less PAD at increasing stimulus intensities was not present, and perhaps even reversed, in other recorded muscles at both the C8-T1 (ADM) and more rostral segmental levels (FCR (C6-C7) and biceps (C5-C6)).

Milosevic et al. also observed incomplete PAD (roughly 40–50%) at multiple hand and arm muscles with a 50 ms interstimulus interval in their cTSS study (Milosevic et al., 2019). Recording over

proximal arm muscles such as biceps, triceps, and extensor carpi radialis, Einhorn et al. did not see significant PAD in 12 of 13 able-bodied volunteers undergoing cTSS at 40 ms interstimulus intervals (Einhorn et al., 2013). The stimulus intensity in that study was 120% of motor threshold, a level at which we observed depression in the APB but not other muscles. In summary, these data argue that the posteroanterior cTSS configuration used here caused mixed activation of afferent and efferent axons to the APB muscle at lower stimulus intensities, and predominantly efferent axon activation at higher stimulus intensities (Danner et al., 2011; Gerasimenko et al., 2015; Sayenko et al., 2015). In individuals with varying degrees of cervical spinal cord injury and post-surgical changes, the transition from dorsal to ventral root activation seems to occur at more variable intensity levels.

4.5. Limitations

In addition to the relatively small number of subjects, especially those with ALS, tested in this study, there was variability in the number of subjects that underwent each testing condition. This especially limited the power of observations regarding proximal arm muscle responses. The ALS observations can only be considered exploratory or demonstration of principle. Furthermore, motor thresholds were determined only for the 'target' APB muscle, which was defined as the dominant hand for AB subjects and generally the stronger hand for SCI or ALS subjects. Given that cTSS activates muscles bilaterally at multiple levels, it would be informative to systematically define cTSS thresholds at each muscle and conduct parallel experiments at multiples of each muscle's individual threshold. Likewise, peripheral motor conduction times were not measured at more proximal muscles in this study. High-intensity electrical stimulation at Erb's point would have provided more direct evidence to support our conclusions in the response latency experiments (Mills and Murray, 1986). Finally, subject heights and neck circumferences were requested retrospectively, with incomplete responses.

Despite these limitations, our study is the first to our knowledge to apply cTSS using a posteroanterior configuration and report safety and preliminary mechanistic outcomes in subjects with SCI and ALS. The posteroanterior cTSS configuration is well tolerated and easily activates upper extremity muscles in individuals with or without responses to transcranial magnetic stimulation. Posteroanterior cTSS likely activates motor neurons both transsynaptically via large-diameter afferents, and non-synaptically via efferent motor axons. Due to the non-invasive nature of this form of stimulation, it will be critical to model how the resulting electrical fields travel through the multiple layers of tissue and fluid between the surface electrodes and neural structures. Ongoing experiments are investigating how cTSS interacts acutely with other forms of non-invasive neurostimulation and with concurrent volitional muscle contraction.

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Declaration of Competing Interest

The investigators have no conflicts of interest to declare.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinph.2019.11.056.

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